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Improving post-harvest survivability of southern rock lobster in a changing environment

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In submitting this report, the researcher has agreed to FRDC publishing this material in its edited form.

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Executive Summary

What the report is about

This report details the results of a multifaceted research program led by the Institute for Marine & Antarctic Studies, University of Tasmania in collaboration with the School of Animal and Veterinary Sciences, The University of Adelaide. The work was undertaken to better evaluate post-harvest mortality of Southern Rock lobster (SRL) and investigate tools and processes to improve lobster holding within the SRL processing and exporting industry sector. The research was conducted to investigate a recent apparent increase in post-harvest mortality of SRL across the entire SRL industry sector in Tasmania, South Australia and Victoria. The research program was comprised of dedicated investigations of the physiology and pathology of moribund lobsters and the epidemiology (or patterns and associated factors) of lobster mortality across the industry sector and within case facilities. Complimentary research was conducted to inform and educate industry on "best practices" for post-harvest maintenance of live SRL and to develop practical extension tools to determine quality and vitality of captive SRL stock.

Background

The export of southern rock lobsters (SRL, *Jasus edwardsii*) from southern Australia to markets in Asia is one of Australia's most valuable fisheries. The export industry is an important component of the market supply chain as it links the fishery with the international markets. During the 2016 season, SRL holding and exporting facilities in southern Australia apparently experienced high levels of mortality which far exceeded normal/historical levels of mortality. Levels of mortality was reported as very severe with losses in the hundreds of $1,000s reported by several exporting companies. High levels of post-export mortality have also been described which was damaging to the reputation of the Australian Southern Rock Lobster brand. Considering the apparent severity and widespread nature of this event, an in depth and concerted effort was warranted to determine the cause of the mortality and to develop strategies to minimise its impact in future fishery seasons.

Here we present a 2-year study which focused on characterising the mortality conditions in SRL holding and the development of mitigating strategies to minimise its impact on the lobster exporting and fisheries industries. This project used the disciplines of epidemiology, physiology and pathology which together examined distinct but complimentary lines of investigation which directly assist and build on each other with the aim of establishing the cause(s) of the mortality.

Aims/objectives

1. Undertake an epidemiological investigation to describe the magnitude of the event and to identify potential environmental and management risk factor(s) associated with increased mortality;

2. Examine the underlying physiological processes or mechanisms resulting in lobster mortality and potential links with marine biotoxins;

3. Full review of the pathology from both the Tasmanian and South Australian mortality events during the 2016 season as well as further characterisation of any significant pathologies (e.g. antennule gland changes) observed in these investigations as well as further pathological investigations for the 2017season.

Methodology

Lobster haemolymph biochemistry studies investigated the physiological status of healthy and moribund lobsters to determine whether the underlying cause of mortality could be identified. Comparisons based on holding time within facilities and a reflex-based vitality score were made over a range of 22 biochemical parameters of collected haemolymph samples. These parameters included electrolytes, minerals, ions, metabolites and enzymes.

Immunology research characterized the immune response of healthy and moribund lobsters to elucidate if unexplained mortality in commercial holding was likely resulting from disease infection. The research developed and validated novel immunological methodologies for SRL. Immunological findings from moribund lobsters were compared to newly landed apparently healthy lobsters (negative control), apparently healthy lobsters after extended in holding and lobsters with a known disease infection (shell necrosis- positive control).

A sector wide survey was conducted to explore the magnitude of sub-optimal survival experienced by facilities and to identify potential associated management and capacity related risk factors. A custom questionnaire was designed to collect information on capacity, bio-filter, water and stock management within SRL holding facilities. Facility visits and face-to-face interviews with facility manager/owners were conducted in South Australia, Victoria, and Tasmania. In absence of reliable stock and survival data across surveyed facilities, the case definition for sub-optimal survival was based on the perceived change in survival rates in recent seasons.

Cohort studies using on stock and mortality data were conducted at two SRL volunteering holding facilities during the 2016-17 fishing season to investigate batch-level factors associated with survival. In one of these facilities, a data collection system was designed to trace incoming stock batches from arrival until exit.

Microbiological assessments were undertaken of SRL showing morbidity or mortality. Submissions underwent a full diagnostic work up consisting of total haemocyte counts, haemolymph biochemical analysis, microbiological analysis, gross necropsy and histological assessment.

The diagnostic pathology component of the project investigated whether there was a common pathology seen in moribund animals. The work was undertaken using a common veterinary diagnostic format for each submission. Each SRL was treated as a unit of investigation and a range of diagnostic modalities were performed on each animal (i.e. gross examination, haemolymph collection and analysis, microbiological examination of haemolymph and gross lesions, histopathology and virus rule out). The methodology for these examinations is detailed within another chapter of this project report entitled ‘Southern Rock Lobster (SRL) (*Jasus edwardsii*) Health Assessment Procedure Manual’.

Clinical chemistry investigations were conducted in-order to refine health assessments of SRL. A clinical biochemical reference interval for haemolymph was developed using 34 healthy SRL. To compliment this, 32 chemical parameters in 8 specific tissues of 23 SRL were measured to enable

appropriate interpretation of the various parameters. Additionally, a multi-laboratory validation was undertaken to strengthen the accuracy of results and the species-specific reference intervals produced.

A comparison of a range of water quality testing approaches was conducted to provide the industry with information about their fitness-for-purpose. The study compared the accuracy, ease-of-use and cost of methods raging from cheap and simple test kits to more sophisticated spectrophotometers across key water parameters including pH, ammonia, nitrite, nitrate, alkalinity and phosphorus.

Analysis was conducted to evaluate the novel use of a handheld lactate meter as a practical stress test for the SRL fishing and processing industry sectors. Lactate builds up in SRL as a by-product of stressors such as excessive activity, emersion, temperature or hypoxia stress and measurement of lactate concentration in the haemolymph is a useful diagnostic marker for stress and a prognostic marker for mortality in lobsters.

The best practice guide was developed to standardise optimum handling and processing of SRL during post-harvest processes by the SRL processing and export industry sector. The document provides a guideline for industry best practice from landing to international live product export and presents recommendations for best practice during key industry practices including handling, periods of emersion, land transport, grading, holding and finally air freight transport. The recommendations made to industry in this report incorporate the FRDC project findings. The guide also highlights key knowledge gaps in industry processes and provides direction for future research priorities. The best practice guide has been presented to the FRDC SRL Clean Green Program (2017-224) and is being used as the basis for the development of a processors sector operations standard and audit guideline.

The project presented Best Practices Workshops to the SRL Processors Industry. These one-day industry workshops were offered to SRL industry members in Hobart and Mount Gambier in October 2018. The workshop covered the current state-of-the-art knowledge of a wide range of critical lobster holding industry principles, presented an update on the FRDC project progress, provided an analysis of water quality measurement tools and presented a new tool for SRL stress assessment (hand held lactate meter). The workshops also gathered feedback from industry members about industry concerns and future research priorities.

Results/key findings

Haemolymph biochemistry showed significant profile variability within and between sampled animals, including reference lobsters experiencing no mortality. The absence of distinct biochemical profile between animals group suggested that the experienced mortalities was not the result of a single cause or a single pathogen. Findings indicate an association between low haemolymph pH with low lobster vitality or time in holding suggesting a lack of capacity for lobsters to recover from stressful post-harvest practices that induce metabolic stress, such as transport emersion and handling. Water testing at lobster holding facilities found that while nutrient levels were generally within acceptable range, pH, salinity and possibly alkalinity show considerable variability between facilities and from sea water reference ranges. These findings suggest a synergistic role of the stress incurred through post-harvest practices and sub-optimal physicochemical water quality on SRL mortality in holding.

Immunological findings show a distinct immunological response of lobsters with a known disease condition compared to the other lobster groups investigated. Finding suggest unexplained moribund lobsters were not suffering from pathogenic bacterial infection and that these lobsters were moribund for other non-infectious reasons, such as inappropriate water quality or handling practices.

The industry wide survey found that sub-optimal survival was reported in 42% of participating facilities. A comparison across facilities revealed a wide range of holding practices and that facilities with intensive stocking management were more at risk of experiencing lower survival during the 2015-16 season. However, further within facility investigation is needed to identify which specific practices are leading to sub-optimal survival.

Batch level investigations found that sub-optimal survival was associated with post-landing transport time or distance suggesting a need to develop improved systems and technologies to reduce emersion and transport related physiological stress. Sub-optimal survival was also associated with season (being worse during the warmer months), lobster size, lobster colour and stocking volume. Industry implication associated with these risk factors are discussed.

Microbiological assessments from haemolymph culture across submissions over 2016 -2017 frequently found *Vibrio spp* and specifically *Vibrio tapetis* (*V. tapetis*) in a number of cohorts. Genetic phylogeny of the *V. tapetis* revealed associations with a point source and there were no significant associations between *Vibrio* isolation and morbidity and mortality in submitted animals (i.e. there were a range of pathologies associated).

Pathological investigation found that despite a range of pathologies being observed in submitted SRL samples, there was no common pathology observed across states and facilities. Poor health and survivability were the only clinical presentations noted by submitters and whilst this may be common to all diagnostic cases there could be a range of causes for this presentation in the SRL.

Clinical chemistry results showed that tissue enzyme activity of SRL did resemble that of terrestrial vertebrates and other crustaceans however significant differences were also observed. In determining a clinical biochemical reference interval chemical changes were noted in some analytes when haemolymph samples were frozen at -80ºC compared to fresh. Therefore, separate reference intervals were created for these analytes to ensure adequate interpretation of fresh and frozen SRL haemolymph biochemistry.

Water quality testing comparison identify the strengths and weaknesses in the fitness-for-purpose of each approach. The findings show that the API test kits represent an easy to use, economic and moderately accurate tool for regular water quality analysis in routine. For facilities looking to advance their accuracy and precision, the YSI and Hach spectrophotometers offer highly valid results. Recommendations on the parameters and frequency of testing are made to provide the SRL industry with a framework for developing improved water monitoring regimes.

Lobster haemolymph lactate concentrations reported by the Lactate Scout+ handheld meter correlated strongly with the lactate concentrations measured by reference laboratory procedures. The results suggest that handheld lactate meters can be developed as useful and practical tool to measure lobster stress condition at any stage of post-harvest chain of custody. Recommendations are provided for the further validation of the lactate and the refractive index assessment tools to provide industry with superior evaluation matrix for determining the condition and competency of lobsters for holding and live export.

Implications for relevant stakeholders

Collectively, the physiological and pathological investigations did not support the systematic association of a detectable crustacean pathogen with lower post-harvest SRL survival. However, a field survey suggested a limited holding capability in some exporting facilities leading to sub-standard stock survival and impacting commercial processes. Anecdotal reports from industry suggest an increased sensitivity of lobsters in recent seasons and the project hypothesis is that the animals are experiencing reduced holding capacity and increased mortality rates post capture as a result higher physiological stress, reduced immune function and then overwhelming infections with secondary bacteria or loss of physiological maintenance leading to death. However, further investigations, particularly on the role of potential unknown viral pathogens, is required to completely rule-out an infectious aetiology.

The cause for the apparent sensitivity remains unclear, which could be related with environmental stress, such as increasing water temperatures. The south east coast of Australia has been identified as an ocean warming hotspot. The impact of this environmental change on SRL physiology currently remains unclear and should form the basis of future research. The project is particularly concerned that environmental change may be impacting lobster moult cycles resulting in weak pre- and post-moult lobsters being landed during the fishing season. Currently there are no practical and accurate methods available for industry to identify pre- and post-moult lobsters. The project strongly recommends that improved tools for the assessment of lobster moult cycle and stress condition are developed and made available to industry stakeholders across the entire post-harvest chain of custody.

Investigation revealed considerable differences in industry live lobster management practices and apparent levels of mortality across the sector. These findings suggest a role of post-harvest practices for improving lobster performance in holding. Physiological and epidemiological investigation particularly highlight the potential for transport related emersion, stocking practices and sub-optimal physicochemical water quality (particularly low pH) to impact SRL mortality in holding. Mortality appears most severe during the warmer months during summer likely due to the heightened metabolic demands of lobsters associated with higher temperatures. The project strongly recommends that the Australian SRL industry focusses dedicated effort towards optimising post-harvest practices from wild capture to international export.

The project has taken significant steps towards facilitating improved industry practice through:

- Initial validation of improved lobster quality assessment tools, handheld lactate meter
- Assessment of water quality measurement tools
- Industry education on best practices through industry workshops
- The development of an SRL processors industry best practice guide
- Development and validation of new immune function assays for SRL
- Development of SRL haemolymph biochemical profiling capacity in Australia
- Development of an SRL health assessment procedure manual

In conclusion, the SRL industry is in a period of change and the industry must adapt to this change. The industry is changing in terms of its business model, political atmosphere and physical

environment, all of which heighten the requirement for optimal post-harvest processes. The increased value of lobsters and reliance on live export has meant that even low levels of stock losses can have significant financial or SRL brand consequences. The political atmosphere is changing as consumer perceptions on the health and welfare of lobsters is increasingly becoming an important marketing issue. Finally, SRL exists in a global warming hot spot which appears to be placing new challenges for appropriate post-harvest processes. It is clear that the ultimate outcome for a lobster is dependent on the sum of its experiences through the entire post-harvest chain of custody. This project has focused on practices by the processing and holding industry sector. For optimum performance of SRL from capture to market, requires further research on other aspects of post-harvest processes, including the fishing industry and post export maintenance sectors.

Anecdotal evidence suggests the industry has accepted the need for improve industry practices and several companies have made efforts to improve operations. Feedback from industry suggests that levels of mortalities across the sector have reduced in recent seasons which may be because of these improved maintenance practices. Attached to this final report is an impact statement from one of Australia's largest SRL processors and export companies which details how the company pro-actively acted on project findings/recommendations and implemented numerous changes to company practices. In the most recent 2018-19 season, this company has experienced a 50% reduction in mortality that may be attributed to these improved practices. A 50% reduction to mortality loss across the industry sector represents a FRDC return for investment of 3-fold in just a single year and 30-fold over a decade.

Recommendations

The project findings suggest a need for improved industry practices, particularly in the key areas of:

- Post capture transport, particularly on land dry transport procedures
- Post transport recovery and purging procedures
- Holding facility aquaculture systems
- Water quality monitoring and maintenance
- Live lobster handling procedures
- Data collection and stock traceability
- Stock quality assessments

Details regarding these recommendations are provided in this report and to industry within the "Best Practice" guide.

The project gathered feedback from industry members about industry concerns and future research priorities. The project identified 19 knowledge gaps or potential future research priorities regarding best post-harvest practice for live SRL. Written feedback on priority ranking of these research priorities were received from industry participants. Based on this feedback the top ranked future research priority for the post-harvest maintenance of live SRL was:

- The development of improved tools and validation of condition/vitality assessments and relationship to survivability during holding, including Brix, lactate meter, reflex responses

Further to the ranked priorities, another priority which was not ranked at the industry workshop but was brought up by industry participants as a high priority was:

- The assessment and refinement of on-vessel handling and maintenance processes to improve post capture lobster condition/vitality/survivability

The project supports these two research priorities as they extend best practice across the entire chain of post-harvest custody from capture to export and will result in tools that will inform fisherman and processors on the quality of the stock and suitability of maintenance practices. We consider the next most important research priority would be the development of improved transport technologies and procedure to limit emersion stress on lobsters.

Keywords

Southern Rock Lobster, *Jasus edwardsii*, post-harvest, processors and exporting sector, physiology, pathology, health, epidemiology, aquaculture systems.

Contextual Statement

Result chapters reported within this document have been structured as individual scientific manuscripts be submitted to separate journals or as activity reports. These chapters have been written in the structure used for the targeted journals and include a specific abstract, introduction and methods section for each manuscript. However, the formatting of each chapter has been updated to reflect a consistent style throughout this report. Each chapter begins with a preface highlighting related project objection. The report is prepared in the style used by the FRDC for Final Reports and includes a common introduction, experimental approach (in lieu of a specific methods chapter which is detailed within individual manuscripts) and discussion section.

Introduction

The export of southern rock lobsters (SRL, *Jasus edwardsii*) from southern Australia to markets in Asia is one of Australia's most valuable fisheries and currently accounts for approximately 3,500-4,000 tonnes with a gross revenue of approximately AUD200 million. The export industry is an important component of the market supply chain as it links the fishery with the international markets. The ability to maintain or hold lobsters for extended periods is imperative for the industry to capitalize on fluctuating market demand and gain maximum return for the fishery product. During the 2016 season, SRL holding and exporting facilities in southern Australia apparently experienced high levels of mortality which far exceeded normal/historical levels of mortality. Mortality was characterized by a sudden loss of vitality followed by death after several days in holding without other signs of gross pathology. The mortality did not appear to discriminate between sex, size, colour or source of lobsters, with only time in holding, appearing to be a factor affecting rate of mortality. Degree of mortality was reported as very severe and in excess of 10% of pre-export stock per day in some facilities with losses in the hundreds of $1,000s reported by several exporting companies. As a consequence, the industry has been forced to change operating practices and on-sell lobsters soon after landing placing further strain on revenue. High levels of post-export mortality have also been described by purchasers which was damaging to the Australian Southern Rock Lobster brand. This damage has been reported to have already resulted in a significant drop in the international price for Australian lobsters which combined with losses due to mortality is placing significant strain on the viability of the Australian lobster export industry. It is feared that continual poor performance of exported live lobsters may trigger key Asian markets to place a moratorium on the Australian product that could jeopardize the entire fishing industry. Considering the severity and widespread nature of this event an in depth and concerted effort is warranted to determine the cause of the mortality and to develop strategies to minimize its impact in future fishery seasons.

Lobster mortality events at holding facilities in Southern Australia were independently and repeatedly investigated during 2016. The Institute for Marine & Antarctic Studies (IMAS) and Biosecurity Tasmania investigated a large mortality event at holding facilities in the north-west of Tasmania during January and February, 2016. This investigation focused on pathology and toxicology but failed to find any evidence of disease agents, tissue dysfunction or metal toxicity. The University of Adelaide investigated a similar problem at facilities in South Australia. Similarly, this investigation found no consistent evidence of pathology which could explain the high levels of mortality. In May 2016, IMAS and Biosecurity Tasmania investigated a Hobart based event and again found no clear evidence of a causative agent or physiological dysfunction. These findings suggested a complex and undefined cause to the problem which required intensive investigation in order to pin point the likely cause.

The severity of the problem prompted an exporter meeting which was held at Tasmanian Seafood Industry Council on the 26th May 2016. The three largest exporting companies all reported unusually high and debilitating mortality during the 2016 season. High levels of mortality were also reported by several smaller exporters from across both Tasmania and South Australia. The apparent wide spread nature of this mortality indicated that this was an industry wide problem which had the potential for significant flow-on effects to the Australian lobster fishing industry.

Here we present a 2-year study which focused on characterizing the mortality conditions in SRL holding and the development of mitigating strategies to minimize its impact on the lobster exporting and fisheries industries. This study examined the 3 areas of epidemiology, physiology and pathology and involved expert collaborators from IMAS and The University of Adelaide. These three research areas examined distinct but complimentary lines of investigation which directly assist and build on each other with the aim of establishing the cause of the mortality. Collectively they examined the three key areas of the mortality condition including, the environment in the epidemiological investigations, the host in the physiological investigations and the potential of a causative agent in the pathological investigations. Considering that the preliminary investigations failed to establish many leads as to the cause of the mortality this holistic approach was imperative to disentangle this unresolved mortality condition.

Objectives

Undertake an epidemiological investigation to describe the magnitude of the event and to identify potential environmental and management risk factor(s) associated with increased mortality

Examine the underlying physiological processes or mechanisms resulting in lobster mortality and potential links with marine biotoxins

Full review of the pathology from both the Tasmanian and South Australian mortality events during the 2016 season as well as further characterisation of any significant pathologies (e.g. antennule gland changes) observed in these investigations as well as further pathological investigations for the 2017season.

A project variation was approved on the 9/07/2018 to remove the research on the "links with marine biotoxins) to be replaced with;

- An assessment of methods for water quality testing in Southern Rock Lobster holding
- An assessment of a practical lobster stress indicator tool (lactate meter)
- Industry "Best Practice" education workshops in Tasmania and South Australia.

Redirection of efforts were based on project findings on the role of management practices on lobster performance and agreed at the project steering committee held in March 2018. Biotoxin analysis was removed as a priority as this research is now being addressed in the FRDC project 2017-086, "Improved risk management of paralytic shellfish toxins in Southern Rock Lobster". Project PI (Fitzgibbon) is heavily involved in the biotoxin research which involves large experimental trial examining the impacts of biotoxin on SRL health, physiology and performance.

A project variation was approved on 18/01/2019 which involved additional extension outputs from the project as follows;

- Basic Best Practice Guide for SRL Post-Harvest Operations (water quality, tank and stock management etc)
- A SRL Post-Harvest Best Practice Standard (this will inform further alterations to the current post-harvest portion of the Clean Green Standard)
- Relevant Templates for Data Collection, focussed on two areas: quality parameters and traceability parameters

Method

At the beginning of the project a Steering Committee was formed to seek expert guidance and ensure project progress communication to the relevant stakeholders. The steering committee was comprised of representatives from Southern Rocklobster Limited, The Tasmanian Rock Lobster Processors Association, The South Australian Rock Lobster Advisory Council, The Victorian Rock Lobster Association, Primary Industries and Regions SA, Department of Primary Industries Water and Environment, and Agriculture Victoria. The committee met on an annual basis throughout the project.

To address the aims of this project a diverse range of experimental methodological approaches were used. Due to the diversity and complexity of methodological approached employed detailed methods are provided within the individual results chapters.

Results 1: Biochemical Investigations

Preface

This aspect of the project was undertaken to partly address objectives 2 of this study:

- Examine the underlying physiological processes or mechanisms resulting in lobster mortality

Manuscript information

Investigating the physiological basis of post-harvest mortality in Southern Rock Lobsters (*Jasus edwardsii*)

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Abstract

Post-harvest mortality in rock lobster fisheries represent a substantial economic loss to the industry and can damage the fishery brand. In this study, unexplained mortality events in industry processing facilities holding Southern Rock Lobster (*Jasus edwardsii*) were investigated to characterise and compare the physiological status of healthy and moribund lobsters to determine whether the underlying cause of mortality could be identified. Comparisons based on holding time within facilities and a reflex-based vitality score commonly used in the industry were made over a range of 22 biochemical parameters of collected haemolymph samples. These parameters included electrolytes, minerals, ions, metabolites and enzymes. Every experiment, including a baseline experiment without mortalities, showed significant changes in some aspect of haemolymph biochemistry, with the breadth of biochemical changes varying between experiments. This lack of a consistent response between experiments indicated that the mortalities were not the result of a common cause such as a pathogen, and that they were likely the result of local water conditions or lobster handling practices. One consistent finding between experiments was the association between low haemolymph pH with low lobster vitality or time in holding. These findings suggest a lack of capacity for lobsters to recover from stressful post-harvest practices that induce metabolic stress such as emersion and handling. Water testing at lobster processing facilities found that while nutrient levels (i.e. ammonia, nitrite, nitrate) were generally within acceptable limits, physicochemical properties such as pH, salinity and possibly alkalinity show considerable variability between facilities and, more importantly, from sea water reference ranges. These findings suggest a synergistic role of the stress incurred through post-harvest practices that result in metabolic acidosis and sub-optimal physicochemical water quality on SRL mortality in holding. These findings highlight a severe knowledge gap

regarding the chronic effects of holding conditions and the synergistic effects of stressors throughout the harvest and post-harvest process. Further research into these areas will provide much needed evidence for the development of industry best practices to increase efficiency through the reduction of avoidable post-harvest mortalities.

Introduction

Rock (or spiny) lobsters are important fisheries around the world, representing the highest value single species fishery for a number of countries (Winterbottom et al., 2012) due to its status as a luxury seafood item. This economic value has important socioeconomic impacts, as rock lobster fisheries provide a high quality food source domestically, a high value export good and generate direct and indirect employment (Winterbottom et al., 2012).

In Australia, spiny lobster fisheries are worth nearly AU$700 million, accounting for nearly 40% of the total wild caught fisheries production by value (Mobsby and Koduah, 2017). The Southern Rock Lobster (SRL; *Jasus edwardsii*) is an important component of the fishery industry of three states: South Australia, Tasmania and Victoria. Together, these fisheries produced an annual catch of between 4,500-5,000 tonnes historically in the 1980s-1990s (Linnane et al., 2013) and around 3,100 tonnes more recently (Plagányi et al., 2018). The recent decrease in annual total allowable catch for the industry is the result of a strictly regulated quota system which is intended to maximise rents from the fishery via payments from fishers to quota owners rather than tonnage of catch (Gardner et al., 2015). Rents (and economic yield) currently equate to more than 60% of the revenue, which is achieved by maintaining small catches, high catch rates, efficient harvesting and high market price (Ogier et al., 2018), all of which is enhanced by reducing mortalities.

It is the latter that will be focused on here, as waste in the lobster industry represents mortalities occurring along the supply chain and the SRL industry relies heavily on the overseas live export market, with China as the destination for a majority of the production (Norman-López et al., 2014). Losses at processors and the arrival of moribund or deceased lobsters decrease confidence in the quality of the industry and damage the reputation of the brand. The impact of brand damage was demonstrated recently when the SRL processing industry experienced a series of large scale mortality incidents over the 2015-2016 fishing season that led to a decrease in wholesale price that cost the industry AU$25 million (Simon et al., 2016).

Research into reducing within the SRL fishery has historically focused on quantifying the impacts of emersion on a variety of physiological parameters (Taylor and Waldron, 1997; Morris and Oliver, 1999a; b; Speed et al., 2001), as air exposure is a common stressor during catching, processing and live export. Following the 2015-2016 mortalities, Simon et al. (2016) quantified the effect of emersion on an extensive suite of haemolymph biochemistry parameters to evaluate observation from the SRL industry that brindle lobsters that come from deeper habitats and have a mottled white and red shell are more sensitive to the stress of transport than red lobsters from shallow habitats. While no difference was found between the colour forms, the authors reported a range of differences in haemolymph biochemistry associated with mortality.

While the type of blood biochemistry panel used by Simon et al. (2016) has routinely been used to assess condition in mammals, its development for use in marine invertebrates is recent. Haemolymph biochemistry analysis has been used to attempt to predict mortality in spiny lobsters (Paterson et al., 2005), evaluate nutritional condition (Simon et al., 2015) and evaluate SRL exposed to seismic air gun signals (Fitzgibbon et al., 2017).

Here, mortality events in SRL processing facilities was investigated using the haemolymph biochemistry approach established by Simon et al. (2016) and used by Fitzgibbon et al. (2017) to quantify the impact of seismic surveys on SRL. Utilising both experimental and reactive approaches, the haemolymph biochemistry of healthy and moribund lobsters was compared to test whether a consistent physiological basis for mortality could be identified. In a pair of experiments, lobsters were sampled weekly to evaluate how haemolymph biochemistry changed over the course of holding time. In one of these experiments, the final sample week coincided with a mortality event at the facility. In addition to the experimental approach, a pair of reactive sampling experiments were

conducted at facilities experiencing mortalities to allow for comparison with haemolymph parameters from healthy lobsters.

Materials and methods

Experimental design

Experiment 1: Baseline holding of lobsters

To develop a baseline understanding of the impact of holding in the processing facility may have on haemolymph biochemical parameters, haemolymph samples from 34 male lobsters were collected over the course of 4 weeks according to the sampling procedure described below. Sampling was conducted in June and July 2016 at a single Tasmanian processing facility and stocked according to standard facility procedures. Lobsters were placed into vented crates at a density of 12 lobsters per crate and maintained in the facilities recirculating system at a water temperature of 12°C. The first sample point was one day after arrival at the facility to allow the lobsters to acclimate following transport to and processing at the facility. A total of 13 lobsters were randomly selected and sampled, after which they were euthanised via immersion in an ice/sea water slurry. A further 6 lobsters were randomly selected, sampled and then euthanised after a nominal week (8-10 days post processing), two weeks (15-17 days) and three weeks (22-23 days) and then 3 individuals were sampled at four weeks (28 days).

Experiment 2: Transported moribund lobsters

During a 2016 mortality event, 8 moribund lobsters (7 male, 1 female) and 4 apparently healthy lobsters (4 male), as assessed using a behavioural vitality score (Stoner, 2012), were obtained from a Tasmanian processor. These lobsters were packaged for transport following the industry standard procedure for live export described by Simon et al. (2016), in which lobsters were packed into the bottom of a 20 L polystyrene box lined with a foam, wood wool was placed on top of those lobsters, another layer of lobsters were packed into the box, a second layer of wood wool was added and then two 1 L ice packs were placed on top. Packed as such, the lobsters were then transported to the Biosecurity Tasmania Animal Health Laboratory in Mt Pleasant, Tasmania for assessment. As a part of this assessment, haemolymph samples were taken according to the procedure described below and processed as in the previous experiments. As these lobsters were emersed for *ca*. 2.5 h during transportation, the blood parameter results from this experiment cannot be directly compared to those of the other experiments comprising this study.

Experiment 3: Weekly sampling culminating in mortality event

Beginning in December 2017 at a lobster processing facility in Tasmania, a cohort of 36 male lobsters obtained from a single source were stocked according to standard facility procedures as previously described. Lobsters were tagged and placed into vented crates at a density of 12 lobsters per crate and maintained in the facilities recirculating system at a water temperature of 12°C. From this cohort, 5 lobsters were randomly selected and tagged for serial sampling. Over the 4 weeks the experimental sampling was conducted, the 5 tagged lobsters and 5 fresh, randomly selected lobsters from the cohort were sampled according to the procedure described below. In the final week of the experiment, sampling was conducted two days early as a mortality even was occurring. As lobsters were removed each week, new lobsters were added to the crates to maintain a constant stocking density. These lobsters were not tagged in order to differentiate them from the study sample.

In this experiment water quality testing was performed each week as lobsters were sampled. Water from the recirculating system was tested for pH using a Testo 205 handheld pH probe. Temperature and dissolved oxygen were measured using a Hach IntelliCal Rugged optical LDO probe (LDO101) and salinity was measured using a Hach IntelliCal Rugged Conductivity probe (CDC401), both of which were connected to a Hach HQ40d meter. Ammonia (Hach Nitrogen-Ammonia TNT AmVer

Low Range 2604545), nitrite (Hach NitriVer 3 TNT Low Range 2608345) and nitrate (Hach NitriVer 5 Nitrate Reagent Powder Pillows 2106169) were measured on a Hach DR1900 spectrophotometer.

Experiment 4: Mortality event reactive sampling

In April of the 2016-2017 season, reactive sampling was conducted at five different processing facilities in the Tasmanian and South Australian SRL fisheries as they were experiencing mortality events. In each facility, 5 moribund lobsters (4 male and 1 female in all cases) were sampled for haemolymph biochemistry as described below.

At each facility reactively sampled following mortality events, water quality was analysed as above in Experiment 3. In addition, facility visits were conducted across the Tasmanian and South Australian Southern Zone processing industry. During these visits, water quality was analysed as described in Experiment 3.

Table 1. Summary of sample sizes for each treatment and lobster size, weight, sex and moult stage for each experiment in this study. N.B. Moult status estimated through time of year lobsters were caught, pigmentation of haemolymph and observation of carapace fouling by epibionts.

Experiment	Treatment/Site	Number of lobsters	Carapace length (mm)	Weight (g)	Sex	Moult stage (Estimated)
1	Week 1	13	114.8 ± 0.1	716.8 ± 16.1	13 ♂	Late intermoult (C_4)
	Week 2	6	n.d.	n.d.	6 ♂	Late intermoult (C_4)
	Week 3	6	n.d.	n.d.	6 ♂	Late intermoult (C_4)
	Week 4	6	121.5 ± 3.8	822.0 ± 63.6	6 ♂	Late intermoult (C_4)
	Week 5	3	120.7 ± 4.3	862.3 ± 83.7	3 ♂	Early premoult (D_0-D_1)
2	Moribund	9	134.4 ± 0.4	1059.3 ± 76.3	8 ♂ 1 ♀	All males intermoult (C_4), female premoult (D_0-D_1)
	Healthy	4	137.5 ± 0.8	1825.0 ± 355.0	4 ♂	All intermoult (C_4)
3	Week 1	10	118.9 ± 2.8	883.2 ± 68.3	10 ♂	All intermoult (C_4)
	Week 2	5	114.3 ± 2.1	674.7 ± 23.1	5 ♂	All intermoult (C_4)
	Week 3	5	113.0 ± 0.8	728.7 ± 19.6	5 ♂	All intermoult (C_4)
	Week 4	5	142.4 ± 3.5	1504.8 ± 129.7	4 ♂ 1 ♀	All intermoult (C_4)
4	Site 1	5	1126.2 ± 89.1	n.d.	4 ♂ 1 ♀	All intermoult (C_4)
	Site 2	5	1172.0 ± 101.8	n.d.	4 ♂ 1 ♀	All intermoult (C_4)
	Site 3	5	n.d.	n.d.	4 ♂ 1 ♀	All intermoult (C_4)
	Site 4	5	1492.6 ± 514.8	n.d.	4 ♂ 1 ♀	All intermoult (C_4)
	Site 5	5	862.3 ± 83.7	n.d.	4 ♂ 1 ♀	All intermoult (C_4)

Sampling

From each individual in the 4 experiments comprising this study, a 3.5 ml haemolymph sample was collected from the base of the distal walking leg using a sterile 5 ml syringe fitted with a 26 gauge needle. Syringes and needles were pre-chilled on ice to prevent coagulation. Haemolymph samples were aliquoted into three 1.5 ml centrifuge tubes.

From the first tube, pH was measured using a Testo 205 temperature compensated pH probe and the refractometery index was measured using a Hanna Instruments digital refractometer zeroed using distilled water (HI96801). The other two haemolymph tubes were centrifuged at 10,000 x *g* for 5 mins, after which the supernatant was removed using a pipette, transferred to a 2 ml cryotube and stored on ice while transported to the laboratory at the Institute for Marine and Antarctic Studies Taroona facility (ca. 2 h), where they were frozen and stored in a -80°C freezer. Samples were then shipped in a dry shipper charged with liquid nitrogen to the Diagnostic Services laboratory at the Atlantic Veterinary College, University of Prince Edward Island, Canada, and analysed using a Cobas c501 automated biochemistry analyzer (Roche Diagnostics Corporation, Indianapolis, IN, USA) for a full blood profile consisting of the electrolytes (mmol L−1) sodium (Na), chloride (Cl), potassium (K), magnesium (Mg) and bicarbonate (bicarb); minerals (mmol L−1) calcium (Ca) and phosphorus (P); metabolites (mmol L−1) glucose (Gluc), lactate (Lact), cholesterol (Chol), triglyceride (Trig), total protein (TP, in g L−1), urea, and uric acid (Uric, in μmol L−1); enzymes (U L−1) lipase (LIP), amylase (AMY), alanine (ALT) and aspartate (AST) aminotransferases, alkaline phosphatase (ALP), sorbital (SDH) and glutamate (GDH) dehydrogenases, and γ-glutamyl transferase (GGT). Several metabolite (i.e. lactate) and enzyme (i.e. amylase, lipase, alanine, aspartate aminotransferase, alkaline phosphatase and sorbitol dehydrogenase) results indicated that either due to the time on ice during processing or holding time in the -80°C freezer, the results were not reliable and were thus excluded from analysis.

Statistical analyses

Data from Experiment 1 were analysed using two-way ANOVA with holding time and vitality score as factors. Considering that the holding times of this study (maximum 4 weeks) were modest compared to the potential holding times over which *J. edwardsii* can be held, no relationship between holding time and vitality was expected and the interaction of the factors was excluded *a priori*. For all ANOVA analyses, normality was tested using Shapiro-Wilk tests and equality of variance was tested using Levene's test prior to conducting the analysis. If assumption of normality or equality of variance tests was violated, data were transformed as required using Tukey ladders of power (transformTukey in rcompanion package). If no appropriate transformation could be found, data were analysed using permutational ANOVA. Where either factor was found to have a significant effect, TukeyHSD post-hoc comparisons were conducted. All analyses were performed using R v3.4.1 (R Foundation for Statistical Computing).

Data from Experiment 2 were analysed using two-sample Welch t-tests to accommodate for the differing sample size between healthy and moribund treatments.

Data from Experiment 3 were analysed using one-way ANOVA with holding time as the factor. Vitality score was not used as a factor in this analysis as the only occurrence of vitality scores below 4 or 5 was the final week during a mortality event, making it unlikely that vitality was independent from holding time in this case. Otherwise, these analyses were conducted as described above for Experiment 1.

In Experiment 4, to determine whether moribund lobsters reactively sampled from mortality events had similar haemolymph parameters, two-way ANOVA with holding site and vitality score as the factors were used. Because all moribund lobsters were sampled during mass mortality events, there

was no reason to link vitality score with holding site, therefore the interaction factor was *a priori* dropped. Again, these analyses were otherwise conducted as described for Experiment 1.

Results

Experiment 1: Holding facility baseline

All mean values ± s.e.m. and statistic values of physicochemical, electrolyte and minerals in response to holding time and vitality score are given in Table 2 and Table 3, respectively. All mean values ± s.e.m. and statistic values of metabolites and enzymes in response to holding time and vitality score are given in Table 4 and Table 5, respectively.

Both holding time and vitality score were found to significantly affect haemolymph pH (Tables 1 and 2). Post-hoc analysis showed a significant increase in pH at week 4 relative to week 1 in response to holding time and failed to indicate any significant differences between vitality scores, though low vitality was associated with a low pH and high vitality was associated with higher pH.

Refractive index did not show a response to either holding time or vitality.

Amongst haemolymph electrolytes and minerals, neither holding time nor vitality score, respectively, had an effect on the haemolymph concentration of sodium, potassium, sodium:potassium ratio, chloride, osmolarity, bicarbonate, calcium, phosphorus or magnesium.

Total haemolymph protein (Tables 4 and 5), which was not affected by holding time, showed a significant response to vitality score, with greater protein in the weaker 1+2 lobsters relative to lobsters scored as 3, 4 and 5. This appeared to have been driven by a significant increase in globulin content in response to vitality score, as 1+2 score lobsters had significantly greater levels that 3, 4 and 5 lobsters. Holding time did not have a significant effect on globulin and neither holding time nor vitality significantly affected albumin or the ratio between albumin and.

Uric acid showed a significant response to holding time but not vitality, with a non-significant decrease in levels over weeks 1-4 followed by a significant increase in week 5 over the previous week. Glucose level was not affected by holding time but responded significantly to vitality, with the 1+2 lobsters showing a significantly greater concentration compared to lobsters with scores of 3, 4 and 5. Holding and vitality had no effect on cholesterol levels or triglyceride in this experiment.

γ-Glutamyl transferase was not affected by holding time or by vitality score, whereas glutamate dehydrogenase (GDH) showed a significant response to holding time and to vitality score. For the former, GDH levels showed a peak at week 3, which was significantly greater than week 1, which was followed by a return to levels similar to week 1 in subsequent weeks. In response to vitality, weak 1+2 scored lobsters showed significantly higher GDH activity than 3, 4 and 5 lobsters.

Table 2. Effect of holding time on physicochemical, mineral and ion properties of a cohort of healthy lobsters sampled over a five-week period, presented as means ± s.e.m. Significant differences between holding weeks, determined using two-way ANOVA with holding time and vitality as factors, are indicated by differing letters.

Week	pH	Brix	Na	K	Na:K	Cl	HCO_3	Ca	Phos	Mg	Osm
1 n=11	7.55 ± 0.03 a	13.0 ± 0.4	494 ± 6	7.9 ± 0.5	64 ± 3	494 ± 7	3.4 ± 0.3	17.44 ± 0.18	1.05 ± 0.18	10.51 ± 0.62	969 ± 14
2 n=9	7.56 ± 0.04 a	12.9 ± 0.7	496 ± 9	8.1 ± 0.3	62 ± 2	494 ± 11	3.0 ± 0.4	17.19 ± 0.28	1.40 ± 0.09	9.41 ± 0.30	989 ± 20
3 n=11	7.63 ± 0.04 ab	13.3 ± 0.6	509 ± 4	7.6 ± 0.3	68 ± 2	505 ± 7	3.7 ± 0.3	17.84 ± 0.42	0.88 ± 0.18	9.98 ± 0.37	1019 ± 9
4 n=6	7.74 ± 0.03 b	13.1 ± 0.6	496 ± 7	7.1 ± 0.3	70 ± 2	492 ± 8	3.2 ± 0.2	18.82 ± 0.33	1.25 ± 0.15	9.77 ± 0.25	986 ± 15
5 n=3	7.65 ± 0.01 ab	14.5 ± 0.8	504 ± 9	7.1 ± 0.3	72 ± 3	500 ± 11	3.3 ± 0.3	18.88 ± 0.75	1.31 ± 0.05	9.61 ± 0.54	1009 ± 18
Statistic	F(4,31)=5.04 P=0.003	F(4,31)=0.548 P=0.70	F(4,31)=1.19 P=0.33	F(4,31)=1.06 P=0.39	F(4,31)=1.99 P=0.12	F(4,31)=0.55 P=0.70	F(4,31)=0.83 P=0.52	F(4,31)=1.11 P=0.37	F(4,31)=1.51 P=0.22	F(4,31)=0.70 P=0.60	F(4,31)=2.20 P=0.093

Table 3. Effect of vitality score on physicochemical, mineral and ion properties of a cohort of healthy lobsters sampled over a five-week period, presented as means ± s.e.m. Significant differences between vitality scores, determined using two-way ANOVA with holding time and vitality as factors, are indicated by differing letters. Note that due to low sample size, lobsters scored as 1 and 2 were pooled together.

Vitality	pH	Brix	Na	K	Na:K	Cl	HCO_3	Ca	Phos	Mg	Osm
1 (n=4) + 2 (n=1)	7.47 ± 0.07	12.3 ± 0.9	499 ± 9	7.6 ± 0.4	67 ± 3	491 ± 11	4.3 ± 0.6	18.29 ± 0.53	1.11 ± 0.79	10.02 ± 0.79	990 ± 23
3 n=9	7.72 ± 0.03	13.4 ± 0.6	504 ± 4	7.2 ± 0.1	71 ± 1	503 ± 5	3.2 ± 0.2	18.67 ± 0.35	1.25 ± 0.27	9.66 ± 0.27	1005 ± 10
4 n=5	7.59 ± 0.02	13.3 ± 1.3	489 ± 12	7.4 ± 0.5	67 ± 4	484 ± 16	2.6 ± 0.4	16.53 ± 0.73	1.15 ± 0.43	9.78 ± 0.43	979 ± 25
5 n=21	7.60 ± 0.02	13.3 ± 0.3	500 ± 5	8.0 ± 0.3	64 ± 2	499 ± 6	3.3 ± 0.2	17.63 ± 0.14	1.08 ± 0.35	10.08 ± 0.35	991 ± 11
Statistic	F(3,31)=3.89 P=0.011	F(3,31)=0.161 P=0.2	F(3,31)=2.31 P=0.08	F(3,31)=1.32 P=0.28	F(3,31)=1.02 P=0.41	F(3,31)=2.46 P=0.066	F(3,31)=1.75 P=0.16	F(3,31)=2.01 P=0.12	F(3,31)=0.33 P=0.86	F(3,31)=0.81 P=0.53	F(3,31)=1.96 P=0.125

Table 4. Effect of holding time on metabolite, protein and enzyme concentrations of a cohort of healthy lobsters sampled over a five-week period, presented as means ± s.e.m Significant differences between holding weeks, determined using two-way ANOVA with holding time and vitality as factors, are indicated by differing letters.

Week	Glucose	Protein	Alb	Glob	A:G	Uric	Chol	Trig	GGT	GDH
1 n=11	0.5 ± 0.03	68 ± 3	7 ± 1	61 ± 3	0.12 ± 0.02	29 ± 5 ab	0.43 ± 0.04	0.41 ± 0.04	1.8 ± 0.3	5.5 ± 0.7 a
2 n=9	0.6 ± 0.02	69 ± 3	7 ± 1	62 ± 3	0.12 ± 0.02	21 ± 3 ab	0.46 ± 0.03	0.46 ± 0.04	2.6 ± 0.3	6.7 ± 0.8 ab
3 n=11	0.6 ± 0.06	69 ± 6	9 ± 1	61 ± 5	0.15 ± 0.02	19 ± 4 ab	0.50 ± 0.05	0.39 ± 0.04	2.8 ± 0.4	9.0 ± 1.2 b
4 n=6	0.5 ± 0.04	75 ± 3	10 ± 1	65 ± 2	0.15 ± 0.01	14 ± 6 a	0.56 ± 0.07	0.52 ± 0.04	2.0 ± 0.5	8.0 ± 0.4 ab
5 n=3	0.5 ± 0.06	80 ± 8	8 ± 2	72 ± 6	0.11 ± 0.02	52 ± 26 b	0.62 ± 0.04	0.56 ± 0.06	2.3 ± 0.9	6.0 ± 1.2 ab
Statistic	F(4,31)=0.10 P=0.98	F(4,31)=1.02 P=0.41	F(4,31)=1.16 P=0.35	F(4,31)=0.96 P=0.44	F(4,31)=1.19 P=0.33	F(4,29)=3.23 P=0.026	F(4,31)=1.74 P=0.17	F(4,30)=2.15 P=0.099	F(4,31)=1.70 P=0.18	F(4,31)=3.09 P=0.030

Table 5. Effect of vitality score on metabolite, protein and enzyme concentrations of a cohort of healthy lobsters sampled over a five-week period, presented as means ± s.e.m. Significant differences between vitality scores, determined using two-way ANOVA with holding time and vitality as factors, are indicated by differing letters. Note that due to low sample size, lobsters scored as 1 and 2 were pooled together.

Vitality	Glucose	Protein	Alb	Glob	A:G	Uric	Chol	Trig	GGT	GDH
1 (n=4) + 2 (n=1)	0.8 ± 0.05 a	82 ± 7 a	8 ± 2	73 ± 5 a	0.11 ± 0.02	25 ± 5	0.58 ± 0.06	0.49 ± 0.02	2.4 ± 0.4	10.8 ± 2.2 a
3 n=9	0.5 ± 0.04 b	73 ± 4 b	8 ± 1	65 ± 3 b	0.13 ± 0.02	25 ± 12	0.54 ± 0.05	0.49 ± 0.04	2.4 ± 0.5	8.0 ± 0.8 b
4 n=5	0.6 ± 0.04 b	60 ± 8 b	8 ± 1	52 ± 7 b	0.16 ± 0.02	18 ± 4	0.37 ± 0.06	0.36 ± 0.07	2.4 ± 0.2	5.4 ± 1.0 b
5 n=21	0.5 ± 0.02 b	69 ± 2 b	8 ± 1	62 ± 2 b	0.13 ± 0.01	26 ± 3	0.47 ± 0.05	0.43 ± 0.03	2.2 ± 0.3	6.3 ± 0.4 b
Statistic	F(3,31)=4.45 P=0.006	F(3,31)=4.07 P=0.009	F(3,31)=0.83 P=0.52	F(3,31)=4.45 P=0.006	F(3,31)=1.13 P=0.36	F(3,29)=1.99 P=0.12	F(3,31)=2.49 P=0.064	F(3,30)=1.89 P=0.14	F(3,31)=0.92 P=0.47	F(3,31)=4.13 P=0.009

Experiment 2: Post-transport mortality event

Amongst the physicochemical, electrolyte and mineral parameters (Table 6), potassium was the only to significantly differ, as moribund lobsters showed a 17% lower level than healthy lobsters. This change also drove a significant increase in Na:K ratio, which was 25% higher in moribund lobsters. No difference was observed in haemolymph pH, refractive index, sodium, chloride, bicarbonate, calcium, phosphorus, magnesium or osmolarity.

Amongst metabolites and enzymes (Table 7), only γ-glutamyl transferase was found to differ significantly between treatments, with moribund lobsters demonstrating an increase of 2.6 times over that of healthy lobsters. No difference was observed in the concentration of glucose, total protein, albumin, globulin, uric acid, cholesterol, triglycerides or glutamate dehydrogenase.

Table 6. Effect of vitality score on physicochemical, mineral and ion properties compared between a cohort of healthy lobsters and a cohort of moribund lobsters reactively sampled during a mass mortality event. Data are presented as means ± s.e.m. and parameters with significant differences between treatments, determined by comparison using Welch two sample t-test, are indicated by bold text.

Status	pH	Brix	Na	K	Na:K	Cl	HCO_3	Ca	Phos	Mg	Osm
Healthy n=4	7.25 ± 0.03	11.8 ± 1.5	505 ± 7	9.2 ± 0.4	55 ± 3	505 ± 9	5.0 ± 1.6	18.2 ± 0.91	1.61 ± 0.33	9.36 ± 0.43	1012 ± 15
Moribund n=8	7.33 ± 0.03	11.8 ± 0.2	518 ± 5	7.6 ± 0.2	69 ± 2	522 ± 6	4.1 ± 0.2	17.9 ± 0.84	1.00 ± 0.15	8.61 ± 0.92	1038 ± 9
Statistic	t=1.70 df=6.59 P=0.13	t=-0.06 df=3.07 P=0.95	t=1.46 df=5.42 P=0.20	t=-3.53 df=5.55 P=0.014	t=25.71 df=11 P<0.001	t=1.50 df=5.71 P=0.19	t=-0.52 df=3.06 P=0.64	t=-0.24 df=7.92 P=0.82	t=-1.68 df=4.30 P=0.16	t=-0.75 df=9.34 P=0.47	t=1.46 df=5.42 P=0.20

Table 7. Effect of health status on metabolite, protein and enzyme concentrations compared between a cohort of healthy lobsters and a cohort of moribund lobsters reactively sampled during a mass mortality event. Data are presented as means ± s.e.m. and parameters with significant differences between treatments, determined by comparison using Welch two sample t-test, are indicated by bold text.

Status	Glucose	Protein	Alb	Glob	A:G	Uric	Chol	Trig	GGT	GDH
Healthy n=4	0.8 ± 0.20	69 ± 11	7 ± 1	62 ± 11	0.13 ± 0.03	76 ± 25	0.77 ±0.25	0.70 ± 0.23	2.0 ± 0.4	11.3 ± 2.5
Moribund n=8	0.6 ± 0.13	63 ± 5	6 ± 1	56 ± 4	0.11 ± 0.01	28 ± 5	0.48 ± 0.07	0.47 ± 0.25	5.3 ± 1.0	12.5 ± 1.8
Statistic	t=-1.27 df=3.86 P=0.27	t=-0.51 df=4.17 P=0.63	t=-0.56 df=7.85 P=0.59	t=-0.47 df=3.87 P=0.67	t=-0.59 df=3.44 P=0.59	t=-1.91 df=3.29 P=0.14	t=-1.13 df=3.51 P=0.33	t=-0.90 df=4.23 P=0.42	t=3.07 df=9.01 P=0.013	t=0.41 df=6.09 P=0.70

Experiment 3: Holding and mortality event

Table 8 gives the physicochemical, mineral and electrolyte results for Experiment 3. Haemolymph pH was found to significantly differ with holding, with high pH recorded at week 1, a moderate pH at week 2, a return to high pH levels at week 3 and a low pH at week 4. Refractive index did not significantly differ by holding time.

Na concentration was significantly affected by holding time, with an increase of around 6-7% observed at week 3 relative to those of week 1 and week 4. Holding time was also a significant factor in haemolymph K concentration, with a significant 20% decrease observed at week 4 relative to week 3 levels. These differences in Na and K appeared to offset each other, as the ratio of Na:K did not significantly differ between sample weeks.

Haemolymph bicarbonate levels responded significantly to holding time, as lobsters sampled at week 4 had a 64% reduction in bicarbonate compared to week 1 lobsters and a 66% reduction compared to week 3.

Calcium levels were significantly affected by holding time, with lobsters in week 3 showing a significant, 11-12% increase over the previous two weeks before returning to a moderate level in week 4 that was not different from any of the previous sampling times.

Magnesium levels were significantly affected by holding time, with weeks 1 and 2 showing similar levels, followed by a 20% increase in week 3 to a level significantly greater than all other sampling points and then a subsequent 40% decrease in week 4 to a level significantly lower than all other sample points.

Chloride and phosphorous concentrations were not affected by holding time in this experiment.

Holding time was a significant factor in haemolymph osmolarity, with lobsters sampled in week 3 showing a significantly higher level than all other weeks. No other differences were observed between treatments.

Amongst haemolymph metabolites and enzymes (Table 9), no difference was found between sample weeks for glucose or total protein or globulin. Albumin levels, however, were found to differ significantly, with a marked decrease in week 4 compared to levels observed in week 1 and 3. This resulted in a significant difference in albumin:globulin ratio, with week 4 recording a significant 60-70% reduction from the levels of the three previous weeks.

Uric acid responded significantly to holding time, with a 2- and 3-fold increase observed at week 3 relative to week 1 and 2 values, respectively, followed by a return to a moderate level similar to the initial weeks during week 4. Neither cholesterol nor triglycerides were significantly affected by holding time.

Both γ-glutamyl transferase (GGT) and glutamate dehydrogenase (GDH) were significantly affected by holding time, as lobsters in week 4 had a level of GGT >10 times that of the previous three weeks and a level of GDH >3 times that of the previous three weeks.

The water quality (Table 10) at the facility, specifically temperature, salinity, dissolved oxygen and nutrient levels were all at levels acceptable to industry guidelines across the sampling series. System water pH, on the other hand, was considerably lower than that of seawater (pH=8.15).

Table 8. Effect of holding time on physicochemical, mineral and ion properties of a cohort of healthy lobsters sampled over a four-week period culminating in a mass mortality event (week 4), presented as means ± s.e.m. Significant differences between holding weeks, determined using one-way ANOVA, are indicated by differing letters.

Week	pH	Brix	Na	K	Na:K	Cl	HCO_3	Ca	Phos	Mg	Osm
1 n=10	7.68 ± 0.04 a	10.1 ± 0.9	452 ± 7 a	6.5 ± 0.2 ab	70 ± 2	441 ± 9	7.8 ± 0.4 a	17.80 ± 0.25 a	0.47 ± 0.19	8.45 ± 0.29 a	864 ± 13 a
2 n=5	7.52 ± 0.07 ab	9.3 ± 1.4	459 ± 8 ab	6.6 ± 0.4 ab	71 ± 5	454 ± 12	6.2 ± 0.6 ab	17.55 ± 0.46 a	0.32 ± 0.25	8.23 ± 0.29 a	878 ± 14 a
3 n=5	7.70 ± 0.02 a	11.4 ± 1.0	484 ± 7 b	7.0 ± 0.7 a	71 ± 6	478 ± 9	8.4 ± 0.8 a	19.79 ± 0.66 b	0.92 ± 0.27	10.10 ± 0.36 b	969 ± 15 b
4 n=5	7.47 ± 0.02 b	10.8 ± 1.0	454 ± 4 a	5.5 ± 0.2 b	84 ± 3	448 ± 3	2.8 ± 0.9 b	18.70 ± 0.55 ab	0.85 ± 0.30	6.01 ± 0.45 c	867 ± 8 a
Statistic	F(3,21)=5.31 P=0.007	F(3,21)=0.60 P=0.62	F(3,21)=3.62 P=0.030	F(3,21)=3.16 P=0.046	F(3,21)=3.00 P=0.053	F(3,21)=2.76 P=0.068	F(3,21)=9.54 P<0.001	F(3,21)=4.91 P=0.01	F(3,21)=2.29 P=0.11	F(3,21)=18.46 P<0.001	F(3,21)=11.07 P<0.001

Table 9. Effect of holding time on metabolite, protein and enzyme concentrations of a cohort of healthy lobsters sampled over a four-week period culminating in a mass mortality event (week 4), presented as means ± s.e.m. Significant differences between holding weeks, determined using one-way ANOVA, are indicated by differing letters.

Week	Glucose	Protein	Alb	Glob	A:G	Uric	Chol	Trig	GGT	GDH
1 n=10	1.3 ± 0.45	50 ± 6	9 ± 1 a	42 ± 5	0.23 ± 0.03 a	10 ± 3 a	0.42 ± 0.06	0.43 ± 0.09	2.2 ± 0.3 a	4.0 ± 0.7 a
2 n=5	0.9 ± 0.14	46 ± 10	7 ± 1 ab	39 ± 9	0.19 ± 0.02 a	15 ± 9 a	0.25 ± 0.05	0.24 ± 0.09	1.8 ± 0.8 a	5.8 ± 1.0 a
3 n=5	1.1 ± 0.20	67 ± 9	10 ± 2 a	57 ± 8	0.18 ± 0.01 a	33 ± 6 b	0.43 ± 0.09	0.42 ± 0.12	2.2 ± 0.5 a	8.4 ± 1.6 a
4 n=5	2.2 ± 0.95	56 ± 7	3 ± 1 b	53 ± 6	0.07 ± 0.01 b	15 ± 5 n=4 a	0.39 ± 0.07	0.40 ± 0.14 n=4	23.6 ± 7.0 b	22.2 ± 12.8 b
Statistic	F(3,19)=0.49 P=0.70	F(3,21)=1.20 P=0.34	F(3,21)=3.96 P=0.022	F(3,21)=1.45 P=0.26	F(3,20)=10.92 P<0.001	F(3,20)=4.70 P=0.012	F(3,21)=1.62 P=0.22	F(3,20)=0.81 P=0.50	F(3,12.69) P<0.001	F(3,21)=6.44 P=0.004

Table 10. Water quality data collected at the time of haemolymph sampling in a cohort of healthy lobsters sampled over a four-week period culminating in a mass mortality event (week 4).

Week	pH	Temp (°C)	Salinity (ppt)	Dissolved O_2	Ammonia (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)
1	7.70	9.1	34.1	90.7%	0.1	2	55
2	7.60	9.0	33.9	92.3%	1.1	7	60
3	7.39	9.1	34.4	92.0%	0.0	6	95
4	7.71	9.6	34.2	89.2%	0.0	4	60

Experiment 4: Reactive sampling

Comparing reactive samples collected from moribund lobsters during mass mortality events showed that the facility in which the lobsters were held had a substantial role on the haemolymph biochemistry (Tables 11, 12). Site was a significant factor for haemolymph pH, with lobsters from site 4 recording a lower pH than those from sites 1 or 2. Refractive index was not affected by facility site.

Sodium, potassium and the ratio of sodium to potassium were all significantly affected by facility site. Lobsters from sites 2, 3 and 4 had similar sodium concentrations, whereas sodium in site 1 lobsters was around 10% lower than those from site 3, a difference that was significant. Site 5 lobsters were also significantly lower than both site 3 and site 4, by a difference of around 16-17%. Site 5 lobsters also had a significantly lower potassium level than all other sites, which were similar to each other, with the difference ranging from 20-30%. This led to site 5 lobsters having the highest Na:K ratio of all sites while the other four sites showed a similar level. Chloride concentrations followed this pattern, with sites 2, 3 and 4 similar, site 1 demonstrating a moderate 13% reduction that was significantly lower than site 3 and site 5 the lowest at a level, 14-20% lower than at sites 2, 3 and 4. Calcium was significantly higher in lobsters from site 4 than in any of the other sites, but a factor of at least 26%. Magnesium concentration was similar at sites 1, 2, 3 and 4 and significantly lower in site 5 lobsters compared to sites 1, 3 and 4. These differences in electrolytes in minerals resulted in a significant difference in haemolymph osmolarity between sites, with site 5 lobsters 15-20% lower than the other four sites, which were all similar.

Bicarbonate and phosphorus did not show a significant response to site. Similarly, none of the metabolites, glucose, protein, albumin, globulin, albumin:globulin ratio, uric acid, cholesterol or triglycerides, showed a response to holding site.

The two enzmyes compared in this study, gamma-glutamyl transferase and glutamate dehydrogenase were both significantly different across sites, with GGT significantly lower in lobsters at site 1 than at sites 2 and 5 and site 5 also significantly higher than sites 3 and 4 whereas GDH was significantly higher at site 2 than at site 1.

Water quality (Table 14) at the sites sampled showed some similarities, in that all five showed pH levels lower than that expected from seawater, all sites kept their systems chilled to a similar temperature and all sites had water with lower salinity than expected from seawater. Nutrient levels varied between sites, though levels were broadly within industry guidelines for acceptable levels.

In contrast to the substantial response to holding site, vitality score (Tables 14, 15) was a significant factor in only haemolymph calcium and glucose concentrations. For calcium concentration, lower vitality was associated with higher Ca levels, as scores 1, 2 and 3 were significantly higher than 4. For glucose, lobsters with a vitality score of 2 had a significantly higher level than those with a 3. No other parameters showed a significant response to vitality score.

Table 11. Haemolymph electrolyte, ion and mineral parameters of lobsters reactively sampled during a mortality event compared by holding facility. Significant differences between holding weeks, determined using one-way ANOVA, are indicated by differing letters.

Site	pH	Brix	Na	K	Na:K	Cl	HCO_3	Ca	Phos	Mg	Osm
1 n=8	7.51 ± 0.04 a	13.5 ± 0.8	492 ± 8 ac	7.1 ±0.3 a	70 ± 2 a	483 ± 10 ac	3.8 ± 0.4	17.34 ± 0.65 a	1.15 ± 0.25	9.98 ± 0.54 a	979 ± 18 a
2 n=5	7.66 ± 0.02 a	10.9 ± 1.4	513 ± 8 ab	7.1 ± 0.4 a	73 ± 3 a	517 ± 12 ab	4.2 ± 0.7	17.86 ± 0.44 a	0.68 ± 0.29	7.78 ± 0.31 ab	981 ± 16 a
3 n=4	7.51 ± 0.08 ab	12.6 ± 1.8	547 ± 30 b	7.8 ± 0.6 a	71 ± 3 a	553 ± 42 b	5.8 ± 1.2	16.66 ± 0.64 a	0.98 ± 0.30	10.77 ± 1.57 a	1046 ± 57 a
4 n=5	7.33 ± 0.05 b	13.6 ± 1.3	538 ± 13 ab	7.7 ± 0.5 a	70 ± 3 a	556 ± 15 b	2.8 ± 0.5	23.77 ± 0.91 b	0.89 ± 0.16	9.87 ± 1.30 a	1029 ± 24 a
5 n=5	7.47 ± 0.02 ab	10.8 ± 1.0	453 ± 4 c	5.5 ± 0.2 b	84 ± 2.5 b	448 ± 3 c	2.8 ± 0.9	18.70 ± 0.55 a	0.85 ± 0.30	6.01 ± 0.45 b	867 ± 8 b
Statistic	F(4,19)=7.20 P=0.001	F(4,19)=1.26 P=0.32	F(4,19)=7.52 P<0.001	F(4,19)=5.31 P=0.005	F(4,19)=4.49 P=0.010	F(4,19)=10.44 P<0.001	F(4,19)=2.43 P=0.083	F(4,19)=21.29 P<0.001	F(4,19)=1.59 P=0.22	F(4,19)=5.96 P=0.002	F(4,19)=6.39 P=0.002

Table 12. Haemolymph metabolite and enzyme parameters of lobsters reactively sampled during a mortality event compared by holding facility. Significant differences between holding weeks, determined using one-way ANOVA, are indicated by differing letters.

Site	Glucose	Protein	Alb	Glob	A:G	Uric	Chol	Trig	GGT	GDH
1 n=8	0.8 ± 0.03	73 ± 7	77 ± 2	66 ± 6	0.11 ± 0.02	24 ± 4	0.53 ± 0.05	0.44 ± 0.04	2.6 ± 0.3 a	9.4 ± 1.6 a
2 n=5	1.3 ± 0.43	52 ± 10	4 ± 1	50 ± 12	0.09 ± 0.01	17 ± 5	0.42 ± 0.08	0.40 ± 0.10	15.2 ± 2.6 bc	34.2 ± 8.3 b
3 n=4	0.72 ± 0.07	69 ± 13	8 ± 3	61 ± 11	0.12 ± 0.05	25 ± 11	0.49 ± 0.11	0.50 ± 0.15	7.0 ± 3.0 ab	13.5 ± 3.0 ab
4 n=5	1.0 ± 0.21	78 ± 10	8 ± 2	70 ± 9	0.11 ± 0.02	19 ± 5	0.80 ± 0.19	0.83 ± 0.22	5.8 ± 2.4 ab	21.0 ± 4.2 ab
5 n=5	2.2 ± 0.95	56 ± 7	3 ± 1	53 ± 6	0.07 ± 0.01	15 ± 5	0.39 ± 0.07	0.40 ± 0.14	23.6 ± 7.0 c	22.2 ± 12.8 ab
Statistic	F(4,19)=0.34 P=0.85	F(4,19)=1.56 P=0.22	F(4,19)=1.72 P=0.19	F(4,18)=1.00 P=0.43	F(4,17)=0.47 P=0.75	F(4,18)=0.78 P=0.55	F(4,19)=2.06 P=0.13	F(4,18)=1.53 P=0.24	F(4,19)=7.89 P<0.001	F(4,19)=3.58 P=0.024

Table 13. Water quality analysis conducted at each site reactively sampled during a mortality event.

Site	pH	Temp (°C)	Salinity (ppt)	Dissolved O_2 (% Saturation)	Ammonia (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)
1	7.75	9.4	30.4	101.3	0.03	4.5	63
2	7.74	11.6	30.5	102.6	0.10	4.0	19
3	7.66	10.1	29.7	97.4	0.10	3.8	52
4	7.54	9.1	31.2	101.1	0.00	7	168
5	7.62	9.6	31.1	92.0	0.30	5	291

Table 14. Haemolymph electrolyte, ion and mineral parameters of lobsters reactively sampled during a mortality event compared by vitality score. Significant differences between holding weeks, determined using one-way ANOVA, are indicated by differing letters.

Vitality	pH	Brix	Na	K	Na:K	Cl	HCO3	Ca	Phos	Mg	Osm
1 n=4	7.51 ± 0.07	13.1 ± 0.6	492 ± 7	7.20 ± 0.2	69 ± 2	482 ± 8	4.1 ± 0.7	18.52 ± 0.62 a	0.95 ± 0.34	10.55 ± 0.75	974 ± 22
2 n=12	7.44 ± 0.05	11.6 ± 0.8	497 ± 11	6.65 ± 0.35	76 ± 3	500 ± 14	3.4 ± 0.5	19.27 ± 0.64 a	0.93 ± 0.17	7.27 ± 0.40	954 ± 22
3 n=9	7.56 ± 0.04	12.5 ± 1.1	529 ± 17	7.60 ± 0.43	71 ± 2	537 ± 22	4.4 ± 0.7	19.13 ± 1.30 a	0.96 ± 0.23	10.07 ± 1.00	1017 ± 32
4 n=2	7.55 ± 0.05	15.5 ± 0.6	474 ± 27	6.30 ± 0.0	75 ± 4	460 ± 34	3.2 ± 0.5	17.49 ± 0.41 b	0.77 ± 0.5	10.59 ± 0.78	949 ± 54
Statistic	F(3,19)=2.75 P=0.071	F(3,19)=0.57 P=0.64	F(3,19)=0.70 P=0.57	F(3,19)=1.35 P=0.29	F(3,19)=0.98 P=0.42	F(3,19)=1.36 P=0.28	F(3,19)=0.20 P=0.89	F(3,19)=4.95 P=0.010	F(3,19)=1.49 P=0.25	F(3,19)=2.68 P=0.076	F(3,19)=0.69 P=0.57

Table 15. Haemolymph metabolite and enzyme parameters of lobsters reactively sampled during a mortality event compared by vitality score. Significant differences between holding weeks, determined using one-way ANOVA, are indicated by differing letters.

Vitality	Glucose	Protein	Alb	Glob	A:G	Uric	Chol	Trig	GGT	GDH
1 n=4	0.8 ± 0.04 ab	87 ± 7	10 ± 2	77 ± 4	0.12 ± 0.03	27 ± 6	0.62 ± 0.06	0.51 ± 0.01	2.7 ± 2	12.5 ± 1.8
2 n=12	1.8 ± 0.42 b	62 ± 5	4 ± 1	59 ± 5 n=11	0.08 ± 0.01 n=10	17 ± 3 n=11	0.51 ± 0.05	0.59 ± 0.12 n=11	12.1 ± 2.1	22.4 ± 5.3
3 n=9	0.7 ± 0.06 a	65 ± 9	6 ± 2	59 ± 8	0.10 ± 0.02	20 ± 5	0.55 ± 0.13	0.45 ± 0.10	12.3 ± 5.3	20.6 ± 6.3
4 n=2	0.7 ± 0.0 ab	58 ± 23	8 ± 3	51 ± 20	0.15 ± 0.01	26 ± 5	0.35 ± 0.8	0.37 ± 0.16	2.5 ± 0.5	6.0 ± 3.0
Statistic	F(3,19)=3.19 P=0.047	F(3,19)=1.29 P=0.31	F(3,19)=1.11 P=0.37	F(3,18)=1.01 P=0.41	F(3,17)=1.72 P=0.20	F(3,18)=0.32 P=0.81	F(3,19)=0.94 P=0.44	F(3,18)=0.72 P=0.55	F(3,19)=0.06 P=0.98	F(3,19)=1.00 P=0.41

Comparing water quality data from processors across the Southern Rock Lobster fishery (Table 16) showed a considerable variation in water parameters between individual sites. Water pH ranged from 7.25-7.87, all of which are somewhat more acidic than would be expected of seawater. Salinity tended to be closer to that of seawater (*ca.* 35 ppt), though some sites showed reduced salinity. Dissolved oxygen was generally at an acceptable level and reductions tended to be transient and in response to events like adding stock or the stoppage of aeration during tank maintenance. Nutrient levels were variable, with a majority of sites found to have low nutrient loads in their water while a number of sites had unacceptably high nutrient loads.

Table 16. Water quality testing results from 16 processor facilities across the Tasmanian and South Australian Southern Zone Southern Rock Lobster fishery.

State	pH	Temp (°C)	Salinity (ppt)	Dissolved O_2 (% Saturation)	Ammonia (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)
SA	7.60	12.0	34.5	98.6	0.05	5	228
	7.70	10.8	29.9	103.6	0.00	3	110
	7.20	14.3	n.d.	89.9	0.05	6	18
	7.25	14.2	n.d.	92.1	n.d.	n.d.	n.d.
	7.69	13.8	n.d.	95.6	0.04	0.032	0.24
	7.40	15.6	31.8	94.4	0.015	0.06	1.75
	7.45	13.8	37.8	89.8	0.123	0.137	0.64
TAS	7.39	8.9	35.4	100	0.00	0.082	9.71
	7.81	15.5	33.3	91.2	0.14	0.026	2.4
	7.87	15.3	34.5	87.3	0.24	0.045	0.8
	6.79	15.8	36.5	79.4	0.09	0.007	13
	7.28	15.1	36.6	89.8	0.16	0.07	8.5
	7.71	13.8	34.6	100.0	0.14	0.0	3.3
	7.75	14.1	34.9	98.0	0.01	0.0	3.1
	7.10	13.1	35.1	93.0	0.19	0.036	7.7
	7.28	14.6	34.1	88.4	0.55	0.58	5.8

N.B.: Entries marked n.d. indicates no data available

Discussion

In this study, the haemolymph biochemistry of the Southern Rock Lobster (*Jasus edwardsii*) was investigated to determine whether any physicochemical, electrolyte, metabolite and enzyme parameters were useful indicators of mortality in the context of processing facilities.

Physicochemical parameters

Haemolymph pH is an important indicator of physiological condition, as it demonstrates a well-characterised, rapid response to stressors and recovers when the stressor is removed. This response, comprised of a combination of respiratory and metabolic acidosis, is a decrease in pH due to build-up of CO_2 and acidic metabolic by-products (e.g. lactate), respectively, in the haemolymph. This response is evidence of physiological stress (Paterson et al., 2005) and can be an indicator of mortality (Simon et al., 2016). Indeed, vitality score was a factor in Experiment 1 and when Experiment 1, 3 and 4 were pooled, as low vitality was associated with a more acidic pH. Furthermore, in Experiment 3, pH was lowest in the final week of the experiment, in which moribund lobsters were sampled.

Haemolymph pH was also significantly affected by holding time, with depressed levels in the initial two weeks of Experiment 1, as the capture, transport and processing aspect of lobster fisheries involve handling and emersion. Lobsters may take up to 96 h to recover from acidosis (Fotedar et al., 2006; Simon et al., 2015; Simon et al., 2016), so by the second week of holding, the haemolymph pH should be expected to return to unstressed levels. It is not clear why haemolymph pH remained low but is likely the result of some other aspect of processing in the facility, such as emersion during the grading process in which lobsters are sorted by weight, during the moving of stock or maintenance of tanks or due to changes in the water parameters of the holding tank. The impact of these external factors is further demonstrated in Experiment 4, where site was a significant factor affecting haemolymph pH but vitality was not.

It is likely that the system water pH levels, which were considerably lower than seawater in nearly all the systems measured, had some effect on haemolymph pH, though it is not clear what that effect might be. The ability of crustaceans to buffer haemolymph is well understood (see below), but the impact of chronic exposure to acidic water has not been studied. It is possible that holding lobsters in reduced pH water may have a detrimental effect, as lobsters may incur the energetic demands of maintaining acid-base balance. Given how common low pH was in these facilities, there is a clear need to better understand what is contributing to the low pH levels and whether chronic exposure to low pH levels has an impact on SRL.

Refractive index measures the concentration of protein in the haemolymph and has been shown to correlate well with nutritional condition in SRL (Simon et al., 2015) and in other lobsters (Ciaramella et al., 2014) and other crustaceans (Moore et al., 2000), with low values reflecting poor nutritional condition. The amount of protein in the haemolymph is strongly influenced by moult cycle, with increasing levels approaching the moult as nutrient stores are mobilised followed by a rapid reduction immediately after the moult as the haemolymph is diluted by the uptake of water (Smith and Dall, 1982). In contrast to the findings reported by Simon et al. (2016), in which an elevated refractive index was associated with mortality following extended emersion, refractive index was not a significant factor in any of the experiments of the present study, indicating that it was not an effective indicator of mortality. These contrary results may be explained by the consideration that sampling of moribund lobsters in the present study was opportunistic and did not control for moult cycle.

Electrolytes

Generally, lobsters have limited osmoregulatory capacity and largely conform to the ionic concentrations of the water they are in. Sodium and chloride ions are, perhaps unsurprisingly, tightly coupled in lobster haemolymph under normal conditions (Dove et al., 2005) and, along with calcium

concentration, respond readily to environmental conditions (Dall, 1974) or stress (Paterson and Spanoghe, 1997; Paterson et al., 2005), whereas phosphorus, magnesium and potassium concentrations, on the other hand, are more tightly regulated and do not respond to changes in water osmolarity (Speare et al., 1996; Paterson and Spanoghe, 1997; Paterson et al., 2005). Increases in some regulated electrolytes, such as potassium and magnesium, have been suggested to be from cellular leakage, indicating cellular damage (Paterson et al., 2005). On the other hand, a majority of haemolymph phosphorus in crustaceans is present as phospholipids and has been found to correlate with triglycerides and cholesterol, with moult cycle and nutritional condition driving concentrations rather than cell damage (Simon et al., 2015). Similarly, bicarbonate levels in the haemolymph are linked to the concentrations of magnesium (Whiteley and Taylor, 1992; Simon et al., 2016) and calcium (Burnett, 1988; deFur et al., 1988), as these minerals are the cations mobilised with bicarbonate from the cuticle to buffer against haemolymph acidosis.

Much of the work investigating haemolymph constituents in crustaceans has focused on the effects of emersion which affects haemolymph homeostasis primarily through dehydration and the maintenance of haemolymph acid-base balance. The results reported here do not indicate that any one mechanism was affecting the lobsters in the present study, as perhaps the most notable characteristic here was the lack of similarity in the responses of each experiment. In Experiment 1, electrolyte concentrations were not affected by either vitality score or holding time, indicating that these lobsters should indeed serve as an effective baseline for healthy lobsters processed in accordance with industry standards. In Experiment 2, potassium levels in moribund lobsters were found to be decreased relative to healthy lobsters, which also altered the sodium:potassium ratio between these lobsters. Although the emersion during transport of these lobsters complicated comparisons between this experiment and the others of this study, it is important to note that the potassium level of the healthy lobsters in this experiment was substantially higher (*ca*. 25% higher) than that observed in healthy lobsters in the other experiments, whereas potassium in the moribund lobsters in this experiment was similar to that of healthy lobsters in the other experiments. Considering that increases in potassium indicate cellular damage and are associated with mortality (Paterson et al., 2005), a potential mechanism to explain the increase in the healthy lobsters is that these lobsters responded to emersion during transport with increased respiration, hypercapnia and acidosis leading to damage of the gill tissue (Taylor and Waldron, 1997; Morris and Oliver, 1999a), whereas the moribund lobsters did not have the physiological capacity respond to the added stress.

In Experiment 3, electrolyte and mineral levels were affected in two of the four weeks comprising the sampling schedule. In week 3, sodium, calcium and magnesium were significantly elevated, which also drove an increase to haemolymph osmolarity. In week 4, pH, potassium, bicarbonate and magnesium levels were depressed, while sodium, calcium and osmolarity had returned to levels similar to those observed in the initial two weeks. These results suggest that the lobsters sampled in week 3 had experienced osmotic stress that resulted in dehydration or an analogous response and that by week 4, the response to that stress left the lobsters physiologically compromised. Given the results of Experiment 1 indicate that holding time within a facility should not be expected to play a role in such a response, external factors within holding must be considered. The results of Experiment 4, in which moribund lobsters across several holding facilities were reactively sampled in response to mass mortality events, provide some insight into these factors. In this experiment, a range of haemolymph parameters varied in response to sampling site but not by vitality score. Indeed, only calcium levels were a significant factor when vitality was considered, with a small number (n=2) of relatively strong lobsters with a vitality score of 4 showing somewhat lower levels of calcium in the haemolymph than lobsters scored 1, 2 or 3. The considerable variation between facilities indicates two important considerations regarding these mortalities: first, that the lack of a consistent response between facilities indicated that mortality was not being caused by a singular cause, e.g. a response to a pathogen, and, second, that significant variations in haemolymph parameters that are not tightly regulated within lobsters (i.e. Na, Cl) indicate that there is substantial variation in the environmental conditions between facilities. Measurements of water quality of these facilities taken concurrently with sampling showed that in all cases, although the facility water had acceptable nutrient loads (i.e.

low ammonia, nitrite and nitrate levels), it differed from natural seawater considerably, mainly through reduced pH and potentially alkalinity (RDD & QPF, unpublished data). There is currently no data available on the chronic effects of water quality in rock lobsters so it was not possible to draw any definitive conclusions as to whether the physiological responses of haemolymph minerals and electrolytes observed in these experiments were the result of environmental stress for the facilities' water, but the lack of a conserved response across facilities eliminated a single, common factor such as a pathogen from consideration.

Metabolites

Hyperglycaemia, or increased glucose concentration, is a common response to a range of stressors, including emersion (Lorenzon et al., 2007), parasite infestation (Stentiford et al., 2001), and thermal and salinity stress (Chang et al., 1998). Viral infection, on the other hand, has been shown to decrease haemolymph glucose (Li et al., 2008), reflecting a depletion of energy stores and metabolic exhaustion. In the present study, glucose levels, even in moribund samples, were generally comparable to control treatments in other studies of stress in lobsters (Morris and Oliver, 1999a; Lorenzon et al., 2007; Simon et al., 2016). In Experiment 1, lobsters with low vitality scores were found to have elevated haemolymph concentrations, consistent with the general crustacean response to stress. It is important to note that this elevated level was quite modest compared to the increases reported in response to emersion stress in SRL (Morris and Oliver, 1999a; Simon et al., 2016), indicating that this was likely not indicative of some severe stress response in this experiment, which was a baseline and did not involve any mortality events.

Protein levels fluctuate with moult cycle, building up as lobsters approach moulting and then falling drastically with the influx of water post-moult (Dall, 1974). The two constituent protein assayed in the present study were "albumin" and globulin. Albumins do not actually occur in lobsters, and the measurement is better described as non-globulin protein, primarily comprised of haemocyanin (Dove et al., 2005), the copper-containing blood protein that carries oxygen in the haemolymph of invertebrates and is the functional analogue to vertebrate haemoglobin. Globulins are an assemblage of serum proteins that perform a number of roles in mammals, such as immune function, blood clotting and liver function, though their role in crustaceans has not been well studied. In all of the experiments comprising the present study, albumin protein was about the same as reported for juvenile SRL whereas globulin, and as a consequence, total protein, were considerably higher (Simon et al., 2015). SRL also showed that feeding significantly increased total protein and emersion had no effect (Simon et al., 2016). Viral infection did not impact protein levels in *Panulirus argus* (Li et al., 2008), suggesting that protein levels may not be effective indicators of health status.

Indeed, in the present study, only one experiment showed a difference in total protein levels, which was the baseline Experiment 1 without any associated mortality. In this experiment, low vitality lobsters were found to have significantly higher total protein levels due to a significantly higher globulin concentration. As this experiment was not associated with mortality and considering the time of year these lobsters were sampled, it is likely that these differences were a function of the moult cycle, with weak, low vitality lobsters showing high protein levels due to dehydration as they approach moulting (Dall, 1974).

In Experiment 3, the albumin:globulin ratio was low in the moribund lobsters sampled in week 4, which was driven by a significantly reduced albumin level. This drop in "albumin" was not observed in any other experiment in the present study, so while it is possible that it was related to the moribundity in these lobsters, as such a drop would be detrimental to oxygen transport, it was not a generalised response across experiments in this study and was likely the result of some local environmental condition.

Cholesterol and triglyceride concentrations in SRL have been found to correspond to nutritional condition (Simon et al., 2015) but were not affected by emersion (Simon et al., 2016), leading to the

conclusion that these parameters are useful for tracking nutritional status without the stresses of catching and handling confounding the results. The results presented here are in agreement with that conclusion, as there were no differences found in these metabolites in any of the experiments comprising this study, which is not surprising given that lobsters in this study were held in a fasted state.

Uric acid is produced from the breakdown of purines in food and as part of the turnover of nucleic acids and converted to ammonia for excretion through the gills (Claybrook, 1983). It has also been found to increase as lobsters approach moulting (Simon et al., 2015), though this was observed in juvenile SRL and may not necessarily occur in adults. Emersion has also been shown to increase uric acid concentration in SRL (Simon et al., 2016), as excretion is not possible in the air, leading to accumulation.

Uric acid generally showed a high level of variation between individuals within treatments in each of the experiments in the present study, with significant differences found in two experiments. The first, in Experiment 1, showed that uric acid was affected by holding time, with lobsters sampled in week 5 showing significantly elevated levels. However, this was an artefact of low sample size (n=3) and one extreme outlier, which had a concentration 4 times greater than the other two lobsters at that week, both of which were on par with the lobsters sampled at other weeks and in the other experiments reported in the present study. The most likely cause for this sudden increase in a single individual is cannibalism, which is known to occur in holding conditions. In Experiment 3, uric acid was significantly elevated in week 3 of the experiment and was again largely the product of somewhat extreme values in two individuals. While two cases of lobsters feeding through cannibalism cannot be dismissed, the osmotic stress demonstrated in these lobsters may indicate they suffered a decrease in gill function and the capacity to excrete waste. It is also possible that these impacts were simply the result of some process involved with the holding of the lobsters, such as emersion during movement between tanks. Developing a better understanding of how industry practices may impact physiological function requires further study and will be an essential element for the development of best practices for the SRL industry in particular and all fishing and aquaculture industries more broadly.

Enyzmes

γ-Glutamyl transferase (or transpeptidase) is a membrane bound enzyme that plays an important role in the metabolism and transfer of glutathione, an antioxidant that functions in numerous roles throughout a range of tissues across animals, plants, bacteria and archaea (see review by Meister and Anderson, 1983)). In humans, elevated levels of GGT are considered a marker for oxidative stress resulting from liver damage, heavy metal toxicity and a range of diseases including cancer and heart disease (Fentiman, 2012). Compared to vertebrates studies, data on the role and function of GGT in marine invertebrates is scarce, but it has been reported that GGT activity was highest in the waste excreting antennal gland in the freshwater crayfish *Procambarus clarkii* (Almar et al., 1988), with relatively high levels also found in the hepatopancreas, which is involved in digestion. Furthermore, GGT in the hepatopancreas of *P. clarkii* decreased significantly in response to fasting, suggesting it is not exclusively involved in secretory function, but in absorption as well (Almar et al., 1988). Considering the role of GGT in secretory and absorptive systems in vertebrates (Meister and Anderson, 1983), an analogous role appears likely.

In the present study, GGT was significantly elevated in moribund lobsters in Experiments 2 and 3. In Experiment 4, holding facility was a significant factor, with two sites showing elevated levels, both of which correlated to facilities with significant changes in electrolyte concentration. In previous assays of GGT activity in SRL, lobsters left moribund following emersion showed a significant increase over controls (200-300 U/L in moribund) with levels much higher (200-300 U/L in moribund) than observed in the present study (2-24 U/L) (Simon et al., 2016) and in an experiment evaluating the effects of seismic exposure on SRL, GGT was recorded with comparatively low

activities (0.86-2.00 U/L) with exposure having no effect on activity levels (Fitzgibbon et al., 2017). Infection with PaV1 virus failed to elicit any response in the Caribbean spiny lobster *Palinurus argus* (Li et al., 2008) and exposure to sea lice pesticide did not affect GGT in the American lobster *Homarus americanus* (Dounia et al., 2016). These results indicate that GGT is likely upregulated in response to oxidative stress, as it is in mammals (Zhang et al., 2005), making it a potentially interesting, though somewhat specific marker of stress for marine invertebrates requiring further study to characterise activity levels, functions and its usefulness as an indicator of stress and mortality.

Glutamate dehydrogenase (GDH) likely originates in the hepatopancreas or muscle tissue (Battison, 2006) and is central to amino acid and ammonia metabolism, as it is responsible for catalysing the transdeamination of glutamate to form NH4+ and α-ketoglutarate, with the former is excreted as waste and the latter used as a substrate in the Krebs cycle and in the synthesis of non-essential amino acids (Claybrook, 1983). This reaction is reversible, and in the opposite direction, results in the synthesis of glutamate, which is itself a substrate for the synthesis of alanine and proline, which crustaceans use for osmoregulation through the accumulation of these (and other) osmolytes as a balance against the osmotic pressure of seawater (Wright, 1995). In the American lobster *Homarus americanus*, baseline glutamate dehydrogenase activity levels span a range of 10-34 U/L (Battison, 2006), and in the present study, *J. edwardsii* demonstrated a largely similar range, with a tendency toward the lower end.

Although GDH activity levels were significantly different in several of the experiments in the present study, its usefulness as an indicator of mortality is not entirely clear. Elevated activity levels were associated with low vitality in Experiments 1 and 3, the latter of which sampled moribund lobsters, but not in Experiments 2 and 4, both of which also sampled moribund lobsters. Furthermore, in Experiment 4, although vitality was not a significant factor in GDH activity, holding facility site was, which suggests GDH activity may be reflecting water osmolarity differences more than directly reflecting health status. Along similar lines, in a previous study on the effects of seismic air gun exposure on *J. edwardsii*, GDH was found to increase significantly in lobsters 120 days post-exposure, though the increase was observed in both control and exposed treatments (Fitzgibbon et al., 2017), indicating it was not related to the exposure. Changes in salinity have previously been shown to increase GDH levels in two species of prawn, with both the magnitude and the speed of the response corresponding to the magnitude of the salinity change (Li et al., 2011; Wang et al., 2012). Furthermore, Li et al. (2011) suggest that the utilisation of amino acids for osmoregulation through GDH activity may negatively impact growth due to the reduced capacity for amino acid metabolism when they are preferentially being used as osmolytes, a factor that could also play a role in SRL that are held fasted and being subjected to osmotic stress. With this in mind, it GDH activity and its role in osmoregulation in SRL may be an important indicator of stress in holding and a better understanding of this parameter is warranted.

Conclusion

Comparison of the lobsters sampled in the course of the present study demonstrated that post-harvest mortality in lobster processing and holding facilities did not have a common, systemic basis indicative of disease such as a virus or pathogenic microbial infection. Rather, mortality events appear to have disparate causes that were facility and temporally specific. These results suggest that some element of facility practices underpinned these mortality events. Consideration of the nature of the physiological responses for each parameter in each experiment, which have been summarised in Table 17, along with familiarity with the practices of the Southern Rock Lobster industry and the reported data on water quality tests conducted at these facilities suggests that the underlying issue is one of water management. Although nutrient levels (i.e. ammonia, nitrite, nitrate) within facilities were generally good, physicochemical properties such as pH and salinity show considerable variability between facilities and, more importantly, from sea water reference ranges. A limited set of alkalinity measurements (data not shown) suggest highly variable levels, making understanding the

levels across the industry and their impact on lobsters, particularly during chronic exposure, an important area to investigate. A further consideration is that practices that leave lobsters emersed for extended periods of time may be contributing to the overall stress load, potentially exacerbating impacts from water quality. Although it is not possible to definitively conclude from the present study that mass mortality events caused, in part or in full, by these variations in water parameters and handling stress, these remain the most probably candidates. These findings highlight a severe knowledge gap regarding the chronic effects of holding in these conditions and the synergistic effects of stressors throughout the harvest and post-harvest process. Further research into this space will provide the evidence necessary to develop recommendations for industry best practices and improve the efficiency of the Southern Rock Lobster industry.

Table 17. Effect of vitality score on lobster haemolymph biochemical parameters across the four experiments comprising this study. Dashes indicate that no change was observed and arrows indicate a significant change in the parameter in the direction indicated by the arrow. Where different cohorts (i.e. two groups of lobsters not sharing a vitality score) demonstrated changes in differing directions, the change demonstrated by the lower vitality score (i.e. weaker lobster) is shown.

Parameter	Experiment 1	Experiment 2	Experiment 3	Experiment 4
pH	↓	—	↓	—
Refractive index	—	—	—	—
Na	—	—	—	—
K	—	↓	↓	—
Na:K	—	↑	↑	—
Cl	—	—	—	—
HCO_3	—	—	↓	—
Ca	—	—	—	↑
Phos	—	—	—	—
Mg	—	—	↓	—
Osm	—	—	—	—
Glucose	↑	—	—	↑
Protein	↑	—	—	—
Albumin	—	—	↓	—
Globulin	↑	—	—	—
A:G	—	—	↓	—
Uric	—	—	—	—
Chol	—	—	—	—
Trig	—	—	—	—
GGT	—	↑	↑	—
GDH	↑	—	↑	—

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Results 2: Immunology Investigations

Preface

This aspect of the project was undertaken to partly address objectives 2 of this study:

Examine the underlying physiological processes or mechanisms resulting in lobster mortality

Manuscript information

The impact of holding stressors on the immune function and haemolymph biochemistry of Southern Rock Lobsters (*Jasus edwardsii*)

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Abstract

Lobsters are fished world-wide due to their status as a high value, luxury seafood. A large proportion of the product is sold via live export, with lobsters subject to a range of stressors during holding post-capture. Improving the current understanding of the immune response to these stressors assists in improving efficiency and reducing loss in the chain between capture and consumption. In this study, the immune status of four treatment groups of Southern Rock Lobster (*Jasus edwardsii*) were studied: controls recently landed from a fishing boat, lobsters displaying advanced shell necrosis, lobsters in an unexplained moribund state and lobsters held in a processing facility for 10 weeks in standard conditions (i.e. high density, fasted). A total of 15 immune parameters and 19 haemolymph biochemical parameters were assayed. Phenoloxidase activity was only sporadically observed in haemocyte lysate and was consistently observed at a low level in the plasma with no difference between treatments for either. Haemocyte lysate prophenoloxidase activity was detected in most individuals, with no differences found between treatments. Prophenoloxidase in the plasma showed the highest level of activity, with the shell necrosis treatment demonstrating an elevated activity level relative to the other three treatments. Cell viability was not affected in any treatment. Lobsters with shell necrosis had a reduced capacity for phagocytosis, a significantly higher total haemocyte count, fewer hyalinocytes and more granulocytes and semigranulocytes. Fasted lobsters showed an opposite shift, with significantly more hyalinocytes compared to the other treatments and very few granulocytes and semigranulocytes. The balance of a range electrolytes, minerals metabolites and enzymes were affected in shell necrosis and fasted treatments, raising them as potential markers for immunocompromised lobsters. Multivariate analysis of all assayed parameters showed that all individuals in the necrosis treatment showed a similar, distinct immune response and that the fasted treatment, along with one control and one moribund individual, showed a separate intermediate response. The remainder of the control and moribund lobsters demonstrated a distinct “non-response” in comparison. These results offer a characterisation of the physiological response to common challenges during post-capture holding of rock lobsters, demonstrating the differential

response to pathogenic bacterial infection, long term fasting, non-specific moribundity and the stress of capture and transport.

Keywords: crustacean, physiology, hemocytes, innate immunity, aquaculture

Abbreviations

SRL – Southern rock lobster

PO – phenoloxidase

ProPO – prophenoloxidase

HLS – haemocyte lysate supernatant

CL – carapace length (mm)

CTL – control treatment

NEC – necrosis treatment

MOR – moribund treatment

FAST – fasted treatment

Introduction

The crustacean immune system is a nonspecific, innate system, with responses including clotting, recognition of foreign particles, phagocytosis, melanisation and cytotoxicity mediated by the functions of circulating haemocytes. Three main crustacean haemocyte cell types are widely recognised: hyalinocytes, semigranulocytes and granulocytes [1]. Hyaline cells are small and spindle shaped, lacking cytoplasmic granules and are considered the primary phagocytic cell type [2]. To deal with foreign particles that are too large to be phagocytosed, the crustacean immune system has a chemical response – the phenoloxidase (PO) system. This system is mediated by the granulocytes, which contain a large number of cytoplasmic granules that contain prophenoloxidase (proPO), the zymogen precursor to PO, and the semigranulocytes, which contain a variable number of small cytoplasmic granules containing PO activating enzymes. When the PO system is initiated through the detection of lipopolysaccharides, β-1,3-glucans or peptidoglycans [3], the granulocytes and semigranulocytes degranulate and exocytose proPO and the activating enzymes, respectively [4]. Factors released as a part of the activated PO system function to aggregate semigranulocytes onto the foreign cell, creating a nodule and encapsulating the cell, and then it is destroyed through the generation of cytotoxic quinones and melanin [2].

Understanding the immune function of crustaceans can be an important tool for enhancing the value of wild catch fisheries. Crustaceans are subjected to a range of stressors as they pass through the supply chain, including injury or limb loss, emersion, changes in water quality and fasting during transport and holding. Of particular interest are rock lobster (Palinuridae) fisheries, as these are considered a high value luxury seafood commodity and are fished throughout the tropical and temperate seas around the world, with an annual global value of over US$2 billion [5]. Much of this value is reliant on the supply of live product to overseas markets. Australia has four rock lobster fisheries, the Western Rock Lobster (*Panulirus cygnus*), the Tropical Rock Lobster (*Panulirus ornatus*), the Eastern Rock Lobster (*Sagmariasus verreauxi*) and the Southern Rock Lobster (SRL; *Jasus edwardsii*). The latter industry is important in the Australian states of South Australia, Tasmania and Victoria, comprising 28%, 11% and 27% of those states' total gross fishery revenue, for a combined US$160 million from an annual catch of 3000 tonnes [6]. The annual total allowable catch for the industry is strictly regulated via a quota system which is intended to maximise rents from the fishery (seen as payments from fishers to quota owners) rather than tonnage of catch [7]. Rents (and economic yield) currently equate to more than 60% of the revenue, which is achieved by maintaining small catches, high catch rates, efficient harvesting and high market price [8], all of which is enhanced by reducing mortalities. Fewer mortalities effectively raises the productivity of the system which means that harvesting is more efficient. Mortalities in the supply chain also affect price because the SRL industry is reliant on live export to sell over 75% of the annual catch, predominantly to China [9]. In this market the presence of mortalities has greater impact than the direct loss of product because of the effect of reputational damage and also by limiting the ability of processors to hold product to time peaks in market demand.

To improve the understanding of how catching and processing affects the immune function of SRL held in processing facilities, the present study compared a range of immune and biochemical parameters between lobsters that experienced one of several common stresses. First, a cohort of lobsters recently caught, transferred from a fishing boat and processed through a facility served as a control to which other treatments could be compared. Having this control group go through the process of transport, grading and purging at the facility was important to reflect the treatment that all lobsters entering into a processing facility will have undergone. Second, a cohort of lobsters showing advanced shell necrosis was analysed to provide a positive control. Shell necrosis is a common bacterial infection that presents initially as black (e.g. melanised) pitting of the shell surface that can progress to large lesions penetrating into the body [10]. Third, a cohort of lobsters held in standard conditions at a processing facility for 10 weeks was evaluated to determine the effect of long-term

fasting on immune function. Although 10 weeks of holding represented an extreme situation within current industry practices, SRL have shown considerable ability to survive fasting, with one study reporting the loss of 35% of stock following 30 weeks of fasting [11]. Finally, a cohort of moribund lobsters that appeared healthy when originally processed into the facility and did not demonstrate any outward signs that explained their decline in health were analysed. To compare these treatments, a range of haemolymph parameters were assessed including the pH and refractive index, phenoloxidase and prophenoloxidase activities in both the haemocytic and plasma fractions of the haemolymph, haemocyte viability, phagocytic capacity, total and differential haemocyte counts and protein concentrations of the haemolymph. In addition, haemolymph biochemistry panels previously used to assess stress in SRL [12, 13] were performed, quantifying concentrations of electrolytes and metabolites. Comparing these parameters between lobsters experiencing known and unknown stressors was done to provide a more thorough understanding of immune function in SRL, particularly in regards to the immunological stresses they face following capture and holding.

Methods

Animals and experimental conditions

All laboratory work was conducted at the University of Tasmania's Institute for Marine and Antarctic Studies (IMAS) site at Taroona, Tasmania. For lobsters obtained from processing facilities, lobsters were packaged at the facility according to industry standard procedures into a polystyrene box and covered with wood wool and transported to IMAS. Upon arrival, lobsters were placed into a tank of seawater obtained from the flowthrough system (temperature *ca.* 16°C) and allowed to acclimate for 1 hour prior to sampling with a battery operated air pump providing aeration (Aqua One 250C, 150 L/h). Following sampling, lobsters were euthanised via immersion in an ice/seawater slurry.

Four treatments of lobsters were compared in this study: control (CTL), shell necrosis (NEC), fasted (FAST) and moribund (MOR). CTL lobsters (n=5 males, 113 ± 1 mm mean carapace length (CL) ± SEM and 695 ± 23 g mean mass ± SEM) were obtained from a local lobster processing facility 1 day after they had been landed from a fishing vessel to represent the immune function of lobsters in the wild with a brief acclimation time to recover from the stress of capture and transport. NEC lobsters (n=5 males, 116 ± 2 mm CL and 789.9 ± 26.7 g mass) displayed advanced shell necrosis following 6 months holding in tanks at IMAS and were used to serve as a positive control to analyse the response of lobsters suffering a challenge to the immune system. FAST lobsters (n=5 males, 111 ± 2 mm CL and 662.7 ± 26.3 g mass) were obtained from a local processing facility following 10 weeks of holding in conditions standard to the industry: 12°C water temperature, contained crates at high density (approximately 20 lobsters per m^2) and not fed for the duration of holding. This treatment was included to evaluate the impact of long term holding and fasting on the immune function of lobsters. Finally, MOR lobsters (n=3 males, 112 ± 0.3 mm CL and 661.3 ± 11.5 g mass) were opportunistically collected following two weeks of holding at the late stages of a mortality event at a local processor to characterise whether moribund lobsters demonstrated any immune response that may be indicative of a pathogenic causative agent. These lobsters had no outward signs of injury or infection that would suggest a cause for their state and had previously been assessed with a high vitality score at landing to the processing facility. CTL and MOR lobsters were sampled in August and September 2017 and were identified as intermoult based on the lack of epibiotic growth on their carapaces indicating they had previously moulted. NEC lobsters were known to have not moulted based on observations during their holding at IMAS and thus were considered to be approaching pre-moult status. FAST lobsters were sampled in May 2018 and were considered to be approaching pre-moult status based moult timing in *J. edwardsii* [14].

Collection of haemolymph

Lobsters were removed from their holding tank, placed ventrum up on a bench and haemolymph was drawn from the sinus at the base of the posterior walking leg using a pre-chilled 1 ml syringe fitted

with a 22 ga needle. A sample of haemolymph was measured for pH using a handheld pH probe (Testo, 205) and refractive index (Brix) using a digital refractometer zeroed with distilled water (Hanna Instruments, HI96801). Haemolymph was aliquoted at a 1:1 ratio into pre-chilled citrate-EDTA anticoagulant (0.45 M NaCl, 0.1M glucose, 30 mM trisodium citrate, 26 mM citric acid, 10 mM EDTA, pH 4.6; Smith & Soderhall 1991; Perdomo-Morales et al. 2007) for the phenoloxidase, viability and phagocytosis assays, at a 1:1 ratio into pre-chilled N-ethylmaleimide anticoagulant (NEM; 0.2 M N-ethylmaleimide in 0.45 M NaCl; [15]) or at a 1:2 ratio into Lillie's formal calcium (1.3 M formalin, 126 mM calcium acetate) for total and differential cell counts.

Phenoloxidase and prophenoloxidase assay

Spectrophotometric assays of PO (EC 1.14.18.1) and proPO were performed on haemolymph lysate and plasma following the microplate method described by Hernández-López [16] and Perdomo-Morales et al. [15] by measuring the formation of dopachrome through the reaction of phenoloxidase with L-dihydroxyphenylalanine (L-DOPA). The PO assay was optimised for haemolymph and plasma volume, substrate concentration and duration of steady-state kinetic reaction. The Total proPO (PO+proPO) assay was optimised for haemolymph and plasma volume, substrate concentration, trypsin concentration for activation, trypsin activation incubation time and duration of steady-state kinetic reaction. All assays were performed in triplicate with sodium cacodylate buffer (10 mM sodium cacodylate, 450 mM NaCl, 10 mM $CaCl_2$, 26 mM $MgCl_2$, pH 7.8) added to bring to total reaction volume in the well to 200 µl. Calculation of proPO activity was determined for HLS and plasma by subtracting PO activity from Total PO activity for each fraction.

Haemocyte lysate was prepared by first centrifuging the haemolymph suspension in citrate-EDTA at 800 x *g* at 4°C for 10 mins. The supernatant was discarded and the cell pellet was washed twice with sodium citrate-EDTA. The cell pellet was then resuspended using 0.45 M NaCl, pH 7.8 and transferred into a glass Dounce tissue grinder that had been chilled on ice. The homogenate was then transferred to a microfuge tube and centrifuged at 10,000 x *g* at 4°C for 10 mins. The resulting haemocyte lysate was removed from the cell pellet and use for PO and proPO assays.

Haemolymph plasma was prepared as described by Perdomo-Morales et al. [15], with NEM used as an anticoagulant to avoid isoelectric precipitation of proteins in the plasma. After collection, the haemolymph-NEM suspension was centrifuged at 800 x *g* at 4°C for 10 mins and the supernatant removed from the cell pellet for use in PO and proPO assays.

For the PO assays, 50 µl haemolymph lysate or 25 µl plasma were reacted with 15 mM L-DOPA (concentration in well) and the formation of dopachrome was determined by reading optical density at 492nm absorbance for 20 mins. For proPO assays, 50 µl haemolymph lysate and 25 µl plasma were activated using an in-well concentration of 0.1% trypsin, with the reaction incubated at room temperature for 20 mins. Then, L-DOPA was added at a concentration in the well of 15 mM and the reaction was read at 492nm for 20 mins. One unit of PO activity (U) was expressed as the increase of 0.001 in optical density at 492nm per minute.

Cell viability

Cell viability was determined using a Live/Dead stain kit (Molecular Probes, Inc.) containing ethidium homodimer-1 (EthD-1) and calcein-AM dyes to identify dead and live cells, respectively. Eth-D1 is capable of penetrating the compromised cell membranes of dead cells to bind to nucleic acids and fluorescing red (>600nm), whereas live cells exclude the dye and no fluorescence is produced. Extracellular calcein-AM does not fluoresce, but fluoresces green (530nm) when it permeates cell membranes and is hydrolysed within the cell. The assay was optimised for dye concentration to ensure intense EthD-1 fluorescence and minimal calcein-AM fluorescence in cells killed with 70% methanol and minimal EthD-1 fluorescence and intense calcein-AM fluorescence in

a sample containing live cells. Incubation time was then optimised to ensure haemocytes had an adequate amount of time to take up the dyes.

Haemolymph diluted 1:1 in citrate-EDTA was centrifuged at 800 x *g* at 4°C for 10 mins. The plasma supernatant was removed from the cell pellet and discarded, then the cell pellet was resuspended in an equivalent volume of citrate-EDTA buffer. The cell suspension was plated in a black-walled microplate in triplicate and 50 µl each of 28 µM EthD-1 and 20 µM calcein-AM were added. The plate was incubated in the dark for 60 mins. A subsample of the cell suspension was incubated with 70% methanol for 30 mins to kill the cells, then this sample was plated and incubated in the same manner. Plates were read for fluorescence at 530/645nm for EthD-1 and 485/530 for calcein AM. The dead cell sample was used as a known sample against which the percentage of dead cells could be quantified.

Phagocytosis

To test the competency of haemocytes, the phagocytic capacity was measured following the method of Oweson et al. [17]. A commercial preparation (BioParticles, MolecularProbes, Inc.) of yeast zymosan particles labelled with fluorescein-5-isothiocyanate (FITC) were diluted in citrate-EDTA buffer with 2 mM sodium azide to a concentration of 1 x 10^7 particles ml^{-1}. Haemolymph was diluted 1:1 using citrate-EDTA, allowing for 20-50 zymosan particles per haemocyte. Haemocytes and zymosan particles were added to wells of a black walled microplate and incubated in the dark at room temperature for 75 mins, after which 100 µl 0.4% trypan blue was added and incubated for a further 10 mins to quench extracellular fluorescence. The plate was then read for fluorescence at 485/528nm. The average relative fluorescence units (RFU) from five measurements per individual and the fluorescence of a zymosan positive control without haemocytes were used to calculate the phagocytic index: $PI\% = [(RFU_{haemocytes} / RFU_{zymosan}) \cdot 100]$.

Total and differential haemocyte counts

Haemolymph diluted 1:2 in Lillie's formol calcium was used for total haemocyte counts (THC) and differential haemocyte counts (DHC). For THCs, haemocytes were counted using an improved Neubauer counting chamber at 40x magnification. For DHCs, haemolymph smears were prepared on slides, fixed with methanol, stained using May-Grünwald Giemsa and 200 cells per slide were identified as hyalinocytes, semi-granulocytes or granulocytes.

Protein assays

Protein content of the haemolymph lysate and plasma components used in the phenoloxidase assay and the whole haemolymph in citrate-EDTA anticoagulant used in the phagocytosis assay were determined using the Bradford method [18]. Haemolymph lysate was used undiluted, plasma was diluted by a factor of 1:280 and whole haemolymph was diluted by a factor of 1:400. For each sample, 5 µl was plated, to which 250 µl Bradford reagent (Sigma Aldrich) was added. The plate was incubated at room temperature for 10 mins and then read for absorbance at 600nm. Protein content of these samples was determined against a bovine serum albumin standard curve and expressed as mg ml^{-1}.

Biochemical analysis

Haemolymph sampling for biochemical analysis has been described previously in reference (Fitzgibbon et al. 2017). Briefly, two 1.5 ml samples collected using pre-chilled syringes and dispensed into centrifuge tubes. They were centrifuged at 10,000 x *g* for 5 mins at 4°C, after which the supernatant was removed using a pipette, transferred to a 2 ml cryotube, frozen and stored in a -80°C freezer until they were shipped in a dry shipper charged with liquid nitrogen to the Diagnostic Services laboratory at the Atlantic Veterinary College, University of Prince Edward Island, Canada, and analysed using a Cobas c501 automated biochemistry analyzer (Roche Diagnostics Corporation,

Indianapolis, IN, USA) for a full blood profile consisting of the electrolytes (mmol L^{-1}) sodium(Na), chloride (Cl), potassium (K), magnesium (Mg) and bicarbonate (bicarb); minerals (mmol L^{-1}) calcium (Ca) and phosphorus (P); metabolites (mmol L^{-1}) glucose (Gluc), lactate (Lact), cholesterol (Chol), triglyceride (Trig), total protein (TP, in g L^{-1}), urea, and uric acid (Uric, in μmol L^{-1}); enzymes (U L^{-1}) lipase (LIP), amylase (AMY), alanine (ALT) and asparate (AST) aminotransferases, alkaline phosphatase (ALP), sorbital (SDH) and glutamate (GDH) dehydrogenases, and gamma-glutamyl transferase (GGT).

Statistical analysis

All results from the measurement of haemolymph parameters are presented as univariate scatter plots that show the treatment mean and individual distributions as suggested for low sample size data by Weissgerber et al. [19].

All haemolymph parameters were checked for normality and equality of variance using Shapiro-Wilks tests and Levene's tests, respectively, transformed as needed and then compared using analysis of variance (ANOVA) followed by Tukey HSD post-hoc multiple comparison tests for significant results (α=0.05). All analyses were performed in R v. 3.4.1.

Data were compared using multivariate analysis in Primer-6 with PERMANOVA+. First, data were normalised to account for the varying units and scales of the immune and biochemical parameters comprising the data. A resemblance matrix using Euclidian distance was then used to generate a multidimensional scaling plot (MDS). Complete linkage CLUSTER analysis was performed to determine the relationships between individuals and the similarity between treatments was analysed using ANOSIM.

Results & Discussion

Physical and biochemical parameters

The lobsters in each treatment did not vary significantly in carapace length ($F(3,14)=2.03$, $P=0.156$), but did in weight ($F(3,14)=6.08$, $P=0.007$), as NEC lobsters had a significantly greater weight than NEC, MOR and FAST treatments. This is not surprising, given the long holding time (6 months) prior to sampling for this treatment, in which they were fed a nutrient rich diet of mussels on a regular basis. In comparison, CTL lobsters were freshly caught from the wild, MOR were had been held fasted within the processing facility for 2-3 weeks and FAST were fasted for 10 weeks during holding at the processing facility.

Haemolymph pH (Fig. 1a) in lobsters from the CTL treatment, with a mean of 7.30 ± 0.06, was significantly lower ($F(3,14)=11.411$, $P<0.001$) than that of lobsters from the other three treatments, which ranged from 7.55 ± 0.02 in FAST lobsters to 7.63 ± 0.05 in NEC lobsters. The relatively acidic pH of the CTL treatment reflects a well characterised response indicative of a lack of full recovery from the stresses resulting from capture and transport to the processing facility. In previous studies on a range of lobster species, clearance of the built-up lactate that depressed haemolymph pH required *ca.* 96 h of holding after periods of emersion [13, 20, 21].

Brix index (Fig. 1b) was also significantly different ($F(3,14)=28.07$, $P<0.001$), as CTL and MOR lobsters had similar means, whereas NEC and FAST lobsters had values 1.7 and 1.5 times greater than CTL lobsters and 2.2 and 2 times greater than MOR, respectively. The comparatively high Brix values of NEC and FAST lobsters can be explained by an increase in haemolymph protein levels due to their pre-moult status compared to the inter-moult status of the other three treatments [22, 23], and offers further support for the superior nutritional condition of the NEC treatment [22, 24].

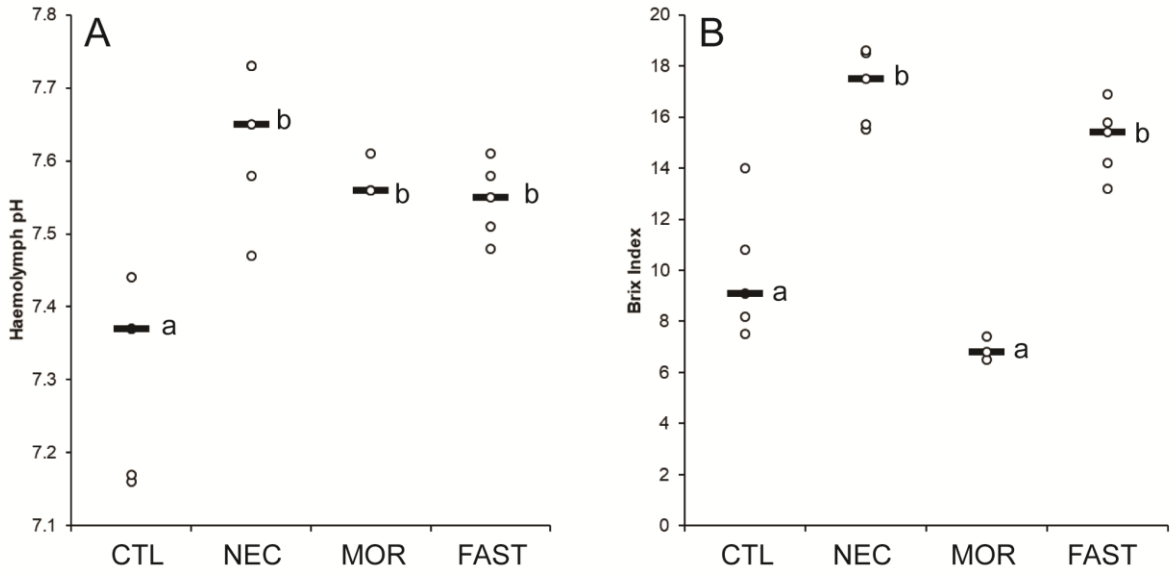

Figure 1. pH and refractive (Brix) index of lobster haemolymph. A) Univariate scatterplot showing mean and distribution of haemolymph pH of control (CTL), shell necrosis (NEC), moribund (MOR) and fasted (FAST) treatments; B) Univariate scatterplot showing mean and distribution of haemolymph refractive index expressed in % Brix of CTL, NEC, MOR and FAST treatments. Each circle indicates an individual within a treatment with the treatment mean indicated by a horizontal bar. Means sharing a letter are not statistically different.

Phenoloxidase system

Phenoloxidase (PO) and its intermediate products are highly reactive and toxic, requiring tight control of the cascade system that initiates an immune response [25], thus, high levels of PO activity are not generally expected to be found in the haemolymph. Furthermore, in contrast to most other crustaceans, in which PO/proPO activity is located in the haemocytes [2], there is some debate over whether this holds true for rock lobsters, with conflicting reports of activity solely in the plasma [16] or in both the plasmatic and haemocytotic fractions [15]. In the present study, PO was found to be largely absent from the haemolymph lysate (HLS; Fig. 2a) with no detectable PO activity in 72% of the individual lobsters assayed. Specifically, no CTL individuals displayed activity, one MOR lobster showed modest activity and two individuals each from NEC and FAST treatments showed detectible activity. Due to the frequency of no detected activity, these data were not statistically analysed. Trypsin activated proPO activity was more prevalent in the HLS (Fig. 2b), with only 28% of the lobsters assayed in this study showing no detectible activity, including three CTL lobsters, one NEC lobster and one MOR lobster. No statistical difference in activity level was found between treatments ($F(3,14)=1.76$, $P=0.20$).

PO was consistently present in the plasma fraction (Fig. 2c) of the haemolymph at modest levels in all four treatments, with no significant difference between them ($F(3,14)=0.72$, $P=0.55$), which is again not surprising due to its reactivity. Indeed, this detectible activity was likely the result of auto-activation during the assay due to the sensitivity of the proPO system mediating semigranulocytes and granulocytes [26]. Separating cell types has been shown to be an effective approach to preventing auto-activation of the proPO system [27]. ProPO had the strongest activity in the plasma, with activities that differed significantly ($F(3,14)=6.59$, $P=0.005$), as NEC lobsters had more than double the activity of both CTL and MOR lobsters, demonstrating an upregulation of the cells that mediate the proPO system in response to the bacterial infection causing the necrosis (see section 3.4). ProPO activity levels comprised 93-100% of the Total PO (PO+proPO) activity in the HLS by treatment and 96-99% of Total PO activity in the plasma by treatment, thus Total PO results (not shown) were similar to that of plasma proPO, with no significant difference found between treatments in the HLS ($F(3,14)=1.55$, $P=0.25$) and a significantly higher activity level in NEC lobsters compared to both CTL and MOR lobsters ($F(3,14)=6.6$, $P=0.0053$).

From these results, it is apparent that PO/proPO expression is not as uniform as previously suggested, with activity either in the plasma or in the haemocytes. Rather, these responses suggest an intermediate situation, in which HLS from healthy SRL from the CTL treatment did not demonstrate either PO or trypsin-activated proPO activity, but in lobsters facing a challenge to the immune system, the PO/proPO system demonstrated a facultative function, particularly with regards to HLS proPO activity. Further study is needed, as it has previously been reported that *J. edwardsii* demonstrated trypsin activated proPO activity in both healthy control and tail-fan necrosis infected treatments, with the former demonstrating an activity level 50% greater than the latter, which the authors attributed to the TFN infected lobsters being immunocompromised [28]. This runs counter to the findings presented here, both with regard to the presence of proPO in healthy SRL and suggestion of reduced capacity in SRL with bacterial infections.

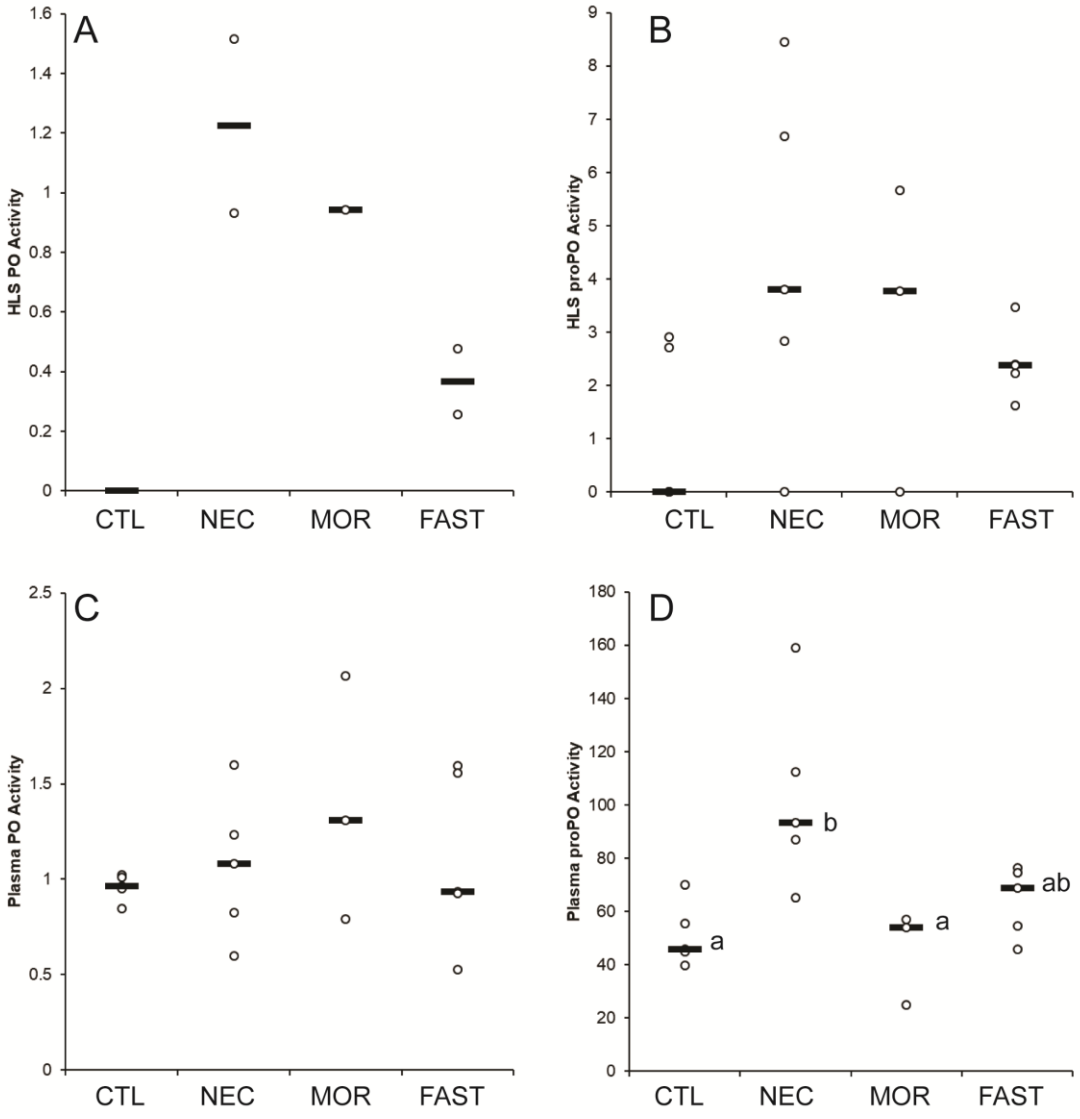

Figure 2. Phenoloxidase system activity in haemocytic and plasma fractions of lobster haemolymph. A) Phenoloxidase (PO) activity, expressed in U (change in 0.001 optical density min^{-1} at 495 nm), in the haemolymph lysate supernatant (HLS); B) pro-PO activity following trypsin activation in the HLS; C) PO activity in the plasma; and D) pro-PO activity following trypsin activation in the plasma of control (CTL), necrosis (NEC), moribund (MOR) and fasted (FAST) treatments. Each circle indicates an individual within a treatment with the treatment mean indicated by a horizontal bar. Means sharing a letter are not statistically different.

Haemocyte numbers and function

Haemolymph is the invertebrate analogue to vertebrate blood, comprised of a haemocytic (cellular) fraction and a humoral (plasma) fraction. Soluble immune factors, such as lectins, pattern recognition proteins and antimicrobial proteins, are also transported via the haemolymph are outside the scope of this study but have been thoroughly reviewed in general [29] and specifically in lobsters [30]. In decapod crustaceans like lobsters, haemolymph performs a range of physiological functions, including the transport of gasses, nutrients, wastes and most relevant here, facilitating the response of the innate immune system. The latter is done largely through the movement of haemocytes around the body.

The innate immune system of lobsters has two mechanisms for dealing with foreign particles such as microbes: phagocytosis and encapsulation [27, 31]. Phagocytosis is the process by which individual haemocytes, primarily the hyalinocyte cell type, engulf and destroy foreign cells. When microbes are too large to be phagocytosed, multiple haemocytes, primarily from the granulocyte and semi-granulocyte cell types, encapsulate the pathogen to seal it off and degranulate to initiate the phenoloxidase cascade, destroying the pathogen without affecting surrounding self tissue.

Following stimulation of an immune response, the number and proportion of haemocytes can vary, generally with a decrease in overall cell numbers and a shift in type dependent upon the response followed by a substantial increase in cell populations as haemocytes are generated through haematopoiesis [27]. Crustaceans have also demonstrated similar shifts in haemocyte populations in response to a range of extrinsic and intrinsic factors including environmental stress, moult cycle and nutritional state. Thus, the number, cell type proportion and function of haemocytes can be powerful indicators of immune status in lobsters.

Haemocyte viability (Fig. 3a), calculated from the proportion of cells demonstrating ethidium homodimer-1 fluorescence compared to a sample of cells permeabilised by incubation in 70% methanol, showed that all treatments had a similar level of live and dead cells with 80-83% live cells and no significant difference between treatments ($F(3,14)=1.32$, $P=0.31$). These results are generally on par with previous measurements of haemocyte viability in rock lobster, which reported about 10% dead haemocytes [28, 32], with the difference in dead cells likely an artefact of the more extensive handling required by the microplate assay used in the present study compared to the results of flow cytometry assays. Regardless of method, haemocyte viability does not appear to show sensitivity to the stressors investigated here.

Phagocytotic index (Fig. 3b) was significantly different between treatments ($F(3,14)=5.85$, $P=0.008$), with NEC showing a reduced capacity for taking up fluorescent-labelled zymosan particles compared to MOR and FAST lobsters. Decreases in phagocytotic capacity are not uncommon in crustaceans [33], but neither fasting nor the unidentified stresses affecting the MOR lobsters had an impact in the present study, as neither treatment was found to differ significantly from CTL lobsters.

Total haemocyte counts (Fig. 4a) were significantly different between treatments ($F(3,14)=4.83$, $P=0.016$), as NEC lobsters were found to have significantly higher THC than both MOR and FAST treatments with over 3-fold more haemocytes.

The THC levels for all four treatments reflect previously reported ranges for SRL [12, 13] and rock lobsters more broadly [34, 35]. However, THCs of the CTL and NEC treatments were considerably higher than the values reported by Zha et al. [28], in which a control SRL cohort were reported to have <40% of the number of haemocytes of the controls in the present study and the TFN-infected treatment only 15% of the NEC treatment reported here. Crustaceans tend to show a high level of haemocyte variation between individuals, both in terms of total number and proportion of cell type, with a range of biotic and abiotic influences identified [27, 35]. As such, the substantial differences

between the Zha et al. [28] study and the present study can probably be attributed to some combination of variations in season, moult cycle, nutrition, weather, stress or a range of other factors.

Another substantial difference in the response to pathogenic bacterial infection in SRL reported by Zha et al. [28] was that TFN-infected lobsters were found to have a significantly reduced haemocyte count compared to controls, whereas in the present study, necrosis infection resulted in a significantly greater count. The generalised haemocytic response to immune insult is characterised by an interplay between cell synthesis, or haematopoiesis, and the loss of cells through immune response functions including cell autolysis, phagocytosis or proPO system activation. This response leads to a rapid decrease in THC followed by a subsequent stimulation of haematopoiesis and haemocyte proliferation, termed a 'left shift' response [36-38], which was seen in the present study in the NEC treatment. In contrast, the relatively low THC in MOR and FAST treatments reflected the comparatively long holding time without feeding [39, 40], as haematopoiesis requires energy expenditure [31] and may be facultative during times of poor nutrition [39, 41].

Differential haemocyte counts (Fig. 4a-d) were significantly different between the four treatments for the percentage of hyalinocytes ($F(3,14)=25.84$, $P<0.001$), the percentage of semigranulocytes ($F(3,14)=29.64$, $P<0.001$) and the percentage of granulocytes ($F(3,14)=4.19$, $P=0.026$). For hyalinocytes, CTL and MOR had a similar proportion of cells, NEC had significantly fewer than all other treatments and FAST had significantly more than all other treatments. Correspondingly, NEC had more semigranulocytes than all other treatments, FAST had fewer semigranulocytes than all other treatments and CTL and MOR were again similar. For granulocytes, FAST had the lowest proportion and significantly fewer than NEC. CTL and MOR had a similar, intermediate number of granulocytes.

Previous studies have reported a wide range proportions of the three cell types in healthy rock lobsters, with a haemocyte composition at an approximately 60%:30%:10% ratio reported in the California rock lobster (*Panulirus interruptus*) [42], a roughly 60%:5%:35% ratio in the European rock lobster (*Panulirus elephas*) [43] and in Western Rock Lobster (*Panulirus cygnus*), a range from 75%:20%:5% [20] to 30%:60%:10% [34, 44]. In lobsters subject to stress, these ratios generally shifted to a more haemocyte dominated ratio (70%:4%:26%) in *P. elephas* [43] and in *P. cygnus* with 80%:15%:5% [20] and 35%:52%:13% [34].

There appears to be a continuum of immune response to stress in rock lobsters that manifests as a trade-off between hyalinocytes and the two granular cell types. At one end of the response continuum, FAST lobsters showed an almost complete shift toward hyalinocytes, which comprised *ca.* 90% of the haemocytes in this treatment, at the cost of semigranulocyte (5%) and granulocyte (5%) proportions. It seems likely that this response was underpinned by the balancing of the energetic costs of immune system response against the energetic constraints of long term fasting, but it was not possible to draw any solid conclusions due to the paucity of data on the metabolic costs of immune responses in invertebrates in general [45] and, more specifically, the lack of data on the effect of long term fasting on DHC in crustaceans.

At the opposite end of the spectrum, as seen in some *P. cygnus* results [20, 34] and in the NEC lobsters in the present study, the response to stress or immune challenge is characterised by a preponderance of the two granular cell types and a decrease in hyaline cells. Indeed, a strong negative correlation was found between hyalinocytes and semigranulocytes in the present study across all treatments, with an R^2 value of 0.95 (Fig. 5a). The relationships between hyalinocytes and granulocytes (Fig. 5b) was also negative, but weaker ($R^2=0.57$; Fig. 5b). This trade off, in which hyalinocytes are downregulated and granular cells are upregulated, drives a shift in immune response from the hyaline cell mediated phagocytosis toward a granulocytic proPO system focused response. The strong proPO activity of the NEC treatment supports this hypothesis, given that the granular cell types are the storage site for the PO precursor and mediator of its activation. In addition, the significantly fewer proportion of hyalinocytes in the NEC lobsters relative to the MOR and FAST

treatments implies a significantly reduced capacity for phagocytosis [2, 39]. Furthermore, it must be noted that, although NEC lobsters had the lowest proportion of hyalinocytes of the four treatments in this study, their substantially greater THC means they had more in number than the other treatments, yet these cells were less “active” in phagocytosis than those of other treatments.

There is some evidence to suggest (amongst the few crustacean taxa studied) that hyaline cells are an intermediate step in the differentiation of phagocytic semigranular cells and that a second sub-type of non-phagocytic semigranular cell develops into granular cells [37, 46]. Differentiation would presumably be mediated by some yet to be identified haemolymph-borne factor, which would explain both the trade-off relationship of the cells and the functional shift from phagocytosis to proPO despite the relatively large number of hyaline cells. This cell differentiation hypothesis still requires further experimental validation and is based primarily on work in two crustacean taxa (the black tiger prawn *Peneaus monodon* and the freshwater crayfish *Pacifastacus leniusculus*). Both the prawn and the crayfish have haemocyte compositions dominated by semigranular and granular cells, with semigranulocytes serving as the primary phagocytes rather than the hyaline cells, so it is unclear what implication that may have for the hyalinocyte dominated rock lobster system. Thus far, rock lobster hyalinocytes and semigranulocytes have both been reported to demonstrate phagocytic [47] and cytolytic capabilities [48], implying that these cells may be different stages of the same lineage, although the specific function and overall interplay between haemocytes in the immune response of rock lobsters remains unclear. Clearly, further study is needed with a specific focus on rock lobsters, to better characterise the function of the immune system with reference to the aspects that are distinct from prawn, freshwater crayfish and clawed homarid lobsters.

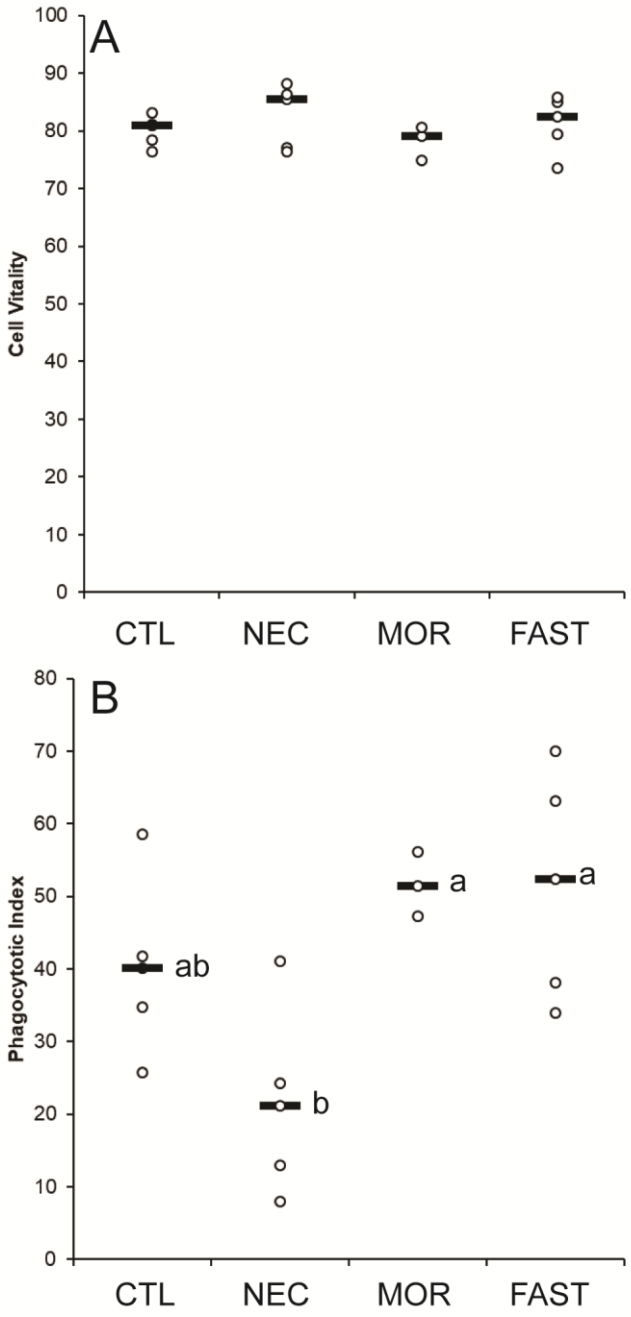

Figure 3. Cell function in lobster haemolymph. A) Cell vitality, measured as a percentage of live cells, and B) Phagocytotic index, measured as a percentage of phagocytosed particle, of control (CTL), necrosis (NEC), moribund (MOR) and fasted (FAST) treatments. Each circle indicates an individual within a treatment with the treatment mean indicated by a horizontal bar. Means sharing a letter are not statistically different.

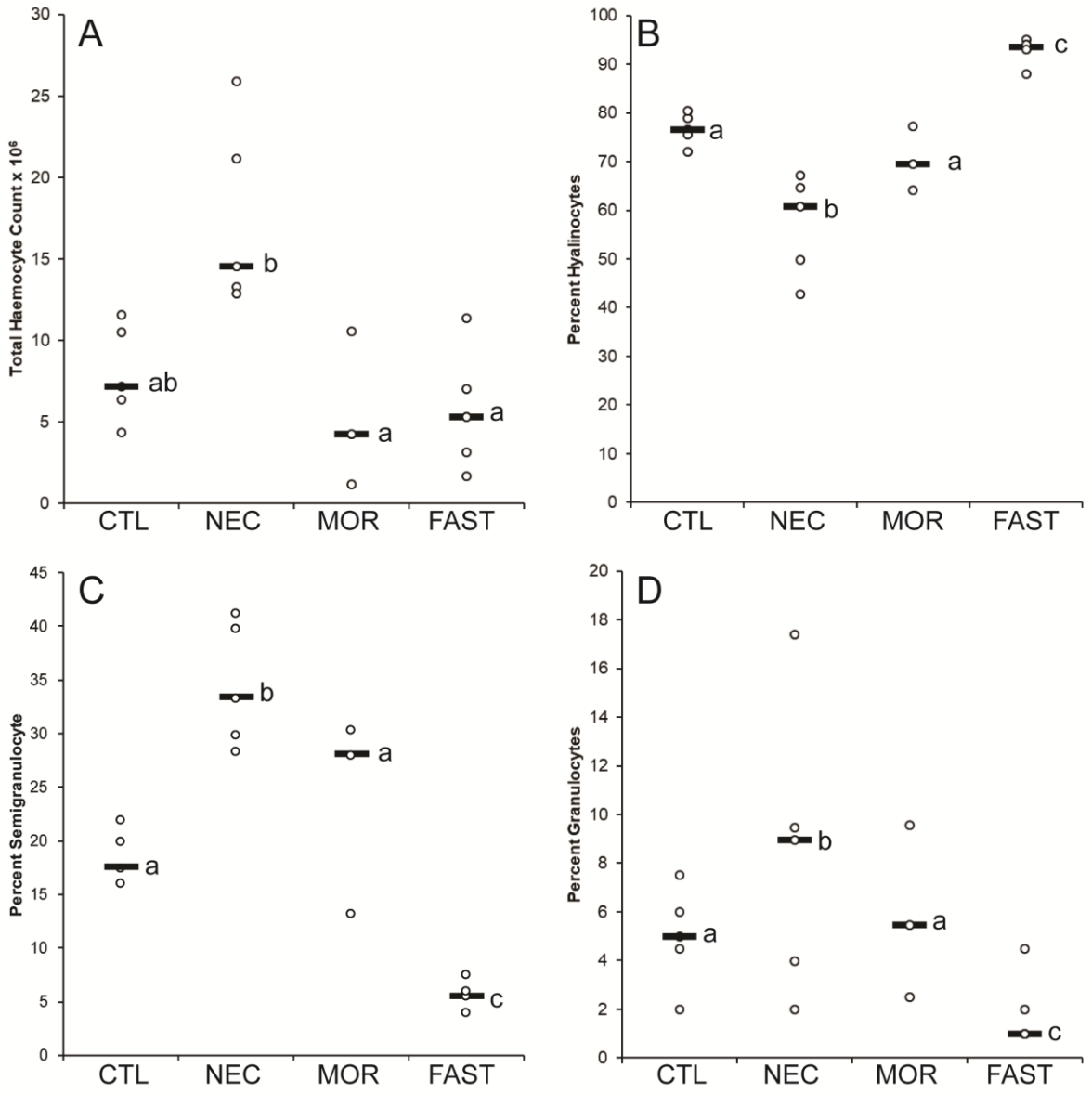

Figure 4. Haemocyte numbers in lobsters. A) Total haemocyte counts; B) Percentage of hyalinocytes; C) Percentage of semigranulocytes and D) Percentage of granulocytes in control (CTL), necrosis (NEC), moribund (MOR) and fasted (FAST) treatments. Each circle indicates an individual within a treatment with the treatment mean indicated by a horizontal bar. Means sharing a letter are not statistically different.

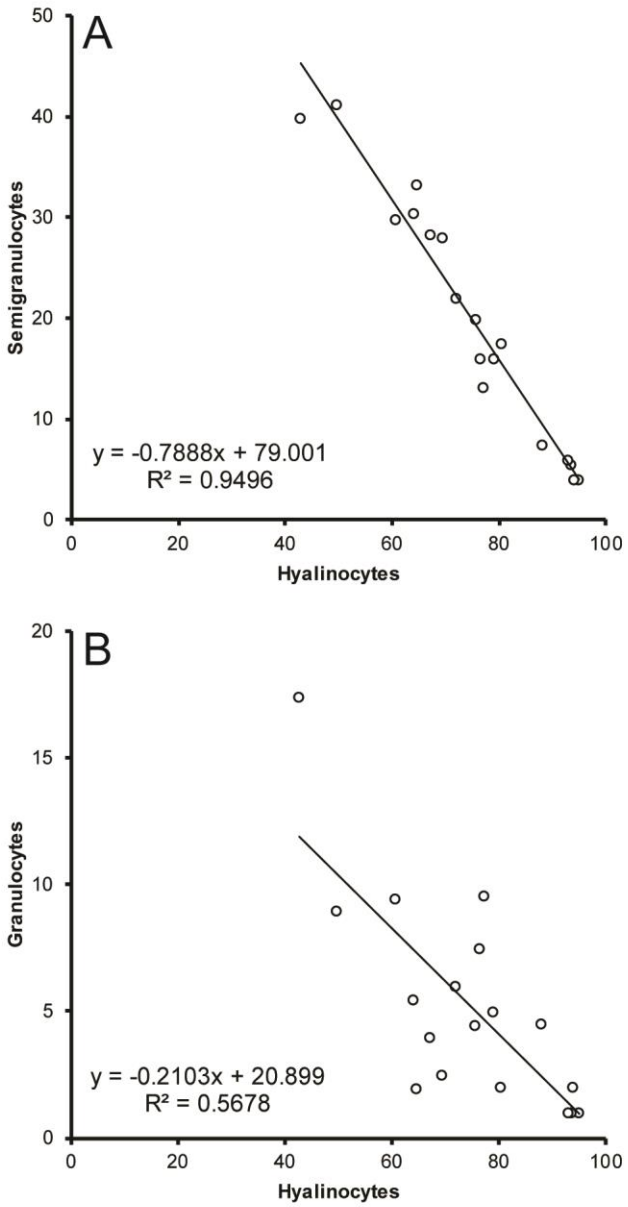

Figure 5. Relationships between cell types. A) Linear regression of semigranulocytes against hyalinocytes across all four treatments demonstrating the strong relationship between the two cell types, B) Linear regression of granulocytes against hyalinocytes across all four treatments demonstrating the weak relationship between the two cell types.

Protein concentration

In the plasma fraction of the haemolymph, measurements of protein levels have frequently been investigated as an indicator of physiological condition. Haemocyanin, the protein that binds oxygen, is the most abundant constituent comprising >60% of total protein levels, with coagulogen, apohaemocyanin, hormones and lipoproteins accounting for the remainder [49]. Protein levels change in response to physiological factors such as moulting, nutritional state and reproductive state and to environmental stressors such as hypoxia, pollution and pathogenic infection. The mechanisms underlying these changes are water uptake, which dilutes the blood and utilisation as a metabolic reserve during starvation [50], both of which result in a reduction in protein levels.

Neither HLS protein concentration ($F(3,14)=3.00$, $P=0.0664$) nor plasma protein concentration ($F(3,14)=1.94$, $P=0.169$) were found to differ between treatments (Fig. 6a, b). Whole haemolymph protein (Fig. 6c) did differ significantly ($F(3,14)=5.46$, $P=0.011$), as NEC lobsters had significantly higher protein levels than CTL lobsters. No difference was found between MOR or FAST lobsters and the other treatments.

Although haemolymph protein concentration is frequently used as a measure of stress in crustaceans, there is evidence to suggest that in rock lobsters, it is not as sensitive as other parameters, including metabolites, THC, DHC and refractive index [44, 50-52]. Previous work showed that tail fan necrosis did not significantly impact protein concentration in either the HLS or plasma of *J. edwardsii* [28], suggesting that the relatively high protein concentration in the whole haemolymph of NEC lobsters seen in the present study was caused by comparatively high nutritional condition relative to lobsters in the other treatments [50, 53].

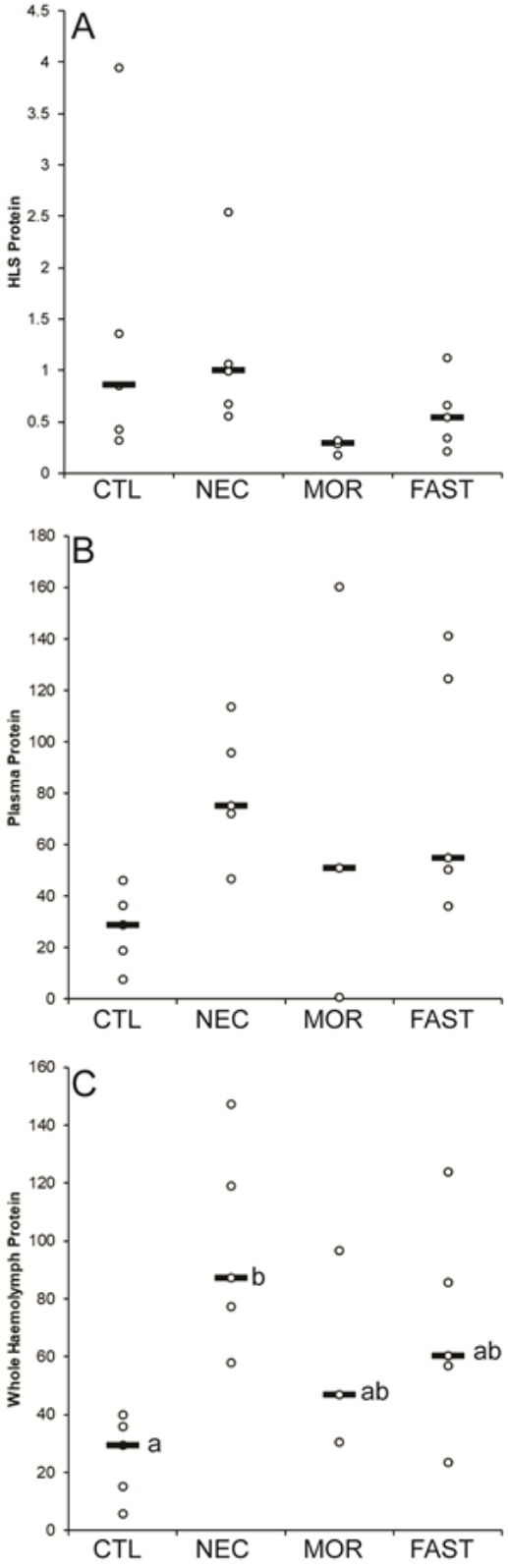

Figure 6. Protein concentration of the lobster haemolymph and its constituents. Protein concentration, expressed in mg/ml, in the A) haemocyte lysate suspension, B) plasma and C) whole haemolymph in control (CTL), necrosis (NEC), moribund (MOR) and fasted (FAST) treatments. Each circle indicates an individual within a treatment with the treatment mean indicated by a horizontal bar. Means sharing a letter are not statistically different.

Haemolymph biochemistry

Haemolymph biochemistry panels, like those commonly used to measure a range of blood parameters in humans, have recently been developed for invertebrates and are becoming increasingly common as a method to characterise how lobsters respond to challenges such as viral infection [47], environmental stressors (e.g. seismic surveys) [12], nutrition [22] and emersion during live export [13].

Amongst electrolytes and minerals (Table 1), Na concentration showed a significant difference between treatments ($F(3,14)=11.32$, $P<0.001$), with the NEC treatment lower than CTL and MOR treatments. Neither K nor ($F(3,14)=1.83$, $P=0.189$) the ratio between Na:K ($F(3,14)=0.85$, $P=0.487$) were significantly different between treatments. The concentration of Cl differed significantly between treatments ($F(3,14)=10.41$, $P<0.001$), with the NEC treatment again having significantly lower levels than CTL and NEC and FAST lower than CTL. The relationship between treatments was similar in Na and Cl, suggesting a common mechanism drove the change. Indeed, decreases in Na and Cl concentration have previously been linked to moribundity in SRL following emersion [13], suggesting that the NEC and FAST treatments were under physiological stress. There were significant differences in the concentration of haemolymph bicarbonate between treatments ($F(3,14)=12.71$, $P<0.001$), with FAST lobsters showing significantly lower levels than all three other treatments and MOR lobsters showing significantly higher bicarbonate than CTL. Bicarbonate has also previously been shown to respond to emersion stress in SRL [13], increasing as a means to buffer falling haemolymph pH as acidic metabolic products build up. However, the MOR lobsters did not have acidic pH levels, making the underlying cause of the observed increase unclear. FAST lobsters had a significantly lower Mg concentration ($F(3,14)=4.08$, $P=0.028$) than both CTL and NEC treatments. Increases in Mg concentrations have been linked to poor condition following transport in lobsters and during moulting [22, 54]. There was no significant difference in the concentration of Ca ($F(3,14)=0.692$, $P=0.572$), Phos ($F(3,14)=1.352$, $P=0.298$) or osmolality ($F(3,14)=1.97$, $P=0.165$) between treatments.

Total protein was significantly different between treatments ($F(3,14)=12.11$, $P<0.001$), with both NEC and FAST lobsters showing significantly elevated levels compared to the CTL and MOR treatments. The measurement of albumin, which in crustaceans more accurately reflects haemocyanin and other high molecular weight protein levels than actual albumin, which they lack [55], was found to significantly differ between treatments ($F(3,14)=23.32$, $P<0.001$), with NEC lobsters higher than CTL and MOR treatments and FAST higher than CTL. Globulin levels were also significantly different ($F(3,14)=9.28$, $P=0.001$), with NEC and FAST treatments showing levels around double those of CTL and MOR. However, the albumin:globulin ratio did not significantly differ between treatments ($F(3,14)=2.80$, $P=0.08$), suggesting that the change in both parameters was coordinated, such as through changes in haemolymph hydration.

Uric acid was significantly elevated in NEC lobsters ($F(3,14)=4.62$, $P=0.02$) relative to CTL lobsters. Glucose concentration did not differ significantly ($F(3,14)=0.87$, $P=0.48$). Cholesterol levels were substantially higher ($F(3,14)=14.83$, $P<0.001$) in the NEC treatment than in the other three treatments by a factor of at least 1.7 (relative to FAST) and as much as 2.5 (MOR). Triglyceride concentrations differed significantly ($F(3,14)=9.22$, $P=0.001$), with NEC and FAST treatments showing more than twice the concentration of both CTL and MOR treatments. The elevated uric acid, triglycerides and cholesterol levels in the NEC treatment were likely a result of the relatively well-fed status of this treatment [22], although the elevated triglycerides in the FAST treatment may indicate some linkage to immune response, as levels should be expected to decrease in fasted lobsters [22].

Although a range of haemolymph enzymes were assayed, only two showed significant differences between treatments, as γ-glutamyl transferase activity levels ($F(3,14)=5.06$, $P=0.014$) showed that NEC and MOR treatments had elevated levels relative to CTL and glutamate dehydrogenase activity levels were significantly higher in NEC and FAST relative to CTL and MOR ($F(3,14)=14.89$,

P<0.001). Both of these enzymes are commonly used in vertebrates (particularly humans) as an indicator of tissue damage and have previously been shown to increase in stressed SRL, with GGT levels correlated to mortality [13], suggesting they may be useful indicators of health status.

Table 1. Biochemical analysis of the electrolytes and minerals, metabolites and enzymes of the haemolymph of lobsters. Mean ± SEM values are given for each treatment. For each parameter, statistically different means as determined using ANOVA are indicated by differing letters. See Section 2.8 for details on parameter abbreviations.

	Na mmol/L	K mmol/L	Na:K	Cl mmol/L	Osm mOsm/kg	Bicarb mmol/L	Ca mmol/L	Phos mmol/L	Mg mmol/L	TPro g/L	Alb g/L	Glob g/L	A:G	Uric µmol/L	Gluc mmol/L	Chl mmol/L	Trig mmol/L	GGT U/L	GD U/L
CTL	508 ± 5 a	7.0 ± 0.5	74 ± 5	513 ± 9 a	1027 ± 5	3.5 ± 0.4 a	16.08 ± 0.42	0.44 ± 0.16	12.34 ± 1.02 a	45 ± 9 a	4 ± 1 a	41 ± 9 a	0.12 ± 0.04	20 ± 2 a	1.0 ± 0.2	0.41 ± 0.06 a	0.30 ± 0.07 a	1.2 ± 0.5 a	6.8 ± 0.7 a
NEC	470 ± 6 b	6.1 ± 0.2	78 ± 2	450 ± 9 b	980 ± 18	3.6 ± 0.2 ab	17.40 ± 1.36	0.80 ± 0.20	12.33 ± 2.18 a	100 ± 5 b	14 ± 1 b	86 ± 4 b	0.17 ± 0.01	34 ± 2 b	0.7 ± 0.1	1.00 ± 0.02 b	0.81 ± 0.04 b	5.4 ± 1.3 b	23.0 ± 5.0 b
MOR	509 ± 7 a	6.4 ± 0.1	80 ± 2	512 ± 16 ac	1029 ± 16	5.1 ± 0.8 b	16.39 ± 1.35	0.46 ± 0.44	9.51 ± 1.52 ab	42 ± 19 a	6 ± 1 a	35 ± 18 a	0.25 ± 0.07	21 ± 1 ab	0.6 ± 0.1	0.39 ± 0.18 a	0.23 ± 0.14 a	6.6 ± 1.8 b	9.3 ± 3.0 a
FAST	487 ± 5 ab	6.1 ± 0.2	81 ± 3	473 ± 7 bc	993 ± 23	2.3 ± 0.1 c	17.95 ± 1.01	0.90 ± 0.03	6.86 ± 0.03 b	84 ± 5 b	9 ± 1 b	75 ± 4 b	0.12 ± 0.01	24 ± 4 ab	0.6 ± 0.1	0.59 ± 0.05 a	0.91 ± 0.18 b	3.0 ± 0.7 ab	23.4 ± 3.2 b

Multidimensional scaling analysis

A multidimensional scaling plot incorporating the 15 haemolymph and immune parameters and 19 haemolymph biochemical parameters quantified in this study was generated to determine the similarity of the individuals within each treatment and the similarity (or lack thereof) between each treatment. Part of the rationale for this was that many of the mechanisms driving changes in biochemistry and haemolymph cytology are unclear in rock lobsters and this scaling enabled all data to be pooled for exploring changes between treatments.

The resultant MDS plots showed a 2D Stress of 0.10 (Fig. 7), indicating a good fit and a 3D stress = 0.06, indicating an even better fit. ANOSIM showed a significant difference between treatments (Global R = 0.659, $P<0.001$; where values of R range from 0 to 1 with 0 representing complete similarity and 1 representing complete dissimilarity), with pairwise comparison showing a high level of dissimilarity between CTL and NEC treatments (R statistic = 0.900). NEC and FAST showed the next highest level of dissimilarity (R = 0.728), followed by CTL and FAST (R = 0.692). MOR and FAST were moderately dissimilar (R = 0.662), as were NEC and MOR (0.662). CTL and MOR showed a low degree of dissimilarity (R = 0.231).

Complete linkage CLUSTER analysis identified two distinct groupings of lobsters in this study (indicated in green on Fig. 7): the first comprised of all five NEC lobsters, all five FAST lobsters plus one each of MOR and CTL lobsters and the second comprised of all four CTL and two MOR lobsters. Within the former cluster, two further clusters were identified, with the first made up entirely of the five NEC lobsters and the second made up of the five FAST lobsters and one each of CTL and MOR treatments. This indicates that the immune function and haemolymph biochemistry of NEC lobsters, the treatment under the greatest immune challenge, showed a distinct response from the other treatments in the study. FAST lobsters, which were also under a comparatively high degree of stress, also demonstrated a response in immune and biochemical parameters, but one that was distinct from that of the NEC treatment. The inclusion of the one individual each from the CTL and MOR treatments in the FAST cluster indicates that these individuals were demonstrating a similar response. The grouping of two MOR individuals with the CTL treatment and the grouping of the third with the FAST lobsters rather than the NEC treatment leads to the conclusion that the moribund lobsters were not suffering some pathological condition, rather, one was likely demonstrating a response characteristic of fasting whereas the other two were in a moribund state due to some other influence, such as stress from being caught or processed, water quality factors or some other impact.

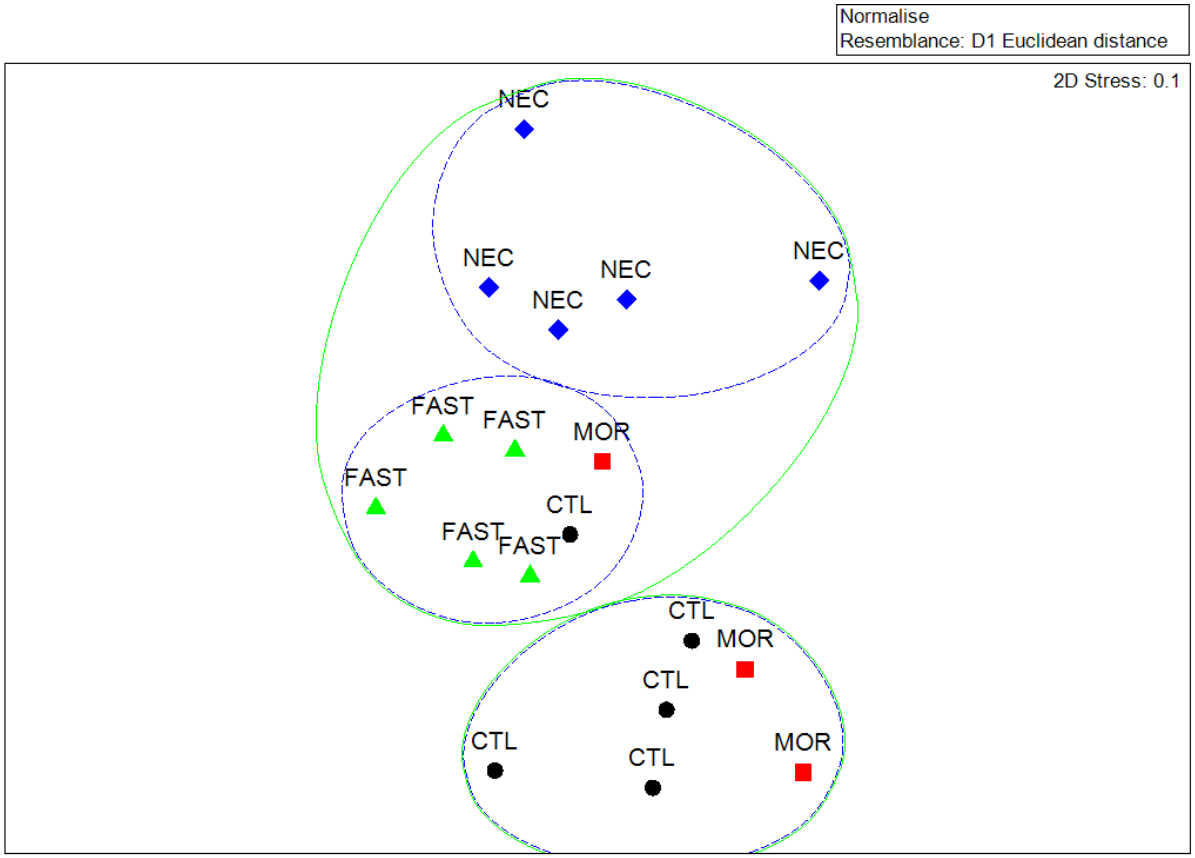

Figure 7. Immune function in lobsters. Multidimensional scaling (MDS) plot analysing the 15 haemolymph physical and immune parameters and the 19 biochemical parameters analysed in the four treatments in this study. The distance between any two individuals on this plot indicates their relative similarity, with increasingly similar points more close together. The 2D stress indicates a good fit and CLUSTER analysis identifies two major groups, encircled in solid green. Within the "response" cluster, two further groups were identifed, encircled in dashed blue, with one comprised entirely of the NEC treatment and the other comprised of the FAST treatment with one individual each of MOR and CTL. Within the separate "non-response" treatment is the remainder of the CTL and MOR treatments.

Conclusions

The aim of this study was to investigate how the immune system of the Southern Rock Lobster would reflect the challenges presented by recent arrival at a processing facility, advanced shell necrosis, long term fasting in industry standard processing facility conditions and being in a moribund state. The shell necrosis treatment served as a positive control, characterising a distinct immune response to a pathogenic infection. This response was typified by an increase in haemocyte numbers and a shift in haemocyte type to a higher proportion of semigranulocytes and granulocytes. Along with this change came a decrease in phagocytosis capacity but an increase in phenoloxidase activity. The fasted treatment was also found to demonstrate an immune response, though the mechanism was quite different. Although fasted lobsters did not demonstrate a change in haemocyte numbers, there was a marked shift in cell type composition, with a high level of hyalinocytes at the cost of semigranulocytes and granulocytes, though this shift did not correspondingly improve phagocytosis capacity or hinder phenoloxidase production.

Of the lobsters in an unexplained moribund state, two of the three sampled showed a "non-response" and were grouped with the control treatment in the multivariate analysis. It appears that these lobsters were not suffering any sort of pathogenic infection that might elicit a response similar to the bacterial infection of the necrosis treatment, and suggests that these lobsters were moribund due to some other cause, such as water quality.

The results of this study indicate that phenoloxidase is indeed present in the plasma, though some activity was observed in the haemocytes as well, particularly in the more immune-stimulated treatments. Of the parameters measured in this study, total and differential haemocyte counts appear to have the most predictive power in regards to health status. Staff can be easily trained to take these measurements, which do not require specialised equipment, potentially making them a powerful tool within the supply chain of lobster fisheries. Other biochemical parameters, such as electrolyte and mineral ion levels, bicarbonate concentration and GGT activity, appeared to be well correlated to immune status, but require further study to form a conclusive link between values and health status, as well as identifying the mechanisms that drive changes.

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Results 3: Facility Level Investigation

Preface

This aspect of the project was undertaken to partly address objectives 1 of this study:

Undertake an epidemiological investigation to describe the magnitude of the event and to identify potential environmental and management risk factor(s) associated with increased mortality

Manuscript information

Risk factor analysis for improving post-harvest survival in Australian southern rock lobster holding facilities

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Target Journal: Journal of Fish Diseases

Abstract

Southern rock lobster (SRL, *Jasus edwardsii*) are caught from the wild waters of southern Australia and forms an important commodity economically for the fisheries industry in Australia. Between landing and export, SRL are held in specialised holding tanks for varying time periods before being exported to China. During the 2015-16 fishing season a higher variation in survival rate was reported by some holding facilities when compared to previous fishing seasons. A multidisciplinary investigation was undertaken to identify possible causes and favouring factors. The study reports the findings of a retrospective study of facility-level risk factors that may contribute to or mitigate survival in SRL.

Materials and methods: A custom questionnaire was built and trialled to collect qualitative and quantitative information on facility's infrastructure and capacity (10 questions), water systems (16 questions), SRL stock and health management (12 questions). Holding facilities with a valid SRL holding licence across South Australia, Victoria and Tasmania were recruited in the study via e-mail and/or telephone. Onsite visits and interviews of consenting facilities, with active holding operations, were conducted. In absence of consistent stock and survival records across surveyed facilities, a facility was defined as a 'case facility' if the manager believed that the stock losses from the 2015-16 fishing season was at least twice the losses from preceding fishing seasons (2013-14 and 2014-15). As the industry consists of a few tens of facilities, an advanced multifactorial analysis could not be conducted. Therefore, facilities were first categorised based on a multivariate analysis and clustering of their responses in questionnaire. Then the association between facility profiles and perceived sub-optimal survival was explored using simple logistic regression models.

Results: Out of the 83 license holders on record, 63 were holding SRL stocks and 52 (83%) consented to participate. Perceived sub-optimal survival was reported in 22 (42%) facilities. The capacity and management practices across the surveyed facilities was highly variable. The cluster analysis for capacity related factors identified clusters of facilities with great differences in terms of SRL holding capacity. Two clusters comprised of low holding capacity facilities with or without modern facilities such as protein skimming unit, disinfection unit and a third cluster with high holding capacity and modern infrastructure. Three clusters for bio-filter management factors differed in terms of their choice to interrupt and clean (or not) the substrate whereas the third cluster had no interruption in bio-filter all year round.

Cluster analysis of water management factors revealed differences in practices relating to frequency of water replacement/addition and water testing across the clusters. Once cluster of facilities had no water quality monitoring whereas managers from other two clusters practiced ad-hoc to regular monitoring. Similarly, managers from one cluster only added, and did not replace, water whereas water was replaced regularly in second cluster or on an ad-hoc basis in the third cluster. The cluster variables for capacity, bio-filter, and water management related factors were not associated with perceived sub-optimal survival.

Cluster analysis of stock management identified two clusters of facilities with intensive and extensive holding practices. The perceived sub-optimal survival was significantly higher in the “intensive holding” facilities compared with the “extensive holding” facilities ($p = 0.002$). Major differences between the two clusters was the greater likelihood for “intensive holding” facilities to process a higher annual tonnage sourced from distant and higher number of sources, purge SRL before grading, stocking SRL using crates (as opposed to allowing SRL to swim freely in tanks), and holding them for a longer period on average. The perceived sub-optimal survival was significantly higher in the “intensive holding” facilities compared with the “extensive holding” facilities.

Conclusion: The SRL holding industry employed highly diverse practices to process and hold SRL. Intensive stocking practices, characterised by increased annual tonnage, were strongly associated with perceived sub-optimal survival within facility. However, identification of specific stock management practices associated with sub-optimal survival will need further research. In addition, routine recording of stock and survival data is highly recommended and may help future research studies on holding survival.

Introduction

Southern rock lobster (SRL, *Jasus edwardsii*) is the second highest valued fishery export commodity in Australia with an approximate annual gross value of AUD 255 million (Mobsby and Koduah, 2017). The SRL fishery industry in Australia is highly regulated by the Australian Government for management purposes. Wild SRL are commercially harvested by along the southern coastal waters of Australia for live export to markets mostly in Asia. Harvested live SRL are transferred and held by licenced operators in specialised holding and export facilities for time periods ranging from days and up to months until they are exported overseas. While mortality (upto 10%) during live shipment is commonly reported by the processors, sub-optimal survival during holding was reported by some facilities at the start of 2015/16 fishing season(Simon et al., 2016). Given the high value of SRL, any reduction in holding survival represent a potential threat to the economical sustainability of a business. The variation in survival rates of SRL during holding and the influencing factors across the industry remains unclear.

Along the supply chain, factors potentially affecting the homeostasis and resilience of SRL to survive post-harvest can be chronologically grouped as (i) pre-capture, (ii) at capture, and (iii) holding factors. The pre-capture factors may include habitat disturbance (e.g. shelter access), food abundance, competition and predation, disease(s), anthropologic disturbances, biological factors (moult stage and nutritional condition) to name a few (Green and Gardner, 2009; Hinojosa et al., 2015; Hooker et al., 1997; Oliver et al., 2005). The capture factors may include fishing methods (e.g. equipment, bait), handling and holding conditions in fishing vessels (Stentiford and Neil, 2000). The holding factors may include transport conditions to and between holding facilities, stock handling, and holding conditions (Paterson and Spanoghe, 1997). At arrival in holding facility, most SRL are mixed with SRL from different origins to accommodate holding capacity and ease stock management. This practice disables the traceability of SRL batches from capture to holding. Therefore, until traceability is implemented in routine SRL holding, it is not possible to investigate potential association between pre-holding factors and holding survival at the industry level.

The holding factors can vary between different holding facilities owing to the differences in management and capacity. Several studies have explored effects of various factors on post-harvest survival (Crear and Forteath, 2000; James et al., 2001; Lorenzon et al., 2007; Paterson and Spanoghe, 1997). However, most of those studies were experimental under controlled conditions which does not reflect real holding conditions under commercial settings. In addition, small scale experiments do not allow the exploration of impact of holding practices across a large number of facilities.

This study aimed at identifying current factors at the facility level potential associated with holding survival using a large scale observational study. An industry-wide survey was conducted to (i) compare holding capacity and management practices across the SRL holding industry and (ii) to investigate any potential association with holding survival. The study results suggest that holding practices vary greatly across the industry and that optimal holding survival was mostly associated with stocking management. This report follows the STROBE-Vet reporting standard for observational study (O'Connor et al., 2016).

Method

Definitions/glossary

Bio-filter - unit containing substrate facilitating bacterial colonisation and growth, and placed along a re-circulating water system for biological treatment of nitrogen chemicals.

Bio-filtration conditioning - commercially available ammonium chloride or other organic material added to a RAS to feed and invoke bacterial growth in its bio-filter.

Bio-filter Seeding - addition of commercially available bacterial culture to a RAS to re-build the bacterial microflora in its bio-filter.

Case facility - a holding facility with SRL stock losses from the 2015-16 fishing season at least twice the perceived losses from preceding fishing seasons (2013-14 and/or 2014-15) based on the memory of the facility manager/owner.

Disinfection unit – a unit placed along a RAS for killing microorganisms present in water.

Flow-through water system - water system with simultaneous entry and exit of pumped-in or gravity-fed seawater into and out of the holding tank.

Holding facility - A land-based building with an active SRL holding commercial licence.

Holding tank - A water enclosure within a holding facility that holds freely moving saltwater.

Holding compartment - A stock enclosure within a holding tank that holds SRL. SRL may be free swimming within a compartment but cannot move between compartments within a holding tank.

Mechanical filter – a unit placed along a RAS for removal of suspended solids present in the water.

Protein skimmer – a unit placed along a RAS for removal of fine or dissolved organic material present in the water.

Purging – process of removal of waste and undigested food by SRL upon arrival at the facility after being transported from source port/facility.

Purging water system – a separate water system used for purging of SRL upon arrival at facility.

Shipping water system – a separate RAS water system for holding SRL prior to packaging for export shipment.

Recirculating aquaculture systems (RAS) – water system in which water is recirculated within holding tank after mechanical and biological treatment.

Water system - a system for water distribution, circulation, and treatment used in holding tanks.

Study design and population

This study was designed as a retrospective, single cohort, observational study to investigate the association between facility-level factors (e.g. practices) and stock relative survival during holding during the 2015-16 fishing season. The target population consisted of SRL holding facilities across Australia with an active SRL holding license during the study period. For each state, a list of licensed SRL holders operating during 2015-16 fishing season were obtained from their respective state authorities (eligible population). Facilities' contact details were obtained by searching publicly available online sources for facility name via a web search engine. The managers were then contacted via e-mail and/or telephone to verify if they were operational (accessible population) and willing to participate in the study (study population). Facilities were visited during the 2017-18 fishing season which included a face-to-face interview and a site tour. Face-to-face interviews using a structured questionnaire were undertaken to collect information about the facility and management.

Questionnaire

The questionnaire included a total of 38 questions (Appendix 1) to collect information on: (1) *holding facility capacity related factors*, such as building characteristics (insulation and floor type), manager education and experience, number and training of permanent and casual staff, holding tank characteristics, primary water system and source, presence of mechanical filter, bio-filtration system, protein skimming unit, disinfection unit, chilling unit, power and water flow alarms in the water system, separate water systems for purging and shipping of SRL; (2) *bio-filtration tank management factors*, such as water flow direction in bio-filter, seeding and bio-filtration conditioning practices, and cleaning and maintenance of bio-filtration tank and it's substrate; (3) *water management factors*, such as pre-treatment of sourced water, cleaning and maintenance of mechanical filter, water addition/replacement, and water monitoring; and (4) *stock management factors and survival performance*, such as annual turn-over or tonnage in 2015-16 fishing season, stock survival in fishing seasons between 2013-2017, location and type of SRL source (fishermen/facility, distance from SRL source (port/facility) to destination holding facility, purging and grading of SRL, stocking method, water temperature in holding tanks, feeding of SRL, frequency of health monitoring, and fate of sick SRL. The questionnaire was first trialled with two volunteer facilities in South Australia, and after minor adjustments, was approved by the Human Research Ethics Committee at The University of Adelaide (approval reference number: H-2017-158).

Data handling and formatting

The responses from the questionnaire paper forms were entered into MS Excel 2016 spreadsheet (Microsoft Corporation, 2016). Secondary variables were generated from the primary variables recorded in the questionnaire (see Appendix-2 for detailed list). For instance, primary variables 'water holding capacity (m^3)' and 'maximum SRL stocking capacity (tons)' were extracted directly from responses in the questionnaire and then combined to create a secondary variable 'maximum stocking density (kg/m^3)'.

Case facility definition

The information on season stock survival, for the study period, 2013-2017, was intended to be collected directly from stock records at each participating facility. However, the SRL stock and mortality data were not consistent within and between facilities. For instance, some managers recorded the mortalities only after experiencing higher losses whereas some managers did not record mortality data at all. In absence of reliable stock and mortality data, surveyed owner/managers were asked to recall stock survival relative to previous fishing seasons. A facility was defined as a case facility if the manager believed that the stock losses from the 2015-16 fishing season was at least twice the losses from preceding fishing seasons (2013-14 and 2014-15).

Statistical analysis

All statistical analyses were performed in R software version 3.4.1 (R Core Team, 2017).

The objectives for the analysis were twofold;

1) To assess diversity in capacity and management practices across holding facilities

2) To identify capacity and/or management practices associated with survival during holding in holding tanks

Descriptive statistics

Association between individual factors and case facility status was assessed using Fisher's exact test. Potential collinearity between categorical factors was explored using Cramer's V correlation coefficient estimated using the 'vcd' package (Meyer et al., 2017). When string collinearity was found (> 0.9 correlation coefficient), the factor which were readily interpretable were favoured over the other correlated factors for further analysis. For example, the material of the holding tank was highly correlated with the internal surface of the holding tank. In this case, the variable 'holding tank material' was retained for further analysis.

Diversity in capacity and management practices

Due to the large number of factors and the relatively low number of facilities surveyed, a conventional multifactorial analysis was not adequate to explore several potential factors at once. Instead a multivariate factor analysis, similar to principal component analysis, was conducted to first reduce the dimension of data (Dohoo et al., 1997). Factor analysis of mixed data (FAMD) was performed using 'FactoMineR' package (Le et al., 2008) for each of the four facility-level factors' groupings of - (1) holding facility capacity; (2) bio-filter management; (3) water management; and (4) stock management factors. The factors were grouped in the four sections to enable FAMD and distinct identification of clusters. The analysis provided reduced number of new uncorrelated factors (or dimensions) that encompasses the information from several original factors. For each factor grouping, the suitable number of dimensions was selected so at least 70% of the total variability in the data was explained. The dimension coordinates calculated for each individual holding facility were used to perform a cluster analysis using partitioning around mediods (PAM) (Maechler et al., 2017) to identify potential group(s) of closely related and/or homogenous holding practices. The practice clusters identified in each factor groups were used to explore potential association with the probability to be a case facility.

Association with facility-level survival

The practice clusters for each factor group were entered into a multivariable logistic regression model to assess whether membership of particular cluster increased the risk of being a case facility. A Chi-squared likelihood ratio test was then performed on the final model to assess significant contribution from a variable to the model.

Results

Out of 63 registered facilities holding a license during the 2015-16 season, 52 (83%) agreed to participate in the survey and were visited between October 2017 and February 2018. A summary of the count facilities contacted, recruited, and visited are reported in Table 1. Figure 1 illustrates the geographical locations of the 52 participating facilities.

Table1. Summary of SRL holding facility/business study recruitment and visits.

SRL facilities/businesses	South Australia	Tasmania	Victoria	Total
Contacted	40	33	10	83
Not eligible[a]	10	7	3	20
Not active/sold[b]	2[c]	2[c]	0	4
Refused participation	0	2	1	3
Recruited	28	22	6	56
Withdrew participation[d]	1	3	0	4
Visited	27	19	6	52

[a] Cooked lobsters immediately upon reception, no live holding tanks on premises.

[b] These businesses were sold and may have been taken over by other businesses.

[c] These businesses were forced to shut down after incurring heavy losses in 2015-16 season.

[d] Owners from these facilities initially agreed for facility visit but later withdrew participation from the study.

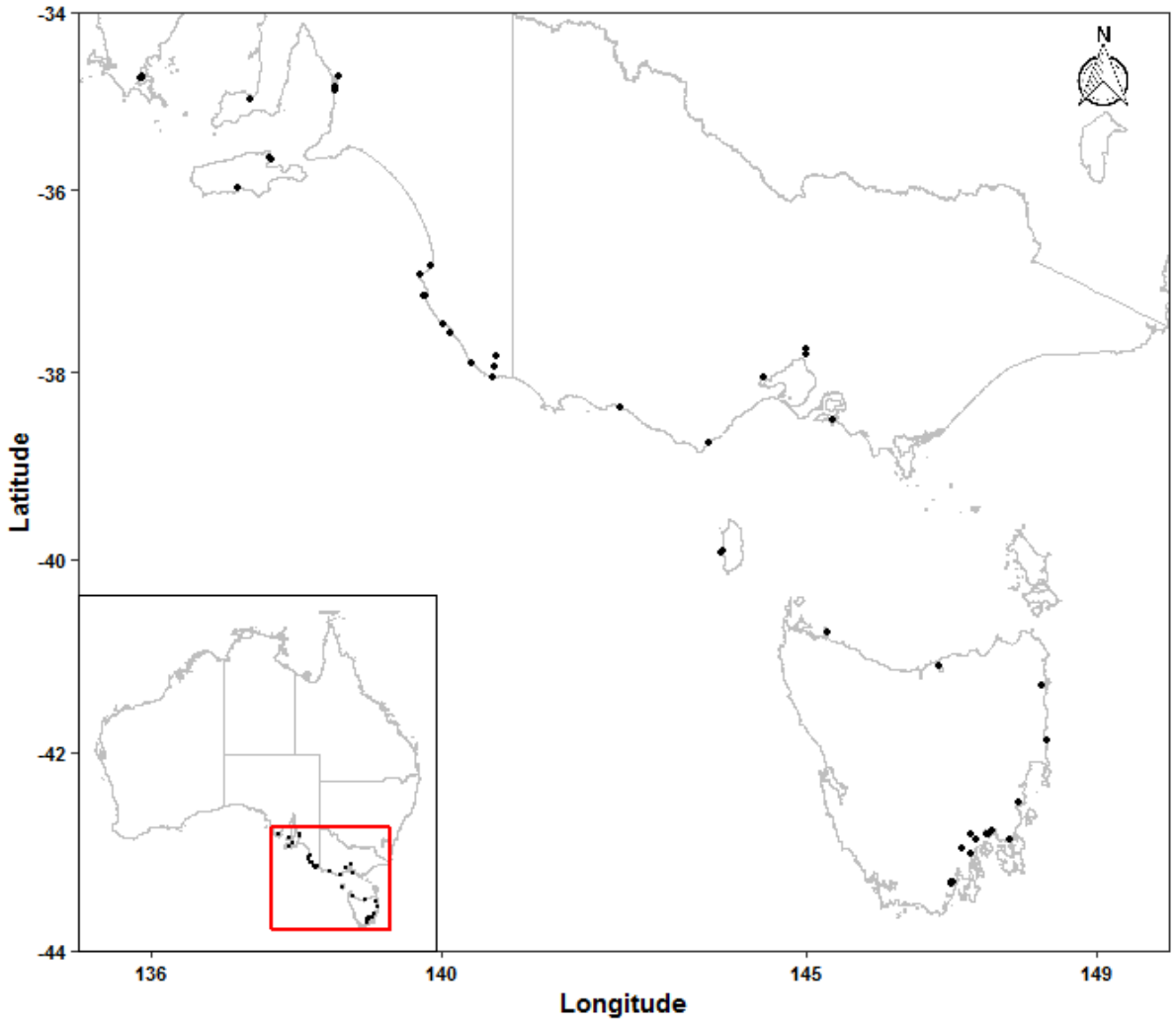

Figure 1. Geographical distribution of participating Australian southern rock lobster holding facilities in South Australia, Victoria, and Tasmania.

The total number of factors available for investigation was 146 (Appendix-2). After investigating for collinearity, 64 factors of interest were selected from the initial pool of 146. The descriptive summaries of qualitative and quantitative selected factors are presented in Appendices 3 and 4.

Managers from 22 out of 52 (42.3%) facilities reported an at least two-fold increase in SRL losses during the 2015-16 fishing season compared with previous fishing seasons (case facilities). In 2016-17 fishing season, 14 (63.6%) case facilities and 5 (16.7%) non-case facilities, as categorised based on the case facility status in 2015-16 fishing season, reported similar SRL losses as observed in 2015-16 fishing season. Overall, 19 (36.5%) facilities reported similar increase in SRL losses, as observed in 2015-16 fishing season.

Factor and cluster analysis

The capacity and management practices across the surveyed facilities was highly variable (Figure 2). No facility had the same capacity or practices than another. There was high variability in the data to explore potential clustering. Therefore, the selected factors belonging to capacity (n = 27), bio-filtration tank (n = 7), water management (n = 14) and stock management (n = 16) practice groups were subjected to factor analysis to reduce variable dimension. Figures 2 to 5 shows the relative proximity of each selected factor level and each individual surveyed facility relative to a pair of selected dimensions. The factor levels and facilities that are closely located to each other are closely related or share similar practices for a given factor grouping. The factor levels which are further away from the origin of dimension axes contributed more to the building of dimension(s).

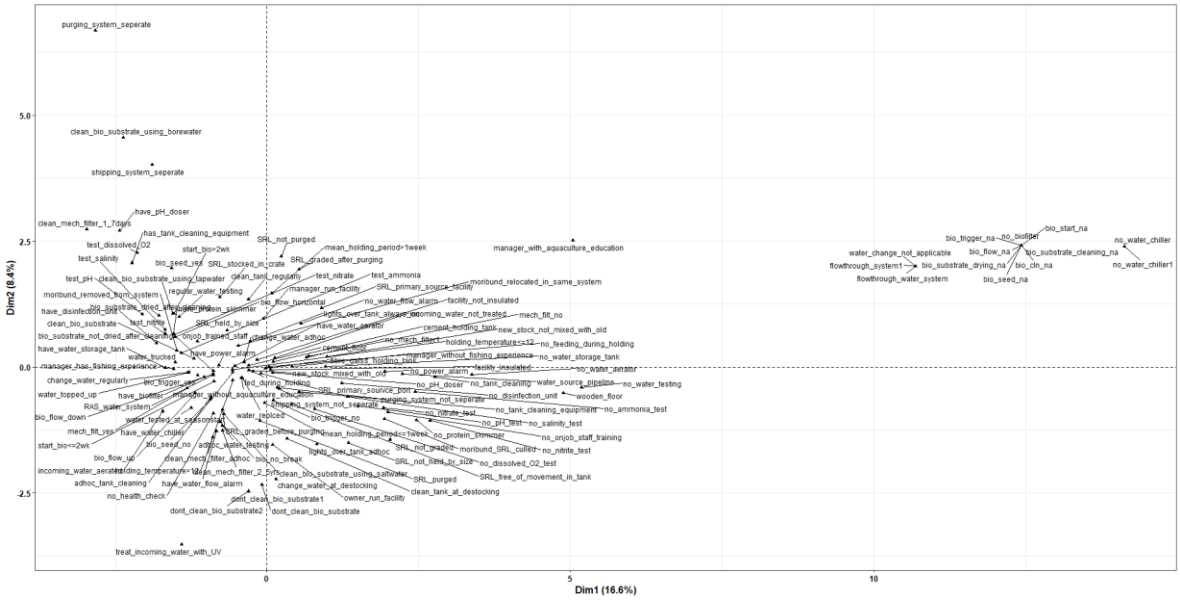

Figure 2. Scatter plot of the distribution of the qualitative factor levels (black dots) according to two dimensions (Dim. 1 and Dim.2) from the factor analysis of mixed data (64 qualitative and quantitative factors).

Factors related to holding facility capacity

The first nine resultant dimensions from the factor analysis for capacity related factors explaining 72%, the total variation were used for cluster analyses. Figure 3 shows coordinates of qualitative capacity related variables on the first and third dimensions.

The cluster analysis identified four major capacity clusters among the 52 surveyed facilities (Table 2). The largest cluster (28 facilities), named ‘Lower capacity modern Recirculating Aquaculture System (LMRAS)’, included mainly facilities with a modern setup of a Recirculating Aquaculture System (RAS) and lower overall tank capacity. Briefly, they were primarily managed by a designated full time manager, had insulation in the roof or walls of building, had a disinfection unit and protein skimmer along the RAS, had holding tanks made up of freezer glass panels, own a vacuum pool machine for cleaning holding tanks and sourced water through contracted truck delivery.

The second cluster (16 facilities), named ‘Lower capacity traditional RAS (LTRAS)’, included mainly facilities with less modern facilities, more traditional RAS water systems and lower overall tank capacity. They were primarily managed by an owner with extensive experience in SRL processing, were less likely to have insulation in roof or walls of building and equipment such as protein skimming or disinfection unit along the RAS, and sourced water through a direct incoming pipeline.

The third cluster (four facilities), named ‘Higher capacity RAS (HMRAS)’, were mainly export facilities with a modern RAS setup and higher holding capacity. They primary had a higher holding volume, number of casual staffs, and had a separate RAS for shipping SRL. The other characteristics of the facilities in this cluster were similar to the facilities belonging to LMRAS cluster.

The fourth cluster (4 facilities), named ‘Flowthrough’, included facilities with a flowthrough water system. They had a relatively low SRL holding volume, were managed relatively less experienced manager, however, with education or formal training in aquaculture.

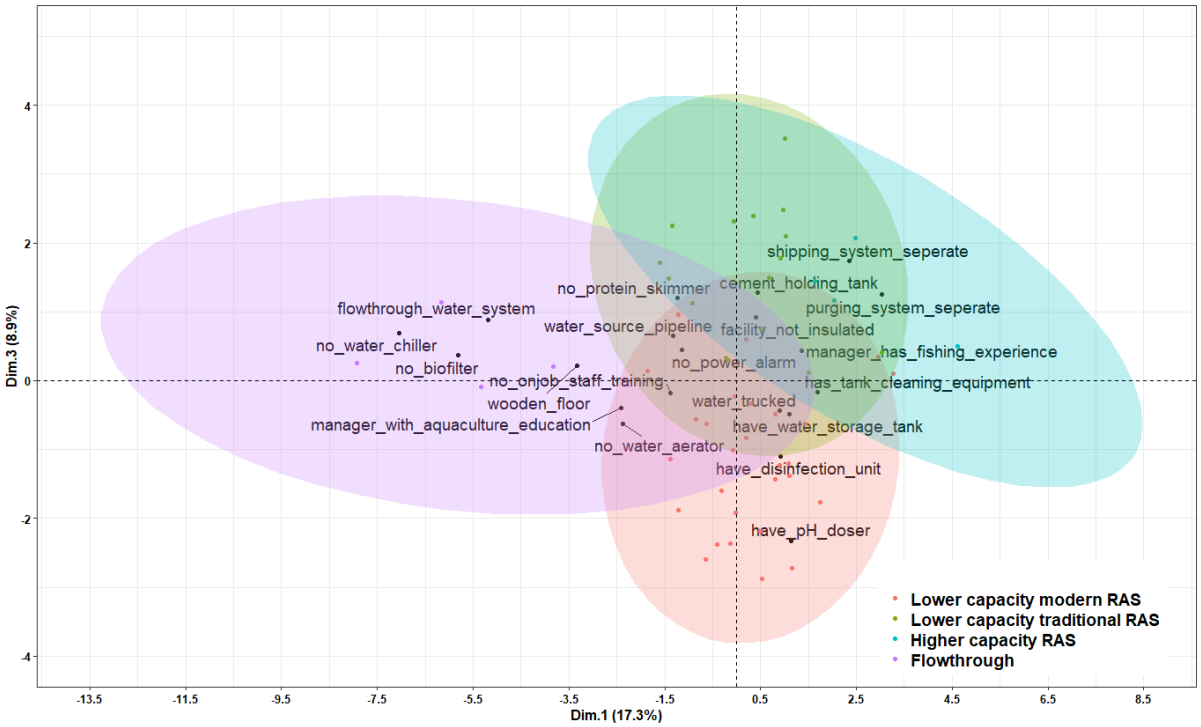

Figure 3. Scatter plot of the distribution of the facility capacity categorical factor levels (black dots) and 52 individual facilities (coloured dots) according to two dimensions (Dim. 1 and Dim.3) out of 9 selected from the factor analysis of mixed data (27 qualitative and quantitative factors). Four facility capacity clusters were identified – facilities with lower holding capacity and modern re-circulation aquaculture system (RAS) (red dots); facilities with lower holding capacity and traditional RAS (green dots); facilities with higher holding capacity RAS (blue dots); and facilities flowthrough water system (purple dots).

Table 2. Description of the clusters of facilities identified from the factor and cluster analysis of capacity related risk factors.

Section, factor	Factor levels	Lower capacity modern RAS (N=28)	Lower capacity traditional RAS (N=16)	Higher capacity RAS (N=4)	Flowthrough (N=4)
Building					
Floor type	Cement	92.9%	100%	100%	75%
	Wooden	7.1%	0%	0%	25%
Insulation (roof and/or wall)	Yes	85.7%	56.2%	50%	75%
	No	14.3%	43.8%	50%	25%
Manager					
Owner manages the facility	Yes	25%	68.8%	25%	25%
	No	75%	31.2%	75%	75%
SRL fishing experience	Yes	17.9%	37.5%	0%	0%
	No	82.1%	62.5%	100%	100%
Total experience in SRL processing (years)	-	15 (9.3)[a]	28 (5) [a]	18 (11) [a]	4 (1) [a]
Experience managing current facility (years)	-	9 (7) [a]	18 (10) [a]	13 (9) [a]	4 (1) [a]
Formal education/training in aquaculture: revalued	Yes	7.1%	0%	0%	50%
	No	92.9%	100%	100%	50%
Staff					

No. of permanent staff (including the manager)	-	3 (2) [a]	4 (2) [a]	3 (1) [a]	2 (1) [a]
No. of casual staff	-	3 (3) [a]	3 (3) [a]	15 (4) [a]	2 (1) [a]
Informal training acquired by staff	Yes	64.3%	68.8%	75%	25%
	No	35.7%	31.2%	25%	75%
Water system					
Holding tank material	Cement	7.1%	43.8%	25%	25%
	Freezer glass panels	92.9%	56.2%	75%	75%
Total volume in holding tanks (m^3)	-	57 (41.3) [a]	54.7 (43) [a]	141.6 (73.7) [a]	10.9 (14.1) [a]
Holding tank cleaning machine	Yes	25%	6.2%	50%	0%
	No	75%	93.8%	50%	100%
Primary water system	RAS	100%	93.8%	100%	25%
	Flowthrough	0%	6.2%	0%	75%
Primary water source	Direct pipeline	25%	56.2%	25%	100%
	Trucked	75%	43.8%	75%	0%
Storage tank	Yes	42.9%	37.5%	25%	(0%
	No	57.1%	62.5%	75%	100%
Presence of mechanical filter in water system	Yes	46.4%	25%	25%	0%
	No	53.6%	75%	75%	100%

Presence of bio-filtration tank in water system	Yes	100%	100%	100%	0%
	No	0 (0%	0%	0%	100%
Presence of pH doser in water system	Yes	7.1%	0%	0%	0%
	No	92.9%	100%	100%	100%
Protein skimmer attached to water system	Yes	82.1%	31.2%	100%	0%
	No	17.9%	68.8%	0%	100%
Presence of disinfection unit in water system	Yes	60.7%	18.8%	25%	0%
	No	39.3%	81.2%	75%	100%
Presence of chilling unit	Yes	100%	100%	100%	50%
	No	0%	0%	0%	50%
Added water aeration	Yes	82.1%	100%	100%	50%
	No	17.9%	0%	0%	50%
Power alarm in facility	Yes	85.7%	56.2%	75%	25%
	No	14.3%	43.8%	25%	75%
Water flow alarm within water system	Yes	42.9%	56.2%	0%	0%
	No	57.1%	43.8%	100%	100%
Separate water system for shipping	Yes	0%	6.2%	100%	0%
	No	100%	93.8%	0%	100%

Separate water system for purging SRL	Yes	0%	0%	75%	0%
	No	100%	100%	25%	100%

[a] Mean (SD) for quantitative risk factors.

There was no significant association between cluster variable for capacity related risk factors and being a case facility (Table 6) ($P = 0.15$).

Factors related to bio-filter management

The first five resultant dimensions from the factor analysis for bio-filter related factors explaining 76%, the total variation were used for cluster analyses. Four facilities without bio-filter were excluded from the factor analysis for bio-filter management factors. Figure 4 shows coordinates from qualitative bio-filter management variables on the first two dimensions. Variables such as cleaning of bio-filter, type of water used for substrate cleaning, and timing of bio-filtration conditioning or seeding before start of the season contributed well to the first dimension. The water flow direction in the bio-filter and the drying of substrate contributed to both first and second dimensions.

The cluster analysis identified three major bio-filter management clusters among 48 of the surveyed facilities (the four flowthrough facility were excluded because they do not use bio-filter) (Table 3). The largest cluster (23 facilities), named 'Interrupt and don't dry substrate between seasons', included mainly facilities that stopped their bio-filter(s) between seasons but did not dry their substrate after cleaning. They also primarily practice late seeding and regular cleaning of substrate with tap water.

The second cluster (16 facilities), named 'Interrupt and dry substrate between seasons', included mainly facilities that stopped their bio-filter(s) between seasons and dry their substrate after cleaning. They were primarily facilities that practice early seeding and regular cleaning of substrate with saltwater.

The third and last cluster (9 facilities), name 'Run all-year around' included mainly facilities that did not interrupt their bio-filter(s) between fishing seasons. Managers form these facilities also conditioned the bio-filter substrate to promote the microflora in the bio-filter, however, the substrate in the bio-filter tank was not seeded with new bacterial culture in majority. The bio-filter tank/substrate was not cleaned in majority of these facilities.

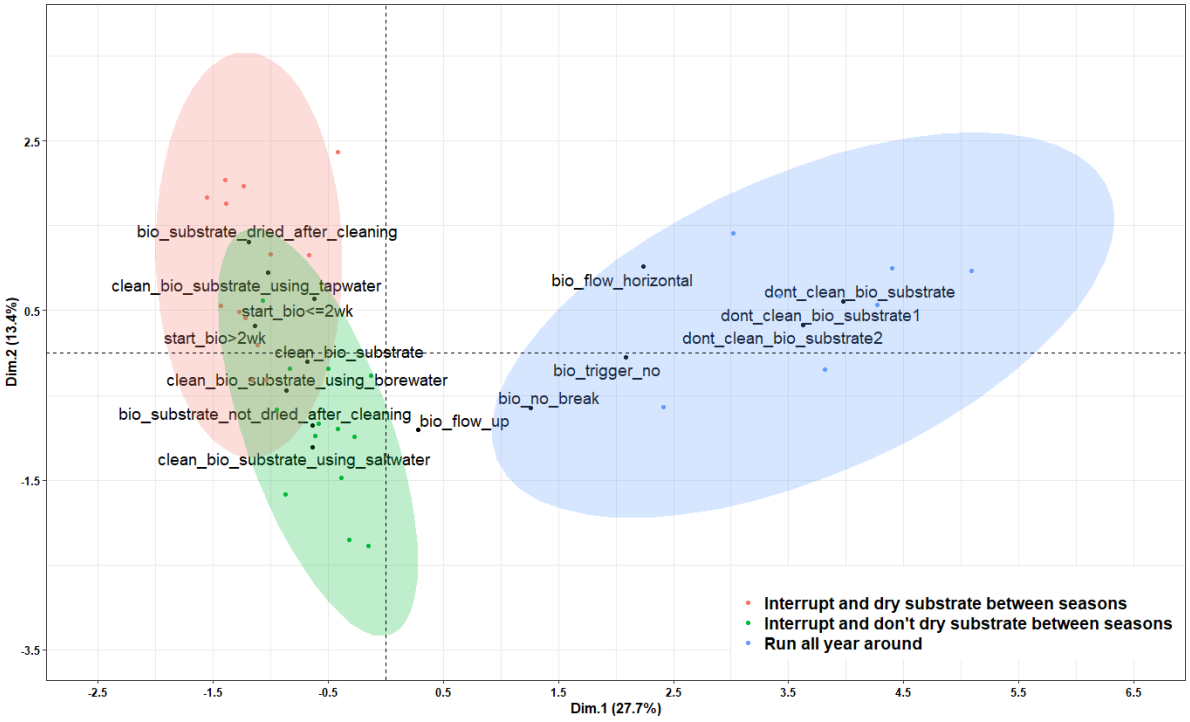

Figure 4. Scatter plot of the distribution of the bio-filer management categorical factor levels (black dots) and 48 individual facilities (coloured dots) according to two dimensions (Dim. 1 and Dim.2) out of 5 selected from the factor analysis of mixed data (7 qualitative and quantitative factors). Three bio-filter management clusters were identified – facilities that interrupt their bio-filter(s) and dry substrate between seasons (red dots); facilities that interrupt their bio-filter(s) but do not dry substrate between seasons (green dots); and facilities that do not interrupt their bio-filter(s) between seasons (blue dots).

Table 3. Description of the clusters of facilities identified from the factor and cluster analysis of bio-filter management factors.

Factor	Levels	Interrupt and don't dry substrate between seasons (N = 23)	Interrupt and dry substrate between seasons (N = 16)	Run all year around (N = 9)
Water flow direction in bio-filter tank	Downwards	34.8%	68.8%	33.3%
	Upwards	4.3%	25.0%	44.4%
	Horizontal	91.3%	6.2%	22.2%
Bio-filtration conditioning	Yes	8.7%	100%	77.8%
	No	13%	0%	22.2%
Seed bio-filter system with artificial media or ammonium chloride	Yes	87%	25%	11.1%
	No	34.8%	75%	88.9%
Start running bio-filter system	≤ 2 weeks before season start	17.4%	25%	11.1%
	> 2 weeks before season start	47.8%	68.8%	0%
	Bio-filter system active all year round	100%	6.2%	88.9%
Clean bio-filter tank/substrate	Yes	0%	100%	22.2% [a]
	No	30.4%	0%	77.8%
Water used for cleaning bio-filter tank substrate	Tapwater	65.2%	75%	0%
	Saltwater	4.3%	18.8%	0%

	Borewater	0%	6.2%	0%
	Never clean bio-filter tank/substrate	0%	0%	100%
Bio-filter tank substrate dried under sun after cleaning	Yes	100%	87.5%	0%
	No	0%	12.5%	0%
	Never clean bio-filter tank/ substrate		0%	100%

[a] only clean the bio-filter tank after transferring the substrate to another temporary tank

There was no significant association between cluster variable for bio-filter management risk factors and being a case facility ($P = 0.054$) (Table 6).

Factors related to water management

The first nine resultant dimensions from the factor analysis for water management related factors explaining 75%, the total variation were used for cluster analyses. Figure 5 shows coordinates of qualitative water management variables on the first two dimensions. The variables related to monitoring of water quality parameters (pH, ammonia, nitrate, nitrite, salinity, and dissolved oxygen) and it's frequency, pre-treatment (UV and aeration) of incoming water, and frequency of cleaning mechanical filter are well represented on the first dimension. Variables such as water addition or replacement and it's frequency are better represented on the second dimension. The quantitative variables for addition and replacement of water are well represented on the second dimension.

The cluster analysis identified four major water management clusters among the 52 surveyed facilities (Table 4). One of the largest clusters (18 facilities), named '*Ad hoc* water quality monitoring and top-up', mainly comprised of practices that cleaned holding tanks frequently, cleaned mechanical filter infrequently, pre-treated incoming water with ultraviolet (UV) light or added aeration, monitored their water quality infrequently and added water in holding tanks when needed.

The other largest cluster (18 facilities), named 'Scheduled water quality monitoring and change', mainly included facilities that cleaned holding tanks frequently, pre-treated incoming water with ultraviolet (UV) light or added aeration, cleaned mechanical filter infrequently, routinely monitored their water quality and replaced water regularly.

The third cluster (12 facilities), named 'No water quality monitoring and ad hoc top-up', consisted of facilities that cleaned holding tanks at destocking, did not pre-treat incoming water, cleaned mechanical filter infrequently, did not or rarely monitored their water quality infrequently and addition of water when needed.

The fourth profile (four facilities), named 'No water change required', consisted of the four flowthrough facilities that cleaned holding tanks regularly or at destocking, did not pre-treat incoming water, and did not require to monitor or change their water.

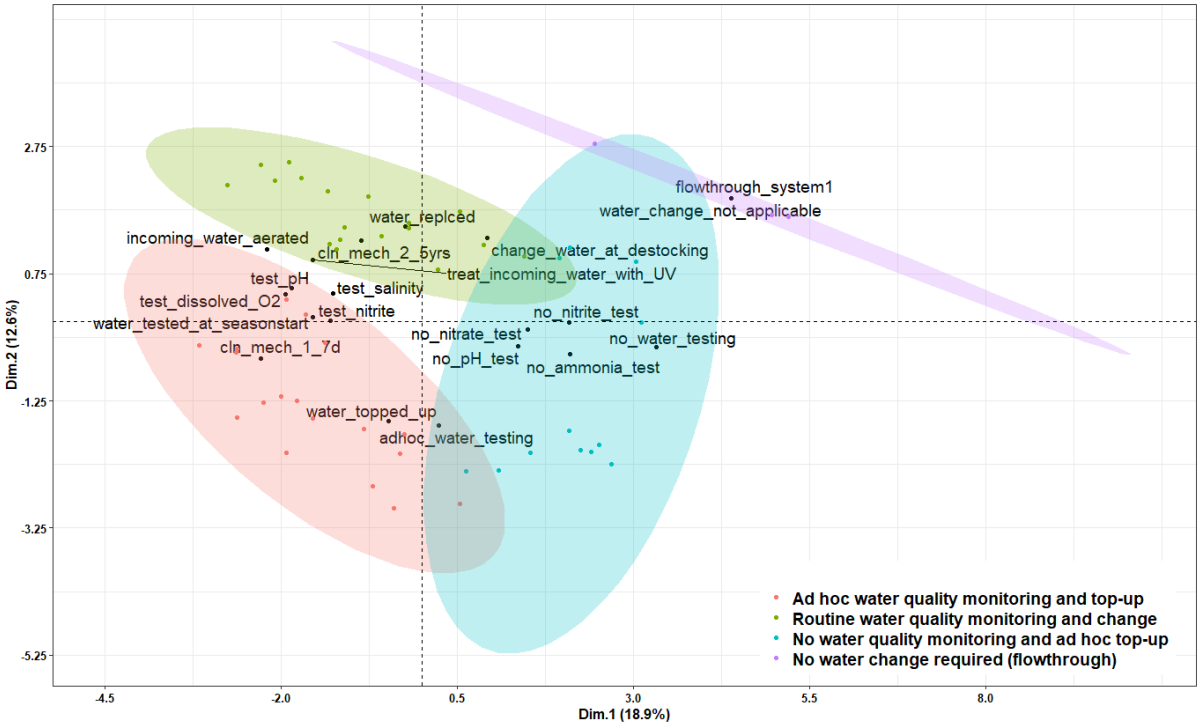

Figure 5. Scatter plot of the distribution of the water management (other than bio-biofilter) categorical factor levels (black dots) and 52 individual facilities (coloured dots) according to two dimensions (Dim. 1 and Dim.2) out of 9 selected from the factor analysis of mixed data (14 qualitative and quantitative factors). Four water management clusters were identified – facilities with *ad hoc* water quality assessment and water to-up (no water change) (red dots); facilities with scheduled water quality assessment and change (green dots); facilities with no water quality assessment and *ad hoc* water to-up (blue dots); and facilities flowthrough water system (no water assessment and change required) (purple dots).

Table 4. Description of the clusters of facilities identified from the factor and cluster analysis of water and water system management risk factors.

Factor	Levels	Ad hoc water quality monitoring and top-up (N = 18)	Scheduled water quality monitoring and change (N = 18)	No water quality monitoring and ad hoc top-up (N = 12)	No water change required (flowthrough) (N = 4)
Holding tank cleaning frequency	Regularly	50.0%	44.4%	33.3%	50%
	Ad hoc	11.1%	33.3%	0%	0%
	At destocking	33.3%	22.2%	50%	50%
	Never	5.6%	0%	16.7%	0%
Pre-treatment of sourced water: type of treatment	Aeration	5.6%	5.6%	0%	0%
	UV	5.6%	5.6%	0%	0%
	No pre-treatment	88.9%	88.9%	100%	100%
Mechanical filter cleaning frequency	Every 1 to 7 days	22.2%	0%	0%	0%
	Every 2 to 5 years	5.6%	16.7%	0%	0%
	Ad hoc	16.7%	16.7%	25.0%	0%
	No mechanical filter	55.6%	66.7%	75.0%	100%
Water addition / replacement frequency	Regularly	61.1%	72.2%	41.7%	0%
	At destocking	0%	11.1%	8.3%	0%

	Ad hoc	38.9%	16.7%	50.0%	0%
	Not required	0%	0%	0%	100%
Mode of water addition	Top up only	100%	0%	66.7%	0%
	Replace with similar volume	0%	100%	33.3%	0%
	Not required	0%	0%	0%	100%
Water addition (%)	-	15.2 (6.9) [a]	0 (0) [a]	6.8 (7.1) [a]	0 (0) [a]
Water replacement (%)	-	0 (0) [a]	59 (32.2) [a]	28.3 (44.7) [a]	100 (0) [a]
Water testing frequency	At season start	27.8%	22.2%	0%	0%
	Regularly	38.9%	77.8%	8.3%	25%
	Ad hoc	33.3%	0%	33.3%	0%
	No water monitoring	0%	0%	58.3%	75%
Water pH monitored	Yes	61.1%	61.1%	0%	0%
	No	38.9%	38.9%	100%	100%
Water salinity monitored	Yes	66.7%	44.4%	16.7%	25%
	No	33.3%	55.6%	83.3%	75.0%
Water ammonia monitored	Yes	88.9%	88.9%	0%	25.0%
	No	11.1%	11.1%	100%	75.0%
Water nitrite monitored	Yes	83.3%	83.3%	16.7%	0%

	No	16.7%	16.7%	83.3%	100%
Water nitrate monitored	Yes	72.2%	83.3%	8.3%	0%
	No	27.8%	16.7%	91.7%	100%
Water dissolved oxygen monitored	Yes	50.0%	22.2%	0%	25.0%
	No	50.0%	77.8%	100%	75.0%

[a] Mean (SD) for quantitative risk factors.

There was no significant association between cluster variable for water management risk factors and being a case facility (P = 0.80) (Table 6).

Factors related to stock management

The first eight resultant dimensions from the factor analysis for stock management related factors explaining 75%, the total variation were used for cluster analyses. Figure 6 shows coordinates from qualitative stock management variables on the first two dimensions. Practices such as purging of SRL in separate system or tank, grading SRL and it's timing, mixing of different SRL class size, and holding period are better represented on the first dimension whereas primary source of SRL is well represented on the second dimension. Health monitoring was well represented on both dimensions.

The cluster analysis identified two stock management clusters among the 52 surveyed facilities (Table 5). The facilities belonging to first cluster (32 facilities), named 'Extensive holding', processed a lower annual tonnage (median = 43.0 tons per year). These facilities were more likely to not purge SRL before grading and/or stocking, mix old and new batches and size classes, and stock SRL with free movement in holding tanks (no crates).

The other cluster (20 facilities), named 'Intensive holding', included facilities that processed a higher annual tonnage (median = 107.5 tons per year) sourced from wider range of fishermen or other facilities and with a longer transport time. These facilities were more likely to receive and purge incoming stock in separate tank before grading, stock SRL in crates and feed SRL when held for a longer period.

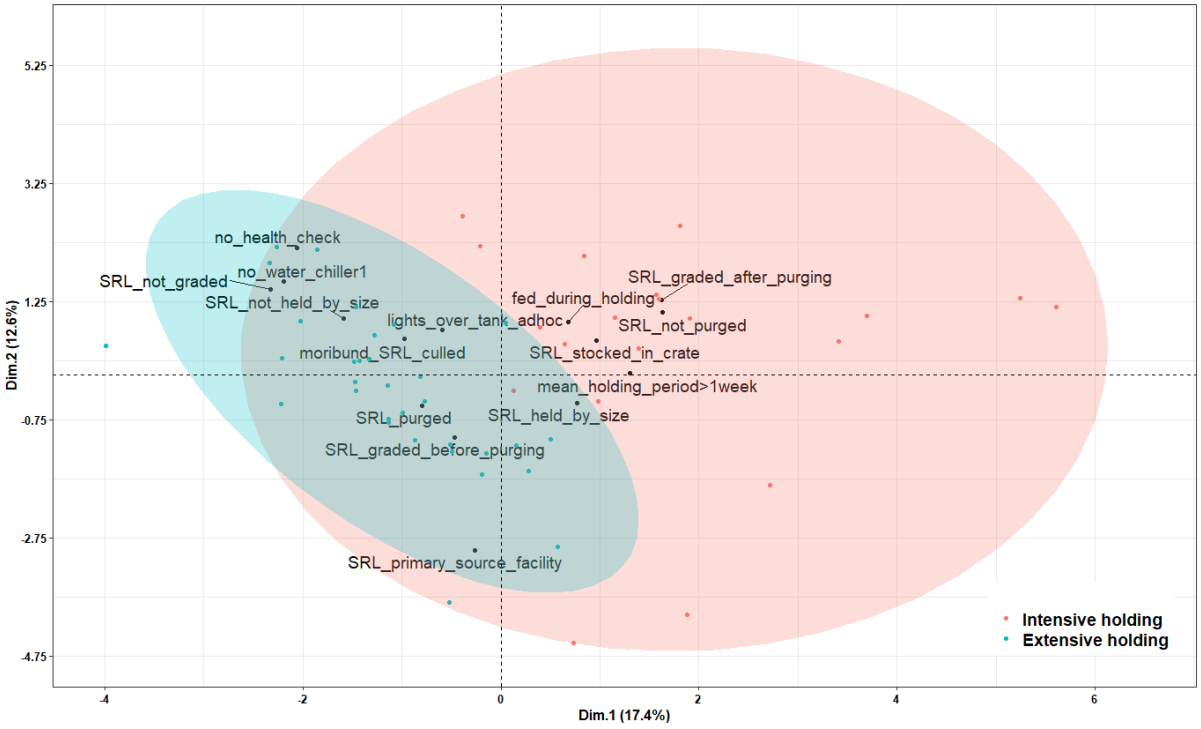

Figure 6. Scatter plot of the distribution of the stock management categorical factor levels (black dots) and 52 individual facilities (coloured dots) according to two dimensions (Dim. 1 and Dim.2) out of 8 selected from the factor analysis of mixed data (16 qualitative and quantitative factors). Two stock management clusters were identified – facilities with intensive holding practices (red dots); and facilities with extensive holding practices (blue dots).

Table 5. Description of the clusters of facilities identified from the factor and cluster analysis of stock management factors.

Factor	Levels	Intensive holding (N = 20)	Extensive holding (N = 32)
Stocking density (kg/m^3)	-	101.7 (34.3 - 446.0) [a]	95.0 (49.6 - 257) [a]
Use of lights over holding tanks	Ad hoc	25.0%	25.0%
	Always on	75.0%	75.0%
Water temperature in holding tanks	≤ 12	75.0%	75.0%
	> 12	20.0%	21.9%
	No chilling unit	5.0%	3.1%
Annual tonnage in 2015-16 fishing season	-	107.5 (4.5 - 500) [a]	43.0 (0.3 - 200) [a]
Primary SRL source	Other facility	10.0%	9.4%
	Ports	90.0%	90.6%
No. of SRL sources	-	6 (4) [a]	3 (2) [a]
No. of fishermen used for sourcing SRL	-	18 (1 - 75) [a]	6 (1 - 23) [a]
Allow purging of SRL in separate system or tank	Yes	75.0%	6.2%
	No	25.0%	93.8%
SRL grading	After purging	75.0%	6.2%
	At arrival in facility	25.0%	75.0%

	Never	0%	18.8%
SRL stocked by class size	No	10.0%	46.9%
	Yes	90.0%	53.1%
Mix old and new batch	Yes	60.0%	87.5%
	No	40.0%	12.5%
SRL stocking method	Crate	60.0%	18.8%
	Free moving	40.0%	81.2%
Average stocking period (weeks)	≤ 1 week	45.0%	81.2%
	> 1 week	55.0%	18.8%
Feed SRL while in holding tanks	Yes	25.0%	6.2%
	No	75.0%	93.8%
Fate of sick SRL	Remains in same system	15.0%	9.4%
	Transferred to other system	20.0%	28.1%
	Culled	0%	6.2%
Mean driving time from source to facility (hrs)	-	1.5 (0.08 – 9.0) [a]	0.7 (0.04 – 5.1) [a]

[a] Median (range) for quantitative risk factors.

There was significant association between cluster variable for stock management risk factors and being a case facility (P = 0.002) (Table 6).

Other factors

The data on water exchange and flow rate could not be collected on a consistent basis for all facilities and hence were excluded in the final analyses.

Association with reported holding survival in 2015-16 fishing season

The cluster variables for capacity related and bio-filter management factor group were not significant in the final multivariable logistic model. The facilities in 'Intensive holding' cluster were more likely to experience perceived sub-optimal survival (OR = 7.0, 95%CI: 2.1-26.13, P = 0.002) compared to the facilities from the 'Extensive holding' cluster. The residual deviance from the fitted final model was significantly lower compared to the null model (P = 0.001).

Table 6. Odds-ratios and *P*-values for unconditional associations between cluster variables and reported holding survival in 2015-16 fishing season.

Risk factor group, cluster	% case facilities	Odds ratio	95% CI	*P*-value
Capacity related				
Lower capacity traditional RAS	18.8	1.00	-	Ref.
Higher capacity RAS	100	na	na	na
Flowthrough	50.0	4.33	0.39 – 51.67	0.217
Lower capacity modern RAS	46.4	3.76	0.95 – 19.08	0.075
Bio-filter management				
Run all year around	22.2	1.00	-	Ref.
Interrupt and dry substrate between seasons	68.8	7.70	1.32 – 66.16	0.35
Interrupt and don’t dry substrate between seasons	30.4	1.53	0.28 – 12.04	0.72
Water management				
No water quality monitoring and ad hoc water top-up	42	1.00	-	Ref.
Ad hoc water quality monitoring and top-up water	50	1.40	0.32 – 6.38	0.65
Scheduled water quality monitoring and change water	39	0.89	0.20 – 4.07	0.88
No water change required (flowthrough)	25	0.47	0.02 – 5.00	0.56
Stocking management				
Extensive holding	25	1.00	-	Ref.
Intensive holding	70	7.00	2.11 – 26.13	0.002

na: not applicable

Discussion

This survey demonstrates considerable diversity in SRL holding systems and practices among the live SRL holding industry sector. Among the management risk group factors examined, only stock management was found to be significantly associated with perceived within facility sub-optimal SRL holding survival in more recent seasons in comparison to previous seasons.

Study limitations

Majority of the data in questionnaire were collected using the respondent's memory (recall). In addition to collecting information particular to the 2015-16 fishing season, the survey visits conducted in a wider timer period (October 2017 to February 2018) could have resulted into potential recall bias from the respondents (Elwood, 2007). However, it was not possible to conduct survey visits in short period of time owing to the difference in timing of state specific fishing seasons.

The findings from this study may be subjected to response bias as not all of the licenced and active facilities were included in this survey (Etter and Perneger, 1997). However, since more than 80% of the facilities from the industry were included in this study, there is likely low limited response bias.

The facilities were classified as case or non-case facility based on the owner's perception of experiencing sub-optimal survival in 2015-16 season compared to previous years. In absence of accurate stock and survival records, the process of classification may have been subjected to misclassification bias. Only a few facilities have had stock and survival data, and hence, it was difficult to estimate survival rates and consequently the level of bias. The case definition included comparison of sub-optimal survival trends across fishing seasons (2013-17) in the same facility. Several facilities lacked routine recording of stock and survival data. Consequently, it was not possible to compare sub-optimal survival trends across facilities.

The formation of cluster of facilities for capacity, bio-filter management, water management, and stock management related factors shows that the practices within the SRL holding industry are highly diverse.

Factors related to bio-filter management

The differences in bio-filter management practices were not associated with sub-optimal survival of SRL in recent seasons. The SRL holding industry is somewhat atypical for aquaculture industries where there is inconsistency on system stock loadings related to fishery open and closed seasons. The facility managers from 'Run all year around' cluster always had the bio-filtration system active and therefore may have had a steady and mature bacterial population required to have an efficient and on-going denitrification process. The shut down and cleaning of bio-filter substrate between seasons may have several benefits including the removal of accumulated excess organic load which can support competing heterotrophic bacteria, increasing the overall system biological oxygen demand, reducing nitrification capacity of the biofilter and harbouring of potentially detrimental pathogens. However, the cleaning of substrate, particularly with tap water and drying after cleaning, as practiced by managers from cluster 'Interrupt and dry substrate between seasons' will likely kill the nitrifying bacteria and impact on the detoxification ability of the bio-filtration system. The Chlorine present in running tap water may serve as a mild disinfectant and drying the substrate after cleaning may additionally deplete the bacterial population present in the substrate. The practice of reloading the substrate, without drying, in the bio-filtration tank after cleaning with fresh saltwater as observed in

the ‘Interrupt and don’t dry substrate between seasons’ cluster may have less chances of complete bacterial flora depletion. Regardless, facilities that clean substrate between seasons will require to reactivate and condition the biofilter in order for it to effectively process nitrogen loads when lobster stocks are returned. The lack of association with increased mortality between bio-filter management clusters may be explained by the complexity of biofilter management processes and maintenance factors.

Risk factors related to water management

The variation in frequency of water monitoring and the mode of water change (addition or replacement) suggests a considerable difference in management in different clusters however again these differences in practices were not associated with sub-optimal survival in recent seasons. The managers from ‘Ad hoc water quality monitoring and top-up’ cluster had the tendency to top up water on a smaller scale (mean = 15.2%) which might be a response to infrequent water testing done on an ad hoc basis in that cluster. Whereas the managers from the ‘Scheduled water quality monitoring and change’ cluster had the tendency to replace water on a larger scale (mean = 59%) and on a regular basis. These results suggest that the practice of regular water quality monitoring encourages more new water exchange which will likely improve overall facility water quality.

The practice of not monitoring the water quality by managers in the ‘No water quality monitoring and ad hoc top-up’ cluster may increase the vulnerability of stock to toxic components in water, mainly ammonia. The facilities in the ‘No water change required’ cluster had flowthrough water system and may not need water quality monitoring as often. Although, the stock in those holding facilities might be at risk of fluctuating ocean water quality. Again, the lack association with increased mortality between water management clusters is likely due to the diversity and complexity of water management processes and resources among industry members.

Risk factors related to stock management

The spread of facilities in both clusters suggests that the facilities in the ‘Extensive holding’ cluster might have had higher consistency across stock management practices compared with the facilities in the ‘Intensive holding’ cluster.

The stock management risk factor group was the only cluster variable that was significantly associated with perceived increasing trend for sub-optimal survival. Finding suggest that facilities in the ‘Intensive holding’ cluster perceived a recent increase in levels of sub-optimal survival compared to the ‘Extensive holding’ cluster. Key stock management differences between the two clusters include a greater likelihood for “intensive holding” facilities to process a larger annual tonnage of lobster sourced from wider range of sources with a longer transport time, purge lobsters before grading, separate lobsters into cohort and size classes, the use of crates as opposed to allowing the lobsters to free swim within tanks and feed SRL when held for a longer period.

High levels of processed stock have the potential to place several pressures on lobster maintenance systems and processes including, greater demands on RAS to maintain adequate water quality and higher pressures on staff to efficiently process and monitor stock. The nutrient load on RAS systems are related to both the stocking load and the physiological status of the cultured animal. Nutrient load on RAS and system oxygen demands increase with stock volume (Crear and Forteath, 2002; Crear and Forteath, 2000). Furthermore, larger stock numbers may place pressure on stock management processes, such as grading, potentially leading to increased air exposure time (Fotedar et al., 2001; Paterson et al., 2005). Air exposure is known to induce physiological stress on lobster and lead to the builds up of ammonia load and cause an oxygen debt. On release back into the water, lobsters will

quickly excrete the accumulated ammonia placing further pressures on RAS (Crear and Forteath, 2000). It is important to note that facilities in this cluster were more likely to source lobsters with longer transit times and from other facilities. Longer air exposure times associated with longer transit times are likely to increase physiological stress on sourced stock. Furthermore, the acquisition of stock from other facilities suggest that stock were handled more than once placing further stress on a potentially already compromised stock. The transfer of stock between facilities is also not recommended due to biosecurity risks associated with the transfer of potential pathogens between facilities.

The sourcing of SRL from nearby locations might be associated with the lower average on shore transport time from source ports to destination facility. Subsequently, the SRL may be exposed to air for a lesser period of time and hence lower stress levels in SRL. Prolonged air exposure has been linked to an increase in physiological stress indicator levels in SRL (Taylor and Waldron, 1997) and other decapod crustaceans (Cheng et al., 2002; Fotedar et al., 2001; Gomez-Jimenez et al., 2000; Lorenzon et al., 2007). Effects of prolonged aerial exposure on survival was evident from a similar experimental study on Norway lobsters (*Nephrops norvegicus*) which reported immuno-suppression and subsequent lower survival as a consequence of prolonged exposure to air, hypoxia, and increasing temperatures (Ridgway et al., 2006).

The managers from 'Intensive holding' cluster held SRL in crates which might be due to management preference or restrictions in available stocking area in holding tanks. Crates have stock management benefits for sorting lobsters and increase tank surface areas potentially allowing for higher tank stocking levels. Crating may also have benefits for lobster stress by providing tractable substrate for lobsters to maintain position (Dall, 1986). However, Crates will disrupt tank water flow mixing which could lead to the creation of flow dead-spots exposing lobster to sub-optimal water quality parameters. Similarly, retention of any organic material, for example dead SRL, SRL body parts, or left over feed, may lead to pockets of poor water quality within creates which in turn may be detrimental to the stock held in holding tanks. On the other hand, stocking SRL freely in holding tanks might be associated with optimal survival. This method of stocking, as practiced by the majority of the facilities in the 'Extensive holding' cluster may lead to less injuries to legs, antennae and body due to overcrowding and/or attacks by fellow SRL. Such injuries can add to the stress levels and/or could lead to sub-optimal survival. However, the impact on crating on lobster post-harvest performance has not been previously examined and requires further investigation.

Facilities in the intensive cluster are more likely to use separate RAS systems to purge lobsters at arrival to the facility. Excretory levels and oxygen demands of lobsters are likely to be greatly enhanced for the period after arrival to facilities due to release of accumulated ammonia associated with handling and air exposure, release of faecal matter associated with feeding at on bait at capture and enhanced oxygen demand due to emersion and feeding at capture. For example, the ammonia excretion rate of SRL is three-times the basal levels after just 30min of emersion and handling (Crear and Forteath, 2002). Spiny lobster oxygen consumption levels can be greatly enhanced for up to two day post feeding (Crear and Forteath, 2000) and for 12 hours post exhaustive handling (Jensen et al., 2013). Purging in a separate system may provide stock benefits by separating existing stock from sub-optimal water quality periods associated with the introduction of new stock to the facility. However the purging period is likely to be a critical period for lobster post capture and transport recovery and purge RAS need to be accurately managed to cope with the incoming load of SRL and swift removal of toxicants (ammonia, nitrate, and nitrite) building up in the system.

The practice of grading SRL at arrival, as practiced mostly by managers in the 'Extensive holding' cluster, suggests that SRL are handled only once after their arrival in the facility as opposed to being handled twice at purging and grading, as practiced by the majority in the 'Intensive holding' cluster. Avoiding excessive handling may reduce stress levels and/or permanent injury which can possibly lead to an increased survival in SRL while in holding tanks (Jussila et al., 1997; Paterson and Spanoghe, 1997). In addition, the minimal handling may also fasten the process of resolution of

disturbed physiological and immunological processes to normalcy (Fotedar et al., 2006). Therefore, avoiding excessive handling may improve survival in stressed SRL.

The proportion of facilities with lighting over the tanks did not differ between the facilities from 'Intensive holding' and 'Extensive holding' clusters. However, with SRL being a nocturnal animal, minimal lighting over the tanks may improve the survival rates as demonstrated in an experimental study which reported an increase in survival in SRL covered with shelters compared to those without shelters (James et al., 2001). However, that study included feeding of SRL in both groups (with and without shelter) which was not observed as a common practice in this study. In addition, exposure to light also increases metabolic demands and likely excretory rates in lobsters which in turn may increase pressure on RAS to maintain the water quality (Briceno et al., 2018).

The difference in the average holding period between facilities in the 'Extensive holding' cluster with lower annual tonnage compared to the facilities in 'Intensive holding' cluster with high annual tonnage might be associated with the market supply and demand chain. Australian SRL is a premium product for the domestic and international markets. The exporters try to exploit the demand and supply chain of the markets to obtain a high price and a better margin. Therefore, there can be situations when processors are forced to hold SRL for longer periods in the holding tanks in response to the market fluctuations. Higher stock levels held for longer period in turn may put pressure on the water system, particularly RAS. Consequently, if the water management during such situations is not optimum, then the practice of holding SRL for a longer period may affect the bio-filtration system and the holding survival of current and incoming stock. A study on western rock lobsters (*Panulirus Cygnus*) showed consistent changes in physiological stress indicators four days after capture (Fotedar et al., 2006). This suggests longer holding periods can affect the stress levels even in controlled conditions.

Conclusion

This study identified that the SRL holding industry employs highly diverse practices to manage SRL. There was a perceived increase in sub-optimal survival across fishing season. The perceived sub-optimal survival was associated with differences in stock management but not with capacity, water management or bio-filtration tank management practices. Facilities with a higher annual tonnage experienced higher perceived sub-optimal survival and were characterised with practices such as prolonged holding period, feeding of stock, increased handling, and purging of SRL in separate tank or system. The impact of individual intensive management practices on lobster stress is not well established and should be further examined. Additionally, water systems should be optimised particularly for high stocking intensity facilities.

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Results 4: Batch Level Investigation, Study 1

Preface

This aspect of the project was undertaken to partly address objectives 1 of this study:

Undertake an epidemiological investigation to describe the magnitude of the event and to identify potential environmental and management risk factor(s) associated with increased mortality

Manuscript information

Investigation of batch-level risk factors for holding survival in Australian southern rock lobster holding facilities

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Target Journal: Journal of Fish Diseases

Abstract

A retrospective investigation into risk factors for post-harvest survival at an Australian southern rock lobster (SRL, *Jasus edwardsii*) holding facility was undertaken using stock records from the 2016/17 fishing season. The facility experienced a cumulative survival of 98.29% and 98.42% based on biomass and count, respectively. Daily sub-optimal survival in a compartment within the holding tank increased during warmer months when stock intakes, and subsequent stocking density, increased rapidly. Survival increased with the decrease in transport distance (and duration) from source facilities. Also, cumulative survival was higher and lower in large (size 'E+') and small (size 'A'), respectively, compared with size 'C' lobsters which may be related, or not, to the stocking density and method during the transport. The water quality may also be of significance (e.g. system capacity), however potential associations could not be investigated in absence of water quality data.

The findings suggest that reduction in on-land dry transport wherever possible and improvement in transport techniques such as better temperature and humidity control, especially during warm months, may help in reducing transport related stress. Further effort should focus on management practices to improve survival during periods of high stocking volumes.

Introduction

Sub-optimal survival in a southern rock lobster holding facility (and across the industry) was reported during the 2015-16 season. The daily stock and mortality data for this facility was extracted retrospectively for further analyses.

The analysis of stock and mortality data covers the period from 2nd September 2016 to 4th August 2017. Retrospective analysis of 2015-16 season could not be analysed as data were not collected or missing.

Materials and methods

Data structure

Retrospective information on the SRL stocked in the facility for a given calendar day was available in MS Excel spreadsheets for each day between 2nd September 2016 and 4th August 2017. Each spreadsheet reported compartment (within a holding tank) level information on the SRL size, source, biomass stocked, holding compartment number, holding tank number, and mortality biomass (Table 1). The information on mortality biomass for each compartment was entered daily by a facility staff member based on findings of dead SRL in the holding tanks.

Data from all spreadsheets was extracted and collated into a single database for analysis. The daily losses and stock count per compartment of a given holding tank was extrapolated from the SRL size class and stocked biomass reported on a given day in a given compartment (Table 2).

Driving time from source to facility was created based on the information for SRL stock sources. The driving time and distance from a given SRL source to facility was estimated using package 'ggmap' in R (Kahle and Wickham, 2013) whereas the rest of the analysis was performed in Stata (v15, StataCorp, College Station, Texas, USA.

Table 1. Structure of the stock and mortality data

Level	Number of groups	Daily observations per group - mean (range)
Holding tank (or system)	4	783 (662-1005)
Holding compartment (within tank)	23 (5-6 per tank)	136 (56-178)

Table 2. Southern rock lobster size class and assumed individual wet bodyweight (Kg).

Size class	Bodyweight in Kg (range)
A	0.5 (>0.4 - ≤0.6)
B	0.7 (>0.6 - ≤0.8)
C	0.9 (>0.8 - ≤1.0)
D	1.25 (>1.0 - ≤1.5)
E-	1.75 (>1.5 - ≤2.0)
E+	2.25 (>2.0 - ≤2.5)
F	2.75 (>2.5)

Data analysis

The outcome was defined as the daily fraction of surviving biomass (*stock survived(Kg)/total stock(Kg)*100*) for a given compartment (within a holding tank).

A fractional response generalised linear regression model was used to assess unconditional association between the daily survival fraction and explanatory variables. The variables with a *P*-value ≤ 0.2 were retained for the multivariable analysis. The full multivariable model was reduced to a final model using backward elimination of non-significant variables at the 5% level.

Results

In total, 527 tons (98.29%) or 412,301 (98.41%) of the held SRL survived and were exported. A summary of SRL stock and survival, based on biomass and count, by holding tank, holding compartment, supplier, arrival month and size held daily and for the entire season are reported in Table 3.

Table 3. Median (range) of stocked SRL biomass (tons) and survival (%) by facility, holding tank, and holding tank compartment. NA – non applicable.

Data level, parameter	**Facility** Median (range)	**Holding tank** Median (range)	**Compartment within tank** Median (range)
Entire season			
Biomass (tons)	536.23	135.52	24.20
	(NA)	(104.64 – 160.56)	(2.61 – 35.95)
Count	418,940	106,962	18,089
	(NA)	(77,012 - 128,005)	(4,512 - 29,926)
Survival (% biomass)	98.29	98.22	98.41
	(NA)	(98.17 - 98.59)	(97.50 - 98.87)
Survival (% count)	98.41	98.40	98.40
	(NA)	(98.20 - 98.50)	(97.60 – 98.90)
Daily			
Biomass (tons)	1.88	0.54	0.15
	(0.04 - 6.74)	(0.004 - 2.17)	(0.001 - 0.51)
Count	1,458	390	107
	(44 - 6166)	(2 - 2,321)	(1 - 852)
Survival (% biomass)	98.93	99.01	100
	(92.25 - 100)	(56.00 - 100)	(33.33 - 100)
Survival (% count)	98.90	99.10	100
	(91.5 - 100)	(85.70 - 100)	(33.33 – 100)

Sub-optimal survival was lower in warmer months (February, March, April, and May) (Figure 1 and Table 4). Increase in stocking volumes was positively associated with sub-optimal survival.). The moving average (± 3 days) increased with increasing lagged (5 days) stock volumes (Figure 2 and Table 4.

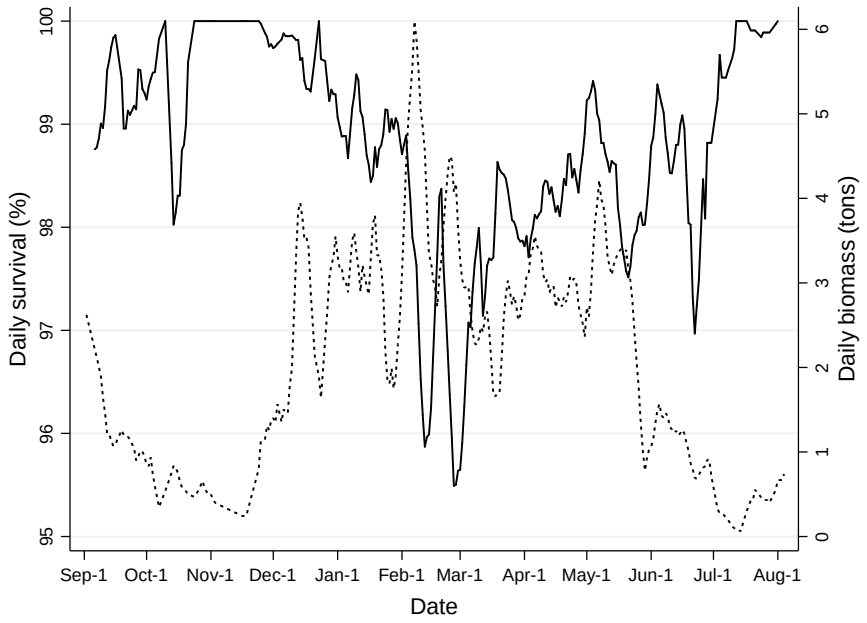

Figure 1. Smoothed (± 3 days) daily held biomass (dashed line) and daily % biomass survival (solid line) at facility level from September 2016 to August 2017.

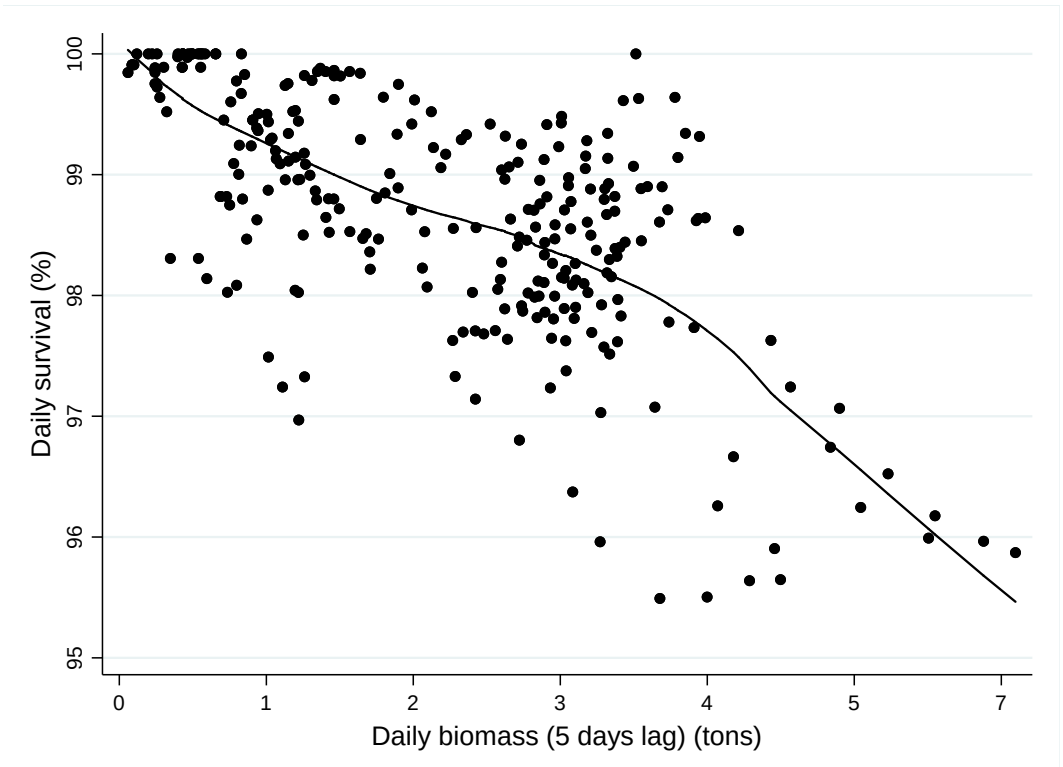

Figure 2. Scatter plot of total stock tons), with five days lag, and survival (% biomass survived) moving average (± 3 days) from September 2016 to Aug 2017.

Unconditional associations

Due to the large number of observations, majority of unconditional associations between explanatory variables and survival were significant (Table 4). However, survival was not different between systems (or holding tanks). Survival was significantly higher in stock arriving from one of the sources (source 5), and 'E-' and 'F' size class had significantly lower survival compared with 'C' (small) sized SRL (Table 4). Survival was lower in months of February, March, April, and May when the air temperature were relatively higher than in July (Table 4).

Table 4. Summary of lobster stock and survival (based on biomass and lobster count) and unconditional associations between independent variables and mortality.

Variable, levels	Number of records	Biomass			Count	
		Total stock (kilos)	Survival (%)	*P*-value	SRL count	Survival (%)
Tank						
A	662	104,639	98.20[#]	-	77,012	98.24
B	1,002	160,555	98.23	0.523	128,005	98.51
C	778	148,519	98.11	0.111	114,006	98.38
D	688	122,513	98.48	0.475	99,917	98.48
Compartment						
A2	157	30,689	97.82	0.494	18,089	98.00
A3	90	4,772	98.52	0.623	7,151	98.60
A4	147	23,803	98.21	0.614	17,462	98.27
A5	141	22,141	98.41	0.587	16,528	98.36
A6	127	23,233	98.42	0.935	17,782	98.17
B1	171	28,791	97.77	0.466	12,555	97.96
B2	177	34,583	97.68	0.606	19,404	97.74
B3	176	12,987	98.62	0.485	17,166	98.60
B4	178	31,150	98.42	0.994	23,346	98.66
B5	150	28,825	98.48	0.557	26,516	98.65
B6	150	24,219	98.83	0.499	29,018	98.94
C1	135	23,652	98.24	0.695	13,155	98.30
C2	152	35,951	97.71	0.444	20,980	98.05
C3	92	7,168	97.27*	0.02	9,117	97.76
C4	146	28,921	98.85[#]	-	18,521	98.91
C5	129	26,977	97.83	0.598	22,307	98.24
C6	124	25,851	98.28	0.628	29,926	98.61
D1	133	19,611	98.57	0.881	11,081	98.76

D2	123	23,070	98.55	0.803	13,362	98.68
D3	56	2,608	97.21	0.619	4,512	97.61
D4	134	26,691	98.62	0.147	19,710	98.63
D5	126	27,621	98.61	0.62	24,948	98.67
D6	116	22,913	98.19	0.738	26,304	98.11
Source (driving hrs to facility)						
1 (6- 7)	470	58,088	98.22*	0.001	55,322	98.64
2 (4- 5)	600	116,146	98.44*	<0.001	97,653	98.47
3 (7-8)	985	213,208	97.64*	<0.001	138,954	97.70
4 (NA)	29	1,221	99.24	0.664	1,059	99.24
5 (3.5-4)	1,046	147,564	98.99#	-	125,952	99.05
Size class						
A	249	14,635	98.18	0.189	29,273	98.21
B	398	62,802	98.64	0.796	89,727	98.65
C	407	60,295	98.93#	-	66,989	98.94
D	678	142,768	98.45	0.233	114,209	98.46
E-	479	92,038	98.05*	0.041	52,594	98.06
E+	453	81,785	98.10	0.371	36,360	98.11
F	466	81,902	97.49*	0.018	29,788	97.54
Arrival month						
Jan 17	371	71,399	98.96	0.075	59,206	99.11
Feb 17	423	110,550	96.97*	<0.001	82,543	97.15
Mar 17	358	72,274	97.66*	<0.001	54,243	97.74
Apr 17	403	81,129	98.27*	<0.001	61,930	98.27
May 17	416	78,380	98.38*	<0.001	57,017	98.51
Jun 17	338	28,449	98.71	0.598	20,366	99.08
Jul 17	113	4,075	99.73*	0.001	3,720	99.68
Aug 17	36	2,865	100.00*	<0.001	2,745	100.00
Sep 16	213	22,718	99.01#	-	13,351	99.12

Oct 16	132	9,215	99.35	0.795	6,215	99.49
Nov 16	60	5,788	99.84*	0.004	6,403	99.89
Dec 16	267	49,385	99.50*	0.040	51,201	99.49

* Significant association with mortality (P<0.05)

Reference group for comparison

Conditional association

Increase in daily held stock was associated with lower survival. Survival was lower in SRL graded as 'A' and higher in 'E+' size class (compared with 'C') (p = 0.039), arriving from distant source (Source 1, 2 and 3 compared with Source 5, P < 0.001) and held in months of January (p = 0.046), February (p < 0.001), March (p < 0.001), April (p < 0.001), May (p < 0.001), November (p = 0.004), and December (p = 0.017) (compared with September month) (Table 5). Survival was not different in other individual tank compartments except in 'D4' (p = 0.12) when compared to 'C4'. There was no evidence of clustering of survival between holding tanks (ICC = 0.01, 95% CI = 0.002 – 0.054) and between different compartments with the holding tank (ICC = 0.01, 95% CI = 0.002 – 0.054). There was no evidence of interaction between source and size class and between arrival month and size class of SRL.

Table 5. Output from the final multivariable model with odds ratios (with 95% CI) for variables significantly associated with survival.

Variable, levels	Odds ratio	95% CI	*P*-value
Stock	0.993	0.992 - 0.994	<0.001
Source			
5	Ref.	-	-
1	0.45*	0.33 - 0.61	<0.001
2	0.51*	0.40 - 0.65	<0.001
3	0.44*	0.35 - 0.57	<0.001
4	0.93	0.32 - 2.70	0.904
Size class			
C	Ref.	-	-
A	0.60*	0.37 - 0.97	0.039
B	1.02	0.72 - 1.45	0.897
D	1.08	0.78 - 1.49	0.662
E-	1.35	0.94 - 1.96	0.100
E+	1.49*	1.02 - 2.22	0.039
F	1.39	0.93 - 2.08	0.108
Arrival month			
Sep 16	Ref.	-	-
Jan 17	0.65*	0.43 - 0.99	0.046
Feb 17	0.23*	0.15 - 0.36	<0.001
Mar 17	0.21*	0.13 - 0.32	<0.001
Apr 17	0.28*	0.18 - 0.43	<0.001
May 17	0.32*	0.21 - 0.49	<0.001
Jun 17	0.68	0.44 - 1.06	0.094
Jul 17	2.22	1.06 - 4.76	0.034
Aug 17	1.00	NA	NA
Oct 16	1.47	0.79 - 2.7	0.224

Nov 16	4.76*	1.64 - 12.5	0.004
Dec 16	1.79*	1.11 - 2.94	0.017
Tank compartment			
C4	Ref.	-	-
A2	0.74	0.41 - 1.32	0.302
A3	0.63	0.3 - 1.32	0.218
A4	1.14	0.65 - 2	0.647
A5	1.30	0.72 - 2.38	0.376
A6	1.06	0.58 - 1.96	0.842
B1	0.85	0.48 - 1.54	0.609
B2	0.85	0.49 - 1.49	0.579
B3	1.05	0.59 - 1.89	0.865
B4	0.94	0.55 - 1.61	0.823
B5	0.84	0.48 - 1.49	0.553
B6	0.85	0.47 - 1.54	0.589
C1	1.04	0.59 - 1.85	0.892
C2	0.87	0.51 - 1.49	0.618
C3	0.70	0.36 - 1.33	0.283
C5	0.72	0.4 - 1.32	0.296
C6	0.65	0.35 - 1.2	0.171
D1	1.54	0.88 - 2.7	0.131
D2	1.15	0.65 - 2	0.642
D3	0.98	0.41 - 2.38	0.973
D4	2.08*	1.18 - 3.57	0.012
D5	1.49	0.83 - 2.7	0.187
D6	1.23	0.67 - 2.27	0.504

Ref. – Reference category

NA – not applicable.

Discussion

Higher survival in SRL sourced from the closest located source (Source 5) may be due to lower transport duration (relative to the distance). Southern rock lobsters from source 5 might have been air exposed for a lesser period of time and hence might have had experienced lower physiological stress. Prolonged air exposure associated with hypoxia and increasing temperature has been linked to an increase in physiological stress indicator levels in SRL (Morris and Oliver, 1999; Simon et al., 2016; Taylor and Waldron, 1997) and other decapod crustaceans (Cheng et al., 2002; Fotedar et al., 2001; Gomez-Jimenez et al., 2000; Lorenzon et al., 2007; Paterson et al., 2005). It is well established that air exposure significantly impairs gas and excretory exchange of lobsters which can disrupt lobster physiological homeostats resulting in hypercapnia, hyperglycaemia, haemolymph acidification, increased levels of lactate and other disruptions to haemolymph biochemistry (Simon et al., 2016; Taylor and Waldron, 1997). Previous studies have demonstrate a positive association between emersion time and mortality of SRL (Simon et al., 2016; Spanoghe and Bourne, 1997) .

Higher survival observed in larger size SRL ('E+') may be explained by their lower mass specific metabolic demands and, therefore, lower vulnerability to metabolic stressors (Crear and Forteath, 2002).

Lower survival observed between January to May may be due to higher water and air temperatures adding to thermic stress to SRL during transport and handling. Decrease in survival following increasing water temperatures in warmer months was reported in a study on American lobster (*Homarus americanus*) (Howell et al., 2005; Pearce and Balcom, 2005). Lobster metabolism and excretory rates are directly related to temperature resulting increased oxygen demand and ammonia excretory rates (Crear and Forteath, 2002; Crear and Forteath, 2000). Higher temperatures at capture and during transport also has the potential for acute thermal acclimation stress when exposed to rapid temperature change resulting fro introduction to cold water in holding tanks within holding facilities.

A lower stocking density may decrease injuries caused by other SRL. Such injuries can further challenge the homeostasis and, subsequently, survival. Additionally, overcrowding contributes to the saturation of the nitrification capacity of the water system and subsequent accumulation of ammonia and nitrite above toxic concentrations.

Water quality data such as levels of ammonia, nitrate, nitrite, temperature, last water change, temperature, and pH were missing and could not be included in the analysis. These parameters are likely associated with SRL homeostasis and mortality.

The holding period in tank (or period of time spent in tank) in facility may also be associated with survival. However, holding period could not be precisely estimated due to major data gaps in the end of holding dates.

Lobster specific variables such as approximate age, colour, and sex of the lobsters may have an effect on post-harvest survival. However, such assessment was out of scope for this analysis because information on age, colour, and sex of SRL was not recorded.

Conclusion

Based on available data, increase in stocking density, SRL size, longer transport duration from source facility, and transport during warmer months were associated with increased mortality. Lobsters transported for shorter duration and held at a lower stocking density survived better during holding. Precautions should be taken when holding SRL during warmer months, particularly for smaller sized SRL.

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Results 5: Batch Level Investigation, Study 2

Preface

This aspect of the project was undertaken to partly address objectives 1 of this study:

Undertake an epidemiological investigation to describe the magnitude of the event and to identify potential environmental and management risk factor(s) associated with increased mortality

Manuscript information

Investigation of batch-level risk factors for holding survival in Australian southern rock lobster holding facilities

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Target Journal: Journal of Fish Diseases

Abstract

For the 2016/17 fishing season, a total of 4,523 (3.1%) southern rock lobsters (SRL, *Jasus edwardsii*) were rejected of the total 147,292 purchased and processed SRL. A cumulative sub-optimal survival of 1.4% (n = 2,008) was observed between grading and packing including the holding period.

At grading sessions, 1% (n = 1,428) of 147,292 purchased lobsters were rejected due to missing legs (32%), crushing (15%) and molting (shelling) signs (22%). Tail damage and inflammation (swelling) was noticed in 3% and 2% of rejected SRL, respectively, whereas 23% rejected SRL did not show any apparent damages or injuries. Of the total rejected SRL, 557 (39%) were found dead at grading. A total of 260 (46.7%) dead SRL at grading had none apparent damages. Survival was lower in 'B' size class SRL compared to 'D' (OR = 0.53 *P* = 0.001) however, it was higher in SRL with transport time ≤1 hour (OR =2.68, *P* = 0.006) compared with ≥1 hour, and in SRL transported between months April to October (2017) (*P* = 3.45, 0.002) compared with those transported between November (2016) to March (2017).

A total of 3,095 (2.1%) SRL were rejected during holding or at packing stages. A majority (n = 2,536, 82%) of the rejections were due to sub-optimal survival (death or mortality). Of the dead SRL, 736 (29%) SRL showed signs of crushing whereas 69% presented none apparent injuries which were defined as sub-optimal survival cases during holding. Of the total (n = 1,740) cases during the entire study period, 1,194 (69%) could be traced back with the grading dataset. Therefore, cumulative survival in the analysed population for the entire study period was 99.14%. Sub-optimal survival rate was higher in red compared to brindle SRL (Sub-optimal survival rate ratio (SRR) = 1.54, P = <0.001), in SRL held between November (2016) to March (2017) (SRR = 1.92, P = 0.017) compared with April to October (2017). Sub-optimal rate was higher and lower in 'D' (SRR = 1.62, P <0.001) and 'E- and E+' (SRR ('E-') = 0.52, P = 0.006 and SRR ('E+') = 0.09, P = 0.015) size class SRL, respectively, compared with 'B'. Sub-optimal rate was higher in SRL held form one day up to one week (SRR = 4.74, P <0.001), whereas it was highest on the day of arrival (SRR = 24.14, P <0.001) compared to those held for more than one week. Sub-optimal survival at grading or at holding was highly correlated (Intraclass correlation coefficient = 0.47) in SRL coming from same batch as identified by the docket number suggesting that the variation in sub-optimal survival between batches of SRL from same fishermen may be a result of varied transport duration. Sub-optimal survival rates during holding were similar in batches of SRL with and without sub-optimal survival at grading suggesting that the factors influencing survival in SRL at grading might be different to those during holding in holding tanks within a facility.

Reduction in land transport time/distance might help to reduce transport related stress. Improvement in management and practices leading to better humidity and temperature control may help in reducing the transport stress especially in warmer months when the metabolic demands in SRL are high. Improvement in management practices of incoming SRL, especially on day of arrival and during warmer months, may help in improving survival during initial stages of holding.

Introduction

Sub-optimal survival in southern rock lobster (SRL) at a facility (and across the industry) was reported during the 2015-16 fishing seasons. A longitudinal study was designed to explore within – facility risk factors associated with sub-optimal. The stock and survival data covers the period from 18th November 2016 to 1st October 2017.

Materials and methods

At grading

Routinely, new arriving stocks of up to 150Kg were transferred into metal basket and then placed into one of two 'purging' tanks overnight to recuperate after the road transport and graded the next day. At peaks of the fishing season, incoming stock were often too large to accommodate prior purging and therefore, SRL were graded on arrival at the facility. Grading involved sorting out of SRL into commercial size classes (class B to F, Table 1) and allocated to crates by size, colour (red or brindle) and by sex when possible up to a certain count to reach a target stocking weight in the crate (varying according to size classes). Lobsters ineligible for holding were rejected by an assessor at grading based on the observation of any apparent damage such as damaged antennae, body, or tail, missing legs, and apparent swelling of soft membranes. The grading assessor also recorded the vigour scores (1-5, Table-2) for the rejected SRL based on visual assessment. For each incoming crate, information on grading date, fisherman name, docket number, shell colour, size, crate number and weight, and number of males and females in the crate were recorded. Additionally, information on port of landing and bait used in fishing pots was available based on the docket number for each incoming batch of SRL.

Table 1. Southern rock lobster size class and wet bodyweight range in Kg.

Size class	Bodyweight range (Kg)
B	>0.6 - ≤0.8
C	>0.8 - ≤1
D	>1 - ≤1.5
E-	>1.5 - ≤2
E+	>2 - ≤2.5
F	≥2.5

Table 2. Description of vigour scores given to rejected southern rock lobsters by an assessor at grading process and during holding.

Vigour score	Description
1	Dead
2	Alive and very weak
3	Alive and weak
4	Alive and strong
5	Alive and very strong

During holding

The SRL stock was transferred into dedicated holding tanks after grading. Individual crates were monitored when possible for damaged and dead SRL. The crates were individually traced using a unique four digit identification number and grading date. Information on damage and vigour scores (Table 2) similar to those used for rejected SRL at grading, were recorded.

SRL were individually assessed at packing for shipping and the information on vigour score and damage findings was recorded when a SRL was rejected.

Data analysis

The data was entered in MS Excel and then transferred to R software (v3.5.1) (R Core Team, 2018) for analysis.

Sub-optimal survival at grading

Dead SRL at grading were identified based on a vigour score of '1'. Dead SRL at grading found with swollen body or no apparent damage were classified as 'case' for sub-optimal survival. The count of SRL that survived until grading was calculated using the data on cases. A mixed effect logistic regression model was used to assess association between the survival at grading and potential explanatory variables. The information on fisherman source and docket (nested within the fishermen) was entered as random effects in the model to account for the correlation between SRL within a given batch and sourced from a given fisherman. The variables with a P-value ≤ 0.2 were used into building a multivariable analysis. The full multivariable model was reduced to a final model using backward elimination of non-significant variables (P-value > 0.05).

Sub-optimal survival during holding

Death during holding and at packing were also classified from on a vigour score of '1' among rejected SRL, respectively. Dead SRL rejected during holding and at packing with swollen body or no apparent reason were defined as SRL 'case' for sub-optimal survival. It was not possible to precisely identify the date of death for rejects. Therefore, the cumulative sub-optimal survival during the entire

holding period was used to investigate potential batch-level factors. A Poisson regression model was built to explore association between sub-optimal survival rate during holding and potential explanatory variables. Fisherman and docket (nested within the fishermen) variables were used as random effects in the model to account for the correlation between SRL within a given batch sourced from a given fisherman. Variables with a *P*-value ≤ 0.2 for the association with outcome were entered in multivariable analysis. The full multivariable model was reduced to a final model using backward elimination of non-significant variables interpreted at the 5% significance level.

Results

Data collation

Datasets from accepted (crate level data) and rejected stocks (individual SRL level data) at grading, holding and packing were merged based on their docket number. The summary of data matching is reported in Table 3. Sixty-nine percent of the rejected SRL during holding and at packing could be traced back to the grading. The packing data could be matched with 34% of crates at grading.

For crates with missing packing dates, the subsequent grading date was used instead assuming SRL from those crates were held in the tanks up to the subsequent grading date. Holding duration could be calculated for a total of 138,060 SRL (94.6%).

Table 3. Summary of datasets available for data analysis and the number (and %) of SRL tracked from spreadsheets with information on rejected SRL to the spreadsheet, named 'GRADING', with all information on incoming SRL.

Source database	**Data level**	**Data type**	**No. of observations**	**No. of SRL**	**% SRL matched with GRADING dataset**
GRADING	Crate	SRL eligible for holding at grading	11,973	1,45,864	-
GRADING_REJECT	SRL	SRL rejected at grading	1,428	1,428	100%
DAILY_REJECT + REJECT_AT_PACKING	SRL	SRL rejected during holding and at packing	3,095	3095	69%
PACKING	Crate	Packing and export logistics	2,209	50,285	34%

Sub-optimal survival at grading

The number of SRL graded on a given day ranged from 12 to 5,057 and averaged at 1238 (median = 870). Incoming SRL were graded over 120 days and the number of SRL batches graded on a given

day varied from 1 to 5 (median = 1). Survival at grading varied from 98.97 to 99.97% on a given day whereas it varied from 95.82 to 99.96 by received batches (Figure 1 and Appendices 5 and 6).

Overall, 1.00% of SRL (n = 147,292) were rejected at grading for various damages as detailed in Table 4. Seventy-two per cent (138/192) of incoming SRL batches had at least one rejected SRL at grading. Of the total rejected SRL, 39% (n = 1,428) were dead at grading and almost half (46.7%) of the dead SRL at grading had no apparent damages. Mechanical injuries including tail damage, missing legs, and crushing by fellow SRL were recorded in 43.7% of the total dead SRL.

Of the total graded SRL, 0.18% (n = 265) were classified as unexplained losses (i.e. cases). More than one case of unexplained mortality were observed in SRL from 82% (31/38) of the fishermen and in 35% (68/192) of the SRL batches arrived at facility.

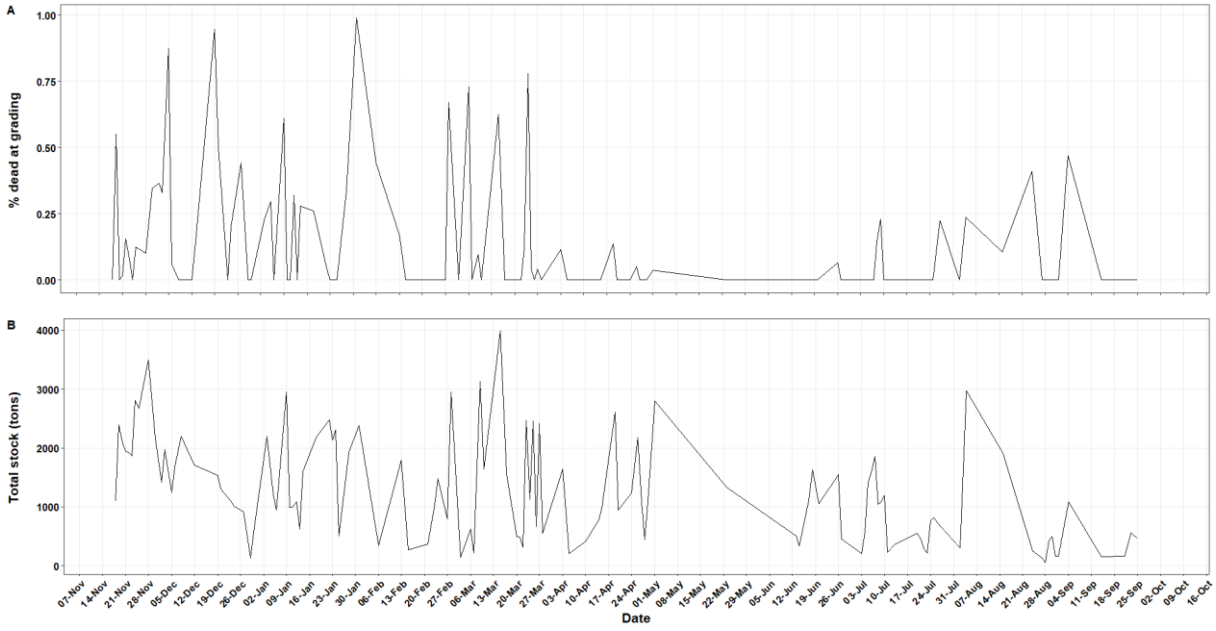

Figure 1. Time series plot of smoothed (± 2 days) per cent mortality (deaths) (plot 'A') and total SRL arrived at the facility from November 2016 to October 2017 (plot 'B').

Table 4. Summary (number and %) and causes of SRL stock rejected at grading.

Damage	Total	% dead	% alive and strong	% alive and weak
None apparent	331	46.7	0.3	12.0
Crushed	220	39.4	0.0	0.0
Molting signs	318	8.6	18.8	37.3
Missing >=3 legs	462	1.6	75.0	40.6
Tail damage	42	1.4	2.7	4.5
Multiple	25	1.3	1.7	2.3
Swollen	30	1.1	1.4	3.5

Table 5. Summary of SRL stock survived at grading and unconditional associations between independent variables and survival at grading.

Variable	Total graded	% survival
Bait		
Blue mackerel	4,650	99.38***
Blue mackerel + couta	53,116	99.84[#]
Blue mackerel + couta + mullet	9,934	99.79
Blue mackerel + couta + salmon	32,267	99.88
Couta + salmon	1,114	99.91
Mullet + salmon	2,291	99.56**
Missing data	43,982	99.82
Sex		
Female	22,948	99.81[#]
Male	106,778	99.79*
Missing data	17,566	99.98
Shell		
Red	77,985	99.82[#]
Brindle	69,307	99.82
Size		
B	93,754	99.79*
C	28,812	99.89
D	18,437	99.82[#]
E-	4,181	99.88
E+	1,343	100
F	765	99.74
Month of arrival		
Nov-16	25,781	99.83**
Dec-16	18,693	99.70**
Jan-17	26,463	99.73***

Feb-17	6,463	99.85**
Mar-17	24,786	99.76***
Apr-17	11,300	99.96#
May-17	4,075	99.98
Jun-17	6,816	99.99
Jul-17	14,440	99.95
Aug-17	6,087	99.84
Sep-17	2,376	99.83
Oct-17	12	100
Season of arrival		
April to October	45,106	99.94#
November to March	102,186	99.77***
Landing port		
1	75,289	99.87
2	22,457	99.76
3	16,681	99.81#
4	11,348	99.76
4	5,085	99.8
6	4,866	99.69
7	2,361	99.07
8	2,249	99.96
9	1,665	99.94
10	1,480	99.93
11	1,364	99.56
12	1,256	99.84
13	677	100
14	414	100
15	12	100
Missing	88	100

Driving time to facility		**100**
<=1 hours	78,806	99.87[#]
>1 hours	68,398	99.76*
Missing	88	100

* Significant association with survival at grading (‘***’ $P < 0.001$, ‘**’ $P < 0.01$ ‘*’ $P < 0.05$)

Reference group for comparison

Multivariable model

‘Bait’ and ‘sex’ variables were not used in the multivariable model due to the higher proportion of missing data. In the final multivariable model, size, driving time to facility, and season were significantly associated with survival at grading. The ‘B’ size class SRL had lower survival than ‘D’ size class SRL (Odds ratio (OR) = 0.53, $P = 0.001$). Higher survival was observed in SRL at grading between April 2017 to October 2017 compared with the season from November 2016 to March 2017 (OR = 3.45, $P = 0.002$) (Table 6). Survival was higher in SRL with transport time to facility of ≤1 hour compared with those with transport time of >1 hour (OR = 2.68, $P = 0.006$).

Survival in SRL was not correlated between different batches (dockets) from the same fisherman (Intra-class correlation coefficient (ICC) = 0). However, a high correlation of survival (ICC = 0.47) was observed between SRL from same batch (docket). This suggests that survival was clustered within SRL arriving under the same batch (docket). Summary of sub-optimal survival at grading by docket number and source port is included in Appendices 6 and 7.

Table 6. Output from the final multivariable model with odds ratios (with 95% CI) for variables significantly associated with survival at grading.

Variable	**Odds ratio**	**95% CI**	***P*-value**
Size			
D	Ref.	-	-
B	0.53	0.36 - 0.78	0.001
C	1.32	0.81 - 2.17	0.275
E-	1.89	0.72 - 50	0.192
E+	NA	NA	NA
F	0.50	0.11 - 2.17	0.353
Driving time to facility			
>1 hours	Ref.	-	-
<=1 hours	2.68	1.32 – 5.44	0.006

Season of arrival			
November to March	Ref.	-	-
April to October	3.45	1.56 – 7.62	0.002

Ref. – Reference category

NA – not applicable.

Sub-optimal survival during holding and until packing

Graded SRL were held in holding tanks for 14 days on an average (range = 0.5 to 175 days, median = 7). The crate weight ranged from 1 to 32 Kg and averaged at 10.4 Kg (median = 8.9). The number of SRL in crate ranged from 1 to 49 and averaged at 12 (median = 12).

A total of 3,095 (2.1%) SRL were rejected during holding or at packing of the 145,864 SRL passed at grading and processed in the facility due to damages as listed in Table 7. A majority (82%) of the rejected SRL were rejected due to mortality.

Of the total cases, 69% (n = 1,194) could be tracked back to their respective crates with a valid grading and packing date (Table 3). Therefore, the cumulative survival during holding in the analysed dataset with 137,840 SRL was 99.14% whereas the true cumulative survival in the entire dataset was 98.74%. Sub-optimal survival was observed in 6.80% (n = 786) of the total 11,078 crate cycles during the entire study period. In crates with sub-optimal survival, the cumulative sub-optimal survival in a given crate varied from 4% to 100% (median = 90%) whereas the sub-optimal survival rate varied from 0.1 to 75 (median = 1.4) per 100 lobster-days.

Table 7. Summary (number and %) of SRL stock rejected during holding and at packing.

Damage	**Total**	**% dead**	**% alive and strong**	**% alive and weak**
None apparent	2,040	66.9	3.6	68.1
Crushed	733	28.6	3.6	1.4
Swollen	70	1.9	1.8	4.1
Missing ≥3 legs	166	1.1	75.0	18.7
Shelling	46	0.9	1.8	4.5
Body damage	18	0.3	10.7	0.8
Antennae damage	11	0.2	3.6	0.6
Tail damage	11	0.1	0.0	1.8

Unconditional analysis

All categorical variables except port and purging tank were significantly associated with sub-optimal survival rate (Table 8). Crate weight, the variable with quantitative information, was negatively associated with sub-optimal survival rate ($P < 0.001$).

Table 8. Summary of SRL stock held and cumulative survival during holding and up to packing and unconditional associations between independent variables and sub-optimal survival rate (no. of cases /1000 lobster-days).

Variable	No. held	No. at risk	Cumulative survival (%)	Sub-optimal survival rate (no. cases /1000 lobster-days)
Bait				
Blue mackerel	4,355	4,318	98.21	1.29*
Blue mackerel + couta	51,051	50,881	99.12	0.66
Blue mackerel + couta + mullet	9,603	9,571	99.08	0.59
Blue mackerel + couta + salmon	29,694	29,615	99.22	0.45#
Couta + salmon	1,075	1,075	99.91	0.17
Mullet + salmon	2,129	2,120	98.92	0.77
Missing	39,933	-	-	-
Sex				
Female	22,584	22,487	99.20	0.62#
Male	98,433	98,053	98.99	0.70***
Missing	16,823	-	-	-
Shell colour				
Red	71,504	71,194	98.97	0.72***
Brindle	66,336	66,164	99.31	0.48#
Size				
B	89,158	88,906	99.26	0.52#
C	26,669	26,588	99.05	0.62
D	16,479	16,341	98.41	0.94***
E-	3,765	3,754	99.44	0.89

E+	1,162	1,162	99.91	0.18
F	607	607	100	0
Season of holding				
April to October	37,814	37,702	99.18	0.44#
November to March	100,026	99,656	98.71	1.01*
Month of holding				
Nov-16	25,163	25,100	99.23	1.13
Dec-16	18,309	18,224	98.35	1.18
Jan-17	26,072	25,905	97.62	1.40*
Feb-17	6,339	6,331	99.46	0.43
Mar-17	24,143	24,096	99.42	0.44
Apr-17	10,676	10,670	99.83	0.07**
May-17	3,495	3,494	99.89	0.02***
Jun-17	6,680	6,675	99.54	0.43#
Jul-17	11,430	11,332	97.81	1.80***
Aug-17	4,702	4,700	99.91	0.06**
Sep-17	831	831	99.76	0.25
Landing port				
1	71,171	70,957	99.19	0.51
2	19,626	19,527	98.95	0.77
3	15,953	15,879	98.83	0.93
4	11,015	11,006	99.75	0.30
5	4,858	4,848	99.59	0.24
6	4,692	4,671	98.98	0.65#
7	2,165	2,156	98.85	0.93
8	2,170	2,156	98.16	1.44
9	1,272	1,272	100	0
10	1,427	1,425	99.72	0.38
11	1,175	1,156	97.11	2.00

12	1,207	1,198	98.09	1.75
13	667	667	99.85	0.22
14	355	353	98.59	0.91
Missing	87	-	-	-
Holding duration				
<=1 day	16,533	16,484	99.32	6.86***
>1 day to <= 1week	47,057	46,940	99.15	1.92
> 1 week	74,250	73,934	98.53	0.62#
Holding tank				
12	1,069	1,067	99.53	4.07***
7	11,181	11,132	98.80	0.79
10	7,554	7,533	99.10	0.71
8	9,814	9,783	98.97	0.70
1	20,239	20,149	98.95	0.67
11	6,056	6,035	99.17	0.65*
4	12,569	12,525	99.16	0.64#
2	15,937	15,876	99.10	0.59
5	11,540	11,508	99.27	0.54*
9	5,660	5,645	99.36	0.53
3	14,604	14,550	99.07	0.52**
6	13,369	13,331	99.42	0.35
0	3,855	3,855	100	0
13	2,071	2,071	100	0
Missing	2,322	-	-	-
Purging tank				
5	180	178	96.67	3.31
8	2,132	2,115	98.36	1.29
4	1,660	1,644	98.19	1.25
3	1,123	1,114	98.31	1.13

11	441	438	98.41	1.06
10	1,487	1,482	99.33	0.70
0	61,560	61,351	99.16	0.61#
1	909	906	99.34	0.61
12	45,620	45,489	99.32	0.44
6	2,112	2,111	99.72	0.25
2	410	409	99.76	0.20
13	2,198	2,197	99.82	0.18
9	500	499	99.60	0.18
7	291	291	100	0
Missing	17,217	-	-	-
SRL from batch with rejections at grading				
No	24,990	24,862	98.82	0.81#
Yes	112,850	112,496	99.20	0.56***
SRL from batch with mortality at grading				
No	73,518	73,223	99.00	0.73#
Yes	64,322	64,135	99.28	0.48***

* Significant association with sub-optimal survival rate during holding in holding tanks ('***' $P < 0.001$, '**' $P < 0.01$ '*' $P < 0.05$)

Reference group for comparison

Multivariable model

The variables such as sex, purging tank, and holding tank were not entered in the multivariable model due to the higher proportion of missing data. Holding period, season, crate weight, size, and shell colour were significantly associated with the sub-optimal survival rate during holding (Table 9).

Sub-optimal survival rates were higher in 'D' size class compared with 'B' size class SRL (P <0.001), in red shell coloured compared with brindle coloured SRL (P <0.001), and in SRL held from November (2016) to March (2017) compared with those held from April to October 2017 (P = 0.017) (Table 6). The sub-optimal survival rate was 24.1 and 4.7 times higher in SRL held for a day (P <0.001) and between two days up to one week (P <0.001), respectively, compared with those held for more than one more week. The variable 'crate weight' was negatively associated with the sub-optimal survival rate in the final model (P = 0.006). However, the association was weak and confounded by SRL size class.

The sub-optimal survival rate in SRL was not correlated between different batches of SRL, based on the docket number, belonging to the same fisherman (ICC = 0). However, a moderate correlation (ICC = 0.21) was observed between SRL within same batch (docket). This suggests that sub-optimal survival was clustered between SRL arriving under same docket as observed at grading. Sub-optimal survival rates during holding and up to packing by fisherman and docket number are presented in Appendices 1 and 2, respectively.

Table 9. Output from the final multivariable model with sub-optimal survival rate ratios (with 95% CI) for variables significantly associated with sub-optimal survival rate during holding and up to packing.

Variable	**Sup-optimal survival rate ratio**	**95% CI**	***P*-value**
Size			
B	Ref.	-	-
C	1.00	0.86 – 1.17	0.994
D	1.62	1.38 – 1.91	<0.001
E-	0.52	0.32 – 0.83	0.006
E+	0.09	0.01 – 0.62	0.015
F	NA	NA	NA
Shell			
Brindle	Ref.	-	-
Red	1.54	1.28 – 1.84	<0.001
Season of holding			
April to October	Ref.	-	-
November to March	1.92	1.13 – 3.27	0.017
Holding duration			
>1 week	Ref.	-	-
<=1 day	24.14	19.16 – 30.42	<0.001
>1 day to <= 1week	4.74	4.05 – 5.55	<0.001
Crate weight	0.95	0.91 – 0.99	0.006

Ref. – Reference category

NA – not applicable.

Interpretation and discussion

Study limitations

Analysis was performed on data from 2016-17 season. Sub-optimal survival rate estimates might have been affected by management bias arising from the implementation of changes in SRL management practices, to improve survival, since the first incidence of sub-optimal survival in 2016-17 season.

The estimates for sub-optimal survival rates during holding may have been affected by information bias as not all of the rejected SRL during holding and at packing could be tracked back to docket (batch) and crate number in the grading dataset. It was not possible to estimate the impact of such bias on the rate estimates. In addition, the estimates for survival at grading and sub-optimal survival rates during holding may have been affected by rejection misclassification bias due to having different assessors assessing the SRL at grading and during holding during the study period.

Sub-optimal survival

Lower survival at grading was observed in 'B' size class SRL, in SRL transported for longer distances, and in SRL transported between months November to March. During holding, survival rate was lower in red SRL, in 'D' size class SRL, in SRL stocked between months November to March. Survival rate during holding was lower in SRL held for up to one week, whereas it was lowest on the day of arrival which might be associated with increased stress levels during capture and post-harvest transport. The water quality may also be of importance (e.g. system capacity), however potential associations with survival rate during holding could not be investigated in absence of complete water quality data.

Higher transport time and distance may add to the stress in the harvested SRL. Higher survival at grading in SRL sourced from nearby locations might be associated with the lower driving time from source ports to destination facility. Subsequently, the SRL may be exposed to air for a lesser period of time and hence lower stress levels in SRL. Prolonged air exposure has been linked to increase in physiological stress indicator levels in SRL (Taylor and Waldron, 1997) and other decapod crustaceans (Cheng et al., 2002; Fotedar et al., 2001; Gomez-Jimenez et al., 2000; Lorenzon et al., 2007). Effects of prolonged aerial exposure on survival was evident from a similar experimental study on Norway lobsters (*Nephrops norvegicus*) which reported immuno-suppression and subsequent lower survival as a consequence of prolonged exposure to air, hypoxia, and increasing temperatures (Ridgway et al., 2006). Studies investigating effects of emersion stress for live international transport have been conducted (Spanoghe and Bourne, 1997), however, the effect of metabolic stress and transport techniques during domestic transport on SRL have not been examined and remains unknown.

Lower survival at grading and during holding observed between months November (2016) and March (2017) may be due to relatively higher water and air temperatures at capture and during subsequent sea and land transport. It is well established that metabolic and excretory rates of lobsters are directly related to temperature which places heightened physiological demand on lobsters during stressful practices such as capture and transport (Crear and Forteath, 2000; Fitzgibbon et al., 2017). Increased oxygen demands of lobsters at higher temperatures may cause the onset of anaerobic metabolism if sufficient oxygen cannot be supplied to the tissues. The capacity of the cardio-respiratory system to supply oxygen is known to be impaired in lobsters during stressful post-harvest practices such as emersion during land transport and in sub-optimal water quality conditions. Anaerobic metabolism will lead to the built up of lactate which is not sustainable and will lead to death if not resolved. Research studies in USA have reported increase in mortalities in response to increasing water temperatures in warmer months (Howell et al., 2005; Pearce and Balcom, 2005).

Small sized SRL have higher mass specific metabolic demands and therefore may be more vulnerable to metabolic stresses (Crear and Forteath, 2002). In addition, restrictions to optimum oxygen uptake during transport conditions may have led to higher stress levels in 'B' size class SRL compared to the 'D' size class SRL leading into lower survival at grading (Crear and Forteath, 2000). However, survival during holding was higher in 'B' size class SRL, compared to 'D' size class SRL which may be due to the claw size difference between the two classes of SRL. Larger claws in 'D' size class SRL may inflict higher injuries to other SRL stocked in the same crate resulting into chronic sub-optimal survival due to injuries. However, such hypothesis might not be true for SRL ('E-' and 'E+') larger than 'D' size class SRL as higher survival rate during holding was observed in those size classes compared to 'B' size class SRL. In addition, the 'B' size class SRL, with higher mass specific metabolic demands (Crear and Forteath, 2002), might not have fully recovered from the transport stress compared to the 'E-' and 'E+' size class SRL resulting into significant holding survival differences between those classes of SRL.

The lower survival rate during holding observed in SRL on the day of arrival suggest mismanagement during capture and/or transport or lack of recovery from the post-harvest handling and transport stress in holding tanks. The stress in SRL may build up due to variety of factors. For example, laboratory investigation following from a major mortality event in American lobster (*Homarus americanus*) on the Long Island reported that interaction between stressors such as poor water quality, lower oxygen saturation in water, and increased temperature were associated with sub-optimal survival in lobsters (Howell et al., 2005; Pearce and Balcom, 2005; Robohm et al., 2005). However, it could be that the incoming SRL stock might have been already stressed during or before capture and the post-harvest activities may have had added to the stress levels eventually affecting survival during holding. For example, the predation of SRL by Maori octopus (*Octopus maorum*) may increase stress levels in survived lobsters within the same pot (Brock and Ward, 2004; Harrington et al., 2006). Consequently, those SRL may have reduced immunity and have reduced chances of survival when held in the facility with varied management conditions (Brock et al., 2003).

The water system used for purging, a separate system or a separate tank, may need to be accurately managed to cope up the incoming load of SRL and swift removal of toxicants (ammonia, nitrate, and nitrite) building up in the system in the first few hours after SRL arrival. During purging, the newly arrived stock may put more pressure on the nitrification capacity in the biofilter tank of water system, particularly recirculating aquaculture system (RAS), for removal of toxicants. Consequently, if the water management during such situations is not optimum, then the accumulation of toxicants in water may affect the bio-filtration system and the holding survival of fresh stock. A study on western rock lobsters (*Panulirus cygnus*) showed consistent changes in physiological stress indicators in first four days after capture in presence of varied water quality (Fotedar et al., 2006). Another study on western rock lobsters reported marked changes in physiological parameters due to emersion in cold water in first hour and that lobsters required at least 24 hours to adapt to conditions in holding tanks (Spanoghe, 1996). The finding of lower survival in the period up to 24 hours of arrival, compared to those held for up to one week or above, suggests that SRL after arrival in facility might still be in process of adapting to the new conditions in holding tanks and those with a higher transport stress may have succumbed to the emersion shock.

The finding of lower survival rates in red coloured SRL, compared to brindle, was contrary to the perception of higher survival in red SRL in the industry. The lower survival rate in red SRL compared with brindle could be due to the pre-capture poor nutritional conditions (Mendo et al., 2016). The survival in red SRL may further reduce during holding due to SRL succumbing to chronic impairments such as immune suppression and a gradual decline in health condition. The effects of pre-capture lower nutritional status on post-harvest survival can be exaggerated by the fact that SRL are not fed during holding. Consequently, heath in SRL may decline with an increase in duration of holding possibly leading to sub-optimal survival. The brindle SRL, on the other hand, might be in better nutritional condition and may have higher post-harvest survival during holding compared with

red SRL. However, actual effects of pre-capture nutritional status on post-harvest survival during holding needs to be examined further.

The negative association between the crate weight and sub-optimal survival might not be biologically plausible. In addition, the estimate of association between crate weight and survival rate was not far away from one which suggests a very low impact on the survival rate estimate. Further, the negative significant association could be merely due to a large sample size.

Though this study did not explore any capture and fishermen related factors there was no correlation between fishermen and sub-optimal survival as indicated by an intra-class correlation coefficient for fishermen (ICC = 0). Sub-optimal survival was not similar between dockets from same fishermen which suggests that the variation in sub-optimal survival between batches from the same fisherman (dockets) may be a result of varied transport durations. This observation was further supported by finding of lower survival in SRL at grading transported for a longer period of time.

The sub-optimal survival rate during holding in SRL from batches with sub-optimal survival at grading was not higher compared with batches without sub-optimal survival at grading. This suggests that the causes of sub-optimal survival at grading might be different to the causes of sub-optimal survival during holding. This observation is likely related to different physiological stressors at different stages of production stages (during capture, post-harvest, and during holding).

Other parameters (variables) affecting survival

The current data analysis did not include important water quality parameters such as levels of ammonia, nitrate, nitrite, temperature, last water change, temperature, and pH. These parameters may be associated with sub-optimal survival, however, associations between these parameters and sub-optimal survival was not possible in absence of accurate and complete water quality data. The water quality and holding period in temporary tanks on catching boats can also have an effect on survival of caught lobsters. However, investigation of those factors were out of scope for this study.

Conclusion

Based on available data, lobster size, longer transport duration from source port to facility, and transport during warmer months were associated with increased sub-optimal survival at grading. Reduction in transport duration and improvement in current transport practices such as better temperature or humidity control (Jussila et al., 2013) may help in improving survival during transport. Further research into advanced transport technologies such as wet vessel transport and trickle systems for southern rock lobster is warranted.

Survival rate during holding was associated with holding during warmer months, SRL shell colour and size and holding period. Finding suggest that the causes of post-harvest sub-optimal survival observed at grading might be different to those leading to sub-optimal survival during holding in the facility. Improvement in management of incoming SRL, especially during warmer months, may help in improving survival during initial stages of holding.

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Results 6: Microbiological Investigations

Preface

This aspect of the project was undertaken to partly address Objectives 3 of this study:

Full review of the pathology from both the Tasmanian and South Australian mortality events during the 2016 season as well as further characterisation of any significant pathologies (e.g. antennule gland changes) observed in these investigations as well as further pathological investigations for the 2017season.

Manuscript information

Importance of bacterial findings in Southern Rock Lobster (*Jasus Edwardsii)* during investigations of poor post capture survivability.

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Target Journal: *Disease of Aquatic Organisms*

Abstract

The southern rock lobster (SRL) is one of the most valuable wild-caught fishery species in Australia. In 2016, a joint government and industry funded two-year project was initiated investigating poor post capture survivability of SRL. A significant component of this project was to undertake pathological assessment of SRL showing ill-thrift or mortality. Submissions underwent a full diagnostic analysis consisting of total haemocyte counts, haemolymph biochemical analysis, microbiological analysis, gross necropsy and histological assessment. Across submissions over 2016 -2017 there was a consistent microbiological finding from haemolymph culture, of *Vibrio* spp and specifically *Vibrio tapetis* in a number of cohorts. 16 Sanger (16S) sequences from bacterial isolates were obtained to investigate the significance and genetic association of the Vibrio species cultured, notably the *V. tapetis* isolates particularly as they related to the source of the animals. Results were also analysed to test the significance of bacterial isolation and poor health in the SRL. Two chi-square tests were performed, the first investigating an associated between *Vibrio* spp isolation and mortality and the second to assess the association between *Vibrio tapetis* specifically and mortality events. Genetic phylogeny of the *V. tapetis* revealed associations with a point source and there were no significant associations between Vibrio isolation and morbidity and mortality in submitted animals (i.e. there were a range of pathologies associated). This is the first publication of an association of *V. tapetis* with morbidity and mortality in SRL.

Background

The southern rock lobster (SRL) is the most valuable wild-caught species produced and the industry is an important contributor to the Australian economy. Currently the export revenue is valued at approximately $675 million with an estimated increase of 4% in the coming season (Department of Agriculture and Water Resources, 2018). This species of spiny lobster is only found in the waters of southern Australia and New Zealand (Department of Agriculture and Water Resources, 2018). Due to the value and limited geographical distribution of the product, sustainable fishing strategies are put in place by the Australian Government to maintain the future of the species as well as the industry. SRL fishing activity is strictly controlled through catch quotas applicable to licensed fishers and businesses 2. The Australian SLR industry members are also actively involved in research based projects aimed at improving the sustainability of the industry (May, *et al*. 2016).

In 2016, a government and industry funded two year project was initiated investigating poor post capture survivability of SRL. This project was divided into three components; a physiological component, an epidemiology component and a pathology component. One of the constituents of the pathology component was disease investigation of post-capture SRL mortality events reported throughout Australia. These submissions underwent a full diagnostic work up consisting of total haemocyte counts, haemolymph biochemical analysis, microbiological analysis, gross necropsy and histological assessment. Across submissions over 2016 -2017 there was a consistent microbiological finding of *Vibrio* spp importantly *Vibrio tapetis (V. tapetis)* from haemolymph culture.

Vibrio species are ubiquitous in aquatic environments and are known to have symbiotic or pathogenic relationships with their hosts (Rodrigues, *et al*. 2015). These microbes have been reported to cause vibrioses in various marine organisms including molluscs and many shellfishes (Rodrigues, *et al*. 2015). *V. tapetis* is commonly known as the causative agent of Brown Ring Disease (BRD) that was first isolated in France in 1989 (Levican, *et al*. 2017). This disease causes massive mortality in molluscs particularly in the production of *Venerupis philippinarus* but has also been reported in other species such as *Venerupis decusssta, Venerupis aurea* and *Tepes rhomboids* (Levican *et al*. 2017, Dias *et al*. 2018). In addition to this *V. tapetis* has also been identified in mortality events occurring in a number different fish aquaculture facilities across the globe with the most recent from Dover sole (*Solea solea)* in Belgium, wedge sole (*Diclogoglossa cuneate)* in Spain, Atlantic halibut (*Hipoglossus hipoglossus)* in Scotland and corkwing wrasse (*Symphodus melops)* in Norway (Levican, *et al*. 2017).

There is a varying degree of virulence of this microbe as isolates from the Atlantic halibut and wedge sole were identified as unable to cause mortality under laboratory conditions (Levican *et al*. 2017). To our knowledge there has been no published information associating *V. tapetis* as a primary cause of mortality in SRL. However based on the variable virulence as well as variability associated with the host of origin, it is important that *V. tapetis* is not dismissed when isolated consistently from mortality events of aquatic species.

The purpose of this work was to investigate the relevance of bacterial findings of *Vibrio* spp in particular *V. tapetis* as a significant cause of post capture mortality in SRL from a statistic and genetic perspective.

Methods & Materials.

Lobster Care and Management.

Lobsters (n = 133) obtained through the project period were submitted by various SRL licenced holding facilities from South Australia (SA) (n=80), Tasmania (TAS) (n=32) and Victoria (VIC)

(n=21). All submissions were sent to the University of Adelaide's Veterinary Diagnostic Laboratory (VDL) located at the universities Roseworthy campus. The purpose of these submissions varied, with facilities submitting lobsters during a mortality period for investigation (n= 87) or for screening purposes (n= 46). Lobsters were submitted live as per export conditions (tightly packed in eskies with ice bricks) through air or bus freight. On arrival each submission was designation an accession number for record and sample keeping purposes. Animals' were refrigerated for a 2 two hour minimum to induce stupor and a full diagnostic work up was carried out which involved an initial haemolymph collection for total haemocyte counts, microbiology and biochemistry. Animals were then subsequently appropriately euthanised for gross necropsy and histological sample collection.

2.1 Microbiology Culture & Identification

Reagents required:

Sheep Blood agar with 2% salt added (SBA2%)

Zobell's Marine agar (ZMA)

Thiosulfate-citrate-bilesalts-sucrose agar (TCBS)

Prior to euthanasia haemolymph was collected from the ventral aspect of the fifth walking leg joint. Approximately 0.2 to 0.3 ml is required for plating on three bacterial growth plates; SBA2%, ZMA and TCBS agar. Haemolymph is spotted on each plate immediately after collection and is then streaked out for distribution over the plate with a sterile loop using the quadrant streaking method a sterile loop (Figure 1). The quadrant streak method is commonly used for most diagnostic specimens to obtain isolated bacterial colonies that can be then either biochemically and/or genetically identified (Markey *et al*. 2013).

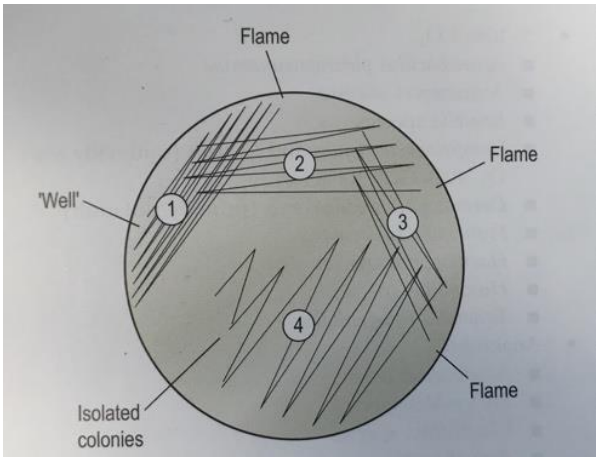

Figure 1: Quadrant Streaking method for obtaining isolated bacterial colonies on agar media.

Plates were then placed in a 25°C incubator for 48 hours of culture. All plates were read and assessed at 24 as well as 48 hours post incubation. Dominate heavy growths of single organisms found were picked and re-plated to obtain pure growth cultures. These samples were re-incubated in the 25°C incubator for another 48 hours on SBA2%, ZMA and TCBS agar plates .Samples that were observed to have pure growth 48 hours post re-incubation were assessed and recorded. TCBS plates of those

pure growth colonies were sent to the aquatic department of the Department of Primary Industries Animal Health Laboratory in Tasmania where bacterial colony identification and typing was undertaken using the Microsys method. Corresponding SBA2% and ZMA plates were refrigerated and stored while identification was underway. Based on species identification results, selected stored SBA2% and ZMA samples were beaded and frozen in a -80°C freezer for archiving and further DNA analysis.

2.2 Bacterial DNA analysis.

16 Sanger (16S) sequences were obtained to investigate the significance and genetic association of *Vibrio species* cultured, notably the *V. tapetis* isolates. Unpurified PCR products were made of selected 2016 and 2017 bacterial samples that were identified as a *Vibrio* sp and also revivable from bead storage at -80°C.

These PCR products were then sent to Australian Genome Research Facility (AGRF) in Adelaide where amplicons were purified and dual direction 16S sequences were formulated [8]. AGRF then proceeded to undertake sequence alignments using ClustalW a multiple sequence alignment program and a Baysian inference of phylogeny was carried out using the MrBayes software program resulting with the production of a phylogenetic tree.

2.3 Statistical Analysis

The purpose of this study is to investigate the role *Vibrio sp* may have in causing reduced post-capture survivability. Therefore an association between bacterial species and mortality events was assessed using a chi-square (x^2) test. This tests gives only evidence of a presence or absence of association however it does not produce a risk ratio or odds ratio (Pandis. 2016)

Two chi-square tests were performed, the first investigating an associated between the *Vibrio sp* and mortality and the second assessed the association between *Vibrio tapetis* specifically and mortality events.

Results.

3.1 Microbiology Culture & Identification

As previously mentioned, a total of 133 lobsters were submitted for diagnostic investigations over the course of this research project. Of that total only one submission (n=8) did not undergo the microbiological culture process. The total number of crustaceans assessed for microbiology over the three years (n=125) and culture results can be found in Table 1, 2, and 3 respectively.

Table 1 shows that a total of 23 SRLs were assessed in 2016 all of which originated from facilities based in SA. Eight lobsters submitted from a mortality event (16-1466) did not undergo microbiological assessment on diagnostic work up.

All fish in assessed in 2017 (Table 2) and 2018 (Table 3) underwent microbiological assessment.

Table 1: Demonstrates the total number of SRLs submitted in 2016 that underwent microbiological assessment and displayed positive bacterial growth on haemolymph microbiological culture.

Accession & Location	Fish number	Microbiological assessment	Microbiological Growth
16-0456 SA Screening (n=4)	1	+	+
	2	+	x
	3	+	+
	4	+	+
16-0544 SA Screening (n=5)	1	+	x
	2	+	x
	3	+	+
	4	+	x
	5	+	+
16-1466 SA Mortality (n=8)	1	x	x
	2	x	x
	3	x	x
	4	x	x
	5	x	x
	6	x	x
	7	X	x
	8	x	x
16-1633 SA Mortality (n=6)	1	+	+
	2	+	x
	3	+	+
	4	+	x
	5	+	+
	6	+	+

\+ Microbiology assessed and or positive growth on culture
x No microbiological assessment undertaken and or no growth post 48hour culture.

Abbreviations: SA – South Australia

Table 2: Demonstrates the total number of SRLs submitted in 2017 that underwent microbiological assessment and displayed positive bacterial growth on haemolymph microbiological culture.

Accession & Location	Fish number	Microbiological assessment	Microbiological Growth
17-0215 TAS Mortality (n=5)	1	+	x
	2	+	+
	3	+	x
	4	+	x
	5	+	x
17-0304 SA Screening (n=5)	1	+	+
	2	+	+
	3	+	x
	4	+	x
	5	+	x
17-0510 SA Screening (n=9)	1	+	+
	2	+	+
	3	+	+
	4	+	+
	5	+	+
	6	+	+
	7	+	+
	8	+	+
	9	+	+
17-0639 SA Mortality (n=12)	1	+	x
	2	+	+
	3	+	+
	4	+	x
	5	+	x
	6	+	x

	7	+	x
	8	+	x
	9	+	x
	10	+	x
	11	+	x
	12	+	x
17-0693 SA Mortality (n=6)	1A	+	x
	1B	+	x
	1C	+	x
	2A	+	+
	2B	+	+
	2C	+	x
17-0702 SA Mortality (n=5)	1	+	x
	2	+	x
	3	+	x
	4	+	x
	5	+	x
17-1375 VIC Mortality (n=6)	1	+	+
	2	+	+
	3	+	+
	4	+	+
	5	+	+
	6	+	x
17-1410 TAS Mortality (n=14)	1	+	+
	2	+	+
	3	+	x
	4	+	+
	5	+	+

	6	+	+
	7	+	+
	8	+	+
	9	+	+
	10	+	+
	11	+	x
	12	+	x
	13	+	+
	14	+	x

+ Microbiology assessed and or positive growth on culture
x No microbiological assessment undertaken and or no growth post 48hour culture.

Abbreviations: SA – South Australia, TAS – Tasmania, VIC – Victoria

Table 3: Demonstrates the total number of SRLs submitted in 2018 that underwent microbiological assessment and displayed positive bacterial growth on haemolymph microbiological culture.

Accession & Location	Fish number	Microbiological assessment	Microbiological Growth
18-0302 SA Mortality (n=20)	1	+	+
	2	+	x
	3	+	+
	4	+	+
	5	+	+
	6	+	+
	7	+	+
	8	+	+
	9	+	x
	10	+	+
	11	+	+
	12	+	x
	13	+	+
	14	+	+
	15	+	+
	16	+	+
	17	+	+
	18	+	x
	19	+	+
	20	+	x
18-0429 - TAS Screening (n=3)	1	+	x
	2	+	x
	3	+	x
18-0568 VIC	1A	+	x
	1B	+	x

Screening (n=6)	1C	+	x
	2A	+	x
	2B	+	x
	2C	+	+
18-0582 – VIC Screening (n=3)	1	+	x
	2	+	+
	3	+	x
18-0732 – TAS Screening (n=2)	1	+	x
	2	+	x
18-0793 VIC Screening (n=6)	1A	+	x
	1B	+	x
	1C	+	+
	2A	+	x
	2B	+	x
	2C	+	x
18-0991 – TAS Screening (n=3)	1	+	x
	2	+	x
	3	+	x
18-1127 TAS Mortality (n=5)	1	+	x
	2	+	x
	3	+	x
	4	+	x
	5	+	x

+ Microbiology assessed and or positive growth on culture
x No microbiological assessment undertaken and or no growth post 48hour culture.

Abbreviations: SA – South Australia , TAS – Tasmania, VIC – Victoria

Isolates from significant microbiological growth at culture were sent for bacterial identification using the Microsys method. Table 4 below serves as an overview of the bacterial species cultured over 2016 and 2017. In addition to this the last column of the table demonstrates which identified bacterial isolates were selected for further genetic investigations.

Table 4: Overview of bacterial colony morphological descriptions, Microsys Identification (ID) as well as participation of samples in genetic assessment for individual SRLs submitted in 2016 and 2017

Accession	Fish No.	Main Colony Growth – Morphological Description	Microsys ID	DNA sequencing
16-0456 Screening	2	No information	*V. tapetis*	Yes
	3	No information	*Vibrio sp*	Yes
	4	No information	*V. tapetis*	Yes
16-0544 Screening	3	No information	*Kocuria Rosea*	No
	5	No information	Glucose non fermenter	No
16-1633 Mortality	1	Yellow & Green Colonies (TCBS)	*V. splendidus type1 &11*	No
	3	Yellow & Green Colonies (TCBS)	*V. splendidus type1 &11*	No
	5	Yellow & Green Colonies (TCBS)	*V. splendidus type1 &11*	No
	6	Yellow & Green Colonies (TCBS)	*V. splendidus type1 &11*	No
17-0215 Mortality	2	No information.	*Vibrio. sp*	No
17-0304 Screening	1	Shiny yellow colony (SBA2%)	*Fermentative non motile organism*	No
	2	Green colony (TCBS)	*No ID*	No
17-0510 Screening	All 9 Fish	Colony 1: Dark grey round & shiny (SBA2%) & Yellow with dark centre (TCBS)	*No ID*	No
		Colony 2: Green with dark centre (TCBS)	*No ID*	No
17-0639 Mortality	2	Dark black pigmented colony (SBA2%)	*No ID*	No
	3	Grey white colony (SBA2%) & Yellow colony (TCBS)	*No ID*	No
17-0693 Mortality	2A	Grey white colony (SBA2%) & Yellow colony (TCBS)	*No ID*	No
	2B	Grey white colony (SBA2%) & Yellow colony (TCBS)	*No ID*	No

17-1375 Mortality	1	Translucent colony (ZMA) & yellow colony (TCBS)	*Vibrio sp*	Yes
	2	Orange translucent colony (ZMA) & Grey colony (SBA2%) and Yellow colony (TCBS)	*V. tapetis*	Yes
	3	Grey colony (SBA2%) and Yellow colony (TCBS)	*V. tapetis*	Yes
	4	Translucent colony (ZMA) & Grey colony (SBA2%) and Yellow colony (TCBS)	*V. tapetis*	Yes
	5	Translucent colony (ZMA) & Grey colony (SBA2%) and Yellow colony (TCBS)	*V. tapetis*	Yes
17-1410 Mortality	1	Translucent colony (ZMA) & Yellow colony (TCBS)	*V. tapetis*	Yes
	2	Green + Yellow + Black Colonies (TCBS)	*No ID*	No
	4	Translucent colony (ZMA) & Yellow colony (TCBS)	*V. tapetis*	Yes
	5	Translucent colony (ZMA) & Yellow colony (TCBS)	*V. tapetis*	Yes
	6	Translucent colony (ZMA) & Yellow colony (TCBS)	*V. tapetis*	Yes
	7	Translucent colony (ZMA) & Yellow colony (TCBS)	*Vibrio sp*	Yes
	8	Grey translucent colony (ZMA) & Yellow colony (TCBS)	*No ID*	No
	9	Translucent colony (ZMA) & Yellow colony (TCBS)	*V. tapetis*	Yes
	10	Yellow + Green colonies (TCBS)	*No ID*	No
	13	Dark green colony (TCBS)	*No ID*	No

Abbreviations: ID – Identification, TCBS - Thiosulfate-citrate-bilesalts-sucrose agar, ZMA - Zobell's Marine agar, SBA2% - Sheep Blood agar with 2% salt, V – *Vibrio*, Sp – species.

The table above shows that no identification was carried out for certain samples from accessions 17-0304, 17-1510, 17-639, 17-0693 and 17-1410. This occurred due human error whereby these samples were accidentally disposed of. It is assumed however that morphologic descriptions of yellow and green pure colonies grown from those submissions on TCBS plates suggests positive growth of *Vibrio species.*

In 2018 all samples with positive growth were sent for identification as shown on table 5 below.

Table 5: Overview of bacterial colony morphological descriptions, Microsys Identification (ID) for individual SRLs submitted in 2018

Accession	Fish No.	Main Colony Growth – Morphological Description	Microsys ID
18-0302 Mortality	1	Yellow + Green colonies (TCBS)	*Vibrio sp*
	3	Grey colony (SBA2%) & Green colony (TCBS)	*Vibrio sp*
	4	Grey colony (SBA2%)	*Phenon 36*
	5	Grey colony (SBA2%) & Green colony (TCBS)	*Vibrio sp*
	6	Grey colony (SBA2%) & Green colony (TCBS)	*Vibrio sp*
	7	Grey colony (SBA2%)	*Vibrio sp*
	8	Grey colony (SBA2%)	*Vibrio sp*
	10	Grey colony (SBA2%) & Yellow colony (TCBS)	*Vibrio sp*
	11	Grey colony (SBA2%)	*Vibrio sp*
	13	White colony (SBA2%)	*Vibrio sp*
	14	Yellow Colony (TCBS)	*Vibrio sp*
	15	Grey colony (SBA2%)	*Vibrio sp*
	16	Cream white colony (ZMA)	*No ID unlikely Vibrio sp.*
	17	Grey colony (SBA2%)	*Vibrio sp*
	19	White colony (ZMA)	*Vibrio sp*
18-0568 Screening	2C	Grey colony (SBA2%) & Yellow colony (TCBS)	*Vibrio sp*
18-0582 Screening	2	White colony (ZMA) & Yellow colony (TCBS)	*Vibrio sp*
18-0793 Screening	1C	No information	*Vibrio sp*

Abbreviations: ID – Identification, TCBS - Thiosulfate-citrate-bilesalts-sucrose agar, ZMA - Zobell's Marine agar, SBA2% - Sheep Blood agar with 2% salt, Sp – species

Bacterial DNA analysis.

From the total number of samples submitted for DNA testing (Table 4) only 10 were viable for 16-S sequencing and phylogenetic tree construction. Figure 1 below represents the Baysian tree topology generated for the data submitted.

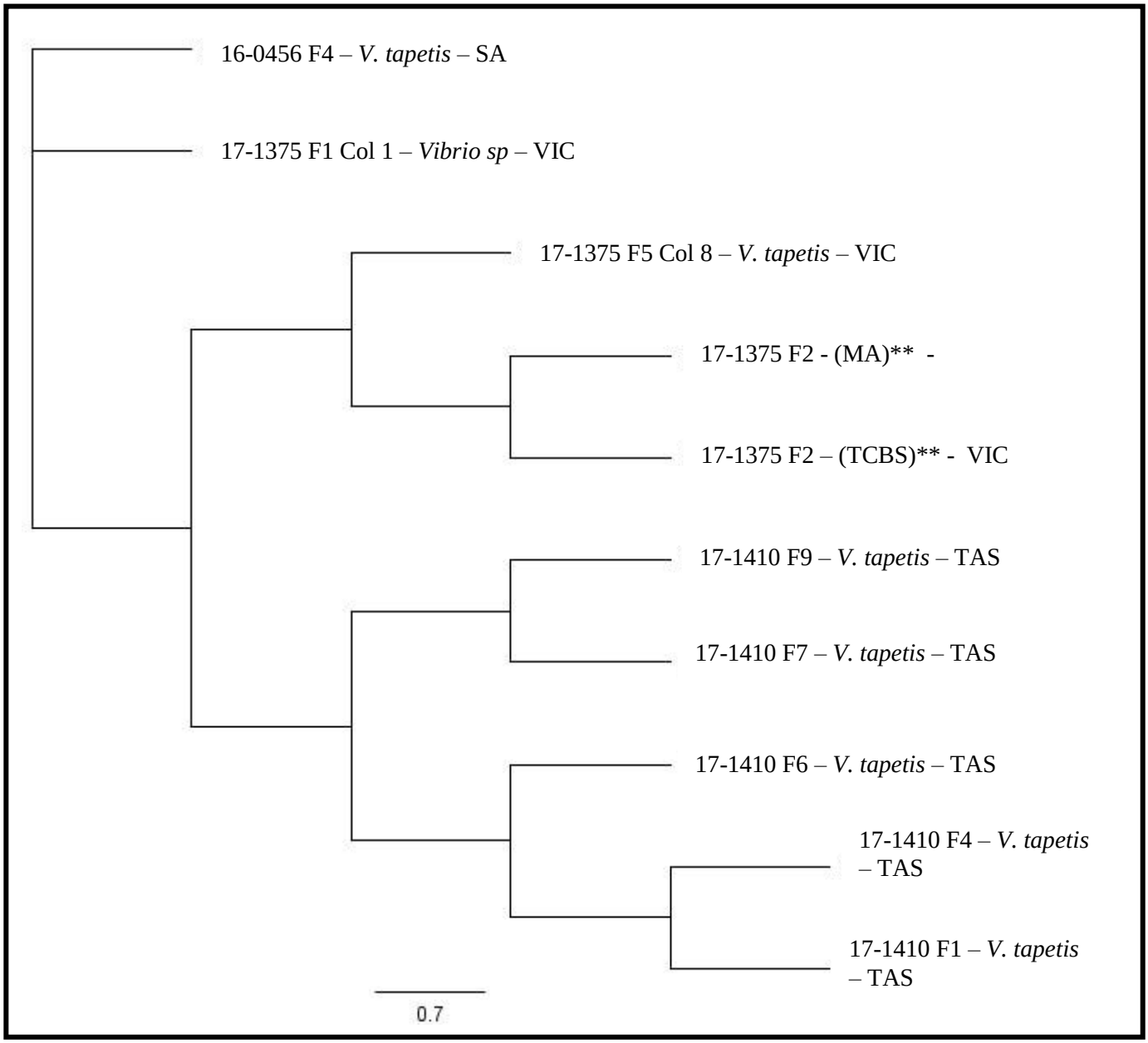

Figure 1: Baysian tree generated from *Vibrio* samples submitted to AGRF.

3.3 Statistical analysis.

The chi square (x^2) test is implemented in two ways to investigate for a statistically significant association between *Vibrio* species and reduced survivability as well as between *Vibrio tapetis* isolated and reduced survivability of SRL. All data complied through 2016 to 2018 were used. It is

important to note that samples that were not identified using the Microsys method but on culture that grew yellow or green colonies on TCBS agar were suspected to be a form of *Vibrio sp.*

The null hypothesis suggests that there is no associated between *Vibrio sp* and survivability events. To test this appropriately the expected frequencies are to be calculated for each cell in a 2 X 2 table. Results were calculated using IBM SPSS Statistics 25 for Windows which not only calculated the chi square value but included nominal measures for association which can be seen in table 8 and 11 for *Vibrio* species and *Vibrio tapetis* respectively.

Table 6: 2 X 2 cross table for Chi Square test assessing the significance of *Vibrio species* as a finding.

	Diagnostic Purpose		
Vibrio Species	Screening	Mortality	Total
Yes	16	36	73
No	35	38	52
Total	51	74	125

Table 7: Chi square results between *Vibrio species* and mortality events.

	Chi-Square Tests	
	Value	P-Value
Pearson Chi-square	3.71[a]	0.054
Continuity Correction[b]	3.03	0.082

Table 8: Results for symmetric measures of association for *Vibrio species.*

	Symmetric Measures	
Nominal by Nominal	Value	P-Value
Phi	0.17	0.054
Cramer's V	0.17	0.054

Table 9: 2 X 2 crosstable for Chi Square test assessing the significance of *Vibrio tapetis* as a finding

	Diagnostic Purpose		
Vibrio tapetis	Screening	Mortality	Total
Yes	2	9	11
No	49	65	114
Total	51	74	125

Table 10: Chi square results between *Vibrio tapetis* and mortality events

	Chi-Square Tests	
	Value	P-Value
Pearson Chi-square	2.56[a]	0.110
Continuity Correction[b]	1.63	0.202

Table 11: Results for symmetric measures of association for *Vibrio tapetis.*

	Symmetric Measures	
Nominal by Nominal	Value	P-Value
Phi	0.14	0.110
Cramer's V	0.14	0.110

Discussion

The taxonomy of *Vibrionaceae* has continued to evolve over the years and represents a large diverse group of bacteria that are ubiquitous in aquatic environments (Carson, *et al*. 2006). Several cultivation- dependant and independent studies have shown close associations between this bacterial species and aquatic animals either as symbionts, normal flora or as pathogens (Carson *et al*. 2006, Thompson *et al*. 2004).

In 2006 the Australian Governments Fisheries Research and Development Corporation submitted a report on developing the national standard diagnostic technology for identifying *Vibrionaceae* of Aquatic animals. *Vibrio tapetis* is documented as a species exotic to Australia as it was not been detected as a primary pathogen in association with aquatic animals (Carson *et al*. 2006, Buller 2014).

Additionally there are no reports of *Vibrio tapetis* implicated as a primary cause of mortality in crustaceans, which is reflected in the statistical analysis of the data above. The Chi square assessment of *Vibrio tapetis* cultures isolated through the time of this project showed no statistical significant association to mortality events with a p-value > 0.05 (Pandis 2016). *Vibrionaceae* as a genus however showed a possibility of significance p-value = 0.05. The degree of association was measured between two nominal variables namely the phi and Cramer's V (Babu *et al*. 2014). These measures of association are scaled reaching a maximum numerical value of 1 to indicate two variables have a perfect relationship with each other and a minimum numerical value of 0 when there is no association between two variables. Based on the results on table 8 a low association of 0. 17 was noted between *Vibrio sp* and mortality cases.

The lack of association observed between *V. tapetis* and *Vibrio sp* with mortality events suggests that these microbes are not the primary cause of death for SRL within this study. However, the fact that these bacterial species were isolated in SRL haemolymph consistently in certain submissions remains a significant finding. *Vibrionaceae* are known to cause disease opportunistically. Commonly, they only exhibit pathogenic capacity if host defences are compromised which may be predisposed from stressful events, physical damage, physical change such as molting and any other event leading to immunosuppression (Carson *et al*. 2006).

It has been suggested that a relationship is present between *Vibrio. sp* and zooplankton forming a biofilm on the exoskeletons of crustaceans and other marine organisms. This symbiosis is said to serve as a survival strategy during starvation or other environmental stressors (Thompson *et al*. 2004). Therefore although *Vibrio sp* are not observed to be a primary pathogen of mortality in SLR the systemic isolation of these ubiquitous microbes do serve as insight, suggesting these animals were compromised in some way allowing these opportunistic bacteria to invade.

The isolation of *V. tapetis* through this project serves as yet another important relevant finding. Based on current literature *V. tapetis* has been documented to be a pathogen isolated causing disease and mortalities in clams (*Venerupis philippinarum* and *Venerupis decussata),* corkwing wrasse (*Symphodus melops),* wedge sole (*Dicologlossa cuneate),* dover sole (*Solea solea),* Atlantic halibut (*Hippoglossus hippoglossus)* and most recently the red conger eel (*Genypterus chilensis)* as well as the fine founder (*Paralichthys adspersus)* (Levican *et al*. 2017, Buller 2014). The presence of *V. tapetis* in SRL during a mortality period may serve as an extension of the known host range and geographic location for this pathogen.

For the genetic analysis component of this study the Baysian tree construction revealed some interesting findings. Distinct clumping of genes were observed for sequences originating from the three different states. Relatability was observed between Victorian and Tasmanian samples submitted with the Victorian samples settled on the upper parts of the branch while the Tasmanian samples took up the bottom branches. It is also important to note that all Victorian and Tasmanian samples submitted for genetic analysis were obtained by SRLs managed by one company. Curiously sample 17-1375 Col 1 that was identified as *Vibrio sp* using the Microsys V36 showed a closer relationship

with *V. tapetis* sample from SA compared to other *V. tapetis* samples submitted. From a genetic component this suggests the presence of *V. tapetis* strains. This information is highlighted in the figure 2 below:

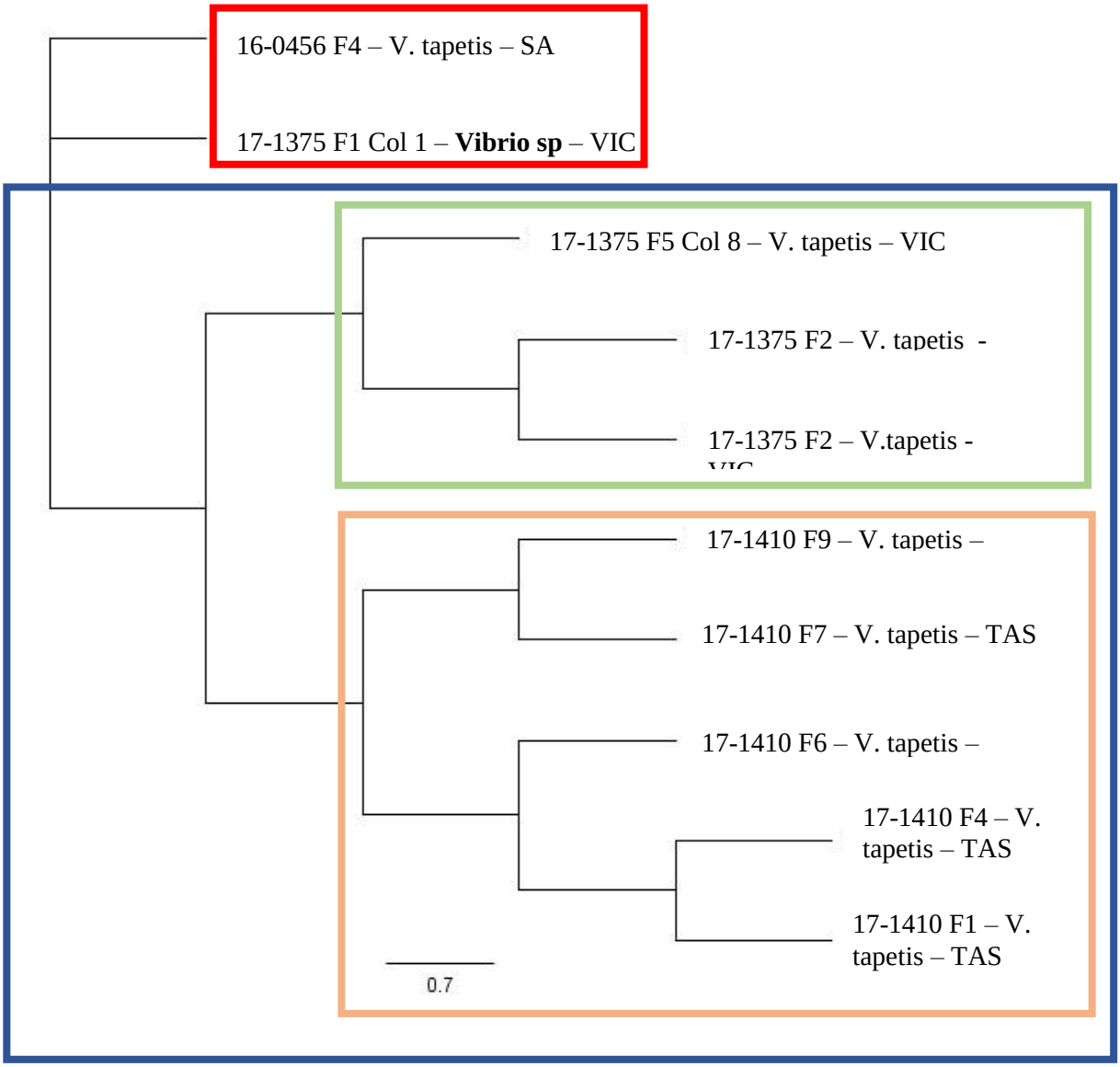

Figure 2: Highlighted associations of Baysian tree generated from *Vibrio* samples submitted to AGRF

To date several known *V. tapetis* strains have been studied to characterise bacterial pathogenicity mechanisms as well as virulence factors Dias *et al*. 2018). The main virulence factors identified were toxins such as haemolysin and cytotoxins as well as other proteins which were observed in a variable manner. An example of this is the Virb4 a component of the type IV secretion system which is systematically present in the genome of pathogenic *V. tapetis* strains but absent in non-pathogenic strains (Dias *et al*. 2018). To date the complete repertoire of genes associated with *V. tapetis* pathogenicity remains largely unknown despite the availability of its full sequence CEC4600^{T} (Dias *et al*. 2018). This stresses the importance of further monitoring and genetic analysis of *V. tapetis* isolated from SRL.

The results of the Baysian tree do also question the phenotypic identification methods of *V. tapetis*. *Vibrionaceae* commonly exhibit a variety of phenotypes between as well as within species. A study conducted 2017 two strains of *V. tapetis* isolated from two native fish species in Chile and found that one strain showed acid production from sucrose which was not observed in the other *V. tapetis* strain (Levican *et al*. 2017). In 2003 similar phenotypic variation was noted where the LP2 *V. tapetis* strain differed from the B1090 strain in the metabolism of ribose, lactose and sucrose (Sigmund *et al*. 2003). This platform of phenotype heterogeneity increases the complexity in the identification process.

The current recommended identification strategies include the use of simultaneous polythetic approach combined with computer assisted probabilistic identification (Carson *et al*. 2006). In Australia a miniaturised commercially available identification matrix was developed specifically for *Vibrionaceae species* associated with a diversity of Australian aquatic animals (Carson *et al*. 2006). It is important to note that this matrix was created at a time were *V. tapetis* was considered an exotic pathogen to Australian shores therefore suggesting this method of identification may not account for the phenotypic variance present in *V. tapetis*.

In order to appropriately understand taxonomy a high level of association between genotypic and phenotypic data is required to obtain mutual consistency between phylogenetic and phenotypic base taxonomies (Carson *et a*l 2006). The discovery of *V. tapetis* is morbid SRL should not be taken lightly this is a microbe that continues to show variation in host range and pathogenicity within those hosts. Based on the phenotypic and genetic data obtained through this study the presence of multiple *V. tapetis* strains is a possibility.

From a commercial and economic perspective, further research appropriately investigating the effects of *V.tapetis* on SRL is imperative. This can be undertaken through controlled infection trials allowing the government and industry to be informed and prepared in a disease outbreak were to occur. Further genetic analysis of this microbe cultured from SRL is also required to develop improved and accurate methods of stain identification. Further research in this area would not only fill the large knowledge gaps present in the study of *Vibrionaceae* but may also contribute in the prevention of mass mortalities in SRL.

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Results 7: Pathology Investigations

Preface

This aspect of the project was undertaken to address Objectives 3 of this study:

Full review of the pathology from both the Tasmanian and South Australian mortality events during the 2016 season as well as further characterisation of any significant pathologies (e.g. antennule gland changes) observed in these investigations as well as further pathological investigations for the 2017/18 season.

Abstract

The underpinning question from the industry during the diagnostic pathology component of this project was whether there was a common pathology seen in affected animals and hence could assumptions be made about a common causality for the poor survival rates seen periodically in wild caught SRL. The work was undertaken using a common veterinary diagnostic format for each submission of animals. Each SRL was treated as a unit of investigation and a range of diagnostic modalities were performed on each animal (i.e. gross examination, haemolymph collection and analysis, microbiological examination of haemolymph and gross lesions, histopathology and virus rule out). The methodology for these investigations is detailed within another chapter of this project report entitled 'Southern Rock Lobster (SRL) (*Jasus edwardsii*) HEALTH ASSESSMENT PROCEDURE MANUAL'.

Introduction

Following are the detailed case summaries of the individual Southern rock lobster accessions examined at the Veterinary Diagnostic Laboratory, University of Adelaide, Roseworthy Campus, South Australia. The methodology for these investigations is detailed within another chapter of this project report entitled 'Southern Rock Lobster (SRL) (*Jasus edwardsii*) HEALTH ASSESSMENT PROCEDURE MANUAL' (Appendix 9). Normal histology and selected histopathology is documented within a separate manual entitled 'Selected histology and histopathology of Southern Rock Lobster (SRL) (*Jasus edwardsii*)' (Appendix 11).

Individual accessional information and results summaries.

Accession number: 16-0456

Origin: Adelaide facility

Species: Southern rock lobster (*Jasus edwardsii*)

Culture Type: Wild caught and tank held for processing and shipment. 4 x Rock lobsters submitted from morning grading and sorting Adelaide facility, for necropsy examination and ancillary diagnostic investigation.

Time and date of death: 23/3/2016

Time and date of post mortem examination: pm/23/3/2016

Carcass condition & preservation: 3 x animals moribund and alive, 1 x Dead on arrival (DOA).

Gross pathology summary:

Fish 1:

Carapace length: 11.5cm and total tail/telson length 18.5cm. DOA.

Swollen intersegmental membranes. Complete loss of left antennae. Minor splitting and melanosis of telson fan.

Fish 2:

Carapace length: 17.3cm and total tail/telson length 24.5cm. Alive and moribund.

Broken antennae right and left. Moderate splitting and focal melanisation of telson. Proximal gut: multifocal yellow orange papillary proliferations on luminal surface. Generally soft watery muscle in tail.

Multifocal erosions and ulcerations and melanisation of walking leg segments.

Fish 3:

Carapace length: 17.8cm and total tail/telson length 26cm. Alive and moribund.

Dorsal longitudinal muscle in tail pale and white.

Lost large oral appendage.

Fish 4

Carapace length: 17.3cm and total tail/telson length 25.3cm. Body weight 2.04kg. Alive and moribund.

Right antennal fracture. And melanisation.

Telson blistering central fans.

Morphological diagnosis

Carapace: Focal to multifocal acute to chronic erosion, oedema and inflammation with melanisation.

Skeletal Muscle: Acute oedematous myositis

Histological summary

Histology Summary of Accession 16-0456 (4 Fish)	
Gills	Fish are observed to have minor to moderate biofouling within the gills. This is associated with the presence of bacterial plaques between the gill filaments. Haemocyte aggregations and mild melanisation was also noted in F3.
Hepatopancreas	Digestive glands were observed to show dilation of luminal area with presence of reactive haemocyte aggregations. Mild vacuolisation of lining cells was noted in F1 and F3.
Nerves	There were degenerated sections of nerve seen in F1 and F2 with the presence of purple amorphous inclusions. No significant changes seen in F3 and F4.
Antennal Gland	No significant pathologic changes seen on histology however all fish were seen to have two distinct sections within the interstitium of the antennal gland that were described as a compact and open interstitium.
Reserve Cell	Moderate amounts of normal reserve cell observed in all fish.

Microbiological Summary

Fish 2: *Vibrio tapetis*

Fish 3: *Vibrio sp.*

Fish 4: *Vibrio tapetis.*

Haemocyte counts

Fish 2 – $2.8x10^6$/ml

Fish 3 – $1.9x10^6$/ml

Fish 4 – $6.95x10^6$/ml

Case summary:

External lesions indicative of trauma and evidence of systemic Vibriosis.

Accession number: 16-0544

Origin: Adelaide facility

Species: Southern rock lobster (*Jasus edwardsii*)

Culture Type: Wild caught and tank held for processing and shipment.

Time and date of death: 06/04/2016

Time and date of post mortem examination: pm/ 06/04/2016

Carcass condition & preservation: 5 x animals moribund and alive. Animals in esky with ice bricks.

Gross pathology summary:

Fish 1:

Body weight: 2.16kg

Carapace length: 17cm and total tail/telson length 25cm. Alive.

Right antenna gland lost half way.

Fish 2:

Body weight: 1.88kg

Carapace length: 14.5cm and total tail/telson length 23.5cm. Alive and moribund.

Loss of left walking legs 4 and 5.

Abnormal gonads – removed for histopathology.

Fish 3:

Body Weight: 2.14kg

Carapace length: 17.5cm and total tail/telson length 25cm. Alive and moribund.

Loss of the rostral tip of right antenna.

Multiple erosions visible on caudal edge of telson.

Antennal gland appears pale and granulomatous.

Fish 4:

Body weight: 2.08kg

Carapace length: 15.5cm and total tail/telson length 24.5cm. Alive and moribund.

Loss of left antenna and loss of left 4th walking leg.

Blister like erosions on telson

Tail membranes appear swollen.

Fish 5:

Body weight: 1.97kg

Carapace length: 15.5cm and total tail/telson length 25.5cm

Loss of 1st right walking leg and loss of 2nd left walking leg.

Lesion present on 1st tail membrane on right hand side (approximately 10mm X 5mm X 3mm) – (uropod)

Ulcerative lesion present on telson.

Antennal glands appear pale

Morphological diagnosis

Telson: Focal to multifocal acute to chronic erosion, oedema and inflammation with melanisation.

Antennal gland: granulomatous adenitis

Histological summary

Histology Summary of Accession 16-0544 (5 Fish)	
Gills	Grade of biofouling ranges between 1 to 3 in fish of this accession. Bacterial presence is seen in all fish ranging from just mild amounts of filamentous plaques to a marked quantities consisting a mix of filamentous and bacillary bacteria with areas of melanised gill filaments. Exception of F5 with no significant findings.

Hepatopancreas	Active digestive glands observed. Some dilation and enlargement of random tubules. Absorptive vesicles are prominent. Exception of F3 as luminal surfaces appeared closed on histology.
Nerves	No significant findings in all fish.
Antennal Gland	Range of different findings observed within fish of this accession. No significant findings noted in F1. F2 to F4 were seen to have disrupted normal architecture with multifocal areas of melanisation and necrosis. In addition there were multifocal areas of blue inclusions within the cytoplasm (apoptotic?). Random areas of the gland appeared coagulative with haemocyte aggregations associated. F5 however appeared to have yellow to green polarising deposits within the epithelium and interstitium that were randomly distributed around the gland. (Urate crystals?)
Reserve Cell	Mild to moderate amounts seen through fish that appeared degenerated and vacuolated.

Microbiological Summary

Fish 3: *Kocuria rosea*

Fish 5: Glucose non-fermenter

Case summary:

Findings are consistent with chronic degenerative change. The general degree of gill filament biofouling is suggestive of long holding periods for these animals within the re-circulating holding tanks. The chemical composition of the bio-refringent inclusions within the antennule glands were not identified.

Accession number: 16-1633

Origin: Port McDonald facility

Species: Southern rock lobster (*Jasus edwardsii*)

Culture Type: Wild caught and tank held for processing and shipment.

Time and date of death: 18/10/2016

Time and date of post mortem examination: pm/18/10/2016

Carcass condition & preservation: 6 x animals moribund and alive. Animals in esky with ice bricks.

Gross Pathology summary:

Fish 1:

Body weight: 0.48kg and total tail/telson length 26cm. Alive on arrival.

Complete loss of Left antennae. All specimens are reddened suspect due to oxidative change. Soft muscle mass in tail muscles. No significant erosions on telson, pleopod, pereopod.

Fish 2:

Body weight: 0.48kg

Carapace length: 10cm and total tail/telson length 27cm. Alive and moribund.

Prominent gonads.

Gill discolouration present.

Fish 3:

Body Weight: 0.67kg

Carapace length: 11.5cm and total tail/telson length 29cm. Alive and moribund.

Prominent gonads.

Gill discolouration present.

Fish 4:

Body weight: 0.51kg

Carapace length: 10.2cm and total tail/telson length 26.5cm. Alive and moribund.

Haemolymph sample was coagulated.

Red discolouration to most organs

Antennal Gland enlarged.

Fish 5:

Body weight: 0.6kg

Carapace length: 11cm and total tail/telson length 29cm. Alive and moribund

Blister present on lateral aspect of right uropod.

Melanisation of left 3rd pleopod.

Fish 6:

Body weight: 0.59kg

Carapace length: 11cm and total tail/telson length 28cm. Alive and moribund

Tail melanisation with pale white discolouration,

Right antennal loss with melanisation present.

Morphological diagnosis

Antennule gland: adenitis

Carapace: mild erosion and melanisation

Histological summary

Histology Summary of Accession 16-1633 (6 Fish)	
Gills	No significant findings and no biofouling observed in F1 to F4 and F6. There was scant grade 1 biofouling noted in F5.
Hepatopancreas	There were decreased to depleted number of absorptive vacuoles seen in F2, F4, and F6 with mild autolysis with regional necrosis present. F1 and F3 tissue appeared to have areas of sloughed epithelium with architectural derangement and tubular lumens were dilated. Marked autolysis was observed in F5 characterised by karyolysis and karyorrhexis.
Nerves	Mild autolysis with no significant findings observed in F1, F4 and F6. There were ganglia with multifocal areas with large central vacuoles observed in F2 with no inflammatory response associated. However in F3 and F5 there is a suspected neuritis with multifocal areas of degenerated ganglia with inflammatory association.
Antennal Gland	No antennal gland sample for F4 to F6. F1 to F3 there was observed to be copious amounts of normal interstitial tissue with no signs of inclusions in the lining cells. There was also mild sloughing of antennal glands into the lumen with no inflammatory response noted in F1 and F2. The lining cells of a section of gland tissue in F1 was noted to show swelling with karyolysis present.
Reserve Cell	Mild to moderate amounts of degenerated and vacuolated tissue in F1 to F3 and F6. Copious amounts of normal tissue seen in F4 and F5.

Microbiological summary:

Light to moderate growth of organisms from all samples

Two organisms were present in all four samples – identified as:

Vibrio splendidus type 1

Vibrio spendidus type 11.

Case summary

Significant findings are consistent with longer holding periods (i.e. biofouling) with autolytic changes apparent in some antennal gland. The neuritis and neuronal vacuolation is worthy of note and interpretation of cause is not possible. The isolation of *V. splendidus* may indicate a possible focal bacterial reaction within the nerves.

Accession number: 17-0340

Origin: Adelaide facility

Species: Southern rock lobster (*Jasus edwardsii*)

Culture Type: Wild caught and tank held for processing and shipment.

Time and date of death: 02/03/2017

Time and date of post mortem examination: pm/ 02/03/2017

5 x Rock lobsters submitted from morning grading and sorting Adelaide facility, for necropsy examination and ancillary diagnostic investigation.

Gross pathology summary:

Fish 1:

Body weight: 2.57kg

Carapace length: 18.5cm and total tail/telson length 44.5cm.

Left and right antenna broken.

Ulceration present on uropod. Yellow antennal glands.

Fish 2:

Body weight: 3.13kg

Carapace length: 19cm and total tail/telson length 34cm.

Dark blue/ grey lesion around heart, GIT and ventral nerves.

Enlarged antennal glands that are yellow to orange in colour.

Fish 3:

Body Weight: 2.62kg

Carapace length: 15.5cm and total tail/telson length 45cm.

Fractured distal end of right pleopod 1 with moderate melanisation present.

Loss of small part of caudal edge of first segmental part with some healing evident.

Abrasions present on caudal end of tail.

Evidence of minor focal melanisation on right ventral aspect of last intersegment unit.

Abnormal tissue present cranio-dorsal to hepatopancreas.

Tail weight: 0.64kg

Fish 4:

Body weight: 2.71kg

Carapace length: 19cm and total tail/telson length 45cm.

Dorsal aspect of carapace tail junction is oedematous.

Segmental bulging / swelling of first two cranial segments of tail.

Left antenna loss – appears as old wound.

Tail weight: 0.60kg

Fish 5:

Body weight: 0.59kg

Carapace length: 12.5cm and total tail/telson length 29cm

Right 4th pleopod missing

Fractured left 3rd pleopod at distal most joint.

Ulcerations present on tail

Internals very friable

Tail weight: 0.18kg

Morphological diagnosis:

Carapace: mild to marked erosion and ulceration with focal melanisation

Intersegmental protrusion and oedema.

Histological summary:

Histology Summary of Accession 17-0340 (5 Fish)	
Gills	Significant grade 3 to 4 biofouling observed in F1 and F2 with marked amounts of bacterial plaques within lumens between gill filaments. Protozoa, ciliates and leucothrix presence with erosion and melanisation of chitin observed. Numerous haemocyte aggregations observed. Minimal to no biofouling observed in F3 to F5. There was an abnormal glandular organ seen in the gill raker of F4.
Hepatopancreas	Tissue of F1 and F2 showed dilated lumens with mild amount of debris and digestion within lumens. Height of luminal walls are low with marked number of absorptive vacuoles and multifocal areas of haemocyte aggregation suggesting a degree of interstitial hepatopancreatitis in these two fish. F3 to F5 were observed to have occasional dilation of hepatopancreatic cell with mild numbers of absorptive cell vacuoles.
Nerves	No sample for F5. No significant findings in F2 and F3. There were large vacuolisation of axons of the ganglia with sections of haemocyte aggregations observed in F1. Haemocyte aggregations also observed surrounding ganglia in F4. A section of F4 tissue showed organised melanised granulomas with extensive focal necrotising neuritis. Reserve cell associated with this nerve is vacuolated with a necrotic lesion that is pseudo-encapsulated.
Antennal Gland	F1 to F3 appear to have a granular pink matrix in interstitium of gland. There is squamous metaplasia of cuboidal cells with marked rhomboid eosinophilic cytoplasmic inclusions observed in F1, F3 and F4. There are dilated areas with mild foci and melanisation noted in F3. F5 tissue showed general autolysis across the sample.
Reserve Cell	Varied from depleted atrophic amounts to ample normal amounts.

Microbiological summary:

Fish 1: *Fermentative non- motile organism.*

Fish 2: *Unidentified.*

Haemocyte counts:

Fish 1: $1.9 \times 10^6 cells/ml$

Fish 2: $1.2 \times 10^6 cells/ml$

Fish 3: $1.4 \times 10^6 cells/ml$

Fish 4: $1.5 \times 10^6 cells/ml$

Case Summary:

The key features of pathology in these fish were the chronic active erosive and ulcerative changes in external structures, Grade 3-4 biofouling in two of the fish with associated hepato-pancreatitis and antennal gland cytoplasmic inclusions.

The changes may reflect holding time (i.e. more severe changes in longer held animals). Microbiological results were non-informative.

Accession Number: 17-0510

Origin: Mt Gambier facility

Species: Southern rock lobster (*Jasus edwardsii*)

Culture Type: Wild caught and tank held for processing and shipment.

Time and date of death: 21/03/2017

Time and date of post mortem examination: pm/ 22/03/2017 and am/23/03/2017

Carcass condition & preservation: 9 Live Lobsters. Animals in esky with ice bricks.

Gross Pathology summary:

Fish 1:

Body weight: 0.66kg

Carapace length: 11.8cm and total tail/telson length 30.1cm. DOA.

Released clear orange fluid from GIT when removed from esky.

Loss of 5th right walking leg. No melanisation at site.

Left antenna loss above segmented region with melanisation present.

Right antenna loss at segmented region with no melanisation at site.

Erosions present on uropods.

Erosive lesion also present at intersegmental membrane between segments 1 and 2.

White discolouration to aspects of left antennal gland.

Haemolymph Clotted.

Fish 2:

Body weight: 0.79kg

Carapace length: 12.5cm and total tail/telson length 30.5cm. Live.

Left antenna loss at segmented region and right antenna loss above segmented region. Melanisation present at both lesions.

Loss of 2nd and 3rd walking legs with melanisation at both sites.

Erosions present over pleopods.

Right antennal gland appears paler compared to left.

Discolouration present of cranial and caudal gill sets. Cranial gill sets appear darkened and brown and caudal gills appear to be pink tinged.

Dark brown discolouration evident to hepatopancreas. White paler focal areas visualised more central regions of hepatopancreas

Reserve cell tissue is decreased with white deposits present within tissue.

Flexor muscles appear pale.

Fish 3:

Body Weight: 1.04kg

Carapace length: Unknown and total tail/telson length Unknown. – live

Left antenna loss just above segmented region with signs of melanisation.

Bulging/Swelling of intersegmental membranes

Chronic erosions and ulceration on left uropod

Minor erosions to right uropod and telson.

There is surface melanisation of hepatopancreas with a dark brown membranous change

Dark brown discolouration and enlargement associated with both antennal glands

Discolouration present of right cranial and caudal gill sets. Cranial gill sets appear darkened and brown and caudal gills appear to be pink tinged.

Brown discolouration also associated with left cranial gills.

White plaque like discolouration to reserve cell tissue.

Mild darkening to caudal gastro-intestinal tract.

Generalised orange discolouration to tail muscle.

Fish 4:

Body weight: 0.68kg

Carapace length: 10.5cm and total tail/telson length 29.7cm. DOA

Both left and right antennae lost at segmented region with presence of melanisation.

Loss of 4th right walking leg with no signs of melanisation at site.

Irregular erosive lesions over uropods and telson.

Left antennal gland is pale compared to the right

Discolouration present of right cranial and caudal gill sets. Cranial gill sets appear darkened and brown and caudal gills appear to be pink tinged.

Prominent gonads

Decreased reserve cell tissue.

Haemolymph sample obtained

Fish 5:

Body weight: 0.67kg

Carapace length: 12.1cm and total tail/telson length 31cm. Alive

Irregular erosive lesions on caudal edges of telson and uropods

White plaque like deposits on L and R antennal glands

Cranial gill margins on both left and right sides appear to have dark brown discolouration.

Decreased reserve cell deposits and reserve tissue is soft and friable.

Pink discolouration to dorsal tail muscle compared to ventral tail muscle.

Haemolymph sample obtained.

Fish 6:

Body weight: 0.51kg

Carapace length: 10.1cm and total tail/telson length 27.8cm. Alive.

Right antenna is broken above the segmented area.

Loss of walking leg 5 on the right side and loss of walking legs 3,4, and 5 on the left side.

There is chronic ulceration and erosion of caudal edges of telson and uropods.

There is a fluid filled blister like lesion on the last left pleopod. Biofouling is also apparent over pleopods.

Right and left antennal glands appear pale yellow.

Discolouration to both right and left gill sets. Dark brown discolouration to cranial gill margins and pink tinged areas on caudal gill margins.

Depleted reserve cell deposits.

Fish 7:

Body weight: 1kg

Carapace length: 13.3cm and total tail/telson length 33.2cm. Alive.

Loss of legs 2,3,4 on the right side with signs of early healing.

Loss of leg 5 on the left side with melanisation present at site.

Eroded edge of telson and uropods. Multifocal eroded spots on pleopods

Focal Melanised lesion on fascial plane located on dorsal muscle of tail.

Enlarged haemolymph vessels surrounding reserve cell tissue of walking leg base.

White plaque like deposits on reserve cell tissue.

Fish 8:

Body weight: 1.87kg

Carapace length: 16cm and total tail/telson length 44cm. DOA

Brown dark discharge released from anus when picked up. No haemoplymph sample obtained.

Left antenna is broken above the segmented region with melanisation present at site.

Minor erosions present on caudal edges of telson and uropods.

Left and right antennal gland appear discoloured with pale yellow to white colouring.

Discolouration associated to left gills as cranial gill sets are dark brown compared to caudal gill sets.

Heart tissue is gelatinous in nature.

There is uneven brown to yellow discolouration associated throughout hepatopancreas.

Reserve cell tissue appears discoloured and pale.

Dorsal tail muscle is blue to pink compared to ventral tail muscle that appears pale yellow to white.

Fish 9:

Body weight: 2.55kg

Carapace length: 18.4cm and total tail/telson length 43cm Live

Antennae loss above segmented region with melanisation at site.

Proliferative erosions on uropods.

2 X 5cm ellipsoid dark lobular region found on dorsal muscles around area of the first intersegmented membrane.

Pink discolouration to longitudinal muscles of the tail

No reserve cell tissue found around the legs

Pink gill discolouration at tips and bases of gill sets that extend along whole gill areas on left and right sides

Haemolymph collected and appears to be brown and discoloured.

Morphological diagnosis:

Carapace: Mild focal erosion with melanisation

Antennal gland: adenitis and concretions

Hepatopancreas: Melanising hepato-pancreatitis

Histological summary

Histology Summary of Accession 17-0510 (9 Fish)	
Gills	Most pathology observed in F1 with mild biofouling and multifocal random areas of melanisation and haemocyte aggregation of secondary lamella. Similar signs observed through other fish sporadically.
Hepatopancreas	No significant observations noted in F4 to F6. Varying degrees of vacuolisation, degeneration and autolysis noted in other fish. F1 was observed to have glassy and amorphous pink eosinophilic inclusions with occasional haemocyte thrombi within interstitial septa (viral inclusions?). Mild to depleted reserve cell noted in fish with digestive gland pathology.
Nerves	No significant findings in F2, F4 to F7 and F9. There were no obvious lesions but mild generalised autolysis observed in F8. There is mild vacuolisation of the proximal ends of the axons in one section seen in F3. Depleted reserve cell tissue and degenerative ganglionic neurons and multifocal areas of haemocyte aggregation and melanisation with evidence of advanced atrophy observed in F1.
Antennal Gland	F7 was the only fish with no significant findings. Extensive to moderate zones of necrosis and melanisation of lining cells with interstitial oedema and streaming of mixed populations of haemocytes seen through most fish. Severe chronic melanising adenitis of the antennule gland within a capsule seen in F3. Reserve cells within tissue is vacuolated and degenerative. Moderate to high levels of crystalline eosinophilic cytoplasmic inclusions within the lining cells of these fish.
Reserve Cell	Depleted reserve cell with marked vacuolisation and varying amounts of degeneration.

Haemocyte counts

Fish 2: $1.7 \times 10^6 cells/ml$

Fish 3: $4.9 \times 10^6 cells/ml$

Fish 4: $0.8 \times 10^6 cells/ml$

Fish 5: $3.4 \times 10^6 cells/ml$

Fish 6: $16.8 \times 10^6 cells/ml$

Fish 7: $15.2 \times 10^6 cells/ml$

Fish 9: $2.1 \times 10^6 cells/ml$

Case Summary

All fish had depleted degenerated reserve cell tissue indicating prolonged period of nutritional stress. Individual fish had significant pathologies of hepato-pancreas and antennal gland as well as peripheral ganglion. All unrelated.

Accession number: 17-0639

Origin: Mt Gambier facility

Species: Southern rock lobster (*Jasus edwardsii*)

Culture Type: Wild caught and tank held for processing and shipment.

Time and date of death: 5/4/2017

Time and date of post mortem examination: pm/5/4/2017

Carcass condition & preservation: 3 Separate cohorts. Each cohort contains 4 lobsters packed in an esky with ice bricks. Cohort 1 – All animals alive

Cohort 2 – All Animals alive

Cohort 3 – 3 animals dead on arrival (DOA) and 1 Alive.

Sudden onset of high mortalities this month. This holding facility has not experienced mortalities like this before. No known changes in facility system. Three separate cohorts of lobsters equating to 12 fish submitted for necropsy examination and ancillary diagnostic investigation.

Gross pathology summary:

Fish 1A:

Body weight: 1.11kg

Carapace length: 12.3cm total tail/telson length 34cm. Live.

Pinpoint erosions along caudal edge of uropod and telson.

Pink discolouration to gills and rakers on left and right sides.

Pale streaks evident on cranio-dorsal muscles

Dark brown discolouration of gonads.

Grey to blue discolouration to dorsal tail muscle compared to ventral tail muscle.

High levels of reserve tissue around carapace ganglion and between antennal glands.

Approximately 1ml of haemolymph sampled for further diagnostics. Haemolymph was noted to have a grey to black discolouration.

Fish 1B:

Body weight: 0.49kg

Carapace length: 10.4cm and total tail/telson length 27cm. Live.

Hepatopancreas – mottled colour.

High levels of reserve tissue around carapace ganglion and between antennal glands.

No other significant findings.

Approximately 1ml of haemolymph sampled for further diagnostics.

Fish 1C:

Body weight: 0.81kg

Carapace length: 11.6cm and total tail/telson length 31.5cm. Live

Mild pinpoint erosions along caudal edge of uropod and telson

Dark brown discolouration to cranial gills compared to caudal gills on left and right sides.

Gonads have a dark brown discolouration.

Grey to blue discolouration to dorsal tail muscle compared to ventral tail muscle.

High levels of reserve tissue around carapace ganglion and between antennal glands.

Approximately 1.1ml of haemolymph sampled for further diagnostics.

Fish 1D:

Body weight: 0.48kg

Carapace length: 19.6cm and total tail/telson length 26cm. Live.

No significant findings.

High levels of reserve tissue around carapace ganglion and between antennal glands.

Approximately 1.5ml of haemolymph sampled for further diagnostics

Fish 2A:

Body weight: 0.83kg

Carapace length: 11.6 and total tail/telson length 33.6cm. Live

Loss of all appendages with no melanisation present at areas of dislodgement.

Broken right antenna above the segmented region with evidence of melanisation at site.

There are mild erosions present at caudal edge of uropod and telson. There are minor focal pin point lesions on pleopods.

Left and Right gills appear to have dark brown discolouration on cranial margins compared to caudal margins. There is also a mild pink tinge associated with the caudal gill margins which are not apparent on cranial margins.

Pale yellow discolouration associated with the heart.

Grey to blue discolouration to dorsal tail muscle compared to ventral tail muscle.

Approximately 1.2ml of haemolymph sampled for further diagnostics. There is a green tinge associated to haemolymph colouration.
Fish 2B:

Body weight: 0.46kg

Carapace length: 9.6cm and total tail/telson length: 24.6cm. Live

Right antenna is broken with evidence of melanisation above the segmented region.

Multiple black focal spot like lesions on pleopod. Mild erosions present at caudal edge of uropod and telson.

Pale yellow discolouration associated with both antennal glands.

Dark brown to black discolouration of cranial margins of gills compared to caudally. This is present on both left and right sides.

Hepatopancreas is discoloured with an uneven distribution of yellow to pale yellow.

Grey to blue discolouration to dorsal tail muscle compared to ventral tail muscle.

Approximately 1.15ml of haemolymph sampled for further diagnostics. Cloudy dark grey discolouration associated with haemolymph.

Fish 2C:

Body weight: 0.78kg

Carapace length: 12cm and total tail/telson length: 31.3cm. Live

Melanisation, erosion and ulcerated lesion present on second left uropod.

Very low to little reserve tissue present surrounding carapace ganglion

Pale discolouration associated to the tips of hepatopancreas tubules.

Approximately 1.3ml of haemolymph sampled for further diagnostics. Haemolymph colouration appears normal.

Fish 2D:

Body weight: 0.45kg

Carapace length: 9.9cm and total tail/telson length: 25.5cm. Live

Right antenna is broken above the segmented region and melanisation present at site.

Mild erosions present on caudal edge of telson and uropod

Antennal glands bilaterally appear to have pale yellow discolouration.

Dark brown discolouration to cranial gill margin compared to caudal margins on both left and right sides.

High levels of reserve tissue present around carapace ganglion and between antennal glands.

Grey to blue discolouration to dorsal tail muscle compared to ventral tail muscle.

Mild cloudy grey tinge discolouration associated with haemolymph. Approximately 1ml of haemolymph sampled for further diagnostics.

Fish 3A:

Body weight: 0.80kg

Carapace length: 11.8cm and total tail/telson length: 31.4cm. DOA

Left antenna broken at segmented region with melanisation present. The right antenna is also broken above the segmented region with melanisation present at site.

Loss of left walking legs 4 and 5 with evidence of melanisation at site of dislodgement.

Loss of right walking leg 3 with no evidence of melanisation.

Moderate erosion and ulceration to the telson with similar lesions present on uropods. Blistered lesion present on 2nd right uropod.

Antennal glands bilaterally appear pale yellow.

Brown uneven discolouration observed on hepatopancreas. Tissue is gelatinous in texture and friable.

Similar gelatinous texture associated with the heart.

There appears to be pale brown discolouration to left and right gill sets that is evenly distributed cranially and caudally.

Grey discolouration to dorsal tail muscle compared to ventral tail muscle.

Small amount of haemolymph sample obtained for microbiology investigations.

Fish 3B:

Body weight: 0.84kg

Carapace length: 11.7cm and total tail/telson length 32.6cm. DOA

Right antenna is broken at segmented region with melanisation at site. The left antenna is broken above the segmented region with melanisation at site.

Loss of right 4th walking leg with no melanisation at site.

Loss of left walking legs 1, 2 and 3 with evidence of melanisation at area of dislodgement.

Moderate erosions on caudal edge of uropod and telson. Mild focal lesions present on pleopod.

Antennal glands bilaterally appear pale yellow and tissue is friable upon collection.

Brown discolouration associated to cranial gill margins compared to caudal on both left and right sides.

Heart appears gelatinous in texture.

Cranial muscles appear to be discoloured with a pink tinge colour. Similar pink discolouration present on dorsal tail muscle compared to ventral trail muscle.

No haemolymph sample obtained.

Fish 3C:

Body weight: 0.58kg

Carapace length: 10.9cm and total tail/telson length: 29.2cm. DOA.

Left antenna is broken at segmented region with melanisation present at site.

Right walking legs 3,4,5 and left walking legs 3,4,5 are missing with no melanisation present at dislodgement sites.

Moderate erosive lesions present on telson and uropods. Mild focal lesions present on pleopods.

Reserve cell tissue between antennal gland appear discoloured with a pale yellow colour.

Bilaterally antennal glands also appear pale yellow.

Dark brown discolouration to cranial gill margins compared to caudal margins on both left and right sides.

Heart is gelatinous in texture and tissue is friable upon collection.

Mild to moderate reserve cell tissue present at carapace ganglion.

Pink discolouration of dorsal tail muscle compared to ventral tail muscle.

No haemolymph sample obtained.

Fish 3D:

Body weight: 0.85kg

Carapace length: 11.7cm and total tail/telson length: 32cm. Live

Left antenna broken above segmented region with melanisation present at site.

Mild erosions present on caudal edge of telson and uropods. Similar mild focal lesions present on pleopods.

Pink widespread discolouration to stomach and mouth parts.

Pale yellow discolouration to antennal glands as well as reserve cell tissue between antennal glands.

Dark brown discolouration to cranial gill margins compared to caudal margins on both left and right sides.

Hepatopancreas appears pale yellow with pink striated areas.

Sporadic red discoloured portions of cranial and caudal GIT.

Reserve tissue at carapace ganglion appears pale yellow with multiple focal white plaque like deposits.

Grey discolouration to dorsal tail muscle compared to ventral tail muscle.

Approximately 1.5ml of haemolymph sampled for further diagnostics Haemolymph is cloudy and grey tinged.

Morphological diagnosis:

Carapace: mild erosion

Hepatopancreas: adenitis

Antennal gland: adenitis

Histological summary:

Histology Summary of Accession 17-639 (12 Fish)	
Gills	Cohort 1: No significant findings with no biofouling. Cohort 2: Mild to no biofouling noted in fish 2B to 2D. Advance stage of Bio-fouling present in fish 2A. with evidence of leucothrix infestation, sessile protozoa, ectocommensals (indication held in tank for awhile). Cohort 3: ; Grade of 1 to 2 including leucothrix filamentous bacteria with

	some plaques of other mixed aggregates of bacteria attached to surface of the cuticle of the gills.
Hepatopancreas	Cohort 1: Varying degrees of reserve cell present within interstitium. Some areas of hepatopancreatic tubules are have a more dilated lumen then others and some contain whorls of proteinaceous material. There are areas of hepatopancreas luminal autolysis and associated degeneration of interstitial tissue Cohort 2: Copious amount of reserve cell noted in all but fish 2C where no reserve cell and marked vacuolisation of lining cells was observed. There was a degree of dilation of tubular lumen and reasonable amount of autolysis seen in other fish. Cohort 3: Some dilatation of glandular lumens. There is absorptive cells. Interstitium is amorphous pink to blue where it appears degenerative reserve cells are still there ghosting. Autolytic post mortem change? With a maintenance of integrity of hepatopancreatic tubules. Bacterial plaques present within all lumens of fish 3D.
Nerves	Cohort 1: Autolytic change in nerve ganglion. areas of basophilic granular material. There are small slight preganglionic haemocyte aggregations and occasional dead neurons. There are areas of the peripheral nerve that appears vacuolated with degeneration of neurons. Occasional cytoplasmic vacuolisation surrounded by degenerative reserve cell Cohort 2: Inflammatory cells present surrounding ganglia. Degree of autolysis present. Most neurons appear pink (Uncertain anti or post autolysis). Vacuolated axons and ganglion Cohort 3: There is a degree of autolysis but the nerves themselves appear okay with no inflammatory aggregates. Otherwise no significant findings.
Antennal Gland	Cohort 1: Two distinct type of tissues visualised in all fish. One is more glandular and open in appearance and the other appears compact. Fish 1B appeared to show focal areas of haemocyte aggregations. Focal apoptosis and degeneration of interstitial cells. There was loss of interstitial cells in the open interstitium area and glandular like cells are not present in fish 1C. Lastly fish 1D appeared to have multifocal areas of tubular degeneration and loss of anatomic anatomy within the compact region of antennal gland. Cohort 2: Two distinct type of tissues visualised in all fish. Zonal areas of autolysis present with no inflammatory cell association in fish 2A. Advanced Focal area of autolysis with variable stages of autolysis of sample characterised by variable areas of swelling, degeneration and loss of cell architecture observed in fish 2B. Fish 2C showed coagulative necrosis in large regions with no association to inflammatory cells and copious amounts of crystalline pink material in the cytoplasm of the lining cell. Cohort 3: There are regional areas of autolytic change, characterised by sloughing of epithelium cells into the lumen. Generally speaking the lumens appear dilated on most fish. There is an overall expansion of the luminal

	areas across the antennal gland. There is some aggregation of haemocytes present within the lumens of the compacted region of the gland. Haemolymph within the interstitium appears bright pink and there are occasional areas where there is a suspected aggregation of haemocytes. There is one focal granulomatous change which is characterised by a fibrous tissue capsule and caseous material within the central area. It is well demarcated and is within the interstitial area of the open glandular section of gland. Lots of sloughed epithelial cells within the lumens. Difficult to confirm is this is a toxic insult autolysis?
Reserve Cell	Cohort 1: No significant findings in fish 1A and 1B. Tissue appears degenerated in fish 1C and 1D. Cohort 2: No significant findings in all fish apart from 2D as reserve tissue was degenerated in that sample. Cohort 3: Mild reserve cell tissue present with autolytic change in fish 3A. No significant findings in other fish of this cohort.

Haemocyte counts:

Fish 1A: $10.8 \times 10^6 cells/ml$

Fish 1B: $9.4 \times 10^6 cells/ml$

Fish 1C: $7.9 \times 10^6 cells/ml$

Fish 1D: $8.3 \times 10^6 cells/ml$

Fish 2A: $9.4 \times 10^6 cells/ml$

Fish 2B: $19.6 \times 10^6 cells/ml$

Fish 2C: $5.0 \times 10^6 cells/ml$

Fish 2D: $18.8 \times 10^6 cells/ml$

Case summary:

There were moderate amounts of biofouling of the gills suggestive of holding facility issues or prolonged holding. Generally pathologies were mild in nature with no indication of an underlying cause.

Accession number: 17-0693

Origin: Mt Gambier facility

Species: Southern rock lobster (*Jasus edwardsii*)

Culture Type: Wild caught and tank held for processing and shipment.

Time and date of death: 12/4/2017

Time and date of post mortem examination: pm/12/4/2017

Carcass condition & preservation: 2 Separate cohorts. One containing 3 lobsters and the other containing 2. Packed in an esky with ice bricks. Cohort 1 – All animals alive

Cohort 2 – All Animals alive

Gross pathology summary:

Fish 1A: Green zip tie on left antenna.

Body weight: Not obtained

Carapace length: Not obtained total tail/telson length: Not obtained. Live.

Melanised tip of 2nd right walking leg? Or antenna?

Mild erosive and ulcerative lesions with evidence of melanisation found on uropods and telson

Shell is palpably soft.

There is pink discolouration to gill tips of the left gill set.

Low levels of reserve cell tissue found surrounding carapace ganglion.

Haematology:

Haemolymph: Grey tinged

0.5mls placed with cacodylate buffer

1ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

No clot > 5 minutes.

Fish 1B: Yellow zip tie on left antenna

Body weight: 1.39kg

Carapace length: 13.4cm and total tail/telson length 35.9cm. Live.

Left antenna broken and stripped at the start of segmented region

Mild to moderate erosive lesions present on uropods and telson

Pink tinge discolouration associated to cranial and caudal abdominal muscles as well as to ventral and dorsal tail muscles.

Shell is palpably soft.

Both gills sets appear to show dark brown discolouration to cranial margins compared to caudal margins.

Haematology:

Haemolymph: orange tinged and cloudy

0.5mls placed with cacodylate buffer

1ml collected as fresh sample

Clotting time: 3.05minutes.

Fish 1C: Yellow and red zip tie on Left antenna

Body weight: 1.31kg

Carapace length: 14.4cm and total tail/telson length 36.7cm. Live

Left antenna broken at rostral tip with melanisation present at site.

Loss of 4th right walking leg with no evidence of melanisation.

Minor erosive lesions present on caudal edge of uropods and telson.

Both left and right gill sets show dark brown discolouration to cranial gill margins compared to caudal margins.

Blue to grey discolouration associated to dorsal tail muscle compared to ventral tail muscle.

Haematology:

Haemolymph: Grey and clear

1mls placed with cacodylate buffer

1ml collected as fresh sample

No clot >5mins

Fish 2A: White zip tie on right antenna and black zip tie on left antenna.

Body weight: 1.03kg

Carapace length: 13.2cm and total tail/telson length 32.5cm. Live.

Loss of 3rd right walking leg with no melanisation present at site.

Shell appears hard – normal

Large antennal glands

Right gills ? dark discolouration to tip?

Prominent white ? walking legs.?

Haematology:

Haemolymph: Dark grey tinged and clear

0.5mls placed with cacodylate buffer

1.2mls collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

No clot > 5mins

Fish 2B: Green zip tie on right antenna

Body weight: 0.92kg

Carapace length: 11.9 and total tail/telson length 32.9cm. Live

Left antenna broken at segmented region with melanisation present.

Right antenna broken just over the level of segmented region with evidence of melanisation.

Marked erosive and ulcerative lesions present of caudal edge of uropods and telson

Reserve cell tissue surrounding antennal glands appear pale yellow.

Both left and right gill sets show dark brown discolouration on cranial margins compared to caudally.

General brown to yellow discolouration associated with hepatopancreas.

Pale and reduced reserve cell tissue surrounding carapace ganglion. There also appears to be multiple focal white plaque like deposits found on reserve cell tissue surface.

Haematology:

Haemolymph: Light grey and clear

0.5mls placed with cacodylate buffer

0.6mls collected as fresh sample

No clot > 5mins.

Morphological diagnosis:

Carapace: Marked erosive and ulcerative lesions present of caudal edge of uropods and telson

Skeletal muscles: Mild myositis

Histological summary:

Histology Summary of Accession 17-0693 (5 Fish)	
Gills	None to mild bacterial biofouling with filamentous bacteria present. There are a mild number of cross sections of metazoans at the base of primary lamellae on the cranial aspect of the gill filaments. Within the gill raker of fish 1B and 2A there are multifocal circular to ovoid structure that in cross section appear as if metazoans embedded in the subcuticular tissue. Higher concentrations found at distal and proximal end of the gill raker. Moderate amount of reserve cell surrounding these organisms. These organisms appear to have coelomic cavities.
Hepatopancreas	Dilatation of hepatopancreatic lumens with minor levels of absorptive vacuoles within digestive gland of fish 1A and 2B. Depleted to moderate reserve cell tissue noted in organ.
Nerves	In fish 1A there are multiple vacuolisation within nerve coming from one section of ganglia. Neuronal bodies appear normal. Reserve cells associated are atrophic and vacuolated. No significant findings in other fish.
Antennal Gland	Mild to high density eosinophilic cytoplasmic inclusions seen in all fish. Focal areas of apoptosis within the lining cells of the antennal gland noted in fish1B. Mild degree of autolysis noted in fish 2A and 2B.
Reserve Cell	Large variation from depleted vacuolated tissue to copious normal amounts.

Haemocyte counts:

Fish 1A: $1.7 \times 10^6 cells/ml$

Fish 1B: $2.7 \times 10^6 cells/ml$

Fish 1C: $4.4 \times 10^6 cells/ml$

Fish 2A: $13.6 \times 10^6 cells/ml$

Fish 2B: $3.3 \times 10^6 cells/ml$

Case Summary:

Generally the pathology mild with indications of antennal gland atrophy and degeneration.

Accession number: 17-0702

Origin: Mt Gambier facility

Species: Southern rock lobster (*Jasus edwardsii*)

Culture Type: Wild caught and tank held for processing and shipment.

Time and date of death: 13/04/2017

Time and date of post mortem examination: pm/ 13/04/2017

Carcass condition & preservation: 5 Live Lobsters. Animals in esky with ice bricks.

Gross pathology summary:

Fish 1: Green zip tie on Right Antenna; Black zip tie on Left Antenna.

Body weight: 0.55kg

Carapace length: 10cm and total tail/telson length 26.4cm. Live.

Both antenna broken above segmented region with melanisation present.

Mild erosions on caudal edge of uropods and telson.

There is two focal white plaque like deposits on right antennal gland. Left antennal gland appears normal.

Cranial gill margins appear darker brown compared to caudal margins on both left and right gill sets.

Moderate amount of reserve cell present surrounding carapace ganglion.

Haematology:

Haemolymph appears clear with a grey to black tinge

0.5ml placed with cacodylate buffer

0.5ml collected as fresh sample – *(sample clotted unable to obtain biochemistry)*

Clotting time 3.12 minutes

Fish 2:

Body weight: 0.64kg

Carapace length: 10.5cm and total tail/telson length 27.9cm. Live.

Right antenna broken above segmented region with melanisation present.

Mild to moderate erosions present on caudal edge of uropods and telson.

There is a pink tinge of discolouration to cranial and caudal abdominal muscles as well as ventral and dorsal tail muscles.

Cranial gill margins appear dark brown compared to caudal gill margins on both left and right gill sets

Red tinge of discolouration associated with cranial GIT.

General discolouration to hepatopancreas with dark yellow to yellow colouring.

Haematology:

Haemolymph appears orange and cloudy

0.5ml placed with cacodylate buffer

0.7ml collected as fresh sample

Clotting time 3.25 minutes.

Fish 3: Red zip tie on left antenna; Black zip tie on right antenna.

Body Weight: 1.19kg

Carapace length: 12.7m and total tail/telson length 33.3cm. Live

Left antenna loss just at segmented region with signs of melanisation.

Mild to moderate erosion on caudal edge of uropods and telson.

Cranial abdominal muscles appear gelatinised with a clear yellow discolouration.

High level of reserve cell tissue present surrounding carapace ganglion as well as between antennal glands.

Right antennal gland appears pale yellow and discoloured. There is also a general white plaque like deposit found on the rostral surface of the gland.

Left antennal gland appears pale yellow with similar white plaque like deposits found along the lateral margin of the gland.

Brown to black granular content observed within the stomach.

General grey to brown discolouration associated with hepatopancreas.

On left and right gill sets, the cranial gill margins appear dark brown compared to caudal gill margins. There is also a pink tinge associated with caudal gill margins.

Pink to grey discolouration of dorsal tail muscle compared to ventral tail muscle.

Haematology:

Haemolymph appears light grey and clear.

0.5mls placed with cacodylate buffer

0.7mls collected as fresh sample -

Clotting time 5 minutes.

Fish 4: 2 Black zip ties on right antenna; 1 black zip tie on left antenna

Body weight: 0.57kg

Carapace length: 10.1cm and total tail/telson length 27cm. Live.

Left antennae broken at segmented region with melanisation present at site.

Right antenna broken on rostral tip with no melanisation present.

Loss of 2nd left walking leg with melanisation present at site.

Mild erosive lesions on caudal edge of uropods with more chronic ulcerative lesions found on the telson.

General pink discolouration associated with cranial and caudal abdominal muscles as well as ventral and dorsal tail muscles.

Similar pink discolouration observed to cranial and caudal GIT.

Cranial margins of right and left gill sets appear dark brown compared to caudal margins. There is also a mild generalised pink tinge to gills.

Haematology:

Haemolymph appears orange and cloudy

0.5mls placed with cacodylate buffer

0.7mls collected as fresh sample

Clotting time 3.40 minutes.

Fish 5: Black zip tie on left antenna.

Body weight: 1.12kg

Carapace length: 12.2cm and total tail/telson length 35.3cm. Live

Left antenna broken above segmented region with no melanisation noted.

Loss of 5th right walking leg with melanisation present at site.

Swelling and bulging present of intersegmented regions on ventrum

Chronic erosive and ulcerative lesions present on uropods and telson. Mild erosions found on pleopods.

General black discolouration to left antennal gland and smaller in size compared to right antennal gland.

Right gland also appears abnormal with a yellow to orange discolouration. There is also multiple focal white plaque like spots covering the rostral surface of the gland.

Reduced reserve cell tissue found between antennal glands as well as surrounding carapace ganglion.

Both left and right gill sets appear to have marked dark brown discolouration associated with cranial gill margins.

General brown to yellow discolouration evident to hepatopancreas.

Pink discolouration to dorsal tail muscle.

Haematology:

Haemolymph: Dark grey to black and cloudy

0.5mls placed with cacodylate buffer

0.8mls collected as fresh sample. *(sample clotted unable to obtain biochemistry)*

Clotting time 4.47 minutes.

Morphological diagnosis:

Carapace: mid erosive inflammation

Antennal gland: adenitis

Histological summary:

Histology Summary of Accession 17-0702 (5 Fish)	
Gills	Multifocal areas of melanisation of the tips of the secondary lamellae. High levels of reserve cells. No biofouling. In F4 there were Multifocal aggregations of circular structures with a bilaminar appearance. Possible central digestive organ. These are found under the germinal epithelium of carapace, Moderate amount of reserve cell present. (Normal structure or metazoan?)
Hepatopancreas	No significant findings in F1 and F2. Dilated tubular lumens with moderate amounts of reserve cell in organ and moderate amounts absorptive vacuoles noted in F3, F4 and F5.
Nerves	No significant findings.
Antennal Gland	Chronic active multifocal necrosis with “fibrosis” and melanisation noted in F1, F3 and F4. There are zonal areas of eosinophilic cytoplasmic inclusions ranging in size and shape from cuboidal to round or ovoid. Some of the ovoid ones are large and multifaceted. F5 appeared to have Focally extensive areas of birefringent crystalline deposits within the lumen of the tubules associated with focal erosion and ulceration with occasional metaplasia of the lining cells. In the interstitium there are also pigmented granulated aggregates that are not birefringent and are also encapsulated with fibroblastic capsules.

	These look synonymous with melanomacrophage centres in teleost fish. Important to note that o eosinophilic cytoplasmic inclusions were observed in this fish as well as F2.
Reserve Cell	No significant findings.

Haemocyte counts:

Fish 1: $12.6 \times 10^6 cells/ml$

Fish 2: $5.8 \times 10^6 cells/ml$

Fish 3: $12.7 \times 10^6 cells/ml$

Fish 4: $1.6 \times 10^6 cells/ml$

Fish 5: $0.9 \times 10^6 cells/ml$

Case Summary:

Generally gross pathology is mild and non-specific. The significant pathologies were found in antennal glands and were marked chronic and active. No cause identified.

Accession number: 17-1375

Origin: Melbourne facility

Species: Southern rock lobster (*Jasus edwardsii*)

Culture Type: Wild caught and tank held for processing and shipment.

Time and date of death: 21/7/2017

Time and date of post mortem examination: pm21/7/217

Carcass condition & preservation: 5 X animals alive and 1 X DOA. Animals in esky with ice bricks.

Sudden mortality event over the past week. Owners claim that the lobsters affected have been transported from Tasmania while Victorian lobsters at the facility are fine.

Gross pathology summary:

Fish 1:

Body weight: 1.10kg

Carapace length: 17.1cm and total tail/telson length 32.3cm. Alive.

Right and left antenna broken with melanisation present.

Mild to moderate lesions on caudal edges of telson and uropods.

Pink tinge present to the edges of gills. Dark gills.

Good reserve cell levels

Soft shell and lobster in poor body condition.

Antennal glands appear to be small.

Haematology:

Haemolymph: Grey tinged

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 2.42 minutes.

Fish 2:

Body weight: 1.10kg

Carapace length: 12.2cm and total tail/telson length 32.5cm. Alive.

Right and left antenna broken with melanisation present

Mild to moderate erosions and blisters present on caudal edges of telson and uropods.

Loss of left walking leg 3 with melanisation present at site.

Moderate to low reserve cell aggregates.

No gut contents

Hepatopancreas is pale

Large antennal glands

Lobster has soft shell and appears to be in poor body condition.

Haematology:

Haemolymph: Grey but clear

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 0.40 minutes

Fish 3:

Body weight: 1.15kg

Carapace length: 13cm and total tail/telson length 33.7cm. Dead on arrival (DOA)

Right antenna is broken over the segmented region with melanisation present.

Left antenna is broken below the segmented region with no melanisation present.

Mild to moderate erosions on caudal edges of telson and uropods.

Antennal glands bilaterally appear large with pale yellow discolouration.

Both gills sets appear to have widespread dark brown discolouration.

Poor body condition noted with good reserve cell levels.

Pink tinge associated with cranial and caudal abdominal muscles.

Heart is gelatinous in nature and friable – considered to be associated with being DOA.

Dorsal tail muscle is discoloured as it is pink tinged compared to ventral tail muscle.

Haematology:

Haemolymph: Orange but clear

0.4mls placed with cacodylate buffer

0.4ml collected as fresh sample - *(biochemistry obtained)*

Clotting time: No clot > 5mins

Fish 4:

Body weight: 1.29kg

Carapace length: 13.3cm and total tail/telson length 34cm. Alive.

Left and right antenna appear broken above segmented region with melanisation present at site.

Mild to moderate erosions on caudal edges of telson and uropods.

There is a brown ulcerated lesion on the ventral aspect of the 2nd intersegmental membrane approximately 0.5cm in length.

Intersegmental membranes have an abnormal bulging, and swollen appearance.

There is loss of right 4th walking leg with melanisation present at site.

Both L and R gill sets appear to have widespread dark brown discolouration.

Good reserve cell levels.

Haematology:

Haemolymph: Grey and cloudy

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 4 minutes

Fish 5:

Body weight: 1.07kg

Carapace length: 11.8cm and total tail/telson length 32.2cm. Alive but moribund.

Left antenna broken above segmented region with melanisation present.

Right antenna broken at the rostral tip with melanisation present.

Mild erosions on caudal edges of telson and uropods.

Pale yellow discolouration to both antennal glands.

Both L and R gill sets appear to have dark brown discolouration to cranial gill margins and compared to a pale brown colouration associated to caudal gill margins.

Dorsal tail muscle is pink tinged compared to ventral tail muscle.

Haematology:

Haemolymph: Orange tinged but clear

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 1 minute.

Fish 6:

Carapace length: 13.2cm and total tail/telson length 33.2. Alive.

Left antenna broken at segmented region with melanisation present at site.

Right antenna broken over segmented region with melanisation present.

Mild to moderate erosions on caudal edges of telson and uropods.

Blister and moderate necrotic erosion on L and R pleopod 3 as well as on R pleopod 4.

Antennal glands missing? With melanisation present?

Left 4th walking leg missing with melanisation present at site.

Dark discolouration associated with hepatopancreas. -??

Eroded uropods and telson.

Soft shell.

Haematology:

Haemolymph: Grey and cloudy

0.5mls placed with cacodylate buffer

0.8ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 0.30 minutes.

Morphological diagnosis:

Gills: Mild branchitis

Carapace: Mild uropod and telson erosions.

Histological summary:

Histology Summary of Accession 17-1375 (6 Fish)	
Gills	Multifocal melanisation of secondary lamellae observed in F1, F4. Grade of biofouling varies in accession; Grade 0 in F2, F3 and F5. Grade 1 to 2 in F1 and F6. Grade 2 – 3 in F4.
Hepatopancreas	Dilated tubules generalised with mild to moderate amounts of reserve cell tissue in F1, F2, and F4. Similar findings noted in F6 however reserve cell tissue is depleted. The digestive gland of F3 is severely autolytic. F5 has similar findings with mild zones of autolysis.
Nerves	Vacuolisation in one of the neuronal bodies noted in F3. No significant findings found in other fish.
Antennal Gland	Extensive cytoplasmic inclusions within gland noted in all fish. Lining cells appear shrunken with dilation of tubular lumens in F1 and F5. Multifocal haemocyte aggregations in the interstitium where some are encapsulated and melanised in F2. Severe autolysis noted in F3. F4 and F6 appeared to show loss of height of lining cells with presence of dilated tubular lumens.
Reserve Cell	Autolytic and vacuolated tissue of F3 and F4. No significant findings in other fish.

Haemocyte counts:

Fish 1: $5.9 \times 10^6 cells/ml$

Fish 2: $7.3 \times 10^6 cells/ml$

Fish 3: $0.6 \times 10^6 cells/ml$

Fish 4: $8.8 \times 10^6 cells/ml$

Fish 5: $3.4 \times 10^6 cells/ml$

Fish 6: $5.9 \times 10^6 cells/ml$

Microbiological summary:

Fish 1: *Vibrio Sp.*

Fish 2: *Vibrio tapetis*

Fish 3: *Vibrio tapetis*

Fish 4: *Vibrio tapetis*

Fish 5: *Vibrio tapetis*

Fish 6: *No growth post 48 hours.*

Case summary: Despite the uniform microbiological isolation the general pathology in all animals is mild with limited host response usually found in septicaemia's.

Accession number: 17-1410

Origin: Tasmanian facility

Species: Southern rock lobster (*Jasus edwardsii*)

Culture Type: Wild caught and tank held for processing and shipment.

Time and date of death: 26/7/2017

Time and date of post mortem examination: 26/7/2017 to 27/7/2017

Carcass condition & preservation: 11 X Alive and 3 X DOA. Animals in esky with ice bricks.

Sudden high mortality event. Similar event occurred to associated Victorian facility who claim it was lobsters from this source that were dying off.

Gross pathology summary:

Fish 1: Tag - 003183

Body weight: 0.66kg

Carapace length: 11cm and total tail/telson length 27.8cm. Alive but moribund.

Poor body condition

Right 3rd walking leg missing with no melanisation present.

Mild to moderate erosions on caudal edges of telson and uropods.

Left gills appear darker brown cranially compared to paler brown caudally, similar discolouration observed on the right gills but to a lesser extent.

Good reserve cell levels

Dorsal tail muscle is pink tinged compared to ventral tail muscle.

Haematology:

Haemolymph: Grey and cloudy

0.5mls placed with cacodylate buffer

0.2ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 2.48 minutes.

Fish 2: Tag – 003188

Body weight: 0.69kg

Carapace length: 11.3cm and total tail/telson length 29cm. Alive.

Both antenna broken over segmented region with melanisation present at site.

Marked melanisation and erosion of the left lateral uropod. Uropod appears necrotised and degenerated. Mild erosions present on caudal edges of other uropods and telson.

Swelling apparent of intersegmental membrane 1 – grey tinge associated to swollen area.

Dark brown discolouration to cranial gills compared to pale brown colouration of caudal gills on L and R sides.

Hepatopancreas is not uniformly coloured with a brown to yellow colouration and tissue appears moulted and friable.

White plaque like deposits present on ventral reserve cell location.

Dorsal tail muscle is pink tinged compared to ventral tail muscle.

Haematology:

Haemolymph: Grey and cloudy

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(biochemistry obtained.)*

Clotting time: 1.05 minutes.

Fish 3: Tag – 003185

Body weight: 0.72

Carapace length: 10.8cm and total tail/telson length 28.9cm. Alive.

Both antenna broken above the segmented region with melanisation present.

Swelling and grey discolouration observed on intersegmental membrane 1.

Mild erosions of caudal edges of telson and uropods.

Widespread dark brown discolouration to gills on both L and R sides.

Good reserve cell levels.

Dorsal tail muscle appears pink tinged compared to ventral tail muscle.

Haematology:

Haemolymph: Grey and clear

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 1.18 minutes.

Fish 4: Tag – 003189

Body weight: 0.62

Carapace length: 10.8cm and total tail/telson length 28cm. Alive but moribund.

Moderate to poor body condition.

R and L antenna broken above segmented region with melanisation present at site.

Loss of Left walking leg 1 with no melanisation present.

Moderate erosive lesions on caudal edge of uropods. Similar lesions on telson that extending cranially.

Mild erosive lesions present on edges of pleopods.

Both L and R antennal glands are dark yellow in colour however left gland appears smaller in size compared to the right.

Low reserve cell levels.

Widespread dark brown discolouration to gills on both L and R sides.

Hepatopancreas appears abnormal with a pale yellow to white colouration.

Pink discolouration associated to cranial abdominal muscles as well as dorsal tail muscle compared to ventral tail muscle.

Haematology:

Haemolymph: Grey clear

0.2mls placed with cacodylate buffer

0.4ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 2.40minutes.

Fish 5: Tag – 003190

Body weight: 0.66kg

Carapace length: 10.4cm and total tail/telson length 27.4cm. Alive.

Moderate to poor body condition.

L and R antenna broken above segmented region with melanisation present

Moderate to marked erosions present along caudal edges of telson and uropods.

Antennal glands appear small bilaterally.

Shell is soft.

Right gills appear dark brown cranially compared to a pale brown appearance caudally

Left gills appear to have widespread dark brown discolouration.

Widespread pale brown discolouration to hepatopancreas.

Haematology:

Haemolymph: Grey and cloudy

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 3.18 minutes.

Fish 6: Tag – 003191

Body weight: 0.64kg

Carapace length: 11cm and total tail/telson length 28.5cm. DOA.

Poor body condition.

Right antenna broken above segmented region with melanisation present.

Moderate to marked ulcerative lesions on telson and uropods.

No reserve cell tissue found cranially however normal levels on ventral aspect of carapace.

Gills bilaterally have dark brown discolouration cranially compared to a pale brown caudally

Pink tinge associated to all abdomen and tail muscles.

Heart and hepatopancreas appear normal but tissue is very friable.

Haematology:

Haemolymph: Grey and cloudy

0.1mls placed with cacodylate buffer

No fresh sample collected

Clotting time: Insufficient sample to test.

Fish 7: Tag – 003195

Body weight: 0.67kg

Carapace length: 10.9cm and total tail/telson length 29cm. Alive but moribund

Poor body condition

Left antenna broken at segmented region with melanisation present.

Loss of left walking legs 3 and 4 with melanisation at site.

Mild to moderate erosions on caudal edges of telson and uropods.

Brown discolouration associated to cranial gill sets compared to pale brown caudal gills on both L and R sides.

Pale brown to yellow discolouration of hepatopancreas. Tissue appears to have loss its integrity and is very friable on sample collection.

Heart appears small.

Haematology:

Haemolymph: Orange tinged and clear

0.6mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(biochemistry obtained)*

Clotting time: 4.15 minutes.

Fish 8: Tag – 003193

Body weight: 0.67kg

Carapace Length: 11.1cm and total tail/telson length 29.2cm. Alive but moribund.

Note that prior to necropsy there was moderate amounts of clotted haemolymph attached to external portion of appendage 2 and 3 on the left side. (*Uncertain if this was from a different fish)*

Moderate body condition and shell is soft.

Right antenna broken above segmented region with melanisation present.

Left antenna broken at segmented region with melanisation at site.

Loss of appendage 3 and 4 on the right side with no sign melanisation.

Mild erosions on caudal edge of telson and uropods

Right antennal gland tissue is friable with widespread brown to pink discolouration through the gland. Left antennal gland appeared normal.

Dark brown discolouration to cranial and caudal gill margins on L and R sides.

Good reserve cell level.

Pink discolouration to dorsal tail muscle compared to ventral tail muscle.

Haematology:

Haemolymph: Grey and cloudy

0.5mls placed with cacodylate buffer

0.3ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 0 minutes. *(Clotted instantly)*

Fish 9: Tag – 003201

Body weight: 0.64kg

Carapace Length: 10.5cm and total tail/telson length 28.5cm. DOA

Poor body condition

Mild to moderate erosions on telson and uropods.

There is dark brown discolouration to cranial gill margins compared to a pale brown colour on caudal margins observed on L and R sides.

Gelatinous clotted haemolymph surrounding internal organs.

Dark yellow discolouration to hepatopancreas and tissue is very friable on collection

Depleted reserve cell tissue.

Haematology: *Haemolymph clotted – unable to obtain sample*

Fish 10: Tag – 003194

Body weight: 0.56kg

Carapace Length: 10.2cm and total tail/telson length 27.8cm. Alive

Good body condition with moderately soft shell

Both L and R antenna broken above segmented region with melanisation at site.

Loss of left 4th walking leg with melanisation present.

Loss of right 3rd walking leg with no signs of melanisation.

Mild erosion on caudal edge of telson and uropods.

Widespread dark brown discolouration to gills on L and R sides.

Hepatopancreas is very friable with a dark brown abnormal colouration.

Pink tinge associated with cranial abdomen muscles.

Heart appears mildly small compared to expected size.

Depleted reserve cell tissue.

Haematology:

Haemolymph: Orange tinged and cloudy

0.5mls placed with cacodylate buffer

0.7ml collected as fresh sample - *(biochemistry obtained)*

Clotting time: 2.51 minutes.

Fish 11: Tag – 003186

Body weight: 0.60

Carapace Length: 10.6cm and total tail/telson length 28.2cm. Alive

Poor body condition. Moribund on post mortem.

Both R and L antenna broken above segmented region with melanisation at site.

Left 3rd walking leg lost with no melanisation visible.

Mild to moderate erosions on caudal edge of telson and uropods. Fluid filled blister also present on telson.

Widespread dark brown discolouration to gills on L and R sides.

Hepatopancreas has a dark brown discolouration and tissue is very friable on collection.

Heart appears small compared to expected size.

There are multifocal white plaque like deposits observed on reserve cell tissue.

Haematology:

Haemolymph: Grey and cloudy

0.5mls placed with cacodylate buffer

0.6ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 4.31 minutes.

Fish 12: Tag – 003184

Body weight: 0.76kg

Carapace Length: 11.4cm and total tail/telson length 29.8cm. DOA

Poor body condition and hard shell

Left walking leg 4 is lost with melanisation present at site.

Mild erosive lesions on caudal edge of telson and uropods.

Mild brown discolouration to cranial gills compared to pale brown caudal gills on L and R sides.

Hepatopancreas appears discoloured with multiple dark brown spots randomly distributed through the organ.

Dorsal tail muscle is pink tinged compared to ventral tail muscle.

Haematology: *Haemolymph clotted – unable to obtain sample*

Fish 13: Tag – 003192

Body weight: 0.65kg

Carapace Length: 11cm and total tail/telson length 28.1cm. Alive

Good body condition

Both L and R antenna are broken above segmented region with melanisation at site.

Mild erosions on caudal edge of telson and uropods.

Widespread dark brown discolouration to gill sets on L and R sides.

Dark brown discolouration with a melanised appearance associated with hepatopancreas. Tissue is friable on collection.

Depleted reserve cell tissue with multifocal while plaque like deposits noted on left side of ventral area.

Haematology:

Haemolymph: Orange tinged and clear

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 3.25 minutes.

Fish 14: Tag – 003187

Body weight: 0.61kg

Carapace Length: 10.3cm and total tail/telson length 27.6cm. Alive

Loss of walking appendage 1, 4 and 5 on left side as well as appendage 1 on right side with no melanisation evident.

Mild erosions on caudal edge of uropods and telson.

Left antennal gland is melanised with predominantly a dark brown discolouration and a mild pink tinge to the tissue.

Right antennal gland is predominantly pink tinged with brown discolouration to the medial edge. There is also a large white plaque like deposit on the caudal half of the gland.

There is a focal region of melanisation on the left dorsal portion of the hepatopancreas. Region has a red to brown discolouration.

Heart appears small compared to expected size.

Dorsal tail muscle is pink tinged compared to ventral tail muscle.

Haematology:

Haemolymph: Grey and cloudy

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 3.30 minutes.

Morphological diagnosis:

Gills: Obstructive bronchitis

Carapace: mild multifocal erosions with melanisation.

Hepato-pancreas: Hepato-pancreatitis

Histological summary:

Histology Summary of Accession 17-1410 (14 Fish)	
Gills	No significant findings were noted in F2, F6. F7. F9, F13 and F14. No biofouling was noted on F12 however there were multifocal areas on the primary filament of micro-granulomas some of which were associated with bacteria within the haemolymph channel (suspected post mortem change). Previously described inclusions that were suspected to be organisms were noted in F1 and F10 as well as in previous accessions can now be concluded to be normal setae cut at an angle. Minor grade 1 bacterial biofouling was seen in F5 and F8 with multifocal areas of melanisation of the secondary lamellae. Grade 2 biofouling consisting filamentous bacteria as well as with protozoan presence was observed in F3. F4 and F11. A chronic multifocal to granulomatous melanising bronchitis was noted on F4. Overall there was

	mild to moderate reserve cells associated.
Hepatopancreas	Moderate amounts of reserve cells in the interstitium and area that appears autolysed as well as some autolytic samples with no significant findings noted for F1, F5, F8 to F9 and F12. In F2 and F3 there was generalised dilation of digestive gland tubules with minor amounts of reserve cell tissue in the interstitium. Areas of reactive haemocyte aggregations noted in F4 and F6. For F10, F11, and F13 there were depleted reserve cells with extensive dilation of tubules as well as moderate amounts of vacuole presence in luminal walls. A focally extensive area of chronic necrotising melanising hepatopancreatitis was noted in F14.
Nerves	No significant findings observed in F1 to F3, F7, F8, F10 to F12 and F13. There are a number of sections where ganglia have a vacuolated nucleus with fragmentation of myelin (myelin vacuolisation?) noted in F4 and F5. F6 was observed to have degeneration and apoptosis of neuronal ganglia (Autolytic?). Mild amounts of vacuolisation in the neuronal bodies that are most likely due autolytic change was noted in F9 and F13.
Antennal Gland	Generalised marked number of eosinophilic cytoplasmic inclusions with mild to moderate autolysis and no other significant findings noted in F1, F2, F3, F9, F10 and F13. In F4 and F11 there is elongation of the lining cells with occasional areas with cytoplasmic inclusions and it appears as if there is loss of lining ells into the lumen (autolysis/necrosis?) Cytoplasmic inclusions and a focal granulomatous melanised lesion as described in F5. Generalised large intra-cytoplasmic inclusions were noted in F6 while generalised necrosis with significant cytoplasmic inclusions were observed in F7. Chronic active areas of multifocal to coalescing melanised granulomas indicating of coagulative inflammation with intra-lesional plaques of bacteria and haemocyte infiltration were noted in F8, F12 and F14. In F8 these bacteria were rods.
Reserve Cell	No significant findings in F1, F7, F8 and F11. Moderate to low amounts of normal tissue in F5 and F6. Depleted, degenerated and autolytic samples that are vacuolated noted in F2 to F4, F9, F10 and F12 to F14.

Microbiological summary:

Fish 1: *Vibrio tapetis*

Fish 2: No growth post 48 hours

Fish 3: No growth post 48 hours

Fish 4: *Vibrio tapetis*

Fish 5: *Vibrio tapetis*

Fish 6: *Vibrio tapetis*

Fish 7: *Vibrio Sp.*

Fish 8: No growth post 48 hours

Fish 9: *Vibrio tapetis*

Fish 10: No growth post 48 hours

Fish 11: No growth post 48 hours

Fish 12: No sample

Fish 13: No growth post 48 hours

Fish 14: No growth post 48 hours

Haemocyte counts:

Fish 1: $1.9 \times 10^6 cells/ml$

Fish 2: $9.0 \times 10^6 cells/ml$

Fish 3: $4.3 \times 10^6 cells/ml$

Fish 4: $1.3 \times 10^6 cells/ml$

Fish 5: $3.7 \times 10^6 cells/ml$

Fish 6: $0.5 \times 10^6 cells/ml$

Fish 7: $2.7 \times 10^6 cells/ml$

Fish 8: $5.4 \times 10^6 cells/ml$

Fish 10: $4.4 \times 10^6 cells/ml$

Fish 11: $3.5 \times 10^6 cells/ml$

Fish 13: $2.3 \times 10^6 cells/ml$

Fish 14: $3.4 \times 10^6 cells/ml$

Case Summary:

The significant pathologies appear in the hepato-pancreas and the antennal gland with changes consistent with those seen in other crustacea with bacterial septicaemias.

Accession number: 18-0302

Origin: South Australian facility

Species: Southern rock lobster (*Jasus edwardsii*)

Culture Type: Wild caught and tank held for processing and shipment.

Time and date of death: 19 & 20/2/2018

Time and date of post mortem examination: 19/2/2018 to 20/2/2018

Carcass condition & preservation: 15 X Alive and 5 X DOA. Animals in esky with ice bricks.

Sudden high mortality event.

Gross pathology summary:

Fish 1: Tag - 00070

Body weight: 0.83kg

Carapace length: 11.4cm and total tail/telson length 29.3cm. Alive.

Erosions on caudal margins of telson and uropods.

Left antenna broken above segmented region with melanisation present.

There is pale discolouration and translucent appearance to both antennal glands

The hepatopancreas appears to be pale brown.

Haematology:

Haemolymph: Turbid with a yellow tinge associated

0.6mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: >5 minutes no clots

Fish 2: Tag – 00050

Body weight: 0.62kg

Carapace length: 10cm and total tail/telson length 27.3cm. Alive.

Broken right antenna over segmented region with melanisation present.

No missing legs.

Mild erosions on caudal edge of telson and uropods

Shell is soft

Haematology:

Haemolymph: Cloudy orange

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(biochemistry obtained.)*

Clotting time: 3 minutes.

Fish 3:

Body weight: 0.61

Carapace length: 11.2cm and total tail/telson length 29.1cm. Alive.

Small blisters and erosions present on the caudal edge of the telson and uropods.

No missing legs.

Broken antenna above the segmented regions with melanisation present at site.

There is a pale-ness to the longitudinal muscle on the ventral part of tail.

Fish is soft shelled however is showing good tail response.

Evidence of cannibalism present with lobster material present within mouth parts. Shell bits also present within and throughout caudal GIT.

No cranial reserve cell and poor reserves on ventral caparace.

Both antennal glands are pale and translucent in appearance

Both right and left gills appear to show dark brown discolouration to cranial margins compared to a paler brown to white caudally.

The reproductive tract is a pale yellow colour.

There is also brown discolouration associated with the hepatopancreas.

Haematology:

Haemolymph: Cloudy with a slight yellow tinge

0.6mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 2.15 minutes.

Fish 4:

Body weight: 0.72

Carapace length: 10.9cm and total tail/telson length 30cm. Alive.

Good flap response present

Mild random erosions on telson and uropods

Walking legs 3 and 4 on the left are missing as well as leg 4 on the right – all sites have melanisation present.

Both left and right antenna are broken above segmented region with melanisation present.

There is a pale translucent appearance associated with both left and right antennal glands.

Adequate reserve cell present cranially and ventrally.

Reproductive tract is pronounced in size and is orange in colour.

There is a brown patchy discolouration associated with the hepatopancreas. Hepatopancreas tissue is very friable upon collection.

Haematology:

Haemolymph: Clear with slight yellow tinge associated

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(biochemistry obtained)*

Clotting time: >5minutes no clot.

Fish 5:

Body weight: 0.61kg

Carapace length: 10.1cm and total tail/telson length 27.6cm. Alive.

Weak tail flap response and fish is soft shelled.

There are linear erosions present on telson and uropods.

Left antenna is broken over the segmented region with melanisation present at the site.

Evidence of cannibalism present with lobster material found within mouth parts. Pink shell material present in the mid section of caudal GIT only.

Both antennal glands appear shrunken in size.

Left and right gill margins are pale.

The hepatopancreas and reproductive tract are bright yellow in colour.

Haematology:

Haemolymph: Cloudy and white

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 2.26minutes.

Fish 6:

Body weight: 0.67kg

Carapace length: 10.2cm and total tail/telson length 29.3cm. Alive.

Loss of right antenna at the segmented region with melanisation present at site.

Loss of walking leg 5 on the right side with no melanisation at site.

Mild erosions with ulcerations on caudal aspect of telson and uropods.

Fish is in low body condition as it is thin and pale with a poor tail flap response.

Reproductive tract is enlarged and orange in colour.

Both right and left antennal glands appear pale

There is adequate reserve cranially and ventrally

The cranio-dorsal carapace muscle appears pink tinged.

There is dark brown discolouration associated with the hepatopancreas.

Dark brown discolouration also visible on cranial aspect of gill margins compared to caudal margins on the left side of fish. This dark discolouration is widespread on the right side of fish.

Pink discolouration is also visible on the dorsal tail muscle compared to the ventral tail muscle.

Haematology:

Haemolymph: Cloudy and orange tinged

0.5 mls placed with cacodylate buffer

0.5ml of fresh sample collected *(biochemistry obtained)*

Clotting time: 4.09 minutes.

Fish 7:

Body weight: 0.70kg

Carapace length: 11.5cm and total tail/telson length 29.5cm. Alive.

Poor body condition – soft shelled.

Missing 3rd walking leg on the left side with minimal melanisation at site.

Loss of both antennae above segmented region with no melanisation at site.

Ulcerated telson with blistering evident on uropods.

There is swelling over intersegmented junction of telson/uropod

There is material within the cranial and caudal GIT that is pink tinged. No cranial reserve cell present.

Both left and right antennal glands appear translucent and discoloured.

Left and right gill margins are also discoloured with dark brown discolouration on cranial margins and paler brown on caudal margins

The hepatopancreas is bright yellow.

There is ample reserve cell tissue found on the mid ventral aspect of the carapace.

The reproductive tract of the animal is translucent and small.

Haematology:

Haemolymph: Orange and cloudy

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 1.23 minutes.

Fish 8:

Body weight: 0.75kg

Carapace Length: 11.5cm and total tail/telson length 31.2cm. Dead on arrival (DOA).

Right antenna broken over segmented region with melanisation at site.

Loss of of walking leg 1, 3 and 4 on the left side with melanisation present on all sites.

Moderate erosions on caudal aspect of telson and uropods.

Dark brown discolouration to the hepatopancreas.

There are white plaque like deposits on the ventral margin of the left antennal gland that is also smaller in size compared to the right antennal gland.

Both left and right gill margins are discoloured with brown discolouration to cranial aspects of gills compared to caudally.

The reproductive tract is small and translucent in this animal.

Moderate reserves found cranially and along the mid ventral aspect of the carapace.

Haematology:

Haemolymph: Clear and white

0.5mls placed with cacodylate buffer

0.9ml collected as fresh sample - *(biochemistry obtained)*

Clotting time: 48 seconds.

Fish 9:

Body weight: 0.64kg

Carapace Length: 10.8cm and total tail/telson length 20.5cm. Alive.

Haematology:

Haemolymph: Cloudy and orange

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 3.5 minutes.

Fish 10:

Body weight: 0.63kg

Carapace Length: 10.2cm and total tail/telson length 27.3cm. Alive

Soft shelled animal

Loss of walking leg 4 and 5 on the right side with no melanisation at sites

Minor erosions on caudal aspect of telson and uropods

There is dark brown discolouration throughout gill margin on left and right sides.

Hepatopancreas is bright yellow

Little to no reserve cell tissue.

Haematology:

Haemolymph: Grey and turbid

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(biochemistry obtained)*

Clotting time: 1.38 minutes

Fish 11:

Body weight: 0.71

Carapace Length: 12cm and total tail/telson length 30.5cm. DOA

Poor body condition and soft shelled.

Foul smelling once cut into

Gross mild erosions on telson and uropods

Shell is thin and pale couloured

Hepatopancreas has pale discolouration

Reserve cell deposit?

Haematology:

Haemolymph: Turbid and orange tinged

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(biochemistry obtained)*

Clotting time: 43 seconds

Fish 12:

Body weight: 0.51kg

Carapace Length: 9.7cm and total tail/telson length 25.2cm. Alive

No limb loss

Right antenna broken at rostral tip of non-segmented region with melanisation present

Haemolymph clotted

Haematology:

Haemolymph: Cloudy and grey tinged

0.5mls placed with cacodylate buffer

0.25ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 43 seconds

Fish 13:

Body weight: 0.95kg

Carapace Length: 13.8cm and total tail/telson length 34.6cm. Alive but moribund

Loss of antenna on the left side at the segmented region

Soft shelled and darkly coloured animal

Blistering of uropods and telsons with moderate erosions on caudal aspects.

Haematology:

Haemolymph: Clear

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(biochemistry obtained)*

Clotting time: 4.05 minutes.

Fish 14:

Body weight: 0.64kg

Carapace Length: 12cm and total tail/telson length 29.9cm. Alive

Broken left antenna above segmented region with melanisation at site.

Loss of 4th walking leg on the right.

Large circular loss of telson tissue with mild erosions on other uropods

There is a pink tinge associated to cranial carapace muscles as well as tail muscles

The hepatopancreas is bring yellow in colour

Widespread pink discolouration is present to gill margins on left and right sides

Unable to visualise a reproductive tract.

Haematology:

Haemolymph: Orange and cloudy

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(biochemistry obtained)*

Clotting time: 1.23 minutes.

Fish 15:

Body weight: 0.78kg

Carapace Length: 13cm and total tail/telson length 30.5cm. Alive

Pale lobster. Both antenna fractured at non segmented regions. There are organised melanised erosions on the caudal edge of the uropods and telson.

No cranial reserve tissue visible. There is brown discolouration associated to the hepatopancreas.

The reproductive tract is translucent clear and small in size.

Right and left gill margins are discoloured, with brown discolouration cranially and a paler brown caudally.

There are moderate reserves cells present along mid ventrum.

Brown material is randomly present within caudal GIT.

Haematology:

Haemolymph: Grey and cloudy

0.4mls placed with cacodylate buffer

0.4ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 3.57 minutes.

Fish 16:

Body weight: 0.66kg

Carapace Length: 11.5cm and total tail/telson length 31cm. Alive

Poor condition and has a soft shell.

Loss of third and forth walking leg on the right and the forth walking leg on the left with melanisation present at all sites.

Loss of both antenna above segmented region with melanisation present.

There is brown material present within mouth parts.

Brown discolouration noted on cranial margin of left and right gills with a paler brown noted on caudal margins.

Hepatopancreas tissue is very friable in nature.

The reproductive tract is enlarged and orange.

Ample reserve cells associated cranially and along the mid – ventrum.

There is pink discolouration associated with the tail muscle.

Haematology:

Haemolymph: Orange and cloudy

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(sample clotted on board but obtained some biochemistry values)*

Clotting time: 30seconds.

Fish 17:

Body weight: 0.60kg

Carapace Length: 11.1cm and total tail/telson length 30.3cm. Alive moribund

Poor condition – soft shelled.

Loss of 5^{th} walking leg on the right as well as 3^{rd}, 4^{th} and 5^{th} walking leg on the left with melanisation present on all sites.

The right antenna is broken at the segmented region with no melanisation present.

Reproductive tract is enlarged and orange to brown in colour

There is dark brown material present throughout cranial and caudal GIT

Dark brown discolouration associated to cranial gill margins compared to a pale brown caudally on left and right sides.

The hepatopancrease is pale yellow and discoloured

There is pink tinged discolouration associated to dorsal tail muscle compared to ventral tail muscle.

Haematology:

Haemolymph: Dark turbid and orange tinged

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 4.02minutes.

Fish 18:

Body weight: 0.57kg

Carapace Length: 11.5cm and total tail/telson length 28cm. DOA

Dark coloured fish.

Severe erosions of the lateral left uropod with melanisation. There are more chronic like lesions present on caudal edges of telson and uropods.

Fractured left antenna at the junction where segmented region meets non segmented region with melanisation present at site.

When pithed large amounts of foamy gas bubbles were expressed from site of entry.

Both antennal glands appear white and discoloured.

There is a moderately enlarged reproductive tract that is pale yellow in colour.

Dark brown discolouration associated to the hepatopancreas.

Mild to depleted reserve cell tissue at both sites.

The heart appears very enlarged and gelatinous in nature.

Haematology:

Haemolymph: Grey and cloudy

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 47 seconds

Fish 19:

Body weight: 0.61kg

Carapace Length: 11.5cm and total tail/telson length 29.5cm. DOA

Poor condition and soft shelled animal.

There are large blisters present on all telson and uropods with evidence of melanisation.

Loss of 4th left walking leg with no melanisation on site.

Broken left antenna above the segmented region with melanisation present

Both left and right antennal glands appear brown and discoloured. White plaque like deposits were also visualised on the right antennal gland.

Adequate reserve cells found at both sites.

The reproductive tract is orange in colour and is moderately enlarged.

Mouth part is filled with brown material.

Dark brown discolouration associated to cranial left and right gill margins.

The hepatopancreas appears dark brown and discoloured with a patchy appearance.

Cranial GIT appears distended and fill of brown material.

Haematology:

Haemolymph: Orange and cloudy

0.5mls placed with cacodylate buffer

0.5ml collected as fresh sample - *(biochemistry obtained)*

Clotting time: 2.30 Minutes.

Fish 20:

Body weight: 0.49kg

Carapace Length: 11.5cm and total tail/telson length 20.5cm. DOA

Loss of walking leg 3, 4 and 5 on the left leg with melanisation present at all sites.

There are blisters and linear erosions present on uropods.

Hepatopancreas is pale and tissue is friable on collection.

No obvious reproductive tract visible. Material within heart is clotted.

Haematology: Haemolymph not collected.

Morphological diagnosis:

Carapace: mild erosion and uropod and telson blistering

Antennal gland: adenitis and atrophy

Reserve cells deposits: Atrophy

Skeletal muscle: focal myositis

Hepatopancreas: adenitis

Histological summary:

Histology Summary of Accession 18-0302 (20 Fish)	
Gills	Varying degree of biofouling was observed through this accession ranging from 0 to 3. Multifocal areas of melanisation of the tips of secondary lamellae were noted on some fish. Extensive multifocal areas appears fibrosed in F6 with scant basophilic cytoplasmic inclusions noted in fibrosed areas. Varying degree of metazoans, amplipods and swimming ciliates were observed throughout the accession.
Hepatopancreas	Varying amounts of reserve tissue were noted throughout this accession ranging from no reserve tissue to copious amounts. The most notable finding observed was dilated tubules with marked vacuolisation of the lining cells with F13 also showing areas of degenerative tissue. Abnormal dilation of tubules were noted in F1, F2, F3, F8, F10, F11, F12, F15, and F16. Samples from F20 and F19 were autolytic.
Nerves	F19 showed axon clumping and degeneration with haemocyte focci observed within the nerve themselves. This clumping was also observed in with F8 that appeared to have vacuolisation of nerve tissue similar to that in F13 and F12. Haemocyte aggregations were noted in F10 and F5 within the peri-neural area. No significant findings were observed in other fish of this accession.
Antennal Gland	No significant findings were observed in F1, F2, F3, F7, F10, F12, F13, F15, F16 and F18. Autolysis was noted in F11 and F20. Haemocyte infiltrations and aggregations were observed in F17 and more so in F8. Antennal gland necrosis with bacterial plaques in addition to loss of tissue architecture were described in F14 and F19. Basophilic nucleic inclusions were observed in F4 and these are suspected to be apoptotic cells rather than viral inclusions. Birefringent crystals were observed under polarised light within tissue of F6.
Reserve Cell	No significant findings in all fish apart from F5 and F11 where reserve tissue appeared vacuolated.

Microbiology Summary:

Fish 1: *Vibro species*

Fish 2: *No growth after 48 hours*

Fish 3: *Vibro species*

Fish 4: *Phenon 36 – Unidentified and unlikely Vibrio*

Fish 5: *Vibro species*

Fish 6: *Vibro species*

Fish 7: *Vibro species*

Fish 8: *Vibro species*

Fish 9: *No growth after 48 hours*

Fish 10: *Vibro species*

Fish 11: *Vibro species*

Fish 12: *No growth after 48 hours*

Fish 13: *Vibro species*

Fish 14: *Vibro species*

Fish 15: *Vibro species*

Fish 16: *Unidentified and unlikely Vibrio*

Fish 17: *Vibro species*

Fish 18: *No growth after 48 hours*

Fish 19: *Vibro species*

Haemocyte counts:

Fish 1: $3.3 \times 10^6 cells/ml$

Fish 2: $4.1 \times 10^6 cells/ml$

Fish 3: $1.0 \times 10^6 cells/ml$

Fish 4: $1.7 \times 10^6 cells/ml$

Fish 5: $1.9 \times 10^6 cells/ml$

Fish 6: $2.8 \times 10^6 cells/ml$

Fish 7: $7.9 \times 10^6 cells/ml$

Fish 8: $1.4 \times 10^6 cells/ml$

Fish 9: $2.8 \times 10^6 cells/ml$

Fish 10: $1.4 \times 10^6 cells/ml$

Fish 11: $0.8 \times 10^6 cells/ml$

Fish 12: $5.8 \times 10^6 cells/ml$

Fish 13: $1.7 \times 10^6 cells/ml$

Fish 14: $9.0 \times 10^6 cells/ml$

Fish 15: $1.9 \times 10^6 cells/ml$

Fish 16: $3.5 \times 10^6 cells/ml$

Fish 17: $1.2 \times 10^6 cells/ml$

Fish 18: $0.8 \times 10^6 cells/ml$

Fish 19: $2.0 \times 10^6 cells/ml$

Case Summary:

Most fish in this submission had vibrio isolated from haemolymph suggesting a possible stress with secondary bacterial infection. Dilation of hepato-pancreatic tubules and low reserve cell deposits throughout the accession indicate a poor nutritional plane and long term energy utilisation. The presence of bio-refringent inclusions in the antennal gland indicate possible uric acidosis in one animal and are consistent with debility.

Accession Number: 18-0429

Origin: Tasmanian facility

Species: Southern rock lobster (*Jasus edwardsii*)

Culture Type: Wild caught and tank held for processing and shipment.

Time and date of death: 7/3/2018

Time and date of post mortem examination: 7/3/2018

Carcass condition & preservation: 3x Alive. Animals in esky with ice bricks.

Screening of lobsters. Manager has had no mortality problems in the last 3 years.

Gross pathology summary:

Fish 1:

Body weight: 0.78kg

Carapace length: 11cm and total tail/telson length 30.1cm. Alive.

Fish is in good condition and has hard shell

Fractured right antenna above segmented region with melanisation present at site.

Enlarged brown reproductive tract.

Hepatopancreas has a bright yellow colouring associated.

There is dark brown discolouring present on both left and right cranial gill margins.

Very mild erosions on caudal edge of telson and uropods.

Haematology:

Haemolymph: Orange and cloudy

0.5ml placed with cacodylate buffer

0.5ml collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 4.02mins

Fish 2:

Body weight: 0.99kg

Carapace length: 12.4cm and total tail/telson length 32.3. Alive.

Good body condition score and tail flap response. Hard shelled animal.

Right antenna appears broken above the segmented region with melanisation present at site.

There are mild erosions on the caudal edges of the telson and uropods

Foul pungent smell released as animal was pithed. Brown material present within mouth parts.

The hepatopancreas is bright yellow in colour.

There is a small translucent reproductive tract visible.

Right gill margins are discoloured red. Similar discolouration is seen on the left margin however the red discolouration is associated cranially and a brown discolouration is noticed on the caudal aspect.

Dark brown material present in cranial and caudal GIT.

Haematology:

Haemolymph: Grey clear

0.5mls placed with cacodylate buffer

0.5mls collected as fresh sample - *(sample frozen then clotted unable to obtain biochemistry)*

Clotting time: >5min no clot

Fish 3:

Body weight: 0.69kg

Carapace length: 10.5cm and total tail/telson length 28.3cm. Alive.

Good body condition with strong tail flap response. Hard shelled animal.

Very mild erosions on caudal edge of telson and uropods.

Right gill margins are dark red to brown in colour cranially while the caudal aspect is pale brown.

Left gill margins appear to have widespread dark brown discolouration.

The hepatopancreas has brown to yellow colouring associated.

There was no reproductive tract identifiable.

Cranial muscles appear pale and translucent.

Haematology:

Haemolymph: Cloudy grey

0.5mls placed with cacodylate buffer

0.5mls collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 37 seconds.

Morphological diagnosis:

Carapace: mild erosions

Histological Summary:

Histology Summary of Accession 18-0429 (3 Fish)	
Gills	All fish show moderate amounts of reserve tissue. F1 was observed to have multifocal areas of reactive haemocyte aggregations around the central sinus. F1 and F2 appeared to have multiple cross sections of amplipods and metazoans with no biofouling present. Gill rakers with focal areas of cuticular erosion was noted in F2 while there was zonal areas of coagulative gill necrosis in F3.
Hepatopancreas	Mild to moderate amounts of reserve tissue noted in all fish. No significant findings were observed apart from peculiar colour staining of tissue in F3.

Nerves	Tissue of F1 appeared surrounded by degenerative reserve tissue and no significant findings were observed in other samples.
Antennal Gland	No inclusions bodies were noted in F1 compared to F2 and F3 where eosinophilic inclusions were present. There appeared to be one section of interstitium with haemocyte aggregations in F1 and mild amounts of depleted glandular cells that appeared degenerative in F2.
Reserve Cell	No significant findings.

Microbiological summary:

No growth on all plates after 48hours of incubation.

Haemocyte counts:

Fish 1: $8.0 \times 10^6 cells/ml$

Fish 2: $14.36 \times 10^6 cells/ml$

Fish 3: $17.9 \times 10^6 cells/ml$

Case Summary:

A mild degree of gill biofouling is present and other pathologies are minor in magnitude . Noting cytoplasmic inclusions in the antennal gland.

Accession number: 18-0568

Origin: Melbourne facility

Species: Southern rock lobster (*Jasus edwardsii*)

Culture Type: Wild caught and tank held for processing and shipment.

Time and date of death: 22/3/2018

Time and date of post mortem examination: 22/3/2018

Carcass condition & preservation: 6x Alive. Animals in esky with ice bricks.

Screening of Southern Rock Lobster from distribution depots.

6 fish in total – 3 from each depot. All fish held in common Melbourne depot prior.

Gross pathology summary:

Fish 1A:

Body weight: 0.49

Carapace length: 10cm and total tail/telson length 25.9cm. Alive.

Loss of left antenna at the first segmented junction with melanisation at site.

Mild to no erosions on the caudal edges of the telson and uropods.

Good condition with good flap response and hard shell.

Hepatopancreas has a bright yellow colouration.

Left and right gill margins appear to show widespread brown discolouration.

Material present within the caudal GIT.

Ample reserves found cranially and ventrally.

Haematology:

Haemolymph: Dark grey and cloudy

0.5mls placed with cacodylate buffer

0.5mls collected as fresh sample - *(biochemistry obtained)*

Clotting time: 3.02 minutes

Fish 1B:

Body weight: 1.26kg

Carapace length: 13.2cm and total tail/telson length 33.9. Alive.

Broken right antenna above segmented region

Very mild erosions on uropods and telson

Good condition with good flap response and hard shell.

Loss of 4th right walking leg with melanisation present at site.

Hepatopancreas has a dull yellow to brown discolouration associated and tissue is fragile upon collection.

The reproductive tract is small and translucent in appearance.

Ample reserves stores cranially and along mid ventral carapace.

Haematology:

Haemolymph: Grey and cloudy

0.5mls placed with cacodylate buffer

0.5mls collected as fresh sample - *(biochemistry obtained)*

Clotting time: 1.04 minutes

Fish 1C:

Body weight: 1.26kg

Carapace length:13.3cm and total tail/telson length 34.8cm. Alive.

Animal appears to be in good condition with a hard shell and active tail flap response.

Both left and right antenna appear fractured above segmented region with melanisation present at sites.

There are erosions present on caudal edges of telson and the lateral aspect of the left most uropod.

The heart appears small in size.

The hepatopancreas has a dull yellow to brown patchy discolouration associated.

Mouth parts are full of brown content which is seen in cranial and caudal GIT sections

The reproductive tract is small and translucent.

Left and right gill margins appear to show grey to brown widespread discolouration.

Haematology:

Haemolymph: Light grey

0.5mls placed with cacodylate buffer

0.5mls collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 34 seconds

Fish 2A:

Body weight: 0.55kg

Carapace length: 10.3cm and total tail/telson length 27.2cm. Alive.

Broken left antenna above the segmented region with melanisation present at site.

Very mild erosive lesions present on caudal edges of telson and uropods.

Tail flap response is poor in this fish and its shell is soft – suggesting poor condition

The hepatopancreas appears to show red purple to brown blotchy discolouration.

The reproductive tract was not identified.

There are poor reserve cells found in all stored areas.

Caudal and cranial GIT appear full of content

Haematology:

Haemolymph: Clear with a slight grey tinge associated.

0.5mls placed with cacodylate buffer

0.5mls collected as fresh sample - *(biochemistry obtained)*

Clotting time: >5mins – no clot

Fish 2B:

Body weight: 2.97kg

Carapace length: 17.7cm and total tail/telson length 46.1cm. Alive.

Note: Grey sandy turbid liquid was expressed when animal was pithed during euthanasia. Grey material was foul smelling and texturally appeared course and sand like

Widespread grey discolouration to gill margins on left and right sides.

Reproductive tract appeared translucent and white

Haematology:

Haemolymph: Dark grey tinged and clear

0.5mls placed with cacodylate buffer

0.5mls collected as fresh sample - *(biochemistry obtained)*

Clotting time: 3.52 minutes

Fish 2C:

Body weight: 2.53kg

Carapace length: 17.4cm and total tail/telson length 43.9cm. Alive.

Left antenna broken above segmented region with melanisation present at site.

Right rostral tip of antenna appears broken with melanisation present at site.

Loss of left 3rd walking left with melanisation at site.

Mild erosive lesions present on caudal edges of telson and uropods.

There is an erosive ulcerated lesion present on the inter segmented junction on the ventrum of this fish.

Hepatopancreas appeared pale brown and discoloured.

There are white plaque like deposits present on the mid-ventral carapace reserve cell tissue.

Reproductive tract appears translucent and white.

Haematology:

Haemolymph: Light grey and clear

0.5mls placed with cacodylate buffer

0.5mls collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 4.32 minutes

Morphological diagnosis;

Hepatopancreas: adenitis

Carapace: mild erosion of telson and uropods

Histological Summary:

Histology Summary of Accession 18-0568 (6 Fish)	
Gills	No significant findings observed in F1C and F2C. Filamentous bacteria as well as leukotrix biofouling at grade 1 were noted for F1A, F1B and F2B. A haemocytoma was observed in F1B as well. Metazoans with no biofouling were seen in F2A.
Hepatopancreas	Marked dilation of tubules with varying degree of vacuolisation and reserve tissue was observed in all fish.
Nerves	No significant findings were observed in the F1 cohort. Reduction of blue matrix of the nerve and tissue appears degenerative / autolytic was noted in F2A. Vacuolisation of ganglia was noted in F2B.
Antennule Gland	Dilated appearance associated with the gland as well as marked eosinophilic inclusions with vacuoles throughout the gland. These signs were observed in F1B, F1C, F2B and F2C. No inclusions were observe din F1B and 2A however dilation was still noted.
Reserve Cell	No significant findings were noted for F1A. Degenerative tissue was observed for F1C and F2C. Depleted reserves were recorded for F2A and F2B.

Microbiological summary:

Fish 1A: *No growth after 48 hours*

Fish 1B: *No growth after 48 hours*

Fish 1C: *No growth after 48 hours*

Fish 2A: *No growth after 48 hours*

Fish 2B: *No growth after 48 hours*

Fish 2C: *Vibrio species.*

Haemocyte counts:

Fish 1A: $11.0 \times 10^6 cells/ml$

Fish 1B: $16.7 \times 10^6 cells/ml$

Fish 1C: $10.6 \times 10^6 cells/ml$

Fish 2A: $2.5 \times 10^6 cells/ml$

Fish 2B: $8.2 \times 10^6 cells/ml$

Fish 2C: $5.1 \times 10^6 cells/ml$

Case Summary:

Indications of gill biofouling possibly due to holding environment and other pathologies not significant.

Accession Number: 18-0582

Origin: Melbourne facility

Species: Southern rock lobster (*Jasus edwardsii*)

Culture Type: Wild caught and tank held for processing and shipment.

Time and date of death: 23/3/2018

Time and date of post mortem examination: 23/3/2018

Carcass condition & preservation: 3x Alive. Animals in esky with ice bricks.

Screening of Southern Rock Lobster from previously un sampled depots.

Gross pathology summary:

Fish 1:

Body weight: 0.56kg

Carapace length: 10.4cm and total tail/telson length 27.9cm. Alive.

Soft shelled animal.

Mild erosions on caudal edges of telson and uropods.

Right antenna is fractured above segmented region with melanisation present at site.

Loss of 2nd right walking leg with melanisation at site.

Hepatopancreas has a pale brown colour associated.

The reproductive tract is small and yellow in appearance.

Poor reserve cell tissue.

Cranial and caudal GIT filled with brown content

There is a widespread grey discolouration associated to right and left gill margins.

Haematology:

Haemolymph: Light grey and turbid

0.5mls placed with cacodylate buffer

0.5mls collected as fresh sample - *(biochemistry obtained)*

Clotting time: 15.3 seconds

Fish 2: TAG 690000

Body weight: 0.57kg

Carapace length: 10.6cm and total tail/telson length 27.9cm. Alive.

Mild erosions on the caudal edges of telson and uropods.

Broken left antenna at the segmented junction with no melanisation at site.

Mouth full of content – similar content found in cranial and caudal GIT.

There is a dull brown discolouration associated to the hepatopancreas

The reproductive tract is small and translucent

Moderate reserves stored.

Haematology:

Haemolymph: White and turbid

0.5mls placed with cacodylate buffer

0.5mls collected as fresh sample - *(biochemistry obtained)*

Clotting time: 34.18 seconds

Fish 3:

Body weight: 0.68kg

Carapace length: 10.7cm and total tail/telson length 30cm. Alive.

Moderate to mild erosions on the telson and uropods

Broken left and right antenna above the segmented region with melanisation present at site.

The hepatopancreas is dull brown and mottled in appearance

The reproductive tract has a dull yellow colour associated.

Haematology:

Haemolymph: Yellow to white and cloudy

0.5 mls placed with cacodylate buffer

0.5 mls collected as fresh sample - *(sample clotted unable to obtain biochemistry)*

Clotting time: 2.45 minutes.

Morphological diagnosis:

Carapace: mild erosions and blisters

Histological summary:

Histology Summary of Accession 18-0582 (3 Fish)	
Gills	Reduced reserve cell tissue that is vacuolated with no gill biofouling and oedema present around the central support vessels was observed in all fish.
Hepatopancreas	Generalised dilation of tubules with moderate amounts of absorptive cells in the mucosa noted in F1 and F2 with no significant findings observed in F3.
Nerves	Mild degeneration noted in tissue sample of F2. No significant findings in F1 and F3.

Antennal Gland	Degree of autolysis with apoptotic cells and karyolysis noted in F1 with no significant findings observed in other fish.
Reserve Cell	Depleted in all fish with degeneration observed in F2.

Microbiological summary:

Fish 1: *No growth after 48 hours*

Fish 2: *Vibrio species*

Fish 3: *No growth after 48 hours*

Haemocyte counts:

Fish 1: $11.4 \times 10^6 cells/ml$

Fish 2: $16.8 \times 10^6 cells/ml$

Fish 3: $21.1 \times 10^6 cells/ml$

Case Summary:

No significant findings.

Accession Number: 18-0732

Origin: Tasmanian facility

Species: Southern rock lobster (*Jasus edwardsii*)

Culture Type: Wild caught and tank held for processing and shipment.

Time and date of death: 23/3/2018

Time and date of post mortem examination: 23/3/2018

Carcass condition & preservation: 2x Alive. Animals in esky with ice bricks.

2 Fish that have been held in tanks for 1 month. Sent in for screening. This is part of a surveillance of Tasmanian fish over a period of time where 2 to 3 fish are sent in for screening monthly to monitor presence of Vibrio *tapetis* growth.

Gross pathology summary:

Fish 1:

Body weight: 0.74kg

Carapace length: 11.3cm and total tail/telson length 39.3cm. Alive.

Animal is in moderate body condition with good tail flap response.

There is no leg or antenna loss.

The hepatopancreas has a dull yellow colour associated and the reproductive tract is small and translucent.

Cranial GIT appears to be full of brown content

There is widespread grey discolouration to the right gill margins while the left margin appears to have dark brown discolouration cranially and a lighter brown caudally.

There is ample reserve tissue

Mild erosions noted on the caudal aspects of the uropods and telsons.

The ventral tail muscle appears pink tinged and discoloured compared to the dorsal tail muscle.

Haematology:

Haemolymph: Dark grey and turbid

0.5mls placed with cacodylate buffer

1mls collected as fresh sample - *(biochemistry obtained)*

Clotting time: 1.35 minutes.

Fish 2:

Body weight: 0.72kg

Carapace length: 11cm and total tail/telson length 29.5cm. Alive.

Loss of left antenna at the segmented junction with no melanisation observed at site. There is also a missing 6th walking leg on the right side of the animal with melanisation present.

This is a soft shelled animal that is in a moderate body condition.

The left Antennule gland is enlarged with a translucent appearance associated with the dorsal half compared to the ventral part.

The mouth part of this animal is full of content which is also noted within cranial and caudal GIT.

There is a dull yellow discolouration associated with the hepatopancreas and a white translucent appearance noted of the reproductive tract.

The gills are bilaterally similar in appearance; dark brown cranially with a lighter brown on caudal margins.

The ventral tail muscle of this fish if pink tinged in comparison to the dorsal tail muscle.

There is adequate reserve cell tissue in this animal.

Haematology:

Haemolymph: Dark grey with a yellow tinge and cloudy

0.7mls placed with cacodylate buffer

1.5mls collected as fresh sample - *(biochemistry obtained)*

Clotting time: 4.37 minutes

Morphological Diagnosis.

Antennal gland: adenopathy and adenitis

Histological summary:

Histology Summary of Accession 18-0732 (2 Fish)	
Gills	Copious amounts of reserve tissue present in both fish. Cross section of possible isopod associated with the gill raker was noted in F1. No other significant findings observed.
Hepatopancreas	Dilated tubules. Minimal absorptive vacuoles with copious reserve cells seen in both fish.
Nerves	No significant findings.
Antennule Gland	Copious generalised eosinophilic inclusions with multifocal areas of haemocyte aggregations and inflammation in the interstitial tissue was noted in F1 and only mild presence of eosinophilic inclusions with no other findings observed in F2.
Reserve Cell	Vacuolisation observed in F2 with no other significant findings.

Microbiology:

No growth on all plates after 48 hours of incubation.

Haemocyte counts:

Fish 1: $14.1 \times 10^6 cells/ml$

Fish 2: $5.3 \times 10^6 cells/ml$

Case summary:

No significant findings.

Accession number: 18-0793

Origin: Melbourne facility

Species: Southern rock lobster (*Jasus edwardsii*)

Culture Type: Wild caught and tank held for processing and shipment.

Time and date of death: 18/4/2018

Time and date of post mortem examination: 18/4/2018

Carcass condition & preservation: 6x Alive. Animals in esky with ice bricks.

Screening of Southern Rock Lobster from distribution depots.

6 fish is total – 3 from each depot. No mixing of fish from other depots,

Gross pathology summary:

Fish 1A: Depot 1

Body weight: 0.69kg

Carapace length: 11cm and total tail/telson length 29.3cm. Alive.

Animal has a good tail flap response and is in moderate condition. Soft shelled.

The right antenna is broken with melanisation present at site.

There are moderate erosions to the caudal edge of the telson and uropods with a large blistering lesion on the lateral edge of the left uropod/

The reproductive tract is large and brown in colour while the hepatopancreas is bright yellow in appearance.

Heart appears small.

Pink material noted within the cranial GIT.

Left and right gill margins show widespread dark brown to grey discolouration that appears worse on the left side.

Ample reserve cells cranially and along mid ventrum of carapace.

Haematology:

Haemolymph: Brown to yellow – dark and turbid

0.5mls placed with cacodylate buffer

1.5mls collected as fresh sample - *(biochemistry obtained)*

Clotting time: 40 seconds

Fish 1B: Depot 1

Body weight: 0.84kg

Carapace length: 11.7cm and total tail/telson length 32.4cm. Alive.

Animal has good tail flap response.

The left antenna is broken above the segmented region with no melanisation at site.

Here is dark grey discoloured tinge associated to ventral inter segmented regions with swelling present in those areas.

Mild erosions noted on the caudal edge of the telson and uropods.

The reproductive organ is large with an orange to brown colouration while the hepatopancreas is bright yellow in appearance.

There is widespread dark brown discolouration associated along left and right gill margins.

Ample reserve stores noted.

There is yellow translucent discolouration associated to the tail muscle (ventral and dorsal)

Haematology:

Haemolymph: Grey and cloudy

0.6mls placed with cacodylate buffer

1.5mls collected as fresh sample - *(biochemistry obtained)*

Clotting time: >5 mins no clot

Fish 1C: Depot 1

Body weight: 0.77kg

Carapace length: 11.4cm and total tail/telson length 30.5cm. Alive.

The left antenna is broken at the segmented junction with melanisation at site.

There are mild erosions present on the caudal edge of the telson and uropods.

Animal appears to the in good condition with a vigorous tail flap response.
The reproductive organ is large with a brown colouring associated while the hepatopancreas is a dull yellow.

The right gill margin shows dark brown discolouration cranially with a lighter brown associated to caudal margins while the cranial left gill margin shows a dark brown to black discolouration.

Haematology:

Haemolymph: yellow and cloudy

0.6mls placed with cacodylate buffer

1.5mls collected as fresh sample - *(no biochemistry due to haemolymph clot)*

Clotting time: 20 seconds

Fish 2A: Depot 2 Tag: 000062

Body weight: 0.80kg

Carapace length: 11.3cm and total tail/telson length 30.1cm. Alive.

Animal is in good condition with a vigorous tail flap response associated.

The left antenna is broken with no melanisation at site.

There are mild erosions on the caudal edge of the telson and uropods.

Animal has lost walking legs 2 and 3 on the left side that appear to show the mild melanisation at sites.

The hepatopancreas is a pale yellow and the reproductive tract is translucent clear and small.

Brown content was noted throughout the cranial GIT while there was sporadic zones of content in the caudal GIT.

The caudal aspect of the right gill margin appeared to show linear brown focal spots from top to bottom – this was not seen on the left gill margin.

Reserve cells appear milky white in appearance and animal has poor reserve stores.

Haematology:

Haemolymph: Turbid and white with a slight yellow tinge

0.6mls placed with cacodylate buffer

1.5mls collected as fresh sample - *(no biochemistry due to haemolymph clot)*

Clotting time: 33 seconds

Fish 2B: Depot 2

Body weight: 0.91

Carapace length: 11.9cm and total tail/telson length 31.9cm. Alive.

The left antenna is broken with melanisation present at site.

There are very mild erosive lesions associated with the caudal edges of the telson and uropods

Animal has a good tail flap response and is in moderate body condition with a soft shell.

There are abnormal sporadic red focal spots visible on the surface of the left Antennule gland. Right Antennule gland appears normal.

The hepatopancreas is dark brown and mottled in appearance while the reproductive tract is translucent and small.

Right and left gill margins have dark brown discolouration associated to caudal margins

Moderate to poor reserve stores noted.

Haematology:

Haemolymph: Grey and turbid

0.7mls placed with cacodylate buffer

1.5mls collected as fresh sample - *(no biochemistry due to haemolymph clot)*

Clotting time: 2.58 minutes

Fish 2C: Depot 2 – Tag: 000061

Body weight: 0.81

Carapace length: 11.2cm and total tail/telson length 30.3cm. Alive.

Vigorous tail flap response with hard shell – animal in good condition.

Very mild erosive lesions associated to caudal edge of telson and uropods.

The reproductive organ is large and brown in appearance while the hepatopancreas appears discoloured with a pale yellow colouring and tissue is very friable on collection.

Heart does appear mildly small in size.

Brown gut content present in cranial and caudal GIT- note in caudal areas content has a pink tinge to it.

There are ample reserve tissue stores.

The tail muscle (dorsal and ventral) has a translucent yellow discolouration associated.

Haematology:

Haemolymph: Dark yellow and turbid

0.6mls placed with cacodylate buffer

1.5mls collected as fresh sample - *(biochemistry obtained)*

Clotting time: >5 mins – no clot

Morphologic diagnosis:

Carapace: Mild to marked erosions and blistered of telson and uropods

Histological summary:

Histology Summary of Accession 18-0793 (6 fish)	
Gills	Bacterial plaques with biofouling and a varying degree of reserve tissue was observed in cohort 2. Grade 1 biofouling and melanisation was observed consistently in cohort 1. Note melanisation was also observed in F2C.
Hepatopancreas	No significant findings were observed in F1A, 1B and 2C while there is mild degeneration, dilation and vacuolisation observed in F1C, 2A, and 2B.
Nerves	No significant findings were observed in F1A, F1B, F2B and F2C. Vacuolated tissue was present in F1C and F2A.
Antennule Gland	No significant findings throughout the accession apart from F1A with multifocal areas of crystalline to globoid eosinophilic inclusions in the cytoplasm's and also intraluminally. These lesions were distributed randomly throughout the gland. Within the interstitium focally extensive aggregations of approximately 1 micron in diameter dense blue structures were noted. (remnant nuclei?) (atrophic to degenerate interstitial cells)
Reserve Cell	No significant findings observed in cohort 1. Depleted reserves noted in F2A and 2B and vacuolated reserve seen in F2C.

Microbiology summary:

Fish 1A: *No growth after 48 hours*

Fish 1B: *No growth after 48 hours*

Fish 1C: *Vibrio species.*

Fish 2A: *No growth after 48 hours*

Fish 2B: *No growth after 48 hours*

Fish 2C: *No growth after 48 hours*

Haemocyte counts:

Fish 1A: $11.0 \times 10^6 cells/ml$

Fish 1B: $16.7 \times 10^6 cells/ml$

Fish 1C: $10.6 \times 10^6 cells/ml$

Fish 2A: $2.5 \times 10^6 cells/ml$

Fish 2B: $8.2 \times 10^6 cells/ml$

Fish 2C: $5.1 \times 10^6 cells/ml$

Case summary:
No significant findings

Accession number: 18-0991

Origin; Tasmanian facility

Species: Southern rock lobster (*Jasus edwardsii*)

Culture Type: Wild caught and tank held for processing and shipment.

Time and date of death: 9/5/2018

Time and date of post mortem examination: 9/5/2018

Carcass condition & preservation: 3x Alive. Animals in esky with ice bricks.

2 Fish that have been held in tanks for approximately 2 months. Sent in for screening. This is part of a surveillance of Tasmanian fish over a period of time where 2 to 3 fish are sent in for screening monthly for the presence of *Vibrio tapetis* growth.

Gross pathology summary:

Fish 1:

Body weight: 0.72kg

Carapace length: 11.1cm and total tail/telson length 29cm. Alive.

Both left and right antenna are broken over segmented regions with melanisation at sites.

Mild erosive lesions on caudal edges of telson and uropods

Heart appears enlarged compared to fish size.

There is evidence of cannibalism present with material found within mouth parts along with cranial and caudal GIT.

The hepatopancreas is generally pale yellow in colour with a pink tinge associated medially.

Reproductive organ is translucent and small.

Adequate reserve stores present with white plaque like deposits noted on reserve tissue located along mid ventrum of carapace.

Left and right gill margins are cranially darker in colour compared to caudally – appears more intense on the left side.

Haematology:

Haemolymph: Grey and turbid

0.6mls placed with cacodylate buffer

1.8mls collected as fresh sample - *(biochemistry obtained)*

Clotting time: 1.32 minutes.

Fish 2:

Body weight: 0.74kg

Carapace length: 11.4cm and total tail/telson length 29.4cm. Alive.

Both left and right antenna are broken over the segmented region with melanisation present at sites.

Heart also appears enlarged in this fish.

The hepatopancreas is bright yellow in colour while there is a moderate sized translucent reproductive organ present.

Pink tinged material observed within cranial and caudal GIT

Gill margins appear darker cranially compared to caudally on left and right sides.

There are white plaque like deposits noted on reserve cell tissue located at the mid ventrum of the carapace.

Animal has moderate reserve stores.

Haematology:

Haemolymph: Dark turbid yellow to grey

0.6mls placed with cacodylate buffer

2.1mls collected as fresh sample - *(biochemistry obtained)*

Clotting time: 42 seconds

Fish 3:

Body weight: 0.70kg

Carapace length: 10.2cm and total tail/telson length 28.4cm. Alive.

Broken left and right antenna above the segmented region with melanisation present at sites.

There is a loss of walking leg 3 on the right side with melanisation present.

Mild erosions noted on caudal edges of telson and uropods.

The hepatopancreas is bright yellow in colour and its tissue is friable in nature.

Large translucent reproductive organ present.

Adequate reserve tissue stores found with white plaque like deposits observed on the mid ventrum portion of carapace.

There is abnormal translucency associated with the tail muscle (dorsal and ventral). Material present within cranial and caudal GIT.

Left and right gill margins show darker discolouration cranially compared to caudally.

Haematology:

Haemolymph: Yellow and turbid

0.6mls placed with cacodylate buffer

1ml collected as fresh sample - *(biochemistry obtained)*

Clotting time: Not recorded

Morphological Diagnosis:

Carapace: Mild erosions noted on caudal edges of telson and uropods.

Histological summary:

Histology Summary of Accession 18-0991 (3 Fish)	
Gills	No significant findings in all fish apart from the presence of copepods within F2.
Hepatopancreas	No significant findings observed in F1 while dilated tubules with autolysis and generalised vacuolisation was observed in F2 and F3 respectively.
Nerves	No significant findings in F3. Degeneration of neural bodies was observed in F2 and vacuolisation of tissue observe din F2 and F1.
Antennule Gland	Mild amounts of eosinophilic inclusions observed in F2 however generalised eosinophilic inclusions with sloughing of tissue was observed in F1 and F3.

Reserve Cell	No significant findings in F1 and F3 however reserves were depleted in F2.

Microbiological summary:

No growth after 48 hours

Haemocyte counts:

Fish 1: $22 \times 10^6 cells/ml$

Fish 2: $14.3 \times 10^6 cells/ml$

Fish 3: $21.2 \times 10^6 cells/ml$

Case summary:

No significant findings

Accession number: 18-1127

Origin: Tasmanian facility

Species: Southern rock lobster (*Jasus edwardsii*)

Culture Type: Wild caught and tank held for processing and shipment.

Time and date of death: 30/5/2018

Time and date of post mortem examination: 30/5/2018

Carcass condition & preservation: 5x Alive. Animals in esky with ice bricks.

Increased mortalities were recorded three weeks prior to this submission – approximate loss of 80kg from the 1 tonne of stock. Since then mortalities have tapered down with no mortalities recorded in the last 10 days but facility is wanting fish to be assessed.

Gross pathology summary:

Fish 1: Tag-0868352

Body weight: 0.74kg

Carapace length: 11.2cm and total tail/telson length 29cm. Alive.

Animal has a good tail flap response and is hard shelled.

Left and right antenna are broken above the segmented region with melanisation at site.

There this a moderate amount of erosive lesions present on the caudal edges of the telson and uropods.

The hepatopancreas is yellow in colour while the gonads are small and translucent in nature.

The left and right gills appear to be in poor condition with brown discolouration noted throughout gill margins.

Good reserves present cranially and along the mid-ventrum however there were while plaque like deposits present on the mid ventrum reserve cell tissue.

Haematology:

Haemolymph: Yellow and turbid

0.6mls placed with cacodylate buffer

1.5mls collected as fresh sample - *(biochemistry obtained)*

Clotting time: 2.34 minutes.

Fish 2: No Tag

Body weight: 0.61kg

Carapace length: 10.4cm and total tail/telson length 27.2cm. Alive.

Animal has a good tail flap response and is hard shelled.

The left and right antenna are broken above the segmented region with melanisation at site.

There is loss of the left 3rd walking leg.

Mild erosive lesions present on the caudal edges of the uropods and telson.

The Antennule glands appear pale yellow in this fish.

Discolouration is also noted for the hepatopancreas that appears a pale yellow to cream colour.

The gonad is small and translucent in nature.

GIT content present in the cranial GIT.

Copious reserves present.

Both left and right gill margins appear discoloured with a brown appearance noted throughout

Haematology:

Haemolymph: Yellow and turbid

0.6mls placed with cacodylate buffer

1.5mls collected as fresh sample - *(biochemistry obtained)*

Clotting time: 2.45 minutes.

Fish 3: Tag - 0868349

Body weight: 0.74kg

Carapace length: 10.5cm and total tail/telson length 28.6cm. Alive.

Good tail response.

Broken left and right antenna above segmented region with melanisation at site.

Loss of left walking leg 2. There are moderate erosive lesions present on caudal edges of telson and uropods.

Antennule gland tissue is normal in appearance but friable in nature. There are good reserve cell tissue deposits found cranially and along mid-ventrum.

The hepatopancreas appears pale yellow and animal has a large translucent to white reproductive organ. There is GIT content present in the cranial GIT.

Left and right gill margins are dark brown and discoloured throughout.

The dorsal tail muscle is discoloured with a blue to grey tinge compared to the ventral tail muscle.

Haematology:

Haemolymph: Dark grey to yellow and turbid

0.6mls placed with cacodylate buffer

1.5mls collected as fresh sample - *(biochemistry obtained)*

Clotting time: 1.15 minutes

Fish 4: Tag - 0868350

Body weight: 0.68kg

Carapace length: 10.7cm and total tail/telson length 27.7cm. Alive.

Good tail flap response but carapace shell is soft

The right antenna is broken at the segmented junction while the left antenna is broken above the segmented region with melanisation present at both sites.

Loss of left walking leg 5 noted with melanisation at site.

There are mild erosive lesions present on the caudal edges of the telson and uropods.

The left and right Antennule gland appears discoloured with a pale yellow colouring associated.

Discolouration of the hepatopancreas is also noted as it is a pale cream to white colour.

The reproductive organ is small and translucent.

Brown discolouration present throughout left and right gill margins.

Haematology:

Haemolymph: Grey and turbid

0.6mls placed with cacodylate buffer

1.5mls collected as fresh sample - *(biochemistry obtained)*

Clotting time: 2.13 minutes

Fish 5: Tag - 0868348

Body weight: 0.76kg

Carapace length: 10.9cm and total tail/telson length 29.2cm. Alive.

Animal has a good tail flap response but the carapace is soft shelled.

Both right and left antenna are broken above the segmented region with melanisation present at site.

There is blistering and moderate erosive lesions on the caudal edges of the telson and uropods.

The hepatopancreas a pale yellow colour and the reproductive organ is large and translucent to white.

Both left and right gill margins are brown and discoloured throughout

GIT content present in caudal GIT

There is sporadic white discolouration to dorsal tail muscle.

Haematology:

Haemolymph: Dark grey and turbid

0.6mls placed with cacodylate buffer

1.5mls collected as fresh sample - *(biochemistry obtained)*

Clotting time: 3.56 minutes

Morphological summary:

Carapace: Mild erosions noted on caudal edges of telson and uropods.

Histological summary:

Histology Summary of Accession 18-1127 (5 Fish)	
Gills	Grade 1 fouling observed in F1 and F5, while grade 2 fouling was noted in F2 and F3. Melanisation of gill tips was observed in F4 and F5. Chitinoclastic destruction was observed in F1, F2, and F3.

Hepatopancreas	Dilation of tubules were observed in F1, F2 and F5. Necrosis as well as dilated tubules was noted in F3. Vacuolated tissue with no significant findings was observed in F4.
Nerves	No significant findings observed in all fish.
Antennule Gland	Generalised eosinophilic inclusions were observed in F1, F2 and F3 with mild to moderate inclusions noted in F4 and F5. Degeneration and mild sloughing of tissue was observed in all fish apart from F4.
Reserve Cell	No significant findings were noted in all fish apart from F4 where vacuolisation of tissue was observed.

Microbiology summary:

Fish 1: *No growth after 48 hours*

Fish 2: *No growth after 48 hours*

Fish 3: *No growth after 48 hours*

Fish 4: *No growth after 48 hours*

Fish 5: *No growth after 48 hours*

Haemocyte counts:

Fish 1: $29.5 \times 10^6 cells/ml$

Fish 2: $20.7 \times 10^6 cells/ml$

Fish 3: $22.4 \times 10^6 cells/ml$

Fish 4: $28 \times 10^6 cells/ml$

Fish 5: $28 \times 10^6 cells/ml$

Case summary:

Pathology is suggestive of debility and poor nutritional plane. The gill biofouling is indicative of poor water quality in holding facility.

Overall summary of pathologies seen during the project.

The key question at the start of this project was whether there was an underlying common cause to the poor post capture survival of SRL seen within the industry with variable occurrence. Despite a range of pathologies being observed there was no common pathology observed in SRL submitted from various states and facilities. Poor health and survivability were the only clinical presentations noted by submitters and whilst this may be common to all diagnostic cases there could be a range of causes for this presentation in the SRL. It must be remembered that the pathology of SRL is poorly documented and has only been investigated from wild caught SRL during Handlinger *et al* (2006) study.

As is the case in many aquatic species the association of pathology and cause is poorly demonstrated and so from a diagnostic sense when pathologies are seen causal assumptions cannot made. The summary tables included in this report from each accession of SRL, indicate the organs most commonly affected with pathological change. These are documented in the chapter in this report entitled 'Atlas of Selected Normal Histology & Histopathology of Southern Rock Lobster (Jasus edwardsii)'. Worthy of note were the pathologies observed in the antennal gland across all submission. The true function of this gland remains un-identified but it is generally accepted that it has a range of functions i.e. ionic regulation (osmoregulation) and excretory organ (elimination of nitrogenous end-products) (Factor, 1995; Binns and Peterson, 1969). The three pathologies observed in the organ were bio-refringent mineralised inclusions, basophilic intranuclear inclusions and eosinophilic intracytoplasmic inclusions.

Mineralised inclusions were noted histologically from antennal glands that were grossly atrophic and dark in colour (melanised). Histologically there was marked haemocyte reactions around dilated and degenerate and inflamed tubules. Under polarised light they appeared bio-refringent (Figure 1)

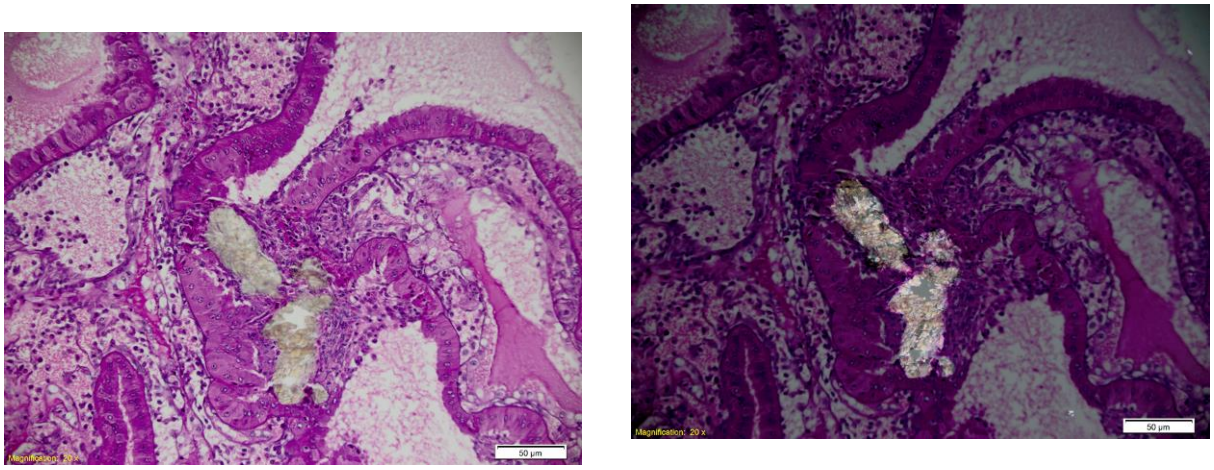

Figure 1 Gold to brown polarising birefringent crystalline deposits within the antennal gland.

These inclusions resemble uric acid crystals, amorphous urates, sodium urate and possibly melamine-cyanuric acid crystals. The latter is most unlikely as a point source in toxicity cases is usually contaminated feedstuffs and as all of these animals are wild caught and not fed during containment.

Research suggests that Lobsters like many aquatic crustaceans are ammonotelic (ammonia is the main nitrogenous waste product) with only minimal amounts of urea and uric acid produced under normal conditions they also have the ability to catabolise uric acid to ammonia. Diets of SRL contain moderate to high levels of purines which are metabolised to uric acid which are then converted to ammonia for excretion. Battison (2012) describes the pathology of subcuticular urate accumulations

in the American lobster (*Homarrus americanus*) which has similar features to the inclusion observed in this study.

The basophilic nuclear changes observed were most likely degenerative changes associated with cell death induced either by bacterial agents or autolysis but had morphological similarity to nuclear changes observed in *Homarrus americanus* experimentally infected with WSSV (Clark *et al*, 2013) and so viral rule out was undertaken due to the agent being OIE List A agent in Australia (Figure 2). Samples were submitted to CSIRO Australian Animal Health Laboratories, Geelong where they were tested for the presence of WSSV using OIE WSSV Real-time PCR diagnostic. All samples were negative for WSSV.

Eosinophilic cytoplasmic inclusions were observed in 55% of lobsters assessed during 2017 and had been previously described in Handlinger *et al* (2006) without an aetiology being identified. There was no gross or histopathology associated with the presence of these inclusions. Samples containing these inclusions were also sent for WSSV rule out and all were negative.

Samples submitted for WSSV rule out were selected on the basis of relevant pathology. (Table 1).

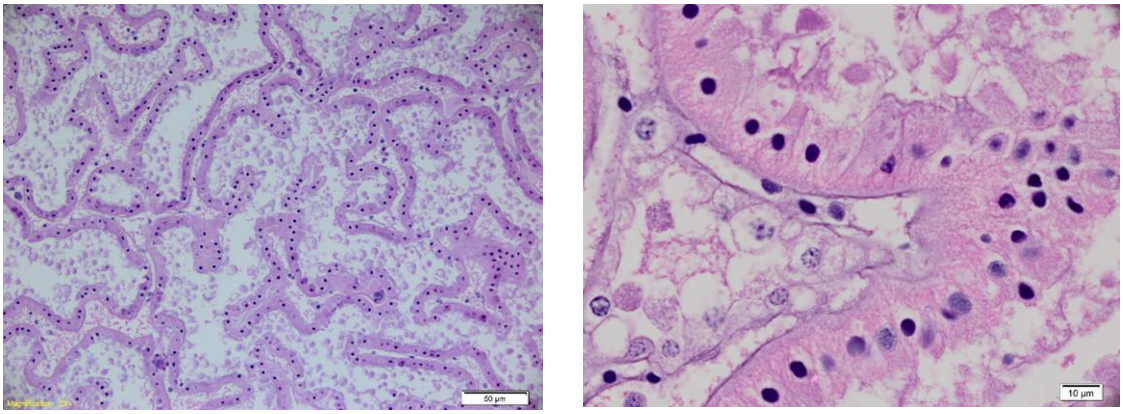

Figure 2. Basophilic nuclear changes and degeneration of antennal gland tubule lining mucosa.

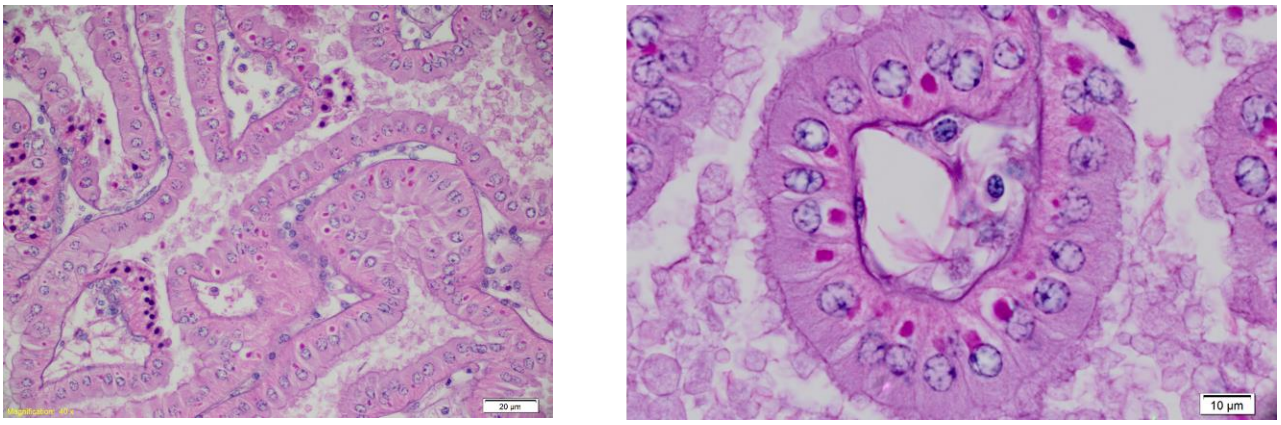

Figure 3 Eosinophilic cytoplasmic inclusions in antennal gland tubules

The presence and importance of bacterial findings re summarised in other sections of this report but apart from the presence of *Vibrio tapetis* there were no significant bacterial associations with mortalities of SRL.

Table 1 Antennal gland samples selected for WSSV rule out

Accession	**Basophilic Inclusions**	**Eosinophilic Inclusions**	**Necrosis**	**Apoptosis**
1. 16-0544 – F2	Blue inclusions in cytoplasm		Areas of coagulative necrosis	
2. 16-0544 – F3			Certain areas appear coagulative	Presence of apoptotic cells
3. 16-0544 – F4			Focal areas of necrosis	Apoptosis
4. 16-1466- F1	Basophilia of the cuboidal lining cells			

5. 16-1466 – F3				Appear mildly atrophied
6. 16-1466 – F8			Focally extensive haem infiltration & necrosis	
7. 16-1633 – F2			Areas of focal necrosis	Characterised by apoptosis
8. 17-0215 – F1		Generalised eosinophilic and cytoplasmic inclusions		Nuclear apoptosis – condensed chromatin
9. 17-0215 – F2			Marked autolysis /necrosis?	
10. 17-0215 – F3		Eosinophilia of epithelium	Focal necrosis	
11. 17-0215 – F5			Focal necrosis of epithelium	
12. 17-0340 – F1			Necrosis of ‘foamy cells’	Atrophy of “foamy cells’
13. 17-0340 – F2			Necrosis of ‘foamy cells’	Atrophy of “foamy cells’
14. 17-0340 – F3		Bight eosinophilic cytoplasmic inclusions		
15. 17-0340 – F4		Full of inclusions & eosinophilic reaction		
16. 17-1510 – F1	Multifocal basophilic intra cytoplasmic inclusions		Extensive zones of necrosis associated with bacterial rods	
17. 17-1510 – F2			Two locally extensive areas of gland necrosis and melanisation	
18. 17-1510 – F3		Lining cells with cuboidal to irregular eosinophilic cytoplasmic inclusions.		

19. 17-1510 – F4			Focally extensive areas of antennal gland necrosis (bacterial plaque associated)	Lining cells appear apoptotic
20. 17-15-10 – F5			Focal areas of necrosis of lining epithelium – minor melanisation	
21. 17-1510 – F6			One section with epithelial necrosis	
22. 17-1510 – F8		Crystalline eosinophilic cytoplasmic inclusions	Generalised necrosis / autolysis?	
23. 17-1510 – F9	Intra-nuclear inclusions (mitotic indices?)	Scant cytoplasmic inclusions	Multifocal areas of necrosis	
24. 17-0639 – F1A			Suspicious areas of necrosis	
25. 17-0639 – F1B				Focal apoptosis / general atrophy
26. 17-0639 – F2C			Coagulative necrosis in large regions	
27. 17-0639 – F3A		Pink inclusions within cytoplasm that appear crystalline		Areas of apoptosis
28. 17-0693 – F1A		Eosinophilic cytoplasmic inclusions		
29. 17-0693 – F2A		eosinophilic cytoplasmic inclusions throughout		Focal areas of apoptosis in lining cells
30. 17-0693 – F3A		Mod – high amounts of eosinophilic inlcusions		

31. 17-0693 – F1B		Intracellular eosinophilic inclusions		
32. 17-0693 – F2B		Random areas with minor cytoplasmic inclusions		
33. 17-0702 – F1	Occasional focal basophilic inclusions	Zonal eosinophilic inclusions ranging in size	Chronic multifocal areas of necrosis with fibrosis	
34. 17-0702- F3		Normal sections have marked number of eosinophilic cytoplasmic inclusions		
35. 17-0702- F4		Moderate levels of cytoplasmic inclusions		
36. 17-1375 – F1		Extensive cytoplasmic inclusions		Lining cells appear shrunken
37. 17-1375 – F2		Generalised eosinophilic cytoplasmic inclusions		
38. 17-1375 – F3		Generalised eosinophilic cytoplasmic inclusions		
39. 17-1375 – F4		Generalised eosinophilic cytoplasmic inclusions		
40. 17-1375 – F5		Marked number of cytoplasmic inclusions		Lining cells appear shrunken
41. 17-1410 – F1		Zonal cytoplasmic inclusions		
42. 17-1410 – F2		Marked cytoplasmic inclusions		
43. 17-1410 – F3		Marked cytoplasmic inclusions		

44. 17-1410 – F4		Multifocal lining cells with cytoplasmic inclusions	Necrosis? Autolysis?	
45. 17-1410 – F5		Generalised cytoplasmic inclusions		
46. 17-1410 – F6		Generalised large intra-cytoplasmic inclusions – appears to have expanded cells itself		
47. 17-1410 – F7		Significant cytoplasmic inclusions	Generalised necrosis – autolysed?	
48. 17-1410 – F8		Mild – mod eosinophilic cytoplasmic inclusions	Focal areas of duct lining necrosis (plaques of intra-lesional rod bacteria)	
49. 17-1410 – F9		Mod cytoplasmic inclusions		
50. 17-1410 – F10		Extensive eosinophilic cytoplasmic inclusions		
51. 17-1410 – F11		High numbers of intra-cytoplasmic inclusions		
52. 17-1410 – F12		Generalised eosinophilic cytoplasmic inclusions		
53. 17-1410 – F13		Generalised eosinophilic cytoplasmic inclusions		
54. 18-0302 – F4	Mod numbers of blue ovoid basophilic inclusions in the lining cells			
55. 18-0302 – F5				Atrophy of luminal cells

56. 18-0302 – F6	Basophilic inclusions present	Scant eosinophilic inclusions		Associated with atrophic cells
57. 18-0302 – F8			Areas of necrosis surrounding glandular lining	
58. 18-0302 – F11		Pink intracytoplasmic inclusions randomly spaced (poor preservation)		
59. 18-0302 – F13				Focal areas of apoptosis within cells with dense chromatic in the nuclei
60. 18-0302 – F14			Half section with coagulative necrosis	
61. 18-0302 – F19			Focal necrosis (bacterial glanitis)	

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Results 8: Clinical Pathological Investigation

Preface

This aspect of the project was undertaken to partly address Objectives 3 of this study:

Full review of the pathology from both the Tasmanian and South Australian mortality events during the 2016 season as well as further characterisation of any significant pathologies (e.g. antennule gland changes) observed in these investigations as well as further pathological investigations for the 2017season.

Manuscript information

Clinical chemistry values and tissue enzyme activities in Southern Rock Lobster (*Jasus Edwardsii)*

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Target Journal: Disease of Aquatic Organisms

Abstract

During 2016 to 2018 a research project was undertaken with the goal of improving post-harvest survivability of Southern Rock Lobster (SRL) in a changing environment. A component of this project was refining methods of disease monitoring and surveillance of SRL. In order to improve health assessments of individual SRL a clinical biochemical reference interval for haemolymph was developed using 34 healthy SRL. To compliment this 32 chemical parameters in 8 specific tissues of 23 SRL were measured as part of this study to enable appropriate interpretation of the various parameters. Additionally a multi-laboratory validation was undertaken to strengthen the accuracy of results and the species specific reference intervals produced. Results showed that tissue enzyme activity of SRL did resemble that of terrestrial vertebrates and other crustaceans however significant differences were also observed. Aspartate Aminotransferase (AST) and Alanine Aminotransferase are examples of analytes that may be used to identify smooth muscle damage in SRL however it is clear that both these measures are not reliable indicators of hepatocellular injury for this species unlike other crustaceans and vertebrates. In determining a clinical biochemical reference interval chemical changes were noted in analytes ALT, AST, magnesium and bicarbonate when haemolymph samples were frozen at -80ºC compared to fresh. Therefore separate reference intervals were created for these analytes to ensure adequate interpretation of fresh and frozen SRL haemolymph biochemistry. This is the first study to determine the clinical chemistry intervals and tissue enzyme distribution in SRL.

Introduction:

The southern rock lobster (SRL), *Jasus Edwarsdii*, is a decapod crustacean of the *Palinuridae* family only found in the waters of southern Australia and New Zealand. In Australia the SRL is the most valuable wild-caught species produced and the industry plays a significant economical role to the country as the current export value of SRL sits at a sum of $675 million for the 2017 – 18 season with a predicted increase of 4% in the coming season (Department of Agriculture and Water Resources, 2018).

All SRL in Australia are wild caught from the ocean then held in commercial facilities prior to being sold or exported. Currently there is no organisation that commercially breeds these crustaceans. In Australia these lobsters are considered to be a well-regulated and sustainable resource. The Australian government undertakes yearly SRL population surveys which are used to set a seasonal quota of catch to which fisherman and businesses must comply. This quota is administered through a system of licenses that can be purchased by commercial industries.

In 2016 a government and industry funded research project commenced with the goal of improving post-harvest survivability of SRL in a changing environment. A component of the project was refining methods of disease monitoring and surveillance of SRL. Currently SRL health is evaluated by subjective assessments of vigour, gross observations of injury and shell condition as well as on objective evaluations such haemocyte counts, clotting times and histological findings. This latter invasive method of disease investigation requires moderate numbers of lobster to be euthanised which deters commercial facilities from undertaking routine health surveillance due to the value of stock. Haemolymph assessment has also been undertaken however this also has some constraints for routine use due to the limits of validated methods and the availability of analytical laboratories who can undertake testing. In addition there are no biochemical reference intervals for SRL published. This limits the capability of interpreting haemolymph results thus impacting the ability to monitor environmental or facility dependent changes in SRL health. Clinical biochemistry measures components of body fluid and serves as an integral component of disease diagnostics in both human and mainstream veterinary medicine (Basti *et al*. 2010). Improved understanding of the biochemical composition of SRL organs and how this is reflected on the haemolymph allows for a more comprehensive and less invasive method of disease diagnosis and surveillance. This will improve methods of diagnostic investigations for SRL but may also encourage those in the industry to undertake more routine health surveillance. We recognize that in veterinary and human medicine, baseline biochemical ranges for individual species can differ based on gender, age, health status and other factors such as culture methods, increasing the complexity of establishing a biochemical reference interval values of "healthy" lobsters (Basti *et al*. 2010).

Objectives: The purpose of this study was to improve health assessments for SRL by firstly determining a clinical biochemical reference interval for SRL haemolymph. To compliment this 32 chemical parameters in 8 specific tissues of *Jasus edwardsii* were measured. This was an essential study to enable the interpretation of various biochemical parameters. SRL haemolymph after culture tends to clot immediately if not kept below 4ºC, redeeming it unsuitable for biochemical analysis. Therefore in order to establish a practical protocol for the collection, preparation and analysis of SRL haemolymph for routine health assessments, duplicate analysis of fresh and frozen haemolymph samples was undertaken for comparison of results. Additionally a multi-laboratory validation was also undertaken to strengthen the accuracy of results and the species specific reference intervals produced.

Materials and Methods:

Tissue enzyme distribution study:

Reagents required:

Phosphate buffered saline solution (PBS)

Standard calibration and analysing reagents required for the Beckman Coulter AU480 Chemistry Analyser

Southern Rock Lobsters (n=23) used for this study were submitted from four catch locations to the University of Adelaide's, Veterinary Diagnostic Laboratory (VDL) for health screening purposes. A complete assessment was undertaken on these animals which involved haemolymph collection for total haemocyte count, bacteriology and biochemistry prior to euthanasia for necropsy assessment.

Animals were deemed "healthy" and appropriate for the tissue enzyme distribution study based on the animal history and a zero bacterial culture for haemolymph. On necropsy, tissue samples (<0.5g) of reserve cell tissue, hepatopancreas, antennal gland, intestinal tract, heart, nerve and muscle were collected and individually placed in 2ml tubes containing 1ml phosphate buffered saline solution (PBS). Each of these tubes had been weighed prior tissue collection and after tissue collection.

Samples were minced within the tubes using mayo scissors then transferred to a glass piston homogenizer and homogenized for 1 minute until no large particles remained. Tissue homogenate supernatants were then produced by centrifugation (10, 000 rpm for 5 minutes) and analysed using Beckman Coulter AU480 chemistry machine for a full biochemistry panel (Table 1) at the VDL located at The University of Adelaide's veterinary campus.

The following 32 analytes were assessed for each tissue sample:

Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)
Bicarbonate (mmol/L)	Glucose (mmol/L)	Urea (mmol/L)
Createnine (umol/L)	Calcium (mmol/L)	Inorganic Phosphate (mmol/L)
Albumin (g/L)	Total Protein – TP (g/L)	Total Bilirubin (umol/L)
Alkaline Phosphatase – Al K Phos (I U/L)	Aspartate Aminotransferase – AST (I U/L)	Alanine Aminotransferase – ALT (I U/L)
Createnine Kinase (I U/L)	Cholesterol (mmol/L)	Amylase (I U/L)
Lipase (I U/L)	Total Bile Acids (umol/L)	Magnesium – Mg (mmol/L)
Gamma Glutamyltransferase – GGT (U/L)	Iron (umol/L)	Triglyceride (mmol/L)
Glutamate Dehydrogenase - GLDH (U/L)	D-3 Hydroxybutyrate (mmol/L)	Uric Acid (umol/L)
Lactate (mmol/L)	Globulin (g/L)	CA : P
Na:K	Anion Gap (mmol/L)	

Table 1: List of analytes assessed for Veterinary Diagnostic Laboratory (VDL) University of Adelaide biochemical profile.

Statistical Analysis

The Shapiro-Wilk test of normality of distribution of data and subsequently the Levene's test were used to assess homogeneity of variances. It is important to note that the Levene's test assumes data-sets are normally distributed and therefore for raw data sets which failed to show a normal distribution pattern the non-parametric Levene's test was performed instead. Using this protocol the effects of

geographic location were investigated for each analyte of each tissue type using IBM SPSS Statistics 25 for Windows.

Establishment of Reference intervals:

To undertake this study “healthy” lobsters were required (as previously described). Haemolymph was obtained from control animals (n=34) that were part of a separate project (FRDC2017-051: Extending Biotoxin Capability and research in Australia through development of an experimental biotoxin contamination facility to target industry relevant issues).

Table 2: List of analytes assessed for CrustiPath biochemical profile.

Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)
Createnine (umol/L)	Glucose (mmol/L)	Urea (mmol/L)
Albumin (g/L)	Calcium (mmol/L)	Phosphate (mmol/L)
Alkaline Phosphatase – ALP (I U/L)	Total Protein – TP (g/L)	Alanine Aminotransferase – ALT (I U/L)
Lipase (I U/L)	Aspartate Aminotransferase – AST (I U/L)	Amylase (I U/L)
Gamma Glutamyltransferase – GGT (U/L)	Cholesterol (mmol/L)	Magnesium – Mg (mmol/L)
Glutamate Dehydrogenase - GLDH (U/L)	Uric Acid (umol/L)	Triglyceride (mmol/L)
Lactate (mmol/L)	Globulin (g/L)	Na:K

These animals were obtained immediately after capture and grading by commercial fisherman, from Port Lincoln (n=18) and Kangaroo Island (n=16). Animals were individually housed (25L recirculating tanks) to reduce stress and to remove opportunities for agonistic and cannibalistic behaviour. Water quality parameters were monitored using API water test kits and Hach Spectrophotometer. The animals were maintained in seawater at recommended water quality for SRL with partial water changes performed as required. All lobsters were individually fed a maximum of two mussels daily (average weight of 1.5 – 2g per day).

Control lobsters were assigned randomly into 5 cohorts (based on time held after capture) and haemolymph samples were obtained (Cohort 1: Day 0, Cohort 2: Day 7, Cohort 3: Day 17, Cohort 4: Day 27 and Cohort 5: Day 41). Animals were anaesthetised in an ice bucket slurry prior to sample collection. Haemolymph samples were collected from the ventral aspect of the 5th walking leg joint and kept on ice to prevent clot formation. Cell free supernatants were then prepared by centrifugation (10,000g for 5 minutes). Three replicates of supernatants from each sample were collected. The first was analysed fresh for a full biochemistry panel using Beckman Coulter AU480 chemistry machine at The University of Adelaide's veterinary diagnostic laboratory while the second and third supernatant replicates were frozen and stored at -80 degrees.

The second replicate of samples were sent to CrustiPath in Prince Edward Island, Canada for biochemical analysis (Table 2) and the third replicate of samples were analysed at the University of Adelaide's veterinary diagnostic laboratory as a comparison to fresh haemolymph biochemical data.

The 32 analytes assessed in the tissue distribution study represented the full biochemistry panel used for samples analysed at The University of Adelaide (Table 1). This deferred slightly to the CrustiPath biochemistry panel of 24 analytes stated above (Table 2).

As control SRL were obtained from two geographic locations, the location affect was investigated for each analyte using the same protocol as the tissue distribution study where the Shapiro-Wild test of normality was used to check data for distribution and pending those results the Levene's test or non-parametric Levene's test was performed to assess homogeneity of variances using IBM SPSS Statistics 25 for Windows. Similarly analysis was undertaken to test variance of frozen replicates, comparison of chemistry analysers (frozen samples) and time of analysis (frozen replicates) for each analyte.

Biochemical information obtained from this study was then used to create replicate specific biochemistry reference range intervals using the Reference Value Advisor V2.1 software package on Microsoft Excel 2018.

Results:

4.2 Tissue Enzyme Distribution Study:

A total of 161 tissue samples were analysed for this study using the Beckman Coulter AU480 Chemistry Analyser. Negative analyte values were zero'ed and these values were not included in calculations for means. Data values that were a naturally occurring zero were included in mean calculations.

Results were graphed (Figure 1 – 5). As the lobsters obtained for this study were acquired from different regions, the data was statistically analysed to account for variance which revealed no significant statistic variance between geographic locations.

Figure one below demonstrates enzyme activity that was observed to be distributed in moderate to high amounts within most organ tissues analysed. Levels of analytes sodium, chloride and phosphate were observed at noticeable levels for all organs. The degree of variation in analyte levels between organs was higher for glutamate dehydrogenase (GLDH) with the lowest activity noted in the hepatopancreas (HP). Mean enzyme levels for urea and magnesium were found to be equally distributed between all organs apart from the reserve cell tissue (RCT) that showed a much lower analyte activity for both measures.

Figure two displays enzyme activity that was distributed in high amounts for two to four organ tissues assessed. Both skeletal and smooth muscle showed similarities as lactate levels were high in both the muscle and heart. AST activity appeared significantly high in the heart as well as the intestinal tract compared to other organ tissues. Mean calcium levels were equally distributed in the nerve, heart and GIT and levels for total bilirubin were consistent in GIT, AG, HP and RCT samples.

For interpreting haemolymph biochemistry results, analytes graphed in figure 1 would be of low use in isolating organ insult as these enzymes are present in significant levels in most SRL organs. Analytes graphed in figure 2 however would be of moderate use in isolating organ insult as significant

analyte levels were only present in a number of organs which would contribute in the diagnostic process.

Figure 1: Mean levels of enzyme activity present in consistent amounts for most organs of Southern Rock Lobster (*Jasus Edwardsii*) (n=23). [GIT: Gastrointestinal tract, AG: Antennal gland, HP: Hepatopancreas, RCT: Reserve cell tissue]

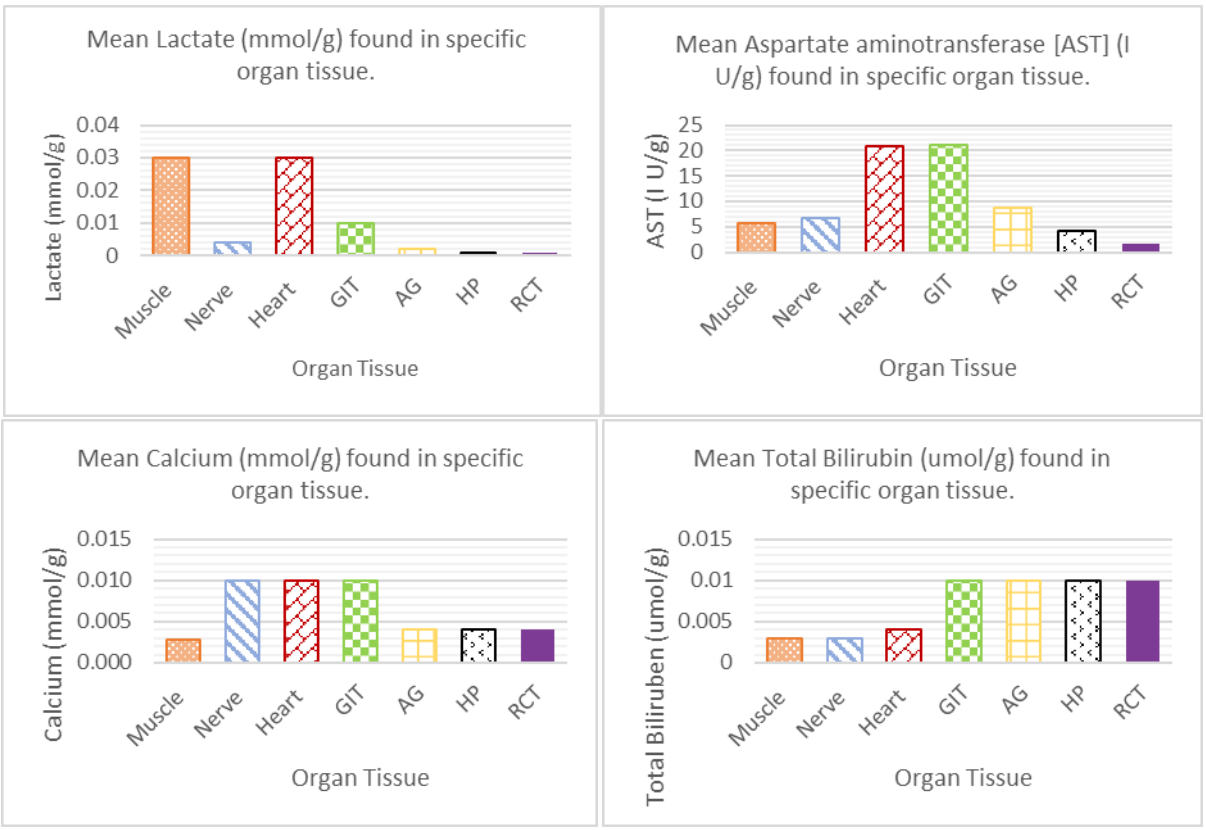

Figure 2: Mean levels of enzyme activity present in higher amounts in a number of organs of Southern Rock Lobster (*Jasus Edwardsii*) (n=23). [GIT: Gastrointestinal tract, AG: Antennal gland, HP: Hepatopancreas, RCT: Reserve cell tissue]

Figures 3 to 6 demonstrates differences in analyte activity within each organ tissue. The nerve tissue appeared to have marked levels of total bile acids and lipase compared to other organs while GGT and uric acid were found in high amounts in the antennal gland. In figure 4 the muscle shows highest levels of bicarbonate, creatinine kinase and potassium. Exceptionally high levels of ALT was observed in the heart compared to all other analysed tissue groups.

Hepatopancreas tissue data in figure 5 was found to have the highest activity for amylase, iron, ALP and creatinine. Lastly reserve cell tissue in figure 6 was the most notable organ analysed as it had marked enzyme levels for total protein, albumin, globulin, triglyceride, glucose and exclusive levels for cholesterol.

The biochemical analyte distribution in these various organ tissues graphed in figure 3 to 6 do provide insight in respective to possible tissue function and will assist in identifying organ dysfunction when interpreting haemolymph biochemistry panels.

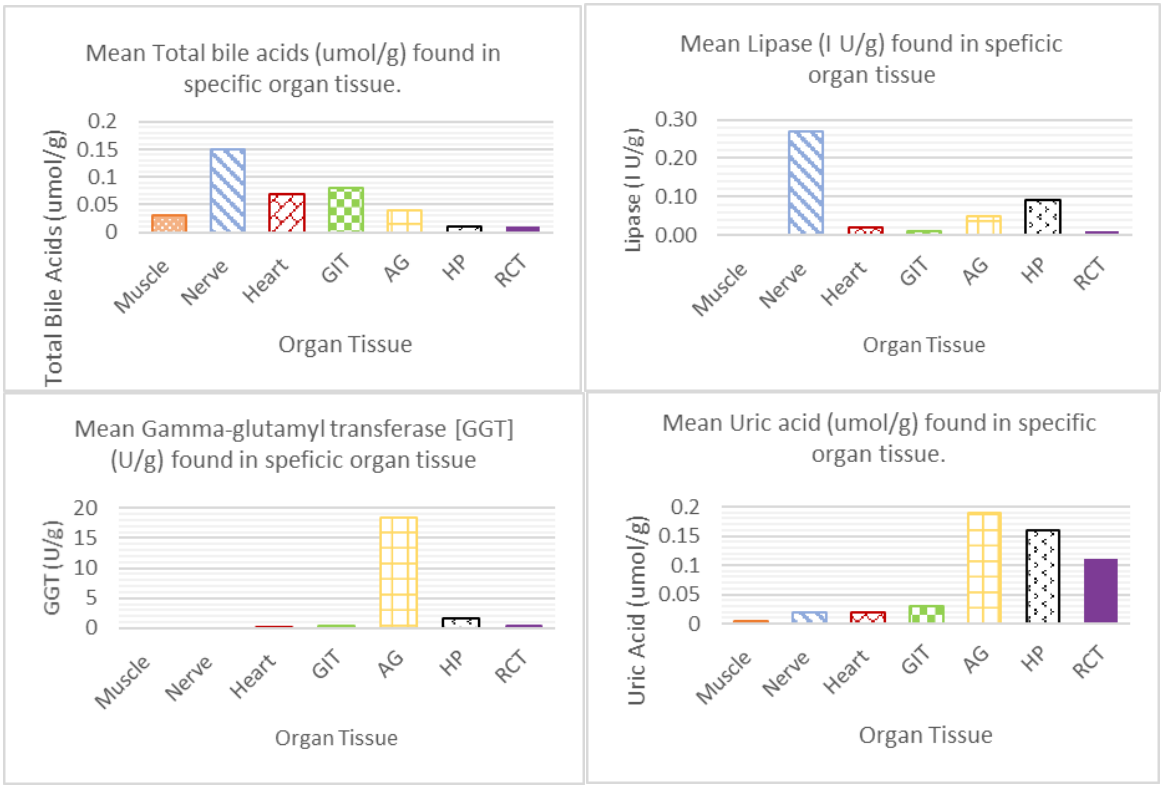

Figure 3: Mean levels of enzyme activity present in higher amounts for one organ of Southern Rock Lobster (*Jasus Edwardsii*) (n=23). [GIT: Gastrointestinal tract, AG: Antennal gland, HP: Hepatopancreas, RCT: Reserve cell tissue]

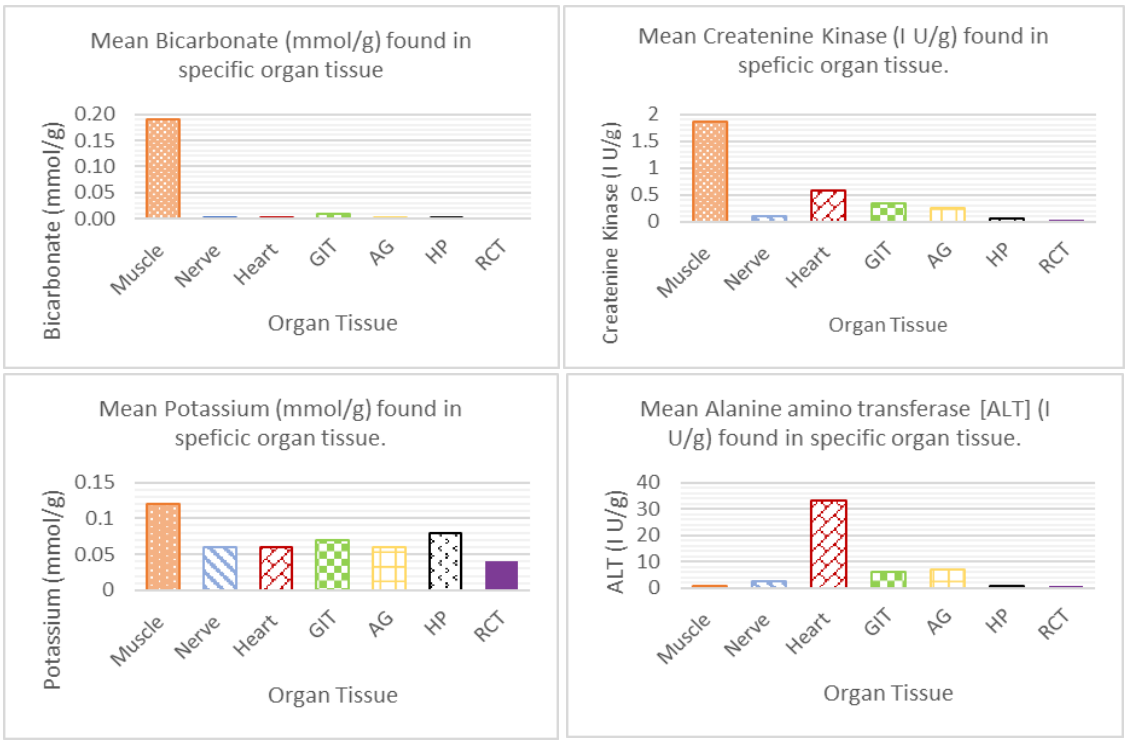

Figure 4: Mean levels of enzyme activity present in higher amounts for one organ of Southern Rock Lobster (*Jasus Edwardsii*) (n=23). [GIT: Gastrointestinal tract, AG: Antennal gland, HP: Hepatopancreas, RCT: Reserve cell tissue]

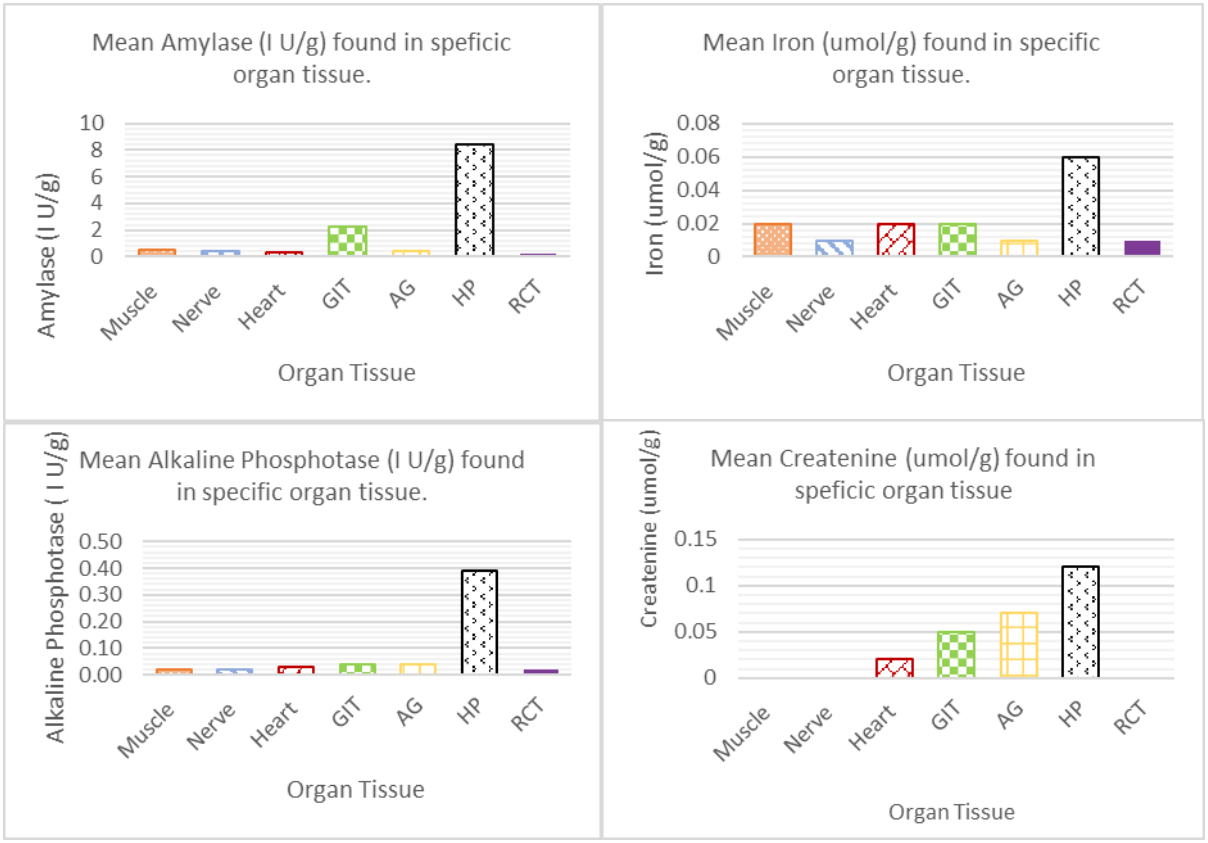

Figure 5: Mean levels of enzyme activity present in higher amounts for one organ of Southern Rock Lobster (*Jasus Edwardsii*) (n=23). [GIT: Gastrointestinal tract, AG: Antennal gland, HP: Hepatopancreas, RCT: Reserve cell tissue]

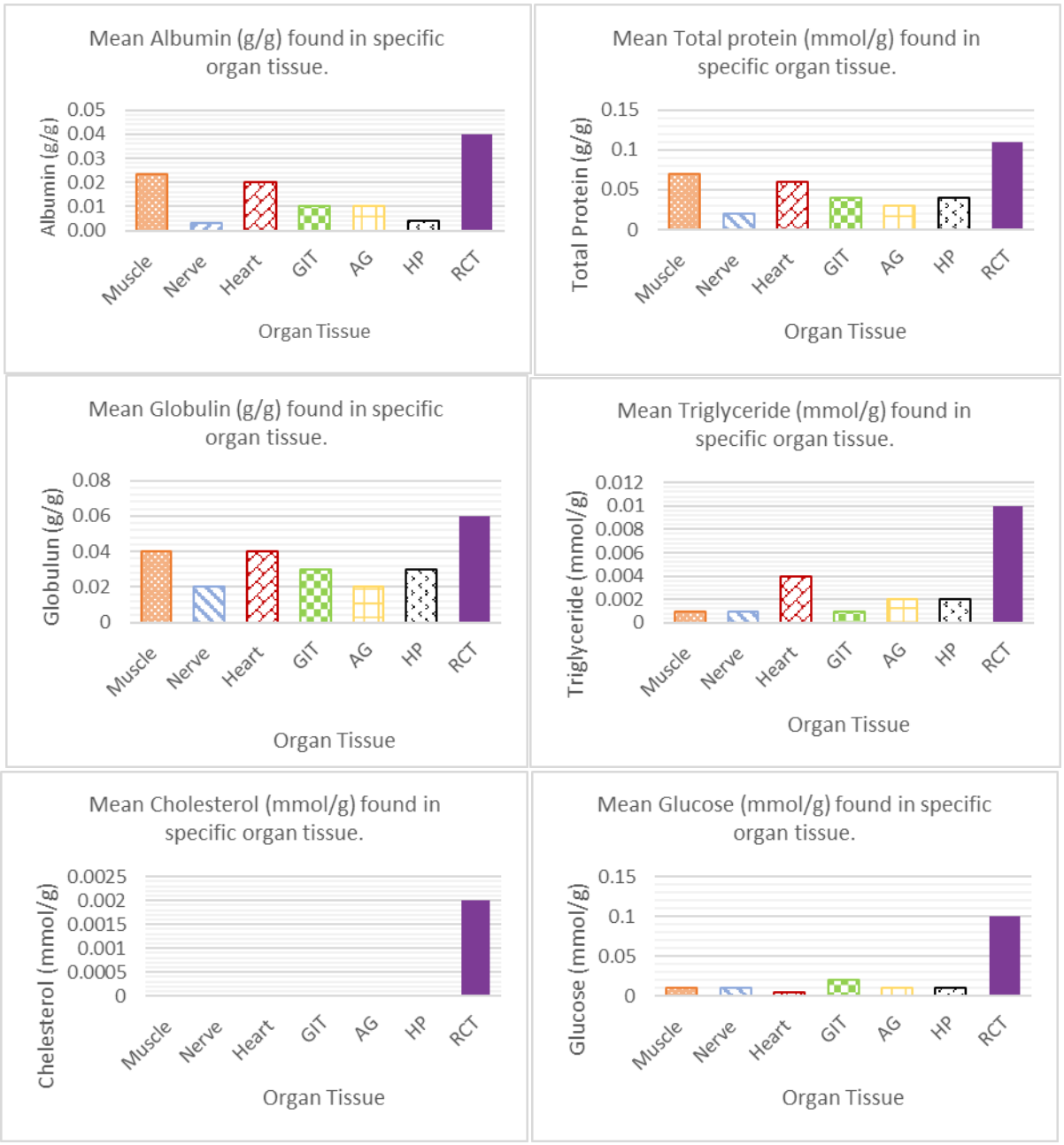

Figure 6: Mean levels of enzyme activity present in higher amounts for one organ of Southern Rock Lobster (*Jasus Edwardsii*) (n=23). [GIT: Gastrointestinal tract, AG: Antennal gland, HP: Hepatopancreas, RCT: Reserve cell tissue]

Establishment of reference range interval:

There was a total of 34 control lobsters in the study however due to the nature of crustacean haemolymph four samples clotted in fresh testing and two samples clotted in both frozen replicates. A total of 62 control haemolymph samples (30 fresh and 32 frozen) were assessed at The University of Adelaide and a total of 32 frozen control haemolymph samples were analysed at CrustiPath, Prince Edward Island.

The Reference Value Advisor software was utilised to evaluate each replicate of samples. All replicate analyte samples sizes were too small (n<40) to compute the non-parametric reference interval therefore the robust method with a Box-Cox transformation was applied to most analytes.

For fresh haemolymph analytes such as chloride and magnesium, results showed an abnormal distribution and a lack of symmetry. Reference intervals were then computed using the robust untransformed standard for chloride and the standard box-cox transformation interval was applied for magnesium Interval ranges were tabulated (Table 3).

The statistical software uses two outlier detection methods; Dixon's range statistic and Tukey's interquartile fences that detected suspected data in a number of analytes (Table 3) (Friedrichs *et al*. 2012). The International Federation of Clinical Chemistry (IFCC) and the Clinical and Laboratory Standards Institute (CLSI) recommends that unless outliers and suspect data are known to be aberrant observations, the emphasis should be on retaining rather than deleting them (Geffré *et al*. 2011). For this reason all data values were retained. Reference intervals for each analyte that had 90% confidence interval (CI) limits that exceeded the width of the interval by 20% or more were also recorded (Table 3).

Results from different samples (fresh vs frozen) showed significant differences for ALT (P=.000), AST (P=.000), ALP (P=.001), creatinine (.001), GLDH (P=.003), inorganic phosphate (P=.000) and magnesium (P=.000). It is important to note that significant values were also observed between bicarbonate (P=.000), anion gap (P=.000) and D3-hydroxybutarate (P=.002) when comparing fresh and frozen samples. These analytes were not part of the Crustipath biochemical profile therefore the difference observed is between fresh and frozen samples analysed at the VDL, Adelaide.

Table 4 below demonstrates further statistical analysis between replicates that was performed on analytes that were found to show variance in the previous table (Table 3). This table shows significant differences observed between fresh and frozen samples analysed at the University of Adelaide for ALT (P=.000) and AST (P=.002). Variance was also noted between frozen replicates analysed in Canada and Adelaide for ALP (P=.000), creatinine (P=.000) and inorganic phosphate (P=.000). Analytes GLDH and magnesium were found to show no variance between replicates when assessed separately.

Analyte	Reference Interval Fresh (n=30)	Reference Interval Frozen Crustipath (n=32) [31.7.18]	Reference Interval Frozen UofA (n=32) [10.8.2018]	P-Value ϕ
Sodium (mmol/L)	429.1 – 559.8* □	452.0 – 564.8 □	465.1 – 572.6 □	NV
Chloride (mmol/ L)	442.1 – 598.9 Ⓟ	427.9 – 566.5	467.6 – 589.2 □	NV
Bicarbonate (mmol/L)	2.6 – 4.7 □	NA	2.9 – 8.8 □	.000
Anion Gap (mmol/L)	0.0 – 155.3 Ⓟ	NA	0.0 – 60.8 □	.000
Potassium (mmol/ L)	8.0 – 14.3 □	7.4 – 13.6 □	7.8 – 14.8 Ⓟ□	NV
Creatinine Kinase (IU/L)	0.0 – 3.6 Ⓟ	NA	0 – 3.8 □	NV
Lactate (mmol/L)	0.1 – 3.0	0.0 – 2.8	0 – 2.8 □	NV
Calcium (mmol/L)	9.3 – 12. 6	13.2 – 18.3 □	11.3 – 12.8 □	NV
ALT (IU/L)	3.1 – 51.4 *□	0 – 2.1 Ⓟ	0 – 3.3 □	.000
AST (IU/L)	2.7 – 103.3 □	0	2.2 - 35.6 *□	.000
Total Bilirubin	0.9 – 4.7 *□	NA	1.0 – 4.5 Ⓟ□	NV
ALP (IU/L)	0.1 – 10.8 □	0	0.0 – 11.4 Ⓟ	.001
Amylase (IU/L)	0 Ⓟ	0 Ⓟ	0.0 – 1.5 *	NV
Creatinine (umol/L)	0.0 – 23.7 □	0 Ⓟ	0.0 – 22.0 *□	.001
Iron (umol/L)	0.1 – 37.6 Ⓟ□	NA	0.0 – 3.0 □	NV
Uric Acid (umol/L)	10.1 – 190.6 Ⓟ□	8.1 – 165.9 □	3.7 – 143.1 Ⓟ□	NV
GGT (U/L)	1.9 – 9.3 □	0.1 – 9.4*	1.5 – 9.4 □	NV
GLDH	0 – 0	3.3 – 32.8 □	1.1 – 52.5	.003
Urea (mmol/L)	0.1 -1.1	0 Ⓟ	0	NV
Inorganic Phosphate	0.0 – 0.5 Ⓟ	0 Ⓟ	0.0 – 0.4 *	.000
Magnesium (mmol/L)	4.4 – 5.5	6.7 – 12.1 *	4.5 – 5.0 □	.000
Total Bile Acids	0 – 1.7 Ⓟ	NA	0.0 – 1.6 Ⓟ	NV
Lipase (IU/L)	0.4 – 6.4	2.6 – 8.4	3.2 – 189.1*	NV
Cholesterol (mmol/L)	0.2 – 1.0 Ⓟ	0.3 – 1.0 □	0.1 – 0.9 Ⓟ	NV
Triglyceride	0.2 – 1.0*	0.3 – 1.0 □	0.2 – 0.9 □	NV

Albumin (g/L)	2.5 – 16.8 □	3.8 – 17.2 □	1.8 – 17.0 □	NV
Glucose (mmol/L)	0.5 – 5.7 □	0.7 – 5.1 □	0.5 – 5.4 □	NV
Total Protein (g/L)	32.3 – 110.0 □	32.4 – 115.9 □	31.4 – 111.5	NV
Na:K	35.6 – 60.7 □	36.6 – 67.0 □	35.3 – 67.3 □	NV
D-3 Hydroxybutyrate	0*	NA	0.0 – 2.0 □	.002
Globulin (g/L)	25.9 – 90.1 □	28.1 – 105.9	25.6 – 90.8 □	NV
Ca: P	24.0 – 166.6 Ⓟ	NA	25.24 – 191.61 Ⓟ□	NV

Table 3: Haemolymph biochemical reference intervals for fresh and frozen southern rock lobster (*Jasus edwardsii)* samples.

ϕ Transformed data with homogeneous variances (Parametric & Non-Parametric Levene's Test) for fresh and frozen samples. NV indicates statistical analysis did not have homogeneous variance (value > .005)

* Suspected defect detected

Ⓟ Possible outliers detected

□ 90% CI limit exceeds width of reference interval by >20%

Abbreviations: ALT- alanine aminotransferase, AST- aspartate aminotransferase, ALP – alkaline phosphatase, GGT- gamma glutamyl transferase, GLDH – glutamate dehydrogenase Na:K – sodium & potassium ratio, Ca:P – calcium and phosphorus ratio, NA – not alanysed. UofA – The University of Adelaide

Analyte	P-Valueϕ of replicate 1* & 3***	P-Valueϕ of replicate 2** & 3***
ALT (IU/L)	.000	NV
AST (IU/L)	.002	NV
ALP (IU/L)	NV	.000
Creatinine (umol/L)	NV	.000
GLDH	NV	NV
Inorganic Phosphate	NV	.000
Magnesium (mmol/L)	NV	NV

Table 4: Statistical analysis between replicates of selected analytes

ϕ Transformed data with homogeneous variances (Parametric & Non-Parametric Levene's Test). NV indicates statistical analysis did not have homogeneous variance (value over .005)

* Fresh haemolymph UoA replicate

** Frozen haemolymph Crustipath replicate

*** Frozen haemolymph UoA replicate

Abbreviations: ALT- alanine aminotransferase, AST- aspartate aminotransferase, ALP – alkaline phosphatase, GLDH – glutamate dehydrogenase, UofA – The University of Adelaide

Discussion:

5.1 Tissue Enzyme Distribution Study:

Clinical chemistry is generally considered as a basic and vital diagnostic tool in both veterinary and human medicine. The purpose of this study was to investigate if this tool could be applied to southern rock lobster haemolymph. In order to assess haemolymph biochemistry panels, an understanding of enzyme distribution within SRL tissue was necessary. A direct correlation is present between enzyme tissue concentration and organ mass, where organs with high concentrations of specific enzymes can be released into serum circulation when organs are damaged or diseased (Hoffman & Solter 2008). In addition to this observing tissue enzyme activity is important as enzymes that are tissue specific in one species may not be so in (Duncan *et al*. 1994b).

While tissue distribution can vary between species there are some common patterns present (Hoffmann & Solter 2008). In veterinary medicine species specific biochemistry panels are available for most domesticated terrestrial vertebrates. These panels tend to include enzymes of skeletal and heart muscle (CK, ALT, AST), liver tissue (ALT, AST, GLDH, ALP, bile acids, bilirubin, cholesterol, lactate, albumin and urea), pancreatic tissue (amylase, lipase, GGT) and kidney tissue (creatinine, and urea) as a means to identify damage or dysfunction to these organs (Battison 2006, Hoffmann and Solter 2008).

Tissue enzyme activity of SRL did resemble that of terrestrial vertebrates however significant dissimilarities were also observed. To our knowledge, only one previous report has been published investigating tissue enzyme distribution of 6 enzymes (amylase, lipase, sorbitol dehydrogenase (SDH), ALT, AST and GLDH) in the American Lobster *(Homarus americanus)* (Battison 2006). Differences of enzyme distribution was observed between these two species of crustacea.

In the American lobster, GLDH was found in highest concentrations within the heart tissue (Battison 2006). This was not the case for SRL as moderate to high concentrations of this enzyme were found in most organ tissues with higher levels observed in the muscle and nerve tissues compared to the heart. Lowest concentrations of GLDH were found in the hepatopancreas. This is a significant difference when compared to other animals where GLDH has been reported to be a sensitive indicator of hepatic injury as it is a mitochondrial enzyme with high activity in peracinar hepatocytes (Peta *et al*. 2007). Reasons for this difference are uncertain. Due to the general distribution of GLDH amongst organ tissues it is not a useful marker for isolating organ dysfunction or disease in SRL. The same conclusion can be made for sodium, chloride, inorganic phosphate, magnesium and urea that were found in moderate to high concentrations in most organ tissues assessed.

In vertebrate species AST and ALT are commonly used as indicators for hepatocellular injury. In the American lobster AST was found in highest concentrations in the heart followed by the hepatopancreas while ALT activity peaked in the heart followed by the proximal intestine (Battison 2006). Mild differences were also observed here between crustacean species as in the SRL, high AST

concentrations were isolated from the heart and GIT with low levels in the hepatopancreas while high ALT concentrations were isolated from the heart only with low levels in all other tissues. In all domestic species the activity of AST is high in the liver as well as muscle, kidney and pancreas (Hoffman & Solter 2008). ALT has been documented to have key roles in liver of domestic species as it contributes to gluconeogenesis and the formation of urea as well as a role in muscle transaminating pyruvate to form alanine (Hoffman & Solter 2008). Parallels can be drawn between domestic species and SRL for using ALT and AST as measures to isolate smooth muscle damage however it is clear that both these enzymes cannot be used as indicators of hepatocellular injury for this species.

Another surprising finding was noted for bile acid activity that appeared highest in SRL nerve tissue. In domesticated species this serves as another indicator of hepatocellular activity as synthesis of primary bile acids is the principal pathway for catabolism of cholesterol which occurs in the liver (Hoffman & Solter 2008). Reasons for this difference is unknown however research suggests that there are alternate pathways of bile acid synthesis one of which is initiated by enzyme cholesterol 24-hydroxylase (CYP46) that is present primarily in the brain (Hoffman & Solter 2008). This may account for the results obtained. The nerve tissue appeared to cultivate the highest activity of lipase as well. Again a dissimilarity is observed here between terrestrial animals and SRL where lipase serves as an enzymatic indicator of pancreatic function.

SRL do not possess the complex kidney organ seen in terrestrial vertebrates. Instead these animals have antennal glands that function in similar ways to a mammalian kidney however in a more primitive nephron form (McMahon 1995). The antennal glands compliment the gills with functions in ion and osmoregulation as well as aiding in the elimination of nitrogenous end products (McMahon 1995). SRL produce urine at a rate of 4.8% of body weight per day and urine nitrogen makes up a total of 11.6% of the total nitrogen loss of the animal (Binns & Paterson 1969). Based on this knowledge it is expected that the antennal gland will have similar enzymatic predictors as that of the kidney. Urea and creatinine were found in antennal tissue however they were also found in high levels in other tissues. GGT a well-established marker for liver and pancreatic disease was found in highest concentrations in antennal gland tissue. Uric acid was also observed in marked levels within this tissue. As crustaceans are ammonotelic, the levels of uric acid observed within the antennal gland serves to suggest that uric acid breakdown occurs in this gland as well as support its function as an excretory organ.

Reserve cell tissue (RCT) found in crustaceans appear to have highest concentrations of proteins, glucose, cholesterol and triglycerides. This suggests an association with RCT and nutritional health as these enzymes are used to assess energy reserves of crustaceans (Ciaramella *et al*. 2014).

Translating the results above into a possible interpretation of haemolymph biochemistry changes would be:

Skeletal and heart muscle analytes: Bicarbonate, CK, ALT, AST and lactate

Hepatopancreatic analytes: Amylase, iron, ALP and creatinine

Antennal gland analytes: GGT and uric acid

RCT analytes: Albumin, globulin, total protein, cholesterol, glucose and triglycerides

5.2 Establishment of reference range interval:

Noteworthy differences were observed for SRL reference intervals from fresh and frozen haemolymph samples for analytes ALT, AST, magnesium, bicarbonate which impacted the anion gap and D3-

hydroxybutyrate. The statistical significance of these results suggest that there is a change in chemical composition of certain analytes in haemolymph when frozen at -80ºC.

In addition to this, a difference can also be observed for analytes ALP, creatinine and inorganic phosphate between samples analysed at CrustiPath and replicates analysed at the VDL. The statistical significance of these analytes suggest there may be differences between chemical analysers used.

GLDH results were not obtained consistently through samples analysed at the VDL, Adelaide. Some samples required dilution as levels were too concentrated to be analysed by the Beckman Coulter AU480 chemistry machine. This proved challenging due to the rapid clotting nature of haemolymph. As a result GLDH readings were not attainable for the majority of the samples analysed. It is due to this reason the statistically significant variation observed between fresh and frozen samples for GLDH is considered inaccurate. Unusual results were also obtained for magnesium that showed a significant difference between fresh and frozen samples as a whole but no statistical significance was observed when replicated were compared to each other individually. The reasoning for this result is uncertain however it may be attributed to the weakness of the Reference Value Advisor software when dealing with small sample sizes with a non-normal distribution (Geffré *et al*. 2011).

The use of the robust method and Box-Cox transformation is currently the international recommendation for determining reference intervals [10]. Due to the small sample size ($n<40$) the reference value advisor software uses the parametric and robust methods from native Box-cox transformed values which is not the gold standard of non-parametric analysis. In addition to this it is recommended that the width of the 90% CI of reference limits should not exceed the width of the reference interval by 20% or more. Based on the intervals recommended on Table 3 there are a number of analyte references that appear to have CI limits that exceeds 20% which increases the uncertainty of results and suggests a need to collect additional reference samples (Friedrichs *et al*. 2012).

While this program automatically performs necessary computations of data sets for the purpose of formatting reference intervals it also has weaknesses (Geffré *et al*. 2011). The Gaussian distribution cannot be achieved in some cases and while other transformations may work better, they are not included in the software (Geffré *et al*. 2011). Alongside this the test for distribution symmetry as the current version of the program includes only a test for normality and it is important to consider that these weaknesses may have affected the validity of the results (Geffré *et al*. 2011). It is also important to note that computing the data is only the last step in determining reference intervals and more critical steps are the selection of reference individuals and the use of quality control analytic procedures (Geffré *et al*. 2009). While attempts were made to sample 'healthy' SRL the ability to accurately quantify the health status of this wild crustacean is exceptionally challenging. Additionally, selection criteria such as sex, molt cycle, geographical factors and time of analysis were not considered in this study.

To our knowledge however, this is the first study to determine clinical chemistry intervals and tissue enzyme distribution in SRL. It has revealed differences in fresh and frozen sample data which is a vital finding as it can impact the diagnosis of disease and population health monitoring. Therefore separate intervals of certain analytes have been created for the interpretation of frozen SRL haemolymph biochemistry (Table 5).

Analyte	Reference Interval for SRL
Sodium (mmol/L)	429 – 560
Chloride (mmol/ L)	442 – 599
Bicarbonate (mmol/L)	3 – 5
Anion Gap (mmol/L)	0 – 155
Potassium (mmol/ L)	8 – 14
Creatinine Kinase (IU/L)	0 – 4
Lactate (mmol/L)	0 – 3
Calcium (mmol/L)	9 – 13
ALT (IU/L)	3 – 51 (0 – 3)*
AST (IU/L)	3 – 103 (0 – 35)*
Total Bilirubin	1 – 5
ALP (IU/L)	0 – 11
Amylase (IU/L)	0
Creatinine (umol/L)	0 – 24
Iron (umol/L)	0 – 38
Uric Acid (umol/L)	10 – 191
GGT (U/L)	2 – 9
Urea (mmol/L)	0 -1
Inorganic Phosphate	0 – 1
Magnesium (mmol/L)	4 – 6 (5 -12)*
Total Bile Acids	0 – 2
Lipase (IU/L)	0 – 6
Cholesterol (mmol/L)	0 – 1
Triglyceride	0 – 1
Albumin (g/L)	3 – 17
Glucose (mmol/L)	1 – 6

Total Protein (g/L)	32 – 110
Na:K	36 – 61
Globulin (g/L)	26 – 90
Ca: P	24 – 167

Table 5: Biochemical clinical range for southern rock lobster (*Jasus edwardsii)*

* Numbers in brackets represent specific intervals to be considered when interpreting frozen samples.

Analyte D3-hydroxybutyrate was not found in individual organs when assessing tissue enzyme distribution and has therefore not been included in the reference interval table above. As results for GLDH were inaccurate it too has not been included in the reference table above however results from the tissue distribution study show it is an important analyte that should be included when analysing haemolymph biochemistry.

To assess the usefulness of the reference interval as a tool we used it to interpret haemolymph biochemistry panels obtained from selected SRL submitted to this FRDC project from mortality events. We found that analyte levels of crustaceans with consistent gross and histopathological lesions on selected organs were we in fact increased on the haemolymph biochemistry panel. This confirmed that this data while quite basic does still serve as a foundation reference resource for *J. edwardsii* haemolymph.

Outcomes & Further Development.

This study has established plasma reference intervals in *J. edwardsii* for a full biochemistry panel assessment from fresh and frozen sampling. As well as this, the distribution of enzymes in SRL tissue was identified allowing for appropriate interpretation of haemolymph biochemistry results. . More information on factors that may affect these enzymes such as a comparison of genders, effect of the molt cycle stages, as well as geographic location is needed. The tissue enzyme distribution study found differences in distribution compared to other terrestrial mammals as well as in other species of crustacea. This highlights the fact that these animals are unique, stressing the importance of SRL research required for a comprehensive understanding of their anatomy and physiology. A thorough investigation is needed to appropriately assess the usefulness of using haemolymph biochemistry as a diagnostic tool as well as creating an efficient applicable method in haemolymph collection. Success in this area would allow for less invasive methods of assessing general lobster health upon arrival to holding facilities which may provide produces useful information when making marketing and management decisions. It would also allow for markedly improved method for efficient surveillance and disease identification. These findings serve as an essential initial step in enabling the interpretation of biochemistry results from *Jasus Edwardsii* which in turn contribute to the clinical disease investigation process as well as to health surveillance schemes

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Ferguson) and the Fisheries Research and Development for funding the industry project. All the members and of the Australian Southern Rock Lobster Processor, Export Industry and SARDI for contacting us and supplying experimental animals/samples. The Tasmanian Rock Lobster Processors Association, The South Australian Rock Lobster Advisory Council, The Victorian Rock Lobster Association and The Australian Southern Rock Lobster Exporters Association for their support and guidance throughout the project. Primary and co-investigators of the FRDC project, Quinn Fitzgibbon, Charles Caraguel, Ryan Day and Kandarp Patel for their cooperation and time spent on the project. Alison Turnbull and her team for providing us haemolymph samples from their FRDC project (FRDC2017-051). The staff at the Veterinary Diagnostic Laboratory at the University of Adelaide for accommodating and allowing us to analyse our samples efficiently.

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Results 9: Methods for Water Quality Testing

Preface

This aspect of the project was undertaken to address variation objectives 1 of this study:

An assessment of methods for water quality testing in Southern Rock Lobster holding

Manuscript information

Evaluating methods for water quality testing in Southern Rock Lobster holding

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Target Journal: Industry report

Abstract

Monitoring water quality is an important aspect of reducing mortality in Southern Rock Lobster processing facilities. A range of methods are available, with varying levels of quality and investment required. The aim of this study was to evaluate a range of water quality testing approaches to provide the industry with information on the effectiveness, difficulty and cost of each approach to enable facilities to make evidence-based decisions on approaches toward improving their ability to measure and monitor the quality of their water. API 5 in 1 strips, API aquarium test kits, YSI Palintest photometer and Hach test photometer kit were compared. All test performed well for pH levels and ammonia, within the ranges relevant to lobster processing facilities. YSI and Hach photometer kits had the best accuracy for nitrite, whereas the two API kits were hard to interpret and overestimated levels at high concentrations. None of the kits showed an adequate sensitivity for nitrate. The Hach phosphorus photometer kit was the most accurate, followed by the YSI photometer kit. For alkalinity, the Hach and API kits were the most accurate. These results indicate that the performance of the API, YSI and Hach kits offer a level of accuracy (and often difficulty) largely corresponding to their cost. For facilities not currently monitoring their water quality, the API test kits represent an easy to use, economic and readily available option to begin a testing water quality. For facilities looking to advance their accuracy and precision, the YSI and Hach photometer kits offer high level results. Finally, recommendations on the parameters and frequency of testing are made to provide the Southern Rock Lobster industry with a framework for developing a water monitoring regime that will facilitate proactive management rather than rote or reactive approaches.

Introduction

The results of the investigation into the haemolymph biochemistry and immune function of moribund lobsters sampled at facilities experiencing mortality events indicated that a pathogen was not likely to be the causative factor. Disruptions in the electrolyte and mineral levels in moribund lobsters suggest that dehydration and water chemistry may have played a role in the mortality events by causing physiological stress. Water quality analysis conducted when these samples were collected suggested that, while nutrient levels were within acceptable ranges, the physical chemistry (e.g. pH, alkalinity) of recirculation systems within the industry are often out of recommended ranges.

Through discussions with industry members, it was apparent that there was a variable level of understanding of water quality parameters and a correspondingly variability in the approaches to water quality testing and management. The present study was undertaken to evaluate several ranges of water quality testing approaches, from economical and readily accessible test kits available at pet shops to expensive specialist equipment (spectrophotometers), with the aim of providing guidance on how processing facilities can improve their water quality monitoring methods. The study assessment included simple API 5 in 1 and API test kits and two photometers (YSI 9500 and Hach DR1900) and examined a range of key water quality parameters including pH, ammonia, nitrite, nitrate, alkalinity and phosphorus (Fig 1)

Figure 1: Test kits and photometers assessed for accuracy, ease of use and costs for water quality analysis.

Methods

pH

A series of pH standards was created using a reference pH electrode (Mettler-Toledo SevenCompact meter with Routine Pro-ISM probe) to measure the adjustment of the pH of 50 ml samples of seawater using hydrochloric acid (HCl) or sodium hydroxide (NaOH). This method was used to make the following standards: 7.00, 7.50, 7.75, 8.00, 8.25, 8.50. The standards were then tested using an API 5-in-1 test, an API high range pH kit, a YSI Phenol Red assay kit (Palintest pH Phenol Red, YAP130) and a Testo 205 handheld pH probe.

Ammonia

Ammonia standards were prepared according to US EPA Method 350.1 (O'Dell 1993a) using a stock solution of anhydrous ammonium chloride (NH_4Cl) in filtered seawater at a concentration of 1.0 mg NH_3-N ml^{-1}. This solution was then further diluted in 50 ml samples of seawater to create the following series of concentrations (mg NH_3-N L^{-1}): 0.00, 0.05, 0.10, 0.50, 1.00, 2.00, 5.00, 10.00. These standards were tested using an API Ammonia test kit, a YSI Ammonia test kit (Palintest Ammonia with conditioner for use with seawater, YAP 152) and a Hach ammonia test kit (Nitrogen-Ammonia TNT AmVer Low Range, 2604545). Ammonia analysis using the API 5-in-1 test was not

performed as this parameter is not available on the test kit. Where standard concentrations exceeded the effective range for a test, the standard was diluted using distilled water.

Nitrite

Nitrite standards were prepared according to US EPA Method 353.2 (O'Dell 1993b) using a stock solution of potassium nitrite (KNO_2) in filtered seawater at a concentration of 1.0 mg NO_2-N ml^{-1}. Stock solution was added to 50 ml filtered seawater to create the following series of concentrations (mg NO_2-N L^{-1}): 0.00, 0.05, 0.25, 0.5, 0.75, 1.50, 3.00, 5.00. These standards were testing using an API 5-in-1 test stick, an API Nitrite test kit, a YSI nitrite test kit (Palintest Nitricol, YAP109) and a Hach nitrite test (NitriVer 3 TNT Low Range, 2608345). Where standard concentrations exceeded the effective range for a test, the standard was diluted using distilled water.

Nitrate

Nitrate standards were prepared according to US EPA Method 353.2 (O'Dell 1993b) using a stock solution of potassium nitrate (KNO_3) in filtered seawater at a concentration of 1.0 mg NO_3-N ml^{-1}. Stock solution was added to 50 ml filtered seawater to create the following series of concentrations (mg NO_3-N L^{-1}): 0.00, 0.0, 0.5, 5.0, 10.0, 75.0, 100.0, 150.0. These standards were testing using an API 5-in-1 test stick, an API Nitrate test kit, a YSI nitrate test kit (Palintest Nitratest, YAP163) and a Hach nitrite test (NitriVer 5 Nitrate Reagent Powder Pillows, 2106169). Where standard concentrations exceeded the effective range for a test, the standard was diluted using distilled water.

Phosphorus

Phosphorus standards were prepared according to US EPA Method 365.3 (Anon 1978) using a stock solution of monopotassium phosphate (KH_2PO_4) that had been dried at 105°C for 1 hour added to filtered seawater at a concentration of 0.1 mg P ml^{-1}. Stock solution was added to 50 ml filtered seawater to create the following series of concentrations (mg P L^{-1}): 0.0, 0.5, 1.5, 3.0, 6.0, 12.0, 25.0, 50.0. These standards were testing using an API Phosphate test kit, YSI low-range and high-range phosphate test kits (Palintest Phosphate LR YAP177, Palintest Phosphate HR YAP114) and a Hach phosphate test (Phosphorus Reactive TNTplus Vial TNT846). Phosphorus analysis using the API 5-in-1 test was not performed as this parameter is not available on the test kit. Where standard concentrations exceeded the effective range for a test, the standard was diluted using distilled water.

Alkalinity

Alkalinity standards were prepared according to US EPA Method 310.2 (Anon 1974) using a stock solution of sodium carbonate ($NaHCO_3$) dried at 250°C for 4 hours and added to distilled water at a concentration of 1.0 mg as $CaCO_3$ ml^{-1}. Stock solution was added to 50 ml filtered seawater to create the following series of concentrations (mg as $CaCO_3$ L^{-1}): 0, 10, 50, 100, 200, 500, 1000. These standards were tested using an API 5-in-1 strip as carbonate hardness (KH), an API Carbonate Hardness (KH) test kit, a YSI alkalinity test (Palintest Alkaphot, YAP188) and a Hach Digital Titrator (1690001) with Alkalinity Reagent Set (2271900).

Each test was performed three times and the results reported as a mean ± SEM. All YSI test kits used in this study were analysed on a YSI 9500 photometer. All Hach test kits used in this study, apart from the alkalinity test, were analysed on a Hach DR1900 spectrophotometer. The Hach alkalinity test was conducted using a Hach Digital Titrator (1690001).

A list of all reagents and equipment, including product codes, used in these trials is provided in Appendix 8.

Results

pH

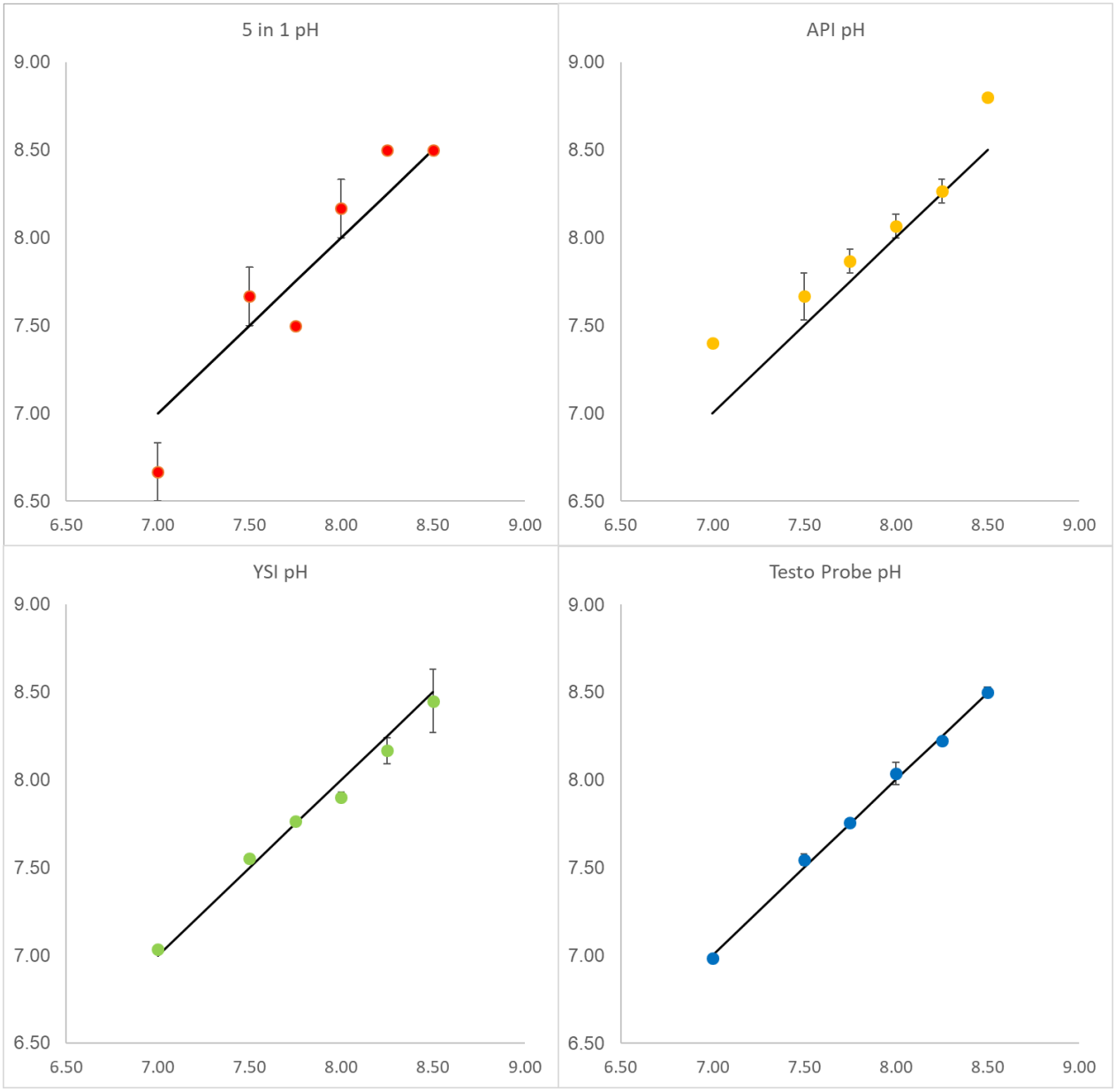

Figure 2. pH test performance of the API 5-in-1 test, API high range pH kit, a YSI Phenol Red assay kit (Palintest pH Phenol Red, YAP130) and a Testo 205 handheld pH probe recorded against standard. The black line indicates the pH of the standard solutions tested and the results of each test are shown as means ± SEM. The further a point is away from the black line, the less accurate the point is. All tests were performed in triplicate; where error bars are not visible, the SEM was too small to represent.

Ammonia

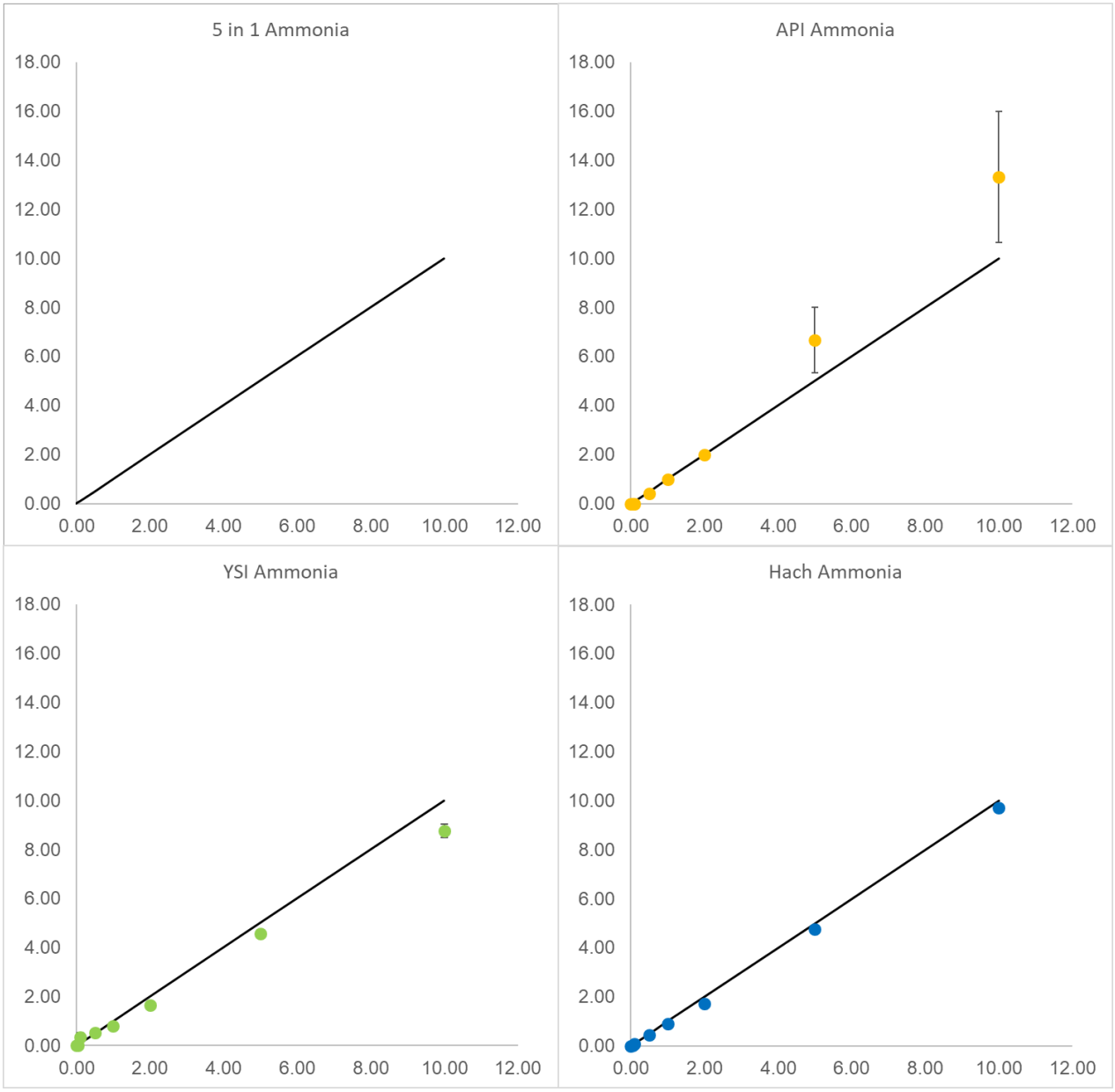

Figure 3. Ammonia test performance of the API Ammonia test kit, a YSI Ammonia test kit (Palintest Ammonia with conditioner for use with seawater, YAP 152) and a Hach ammonia test kit (Nitrogen-Ammonia TNT AmVer Low Range, 2604545) against standards (Note, this analysis is not possible for the API 5-in-1 test). The black line indicates the ammonia concentration of the standard solutions tested and the results of each test are shown as means ± SEM. The further a point is away from the black line, the less accurate the point is. All tests were performed in triplicate; where error bars are not visible, the SEM was too small to represent. Note, the API 5-in-1 test strips did not test for ammonia.

Nitrite

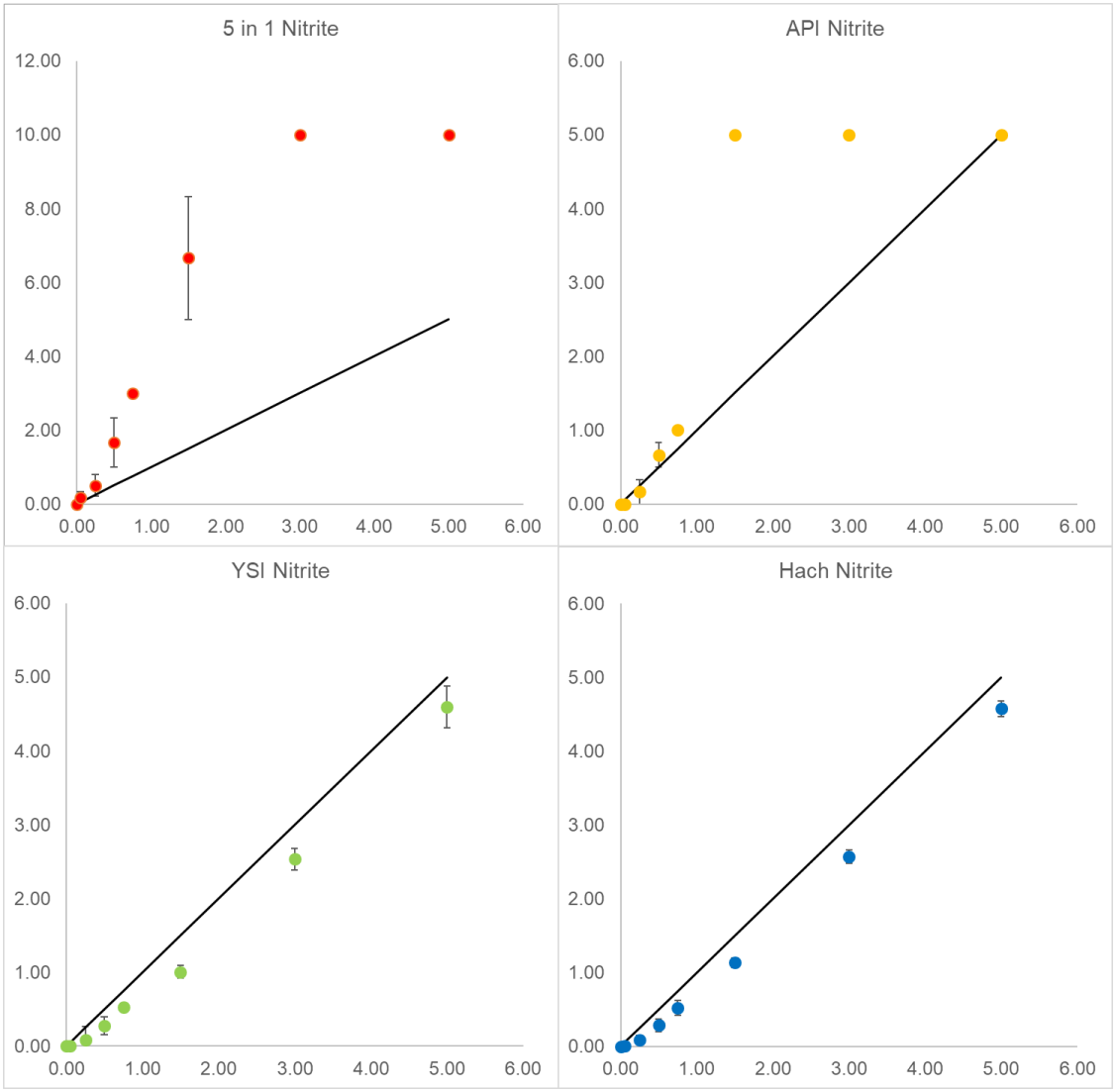

Figure 4. Nitrite test performance of the API 5-in-1 test stick, an API Nitrate test kit, a YSI nitrate test kit (Palintest Nitratest, YAP163) and a Hach nitrite test (NitriVer 5 Nitrate Reagent Powder Pillows, 2106169) recorded against standards. The black line indicates the nitrite concentration of the standard solutions tested and the results of each test are shown as means ± SEM. The further a point is away from the black line, the less accurate the point is. All tests were performed in triplicate; where error bars are not visible, the SEM was too small to represent.

Nitrate

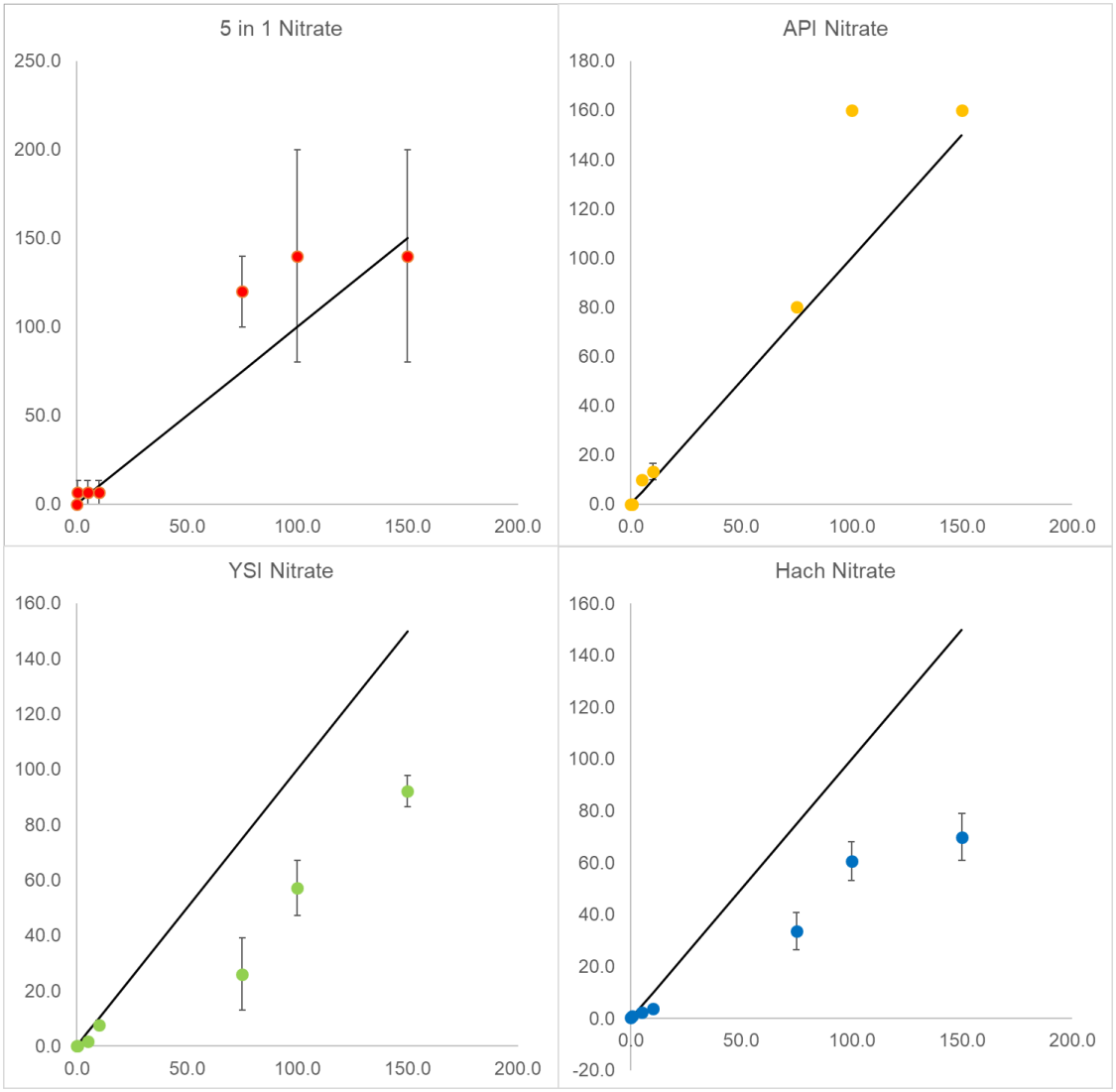

Figure 5. Nitrate test performance of the API 5-in-1 test stick, an API Nitrate test kit, a YSI nitrate test kit (Palintest Nitratest, YAP163) and a Hach nitrite test (NitriVer 5 Nitrate Reagent Powder Pillows, 2106169) recorded against standards. The black line indicates the nitrate concentration of the standard solutions tested and the results of each test are shown as means ± SEM. The further a point is away from the black line, the less accurate the point is. All tests were performed in triplicate; where error bars are not visible, the SEM was too small to represent.

Phosphorus

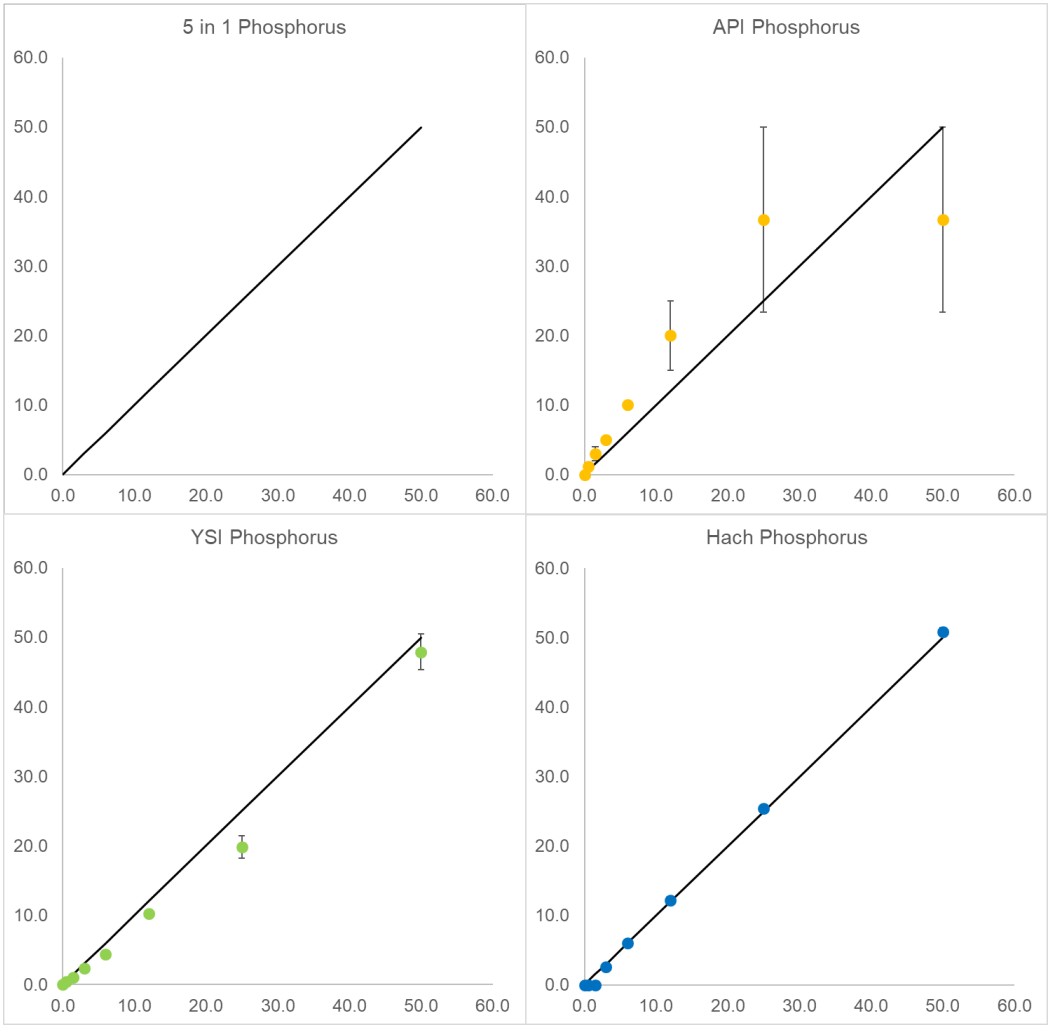

Figure 6. Phosphorus test performance of the API Phosphate test kit, YSI low-range and high-range phosphate test kits (Palintest Phosphate LR YAP177, Palintest Phosphate HR YAP114) and a Hach phosphate test (Phosphorus Reactive TNTplus Vial TNT846) recorded against standards (Note, this analysis is not possible for the API 5-in-1 test). The black line indicates the phosphorus concentration of the standard solutions tested and the results of each test are shown as means ± SEM. The further a point is away from the black line, the less accurate the point is. All tests were performed in triplicate; where error bars are not visible, the SEM was too small to represent.

Alkalinity

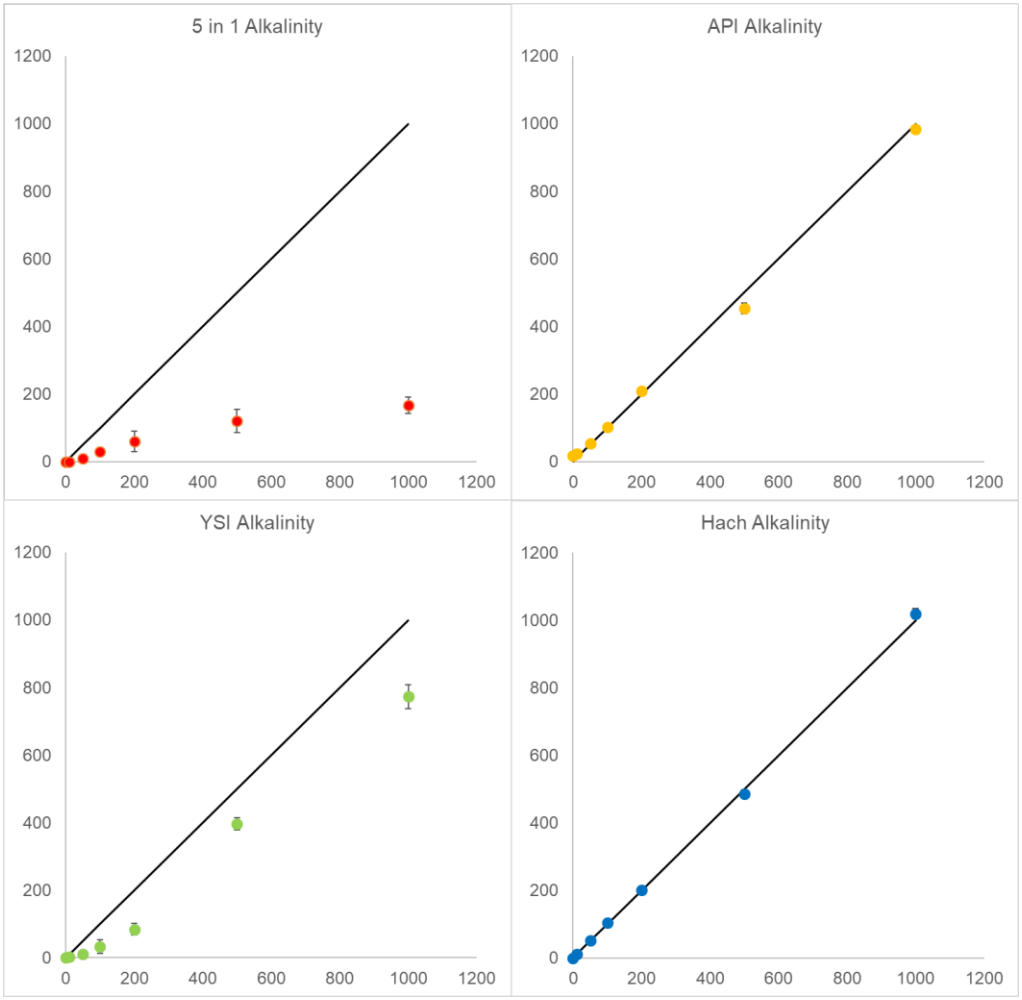

Figure 7. Alkalinity test performance of the API 5-in-1 strip as carbonate hardness (KH), an API Carbonate Hardness (KH) test kit, a YSI alkalinity test (Palintest Alkaphot, YAP188) and a Hach Digital Titrator (1690001) with Alkalinity Reagent Set (2271900) recorded against standards. The black line indicates the alkalinity concentration of the standard solutions tested and the results of each test are shown as means ± SEM. The further a point is away from the black line, the less accurate the point is. All tests were performed in triplicate; where error bars are not visible, the SEM was too small to represent.

Conclusion

pH

The pH of water describes how acidic or alkaline it is. Seawater generally has a pH of around 8.05-8.15. The SRL Clean Green (SRL 2016) program recommends a pH of 7.80-8.20 and the FRDC guide to Optimising Water Quality (Crear and Allen 2002) recommends 7.80-8.40. Tests of pH in facilities ranged from 6.79-7.88, substantially lower than natural seawater. This depression is likely due to the presence of acidic waste products in the water, such as CO_2 and nitrogenous byproducts. Currently, we do not have adequate information on what the thresholds are for acceptable and low or what the effects of chronic exposure to low pH water are in SRL. However, the results of the investigation into haemolymph biochemistry suggest that pH may have played a role in mortality events. As such, accurate monitoring of pH in facilities should be a priority. The results of the pH tests showed that the probe offered the greatest accuracy and precision, that the YSI kit performed well, and that the API kit performed reasonably well, particularly at the levels most relevant to SRL facilities (Fig. 2). It is worth noting that pH probes such as the one evaluated in this study generally compensate readings for the effect of temperature, which is an important consideration for accuracy.

Ammonia

Ammonia is the most important nutrient to monitor and manage, as it is the most toxic and is directly released by lobsters into the water as waste. Ammonia can build up to toxic levels when the biological filtration capacity of the system is exceeded, which can happen after periods of not holding stock or when an unusually large amount of stock is added. Additionally, lobsters that have been emersed build up ammonia due to an inability to release it from the gills, so when they are placed back into water, they can drive a spike as the built-up ammonia is released.

Ammonia also has a relationship with temperature and pH that is important to understand. In water, ammonia can exist in two forms, the toxic unionised NH_3 and the less toxic ionised NH_4. As temperature and pH increase, ammonia is converted from the less toxic form to the more toxic form. However, at the temperatures common to SRL facilities (9-15°C), the proportion of toxic NH3 is generally quite low (Table 1).

Table 1. Relationship between the composition of unionised (percent) by pH and temperature.

pH	Temperature (°C)		
	8	12	16
7.0	0.2	0.2	0.3
8.0	1.6	2.1	2.9
8.2	2.5	3.3	4.5
8.4	3.9	5.2	6.9
8.6	6.0	7.9	10.6
8.8	9.2	12.0	15.8
9.0	13.8	17.8	22.9

Clean Green reccomendations for ammonia are <0.5 mg L^{-1} and Optimising Water Quality recommends <2 mg L^{-1}. Ammonia becomes lethal at concentrations of 20-30 mg L^{-1}, but the effect of chronic exposure to lower levels is not currently known. In facilities tested, ammonia levels ranged from 0.0-0.55 mg L^{-1}.

All three ammonia kits tested performed well, especially at ranges likely to occur in a holding facility (Fig. 3). The Hach kit showed the greatest accuracy, but not without some negative aspects. First, it required a greater degree of accuracy in measuring the water sample volume, requiring a pipette. Second, it took the longest of the kits, at 20 minutes. Third, this kit required the standard to be diluted at the highest concentrations. Finally, the kit contains dangerous goods and the waste must be disposed of appropriately. The YSI kit involved a number of steps to perform, requiring a similar amount of time to complete as the Hach kit. It also required dilutions. The API kit performed well at lower ranges but overestimated and showed considerable variation at higher ammonia concentrations. It was the easiest to perform.

Nitrite

In a recirculation system, the biofiltration of the system converts highly toxic ammonia to moderately toxic nitrite. Clean Green recommendations for nitrite are <1.0 mg L^{-1}, Optimising Water Quality recommends <5 mg L^{-1} and researchers have used 10 mg L^{-1} as for sub-lethal exposure (Battaglene et al. 2004), though the effects of chronic exposure to nitrite are unknown. In facilities, nitrite levels have been found to range from 0.0-0.58 mg L^{-1}. Reduced salinity has been suggested to increase the toxicity of nitrite in lobsters.

For the nitrite tests, the YSI and Hach tests outperformed the API kit and the 5-in-1 strips (Fig. 4). The results of both of the latter tests were difficult to interpret due to poor colour separation on the test cards. Both the Hach and the YSI kits performed equally well and were easy to perform, though they required dilution for higher concentrations.

Nitrate

Nitrate, which is generated by the nitrification of nitrite, is the low toxicity final step of the nitrogen cycle in recirculation systems. Nitrate builds up in systems and must be physically removed via water changes. The toxicity levels, either acute or chronic, of nitrate have not been characterised for SRL, but in prawns, levels above 2,200 mg L^{-1} were found to be toxic (Roman and Zeng 2013). Clean Green and Optimising Water Quality recommend levels of <100-140 mg L^{-1} and <100 mg L^{-1}, respectively. Tested facilities were found to have levels ranging from 0.036 – 13.0 mg L^{-1}.

All of the nitrate tests performed poorly, with variable and inaccurate results (Fig. 5). Given that the low toxicity of nitrate confers a considerable margin for error, the API, YSI and Hach kits performed well enough to be an indicator of levels. Hach and YSI kits required dilution for higher concentrations and the Hach kit generated waste containing cadmium, which is a dangerous good and toxic to aquatic organisms.

Phosphorous

Phosphorus can enter facilities as contamination of the source water from industrial, residential or agricultural run-off or from within the facility through the use of detergents. It is not of high concern in facilities as it has minimal toxicity, however, phosphorous is a limiting nutrient to algal growth, so can fuel algal blooms if tanks receive enough light. It has generally not been found at any measurable level in facilities. In a small number of facilities however, low concentrations of phosphorus (3-7 mg L^{-1} P) have been recorded. In these facilities, source water has been tested and confirmed to not contain phosphorous, indicating that the contamination came from the facility. A characteristic shared by all of the facilities in which phosphorous was detected was that the holding tanks were made of concrete blocks (i.e. Bessar blocks) that were exposed to water. Phosphates are present in concrete and mortar and are likely leaching into the water from exposed blocks.

The Hach phosphorus kit performed the best and was an easy test to perform (Fig. 6). It required dilution at the highest level. The YSI kit performed well and comes in a high range and low range kit.

The low range kit performed better than the high and samples with a high concentration can be diluted to a level compatible with the low range kit.

Alkalinity

Alkalinity is a measure of how well water is buffered against acidic changes to pH – high alkalinity means that addition of acidic lobster waste products will have less of an effect on pH. The alkalinity of seawater is around 116 mg L^{-1} as $CaCO_3$, and Clean Green and Optimising Water Quality both recommend 100-200 mg L^{-1} as $CaCO_3$. Alkalinity was not routinely tested in facilities, but the limited data collected suggests a considerable variation, with values of 68 and 164 mg L^{-1} recorded. The results of the haemolymph biochemistry experiments suggest that alkalinity may play a more important role in lobsters health than previously understood, making this an area of priority for future research.

Of the four alkalinity tests, the Hach digital titrator demonstrated the best performance (Fig. 7), however, it was somewhat difficult to set up, involved the use of concentrated sulfuric acid and the results were relatively difficult to calculate and interpret. It is also important to note that with this test, the results are more precise than the other tests in that carbonate alkalinity and hydroxide alkalinity are calculated.

The next best performance came from the API Alkalinity test. It accurately reflected the concentration of the standards and the results were more readily interpreted than other API test kits due to the test's drastic change in colour. The YSI test was easy to perform, however, it was not as accurate as the other tests and systematically underreported alkalinity. The 5-in-1 strip severely underreported alkalinity.

Two other parameters not evaluated in this study but deserving mention are salinity and dissolved oxygen. Both of these parameters immediately impact lobster physiology and health and degradation of either can rapidly lead to mortality. Salinity can be tested using a hydrometer, and refractometer or a salinity meter, with each representing an improvement in sophistication and accuracy, with a corresponding increase in cost, respectively. Seawater has a salinity of around 35 ppt. Clean Green recommends a salinity of 30-38 ppt, with 35-36 ppt considered optimal. Optimising Water Quality gives a recommended range of 30-38 ppt. In facilities, salinity ranged from 33.3-36.6 ppt. Dissolved oxygen can be measured using two different types of probes – a Clark type electrode and an optical probe. Clark electrodes cost less than optical probes but require routine maintenance to operate properly. Optical probes are more expensive but are more robust and do not require much upkeep. Clean Green recommends that dissolved oxygen levels remain above 70% and Optimising Water Quality recommends a minimum of 70% and a preferred level of at least 80%. In facilities, dissolved oxygen levels ranged from 79.4%-100%.

In summary, the physiology and biochemistry results reported as a part of this project indicate that the processing industry (and the SRL fishing industry) could benefit from a better understanding of water physicochemistry monitoring and management. While there is variability in the current level of knowledge and practices, generally there is a low level of water quality testing. The API kits performed well compared to the more expensive options and are an economically and practically attractive option for facilities that do not currently test their water. For more advanced approaches, the YSI and Hach systems offer increased accuracy and precision commensurate with their respective costs.

Recommendations

For a facility not currently conducting water quality tests, measuring a limited number of parameters or testing only sporadically, we make the following recommendations:

Water in recirculation systems should regularly be tested for the following parameters: ammonia, nitrite, pH, salinity, alkalinity, dissolved oxygen;

Tests should be performed daily during routine operation and more frequently (as much as hourly) during times of high nutrient load, such as during heavy stocking, the beginning of the season or during purging;

Results should be recorded and reviewed to identify trends.

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Results 10: Validation of a Handheld Lactate Meter

Preface

This aspect of the project was undertaken to address variation objectives 2 of this study:

An assessment of a practical lobster stress indicator tool (lactate meter)

Manuscript information

Validation of a handheld lactate meter for measuring haemolymph lactate concentrations in Southern Rock Lobster (*Jasus edwardsii*)

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Target Journal: Industry report

Abstract

Lactate builds up in Southern Rock Lobster as a by-product of stressors such as excessive activity, emersion, temperature or hypoxia stress and measurement of lactate of the haemolymph (blood) is a useful indicator of stress and a potential predictor of mortality in post-harvest crustaceans. Currently, the only way to measure lactate in Southern Rock Lobsters is through a technically difficult and expensive spectrophotometric assay. Handheld lactate meters developed for medical use in humans have been used as an easy and economical way to measure lactate in real-time in animals. Such use requires validation to ensure that the results reported by the meter accurately represent the actual lactate values of the subject animal. To that end, the present study evaluated the accuracy of the Lactate Scout+ meter for measuring haemolymph lactate levels in Southern Rock Lobster. In this study, 5 lobsters were removed from a holding tank and held in air for a period of 6 hours. They were sampled for haemolymph at 0, 1, 2, 4 and 6 hours post-emersion. The haemolymph samples were tested with the Lactate Scout+ handheld meter and a fluorometric laboratory assay. Linear regression analysis showed a strong correlation (R^2=0.91) between the two measurement methods, which indicated that the Lactate Scout+ accurately detected lactate in Southern Rock Lobster haemolymph. This correlation demonstrates the potential for the development of handheld lactate meters as a tool for assessing lobster stress/health in real-time at any point along the supply chain. Recommendations are provided for the further validation of the lactate and the refractive index assessment tools to provide industry with superior evaluation matrix for determining the condition and competency of lobsters for holding and live export. Improved lobster stress assessments will allow for the implementation of better stock management decisions which will reduce stock losses, improve lobster welfare and help ensure that maximum returns are achieved from the fishery product.

Introduction

For lobsters, as in humans, lactate is a by-product of anaerobic metabolism which will accumulate in the haemolymph when the cardio-respiratory system cannot supply sufficient oxygen to support energetic demands (Paterson et al., 2005). Lobsters will resort to anaerobic metabolism during periods of stress such as excessive activity, air exposure, handling, hypoxia, stressful fishery capture practises and inappropriate temperatures, all of which will lead to the build-up of lactate in the haemolymph (Fitzgibbon et al., 2014b; Paterson et al., 2005; Stoner, 2012). Anaerobic metabolism is non-sustainable and if continued, will lead to death (Pörtner, 2010). Lactate accumulation acidifies the haemolymph, which impacts the haemolymph biochemistry as the lobsters strives to maintain its acid-base balance. This process can have detrimental effects on lobsters, make them more susceptible to the stressors associated with harvesting and processing and ultimately cause mortality. Previous research of Western Rock Lobsters (*Panulirus cygnus*) identified lactate accumulation during emersion and associated physiological related effects as major correlates of post-harvest transport mortality (Paterson et al., 2005). Similarly, Simon et al. (2016) suggested the build-up of anaerobic end-products (such as lactate) and nitrogenous wastes as the most likely cause of transport mortality of Southern Rock Lobsters (*Jasus edwardsii*, SRL). The determination of lactate levels of the haemolymph levels is thus frequently used as a stress assessment parameter and has been classified as a potential predictor of post-harvest mortality for crustaceans (Bakke and Woll, 2014; Stoner, 2012).

Currently, the only method for measuring lactate in Southern Rock Lobsters (SRL) is through an expensive and technically difficult spectrophotometric assay. The development of a rapid and easy to use testing approach to quantify the concentration of lactate in the haemolymph has the potential to be a useful tool for lobster fishermen and processors, as it will strengthen the current practice of vitality measurements using visual assessment of vitality with an objective measurement of stress. The development of such a tool would enable the evaluation of lobster health and may serve as a predictor for mortality, which can be used to inform fishing practices, purchasing decisions and processing approaches. Handheld meters developed for point-of-care lactate use in humans have been validated for use in freshwater crayfish (Bonvillain et al., 2013), European lobsters and Norway lobsters (Bakke and Woll, 2014), suggesting that they would be appropriate for use in SRL. To that end, this study was performed to validate the results of a readily available handheld lactate meter (Lactate Scout+, Fig. 1) against a laboratory assay using blood samples collected from SRL.

Figure 1. The Lactate Scout+ handheld meter examined in the present study for its effectiveness for the rapid and easy to use testing approach to quantify the concentration of lactate in Southern Rock Lobster haemolymph.

Methods

Five lobsters were transported to IMAS and allowed to acclimate overnight in a holding tank. The day of the experiment, the lobsters were removed from the tank and placed in a polystyrene box where they were held for the duration of the experiment. A 700 µl sample of haemolymph was taken from the base of the distal walking leg using a pre-chilled 26 gauge needle fitted with a 1 ml syringe at 0 h, 1 h, 2 h, 4 h and 6 h of emersion. From the haemolymph sample, 100 µl was placed on a digital refractometer (Hannah Instruments HI96801) and the refractive index recorded. From this haemolymph, a lactate reading was taken using a Lactate Scout+ handheld meter. The remaining haemolymph sample was placed into a centrifuge tube and centrifuged at 10,000 x *g* for 5 mins at 4°C. 500 µl of the supernatant was transferred to a 10 kD molecular weight cut off spin column (Biovision #1997) and centrifuged at 100,000 x g for 30 mins at 4°C to deproteinate the sample. The filterate was transferred to a cryotube and frozen at -80°C. The frozen filtered haemolymph samples were later defrosted at room temperature and assayed for lactate concentration using a Biovision lactate kit (Biovision, #K607) on a spectrophotometer by measuring fluorescence at 535 nm emission, 590 nm excitation. Results from the handheld meter were compared to the spectrophotometer assay using a linear regression.

Results

Air exposed SRL demonstrated the expected response to emersion, with haemolymph lactate concentration rising in correlation to emersion time. There was a degree of variation between individuals, but when the results from the Lactate Scout+ meter were compared to the laboratory assay, a strong correlation (R^2=0.92) was found (Fig. 2). The Lactate Scout+ meter was found to report concentrations that were approximately 13-14% that of the laboratory assay. Furthermore, below levels of around 8-10 mmol L^{-1}, the Lactate Scout+ reported that concentrations were below the detectible limit.

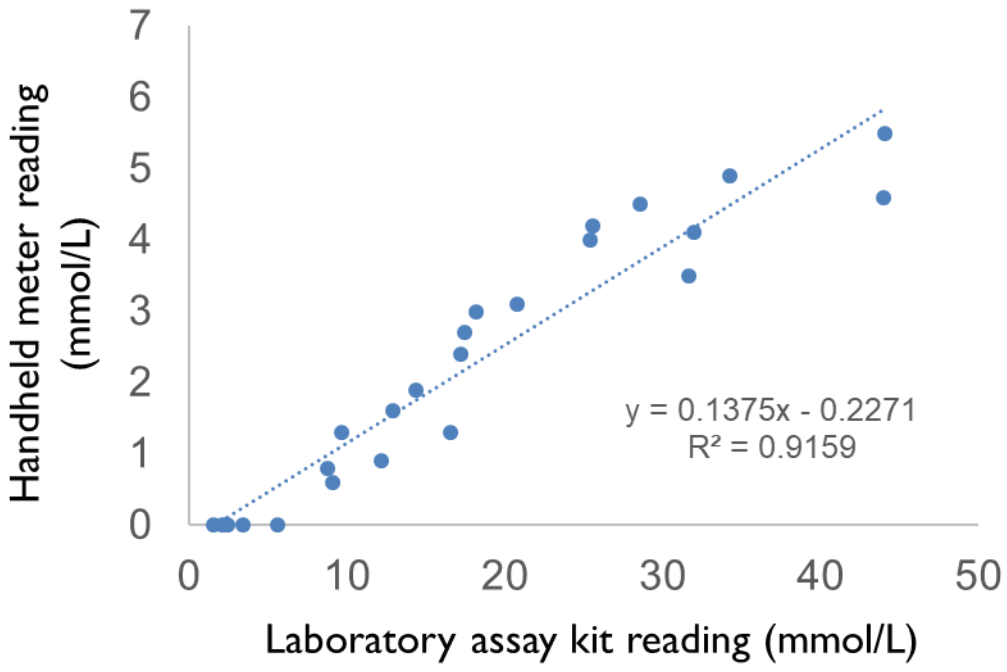

Figure 2. Linear regression of the handheld lactate meter reading against the laboratory assay kit from haemolymph samples collected from emersed Southern Rock Lobster.

Conclusion

The lactate concentrations reported by the Lactate Scout+ handheld meter correlated strongly with the lactate concentrations measured using a fluorometric laboratory assay kit, reporting results that were consistently 13-14% those of the lab kit. The results reported here suggest that handheld lactate meters can be developed as useful and accurate measures of lactate concentration in SRL in real-time for use as both as a useful scientific tool and for the SRL fishing and exporting industries as an effective measure of lobster stress and condition at any stage of post-harvest chain of custody. The post-harvest chain of custody involves several steps where inappropriate handing or maintenance of lobsters can lead to stress which can correlate with poor lobster performance and mortality. A reliable, quick, simple and cheap quantitative measure of stress could therefore be useful to allow an instantaneous measure of stock quality at any step of post-harvest processes to allow for production decisions and countermeasures to be implemented to limit the likelihood of mortality. The Lactate Scout+ handheld meter ($500 per meter) and cost per test ($3.40 per test strip) are relatively inexpensive. Considering the high value of SRL, it would legitimize the use of instrument as a measure of batch level lobster condition (i.e. subsample lobsters from the cohort) or individual lobsters which are thought to be compromised. Furthermore, the instrument only requires a very small sample of lobster haemolymph (0.5-0.6 µl) which is absorbed into the well of the strip through capillary forces. This negates the need for haemolymph sampling with a syringe and ensures self-regulation of the volume applied, both of which act to simplify ease of use and limit negative impacts on the lobster. This research is the first preliminary step towards validating handheld lactate meters as a SRL industry for the assessment of live lobster condition and competency for holding and export. To further develop lactate meters as a useful industry tool, further work is required to;

- further validate the precision of the meters by ensuring that different meters report similar results when used on lobster haemolymph
- more extensively validate its accuracy to measure lactate on more and a wider diversity of lobsters including examining any influence of lobster size, sex and condition
- more extensively validate its accuracy to reflect stress a wider diversity of stress conditions including hypoxia, temperature and handling stress.
- further assess its effectiveness to predict lobster performance/mortality in commercial settings
- refine haemolymph sampling technique to minimize negative impacts on the sampled lobster
- quantify what ranges are acceptable or unacceptable for satisfactory post-harvest lobster performance.

Once further validated as an effective and practical tool to measure stress condition of SRL, it could greatly enhance the ability of SRL fishing and exporting industry to assess live lobster condition and competency for live holding and international export. Instantaneous measures of lobster lactate levels are likely to be effective for identifying individual or cohorts of lobsters which have been exposed to detrimental conditions which induce metabolic stress including excessive activity, prolonged air exposure, poor handling practices, hypoxia, stressful fishery capture practises and inappropriate temperatures (Stoner, 2012). It is envisaged that this tool will be particularly useful for the fishery to assess the quality of captured stock and suitability of on-vessel holding systems, for the assessment of lobster quality at point of landing, during the post-harvest on-shore holding process and before export to international markets. Improved lobster stress assessments will allow for the implementation of better stock management decisions which will reduce industry stock losses, improve lobster welfare and help ensure that maximum returns are achieve from the fishery product.

However, it is noted that metabolic stress, as measurement by lactate, is just one factor that potentially impacts post-harvest performance of SRL and thus it may not explain all cases of poor lobster

performance in industry. Previous research with lobsters and other crustaceans have shown while lactate levels often correlates with post-harvest mortality, the pattern is not consistent between cases (Paterson et al., 2005; Simon et al., 2016; Stoner, 2012). Inconsistency between cases is likely due to the complex and multi-faceted nature for post-harvest mortality of crustaceans and suggests that no single or derived physiological relationship can be used to reliably predict all post-harvest mortality of lobsters. Other factors such as moult stage, nutritional condition, disease and physical damage also have potential to effect lobster post-harvest performance, which may not necessarily induce a lactate response. The most effective process for the condition assessment of live lobster would likely involve several assessments tools broadening the scope of lobster physiological evaluation. Improved measures of lobster moult stage may be particularly important as anecdotal evidence from industry suggest that more out of season moulting is occurring across the fishery sectors possibly in response to changing environmental condition such as increasing water temperatures.

Moulting is a highly energetically demanding process lobster are physiologically compromised and vulnerable to stress leading up to, during and when recovering from the moult (Fitzgibbon et al., 2014a). Following the moult, lobsters soft and vulnerable to physical damage and have poor nutritional condition with limited energy reserve to support starvation during holding (Simon et al., 2015). Pre- and post-moult lobsters have poor capacity to withstand commercial industry practices and the industry should aim to avoid the landing and holding of pre- and post-moult lobsters. The most practical method for industry to determine moult stage of lobsters is through the use of the refractive index (or Brix) of the haemolymph. Refractive index correlates closely with nutritional condition and in-turn moult stage of crustaceans (Simon et al., 2015). Lobster refractive index is easily measured by placing a small droplet of haemolymph on a portable digital refractometer (such as the Hannah Instruments HI96801). The northern hemisphere clawed lobster fishery commonly uses refractive index as a tool to determine quality of stock and has established threshold levels in order to inform production processes (Battison, 2018). While it is well established that refractive index correlates with the nutritional condition of SRL (Simon et al., 2016), an exact correlation with moult stage has yet to be determined. Further research is required to quantify the correlation between refractive index and SRL moult stage in order to provide industry with threshold levels that identify when lobsters may be physiologically compromised and less likely to survive the stresses of industry practices. We recommend the accuracy condition assessment of live SRL would benefit by the combined assessment of lactate and the validation of a refractive index assessment. Together the refined and validated lactate and refractive index assessment tools will provide the SRL industry with a superior evaluation matrix for determining the condition and competency of lobsters for holding and live export.

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Results 11: Best Practice Guide

Preface

This aspect of the project was undertaken to address variation objectives 4 and 5 of this study:

Basic Best Practice Guide for SRL Post-Harvest Operations (water quality, tank and stock management etc)

A SRL Post-Harvest Best Practice Standard (this will inform further alterations to the current post-harvest portion of the Clean Green Standard)

Manuscript information

Best practice guide for Southern Rock Lobster post-harvest holding and processors industry operations

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Target Journal: Industry report

Abstract

The guide was developed to ensure optimum handling and processing of live Southern Rock Lobster (SRL) during post-harvest processes by the SRL processing and export industry sector. The document provides a guideline for industry best practice from landing to international live product export and presents recommendations for best practice during key industry practices including handling, periods of emersion, land transport, grading, during tank holding and finally air freight transport. Industry recommendation incorporate recent findings of the FRDC 2016/235 project and highlights key knowledge gaps in industry processes and future research priorities.

Best practice guide for Southern Rock Lobster post-harvest holding and processors industry operations

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General handling

Background

Careful handling

Lobsters are handled and removed from the water frequently throughout the post-harvest process. Although much of this handling and emersion is necessary, effort should be made to minimise handling as much as possible, because every time a lobster is removed from the water or handled, there is the chance of causing stress or damage. In fact, both emersion and handling have been shown to negatively affect the immune function and vitality scores of lobsters, and lobster subject to both stressors show a greater impact than lobsters subject to only one stressor (Jussila et al., 1999). Furthermore, these impacts last considerably longer than the stresses that created them, as it can take as long as five hours to recover from the physiological impacts after being returned into water (Crear and Forteath, 2000; Taylor and Waldron, 1997). Whenever lobsters must be handled, it should be in a way that minimises the potential for stress and damage, with focus on preventing three negative factors: 1) limb loss, 2) tail flipping and 3) impact from throwing or dropping.

Limb loss

To minimise the risk of limb loss, it is important to consider that crustaceans can actively drop or throw limbs through a reflexive behaviour (autotomy) if they struggle to escape from a threat or can lose limbs passively through breakage (autospasy) at separation points common throughout the species, which is located just prior to the first leg segment (Fig. 1) in rock lobster (Bradstock, 1950). An illustrative example to understand the difference between these two mechanisms is to consider a lobster on the deck of a fishing boat struggling to gain traction and dropping off one or more legs (autotomy) versus lifting a lobster by the leg or having a leg entangled in lobster pot mesh and having it detach (autospasy) (Bradstock, 1950).

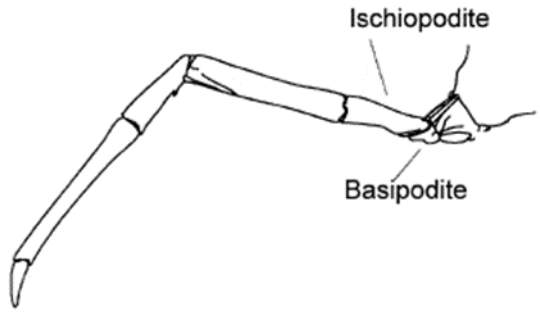

Fig. 1. Autotomy point in the leg of lobsters, which occurs prior to the proximal leg segment between the ischiopodite and the basipodite. Image adapted from Cattaert and Le Ray (2001).

Although the mechanisms of limb loss in SRL have not been thoroughly investigated, it is apparent that legs may be lost through either mechanism whereas antennae are only lost through autospasy (Powrie and Tempero, 2009), meaning they cannot reflexively detach but require an external force to cause them to break off. As such, lobsters should be held by the base of the antennae (horns) or by lightly grasping the carapace. Lifting lobsters by the end of the antennae should be avoided, as this has been shown to contribute to the greatest amount of limb loss in SRL (Davidson and Hosking, 2004). Not only does limb loss reduce the value of an individual lobster, but it can reduce immunocompetence, leaving lobsters with an open wound while at the same time increasing their vulnerability to pathogen entry (Fotedar et al., 2001).

Tail flipping

When using either approach to holding, and whenever handling SRL in general, it is important to limit tail flipping by supporting the tail. Tail flipping is an escape mechanism employed by lobsters that is commonly regarded as evidence of a strong, vigorous lobster. However, while behaviours like tail flipping can be an indicator of stress, they are not good at predicting mortality in lobsters (Simon et al., 2016). Furthermore, tail flipping causes a considerable amount of physiological stress to lobsters, leaving them exhausted, weak and more susceptible to other stresses of the post-harvest process and should be avoided throughout the post-harvest handling chain. While tail flipping is considered the most efficient method of escaping predators for lobsters (Harris and Andrews, 2005), it comes at a substantial cost, as the energy demands of the large tail muscles can quickly outpace the ability of the haemolymph to provide oxygen, shifting the muscles into anaerobic metabolisms which results in the rapid depletion of energy and the build-up of lactate (England and Baldwin, 1983; Gäde, 1984). The build-up of lactate will be discussed thoroughly below in regard to emersion and air exposure of lobsters, but it is indicative of stress, can take a considerable amount of time from which to recover, with one study showing that lobsters recovered from 8 hours of emersion after 24 hours but took 48 hours to recover from 8 hours emersion and tail flipping (Whiteley and Taylor, 1992) and can decrease the quality of the meat of a lobster (Taylor et al., 1997), and thus should be avoided.

Drops, shocks and impacts

The final negative handling factor, impacts from throwing or dropping, is perhaps the easiest factor to manage, as it is simply a matter of care and good animal welfare practices. Considering the number of individual SRL processed each year and the desire to return lobsters to the water as quickly as possible, it is easy to see how speed and efficiency may become the highest priority when handling lobsters. However, shortcuts taken in handling can cause additional stress or damage to lobsters, potentially reducing their value. As aquatic animals, lobsters do not generally have to cope with impacts from drops and, apart from their hard carapace, do not have much protection for their internal organs. During processes such as weighing for grading, it is common for lobsters to be tossed into bins, a practice that has been shown to be associated with a higher rate of injury in the North American clawed lobster industry (Lavallée et al., 2000). Similarly, it is not uncommon for lobsters that are picked up by the antennae to have the antennae break off (as discussed above), resulting in the lobster

falling back into the water or onto the ground. These practices should be eliminated from the processing industry, with a combination of education on careful handling and animal welfare, optimisation of the processing work flow to discourage throwing and dropping and audits of handling practice potential mechanisms for improving on current practices.

Recommendations

- An education program that delivers information on best practice handling for animal welfare for any employee within the supply chain that handles lobsters

Standards

Standard	Auditing Guidelines
All facility staff that handle lobsters shall be trained in animal welfare and handling practices	

Emersion

Background

Physiology

Lobsters breathing is substantially impaired in air, as their gills are adapted for functioning in seawater. When held in air, the gill arches collapse, reducing the surface of exchange, the effectiveness of oxygen uptake and expelling of carbon dioxide (CO2) and ammonia (Morris and Oliver, 1999b; Taylor and Waldron, 1997). The build-up of these waste products (CO2 and ammonia) is problematic for several reasons. First, it causes respiratory acidosis to the animal, as increased levels of CO2, lactate and ammonia cause the pH of the haemolymph to drop, or become more acidic. Low pH levels make the haemolymph less efficient at carrying oxygen throughout the body (Morris and Oliver, 1999a; Vermeer, 1987), forcing the lobster to buffer the haemolymph back into balance by mobilising bicarbonate ions from the shell. SRL have a capacity for buffering haemolymph pH against the build-up of acids for about 4 hours of emersion (Taylor and Waldron, 1997), after which the haemolymph pH becomes increasingly acidic. This is a physiological stressful and energetically demanding process that will reduce the overall resilience and likelihood of survival during holding.

Metabolic stress

Decreased oxygen levels also cause metabolic stress, which occurs when lobsters shift from aerobic metabolism to anaerobic metabolism. Anaerobic metabolism is how energy is generated without using oxygen. Humans experience anaerobic metabolism during short bursts of activity such as sprinting or lifting heavy weights. It is not sustainable over long periods, as it is less efficient than aerobic metabolism, meaning it produces much less energy,

and it results in the build-up of lactate, which impairs muscle function and intensifies the acidification of the blood or haemolymph occurring due to respiratory stress. In SRL, the build-up of lactate has been shown to be correlated with mortality (Simon et al., 2016), making measurement of lactate a potentially useful stress test that can be used by fishermen or processing facility workers to evaluate lobster condition. The build-up of lactate is more persistent than the respiratory stress indicators, with CO2 and pH levels returning to normal within 2-4 hours following return to water, whereas lactate levels remained high for at least 24 hour (Taylor and Waldron, 1997).

Other stressors

While a lack of oxygen and the accumulation of lactate have a strong negative effect on a lobster's vitality, physiological state and likelihood of survival, when additional stressors are added, the negative effect becomes even more potent. Combining handling or strenuous exercise such as tail flipping increases the severity of lactate build-up and increases the time taken to recover (Taylor and Whiteley, 1989; Waldron, 1991). Emersion is unavoidable at certain points in the supply chain, so care must be taken at those points to minimise its impact. When lobsters are emersed on a boat deck or jetty, they are exposed to a range of environmental conditions such as sunlight, wind or rain (freshwater) that can all contribute to further stress.

Keeping lobsters cool, moist and covered is essential for reducing the stress caused by emersion. The rate and capacity of all physiological processes are strongly impacted by temperature which is one of the most dominant environmental factors influencing physiology of aquatic ectotherms, such as lobsters (Fitzgibbon et al., 2017). Lobster oxygen demands and ammonia and excretory rates all increase with temperature (Crear and Forteath, 2002; 2000). For these reasons, efforts should be made to keep lobsters cool at all times, particularly during stressful events such as emersion during transport, as this will act to reduce their metabolism and reduce the build-up of waste products. The optimum temperature for SRL during post-harvest practices is not well established and will likely depend on several factors including ambient temperature at capture and the specific handling and maintenance practices. Too cool a temperature as well as large and rapid change in temperature can also induce stress and be detrimental to crustaceans (Lu et al., 2015; Lu et al., 2016). For this reason, the optimum temperature during emersion would likely be different depending on the season and associated ambient water and air temperatures. While further research is needed to identify optimum temperatures for SRL during post capture transport emersion, 12-14°C is a good target for summer and 8-12°C in winter. Temperatures below 8°C are not recommended as they could be detrimental. Current Clean Green Program standards are that temperature should be between 7-12°C on any transport exceeding one-hour duration.

Stress recovery

Following emersion, lobsters require time in water for recovery. Any time lobsters are held out of water for an appreciable amount of time (i.e. more than 1-2 hours), they should be allowed 24 hours in water to recover before any subsequent emersion or handling. This recovery time achieves two goals. First, this recovery time allows lobsters to purge, or get rid of, the built-up waste products caused by emersion. Secondly, recovery time allows lobsters

to return to a normal physiological state, including a normal pH balance. After wastes are purged and haemolymph pH balance returns to normal, lobsters are more resilient to subsequent handling.

An important consideration to this purging and recovery is that when it happens in a small volume of water and with a large biomass of lobsters, such as a boat hold, or in recirculated system, waste products can rapidly build up and outstrip the biological filtration capacity of the system, lowering the water quality and further stressing the lobsters. Water quality is discussed in detail below, but it is necessary to remember that accumulated wastes must go somewhere. For this reason, using an independent purge tank and water treatment system at the entry of lobsters to a facility is recommended. A well-designed purge tank will be maintained off a separate water source from the main system with a biological filter maintained with the capacity for processing the high concentration of waste products that will be introduced. If the purge tank does not have the capacity for the biological load, toxic ammonia will build up and the lobsters in it will be worse off. Maintaining good water quality in the purge tank will help lobsters to recover more quickly (Battaglene et al., 2004). To maintain the capacity of the biofilter between lobster arrivals, ammonia should be added to the purge tank system during periods of no or very low lobster stocking load, otherwise the bacteria that convert ammonia to less toxic forms will die off during these low periods. This is discussed more fully below in the biofiltration section of Holding stock in facilities.

Recommendations

- Limit emersion to <4 hours in any 24 hour period, provide ample recovery time (24 h) after each incidence of emersion
- Keep lobster moist and cool (8-14°C) for at least 30 minutes before extended emersion or hold at chilled temperature
- Record emersion times for common procedures and use the data to indicate where improvements to the process can be made to minimise emersion

Standards

Standard	Auditing Guidelines
Exposure to sunlight, wind, rain must be avoided any time lobsters are held out of water	
Emersion time should be limited to less than 1 hour in uncontrolled conditions, such as when transferring off vessels	
During emersion, lobsters should be held at 8-14°C and >90% relative humidity	
Following any period of emersion in uncontrolled conditions and greater than 1 hour	

in controlled conditions, lobsters should be given 24 hours recovery time before any further emersion or handling	
Emersed lobsters should be held in purge tanks following transport to a facility and the water quality of the tank monitored to ensure it meets the parameters detailed below in the *Holding stock* section	
The biofiltration system of purge tanks must be maintained to handle the nutrient load produced by a standard lobster arrival	

Receiving stock (Transport and grading)

Background

Getting lobsters from fishing boats into processing facilities requires three distinct processes, each with their own particular risks to lobster health: the transfer from boats to trucks, transport on trucks and transfer into the facility.

Transfer from boats

During the transfer from boats to trucks, the primary risks to stock are emersion, exposure to the elements and handling. Lobsters should be kept in water for as long as possible before transfer off boats. Any time spent on the deck of a vessel or on a wharf is time in which they cannot breathe adequately and are accumulating waste products in their haemolymph, both of which weaken the animal and reduce its chance of surviving holding.

While some emersion is unavoidable, it is important to manage the conditions of this exposure to limit any additional stress factors. Some of this exposure can be prevented by optimising processes and good decision making. For example, transferring lobsters from vessel to truck early in the morning or at night rather than at midday will prevent exposure to intense sun and heat and keeping double handling (swapping lobsters from one container to another during transit) to a minimum will reduce handling stress and time out of water. Transport trucks should be pre-chilled before loading to allow a more thermally stable environment during transport. Other steps include more practical approaches, such as covering lobsters at all times when out of the water, which is important for providing protection from the elements and the maintenance of a moist environment. Exposure to sun, wind and rain can all have a negative impact on lobsters. Sun and wind can cause lobsters to dry out, which, as previously discussed, limits their ability to obtain oxygen, causes the build-up of waste products like lactate and can cause lobsters to drop legs. Sun exposure can also damage rock lobsters' eyes (Meyer-Rochow and Tiang, 1984), as they are nocturnal in the wild and are adapted to low light conditions. The effects of exposure to rain have not yet been studied, but rock lobsters show a poor tolerance to changes in water salinity (Dall, 1974), so exposure to freshwater rain should be avoided. Covering lobsters with a damp length of canvas or hessian is common

throughout the industry as it provides protection from the elements and can be wetted to maintain a moist environment and prevent lobsters from drying out.

Careful handling

As previously discussed, careful handling must be of primary importance. While minimising time spent out of water is important, it must not come at the cost of poor handling. As is the case any time lobsters are handled, some concerns regarding handling include holding lobsters carelessly; throwing, tossing or dropping lobsters; and allowing lobsters to tail flip. Another important concern is avoiding over-packing holding or transport containers. Over-packing can lead to damage, either from crushing or puncture wounds. Such wounds can be difficult to notice when inspecting lobsters but can lead to weakness and mortality later during holding. The Clean Green program standards require that bins are packed no more than 2/3 full in clean, impermeable containers. Providing a surface that the lobster legs can grasp onto may improve welfare, as they may be stressed when unable to gain traction on hard, smooth surfaces (Dall, 1986). Providing tactile substrate for lobsters to grasps will also act to limit lobsters grasping each other which can result physical injuries such as puncture wounds. Furthermore, care must be taken to reduce the potential for lobster feet or other appendages to be damaged if they protrude outside the container. Protruding lobster feet can be broken off when the container is handled inducing stress and reducing the lobster immunocompetence. It is recommended that if using creates whereby lobster feet can protrude and be potentially damaged, hessian cloth is laid on the contained base to limit the likelihood of feet damage and provide a tactical substrate for grasp.

Measuring stress

Given the considerable number of stressors that lobsters may be subject to at this point in the supply chain, it is important to develop tools to measure stress and assess the likelihood of survival through the remainder of the holding process. The identification of lobsters that have not been handled well or are not in a physiological state to withstand the stress of transport would enable processors to make an informed decision as to whether to purchase these lobsters and appropriate strategies for maintenance or sale. Similarly, it would enable fishermen to monitor and demonstrate the quality of their handling procedures and product. Recent research on the FRDC 2016/235 Improving post-harvest survivability project has offered preliminary evidence for the measurement of lactate and refractive index as a potentially powerful diagnostic that provides a measurement of stress and moult cycle in lobsters. How these measurements translate to risk of mortality need further validation research.

Road transport

Once lobsters are packed in crates and placed on trucks, they are better protected from the elements but managing environmental conditions is still important and the longer the journey, the more important it becomes. The FRDC 2016/235 Improving post-harvest survivability project consistently found an association between time and distance of post landing transport

and SRL mortality during holding. Keeping transport time to a minimum is critical for reducing the impact on the lobsters. The Clean Green program requires that transportation of lobsters not exceed 6 hours due to the toll it takes on the lobsters.

Beyond minimising transport time, controlling temperature, moisture/humidity and vibration are the key factors to reducing the impact of transportation on lobsters and delivering strong, healthy lobsters to the processing facility. As discussed previously, keeping lobsters cool is important as it slows their metabolism, reducing the build-up of waste products. While further research is needed to identify optimum temperatures, 12-14°C is a good target for summer temperatures and 8-12°C in winter. Current Clean Green Program standards are that temperature should be between 7-12°C on any transport exceeding one-hour duration.

Humidity is the next important environmental condition factor for trucks. Having a high (>90% relative humidity) is important for preventing lobsters' gills from drying during extended emersion. In the Western Rock Lobster Fishery, trucks have been equipped to spray seawater onto lobsters, with research showing that the practice reduced the accumulation of carbon dioxide and ammonia that occurred during emersion. It did not, however, improve oxygen uptake, as haemolymph lactate levels rose over time (Spanoghe, 1996). Further research is required to determine the optimal approach for delivering healthy Southern Rock Lobster and we recommend that a range of spray or misting systems are investigated to enhance this portion of the supply chain.

Once lobsters are in crates for transport, the crates must be handled with care. Vibration associated with road travel has been shown to add to the physiological stress caused by emersion (Powell et al., 2017). Along similar lines, shocks due to dropping crates or general rough handling are likely to cause similar stress, though little research has been conducted in this area. Data loggers that can measure and record temperature, humidity and vibration are available and could be a useful tool in determining where in the transport chain lobsters are exposed to these stressors, how intense the exposure may be, and provide data necessary to determine what is excessive and how to reduce impact.

Purging

Once lobsters arrive at a facility, it is recommended to place them into water as soon as possible rather than subject them to any further emersion, handling and stress. It is a common, though not universal, practice within the industry to grade lobsters upon arrival at the facility. This handling of lobsters following a long period of transport and emersion may be adding additional stress that could easily be avoided. Instead, upon arrival, immediately placing lobsters into a purge tank may offer an improved approach that allows lobsters to recover from transport. Allowing lobsters to recover in the purge tank for 24-48 hours will enable the flushing of accumulated wastes in the haemolymph and allows the lobsters to recover from physiological disturbance. In addition, using a purge tank separate from the main holding system prevents the flushing of accumulated waste from harming existing stock in the facility. Purge systems also have the additional benefit of providing separate system to allow more gradual thermal acclimation of incoming stock. For example, during the summer months when ambient water temperatures at capture may be >20oC, maintaining the purge system at 14oC will allow an intermediate thermal acclimation period before transfer into cooler holding systems. More gradual temperature acclimation will likely reduce stress on lobsters associated

with sudden temperature changes (Lu et al., 2015). However, it is noted that thermal acclimation will be less important during the winter months where differentials between capture and holding temperature are less profound. After recovery and acclimation in the purge tank, lobsters can then be graded and placed into holding tanks.

Traceability

The development of traceability systems within facilities and across the supply chain is necessary to improve the ability to identify and respond to potential problems. At the simplest level, keeping accurate records of lobsters and their performance in holding allows processors to determine if lobsters coming from a particular tank, fisherman or regions have more problems, giving the processer evidence with which to make effective management decisions. More sophisticated systems can add other elaborations, such as tying information such as water quality testing, evidence of meeting Clean Green standards or any other data to the lobsters they process. A best practice guide to traceability will be generated by the FRDC 2016-177 Traceability systems for wild caught lobster project to provide a framework for the industry to develop these systems.

Recommendations

- Minimise air and sun exposure, keep lobsters covered whenever emersed
- Develop methods to measure lactate
- Research new methods of transport and the physiological impacts
- Sampling at various points in chain will better inform how particular aspects of handling impact lobster health and survivability (Paterson and Spanoghe 1997)

Standards

Standard	Auditing Guidelines
Exposure to sunlight, wind, rain must be avoided any time lobsters are held out of water	
Emersion time should be limited to less than 1 hour in uncontrolled conditions, such as when transferring off vessels	
Lobsters should be held in clean, impermeable bins and not over packed (e.g. no more than 2/3 full)	
Transport time should be limited to under 6 hours, as recommended by current Clean Green	

standards	
Any facility staff handling lobsters during transport shall be trained in handling best practices and animal welfare	
Drops, shocks and impacts shall be avoided during transport, either to individual lobsters or to lobsters packed in bins	
Vibrations should be measured and minimised during road transport	
During emersion, lobsters should be held at 8-14°C and >90% relative humidity	
Following any period of emersion in uncontrolled conditions and greater than 1 hour in controlled conditions, lobsters should be given 24 hours recovery time before any further emersion or handling	
Emersed lobsters should be held in purge tanks following transport to a facility and the water quality of the tank monitored to ensure it meets the parameters detailed below in the *Holding stock* section	
The biofiltration system of purge tanks must be maintained to handle the nutrient load produced by a standard lobster arrival	
Facilities should consult the Traceability best practices guide to be produced by the FRDC 2016-177 Traceability systems for wild caught lobster project to develop procedures for tracking lobsters through the facility	

Holding stock in facilities

Background

As previously discussed, emersion and handling should be minimised and when lobsters are handled, it should be gently. Lobsters arriving at a processing facility are stressed from transportation, so any further stress will have an even greater impact (Taylor and Whiteley, 1989).

Emersion

Upon arrival at a processing facility, it is common in the industry to grade lobsters according to weight. Given the air exposure involved in getting lobsters from the boat to the facility, it is recommended that lobsters be returned to water as soon as possible upon arrival at the facility and that grading be delayed until they have had a chance to recover. Previously, placing lobsters into a purge tank was discussed, as this approach allows them to rid themselves of accumulated waste without increasing the biological load on the main system. If grading is delayed for 24 hours while lobsters are held in the purge tank, it will allow the lobsters to recover from transport and potentially acclimate to holding temperatures before the emersion and handling involved with grading.

Careful handling

During grading, lobsters should be handled carefully, to avoid limb loss, dropping or tail flipping, and quickly. Processes should be optimised so that lobsters are not sitting in crates for extended periods of time out of water. Lobsters should never be tossed or thrown into crates, as this greatly increases the risk of damage or puncture. Forceful squeezing the shell to evaluate moult status should be avoided, as the shell can be cracked, damaging the lobster and weakening it to further handling stresses. Assessment of moult stage is very important consideration for assessment of lobster quality. Pre- and post- moult lobster will perform poorly in post-harvest captivity due to heightened metabolic demands associate with a complex array of energy consuming physiological processes associated with moult preparation and recovery (Fitzgibbon et al., 2014; Simon et al., 2015). Post-moult lobster will also have reduced capacity to withstand starvation and holding due to poor nutritional condition (Simon et al., 2015). The moulting process is a prolonged process and the pre-moult cycle begins well before physical signs of moult progression. Currently there are no practical and accurate methods available for industry to identify pre- and post-moult lobsters. We recommend that improved and more accurate methods are developed to assess SRL moult stage. Measurement of the refractive index of the haemolymph has been shown to be an indicator of moult status (Simon et al., 2015), though the measurement needs further study to identify the thresholds associated with each moult stage for it to be an effective tool for industry.

Vitality

Assessment of vitality is an important component of grading, as it allows for the lobsters unlikely to survive the subsequent stresses of live export to be separated from the more resilient lobsters. Vitality assessments generally rely on the observation of several reflex behaviours and, when conducted by experienced workers in the industry can have a high level of accuracy (Stoner, 2012) (Fig. 2). It is recommended that passive reflexes, such as tail strength and the posture of the antennae and legs be used to assess vitality and that strenuous active measurements like tail flipping be avoided. However, for these visual reflex response assessments to be more accurate for predicting SRL vitality and mortality requires further research. Research has shown that the most effective reflex assessment methods are those which employ numerous simple reflex responses (>6, improved with more) related to likelihood of mortality, which can be easily scored as either absent or present (Stoner, 2012). Selected reflex responses need to be tested in controlled or commercial conditions to determine whether mortality predictions can be made accurately from the reflex-based score.

This statistical relationship between reflex score and observed mortality is describe as a reflex action mortality predictor (RAMP) curve, which once determined can be used to make individual or cohort-based predictions for mortality in holding and inform stock management decisions. RAMP assessment tools have been successfully adapted to numerous commerciality important crustacean species including crab and Norway lobster and, in some cases, adopted by industry to screen product before live transport (Albalat et al., 2017; Kronstadt et al., 2018; Urban, 2015; Yochum et al., 2017). To our knowledge, RAMP curves have not been established for any spiny lobster species. When weak lobsters are identified we recommend that they are removed from the system, killed and sold. We understand that industry can commonly place weak lobsters into "hospital" tanks or partitions of tanks "sickbays". However, we do not recommend this practice as it has a high likelihood of further mortalities which may cause the spread of an infectious disease, increases nutrient and biological oxygen demand (BOD) on water systems by encouraging microbial proliferation and the encourage the inappropriate practice of sale of post-mortem lobsters.

Fig. 2. Current vitality assessment by industry graders informally use reflex responses based on lobster body posture and vigour here shown as strong (left) and weak (right)

Recirculation aquaculture systems

Once lobsters are placed into holding tanks within a facility, the quality of the water they are held in becomes the most important factor affecting their likelihood of survival. It is clear that the existing SRL processing and holding industry utilizes a wide range of aquaculture systems and technologies. Complexity of systems varies widely across holding facilities which ranges from flow-through systems were new water is continually piped from adjacent seawater sources, to in-land recirculation aquaculture systems (RAS) which are reliant on the truck delivery of seawater exchange supplies. Due to the diversity of aquaculture systems employed by industry it is difficult to make generalization about best aquaculture systems and practices. Considering RAS systems are typically more complex and difficult to manage, the following section will concentrate on these processes but many of the fundamental concepts could also be applied to flow-through systems.

Effective RAS systems commonly involved several processes including solid removal, fine and dissolved nutrient removal, biofiltration, aeration or oxygenation, pH control, and finally disinfection, typically in that order (Fig 3).

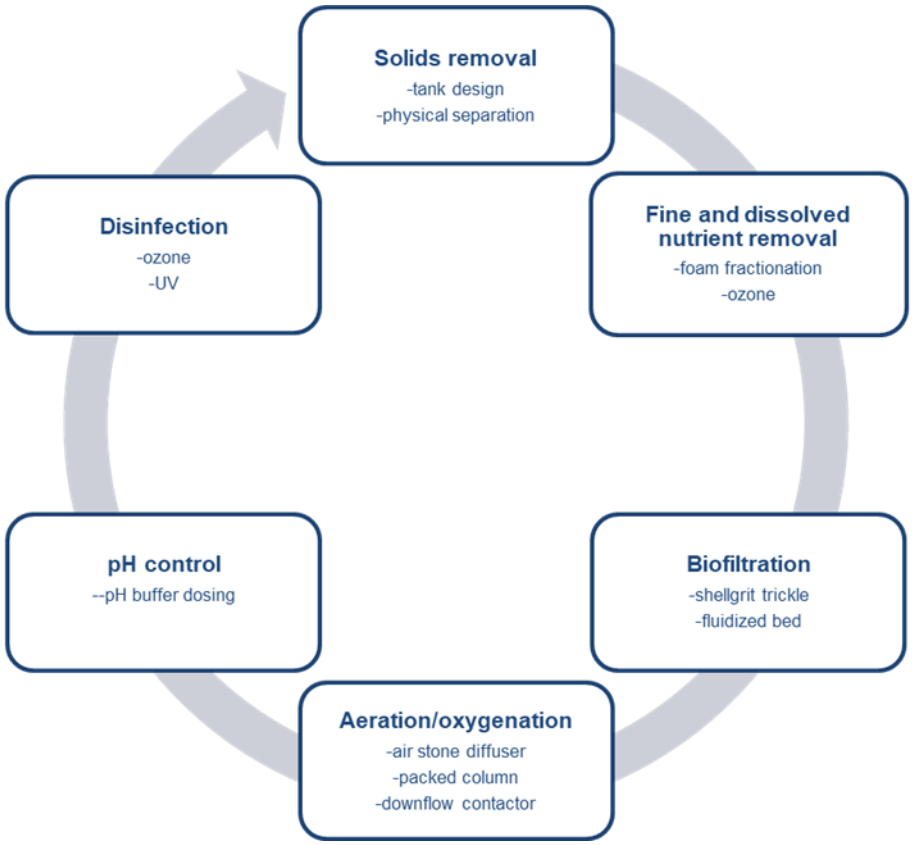

Fig. 3. Typical Systems involved in an effective recirculation aquaculture systems (RAS)

Removal of solid waste

The first step in effective RAS systems is the rapid and efficient removal of solid waste such as faeces, uneaten feed, lost appendages and mortalities. Feeding is not recommended in SRL holding as this increases lobster oxygen demands, excretory wastes and overall demands on RAS systems (Crear and Forteath, 2000; 2002). Despite most facilities not employing feeding, significant amounts of solid waste can accumulate in tank systems, particularly in purge tanks in parts of the fishery where lobsters are quickly transfer from capture to holding. Rapid removal of solid waste in needed because if left, will quickly degrade and become a part of the dissolved nutrient load which is more difficult to remove and will contribute to overall system BOD. In fish aquaculture, solid removal is typically achieved through tank and water flow designs which allow passive removal of settleable solids through tank bottom water flow velocities and subsequent mechanical separation. However, the SRL holding industry typically employs raceway tank designs which have poor self-cleaning properties which is further complicated by lobster stock and commonly, additional tank infrastructure (such as creates) that disrupt water flow patterns. Alternative and better self-cleaning raceway tank designs are available, such as cross-flow and multi-cell raceways (Wong and Piedrahita, 2000) however in the absence of these systems we recommend that tanks are manually cleaned regularly (at least

daily) through sweeping and siphoning. Regular (at least daily) monitoring of stock is also necessary to allow rapid removal or any weak or dead lobsters.

Fine or dissolved organics removal

Once large solids are removed, the next step for water processing is the removal of fine or dissolved organics (less than 30 microns). This is best achieved through effective foam fractionation. Foam fractionation is recommended because it reduces water nutrient load, and BOD, and can assist in oxygenation and water degassing. It is best performed before biofiltration so to limit the amount of organics entering the filter media. When using foam fractionation, routine care should be taken to keep the waste cone clean. Automatic cone flushing is preferable, but care needs to be taken to ensure that fresh cleaning water does not enter RAS water supplies, as this can lower salinity.

Biofiltration

The next step in effective RAS systems is biofiltration. Biofilters are essentially systems that support the growth of beneficial bacteria that removal/convert ammonia excreted from the lobster stock in a process called nitrification. Effective biofilters support the growth of two types of bacteria, Nitrosomonas which coverts ammonia (NH4) to nitrite (NO2) and Nitrobactor which converts nitrite to nitrate (NO3). Both ammonia and nitrite are acutely toxic to lobsters. Lobsters can tolerate much higher levels of nitrate however it will gradually accumulate in working RAS and can typically only be removed by new water exchange. Functioning biofilters are mostly about effective surface area (biomedia) which provides the living space for the bacteria. This living space needs to have direct contact with the RAS water supply. Biofilters should also be kept clean because the accumulation of organic material will encourage the growth of competing bacteria limiting the effectiveness of the biofilters nitrification capacity and potentially being a source for disease. Currently, most SRL processing facilities use trickle or static biofilters with shell-grit as the biomedia. While these biofilters can be effective, common disadvantages of these systems include water channelling through the media and they are easily clogged by organic wastes. Regular cleaning (at least annually) is recommended when using shell-grit as the biomedia. Most modern RAS systems worldwide employ moving or fluidized bed biofilters with plastic biomedia. As in these biofilters the biomedia is constantly moving, it is always in contact with the RAS water and it does not accumulate organic wastes. Moving or fluidized bed biofilters are also considered more efficient than alternative types of biofiltration due to greater effective substrate surface area. Going forward, we would recommend that the industry increases its biofiltration capacity by using moving or fluidized bed biofilters.

Biofilter maintenance

As biofilters are a living system, care must be taken to ensure that they are always healthy and productive. For established biofilters, this is best achieved by ensuring they always have a constant supply of food in the form of ammonia. This food supply is usually provided through excretion from lobster stock. However, during periods of low or no stocking, it is

recommended that RAS systems are fed with an ammonia source such as cloudy ammonia or ammonia chloride, to ensure healthy activity of the nitrifying bacteria. If a biofilter is shut down or intensively cleaned between seasons, it is likely that the nitrifying bacteria will be killed. In these cases, the biofilter will need to be seeded and conditioned before it will be able to effectively process high levels of ammonia. Biofilter seeding products are commercially available which may hasten the conditioning of new biofilters. A recommended alternative for biofilter seeding is the transfer of clean but already conditioned media from an alternative active and healthy biofilter. During closed seasons, we recommend that facilities maintain and feed at least one active biofilter which can be used to seed new filters after cleaning. Once seeded, a new biofilter must be fed and monitored until it is effective of converting sufficient amounts of ammonia to nitrate before lobsters are stocked into the system. In our experience, this can take several weeks to months, particularly for the establishment of Nitrobactor for the conversion of nitrite to nitrate (NO3).

Aeration

Aeration or oxygenation is necessary in RAS for maintaining high levels of oxygen saturation for meeting the lobster and system oxygen demands. If sufficient oxygen is not available to lobsters, they will resort to anaerobic metabolism to support energy demands. Anaerobic metabolism leads to the production of lactate which is not sustainable and will result in death if prolonged. Vigorous aeration in tank systems is recommended for maintaining water oxygen saturation levels during lobster holding. Aeration also acts to circulate tank water assisting the elimination of tank ‘dead’ spots, help with pH control through carbon dioxide degassing and may provide an advantage by providing lobsters cover from stressful visual disturbance from outside activities. Tank oxygen levels should be recorded regularly (at least daily) in all systems and at differing positions within tanks. It is also noted that dissolved gasses such as oxygen and nitrogen can become supersaturated (>100%) in water which can be highly detrimental to lobsters. Supersaturation typically occurs when air is incorporated into the water under high pressure. This can occur due to a leak at the inlet side of a pump or through an air stone being sucked into a pump. Supersaturation can be difficult to measure, however, oxygen saturations levels greater than 100% would suggest a supersaturation problem. In the case of supersaturation, check pumps for leaks and airlines. Oxygenation through the supply of purified oxygen is generally not recommend or considered necessary for lobster holding.

Water pH

pH of RAS commonly decline due to the accumulation of excreted carbon dioxide and nitrogenous waste from the cultured animal. While the chronic tolerance levels of SRL are not well established it is recommended that pH of RAS systems are maintained close to natural seawater levels (pH 8.05-8.15). Finding from the FRDC 2016/235 Improving post-harvest survivability project, found a common trend for low water pH (6.8-7.7) in SRL processing facilities and suggested an association between these acidic conditions and mortality during holding. For these reasons we recommend that SRL holding facilities employ pH control systems by dosing with alkaline chemical buffers. Common buffers used for pH dosing in aquaculture include sodium bicarbonate, sodium hydroxide and calcium carbonate. The differing buffers have differing advantages in terms of ease of use, and health and safety

considerations. A typical pH dosing system requires a mix tank, dosing apparatus (dose pump) and pH control or monitoring system.

Water disinfection

The FRDC 2016/235 Improving post-harvest survivability project also isolated bacteria associated with disease and mortality in some holding facilities. Bacterial disease is best controlled and prevented in RAS though water disinfection techniques. The most common water disinfection techniques for aquaculture include ultra violet (UV) sterilization or ozone disinfection. These disinfection processes are best achieved as the final water polishing step before clean water is returned to the culture tanks. UV sterilization is simple to employ with very few associated risks. However, it most effective in clean water and routine maintenance must be conducted to ensure the lamps are functioning and effective. Ozone is a much more powerful water conditioning and disinfection tool however it comes with considerable more risks in its effective and safe application. Ozonation of seawater produces persistent oxidation by-products which if allowed to build up, will be toxic to lobsters. Ozone gas is also acutely toxic to humans, so it also comes with significant human safety concerns. In our opinion, ozone is a superior water disinfection tool but should only be implemented by experienced users and carefully monitored so not to detrimentally impact the lobsters or facility personnel. We do recommend that UV sterilization is incorporated in to SRL RAS as a simple and low risk disinfection technique.

Temperature

Southern Rock Lobster can tolerate temperatures ranging from 8-23°C, with a suggested optimum holding temperature of 9-13°C (Crear and Allen, 2002). Observations of facilities around Tasmania and South Australia indicate that most recirculating systems keep their water around 9-10°C and flow through systems tend to be slightly warmer, around 12°C, particularly in summer. Cooler temperatures offer benefits, with lobsters experiencing a slowing of their metabolism, reduced activity and reduced aggression. However, large and rapid temperature changes can induce thermal stress, so we recommend gradual temperature acclimation particularly during the summer months when there are large temperature differences between the wild and in holding. We recommend that holding systems are maintained at 8-12°C throughout the year however during the summer months lobsters are provided with at least 24 h acclimation at 12-14oC on entry to the facility.

pH

The pH of water describes how acidic or alkaline it is. Seawater generally has a pH of around 8.05-8.15. The SRL Clean Green program recommends a pH of 7.80-8.20 and the FRDC guide to Optimising Water Quality (Crear and Allen, 2002) recommends 7.80-8.40. the FRDC 2016/235 Improving post-harvest survivability project found pH in facilities generally ranged from 6.79-7.88, substantially lower than natural seawater. This depression is likely due to the presence of acidic waste products in the water, such as CO2 and nitrogenous by-products. Currently, we do not have adequate information on the optimum pH thresholds for

SRL or the impacts of chronic exposure to low pH. However, the investigations by the FRDC 2016/235 Improving post-harvest survivability project suggest that pH may have play a role in SRL mortality events. As such, we recommend that regular (at least daily) monitoring of facility water pH and that facilities aim to maintain water pH in facilities to be at as close to natural seawater pH (8.05-8.15) as possible.

Ammonia

Ammonia is the most important nutrient to monitor and manage, as it is the most toxic and is directly released by lobsters into the water as waste. High levels of ammonia can cause mortality in SRL and sublethal levels can increase aggression levels. Ammonia can build up to toxic levels when the biological filtration capacity of the system is exceeded, which typically occur when volume of stock exceeds the biofiltration capacity, when new biofilters are inadequately seeded and conditioned and when lobsters rapidly excrete large volumes of ammonia after stressful periods on emersion.

Ammonia also has a relationship with temperature and pH that is important to understand. In water, ammonia can exist in two forms, the toxic unionised NH3 and the less toxic ionised NH4. As temperature and pH increase, ammonia is converted from the less toxic form to the more toxic form. However, at the temperatures common to SRL facilities (9-15°C), the proportion of toxic NH3 is generally quite low (Table 1).

Table 1. Relationship between the composition of unionised (percent) by pH and temperature.

pH	Temperature (°C)		
	8	12	16
7.0	0.2	0.2	0.3
8.0	1.6	2.1	2.9
8.2	2.5	3.3	4.5
8.4	3.9	5.2	6.9
8.6	6.0	7.9	10.6
8.8	9.2	12.0	15.8
9.0	13.8	17.8	22.9

Clean Green recommendations for ammonia are <0.5 mg L-1 and Optimising Water Quality recommends <2 mg L-1. Ammonia becomes lethal at concentrations of 20-30 mg L-1 (Battaglene et al., 2004), but the effect of chronic exposure to lower levels is not currently known. The FRDC 2016/235 Improving post-harvest survivability project found that ammonia levels in SRL facilities were typically adequate and ranged from 0.0-0.55 mg L-1.

Nitrite

In a RAS, the biofiltration of the system converts highly toxic ammonia to moderately toxic nitrite. Clean Green recommendations for nitrite are <1.0 mg L-1, Optimising Water Quality recommends <5 mg L-1 and researchers have used 10 mg L-1 as for sub-lethal exposure (Battaglene et al., 2004), though the effects of chronic exposure to nitrite are unknown. The FRDC 2016/235 Improving post-harvest survivability project found that nitrite levels in SRL facilities were typically adequate and ranged from 0.0-0.58 mg L-1. Reduced salinity has been suggested to increase the toxicity of nitrite in lobsters.

Nitrate

Nitrate, which is generated by the nitrification of nitrite, is the low toxicity final step of the nitrogen cycle in RAS. Nitrate builds up in systems and must be physically removed via water changes. The toxicity levels, either acute or chronic, of nitrate have not been characterised for SRL, but in prawns, levels above 2,200 mg L-1 were found to be toxic (Romano and Zeng, 2013). Clean Green and Optimising Water Quality recommend levels of <100-140 mg L-1 and <100 mg L-1, respectively. The FRDC 2016/235 Improving post-harvest survivability project found that nitrate levels in SRL facilities were typically adequate and ranged from 0.036 – 13.0 mg L-1.

Phosphorous

Phosphorus can enter facilities as contamination of the source water from industrial, residential or agricultural run-off or from within the facility through the use of detergents. It is not of high concern in facilities as it has minimal toxicity, however, phosphorous is a limiting nutrient to algal growth, so can fuel algal blooms if tanks receive enough light. The FRDC 2016/235 Improving post-harvest survivability project found low concentrations of phosphorus (3-7 mg L-1 P) in a small number of facilities. In these facilities, source water has been tested and confirmed to not contain phosphorous, indicating that the contamination came from the facility. A characteristic shared by all of the facilities in which phosphorous was detected was that the holding tanks were made of concrete blocks (i.e. Bessar blocks) that were exposed to water. Phosphates are present in concrete and mortar and are likely leaching into the water from exposed blocks.

Alkalinity

Alkalinity is a measure of how well water is buffered against acidic changes to pH – high alkalinity means that addition of acidic lobster waste products will have less of an effect on pH. The alkalinity of seawater is around 116 mg L-1 as CaCO3, and Clean Green and Optimising Water Quality both recommend 100-200 mg L-1 as CaCO3. Alkalinity was not routinely tested in facilities by the FRDC 2016/235 Improving post-harvest survivability project, but the limited data collected suggests a considerable variation, with values from 68 to 164 mg L-1 recorded. The results of the FRDC 2016/235 Improving post-harvest

survivability project suggest that alkalinity may play a more important role in lobsters health than previously understood, making this an area of interest for future research.

Salinity

Salinity can be tested using a hydrometer, and refractometer or a salinity meter, with each representing an improvement in sophistication and accuracy, with a corresponding increase in cost, respectively. Seawater has a salinity of around 35 ppt. Clean Green recommends a salinity of 30-38 ppt, with 35-36 ppt considered optimal. Optimising Water Quality gives a recommended range of 30-38 ppt. The FRDC 2016/235 Improving post-harvest survivability project found that salinity in SRL facilities were typically adequate and ranged from 33.3-36.6 ppt.

Dissolved oxygen

Dissolved oxygen can be measured using two different types of probes – a Clark type electrode and an optical probe. Clark electrodes cost less than optical probes but require routine maintenance to operate properly. Optical probes are more expensive but are more robust and do not require much upkeep. Clean Green recommends that dissolved oxygen levels remain above 70% and Optimising Water Quality recommends a minimum of 70% and a preferred level of at least 80%. The FRDC 2016/235 Improving post-harvest survivability project found that dissolved oxygen levels in facilities were typically adequate and ranged from 79.4%-100%. Levels over 100% have been observed at times and it is important to understand that a reading over 100% does not indicate a faulty meter, but gas supersaturation. Supersaturation is generally caused by a leak in the pumps of a facility, where air is sucked in and mixed into the water by the pump impeller. This causes oxygen and nitrogen levels to increase to potentially dangerous levels. Pumps should be checked regularly, and any evidence of air intake investigated and repaired.

Other holding considerations

Once lobsters are placed into tanks with high quality water, keeping them in that water is the next major consideration. Moving lobsters between tanks should be avoided and transporting between facilities can introduce major biosecurity concerns. While in tanks, lobsters will benefit from being provided cover from visual disturbance. Whether this is a physical structure, such as shade cloth over tanks, or simply surface agitation from aeration, reducing the amount of light and movement lobsters can see will reduce the likelihood of lobsters responding to visual stimuli which will likely reduce stress. Along similar lines, lighting regimes must be considered, though there is currently insufficient evidence to make any conclusive recommendations. However, SRL are typically more active during the night and research has shown that metabolic demands and activity increase during periods of darkness (Crear and Forteath, 2000). For this reason we recommend continual low light levels during holding.

Recommendations

- Development of testing regimes – daily water quality, more frequently during stressful times; recording results; long term development of alarmed, logging approach
- Further research to better define acceptable levels, particularly with an eye towards chronic exposures
- Sampling at various points in chain will better inform how particular aspects of handling impact lobster health and survivability (Paterson and Spanoghe 1997)

Standards

Standard	Auditing Guidelines
Grading should be conducted following a 24 hour recovery period	
Employees involved in the grading process should be trained in handling best practices and animal welfare	
Passive reflex behaviours, such as antennae and leg posture, should be used to assess vitality rather than tail flipping or pinching eyes	
Hospital tanks should not be used in facilities	
Tanks must be cleaned of solid wastes at least daily	
Biofilter systems using shell grit must be cleaned annually	
During periods of low or no stock, biofilters must be maintained or re-seeded and conditioned prior to the introduction of new stock	
Dissolved oxygen levels must be monitored to ensure aeration is adequate (above 80%) and to detect supersaturation (levels above 100%)	
Pumps must be checked on a monthly basis for air leaks that can cause supersaturation	
Water temperature, pH and nutrient levels should be maintained at the levels recommended by Clean Green standards	

Outbound stock (Exporting)

Background

The final component of lobster processing is the preparation for pack out, the packaging of lobsters for transport to overseas markets. Transport from the factory to the destination market is the most stressful part of the process, as it involves handling, extended emersion, transport and exposure to uncontrolled conditions.

Chilling

It is during pack out that the greatest care in handling needs to be taken, as losses during export are the most damaging for the Southern Rock Lobster brand reputation. For this reason, every precaution to avoid limb loss or general damage should be taken.

One of the methods for avoiding damage during pack out is chilling lobsters down. Although methods throughout the industry vary from cold dips to extended chilling times that last several hours, research suggests that long, gradual chilling is the ideal approach (Spanoghe, 1996), as it reduces movement and slows metabolism, lessening the immediate impact of emersion. Shorter chill times appear effective due to immobilisation of the lobsters, but the impact on their haemolymph biochemistry indicates that the treatment is not as effective as longer chill times. Based on research and current practices, it appears that chilling lobsters to 8-10°C over a period of at least several hours is the best approach. During summer, if facility water temperatures are considerably warmer, the warmer end of this range may be more appropriate, as large swings in temperature can be stressful.

Anaesthetic

Some facilities use anaesthetics, such as the Isoeugenol based AQUI-S, instead of chilling. A recent study (Robertson et al., 2018) conducted on the efficacy of AQUI-S suggested that low doses of 40 ppm may be effective in short term immobilisation and the reduction of stress associated with handling but that considerably larger doses (200 ppm) may be necessary for deeper anaesthesia that may improve resilience to the immersion of transport. Unfortunately, this higher dosage is near the estimated 250 ppm maximum dosage tolerated by SRL, leaving little room for error. Furthermore, SRL were found to clear the anaesthetic relatively quickly, particularly when immersed in water, limiting the duration of the anaesthetic effect of AQUI-S. Currently, the only permitted use for AQUI-S in Australian live seafood industry is in the handling and harvesting of salmonids by or under the direction of a registered veterinarian and within the limitations of the APVMA registered product label. Due to this, the SRL industry should never use Isoeugenol based anaesthetics in commercial processing.

Fig. 4. Polystyrene box packed with Southern Rock Lobsters prepared for live export. Image from Green et al. (2011).

Packing

Once lobsters are ready to be packed for export, the next consideration is maintaining optimal conditions within the packing box. Polystyrene (poly) boxes are commonly used throughout the industry due to their insulating abilities (fig. 4). These boxes are packed with ice and wood wool to keep a cool temperature and moist environment. In poly boxes packed in this way and held for a simulated shipping period, temperatures in the box has been shown to quickly drop to >10°C following sealing at pack out, maintain a temperature around 10°C for 40 hours after sealing and then rise to 13-17°C by 64 hours after sealing (Simon et al., 2016). With this increase in temperature came an increase in mortality, from 3% mortality in the 40-hour trial to as much as 63% mortality after 64 hours (Simon et al., 2016). Based on this, keeping the time lobsters are sealed in the poly boxes to under 40 hours is ideal. Lobsters in both 40-hour and 64-hour trials showed evidence of dehydration (Simon et al., 2016), suggesting that steps should be taken to increase humidity in the sealed poly boxes, such as soaking wood wool in seawater before use. Humidity should be maintained at a level greater than 90% in poly boxes, and commercially available temperature and humidity loggers can be used to verify that these conditions are maintained.

Handling

Although it may seem that lobsters packed in poly boxes and surrounded by wood wool and foam sheets are safe and protected, careful handling at this stage remains important. Studies on transportation have shown that vibration emulating that of road transport increased stress on lobsters (Powell et al., 2017). Lobsters will be subjected to a range of vibration and impacts throughout the export process and it is important to keep these to a minimum. Data on the types and intensities of vibration and impacts experienced during the export process are limited, but personal observations have included seeing poly boxes tossed and slammed from the back of a truck onto pallets and lobsters being tossed from poly boxes into tanks at wholesale facilities. Similarly, difficulties with some airlines treating poly boxes poorly have

been communicated by the industry. It is difficult to control these factors that occur outside of the facility, but an important first step is to identify and measure these factors using audits of handling along the supply chain and vibration data loggers in poly boxes.

Recommendations

- Further investigation into the use of anaesthetics and chilling
- Measurements of temperature, humidity and vibration of poly boxes to develop thresholds

Standards

Standards	Auditing Guidelines
Employees involved in the pack out process should be trained in handling best practices and animal welfare	
Lobsters should be prepared for pack out by chilling at 8-10°C for a period of at least 2 hours	
In sealed poly boxes containing lobsters, the temperature and relative humidity should be maintained at 10-12°C and >90%, respectively	
The duration of transportation in which lobsters are sealed in poly boxes should not exceed 40 hours	
Vibration, shock and impact of poly boxes should be monitored and minimised	

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Conclusion

Collectively, the physiological and pathological investigations did not support the systematic association of a detectable crustacean pathogen with lower post-harvest SRL survival. However, a field survey suggested a limited holding capability in some exporting facilities leading to sub-standard stock survival and impacting commercial processes. Anecdotal reports from industry suggest an increased sensitivity of lobsters in recent seasons and the project hypothesis is that the animals are experiencing reduced holding capacity and increased mortality rates post capture as a result higher physiological stress, reduced immune function and then overwhelming infections with secondary bacteria or loss of physiological maintenance leading to death. However, further investigations, particularly on the role of potential unknown viral pathogens, is required to completely rule-out an infectious aetiology.

The cause for the apparent sensitivity remains unclear, which could be related with environmental stress, such as increasing water temperatures. The south east coast of Australia has been identified as an ocean warming hotspot, where rates of warming are up to four times the global average and have recently experienced its longest and most intense marine heatwave ever recorded (Hobday and Pecl, 2014; Oliver et al., 2017; Pecl et al., 2014). The rate and capacity of all physiological processes are strongly impacted by temperature which is one of the most dominant environmental factors influencing the biology and performance of aquatic ectotherms such as lobsters (Fitzgibbon et al., 2017). Temperature is known to be a crucial factor influencing lobster nutritional condition (Fitzgibbon et al., 2017) and seasonal biological processes such as moulting and reproduction (Sachlikidis et al., 2005). The impact of this environmental change on SRL physiology currently remains unclear and should form the basis of future research. The project is particularly concerned that environmental change may be impacting lobster moult cycles resulting in weak pre- and post-moult lobsters being landed during the fishing season. Pre- and post- moult lobsters will perform poorly in captivity due to heightened metabolic demands associated with a complex array of energy consuming physiological processes associated with degradation and reconstruction of the new cuticle (Fitzgibbon et al., 2014; Simon et al., 2015). Post-moult lobster will also have reduced capacity to withstand starvation and holding due to poor nutritional condition (Simon et al., 2015). The moulting process is a prolonged process and the pre-moult cycle begins well before physical signs of moult progression. Currently there are no practical and accurate methods available for industry to identify pre- and post-moult lobsters. The project strongly recommends that improved tools for the assessment of lobster moult cycle and stress condition are developed and made available to industry stakeholders across the entire post-harvest chain of custody.

Investigation revealed considerable differences in industry live lobster management practices and apparent levels of mortality across the sector. These findings suggest a role of post-harvest practices for improving lobster performance in holding. Physiological and epidemiological investigation particularly highlight the potential for transport related emersion, stocking practices and sub-optimal physicochemical water quality (particularly low pH) to impact SRL mortality in holding. Mortality appears most severe during the warmer months during summer likely due to heightened metabolic demands of lobsters associated with higher ambient temperatures. The project strongly recommends that the Australian SRL industry focusses dedicated effort towards optimising post-harvest practices from wild capture to international

export. The project findings suggest a need for improved industry practices, particularly in the key areas of:

- Post capture transport, particularly on land dry transport procedures
- Post transport recovery and purging procedures
- Holding facility aquaculture systems
- Water quality monitoring and maintenance
- Handling procedures
- Data collection and stock traceability
- Stock quality assessments

The project has taken significant steps towards facilitating improved industry practice through:

- Initial validation of improved lobster quality assessment tools, handheld lactate meter
- Assessment of water quality measurement tools
- Industry education on best practices through industry workshops
- The development of an SRL processors industry best practice guide
- Development and validation of new immune function assays for SRL
- Development of SRL haemolymph biochemical profiling capacity in Australia
- Development of an SRL health assessment procedure manual

In conclusion, the SRL industry is in a period of change and the industry must adapt its post-harvest practices to adapt to this change. The industry is changing in terms of its business model, political atmosphere and physical environment, all of which heighten the requirement for optimal post-harvest processes. The value of Australian lobsters has increased by 132% since 2005-06 and now being nearly entirely reliant on the international export of live product (Mobsby and Koduah, 2017). This increased value and demand on the quality of the product has meant that even low levels of stock losses can have significant financial consequences and the arrival of morbid or dead lobsters at international markets can damage the reputation of the SRL industry brand. The political atmosphere is changing as consumer perceptions on the health and welfare of lobsters kept alive in a manner that does not adversely affect food safety and quality is increasingly becoming an important marketing issue (Esposito et al., 2016). Better animal husbandry practices that improve lobster welfare are necessary to ensure Australia's reputation for clean, safe and quality-controlled food production. Finally, SRL exists in a global warming hot spot which appears to be placing new challenges for appropriate post-harvest processes. It is clear that the ultimate outcome for a lobster is dependent on the sum of its experiences through the entire post-harvest chain of custody. In the absence of a traceability system, this project has focused on practices by the processing and holding industry sector. For optimum performance of SRL from capture to market,

requires further research on other aspects of post-harvest processes, including the fishing industry and post export maintenance sectors but this requires tracing individual or batches of lobster from capture to export.

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Implications

The project considers that the lack of finding of an infectious aetiology systematically associated with poor survival is an optimistic outcome for industry as this dismisses potential consumer concerns, negates the need for government or market notification, reduces the need for enhanced biosecurity procedures or restrictions and is potentially easier to resolve through improved lobster maintenance systems and procedures.

However, the findings of the study strongly suggest a need for improved practices throughout the entire post-harvest chain of custody. Recommendation for improved practices are provided in the following section.

Recommendations

A key outcome of the project is the recommendations for improved aquaculture practices in order to limit the impacts of lobster mortality on the SRL industry and brand reputation. Key areas identified for improvement include:

- Post capture transport, particularly on land dry transport procedures: Investigations by the physiological and epidemiological component of the project consistently found an association between post landing transport time or distance and SRL mortality during holding. It is well established that emersion during dry transport induces considerable stress on lobsters. Further research is required to determine the optimal approach for delivering healthy SRL and we recommend that a range of more advanced spray or misting transport systems are investigated to enhance this component of the supply chain.

- Post transport recovery and purging procedures: Epidemiological investigation identified a wide diversity in industry practices and suggest the need for determining the influence of individual practices on lobster performance. Post-transport purging was identified as a key differential among industry participants and a key point in the supply chain where sub-optimal survival is occurring. We recommend that the industry employ purging techniques post-transport as this allows lobsters to purge built-up waste products caused by emersion and allows recovery time for lobsters to return to a normal physiological state before further grading and handling. However, further research is required to determine optimum purging protocols.

- Holding facility aquaculture systems: The SRL processing industry sector generally employs low technology aquaculture systems and there is significant scope for the industry adoption of more advanced systems. Some key areas for advancement are through the use of more modern fluidized bed biofilters and automated pH control. Pathology findings also found an association between novel lobster bacterial disease and mortality during holding. For the control of bacterial disease in holding we recommend that industry more actively utilize water disinfection techniques including ultra violet (UV) sterilization or ozone disinfection.

- Water quality monitoring and maintenance: Maintaining and monitory water quality is of utmost importance for aquaculture management. Epidemiological investigations showed that the degree of water quality monitoring ranged widely among industry participants. We recommend that facilities develop more rigorous water testing regimes and monitor key water quality parameters at least daily and more frequently during stressful times such as purging. The project identified a key lack of knowledge for optimum levels and impacts of chronic exposure for most water quality parameters for SRL. Further research to better define acceptable levels, particularly with regards to chronic exposure is required. Physiological investigation identified water pH as a key water quality parameter that was commonly sub-optimal in SRL facilities. The project recommends that further research is required on the impacts of low pH on SRL and industry employ improved techniques to maintain water pH to be at as close to natural seawater pH as possible.

- Handling procedures: Poor lobster handling techniques can induce physical damage, physiological stress and reduce immunocompetence of lobsters. The project commonly witnessed sub-optimal lobster handling techniques during industry practices. We recommend an education program that delivers information on best practice handling for animal welfare for any employee within the supply chain that handles lobsters.

- Data collection and stock traceability: Epidemiological investigations identified clear inadequacies in industry data collection and traceability systems. The improved development of traceability systems within SRL facilities and across the supply chain is necessary to improve the ability to identify and respond to potential problems.

- Stock quality assessments: Stock quality assessments are essential to allow industry to implement better stock management decisions which will reduce stock losses and improve lobster welfare. The project conducted the initial validation of handheld lactate meters as an industry tool for assessing lobster stress levels. We recommend further validation of the lactate and the refractive index assessment tools to provide industry with superior evaluation matrix for determining the condition and competency of lobsters for holding and live export.

Further development

The project workshops gathered feedback from industry members about industry concerns and future research priorities. The project identified 19 knowledge gaps or potential future research priorities regarding best post-harvest practice for live SRL. Written feedback on ranking of these research priorities were received from more than 35 industry participants (Table 1). Based on this feedback the top priority rank future research from industry for the post-harvest maintenance of live SRL was:

- The development of improved tools and validation of condition/vitality assessments and relationship to survivability during holding, including Brix, lactate meter, reflex responses

Further to these ranked priorities, another priority which was not ranked at the workshop but was brought up by industry participants as a high priority was:

- The assessment and refinement of on-vessel handling and maintenance processes to improve post capture lobster condition/vitality/survivability

The project supports these two research priorities as important future research priorities as they extend best practice across the entire chain of post-harvest custody from capture to export and will result in tools that will inform fisherman and processors on the quality of the stock and suitability of maintenance practices. We consider the next most important research priority would be in the development of improve post capture transport technologies and procedure to limit emersion stress on lobsters.

Table 1: Priority ranking of future research priorities provided by industry participants at the SRL best practice workshops presented in Hobart and Mt. Gambier.

Future research priority	**High Priority (%)**	**Medium priority (%)**	**Low Priority (%)**
Improved tools and validation of condition/vitality assessments and relationship to survivability during holding, brix, lactate meter, reflex	**67.6**	32.4	0.0
Optimum temperature and acclimation rates for SRL in holding and transport	**67.6**	20.6	11.8
Effect of holding industry practices, purging, grading, crating, etc on lobster performance	**64.7**	17.6	17.6
Optimum dry transport methods and transport time thresholds	**61.8**	17.6	20.6
Better methods to determine moult cycle, relationship between Brix index and moult cycle	**58.8**	29.4	11.8
Chronic tolerance and optimal levels for most water and environmental parameters, Nh4, No2, No3, Salinity, Alkalinity	**52.9**	38.2	8.8
Improved understanding of seawater chemistry during holding and more accurate and efficient tools for measurement	**52.9**	29.4	17.6
Effect of a changing environment on lobster physiology (nutritional condition/moult cycles) across the fishery range	**50.0**	35.3	14.7
Continued facilitation by the SRL industry for investigations of morbidity and mortality in animals	**47.1**	32.4	20.6
Preparedness for response to disease outbreaks	**44.1**	41.2	14.7
pH/alkalinity threshold range and optimal pH dosing practices for SRL holding	**41.2**	55.9	2.9
Lobster stock monitoring tools and data collection systems during holding	**41.2**	32.4	26.5
Biosecurity quality control of lobster stocks at the fishery source	**35.3**	41.2	23.5
Optimal and threshold levels of ozonation in SRL holding	**29.4**	44.1	26.5
Benefits of socking or banding to reduce tail flipping and negative physical interactions	**17.6**	29.4	52.9
Maximum starvation time and the effect of moult/nutritional	**17.6**	50.0	32.4

stage and temperature			
The optimal lighting regime during SRL holding	**17.6**	70.6	11.8
Develop and undertake a diagnostic sample collection workshop for industry for on farm health assessments and sample submission for diagnostics	**17.6**	47.1	35.3
Establish haemolymph analysis capacity in country (based at Roseworthy VDL) for access by all of industry	**14.7**	52.9	32.4

Extension and Adoption

Extension

Key to the success and extension of the project was extensive interaction with SRL processing and exporting industry sector stakeholders. This extension was initially facilitated through email to industry members across the sector in southern Australia. Project introductions were followed up through extensive SRL processing facility face-to-face meetings and facility tours. These facility tours and discussions with industry managers were crucial for:

- Developing rapport with the industry stakeholders and making them aware of the project and its aims;
- Surveying the diversity of SRL processing industry practices in relation to live lobster handling and maintenance practices;
- Acquiring collaborative facilities willing to contribute to the project by providing stock management and performance data, contribute to epidemiology surveys, and the supply of pathological samples;
- Disseminating research findings and lobster management advice among industry stakeholders.

Over the course of the project, project investigators visited approximately 95% of active live SRL processing and export facilities throughout Tasmania, South Australia and Victoria (Table 1). Facility tours facilitated collaborative relationships between the project and industry sector members and project investigators subsequently were available for numerous ad hoc consultations through phone and e-mail. The project extended findings to lobster industry representative through presentations to the Sustainable Marine Research Collaboration Agreement (SMRCA), December 2017, the Australian Southern Rock Lobster Exporters Association (ARLEA), August 2018 and regular updates of project progress to the Southern Rocklobster Ltd research, development and extension committee meetings. The project extended it research through consultation and collaboration with the FRDC 2016/177 *Traceability systems for with caught lobster* project and the FRDC 2017/224 *Clean Green Digitisation* project. These collaborations with synergistic FRDC SRL projects led to productive partnerships including the share of resources and staff with the FRDC 2016/177 Traceability project and the incorporation of a research objective (best practice guide) in collaboration with the FRDC 2017/224 Clean Green project. The project also extended findings and concepts for future research direction with the SRL fishing industry sector through meeting with Tasmanian Rock Lobster Fisherman's Association (TRLFA), December 2018 and the project collaborated with the TRLFA in the preparation a fishing industry newsletter in the Tasmanian Seafood Industry News (Volume 16, Feb/Mar, 2019).

Table 1. Summary of company site visit conducted by project investigators

Location	**Company name**
Tasmania	
Hobart	SOUTH AUSTRALIAN LOBSTER COMPANY PTY LTD (SALCO)
	TASSIE LOBSTER PTY LTD
	TONY GARTH SEAFOODS
	MURES FISHING PTY LTD
	MAKO CONSTITUTION DOCK
Sorell	JATALY AUSTRALIA PTY LTD
Dover	CRAIG MOSTYN & CO PTY LTD
	OCEAN BLUE TREASURE PTY LTD
	VAN DIEMAN SEAFOOD PTY LTD
Bicheno	CRAIG MOSTYN & CO PTY LTD
	TRIGONIA SEAFOODS PTY LTD
Dunalley	SOUTHERN UNITED SEAFOOD AUSTRALIA PTY LTD
Huonville	ABTEC PTY LTD.
St. Helens	SALTY SEAS
	NGF PTY LTD
Stanley	HURSEY SEAFOODS
George Town	GEORGETOWN SEAFOODS PTY LTD.
King Island	MD PTY LTD (FACILITY-1)
	MD PTY LTD (FACILITY-2)
South Australia	
Adelaide	FERGUSON AUSTRALIA PTY LTD
	THE FISH FACTORY

	MARK LEE FISH FARM
Mount Gambier	SOUTH AUSTRALIAN LOBSTER COMPANY PTY LTD (SALCO)
	FITZGERALD'S FISH SALE (BEST OCIANA SEAFOOD PTY LTD)
Carpenter Rocks	BEST OCIANA SEAFOOD PTY LTD
Southend	SOUTH AUSTRALIAN LOBSTER COMPANY PTY LTD (SALCO)
Port Macdonnell	FIVE STAR SEAFOODS PTY LTD (FERGUSON AUSTRALIA PTY LTD)
	JOE'S LIVE LOBSTERS PTY LTD
	DW & KA FOX PTY LTD
	PACIFIC SHOJI PTY LTD
Robe	SKY SEAFOODS
	FERGUSON AUSTRALIA PTY LTD
	LIMESTONE COAST SEAFOODS PTY LTD
Mount Schanck	SOUTHERN UNITED SEAFOOD AUSTRALIA PTY LTD
Cape Jaffa	KS & J RAYNER NOMINEES PTY LTD
	SOUTHERN UNITED SEAFOOD AUSTRALIA PTY LTD
Beachport	THE FISH FACTORY PTY LTD
Kingston	LACEPEDE SEAFOODS (SHOPWISE PTY LTD)
Warooka (Yorke Peninsula)	INLAND SEAFOODS
Port Lincoln	SOUTHERN OCEAN ROCK LOBSTER LTD. (SALCO)
	AUSTRALIAN BIGHT SEAFOOD PTY LTD (AUSSEA SEAFOOD)
	THE FISH FACTORY
	MORI SEAFOOD

Kingscote, Kangaroo Island	FERGUSON AUSTRALIA PTY LTD
	THE FISH FACTORY
Vivonne Bay, Kangaroo Island	FERGUSON AUSTRALIA PTY LTD
Victoria	
Warnambool	ALLFRESH SEAFOODS
Apollo Bay	APOLLO BAY FISHERMEN CO-OP (THE HARBOUR FISH SHOP)
San Remo	SAN REMO FISHERMAN'S CO-OP
Avalon	PACIFIC SHOJI PTY LTD (LARA)
Melbourne	SOUTHERN UNITED SEAFOOD AUSTRALIA PTY LTD
	SOUTH AUSTRALIAN LOBSTER COMPANY PTY LTD (SALCO)

An important outcome of the project was the presentation of Southern Rock Lobster Processors Industry, Best Practices Workshops. These one-day industry workshops were offered to SRL industry members in Hobart 11th October 2018 and Mount Gambier 22nd October 2018 and provided a update of project finding and covered current best holding practices for live southern rock lobsters. The workshops were presented by:

- Dr Quinn Fitzgibbon and Dr Ryan Day, University of Tasmania (Lobster physiology and aquaculture);
- Dr Charles Caraguel and Dr Kardarp Patel, University of Adelaide (Aquatic epidemiology);
- Dr Stephen Pyecroft and Johanna Mahadevan, University of Adelaide (Aquatic animal health).

In collaboration with:

- Paul Turner and Luke Mirowski, University of Tasmania, FRDC Traceability Systems Analysis (TSA) for Phase Two Southern Rock Lobster Project;
- Lachlan Bassett and Ross Briggs, Fresh by Design, Aquaculture Products and Systems.

The all-day workshops were attended by more than 60 Australian SRL industry members and presented 180 slides of material covering a wide range of critical lobster holding principles including (Appendix 10):

- lobster biology/physiology at capture, transfer, holding and shipping;
- water quality – principles, monitoring and management;
- aquaculture systems and maintenance;
- handling, feeding and transport practices;
- biosecurity principles;
- traceability – pros & cons;
- lobster health – monitoring and main disorders.

Further to covering the state knowledge for the industry principles, the workshop presented an update on the FRDC project progress, an analysis of water quality measurement tools and presented a new simple tool for SRL stress assessment (hand held lactate meter) (Appendix 10).

Figure 1; Dr Quinn Fitzgibbon presenting and attendees at the Hobart Southern Rock Lobster Processors Industry, Best Practices Workshop

Adoption

At this early stage of project completion, it is difficult to assess and define the adoption of project outcome by the SRL processors and exporting industry. Anecdotal evidence suggests that in general, the industry has accepted the need to improve industry practices and several companies have made efforts to improve operations. Feedback from industry suggests that levels of mortalities across the sector have reduced in recent seasons which may be because of these improved maintenance practices. We have received strong feedback from several industry participants that they have changed, and improved practices based on project recommendations which has resulted in improved lobster performance. Attached to this final report is a letter of support and impact statement from one of Australia's largest SRL processors and export companies. This letter has not been included in this report as it contains company detail that could be considered commercially sensitive. This impact statement details

how the company pro-actively acted on project findings/recommendations and implemented numerous changes to company practices including improvements in water treatment systems, post-harvest operations, cold chain transport and biosecurity. In the most recent 2018-19 season, the company has experienced a 50% reduction in mortality compared to the previous season that may be attributed to these improved practices. The project batch level investigations (Results Chapter 4 and 5) reported a post-harvest mortality rate of approximated 1.5% (1.4-1.7%) which across the SRL industry would represent more than AUD 3.8 million loss of revenue annually (based on an estimated industry value of AUD 255 million, Mobsby and Koduah, 2017). A 50% reduction to this loss to mortality represents a FRDC return for investment of 3-fold in just a single year and 30-fold over a decade. The industry has now formed a national representative body, the Australian Southern Rock Lobster Exporters Association (ARLEA), and we consider that the project has helped to better unite the industry sector.

Project coverage

The project collaborated with the TRLFA in the preparation a fishing industry newsletter in the Tasmanian Seafood Industry News (Volume 16, Feb/Mar, 2019).

Project materials developed

The project has prepared three industry documents which should be published and presented to industry participants these include:

- SRL processors industry best practice guide;
- Assessment of water quality measurement tools;
- SRL health assessment procedure manual.

The project is preparing several manuscripts based on the research chapters for scientific publication. One manuscript has already been accepted for publication.

- Day, R. D., Fitzgibbon, Q. P., Gardner, C. (In Press). "The impact of holding stressors on the immune function and haemolymph biochemistry of Southern Rock Lobsters (*Jasus edwardsii*)." Fish and Shellfish Immunology.

Appendix 1. Custom designed questionnaire used to collect information on capacity and management in surveyed holding facilities.

UNIVERSITY of TASMANIA

IMAS
INSTITUTE FOR MARINE & ANTARCTIC STUDIES

THE UNIVERSITY of ADELAIDE

in conjunction with

Southern Rock Lobster Post-harvest Survival enhancement

Survey questionnaire

Research into solutions

Interviewer: Kandarp Patel (BVSc&Ah, PGDipVPH, PhD)

<u>This survey contains six sections</u>:

Sections 1 and 2 : General and infrastructure information

Sections 3 and 4: Specific information about water and lobster management at the facility

Section 5: Lobster numbers and health

Section 6: Source of data

Questionnaire

SECTION 1: General information

Lobster processing facility/site name:

Contact person: _____

Owner/manager

Processing facility/site **physical address**: _____

_____

_____

_____

Processing facility/site **postal address**: _____

_____

_____

_____

Phone: _____

Fax: _____

Mobile: _____

E-mail: _____

Preferred contact method: Mail/ Phone/ Email

Manager experience and background

Total number of years in lobster **industry**: **Fishing**: _____ year(s), **Processing**:_____ year(s)

Number of years running/managing the present **facility**: _____ year(s)

Formal **education/training** in aquaculture or water systems **Yes / No**

Diploma/Degree: _____, Duration:_____

Certificate:_____, Duration_____

Staff number and background

Number of full- and part- time staff in facility. **Full**-time_____, **Part**-time_____

Busy/peak season		**Off-season**	
Full time	**Part-time**	**Full time**	**Part-time**

Is formal training acquired by staff for working with lobsters: **Yes / No**

If YES: Full time_____, Part-time_____

SECTION 2: Facility infrastructure information

General information

Total number of tanks: _____

Is each tank operated with an independent water system **Yes / No**

If No, no. of systems and **interconnected** tanks: _____

System no.	**No. of interconnected tanks**

Tank **characteristics**:

Tank	**Type; Purging/ Holding/ shipping/**	**Shape**	**Max. stocking density (kgs)**	**No. of compartments/ baskets/ crates**	**Tank material (plastic/ metal/ fibre glass/ cement)**	**Internal lining (smooth/ rough)**	**Volume m^3**	**Length/ diameter (m)**	**Breadth (m)**	**Height (m)**
Tank-1										
Tank-2										
Tank-3										
Tank-4										
Tank-5										
Tank-6										
Tank-7										
Tank-8										
Tank-9										

Tank-10										

Cleaning/removal of hard/visible matter from tank

Frequency:

Daily

Once a week

Fortnightly

Once a month

Once every 6 months

Never

Other: _____

Cleaning method:

Aquatic vacuum siphoning

Manual siphoning

Other:
_____
_____
_____
_____

Lighting over tanks in facility:

Frequency:

Always on

Time on _____

Time off_____

Other: _____

SECTION 3: Water and water system

Type of water system used at facility:

Water system	**No./ Id of tanks**
Flow through	

Recirculation system	

Source/s of water used in lobster holding/shipping tanks

Water source	**Tick**	**Usage in percentage (total 100%)**
Artificial		
Trucked, **location**_____		
Pumped-in (direct line): shore		
Pumped-in: borewell		
Running tapwater		
Other:_____		

If '**Artificial'**, preparation method:

_____
_____
_____
_____

Presence of **settling tank** for incoming water source at facility **Yes / No**

IF YES, a) **Capacity: _____** m^3**,**

b) Number of **days water stored** before being used:_____ days

Any **pre-treatment** of sourced water **Yes / No**

If YES, treatment method:

_____
_____
_____

Settling tank **cleaning** frequency:

Daily

Once a week

Fortnightly

Once a month

Once every 6 months

Never

Other:_____

Cleaning method:

_____
_____
_____
_____

Desaturation and **mechanical** filtration system for water at facility

If YES:

Tank	**Tank id**	**Aerator /water flow unit (Yes /No)**	**Mechanical filter type** **(e.g. sand, drum, percolation, bags, other)**	**Mechanical filter size (microns)**
Tank-1				
Tank-2				
Tank-3				
Tank-4				
Tank-5				
Tank-6				
Tank-7				
Tank-8				
Tank-9				
Tank-10				

Desaturation unit **cleaning** frequency:

Daily

Once a week

Fortnightly

Once a month

Once every 6 months

Never

Other:_____

Never

Cleaning method:

_____
_____
_____
_____

Mechanical filtration system **cleaning** frequency:

Daily

Once a week

Fortnightly

Once a month

Once every 6 months

Never

Other:_____

Cleaning method:

Biofiltration system and **protein skimmer** at facility

Tank	**Tank Id**	**Protein skimmer unit (yes/ no)**	**Biofilter type** **(e.g. shells, bio-balls, shell bags, artificial media, trickle system, fluidized bed)**	**Media / culture used** **(frequency, brand)**
Tank-1				
Tank-2				
Tank-3				
Tank-4				
Tank-5				
Tank-6				
Tank-7				
Tank-8				
Tank-9				
Tank-10				

Cleaning frequency	**Protein skimmer**	**Biofilter**
Daily		
Once a week		
Fortnightly		
Once a month		
Once every six months		
Never		
Other:		

Protein skimmer cleaning method:

_____
_____
_____
_____

Biofilter cleaning and start-up / maintenance method:

_____
_____
_____
_____

_____
___

_____
___

_____
___

_____
___

Water **disinfection** at facility **Yes / No**

If YES, disinfection **method**:

Ozone

UV rays

Other treatment:_____

Other treatment method:

_____

Disinfection frequency	**Time period**
Always on	
Daily	
Once a week	
Fortnightly	
Once a month	
Once every six months	
Other:	

Presence of dedicated water **chilling unit** at facility **Yes / No**

If YES, **Holding** tank temperature maintained at _____^{0}C

Shipping tank temperature maintained at _____^{0}C

Dedicated water **aeration** unit at facility **Yes / No**

If YES, method used and location in water system

Aeration stone, _____

Air cone, _____

Air pipe, _____

Venturi, _____

Other: _____

Recirculation water turn over time and volume

Tank	Pump capacity (Hp or litres/min)	Litres or % volume /hr or day
Tank-1		
Tank-2		
Tank-3		
Tank-4		
Tank-5		
Tank-6		
Tank-7		
Tank-8		
Tank-9		
Tank-10		
Total		

Water changed/added/replaced in the lobster holding tanks **Yes / No**

Water change:

Completely replaced, water source used:_____

Water added only, water source used:_____

Conditional on _____

Frequency	Completely replaced	New water added in (Litres or % volume)	Water taken out (litres or % volume)
Daily			
Once a week			
Fortnightly			
Once a month			
Once every six months			
Other:			

Automation and **alarms** in the water recirculation system **Yes / No**

If YES,

Parameter	**Automation (yes/no)**	**Rate/value maintained**	**Alarm (yes/no)**
Water flow			
pH			
Temperature			
Salinity			
Ammonia			
Nitrite			
Nitrate			
Dissolved O_2			
Ozone			
Power			
Other:_____			

Water **quality monitored** in lobster holding tanks **Yes / No**

If YES, parameters monitored

Parameter	**Method**	**Brand/kit name**	**Checking frequency**
pH			
Temperature			
Salinity			
Ammonia			

Nitrite			
Nitrate			
Dissolved O_2			
Ozone			
Other: **_____**			

Do you have records of **water quality monitoring data** from your facility from 2015 onwards **Yes / No**

SECTION 4: Lobster stock management

Maximum lobster stocking **capacity** of facility:

Busy season: _____ tonnes (or kgs)

Off season: _____ tonnes (or kgs)

Lobster **class** currently held:

Southern rock lobster

Eastern rock lobster (Not to be included in this research)

Other: _____(Not to be included in this research)

Lobster supply **source**:

Port/facility	**No of supplier/fisherman (if port)**	**Kms or time to facility**

Lobsters allowed to **purge** after arrival into the facility **Yes / No**

If YES, purging details:_____

Lobsters purged in	Time period
Crate	
Basket	
Free swimming	

Lobster **grading** and separation **Yes / No**

(If YES) Are lobsters stocked based on grades in separate tanks **Yes / No**

Stocking method:

Crate

Basket

Free swimming

Other: _____

Lobster **holding period** at facility: Avg:_____ weeks and _____ days

Max: _____ weeks and _____ days

Are lobsters fed while being held in the holding facility? **Yes / No**

If YES, type/brand of feed_____

Feeding frequency_____(e.g. twice/week)

SECTION 5: Lobster numbers and health

Are lobsters checked for **sickness** /mortality **Yes / No**

If YES, method:

_____
_____
_____
_____

Frequency	Time of the day (morning/ evening/ fixed time)
Daily	

Once a week	
Fortnightly	
Once a month	
Once every six months	
Other:	

Fate of **sick/moribund** lobsters:

Kept in same tank

Transferred in another (quarantine/ sick bay) tank

Other: _____

<u>Previous laboratory investigations in lobsters</u> over the past five years (2012, 2013, 2014, 2015, 2016).

Yes / No

Month & year	**No sent for investigation**	**Diagnosis (disease/condition)**	**Diagnosis confirmed by veterinarian or Lab. test? (tick (✓) as many apply)**		
			Veterinarian	**Lab**	
				Hemolymph	**Tissue**
Eg Dec 2012	**3**	**High potassium and sodium**		✓	

Did you observe higher than usual mortality (at least twice) in 2015-16 season compared to previous seasons (2012-13, 2013-14, and 2014-15)? **Yes / No**

If YES, did you observe similar levels of mortality in 2016-17 season as 2015-16 season?

Yes / No

Monthly lobster holding **records** from current/previous year/s **Yes / No**

Month	Year 2012-13					Year 2013-14			
	Stock at start (Kg)	Stock out (Kg)	Stock in (Kg)	Stock dead (Kg)		Stock at start (Kg)	Stock out (Kg)	Stock in (Kg)	Stock dead (Kg)
Jan									
Feb									
Mar									
Apr									
May									
Jun									
Jul									
Aug									
Sep									
Oct									
Nov									
Dec									
Total									

Month	Year 2014-15					Year 15-16			
	Stock at start (Kg)	Stock out (Kg)	Stock in (Kg)	Stock dead (Kg)		Stock at start (Kg)	Stock out (Kg)	Stock in (Kg)	Stock dead (Kg)
Jan									

Feb									
Mar									
Apr									
May									
Jun									
Jul									
Aug									
Sep									
Oct									
Nov									
Dec									
Total									

Month	**Year 2016-17**					**Year 2017-18**			
	Stock at start (Kg)	**Stock out (Kg)**	**Stock in (Kg)**	**Stock dead (Kg)**		**Stock at start (Kg)**	**Stock out (Kg)**	**Stock in (Kg)**	**Stock dead (Kg)**
Jan									
Feb									
Mar									
Apr									
May									
Jun									
Jul									
Aug									
Sep									
Oct									
Nov									

Dec									
Total									

SECTION 6: The information provided in this questionnaire was based on:

Written records of facility data

Memory

Mostly memory + a few recorded data

Mostly recorded data + memory

SECTION 7: Comment from facility manager/owner/staff member

Appendix 2. List of facility-level factors generated from information collected during the interview using a custom designed questionnaire.

Section	Factor description
General	State
	Flooring (wood/cement)
	Building insulation (yes/no)
	Building insulation type
Staff	Contact person type (owner/manager/both)
	Fishing experience (years)
	Total SRL processing experience (years)
	Current facility SRL processing experience (years)
	Formal education (yes/no)
	Diploma/degree name
	Degree duration (months)
	Degree type
	No. of full time staff
	No. of part time staff
	Formal training to new staff (yes/no)
	Type of formal training to new staff
Holding tank and system	Total no. of holding tanks
	One tank per system (yes/no)
	Total no of systems
	No of systems with interconnected tanks
	Total no of interconnected tanks
	Purging system separate (yes/no)

	Tank shape
	Maximum stocking capacity all tanks together
	Total no of compartments in holding tanks
	Total volume (all tanks)
	Total surface area (all tanks)
	Tank material
	Internal surface of tank
	Crate length
	Crate width
	Crate height
	Maximum stocking capacity in crate
	Frequency of tank cleaning
	Tank cleaning method
	Other tank cleaning method
	Water lost to manual cleaning or siphoning (yes/no)
	Lighting frequency over tanks
Water system and management	Primary water system
	Primary water source
	Water sourcing location
	Distance from water source to facility
	Time from water source to facility
	Presence of storage tanks for incoming water (yes/no)
	Capacity of storage tanks
	No. of days water stored before being used
	Pre-treatment of sourced water (yes/no)
	Type of pre-treatment
	Storage tank cleaning frequency
	Storage tank cleaning method
	Presence of desaturator in water system (yes/no)
	Presence of mechanical filter in water system (yes/no)
	Mechanical filter type

Mechanical cleaning frequency
Mechanical cleaning method
Presence of carbon filter in water system (yes/no)
Presence of protein skimmer in water system (yes/no)
Presence of bio-filter in water system (yes/no)
Type of bio-filter
Content of bio-filter
- Shellgrit (yes/no)
- Bioballs (yes/no)
- Quartz (yes/no)
- Zeolite (yes/no)
- Coral (yes/no)
- Abalone/oyster/scallop shells
Bio-filter shape
Water flow direction in bio-filter
Bio-filter tank material (concrete/plastic/PVC)
Media culture or ammonium chloride used (yes/no)
Name/brand of media/culture used
Ammonium chloride used to start up bio-filter (yes/no)
Culture used to start up bio-filter (yes/no)
Dead fish used to start up bio-filter (yes/no)
Bio-filter layers
Bio-filter start up before (days)
Bio-filter start up method
Bio-filter pumps running all year round (yes/no)
Bio-filter cleaning frequency
Bio-filter cleaning method
Water used for cleaning
Bio-filter content dried after cleaning (yes/no)
Protein skimmer cleaning frequency
Protein skimmer cleaning method

Presence of disinfection unit (yes/no)
Disinfection method (ozone/UV/no)
Disinfection frequency
Presence of chilling unit (yes/no)
Holding temperature
Shipping temperature
Added aeration (o2 pump/venturi)
Water change frequency
Condition/factor responsible for water change
Water topped up OR replaced
% water change
Power alarm (yes/no)
Water flow alarm (yes/no)
Other alarm
Water quality monitored (yes/no)
Water testing frequency
pH monitored (yes/no)
pH monitoring method
Salinity monitored (yes/no)
Salinity monitoring method
Ammonia monitored (yes/no)
Ammonia monitoring method
Nitrite monitored (yes/no)
Nitrite monitoring method
Nitrate monitored (yes/no)
Nitrate monitoring method
Dissolved oxygen monitored (yes/no)
Dissolved oxygen monitoring method
Ozone monitored (yes/no)
Ozone monitoring method
Chlorine monitored (yes/no)

	Chlorine monitoring method
	Water testing records (yes/no)
Stock management	Annual tonnage in 2015_16 season
	Other species held in holding tank (yes/no)
	Lobster sourced from ports/facility
	Total no of SRL source ports
	Total no of SRL source facilities
	Total number of SRL source fishermen
	SRL purged on arrival (yes/no)
	Purging time (hrs)
	SRL grading (yes/no)
	Grading done at (arrival/destocking/never)
	Stock according to sizes (yes/no)
	Mixing of old batch with new batch (yes/no)
	Stocking method
	Average stocking period (days)
	Average stocking period min
	Average stocking period max
	SRL fed while in tanks (yes/no)
	Feeding frequency
	Food name/brand
	Shipping system separate (yes/no)
	Health and mortality check (yes/no)
	Health check method
	Health check frequency
	No. of times checked
	Fate of sick lobsters
	Previous SRL laboratory submission
	Laboratory submission details
	Holding records available (yes/no)
	Holding record type

Appendix 3. Summary of qualitative holding facility level risk factors related to capacity, bio-filtration tank management, water management and stock management and unconditional association with owner/manager's perceived mortality experience in 2015-16 fishing season.

Group, *sub-group*, factor	Factor levels	N	No. of case facilities	F-test *P*-value
Capacity related risk factor				
Building				
Floor material	Cemented	49	22	0.25
	Wooden	3	0	
Insulation (roof and/or walls)	Yes	38	18	0.34
	No	14	4	
Owner manages the facility	Yes	20	6	0.25
	No	32	16	
Staff				
Manager: SRL fishing experience	Yes	11	7	0.17
	No	41	15	
Manager: Formal aquaculture education/training	Yes	4	3	0.30
	No	48	19	
Informal training acquired by staff	Yes	33	15	0.58
	No	19	7	
Water system				

Holding tank material	Cement	11	6	0.49
	Freezer glass panels	41	16	
Holding tank cleaning machine	Yes	10	5	0.73
	No	42	17	
Primary water system	RAS[a]	49	21	0.63
	Flowthrough	3	1	
Primary water source	Direct pipeline	21	8	0.78
	Trucked	31	14	
Storage tank	Yes	19	9	0.77
	No	33	13	
Presence of mechanical filter in water system	Yes	18	9	0.56
	No	34	13	
Presence of bio-filtration tank in water system	Yes	48	20	1
	No	4	2	
Presence of pH doser in water system	Yes	2	2	0.17
	No	50	20	
Protein skimmer attached to water system	Yes	32	15	0.56
	No	20	7	
Presence of disinfection unit in water system	Yes	21	9	1
	No	31	13	
Presence of chilling unit	Yes	50	22	0.5
	No	2	0	
Added water aeration	Yes	45	19	1
	No	7	3	
Power alarm in facility	Yes	37	16	1
	No	15	6	
Water flow alarm within water system	Yes	21	7	0.39
	No	31	15	
Separate water system for shipping	Yes	5	5	0.01
	No	47	17	
Separate water system for purging SRL	Yes	3	3	0.07
	No	49	19	

Appendix 3. continued

Bio-filtration system related risk factors	Levels	N	No. of case facilities	F-test *P*-value
Water flow direction in bio-filtration tank	Downwards	28	14	0.66
	Upwards	16	5	
	Horizontal	2	1	
	NA	6	2	
Bio-filtration conditioning	Yes	44	19	0.86
	No	4	1	
	NA	4	2	
Seed bio-filtration system with artificial media or ammonium chloride	Yes	8	5	0.38
	No	40	15	
	NA	4	2	
Run bio-filtration system (weeks before season start)	≤ 2	13	5	0.34
	> 2	15	9	
	Bio-filtration system active all year round	20	6	
	NA	4	2	
Clean bio-filtration tank and content	Yes	41	18	0.78
	No	7	2	
	NA	4	2	
Water used for cleaning bio-filtration tank content	Tapwater	19	12	0.02
	Saltwater	18	4	
	Borewater	2	2	
	NA	4	2	
	Never clean bio-filtration tank/content	9	2	
Bio-filtration tank content dried under sun after cleaning	Yes	14	9	0.20
	No	25	9	
	NA	4	2	

Appendix 3. continued

Water management related risk factors	Levels	N	No. of case facilities	F-test *P* value
Holding tank cleaning frequency	Regularly	23	12	0.36
	Ad hoc	8	3	
	At destocking	18	5	
	Never	3	2	
Pre-treatment of sourced water: type of treatment	Aeration	2	1	1
	UV	2	1	
	No pre-treatment	48	20	
Mechanical filter cleaning frequency	Every 1 to 7 days	4	4	0.10
	Every 2 to 5 years	4	2	
	Ad hoc	9	3	
	No mechanical filter	35	13	
Water addition / replacement frequency	Regularly	30	15	0.34
	At destocking	3	0	
	Ad hoc	16	6	
	NA	3	1	
Mode of water addition	Top up only	26	11	0.84
	Replace with similar volume	23	10	
	NA	3	1	
Water testing frequency	At season start	9	5	0.42
	Regularly	23	11	
	Ad hoc	10	2	
	No water monitoring	10	4	
Water pH monitored	Yes	22	11	0.40
	No	30	11	
Water salinity monitored	Yes	23	10	1
	No	29	12	
Water ammonia monitored	Yes	33	15	0.58
	No	19	7	
Water nitrite monitored	Yes	32	15	0.56

	No	20	7	
Water nitrate monitored	Yes	29	12	1
	No	23	10	
Water dissolved oxygen monitored	Yes	14	9	0.06
	No	38	13	

Appendix 3. continued

Stock management related risk factors	Levels	N	No. of case facilities	F-test *P*-value
Use of lights over holding tanks	Ad hoc	13	1	0.004
	Always on	39	21	
Water temperature in holding tanks	≤ 12	39	17	0.75
	> 12	11	5	
	No chilling unit	2	0	
Primary SRL source	Other facility	5	1	0.38
	Ports	47	21	
Allow purging of SRL in separate system or tank	Yes	17	13	<0.001
	No	35	9	
SRL grading	After purging	17	12	0.02
	At arrival in facility	29	8	
	Never	6	2	
SRL stocked by class size	No	17	4	0.08
	Yes	35	18	
Mix old and new batch	Yes	40	17	1
	No	12	5	
SRL stocking method	Crate	18	7	0.77
	Freeswum	34	15	
Average stocking period (weeks)	≤ 1 week	35	9	<0.001
	> 1 week	17	13	
Feed SRL while in holding tanks	Yes	7	4	0.44
	No	45	18	
Fate of sick SRL	Remains in same system	31	14	0.33

	Transferred to other system	6	1
	Culled	13	7

[a] Recirculating aquaculture system

Appendix 4. Summary of facility level quantitative risk factors related to capacity, water management and stock management in case and non-case facilities as determined by owner/manager's perceived mortality experience in 2015-16 fishing season.

Section, risk factor	Case facilities (N = 22)		Non-case facilities (N = 30)		Total	
	Mean (Sd)	Range	Mean (Sd)	Range	Mean (Sd)	Range
Capacity related						
Manager: total experience in SRL processing (years)	15.9 (10.2)	0.3 - 33	20.1 (10.4)	2 - 37	18.3 (10.4)	0.3 - 37
Manager: experience managing current facility (years)	10.2 (8.7)	0.3 - 25	12.9 (9.1)	2 - 30	11.7 (8.9)	0.3 – 30
No. of permanent staff (including the manager)	4 (2.0)[a]	1 - 9 [a]	3 (2.0) [a]	1 - 9 [a]	3 (2) [a]	1 – 9 [a]
No. of casual staff	6 (6.0)[b]	1 - 20 [b]	3 (2.0) [b]	1 - 10[b]	5 (4.5) [b]	1 - 20 [b]
Total volume in holding tanks (m^3)	73.8 (61.8)	2.4 - 242.5	48.6 (36.8)	0.4 - 141.8	59.2 (50.0)	0.4 – 242.5
Water volume per water system	19.8 (20.2)	2.4 - 89.4	21.6 (19.4)	0.4 - 77	20.9 (19.6)	0.4 – 89.4
Water management						
Water addition (%)	14.8 (8.2)	5 - 33	12.7 (6.1)	1 - 25	13.6 (7.0)	1 – 33
Water replacement (%)	68.7 (32.5)	25 - 100	69.7 (34.2)	20 - 100	69.3 (32.8)	20 - 100
Stock management						
Stocking density (kg/m^3)	114.5 (82.8)	40.4 - 445.9	115.1 (51.2)	34.3 - 256.6	114.9 (65.7)	71.6 – 256.6
Annual tonnage in 2015-16 fishing season	124.6 (156.6)	0.5 - 500	67.6 (56.4)	0.3 - 240	91.7 (112.8)	0.3 – 500
No. of SRL sources	6 (5)	1 - 16	4 (2)	1 - 8	4 (3)	1 – 16
No. of fishermen SRL sourced from	17 (18)	1 - 75	10 (9)	0 - 36	13 (14)	1 - 75
Mean driving time from source to facility (hrs)	1.9 (1.6)	0.04 - 4.6	1.7 (2.0)	0.05 – 9.0	1.8 (1.9)	0.04 – 9.0

[a] Facilities without permanent staff were excluded from the analysis. [b] Facilities without casual staff were excluded from the analysi

Appendix 5: Summary of SRL stock rejected and observed dead with no apparent damage at grading and number of SRL at risk, cumulative sub-optimal survival, and sub-optimal survival rate (per 1000 SRL-days) and the percentage of SRL lost from arrival to packing by fisherman.

Fisherman id	At grading			During holding			% SRL lost
	Total graded	% Rejected[a]	% sub-optimal survival[b]	No. at risk	% cumulative sub-optimal survival[b]	Sub-optimal survival rate (no. cases / 1000 SRL-days)	
1	806	0.38	1.36	762	4.12	2.5	5.86
2	2,272	1.41	1.01	2,196	1.63	1.13	4.05
3	1,364	0.51	0.44	1,156	2.89	2.00	3.84
4	2,097	0.86	0	1,994	2.97	1.56	3.83
5	631	1.11	0.16	594	2.49	3.70	3.76
6	1,766	1.53	0.28	1,709	1.91	1.28	3.72
7	6,782	1.8	0.60	6,545	1.07	1.08	3.47
8	377	2.65	0.53	326	0	0	3.18
9	545	2.21	0.18	499	0.40	0.18	2.79
10	2,896	1.21	0.10	2,824	1.34	1.50	2.65
11	1,787	1.79	0.17	1,747	0.46	0.92	2.42
12	1,323	1.29	0.30	1,255	0.79	0.67	2.38
13	6,717	1.04	0.30	6,568	0.94	0.57	2.28
14	1,516	1.12	0.20	1,481	0.94	0.66	2.26
15	2,522	0.79	0.04	2,451	1.38	1.10	2.21
16	3,440	0.96	0.29	3,257	0.83	0.57	2.08

17	3,600	1.47	0.28	3,232	0.31	0.15	2.06
18	207	1.45	0	194	0.52	0.73	1.97
19	13,333	0.54	0.15	12,635	1.17	0.92	1.86
20	7,176	0.98	0.04	6,666	0.84	0.49	1.86
21	2,540	1.54	0	2,478	0.28	0.50	1.82
22	583	0	0.17	501	1.58	1.28	1.75
23	1,964	0.36	0.25	1,942	1.08	0.69	1.69
24	2,281	1.14	0.04	2,231	0.49	0.83	1.67
25	2,068	0.39	0.05	1,866	1.17	0.95	1.61
26	20,428	0.68	0.17	19,664	0.74	0.53	1.59
27	19,092	0.61	0.18	16,299	0.79	0.60	1.58
28	4,012	1	0	3,903	0.54	0.36	1.54
29	469	0.64	0.85	434	0	0	1.49
30	6,488	0.25	0.09	5,784	1.14	0.97	1.48
31	2,638	0.34	0	2,298	1.08	1.14	1.42
32	4,312	0.61	0.02	4,205	0.47	0.41	1.1
33	3,124	0.55	0.19	3,087	0.1	0.08	0.84
34	10,199	0.38	0.04	9,014	0.2	0.07	0.62
35	1,067	0.37	0.19	867	0	0	0.56
36	2,347	0.39	0.04	2,284	0.04	0.02	0.47
37	2,262	0.04	0	2,158	0.23	0.17	0.27
38	173	0	0	165	0	0	0
Missing	88	1.14	0	NA	NA	NA	NA

a – All rejected SRL excluding those found dead with no apparent damage.
b – All dead SRL observed with no apparent damage (sub-optimal survival).

Appendix 6. Summary of SRL stock rejected and observed dead with no apparent damage at grading and number of SRL at risk, cumulative sub-optimal survival, and sub-optimal survival rate (per 1000 SRL-days) and the percentage of SRL lost from arrival to packing by docket number.

Docket	Grading date	Fisherman	Port	At grading			During holding			% SRL lost
				Total graded	% Rejected[a]	% sub-optimal survival[b]	No. at risk	% cumulative sub-optimal survival[b]	Sub-optimal survival rate (no. cases / per 1000 SRL-days)	
1	18/11/2016	16	8	538	1.86	0	528	0	0	1.86
2	19/11/2016	26	10	2981	1.48	0.60	2915	0.38	0.82	2.46
3	20/11/2016	34	7	1646	0.91	0	1613	0.31	0.66	1.22
4	21/11/2016	33	10	1061	0.29	0.09	1057	0.19	0.32	0.57
5	21/11/2016	33	10	649	0.92	0	643	0	0	0.92
6	21/11/2016	28	7	1635	2.14	0	1559	0.64	0.9	2.78
7	22/11/2016	24	3	1114	1.80	0.09	1075	0.09	0.17	1.98
8	22/11/2016	10	7	1359	1.84	0.22	1330	0.23	0.45	2.29
9	23/11/2016	26	7	2236	1.83	0.09	2193	0.09	0.15	2.01
10	24/11/2016	21	7	2540	1.54	0	2478	0.28	0.50	1.82
11	25/11/2016	12	7	1323	1.29	0.30	1255	0.79	0.67	2.38
12	25/11/2016	13	7	1729	0.92	0.12	1688	0.77	1.24	1.81
13	25/11/2016	19	13	1440	0	0	1438	0.49	0.40	0.49
14	28/11/2016	11	13	1787	1.79	0.17	1747	0.46	0.92	2.42

15	28/11/2016	24	6	1167	0.51	0	1156	0.86	1.39	1.37
16	30/11/2016	7	7	2576	3.07	0.35	2462	2.01	2.00	5.43
17	2/12/2016	15	2	266	2.63	0.38	257	0.39	0.41	3.40
18	3/12/2016	26	7	1739	0.46	0.40	1676	1.54	0.98	2.40
19	3/12/2016	2	3	366	1.91	0	350	0.85	0.67	2.76
20	5/12/2016	27	11	446	0.44	1.35	428	4.11	3.32	5.90
21	5/12/2016	15	2	189	1.06	0	184	9.09	14.57	10.15
22	6/12/2016	3	1	331	0.91	0.30	311	2.84	1.96	4.05
23	6/12/2016	10	7	1269	0.71	0	1236	1.05	0.89	1.76
24	8/12/2016	27	11	1125	0.98	0	1109	1.53	0.86	2.51
25	8/12/2016	14	6	930	0	0	918	0.43	0.36	0.43
26	8/12/2016	16	9	99	0	0	98	2.02	1.68	2.02
27	8/12/2016	6	11	919	1.63	0	890	2.88	2.24	4.51
28	12/12/2016	19	13	1041	0	0	1035	1.15	0.64	1.15
29	12/12/2016	2	14	128	0	0	126	4.69	2.41	4.69
30	12/12/2016	15	2	152	0.66	0	150	3.31	1.70	3.97
31	13/12/2016	2	4	340	1.47	0.29	331	3.89	2.09	5.65
32	13/12/2016	16	9	512	0	0	511	0.39	0.16	0.39
33	19/12/2016	27	11	566	1.41	0.53	539	0.19	0.19	2.13
34	19/12/2016	19	13	1335	1.95	1.27	1290	0.39	0.35	3.61
35	19/12/2016	2	14	332	1.21	0.90	325	0	0	2.11
36	19/12/2016	15	7	197	0.51	0	196	0	0	0.51
37	20/12/2016	14	11	586	2.90	0.51	563	1.77	0.97	5.18
38	20/12/2016	16	9	374	2.67	0.27	359	0	0	2.94
39	20/12/2016	6	11	847	1.42	0.59	819	0.85	0.49	2.86
40	23/12/2016	26	7	1853	0	0	1852	0.27	0.20	0.27
41	23/12/2016	16	9	237	1.27	0	233	1.28	1.42	2.55
42	24/12/2016	27	11	706	0	0.28	703	0.14	0.09	0.42
43	24/12/2016	15	2	185	0.54	0	184	0	0	0.54
44	27/12/2016	13	7	1365	1.24	0.44	1341	0.30	0.31	1.98
45	29/12/2016	2	14	95	0	0	95	0	0	0

46	30/12/2016	15	2	163	0	0	162	1.84	0.99	1.84
47	3/01/2017	27	11	588	1.19	0	572	0.17	0.06	1.36
48	3/01/2017	26	10	416	2.89	0.48	395	0.51	1.11	3.88
49	3/01/2017	4	7	1412	1.06	0	1359	1.10	0.62	2.16
50	3/01/2017	16	9	247	1.21	1.62	240	0	0	2.83
51	5/01/2017	22	7	107	0	0.93	105	0.94	1.77	1.87
52	5/01/2017	15	4	214	0	0	210	0	0	0
53	6/01/2017	26	7	978	0.92	0	968	0.41	0.18	1.33
54	9/01/2017	33	10	1414	0.57	0.35	1387	0.07	0.03	0.99
55	9/01/2017	2	14	311	0.96	4.18	293	0.68	0.28	5.82
56	9/01/2017	20	7	1057	1.61	0	1040	0.19	0.13	1.80
57	9/01/2017	15	2	142	0	0	142	0	0	0
58	10/01/2017	23	9	905	0	0	900	1.33	0.77	1.33
59	11/01/2017	27	11	1082	0	0	1064	0.47	0.23	0.47
60	12/01/2017	13	7	1846	1.08	0.33	1800	0.55	0.23	1.96
61	13/01/2017	32	13	920	0	0	899	0.89	0.53	0.89
62	14/01/2017	27	11	1015	0	0	1008	1.67	1.51	1.67
63	14/01/2017	23	9	466	0.43	0	460	1.51	1.18	1.94
64	14/01/2017	2	14	185	1.08	1.62	178	3.33	3.31	6.03
65	14/01/2017	16	9	448	0.89	0.67	438	1.59	1.06	3.15
66	18/01/2017	27	11	844	0.59	0.24	804	2.34	2.80	3.17
67	18/01/2017	2	14	230	1.74	0.43	222	2.67	2.23	4.84
68	22/01/2017	30	7	2601	0.46	0.04	2562	0.55	0.56	1.05
69	23/01/2017	20	7	336	0.60	0	334	0.90	1.59	1.50
70	24/01/2017	32	10	1708	0.41	0	1679	0.65	0.70	1.06
71	24/01/2017	26	7	2228	0	0	2180	3.07	1.62	3.07
72	25/01/2017	28	7	675	0.44	0	670	1.34	0.68	1.78
73	28/01/2017	23	9	339	0.88	1.18	332	0	0	2.06
74	28/01/2017	13	7	1423	0.35	0.14	1401	2.47	0.87	2.96
75	31/01/2017	27	11	567	0.53	2.29	542	2.54	1.23	5.36
76	31/01/2017	4	7	685	0.44	0	635	6.85	3.14	7.29

77	31/01/2017	1	11	806	0.38	1.36	762	4.12	2.50	5.86
78	31/01/2017	10	7	268	0.37	0	258	8.24	5.66	8.61
79	6/02/2017	16	9	457	0.65	0.44	452	0.44	0.23	1.53
80	13/02/2017	27	11	1120	0.71	0.18	1110	0	0	0.89
81	15/02/2017	20	7	335	2.09	0	303	0	0	2.09
82	21/02/2017	16	9	197	0	0	187	0.53	0.44	0.53
83	23/02/2017	27	11	535	0	0	535	0	0	0
84	24/02/2017	25	13	1525	0	0	1475	1.42	1.23	1.42
85	27/02/2017	28	7	1445	0.14	0	1425	0.14	0.11	0.28
86	28/02/2017	35	7	241	0.83	0.41	238	0	0	1.24
87	28/02/2017	23	9	254	0.79	0.39	250	0.80	0.53	1.98
88	28/02/2017	13	7	354	3.39	1.13	338	0	0	4.52
89	3/03/2017	15	8	139	0	0	139	0.72	1.45	0.72
90	6/03/2017	2	14	182	3.85	1.1	173	0	0	4.95
91	6/03/2017	5	4	220	0.46	0.45	216	1.38	2.83	2.29
92	7/03/2017	15	2	143	0	0	143	0	0	0
93	9/03/2017	32	10	1376	1.24	0.07	1339	0	0	1.31
94	9/03/2017	19	13	1591	0	0	1546	3.50	5.32	3.5
95	9/03/2017	20	7	1064	0.85	0.28	1026	0.58	0.76	1.71
96	10/03/2017	15	2	161	3.11	0	155	0.64	1.12	3.75
97	10/03/2017	5	4	134	2.24	0	128	3.82	6.57	6.06
98	15/03/2017	27	11	698	0.57	0	694	0	0	0.57
99	15/03/2017	7	7	1236	0.97	0	1224	0.49	0.53	1.46
100	15/03/2017	7	7	2970	1.04	1.08	2859	0.49	0.49	2.61
101	15/03/2017	15	2	153	0.65	0	152	0	0	0.65
102	17/03/2017	27	11	876	1.83	0	859	0.12	0.14	1.95
103	17/03/2017	37	7	2111	0	0	2064	0.24	0.17	0.24
104	20/03/2017	15	2	134	1.49	0	118	2.50	6.54	3.99
105	21/03/2017	27	11	867	0.69	0	850	0.47	0.39	1.16
106	22/03/2017	15	2	110	0	0	100	0	0	0
107	23/03/2017	19	13	2527	0.35	0.12	2489	0.56	0.68	1.03

108	24/03/2017	17	6	1294	1.16	0.77	1256	0.16	0.13	2.09
109	24/03/2017	3	1	552	0.72	0.91	511	0	0	1.63
110	25/03/2017	36	7	2347	0.39	0.04	2284	0.04	0.02	0.47
111	26/03/2017	19	13	524	0	0	523	0.76	0.35	0.76
112	27/03/2017	15	2	99	0	0	99	0	0	0
113	27/03/2017	26	7	2470	0.45	0.04	2384	0.13	0.05	0.62
114	28/03/2017	20	7	808	0.87	0	789	0.25	0.08	1.12
115	3/04/2017	34	7	2406	0.46	0.12	2266	0.04	0.01	0.62
116	5/04/2017	27	11	290	0	0	281	0	0	0
117	10/04/2017	17	4	125	0	0	105	0	0	0
118	14/04/2017	19	13	693	0	0	684	0.58	0.43	0.58
119	15/04/2017	20	7	864	0.23	0	839	0.24	0.16	0.47
120	19/04/2017	30	7	718	0	0.14	705	0	0	0.14
121	20/04/2017	27	11	1090	0.18	0	1039	0.29	0.16	0.47
122	24/04/2017	32	6	308	0.65	0	288	0.35	0.19	1.00
123	24/04/2017	20	7	497	1.01	0	468	0	0	1.01
124	26/04/2017	19	7	674	0.30	0	643	0	0	0.30
125	26/04/2017	26	7	1090	0.46	0.09	996	0.20	0.09	0.75
126	27/04/2017	17	6	1386	1.59	0	1230	0.24	0.07	1.83
127	27/04/2017	38	4	173	0	0	165	0	0	0
128	27/04/2017	2	14	103	0	0	103	0	0	0
129	28/04/2017	35	7	514	0.19	0	465	0	0	0.19
130	29/04/2017	31	7	369	0	0	369	0	0	0
131	1/05/2017	34	7	1995	0.35	0.05	1757	0.11	0.02	0.51
132	1/05/2017	28	7	257	0	0	249	0	0	0
133	23/05/2017	34	7	1823	0.22	0	1524	0.20	0.08	0.42
134	13/06/2017	27	11	296	0	0	289	0	0	0
135	13/06/2017	31	7	535	0	0	498	0.20	0.19	0.20
136	14/06/2017	27	11	186	0	0	186	0	0	0
137	17/06/2017	20	7	475	0	0	473	0.84	0.55	0.84
138	18/06/2017	26	7	1737	0.23	0	1696	0.77	0.54	1.00

139	20/06/2017	34	7	1525	0.13	0	1501		0.40	0.36	0.53
140	26/06/2017	17	14	226	1.33	0	213		0.47	0.56	1.80
141	26/06/2017	19	7	791	0.25	0	776		0.26	0.28	0.51
142	26/06/2017	26	7	475	0.42	0.21	472		0.64	0.69	1.27
143	27/06/2017	27	11	221	0	0	221		0	0	0
144	27/06/2017	18	11	207	1.45	0	194		0.52	0.73	1.97
145	27/06/2017	5	2	71	1.41	0	70		0	0	1.41
146	27/06/2017	5	2	133	0.75	0	108		6.25	5.71	7.00
147	3/07/2017	22	15	176	0	0	176		0	0	0
148	3/07/2017	17	14	156	0.64	0	155		0	0	0.64
149	4/07/2017	5	2	73	1.37	0	72		0	0	1.37
150	5/07/2017	27	11	1063	0.47	0	934		1.28	2.04	1.75
151	6/07/2017	26	10	1743	0.23	0	1591		0	0	0.23
152	7/07/2017	31	7	1403	0.50	0	1195		0.42	0.52	0.92
153	8/07/2017	19	13	1364	1.91	0	1103		4.11	1.72	6.02
154	8/07/2017	26	7	482	0	0.41	346		1.71	1.24	2.12
155	9/07/2017	30	7	1738	0.12	0.23	1274		4.03	1.66	4.38
156	10/07/2017	22	15	238	0	0	177		2.79	1.15	2.79
157	10/07/2017	20	7	1533	1.30	0	1282		2.48	1.42	3.78
158	10/07/2017	15	2	75	0	0	60		4.92	4.78	4.92
159	10/07/2017	26	7	482	0	0.41	346		1.71	1.24	2.12
160	11/07/2017	5	2	133	0.75	0	108		6.25	5.71	7.00
161	13/07/2017	27	11	384	0.26	0	280		5.19	4.29	5.45
162	17/07/2017	31	7	331	0.60	0	236		7.79	4.43	8.39
163	20/07/2017	27	11	528	0	0	NA	NA	NA	NA	
164	20/07/2017	Missing	Missing	88	1.14	0	87		0	0	1.14
165	21/07/2017	3	1	481	0	0	334		7.20	4.41	7.20
166	22/07/2017	22	4	50	0	0	43		4.55	10.5	4.55
167	22/07/2017	17	14	352	2.84	0	231		1.73	1.53	4.57
168	23/07/2017	20	7	162	0.62	0	67		7.25	6.35	7.87
169	24/07/2017	16	9	283	0	0	185		5.26	5.25	5.26

170	25/07/2017	30	7	1236	0.16	0	1234		0	0	0.16
171	27/07/2017	27	11	439	1.59	0.23	212		0.47	0.42	2.29
172	2/08/2017	27	11	396	1.01	0	291		0	0	1.01
173	4/08/2017	9	13	545	2.21	0.18	499		0.40	0.18	2.79
174	4/08/2017	19	12	1353	0.52	0	1108		0	0	0.52
175	4/08/2017	25	13	543	1.48	0.18	391		0.26	0.16	1.92
176	4/08/2017	29	13	469	0.64	0.85	434		0	0	1.49
177	4/08/2017	8	13	377	2.65	0.53	326		0	0	3.18
178	15/08/2017	27	11	870	1.04	0.11	766		0	0	1.15
179	24/08/2017	20	7	45	0	0	45		0	0	0
180	24/08/2017	35	12	181	0.55	0.55	159		0	0	1.10
181	27/08/2017	27	11	213	0	0	140		0.71	0.68	0.71
182	28/08/2017	16	9	48	0	0	26		0	0	0
183	29/08/2017	17	14	61	3.28	0	42		0	0	3.28
184	30/08/2017	34	7	804	0	0	353		0.28	0.20	0.28
185	31/08/2017	27	11	182	6.04	0	124		0	0	6.04
186	1/09/2017	37	7	151	0.66	0	94		0	0	0.66
187	4/09/2017	27	11	796	0.88	0.50	482		0	0	1.38
188	14/09/2017	27	11	182	0	0	140		0	0	0
189	21/09/2017	35	12	131	0	0	5		0	0	0
190	23/09/2017	30	7	195	0	0	9		0	0	0
191	25/09/2017	27	11	921	0	0	97		0	0	0
192	1/10/2017	22	5	12	0	0	NA	NA	NA	NA	

a – All rejected SRL excluding those found dead with no apparent damage.
b – All dead SRL observed with no apparent damage (sub-optimal survival).

Appendix 7: Summary of SRL stock rejected and observed dead with no apparent damage at grading and number of SRL at risk, cumulative sub-optimal survival, and sub-optimal survival rate (per 1,000 SRL held for a day) and the percentage of SRL lost from arrival to packing by source ports. (Table arranged by descending values for % SRL lost).

Source port id	At grading			During holding			% SRL lost
	Total graded	% Rejected[a]	% sub-optimal survival[b]	No. at risk	% cumulative sub-optimal survival[b]	Sub-optimal survival rate (no. cases / 1000 SRL-days)	
1	75,289	0.76	0.13	70957	0.81	0.51	1.7
2	22,457	0.74	0.24	19527	1.05	0.77	2.03
3	16,681	0.75	0.19	15879	1.17	0.93	2.11
4	11,348	0.89	0.24	11006	0.25	0.30	1.38
5	5,085	0.88	0.20	4848	0.41	0.24	1.49
6	4,866	0.61	0.31	4671	1.02	0.65	1.94
7	2,361	1.53	0.93	2156	1.15	0.93	3.61
8	2,249	0.98	0.04	2156	1.84	1.44	2.86
9	1,665	0.48	0.06	1272	0	0	0.54
10	1,480	1.82	0.07	1425	0.28	0.38	2.17
11	1,364	0.51	0.44	1156	2.89	2.00	3.84
12	1,256	0.72	0.16	1198	1.91	1.75	2.79
13	677	1.48	0	667	0.15	0.22	1.63

14	414	0	0	353	1.41	0.91	1.41
15	12	0	0	0	NA	NA	NA
Missing	88	1.14	0	NA	NA	NA	NA

a – All rejected SRL excluding those found dead with no apparent damage.
b – All dead SRL observed with no apparent damage (sub-optimal survival).

Appendix 8: Water quality test kits and equipment used in this study:

Kit	Product number
API 5 in 1 Test Strips	
API Ammonia	
API Nitrite	
API Nitrate	
API pH	
API Phosphate	
API Carbonate Hardness	
YSI 9500 Photometer	YPT950
YSI Palintest Ammonia Test Kit	YAP 152
YSI Palintest Ammonia Conditioner	YAT1707
YSI Palintest Nitrite Test Kit (Nitricol)	YAP109
YSI Palintest Nitrate Test Kit	YAP163
YSI Palintest pH Test Kit (pH Phenol Red)	YAP130
YSI Palintest Alkalinity Test Kit (Alkaphot)	YAP188
YSI Palintest Phosphate Low Range Test Kit	YAP177
YSI Palintest Phosphate High Range Test Kit	YAP114
Hach DR1900 Portable Spectrophotometer	DR1900-01H
Hach Nitrogen-Ammonia TNT AmVer, Low Range	2604545
Hach NitriVer 3 TNT, Low Range	2608345
Hach NitraVer 5 Nitrate Reagent Powder Pillows	2106169
Hach Sample Cell 1" Square Glass 10 ml matched pair	2495402
Hach Phosphorus (Reactive) TNTplus Vial	TNT846
Hach Digital Titrator	1690001
Hach Alkalinity Reagent Set	2271900
Hach HQ40D Portable Multi Meter (pH, DO, Conductivity/Salinity)	HQ40D53000000
Hach Intellical LDO101 Field Dissolved Oxygen Sensor	LDO10105
Hach Intellical CDC401 Field Conductivity Cell	CDC40105
Hach Intellical PHC101 Field pH Electrode	PHC10105
Testo pH Meter	0563 2051

Appendix 9: ‘Southern Rock Lobster (SRL) (Jasus edwardsii) HEALTH ASSESSMENT PROCEDURE MANUAL’

SOUTHERN ROCK LOBSTER (SRL)

(*Jasus* edwardsii)

HEALTH ASSESSMENT PROCEDURE MANUAL

THE UNIVERSITY of ADELAIDE

Source Reference

Australian Government Department of Agriculture, Fisheries and Forestry 2009, *Operational procedures manual: Destruction* (Version 2.0), Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN), Australian Government Department of Agriculture, Fisheries and Forestry, Canberra, ACT., pp 18.

Bondad-Reantaso, M.G., McGladdery, S.E., East, I., and Subasinghe, R.P. (eds.) Asia Diagnostic Guide to Aquatic Animal Diseases. FAO Fisheries Technical Paper No. 402, Supplement 2. Rome, FAO. 2001. 240 pp.

Buller N.B. Bacteria and Fungi from Fish and Other Aquatic Animals. A practical Identification Manual. Department of Agriculture and Food Western Australia -2nd Edition. 2014., pp 430.

Faisal, M., Hetrick, F.M., eds., 1994. Annual Review of Fish Diseases 4. Permagon, USA, pp. 7-59

FRDC Report. Lobster Autopsy Procedure. Aquatic Science Research Unit, Curtin University of Technology., 93 pp.

Principle

General health testing of Southern Rock Lobster (*Jasus* edwardsii) utilising gross examination, histopathology, microbiology and haematology.

Limitations on Use

Samples should be alive when examined for gross pathological signs. If large numbers of animals are to be sampled or if there is a delay in processing, animals should be kept alive by holding in seawater and maintaining constant aeration and appropriate optimum water quality.

Specific Safety Requirements

- Steel chain mesh glove(s) should be used when handling large crustaceans to prevent injury as well as protective water proof under gloves.

- All waste chemicals should be disposed into an appropriately labelled chemical waste container, for removal and disposal by an authorised chemical removal contractor.

- Always work on an appropriate down-draft histology cut-in bench in the Histology Laboratory when trimming preserved tissues for histology.

Equipment and Material Requirements

Compound microscope	Post mortem knife
Haemocytometer and cover slip	Microscope slides
Pencil	Electronic scales

Pen	Plastic white chopping board
Fish bin – appropriate to the size of the animals	Plastic blue chopping board (photography)
Latex Gloves	Microbiological Disposable loops
Stainless steel chain mesh gloves	500ml Specimen Containers
Ruler or Callipers	25 Gauge Sterile Precision Needles.
Scalpel holder and blades	1ml Syringes
Sharps disposal container	Disposable transfer pipettes
Secateurs (Post mortem scissors)	Electronic Scale
Mayo Surgical Scissors	Razor blade
Micro Iris Surgical Scissors	Histology cassettes
Rat Toothed and Curved tip forceps	2 mL Centrifuge tubes
Plain Capillary tubes	Refractometer

Reagents Requirements *(Appendix B)*

Davidson's fixative

10% Neutral Buffered formalin

70% Ethanol

95% Ethanol

Thiosulfate-citrate-bilesalts-sucrose agar (TCBS)

2% Salt Sheep blood agar (SBA2%)

Zobell's Marine agar (ZMA).

Sodium cacodylate anticoagulant

Southern Rock Lobster Necropsy Method.

Cross reference the Advice Note with the samples, checking that the accession number, species and number of samples match. Attach an Autopsy Worksheet. (*Appendix C)*

Samples are to be placed in the refrigerator (4^{o}C) for approximately 2 hours or ice slurry for 30-60 min to induce stupor. Remove lobster from chilling and place on a dissection board. *Figure 1 (Appendix A)*

Examine, weigh and measure the SRL and record data on the Autopsy Worksheet:

The presence and location of any lesions, blisters, leg or antennae damage

The sex and maturity of the animal

The weight (in kilograms)

Total body length (in centimetres) – see *Figure 2 (Appendix A).*

The carapace length (in centimetres) – see *Figure 2 (Appendix A).*

Signs of possible pre-moult – colour change, exoskeleton hardness

Haemolymph should be taken from the ventral aspect of the 5th walking leg joint using a needle and syringe for microbiological and haematological testing. Collect approximately 1.7ml of haemolymph. Place 0.5ml in a tube with 0.5ml sodium cacodylate buffer for haemocyte counts. 1ml is to be collected and placed on ice as a fresh sample for biochemistry readings and the remaining 0.2ml is to be plated out on each agar plate for microbiology purposes. *Figure 3 (Appendix A)*

Process haemolymph as per instructions found in section 8.

The animal is then pithed for euthanasia using a sharp knife that is thrust downwards just behind the antennae into the central nervous system (cephalic ganglion). Samples required from the proximal region, include an eye and both antennule glands. Dissect and divide each antennule gland equally into two parts. Place one quarter by volume of the tissue into 95% ethanol and store the other quarter as a fresh sample at -80^{o}C. The remainder should be fixed in Davidson solution. *Figure 3 (Appendix A)*

Dissect away all walking legs. If there are melanised lesions present on the legs, place any affected tissues into Davidson solution. Then using a sharp knife cut along the intersegmental membrane and muscle tissue of the carapace and abdomen at the first segmental junction to remove the tail.

Cut through the thoracic region just internal to the two branchiostegal lines (i.e the longitudinal cuts from the distal edge of the carapace to the proximal cut surface, about 1cm on either side of the centre of the carapace).

Remove the left brachiostegite to reveal the gill chambers by cutting along the line and follow the same procedure to remove the right brachiostegite. Gill sets and rakers should also be collected into Davidson solution. *Figure 4 (Appendix A)*

Carefully cut away the exoskeleton from underlying tissues to reveal the heart, hepatopancreas, gastro-intestinal tract, cranial nervous ganglia and gonads. Samples should be taken from each of these organs and placed in Davidson solution. *Figure 4 (Appendix A)*

Cut longitudinally from caudal to cranial along the medio-ventral line of the carapace revealing the muscle, reserve cell tissue and cranial nerve cord that should all be collected into Davidson solution. *Figure 4 (Appendix A)*

Examine the tail and sample any lesions present on the exoskeleton. Make a longitudinal cut of the tail along the ventral and dorsal aspects revealing muscle, reserve tissue, caudal nerve and intestinal tract. Take samples from each and place into Davidson solution. *Figure 5*

(NB. All exoskeleton cuts are aided by use of a sharp secateurs or bone cutters)

(Appendix A)

The following is a summary of organs that should be dissected from the lobster and placed in a large specimen jar containing Davidson's Fixative* (with a ratio of at least 10 volumes of fixative to 1 volume of tissue):

From anterior segment – eye, antennule gland, gills, heart, anterior gut, hepatopancreas, reserve cell tissue and cranial nerve cord.

From tail – ventral nerve cord, caudal gut, and muscle including any pale lesions.

Segment of carapace including muscle

Any other tissues showing signs of abnormalities.

* Davidson's Fixative is used routinely as the preferred fixative for crustaceans.

Samples should be left in Davidson's Fixative for 24 to 48 hours then transferred to 70% Ethanol for a minimum of 24 hours. Samples are then processed as for histology purposes.

Haemolymph Test Procedure.

Total Haemocyte count.

Sodium cacodylate anticoagulant is mixed in with haemolymph

Estimate haemocyte count using a haemocytometer under x100 magnification. Count cells in both grids and use the mean value to calculate haemocytometer count. Refer to formula below:

$$THC = \frac{Cells\ counted \times dilution\ factor \times 1000}{volume\ of\ grid\ (0.1mm3)}$$

Coagulation

Keep whole blood on ice to discourage clotting

Withdraw approximately 30µl into a plain glass capillary tube. Care should be taken to avoid bubbles in haemolymph column.

Immediately after haemolymph is inserted turn the tube into vertical position with the sample at the upper end and start the stop watch.

Allow the tube to remain in this position until haemolymph column has moved to the lower end of the tube under the force of gravity and then invert the tube 180°

Repeat inverting tube until the haemolymph ceases and the time taken for this to occur is defined as the clotting time.

If a clot if not formed by the end of 5 minutes the result is recorded as 'no clot'.

Biochemistry

Keep whole blood on ice to discourage clotting.

Sample is then to be centrifuged at 10,000xg for 5 minutes

Supernatant produced from spin down process is removed and placed on ice for biochemical analysis.

Specific gravity

Keep whole blood on ice to discourage clotting

Open refractometer flap then place a drop of sample on the glass plate and close flap

Looking through the eye piece, old refractometer up towards natural light and read specific gravity level off the scale.

Microbiology

A small amount of haemolymph is spotted on each of the three bacterial growth plates; ZMA, SBA2% and TCBS agar.

Using sterile loop, neat haemolymph should be streaked and distributed on each plate as per standard methods. *Figure 6 appendix B*

Plates are then to be placed in a 25°C incubator for 24 hours

Plates are then read and assessed. *Appendix B*

Dominate heavy growths of single organisms found are picked and re-plated to obtain pure growth for identification and typing.

Pure growth samples are re-incubated in 25°C incubator for 48 hours on ZMA, SBA2% and TCBS agar.

After 48 hours appropriate testing is then undertaken on selected colonies for identification and typing with or without Microsys™.

Appendix.

Appendix A:

Figure 1: Assessment of dorsal and ventral external lesions.

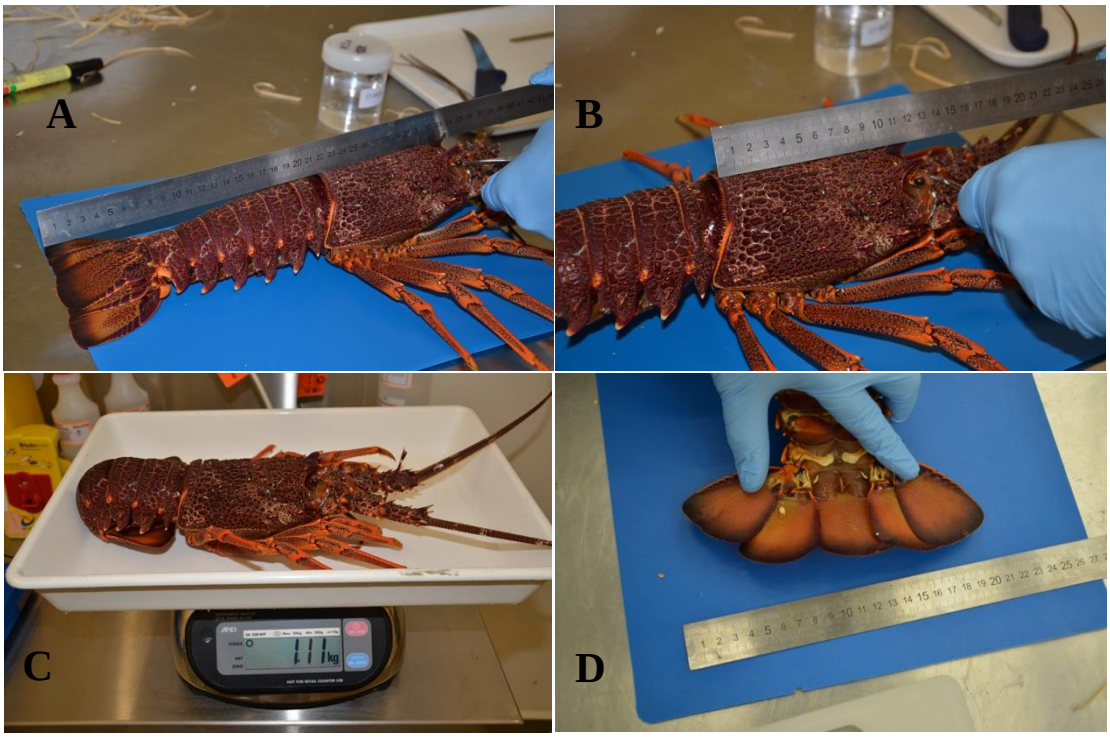


Figure 2: A: Total body length measurement B: Carapace length measurement

C: Body weight measurement D: Assessment of external lesions

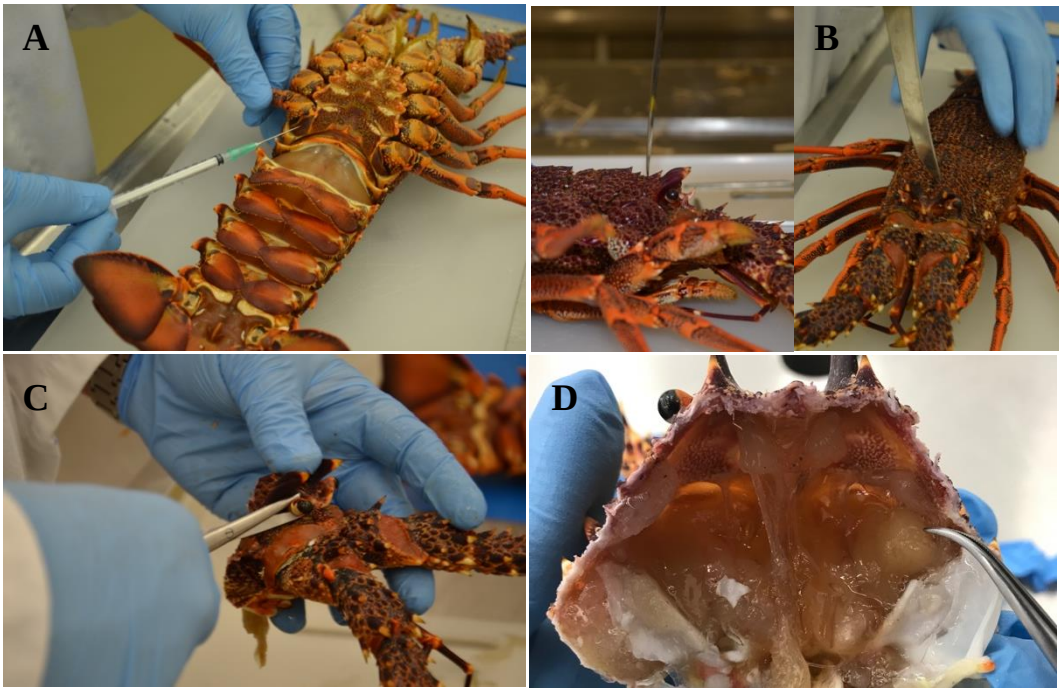


Figure 3: A: Haemolymph collection site B: Knife position to remove proximal area of the carapace. C: Removal of eye D: Location and removal of antennule gland.

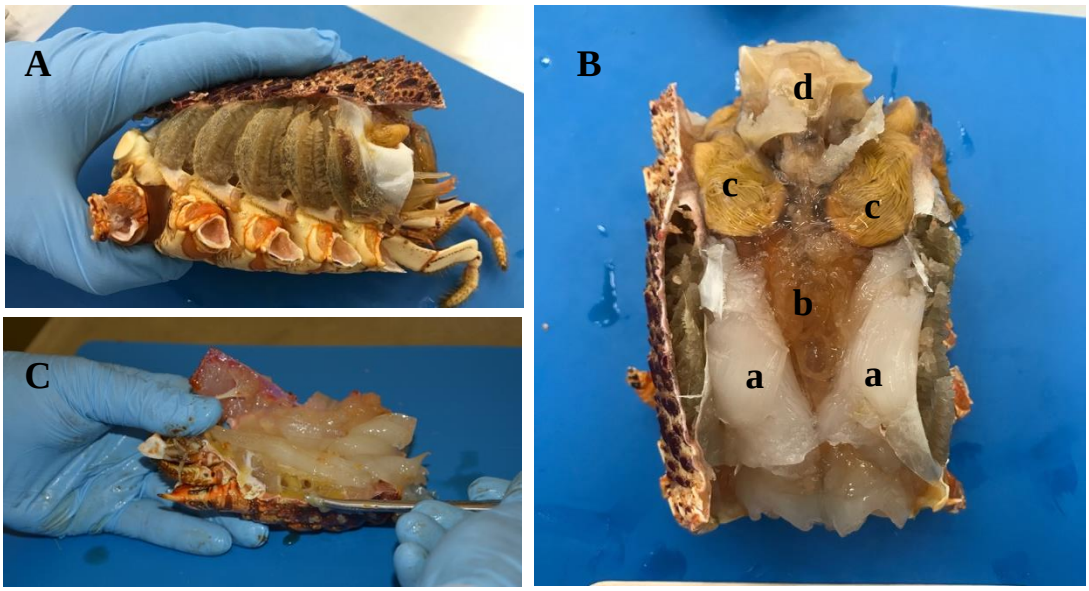


Figure 4: A: Removal of brachiostegite to reveal the gill chambers. B: Tissues underlying exoskeleton revealing a: muscle, b: heart, c: hepatopancreas, d: mouth part. C: Collection of reserve cell tissue located on ventral aspect of carapace.

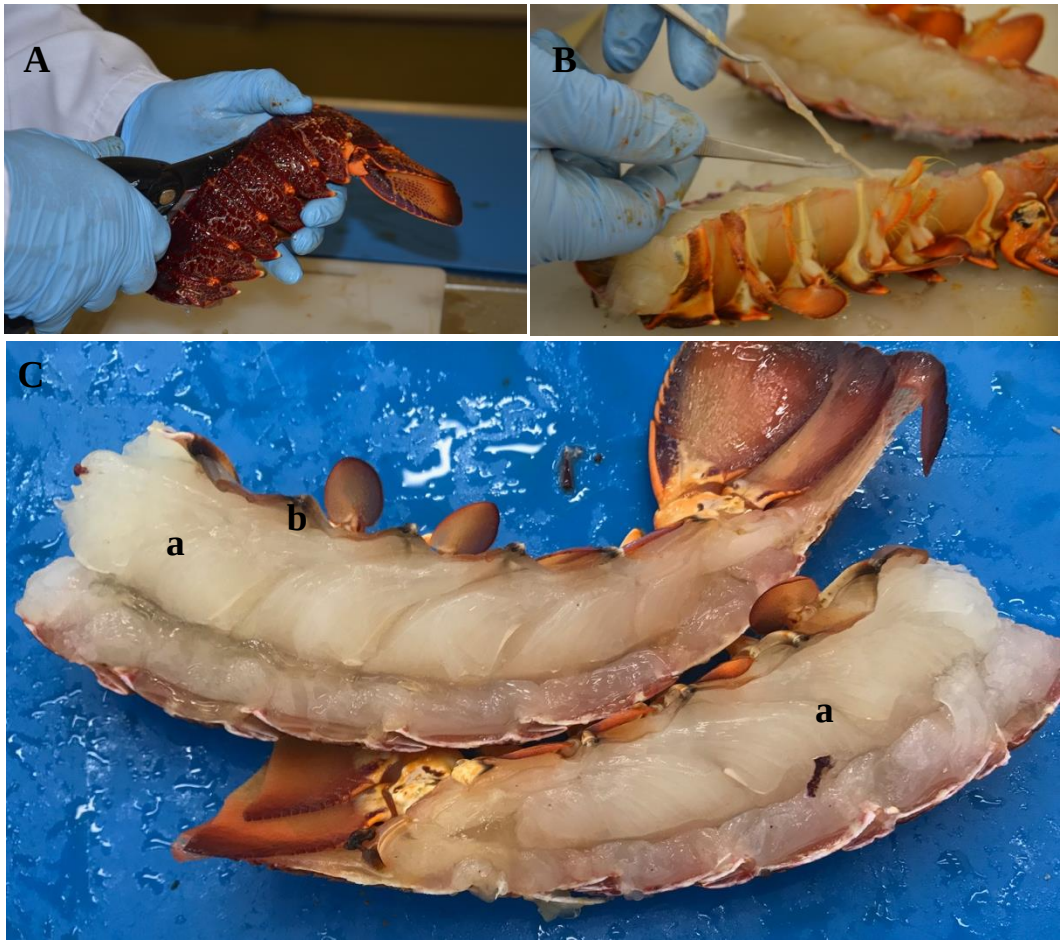


Figure 5: A: longitudinal cut along the dorsal aspect of tail. B: Removal of caudal nerve. C: Lobster tail cut in half revealing a: Muscle, b: Caudal nerve.

Appendix B: (Recipes)

1. Media: Sheep Blood Agar with 1.5% NaCl (SBA +1.5% NaCl)

   Made as per standard method of preparation

   Source Reference: Oxoid Manual, 1990, sixth edition Formulation CM271

FMICG-004 Quality Control of Culture Media and Reagents. 1992, NATA Technical Note #4.

2. Media: Thiosulfate-citrate-bilesalts-sucrose agar (TCBS)

   Made as per standard method of preparation

   Source Reference: Oxoid Formulation CM333

FMICG-004 Quality Control of Culture Media and Reagents. 1992, NATA Technical Note #4.

3. Media: Zobell's Marine Agar (ZMA)

   Made as per standard method of preparation

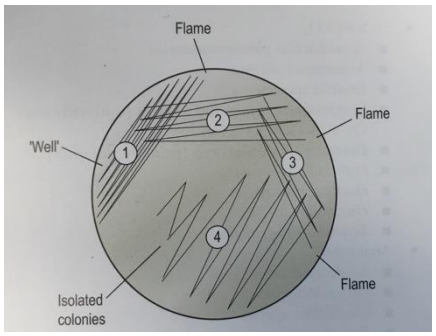
   Source Reference: Amyl Media Technical Data Sheets, 2000.

FMICG-004 Quality Control of Culture Media and Reagents. 1992, NATA Technical Note #4.

4. Sodium cacodylate anticoagulant

4.28g of sodium cacodylate added to 90ml of distilled water, pH adjusted to 7.0, using 1.0M HCL, 400µl of stock 25% glutaraldehyde solution added and volume adjusted to 100ml with distilled water.

5. Streaking of Agar Plates

Figure 

6: Quadrant Streaking method for obtaining isolated bacterial colonies on agar media.

Performed as per standard method

Source Reference: Clinical Veterinary Microbiology. 2nd Ed.

Markey B., Leonard F., Archambault M., Cullinane A., Maguire D. Chapter 2 page 21.

6. Examination of Culture Plates

Performed as per standard method

Source Reference: Bacteria and Fungi from Fish and Other Aquatic Animals. A Practical Identification Manual. 2nd Ed.

Nicky B. Buller. Chapter 2 page 430.

Appendix C: Necropsy Work Sheet

Appendix 10. SRL best practice workshop slides

FRDC PROJECT NUMBER: 2016/235

SRL IPA: Improving post-harvest survivability of southern rock lobster in a changing environment

Southern Rock Lobster Processors Industry Best Practices Workshop

Oct 2018

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Workshop Agenda

October 11 2018, IMAS Taroona		
Time	**Topic**	**Presenter/participants**
9:15-9:30	General introduction/FRDC project update	Q. Fitzgibbon
9:30 -10:45	Lobster biology/physiology	Q. Fitzgibbon
10:45-11:00	Break- morning tea	
11:00-1:00	Aquaculture systems and management	Q. Fitzgibbon/Fresh by Design
1:00-1:30	Break – lunch	
1:30-2:15	Water quality parameters including demonstration of testing products	R. Day/Q. Fitzgibbon
2:15-2:45	Biosecurity	K. Patel
2:45-3:00	Break – afternoon tea	
3:00-3:30	Lobster pathology	S. Pyecroft/J. Mahadevan
3:30-4:00	Traceability	P. Turner/R. Day with participation by FRDC Traceability Systems Analysis (TSA) project
4:00-4:15	Wrap up	Q. Fitzgibbon

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Session 1

FRDC project update

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Investigators

Dr Quinn Fitzgibbon
- Lobster physiology and aquaculture

Professor Caleb Gardner
- Lobster biology and fisheries

Dr Charles Caraguel
- Aquatic epidemiology

Dr Stephen Pyecroft
- Aquatics & Pathobiology

FRDC project conclusions

- Do not support the consistent association of a crustacean pathogen contributing to mortality in holding.
- Identified some potential novel lobster pathogens associate with mortality (including V. tapetis) however occurrence is not consistent across cases so are likely a obligate or opportunistic pathogen of SRL.
- Investigations still suggest a reduced holding capacity of lobsters in some exporting facilities leading to stock losses and impacting commercial processes.
- Investigations suggest an increased sensitivity of lobsters in recent season
- The cause for the apparent sensitivity remains unclear, which could be related with environmental stress, such as increasing water temperatures.

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FRDC project conclusions

- Investigations revealed considerable differences in industry management practices, apparent lobster holding capacity and levels of mortality.
- Initial findings suggests that 'high intensity" facilities (those with high levels of lobster tonnage, densities and handling) are most effected.
- Findings suggest a role of aquaculture practices.
- Further analysis of potential associated industry practice risk factors are underway.
- The project has developed industry tools for improved health monitoring and industry management
  - In facility vitality assessments (i.e lactate)
  - Immune function assays
  - Haemolymph biochemical baseline and profiling capacity in Australia

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FRDC project conclusions

- Lack of an associated pathogen is **good news** for industry
  - Dismisses any potential consumer concerns
  - No need for government or consumer notification
  - No need for enhanced biosecurity procedures/restrictions
  - Potentially easier to solve (i.e. aquaculture improvements not disease management.)

- So where to now?
  - Industry training – workshop, best practice manuals, more focused research?
  - Improved aquaculture technologies
  - Better understanding of tolerance and optimum water quality parameters.
  - Industry tools – water quality and vitality assessment (Brix, lactate, moult stage)
  - Improvements in pre-landing processes - return of weak or moulting lobsters, vessel handling, vessel environmental parameters and logging, packing and dry land transport.
  - Better understanding of the effects of climate change (moulting and nutritional condition)

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Session 1.

Lobster Biology and Physiology

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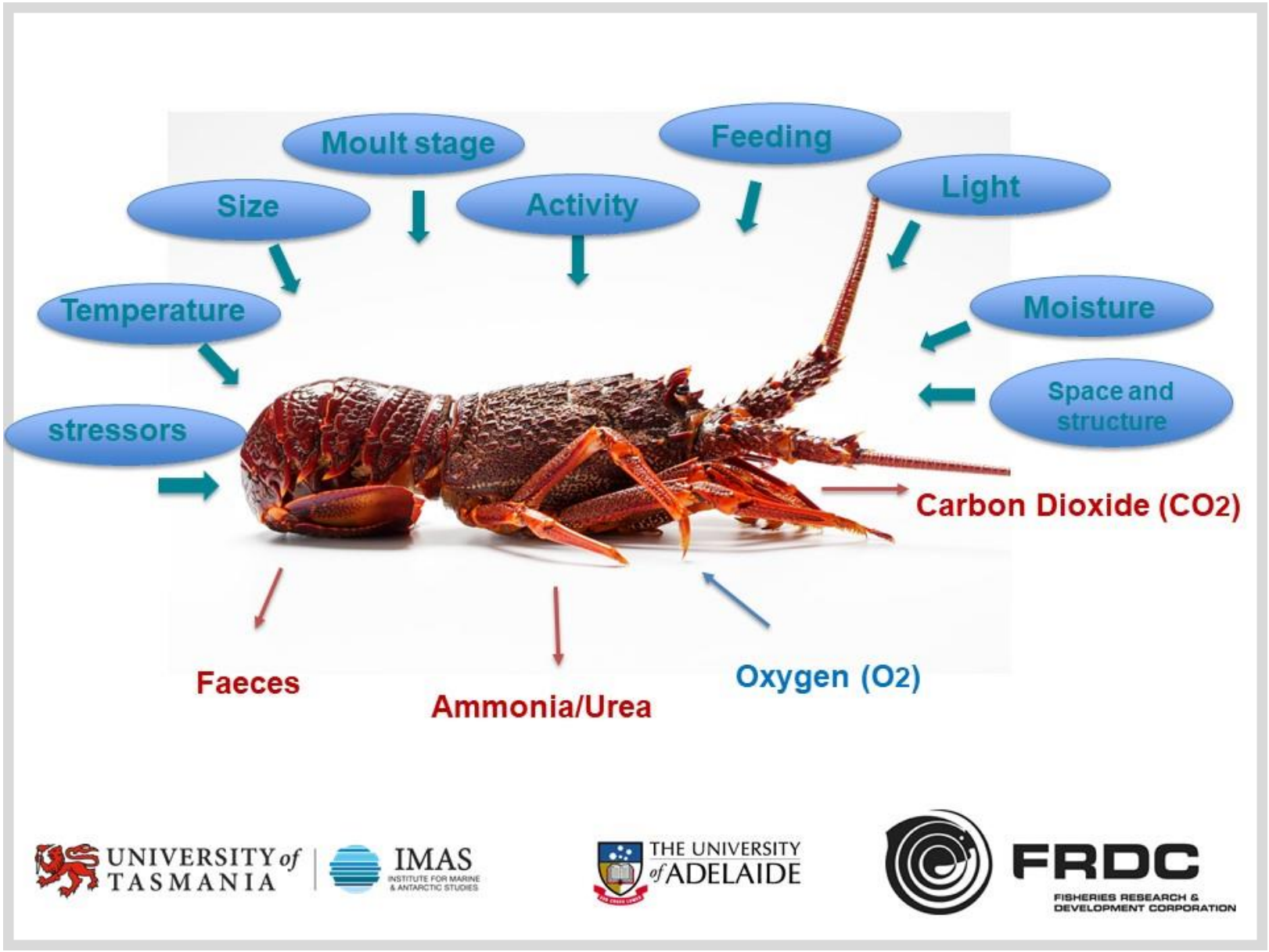
Moult stage
Feeding
Size
Activity
Light
Temperature
Moisture
Space and structure
stressors
Carbon Dioxide (CO2)
Faeces
Ammonia/Urea
Oxygen (O2)
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What lobsters need?

Oxygen

- Oxygen is required to support energy requirements
  - Maintenance energy (standard metabolism)
  - Activity
  - Food digestion and assimilation
  - Growth, reproduction and repair
- If sufficient oxygen is not available, energy requirements will be supported by anaerobic metabolism
  - Leads to the production of lactate
  - Non-sustainable and will result in death if prolonged
- Oxygen is acquired by the circulatory systems through diffusion across the gills
  - Require to be immersed (in water) or at least moist

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What lobsters produce?

Ammonia/Urea

- Nitrogenous excretory as a waste product from protein metabolism
  - Protein turnover and synthesis
- Ammonia the dominant excretory product (80-100%), Urea minor
- Excreted from the circulatory system across the gills
  - Requires to be immersed (in water)
- Toxic if allowed to accumulate in the body or water

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What lobsters produce?

Carbon dioxide

- Byproduct of energy metabolism
- Excreted from the circulatory system across the gills
  - Requires to be immersed (in water) or moist
- Toxic if allowed to accumulate in the body or water
  - Decreases blood (Hypercapnia) or water pH
  - Reduces the oxygen-carrying capacity of the blood

Faeces

- Solid wastes of food digestion and body repair
- Contributes to system nutrient load

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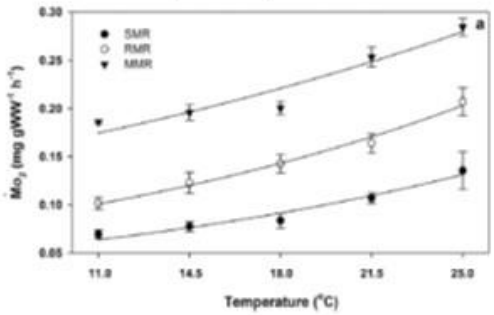
What effects requirement/waste production?

Temperature

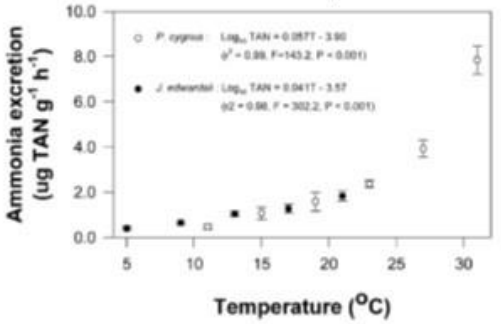
- Dominant environmental factor effecting rates of metabolic functions
  - Energy/oxygen demands and
  - Ammonia and carbon dioxide excretion rates
- In general, energy and excretion demands are halved by every 10°C temperature reduction (Q_{10}=2)

- **Key benefits of reduced temperature during holding and transport!**

Fitzgibbon et al., 2017



Crear and Forteath, 2002



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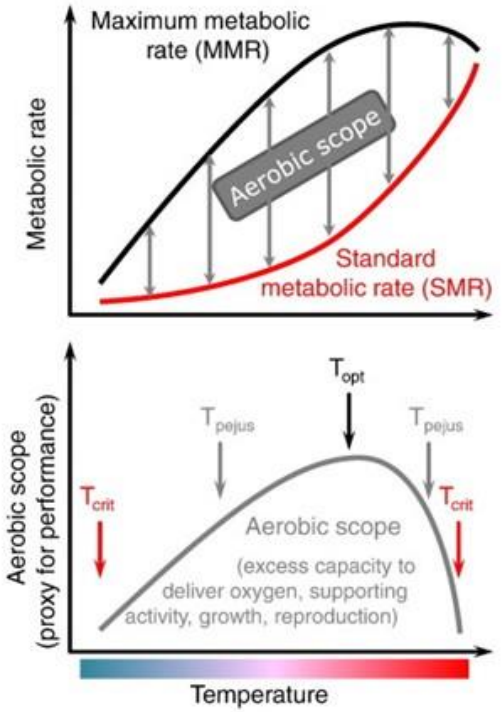
So what is the optimum temperature?

- Has not been well defined for adult southern rock lobster in holding
- Likely the minimal chronic non-lethal temperature
- Also needs to consider the acclimation temperature (summer/winter), rate of temperature change and other environmental stressors
  - Minimum non-lethal temperature may change with season
  - Rapid temperature change can induce stress
  - Thermal tolerance can be influence by environmental stress
- Defined as the lower critical temperature
  - Minimum temperature where there is sufficient aerobic scope to support basal/standard metabolism



Key research question;
Optimum temperature and acclimation rates for SRL in holding and transport

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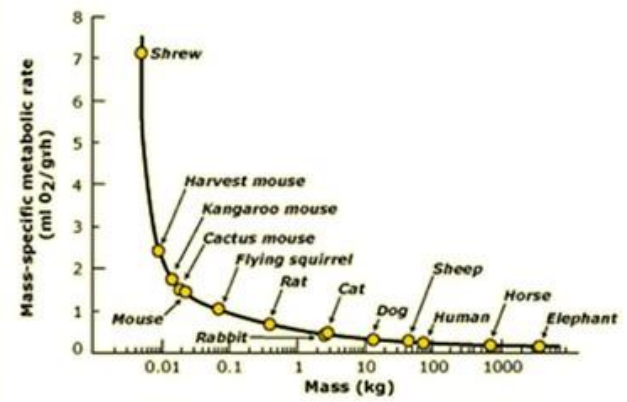
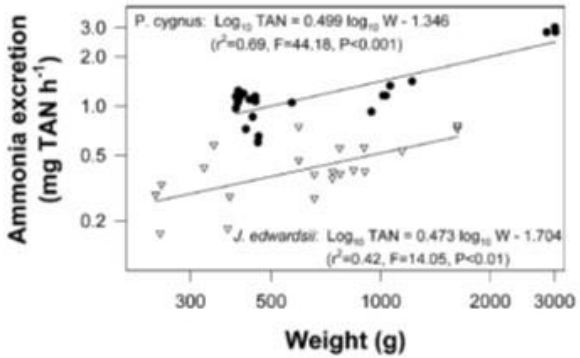
What effects requirement/waste production?

Size

- Body size effects metabolic rates
  - Key concept in animal physiology (mouse to elephant curve)
- Applies to within and across species
- Smaller lobsters have higher mass specific metabolic demands than large lobsters
- Smaller lobster more vulnerable to metabolic stress
- 100 kg of small lobsters requires more oxygen and produces more ammonia than 100kg of large lobsters



Crear and Forteath, 2002



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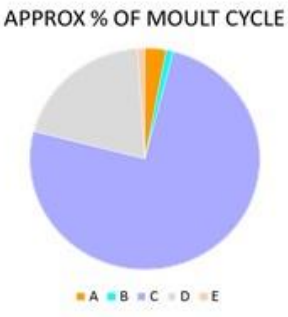
What effects requirement/waste production?

Moult stage

- Critical process of crustacean growth
- 5 stages
  - Stage A – Post-moult (water absorption, exocuticle mineralization)
  - Stage B, C_{1-3} – endocuticle secretion
  - Stage C_4 – Inter-moult (growth and energy storage)
  - Stage D – Pre-moult (resorption of minerals and epidermis retraction)
  - Stage E – Ecdysis (moulting)
- Majority of the cycle the stable intermoult (stage C)
- Moulting cycle effected by;
  - Distribution (location)
  - Lobster size
  - Habitat/Nutritional condition
  - Effect of changing environmental conditions?



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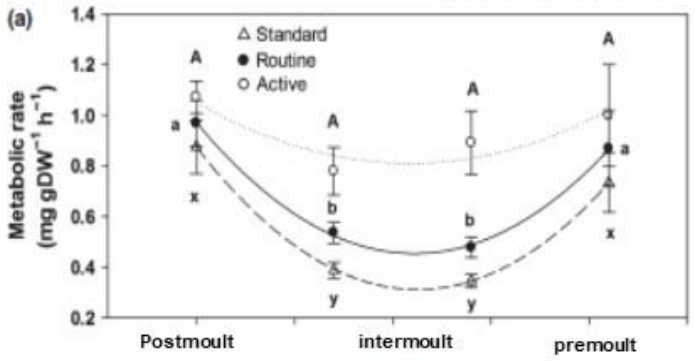
What effects requirement/waste production?

Moult stage

- Highly energetically demanding process
  - Particularly during ecdysis, post-moult and pre-moult
- Post moult lobsters are at poor nutritional condition with limited energy reserves
- Lobsters highly vulnerable to metabolic stress and physical damage during ecdysis, post-moult and pre-moult.
  - Need to avoid taking on lobster stock which are post-moult and pre-moult!

Fitzgibbon et al., 2015



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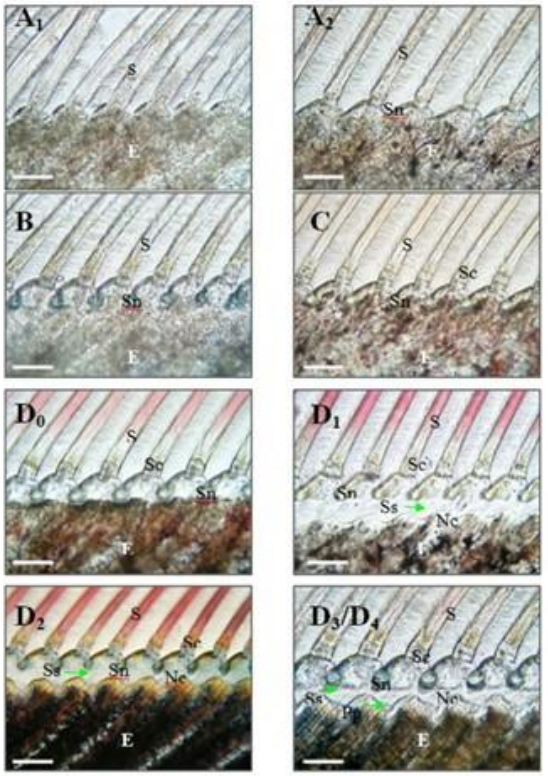
What effects requirement/waste production?

How to determine moult stage

- Shell appearance
  - Orange coloration of abdomen flesh
  - Carapace fouling (tube worm)
  - Can indicate late inter-moult to late pre-moult but unreliable and subjective
- Shell rigidity
  - Soft carapace sides/lower carapace can indicate post-moult
  - Inter-moult overall hard carapace
  - Pre-moult more difficult to determine
  - Suggestion of a brittle crease between the side and lower carapace of pre-moult lobsters?
- Pleopod setal development
  - Can indicate pre-moult stages
  - Somewhat subjective and requires a microscope

Musgrove, 2000



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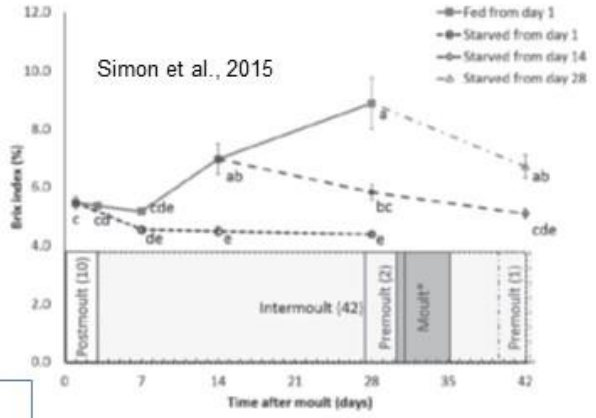
What effects requirement/waste production?

How to determine moult stage

- Refractive index (Brix) of the blood
- Correlated closely with nutritional condition and in turn moult stage
- Brix value determined and used by clawed lobster fishery as a indicator of impending moult (ALMQ project, Battison 2018)
- Clawed lobster processors use brix index to determine lobster with good nutritional condition for long term holding
- Anecdotal evidence used by the NZ SRL industry
- Threshold values and correlation with moult cycle yet to be determine for Australian SRL



Key research question;
Better tools for determining moult stage (Brix etc.) and moulting cycles across the distribution range.

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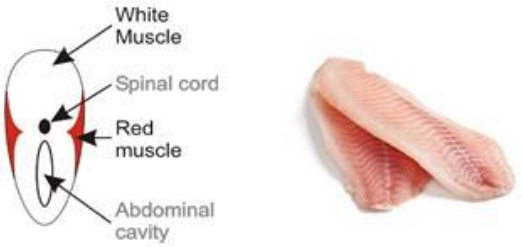
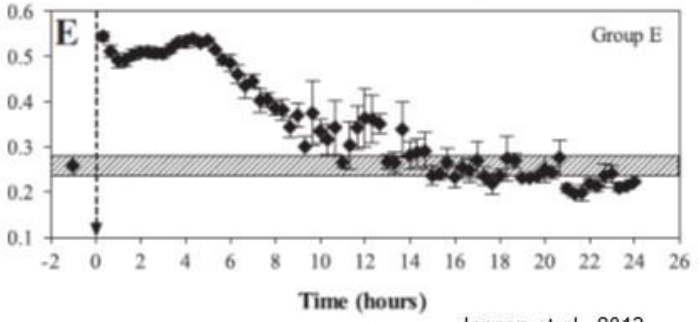
What effects requirement/waste production?

Activity

- Activity increases energy/oxygen demands and rates of excretion
- Lobster tail a powerful anaerobically fueled white muscle
- Tail flipping uses a lot of energy
  - Generates lactate/oxygen debt
  - Can take hours/days for metabolic recovery
- Attempts should be made to reduce/eliminate tail flipping at all stages of handling holding





Jensen et al., 2013

Key research question;
Benefits of socking or banding to reduce tail flipping and negative physical interactions?

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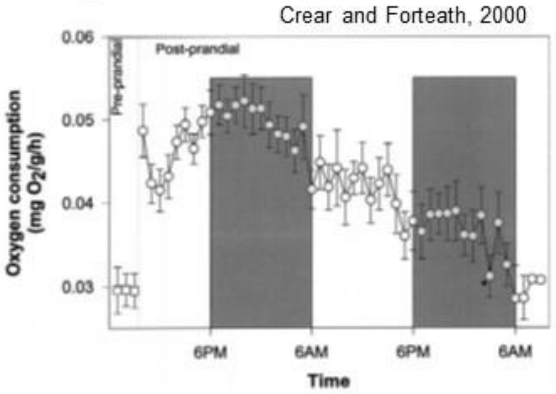
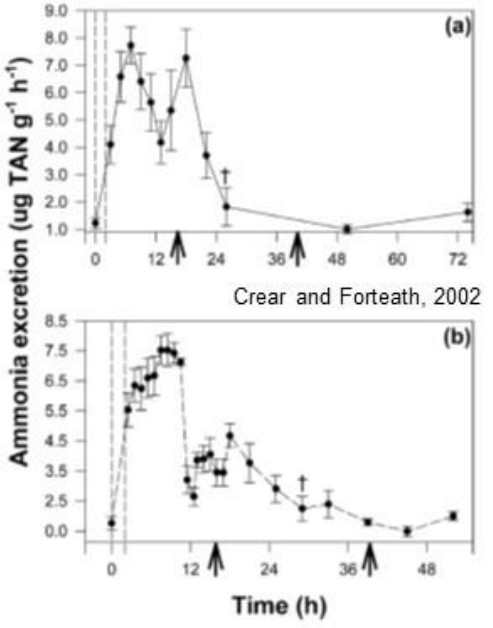
What effects requirement/waste production?

Feeding

- Feeding not recommended as it increases energy/oxygen demands and ammonia/feacal excretion rates
  - Due to energy demands of digestion and nutrient assimilation
  - Elevated metabolism for hours/days
  - Greatly increases RAS nutrient demands
- Lobsters withstand starvation by reducing basal metabolism and utilizing stored energy reserves (lipid then muscle protein)
- Ability to withstand starvation dependent on nutritional condition/moult stage but not well defined
  - Pre-moult poor nutritional condition
  - Lobster in good nutritional condition can withstand longer periods of starvation
  - Temperature dependent (lower better)

Key research question;
Maximum starvation time and the effect of moult/nutritional stage and temperature?

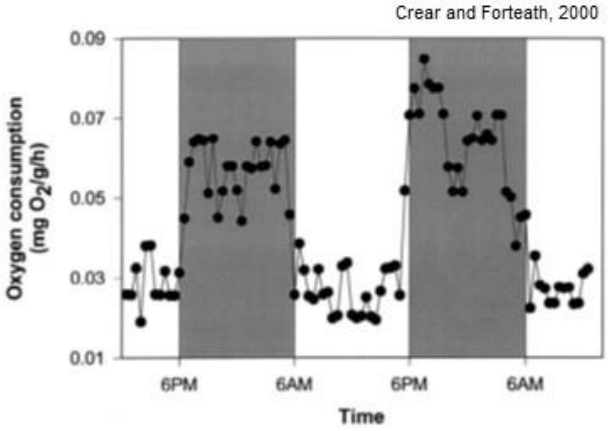


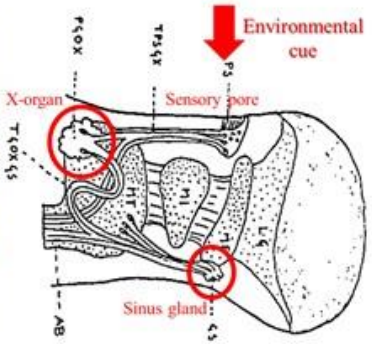


What effects requirement/waste production?

Light

- Lobster have heighten activity levels and energy/oxygen demands at night
  - Naturally nocturnal
  - Suggest a benefit of continual light in holding
- However, light (day length) influences seasonal biological processes
  - Moulting and reproduction
  - Potential to hasten or delay moulting
  - Abnormal day length may induce stress
  - Optimum lighting regime requires further consideration
- Direct sunlight can damage lobster eyes
  - Avoid during transport and holding
- High light levels may induce stress
  - Visual disturbance
  - Hiding





Key research question;
Determine the optimal lighting regime during SRL holding?

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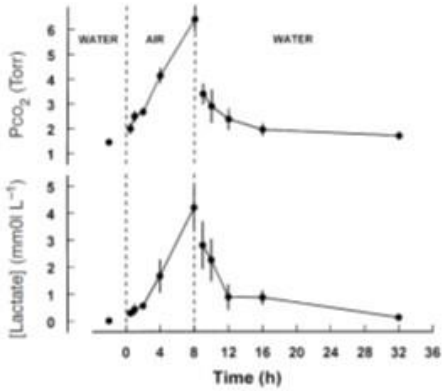
What effects requirement/waste production?

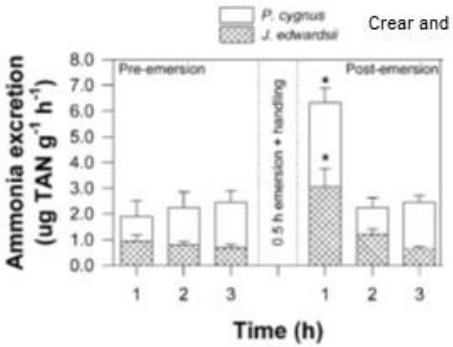
Moisture

- Gas exchange (O_2/CO_2) and ammonia excretion significantly impaired during air exposure
  - Build up of lactate/ammonia
  - Decrease in pH
- Emersion (i.e dry transport) is highly stressful
  - Efforts should be made to limit time out of water
  - Efforts should be made to keep lobsters protected from sun and wind and in cool and moist (humid) environment
  - Cover in wet cloth
  - Spray transport?
- On re-immersion after dry transport
  - Oxygen demands enhanced
  - Ammonia excretion enhanced (system ammonia spikes)
  - Critical time for recovery which can take hours/days
  - Purge systems need to be effective and functional!
  - Allow 24-48 h for recovery

Taylor and Waldron, 1997



Crear and Forteath, 2000



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What effects requirement/waste production?

Space and structure

- Densely packed lobsters compete for oxygen supply and expose each other to waste products
  - Do not overcrowd
  - Provide sufficient water movement through flow or aeration
  - Spread out where possible
- Lobsters prefer structure to hold on to
  - Cannot grip on smooth surfaces
  - Can lead to gripping and damaging other lobsters
  - No structure can lead to stress and heighten oxygen demands
  - Provide hide or substrate structure
  - Creates can provide structure and can help to spread out lobsters but may restrict water mixing

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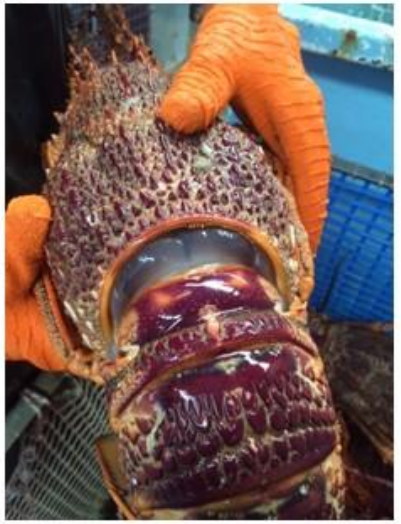
What effects requirement/waste production?

Stressors

- Poor water quality
  - Oxygen, CO_2, ammonia, nitrite, nitrate pH discussed later in the aquaculture and water quality sections
- Salinity another potential problem during vessel/land transport and holding
  - Lobster likely have limited salinity tolerance however more research is required
  - Evaporation during holding increase salinity
  - Fresh water inputs decrease salinity – pH dosing/fractionator flushing
  - Source water – effect of rain run-off/tide/location
  - Exposure from vessel uptake of fresh surface waters
  - Rain during land transport
- Suggested that low or changing salinity leads to swelling
  - Needs to be confirmed
  - Could also be related to moulting



Key research question;
Salinity tolerance and impact of changing salinity on SRL?

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What effects requirement/waste production?

Stressors

- Physical damage
  - Poor handling during capture, transport grading
  - Handle lobsters gently and as few times as possible
  - Crating and foot damage
  - Avoid double handling where possible
  - To handle before or after purging/recovery?
- Visual disturbance
  - Lobster prefer to hide
  - Possible benefit of providing low light, covers or high aeration
- Contaminants
  - Cleaning/disinfection products
  - Toxic metals in aquaculture systems
  - Copper, iron, zinc and aluminum

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Lobster biology and physiology

Conclusions or suggestions for holding and transport

- Numerous factors and processes that contribute to lobster performance
  - Best practices are required at all stages of the chain from capture, transport, holding and export
- Some general conclusions
  - Keep lobster cool at all times. Avoid large temperature changes and allow time for acclimation if needed
  - Keep lobster in water for along as possible. Limit transport times and grading times.
  - When dry transport is required, keep lobster cool, moist, and away from wind, sunlight and freshwater. Cover in wet cloth. Protect feet. Return to water as soon as possible.
  - After dry transport, allow lobster time (24h+) to recover in optimal conditions. Purge systems need to be highly functional.
  - Handle gently and only when needed. Avoid inducing tail flipping
  - Don't feed
  - Maintain optimal water quality including O_2, ammonia, nitriate, nitrate, pH, alkalinity and salinity
  - Do not overcrowd and provide sufficient water flow and mixing

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Lobster biology and physiology

Significant uncertainties

- Chronic tolerance and optimal levels for most water and environmental parameters
- Efficient and accurate moult staging
- Effect of a changing environment on lobster physiology (nutritional condition/moult cycle)
- Vitality/condition/stress assessments
  - Reflex responses, Brix, lactate
  - Practical industry tools
- Accountability and traceability across all industry process sectors
- Influence of differing industry practice and systems

Strong

Weak

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Lobster biology and physiology

Significant uncertainties

- True is that I don't know the best practice for many industry processes
  - Neither do you
  - Many practices based on opinion
  - Why industry practices are so diverse
  - Need more rigorous science
- We are lagging behind our competitor in understanding of industry best practice
  - NZ CRA8 Rock Lobster Industry Association
  - More than 5 years dedicated research effort
  - Results/reports not available (protected)
  - WA industry use larger and more modern facility technologies
- I have more opinions on many industry practices
  - happy to share
- Questions?

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Session 2

Aquaculture systems

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Aquaculture systems

Academic/Commercial Perspectives

Academic boffin perspective

Commercial perspectives

Better understand commercial product, commercial constrains and have the experience, commitment and liability for commissioning commercial systems and processes

fresh by design

RADAQUA

AQUASONIC
Caring for Aquatic Life

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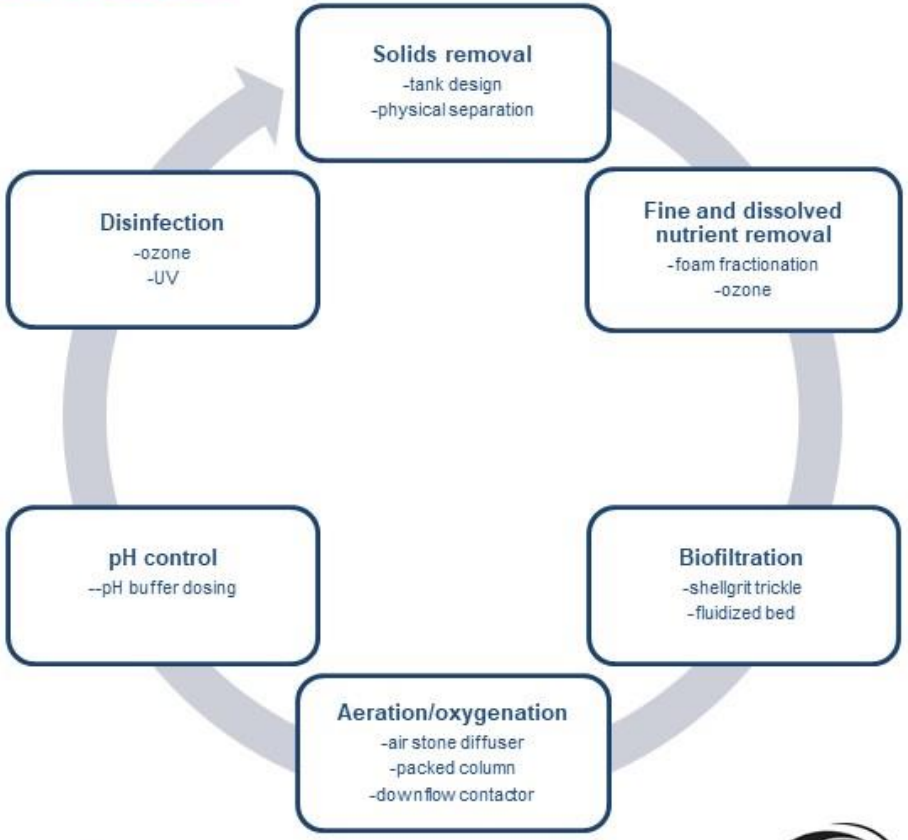
WHO IS ARE WE ?

- Australia's premier Aquaculture Suppliers. Exclusive agents of the world's best Aquaculture products and Aquaculture equipment.
- We have an experienced team that can offer Aquaculture System Design, supply and installation. Including R&D and commercial production systems.
- Provide professional support from aquaculture systems, product installation to our own Veterinary service

How Can We Increase Your Production ???

Aquaculture systems

Main processes



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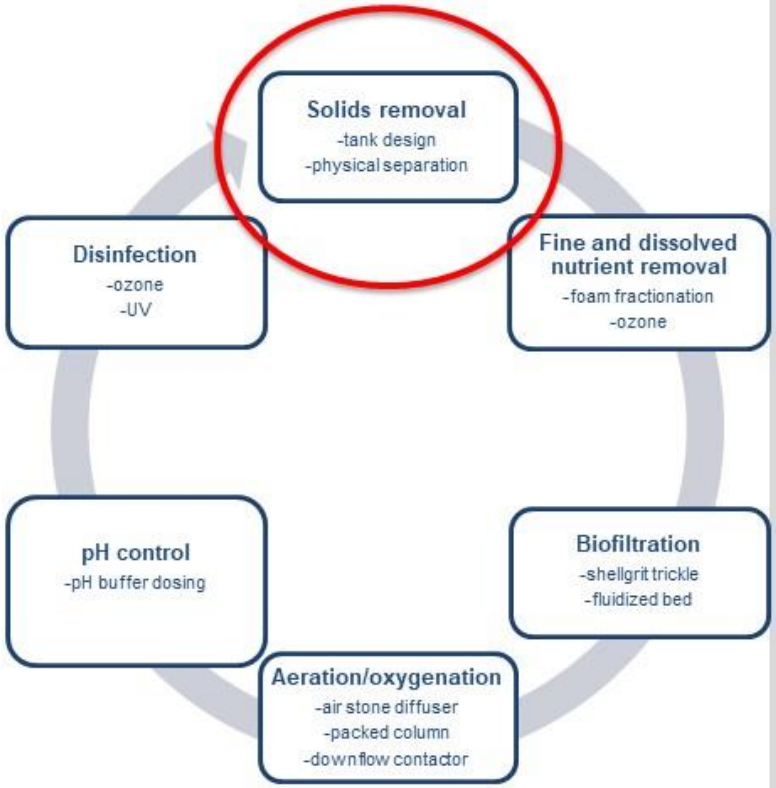
Aquaculture systems
Solids removal

- Solids removal is first critical step in efficient and effective recirculation aquaculture systems
  - Feaces, dead lobsters and body parts, feed?
- Rapid removal before they degrade and become part of the dissolved nutrient/oxygen load
  - More difficult to remove when dissolved
  - Nutrient breakdown consumes oxygen (BOD)
- Amount of solids dependent on;
  - Fishery practices (fishing trip duration, time post capture)
  - Aquaculture practices (feeding)
- Tank design/physical separation



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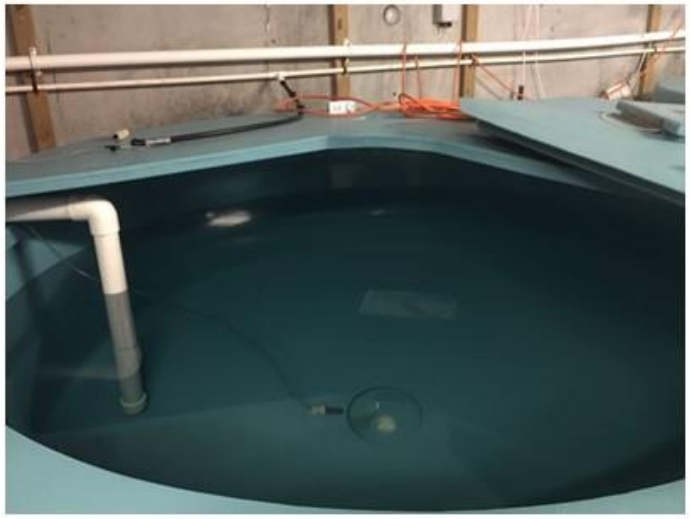
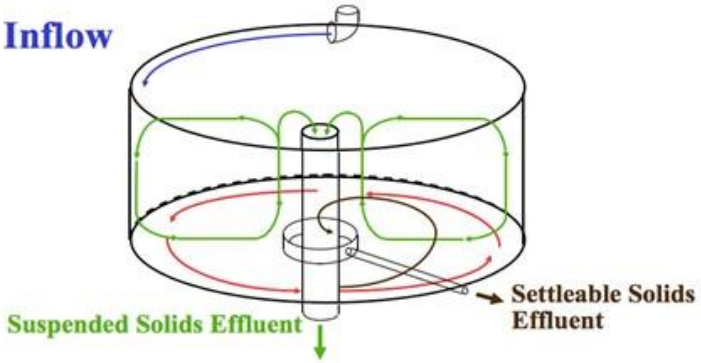
Solids removal

Tank design

- First step is rapid removal of solids from the tank
- In fish aquaculture, achieve by water tank velocities for removal of settleable solids
  - self cleaning tanks
  - Water velocity = 15-20 cm/sec
- Round tanks
  - Primary circular flow
  - Secondary inwards rotational flow
- **But lobster industry uses raceway tanks and, lobsters and tank infrastructure disrupts flow**





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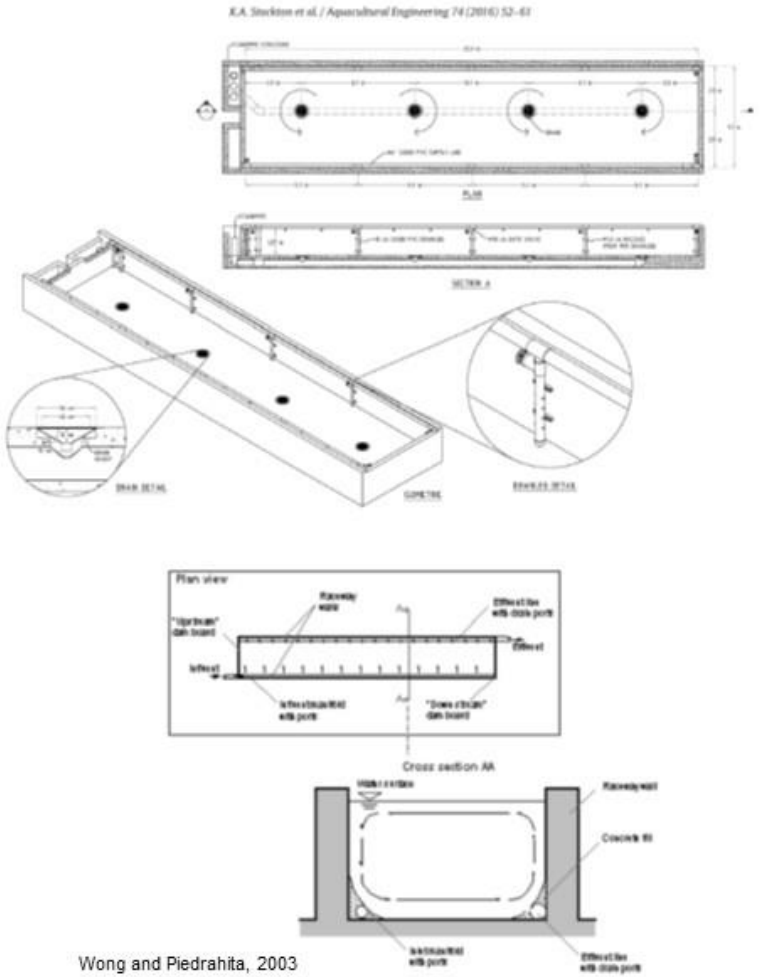
Solids removal
tank design

- Raceways have poor bottom flow rates and are not effective for self cleaning
  - Require physical cleaning (vacuuming etc.)
  - Should be performed regularly
- Alternative better self cleaning raceway designs available
  - Cross-flow raceways
  - Multi-cell raceways
- However designs still need to overcome complications associated with tank infrastructure and benthic lobsters
  - Requires novel solutions for the lobster industry



Wong and Piedrahita, 2003

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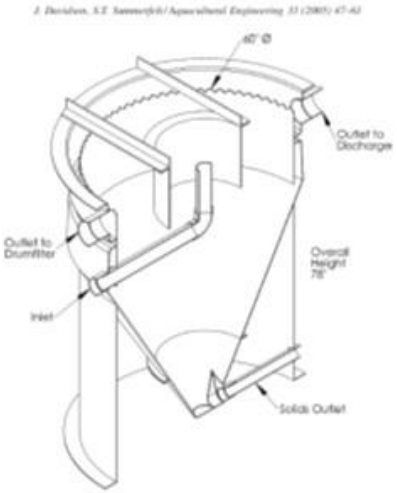
Solids removal

Physical separation

- Once solids are removed from the tank they must be quickly separated from the water
- Numerous options including;
  - Material, swirl/radial flow separators, sand/media filtration, drum screen/film filtration belt, bag filters
- Each option has its advantages and disadvantage
- In general we consider
  - Passive filtration (swirl separation) a good first step where large partials are present, such as uneaten feed
  - Drum filtration to be the gold standard but expensive and can use a lot of water.
  - Media filtration good but needs regular maintenance (back-flushing). Poorly maintained sand filtration can be a source of disease
- Best receive advice on the most appropriate method from a commercial aquaculture systems provider.



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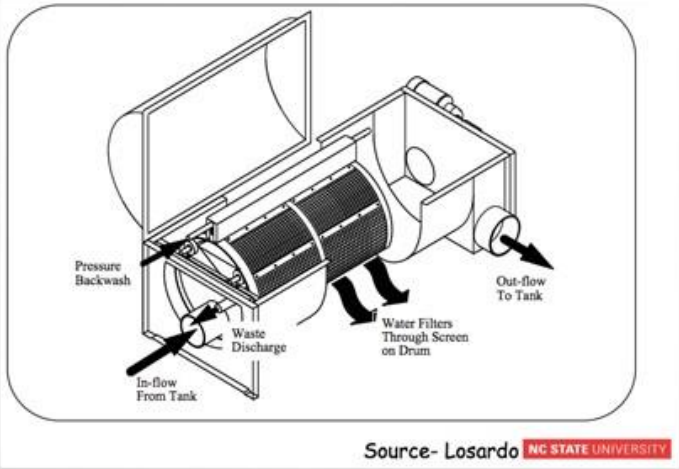
Drum Filter

Drum Microscreen Filters remove 15 - 250 micron sized particles from high flow water systems.



Source- Losardo NC STATE UNIVERSITY

fresh by design

MEDIA FILTERS

Work by allowing water to percolate through beds of media that trap particulates.

ADVANTAGES:

- Durable and easy to clean, as it does not need to be disassembled like other types of filters
- Filter's multiport valve to give you control of water flow through the filtration circuit

DISADVANTAGES:

Filter media needs to be replaced approximately every five years

- Needs to be backwashed regularly, which can use a lot of water
- Generally, requires a larger installation space compared to other filter choices.

FINE SOLID - MEDIA TYPES

Carbon

Zeolite

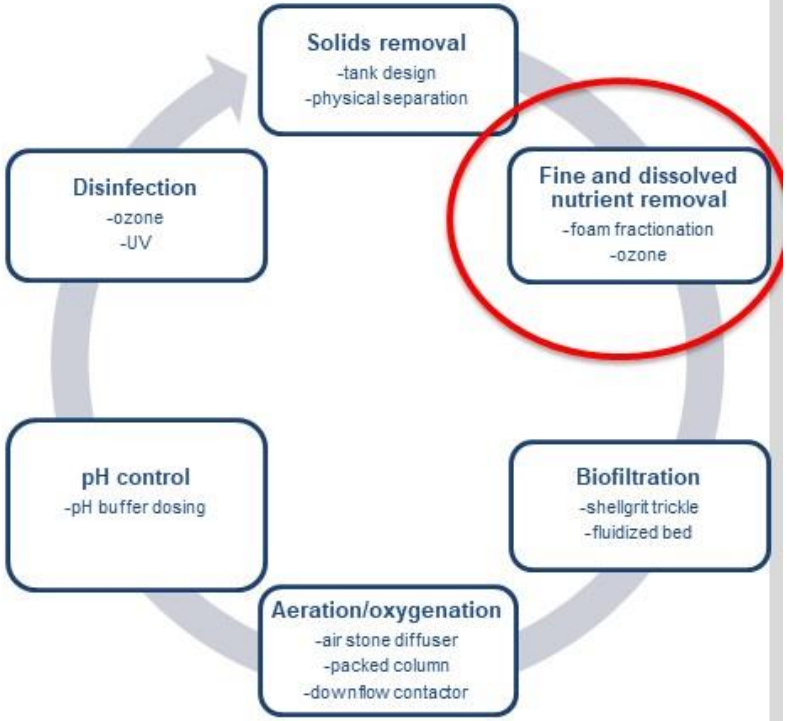
Activated filter media

fresh by design

Aquaculture systems

Fine and dissolved nutrient removal

- Important step for the removal of particles less than 30 microns
- Best achieved by effective foam fraction
  - Reduces water nutrient load
  - Reduced bacteria and biological oxygen demands (BOD)
  - Improves water clarity
  - Best performed before biofiltration
  - Low cost and requires little maintenance
- Ozone will also help but discussed later in the disinfection section



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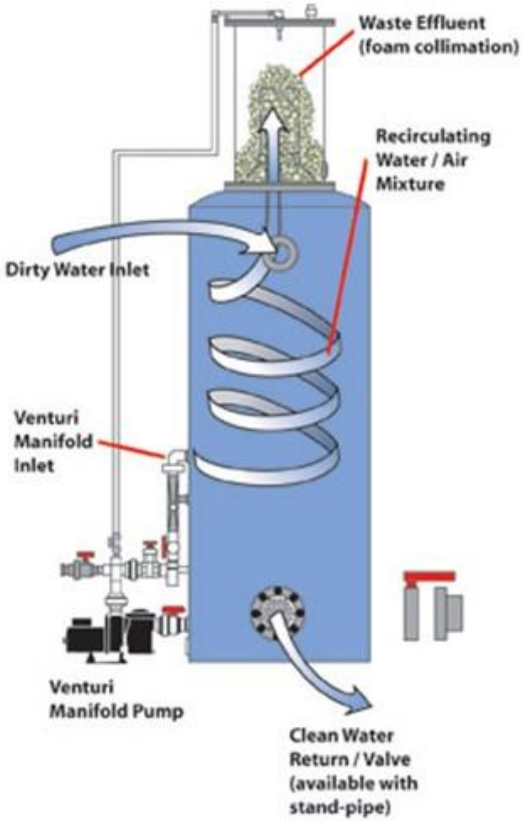
Solids removal

Foam fractionation

- Removes dissolved organic carbon and particulate organic carbon
- Dissolved organic in water are attracted to the air water interface
  - Create foam
- Adds oxygen and can incorporate ozone
- More efficient in seawater
  - pH higher and smaller bubbles
- However, can break up larger particles
  - Should not be first point of contact
- Ensure system is maintained to achieve maximum efficiency
  - Cleaning of the venturi
  - Cone cleaning – automotive flushing best
  - Be careful so that freshwater cleaning water dose not lower system salinity



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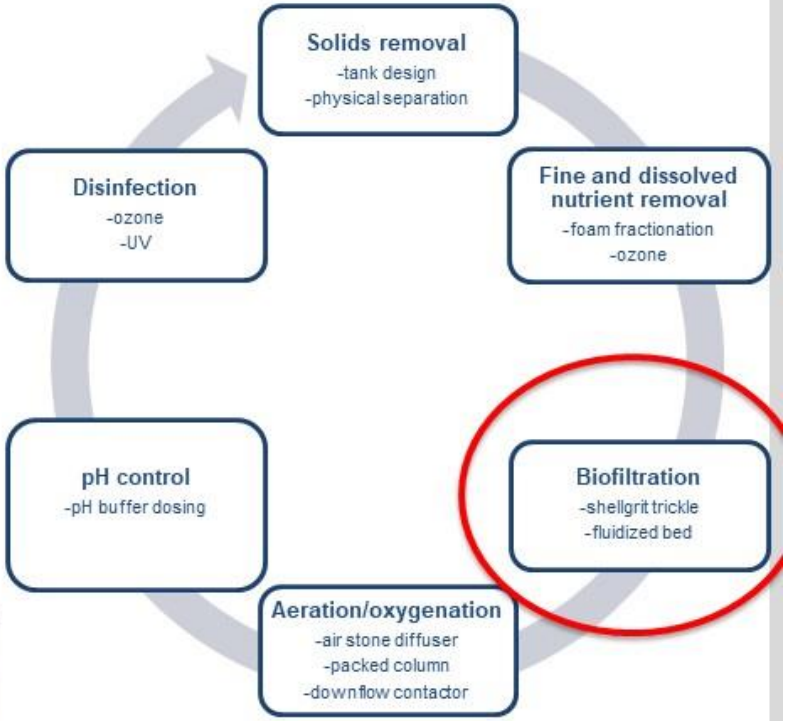
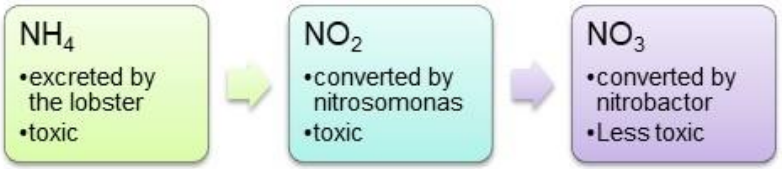
Aquaculture systems
Biofiltration

- Needed for the removal/conversion of ammonia
- Involves two types of bacteria
  - **Nitrosomonas** converts ammonia (NH_4) to nitrite (NO_2)
  - **Nitrobactor** converts nitrite (NO_2) to nitrate (NO_3)
- Activity rate of bacteria effected by temperature
  - Reduced at lower temperature
  - Inactive at >4°C
- Cleaning/disinfecting the biofilter will kill the bacteria
  - Needs to be reseeded/conditioned





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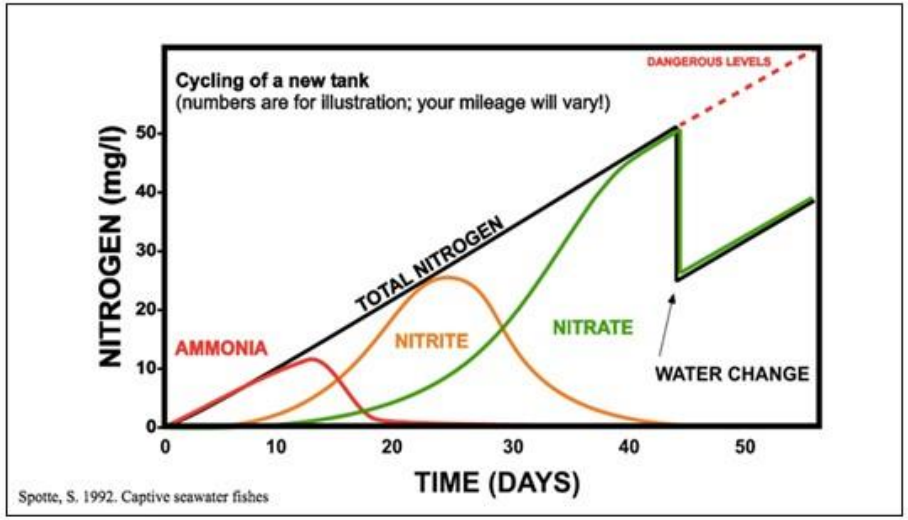
Aquaculture systems
Biofiltration



- Ammonia first to be converted
  - Nitrosomonas colonization first
- Then nitrite peaks
  - Nitrobactor slower and more difficult to achieve colonization
- Nitrate will continue to rise
  - Water exchange the only option

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Aquaculture systems

Biofiltration

- All about effective surface area (biomedia)
  - Living space for bacteria
  - Needs water contact (prevent channelling)
- Reduce bacterial competition
  - Limit organic inputs and collection
- Food for the bacteria
  - Ammonia and nitrate
  - Consistent supply
  - Conditioning – seeding and feeding
- Good living conditions
  - Bissolved oxygen
  - pH, 7.2-8.8 for Nitrosomonas
  - pH, 7.2-9.0 for Nitrobactor
  - Alkalinity >100 mg/l as $CaCO_3$
- Appropriate size for load
  - Needs to consider maximum loads
  - Spike loads (purging)!
- Most/all modern RAS employ fluidized bed/Media Bed Bio Reactor reactors

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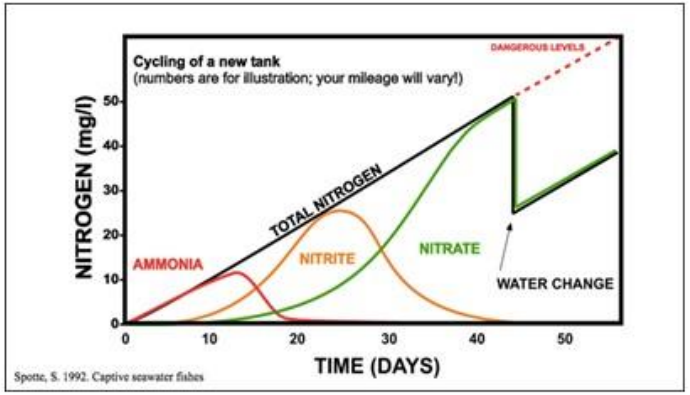
Aquaculture systems

Biofiltration

- Seeding and conditioning can be difficult and takes time
  - More difficult for saltwater than fresh water systems
  - Slower for nitrite than ammonia
  - Not until nitrate is effectively striped is the system ready for stocking
- Can seed
  - Naturally (in seawater) – less reliable
  - Existing conditioned media
  - Commercial seeding products
- Need to feed and demonstrate effective ammonia and nitrite stripping (conditioning)
  - Feed ammonia (eg ammonium chloride, cloudy ammonia)
- Need to calculate potential system ammonia excretion rate of up to 10 ug TAN per gram lobster per hour if stressed or purging (Crear and Forteath, 2002)
- Ammonia feeding calculation need to consider
  - System volume
  - Lobster load
  - Lobster physiological state (stress feeding etc.)
  - Temperature
  - Active constituent of ammonia source



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BIOMEDIA TYPES

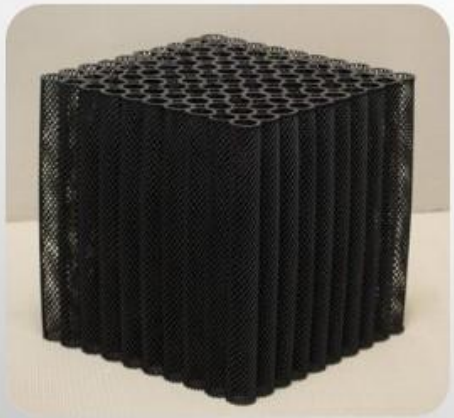
SHELL GRIT

CORAL BONES

PLASTIC MEDIA

BIOMEDIA TYPES

BIOBLOCK

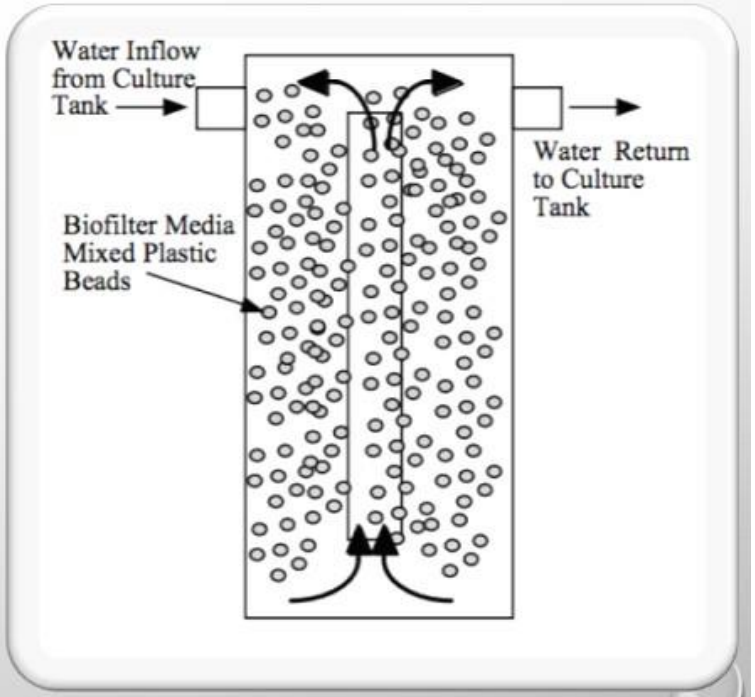


BIOBALLS

SAND

Media Bed Bio Reactor (MBBR)

A cross between up flow plastic bead filters and fluidized bed reactors. These filters use a plastic medium kept in a continuous state of movement.



ADVANTAGES:

- Cheaper construction (Mostly Civil)
- Cheaper MOC (materials of construction)
- Can handle bulk fluids (i.e. can treat large amount of sewage)
- Less skill to run the plant
- Maintenance is quite easy

DISADVANTAGES:

- Cannot produce pure water (particles can escape as they are free moving and not trapped).
- Aeration Lines can get plugged
- Bacterial activity has to be monitored periodically (manually)
- It is still a primitive kind of sewage treatment plant.
- Failure of any mechanical component leads to overall shutdown of the plant.

Trickle Filters

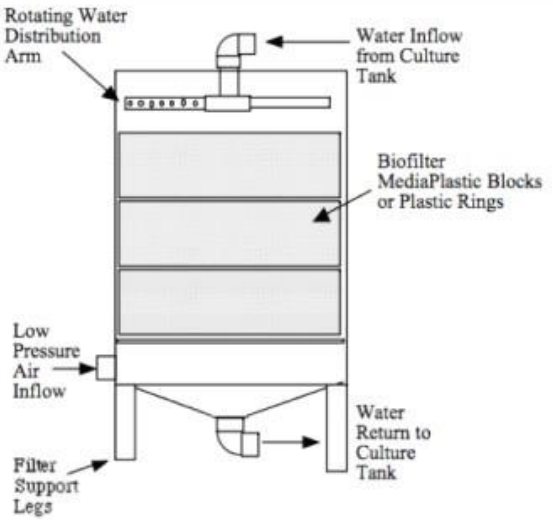
Dirty water is sprayed over a tall unite containing media.
Unlike most filters the trickle filter is not completely submerged in water.

ADVANTAGES:

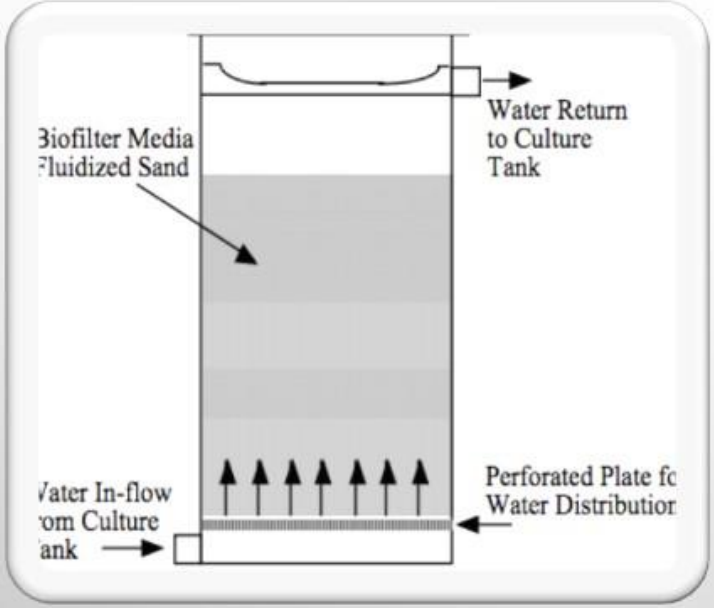
- High process stability due to constant high oxygen levels
- CO_2 removal by degassing
- water cooling in summertime;
- simplicity of design, construction, operation and management.
- Extremely inexpensive.

DISADVANTAGES:

- The relatively low volumetric removal rates.
- Biofilm shedding
- Risk of clogging when not properly designed and operated.



Static Filtration



Advantages:

- Simple Design
- Cheap to make and run
- No moving parts

Disadvantages:

- Clean regularly
- High Labor required for cleaning
- Easily clogged up

REMOVAL RATES

- MOST MEDIA ARE BETWEEN 0.10 AND 0.50 G TAN /M2/ DAY
- FOR COLD SALTWATER WE'D ASSUME 0.2 AND START FROM THERE.
- OXYGEN CONSUMPTION (APPROX. 0.25-0.9 KG PER KG OF FEED) AMMONIA PRODUCTION (APPROX. 0.025 KG TAN/KG OF FEED)
  - EXCRETION RATES VARY ON
    - SIZE – SMALLER ANIMALS RELATIVELY MORE PER KILO WEIGHT
    - TEMPERATURE – HIGHER AT HIGHER TEMPERATURES
    - NIGHT TIME/PHOTOPERIOD
    - STRESS
    - HANDLING/AIR EXPOSURE
    - POST PURGE TIME

WHAT AFFECTS REMOVAL RATES

- BOD – BIOLOGICAL OXYGEN DEMAND – SOLIDS/DIRTY WATER/DISSOLVED SOLIDS/CARBON
  - USING OZONE TO MAINTAIN ALMOST BLUE WATER WILL IMPROVE BIOFILTER PERFORMANCE
- FOR REALLY CLEAN SYSTEMS NEED LOWER REMOVAL AND MORE BIOMEDIA
- HIGHER AMMONIA / MORE LOAD – HIGHER REMOVAL BUT DIRTIER WATER – PUMPING MORE WATER BUT COSTLY
- OVERLOADING/OVERSTOCKING VERSUS SYSTEM DESIGN
- TEMPERATURE/PH/SALINITY CHANGES -

fresh by design

SURFACE AREA

- GRAVEL – 200 M2/M3
- PLASTIC BEADS (BIOBALLS) 1000 - 4000 M2/M3
- FLUIDISED BED REACTOR (FINE SAND) 5,000 M2/M3
- OPERATIONAL FILTER SURFACE AREA VERSUS MEDIA SURFACE AREA
- MBBR – AROUND HALF OF ADVERTISED – SO 700M2/M3 BECOMES 350M2/M3
- FIXED BED + TRICKLE FILTERS – MAYBE 80-90% - SO 300 BECOMES 250-270M2/M3
- AVAILABLE SURFACE AREA/PROTECTED SURFACE AREA – 700M2 BECOMES 500M2/M3
- HIGHER SURFACE AREA = LOWER REMOVAL RATE ?????

fresh by design

BIOFILTER SIZING

4	ug/g/h
1400	kg
134400000	ug/day
134400	mg/day
5600	mg/hr
18000	l/hr
0.31111	mg/l

Removal per pass required/produced by stock in tanks	0.31
Target concentration/clean water out of biofilter /into fish tank	0.30
Treating/Biofilter In concentration	0.61

MAINTAINING BIOFILTER

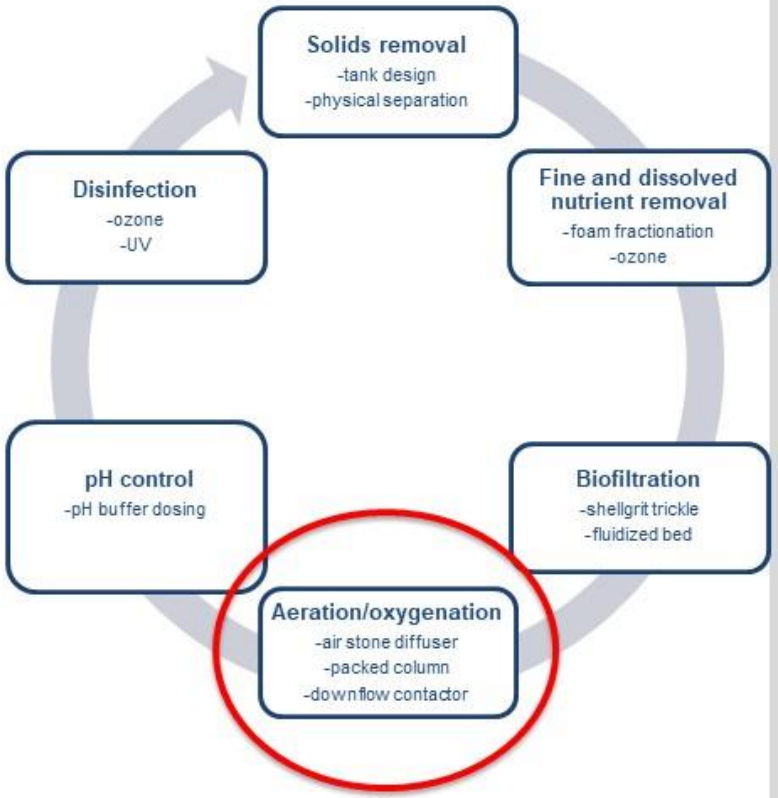
- AMMONIA DOSING
  - USING DOSING PUMP TO MAINTAIN SYSTEM AT OPTIMAL LEVEL
    - CLOUDY AMMONIA
    - AMMONIA CHLORIDE
    - AIM IS TO MAINTAIN AND GROW BACTERIA AND MAINTAIN STABLE SYSTEM
  - OR ADD FEED/WATER FROM OTHER SYSTEMS ALSO
- MAINTAINING CULTURE OF BACTERIA – TO DISINFECT OR NOT – THIS MAINTAINS LOCAL BACTERIA SUITED TO WHAT YOU ARE DOING AND WATER CONDITIONS
- KEEP SMALL NUMBERS OF ANIMALS IN SYSTEM
- MAINTAIN PH AND BICARBONATE LEVEL AT OPTIMAL LEVELS OR LEVELS EXPECTED

fresh by design

Aquaculture systems

Aeration/Oxygenation

- Maintaining high levels of oxygen saturation (>80% saturation) important for meeting lobster and system oxygen demands
- Oxygen saturation levels in water
  - Decreases with increasing temperature.
  - Need higher saturation levels in warm water
  - 6 mgO_2/L = 66% saturation at 10°C
  - 6 mgO_2/L = 81% saturation at 20°C
- Oxygen/gas (nitrogen) supersaturation (>100%) can be detrimental to aquatic organisms
  - Air injection under pressure
  - Leaking pumps at inlet
  - Difficult to measure but indicated by O_2 >100%
  - Excess oxygenation
  - Degassing required – another benefit of moving bed biofiltration



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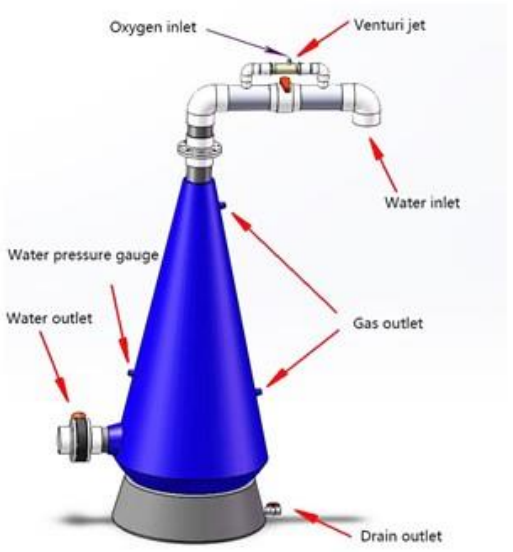
Aquaculture systems
Aeration/Oxygenation

- Aeration
  - Diffused air bubbles (air stones/diffusers)
  - Smaller bubble better
  - Increases oxygen levels and water mixing
  - Provides degassing – CO_2/nitrogen
  - Possible benefit by creating cover for lobsters
  - Needs to consider effects on flow dynamics and breakdown of particular organics
  - Venturi has the potential to supersaturate?
- Oxygenation
  - More efficient
  - Use pure O_2 gas supply or O_2 generators
  - Oxygen contactors, pressurized packed columns
  - Risk saturation
  - Questionable requirement for lobster holding



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Aquaculture systems

pH control

- pH can decline due to CO_2 production by lobster and BOD
- Chronic tolerance levels not well defined
- pH levels maintenance aided by aeration
  - degassing
- Water pH buffering capacity related to alkalinity; quantity bicarbonates, carbonates and hydroxides
- Potential benefit of shell-grit biofiltration media
- pH levels maintaining by dosing with alkaline chemical buffers
  - Sodium bicarbonate
  - Sodium hydroxide
  - Calcium carbonate
- Different buffers have differing benefits in terms of ease of use and health and safety
- Requires a mix tank, dosing apparatus and pH monitoring

Key research question;
pH/alkalinity optimal and threshold levels and optimal pH dosing practices for SRL holding?

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PH CONTROL METHODS

SOLIDS – SODIUM BICARBONATE/LIME

- MIXING VESSEL
- OVERFLOW ON MIXER
- WATER DOSING INTO MIXER WITH SOLENOID CONTROL – VERY SMALL
- PH SENSOR LINKED TO SOLENOID VALVE OPENS AND CLOSES AND DOSE
- CHEAP AND GENERALLY CHEAPER PER DEGREE OF PH CONTROL
- SAFER HANDLING AND CONTROL – CANT DOSE PAST 8.3 OR BURN SKINS (OH+S)
- BULKY
- NEED TO MAINTAIN LOWER PH
- LIME CAN GET MESSY WITH DUST ETC

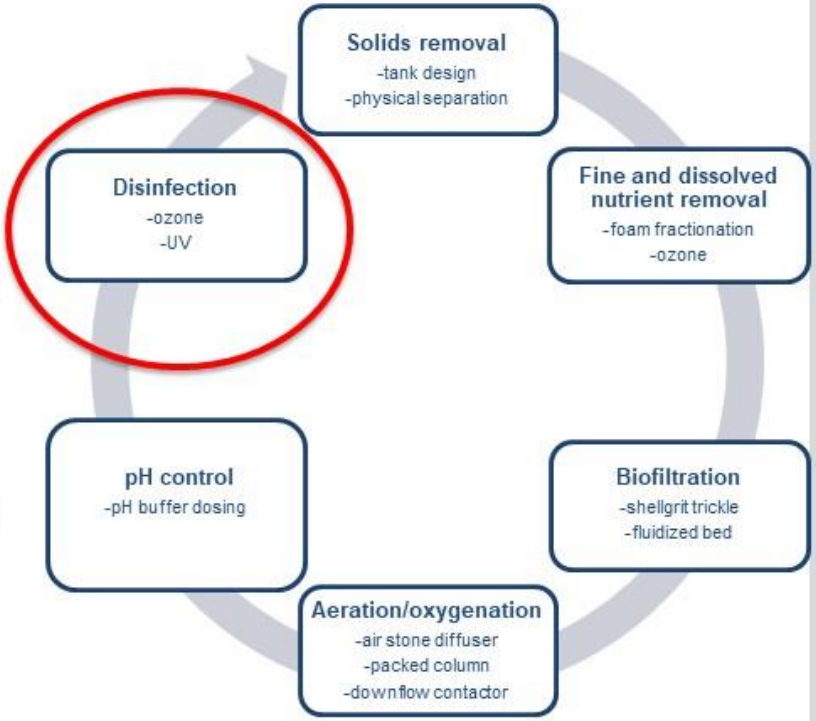
LIQUID - SODIUM HYDROXIDE

- IBC/200L/50L DRUM
- PH SENSOR
- DOSING PUMP WITH HOSES
- EASY TO CENTRALISED FOR MULTIPLE SMALL SYSTEMS
- CHEAPER INITIAL COST TO INSTALL
- OH+S ISSUES – SEVERE BURNS POSSIBLE
- OH+S REQUIREMENTS INCREASE COST – BUNDS, WASH STATIONS ETC
- CAN OVERDOSE THE SYSTEM EASILY
- CAN MAINTAIN HIGHER PH MORE EASILY

fresh by design

Aquaculture systems
Disinfection

- Killing of water-bourn bacterial and viruses
  - Potential disease agent
  - Reduce biological oxygen demand
- Achieved by
  - Ultra violet (UV) sterilization
  - Ozone disinfection
- Final water polishing before delivery to culture tanks



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UV STERILIZATION

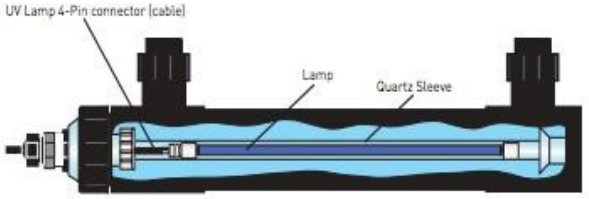
Controls bacteria, micro-algae, fungi, and some viruses in RAS.

ADVANTAGES:

- Eliminate algae and other unwanted organisms (bacteria, fungi, pathogen).
- Reduces water turbidity.
- Chemical free
- Environmentally friendly

DISADVANTAGES:

- Can be expensive depending on the size of the system.
- Extra cleaning: UV light is also only effective in the water being treated is clear
- Lamp must be replaced yearly
- Power consuming (on for 24h)

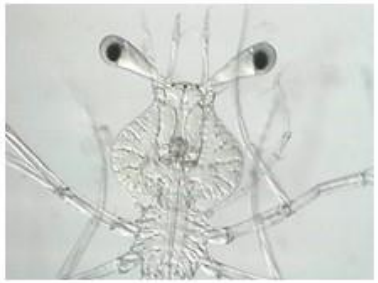


Aquaculture systems

Ozone

- Uses
  - Removal of fine solids
  - Removal of dissolved organics
  - Removal of nitrite
  - Powerful disinfectant
- Advantages
  - Powerful
  - Quick and efficient
  - Increases dissolved oxygen
  - Inexpensive?
- However
  - In seawater ozonation produces persistent oxidation by-products
  - High levels acutely toxic to cultured animals
  - Threshold levels of ozonation oxidation by-products not know for SRL
  - Ozone can also kill people!

Key research question;
Optimal and threshold for ozonation in SRL holding?



Lobster phyllosoma moult deformities

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Aquaculture systems
Ozone

- Created by a ozone generator
  - Passes oxygen through a electrical current
  - Requires a oxygen source (generator, gas supply or O_2 concentrator
- Ozone injected into water through venture
  - Foam fractionator (however not before the biofilter)
  - Contact chambers
- Disinfection property dependent on
  - Amount of ozone
  - Contact time

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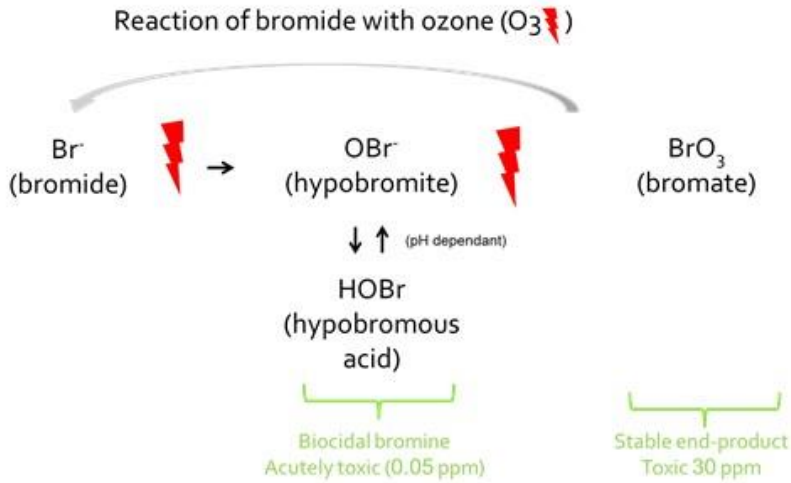
Aquaculture systems

Ozone

- Halogen ions oxidize to halo-oxyions
  - Bromine, Iodine, Chlorine
- Produces oxidation by-products (OBPs)
- Mostly bromine due to high abundance and reactivity



Elements

Symbol	Name	SeaWater Concentration PPM	Symbol	Name	SeaWater Concentration PPM
H+	Hydrogen	Ultra-trace	He	Helium	0.000007600
Cl-	Chloride	19,400.000000000	Ti	Titanium	0.000006500
Na	Sodium	10,800.000000000	La	Lanthanum	0.000005600
Mg	Magnesium	1,280.000000000	Ge	Germanium	0.000005500
S04-	Sulfate	898.000000000	Nb	Nobelium	0.000005000
Ca	Calcium	412.000000000	Hf	Hafnium	0.000003400
K	Potassium	399.000000000	Nd	Neodymium	0.000003300
Br	Bromine	67.000000000	Pb	Lead	0.000002700
C	Carbon	27.000000000	Ta	Tantalum	0.000002500
N2	Nitrogen	8.300000000	Ag	Silver	0.000002000
Sr	Strontium	7.800000000	Co	Cobalt	0.000001200
B	Boron	4.500000000	Ga	Gallium	0.000001200
O2	Oxygen	2.800000000	Er	Erbium	0.000001200
Si	Silicon	2.800000000	Yb	Ytterbium	0.000001200
F	Fluorine	1.300000000	Dy	Dysprosium	0.000001100
Ar	Argon	0.620000000	Gd	Gadolinium	0.000000900
NO3	Nitrate	0.420000000	Sc	Scandium	0.000000700
Li	Lithium	0.180000000	Ce	Cesium	0.000000700
Rb	Rubidenum	0.120000000	Pr	Promethium	0.000000700
P04	Phosphate	0.062000000	Sm	Samarium	0.000000570
I	Iodine	0.058000000	Sn	Tin	0.000000500
Ba	Barium	0.015000000	Ho	Holmium	0.000000360
Mo	Molybdenum	0.010000000	Lu	Lutetium	0.000000230
U	Uranium	0.003200000	Be	Beryllium	0.000000210
V	Vanadium	0.002000000	Tm	Thulium	0.000000200
As	Arsenic	0.001200000	Eu	Europium	0.000000170
Ni	Nickel	0.000480000	Tb	Terbium	0.000000170
Zn	Zinc	0.000350000	Hg	Mercury	0.000000140
Kr	Krypton	0.000310000	Rh	Rhodium	0.000000080
Cs	Cesium	0.000306000	Te	Tellurium	0.000000070
Cr(VI)	Chromium	0.000210000	Pd	Palladium	0.000000060
Sb	Antimony	0.000200000	Pt	Platinum	0.000000050
Ne	Neon	0.000160000	Bi	Bismuth	0.000000030
Se	Selenium	0.000155000	Th	Thorium	0.000000020
Cu	Copper	0.000150000	In	Indium	0.000000010
Cd	Cadmium	0.000070000	Au	Gold	0.000000010
Xe	Xenon	0.000066000	Ru	Ruthium	0.000000005
Al	Aluminum	0.000030000	Os	Osmium	0.000000002
Fe	Iron	0.000030000	Ir	Iridium	Ultra-trace
Mn	Manganese	0.000020000	Ra	Radium	Ultra-trace
Y	Yttrium	0.000017000	Rn	Radon	Ultra-trace
Zr	Zircon	0.000015000	Fr	Francium	Ultra-trace
Tl	Thallium	0.000013000	Ac	Actinium	Ultra-trace
W	Tungsten	0.000010000	Pa	Protactinium	Ultra-trace
Re	Rhenium	0.000007800			

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Aquaculture systems

Ozone

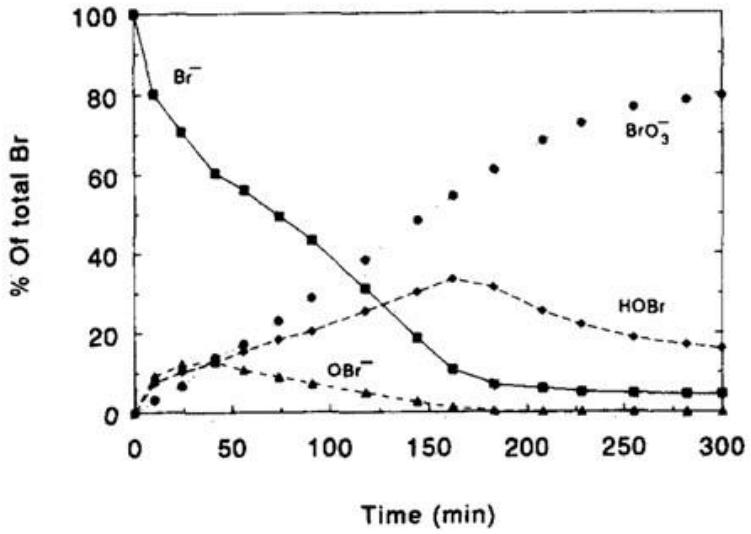

Fig. 1. Bromine speciation vs time during ozonation of 722 μM NaBr: ■, Br^-; ▲, OBr^-; ◆, HOBr; ●, BrO_3^-

Grguric et al., 1994

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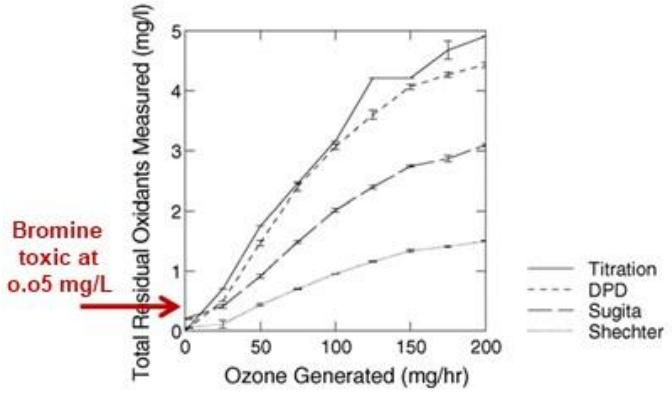
Aquaculture systems

How to measure and control ozone

- DPD colourmetric method
- Fast and straight forward
- Commercially available tests
  - Hach, Palintest, LaMotte
- Measures total residual oxidants
- However, high minimum measurement
  - 0.5mg/L
  - These levels may already be toxic to lobsters?



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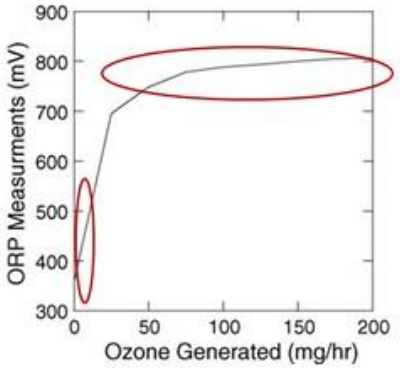
Aquaculture systems

How to measure and control ozone

- Oxidation/reduction potential (ORP)
- Inline meter – real time
- Commercially available probes
  - Foxboro, Pinoints ect.
- Can be connected to a controller
- However,
  - Probes need continual maintenance/cleaning/calabration
  - Can be unreliable at low levels.
  - Never rely on one probe
  - ORP plateaus at high levels



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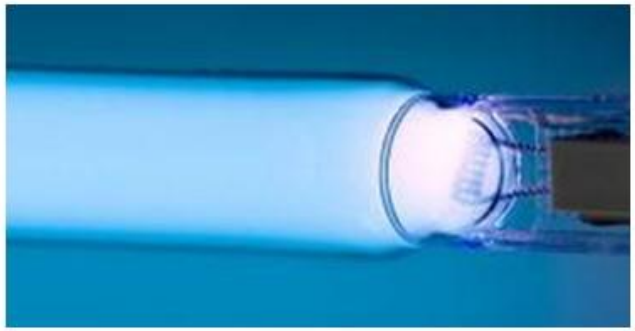
Aquaculture systems
bromine removal

- Time and aeration
  - Requires large water storage
- Granular activated carbon
  - Oxidation substrate
  - Requires regular replacement
- UV irradiation
  - O_3 destroyed by high intensity UV



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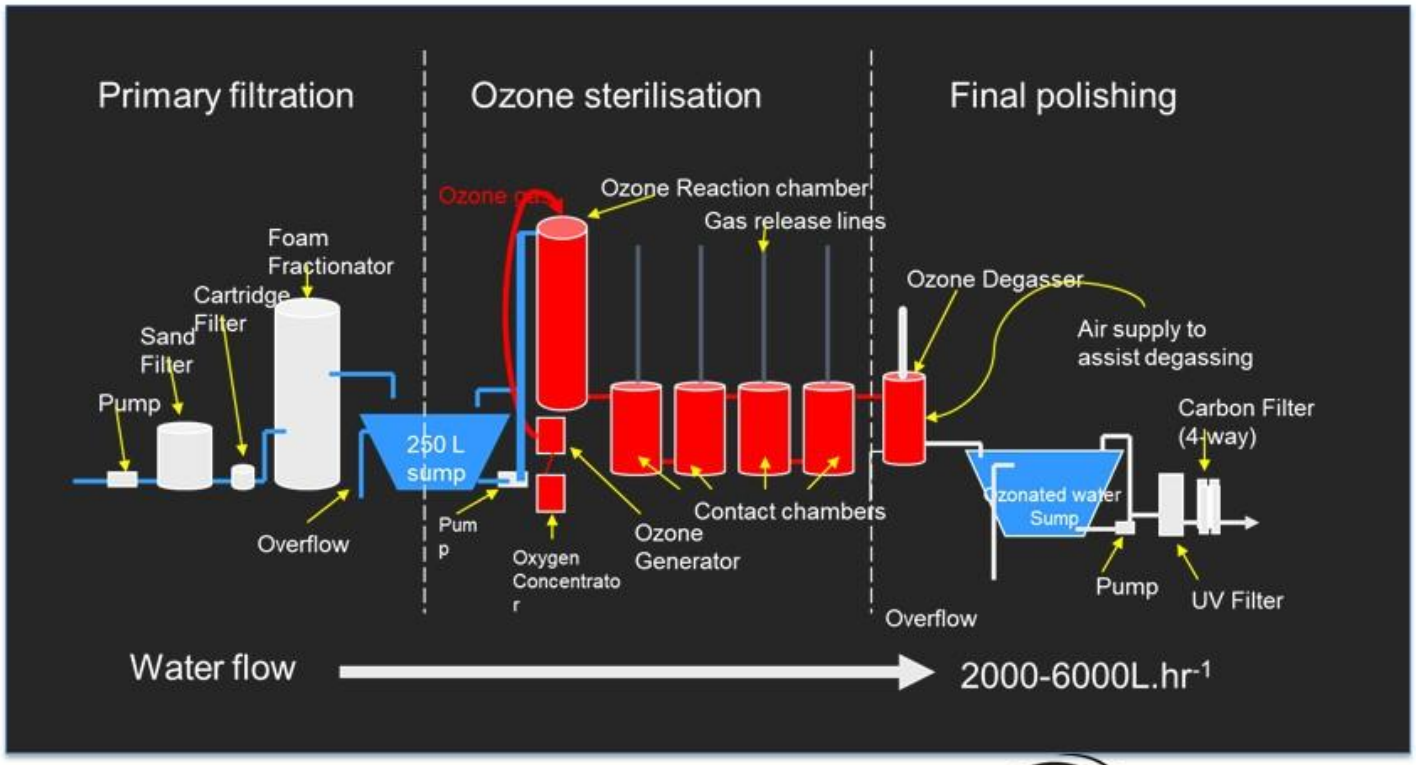
Aquaculture systems

Ozone system set up



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Aquaculture systems

Ozone conclusions

- Ozone a powerfully water conditioning and sterilization tool
  - Likely to resolve any disease/toxin concerns
- However needs commitment, effort and understanding to effectively implement
  - Significant risk
  - Use professional advice
  - Rigorous monitoring
- Introduce after biofiltration
- Strip toxins before introduction to tanks or use very low levels
  - Question the effectiveness of bust treatments
- Be aware of and manage health and safety concerns
  - O_3 generation in well ventilated areas – best outside
  - O_3 corrosive - use appropriate O_3 fittings and lines
  - Maintenance of fitting and lines (check valves, delivery lines)
  - Use O_3 monitors/alarms and gas masks when required

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SHOCK LOADING

- WATER EXCHANGE
  - TEMPERATURE CONTROL OF INCOMING WATER AND SYSTEM WATER
  - WATER STORAGE
  - TREATMENT OF SYSTEM WATER AND RE-USE
- SOLIDS REMOVAL (FINE AND LARGE SOLIDS)
  - MEDIA FILTER, DRUMFILTERS, FRACTIONATORS, SIEVE/PARABOLIC SCREENS, FIXED BED FILTERS, BEAD FILTERS
- BIOFILTER PREPARATION
  - SEEDING AND POST STOCKING SEEDING
  - CYCLING PRE STOCKING
- USING SMALLER HIGH STOCKED PURGE SYSTEMS – COST BENEFITS
- OZONE
  - PLC CONTROLLED AND MULTIPLE FAILSAFES

fresh by design

Aquaculture systems

Flow through

- Advantages
  - Less infrastructure/processes
  - Easier for maintenance of nutrient/biological loads
- Disadvantages
  - Temperature control (costly)
  - Ambient fluctuations (salinity/temperature)
  - Waste water treatment/discharge (biosecurity/environmental restrictions)
  - Suggest that going forward there will be increasing political pressure for all on shore aquaculture to treat water discharge and thus require recirculation technologies
- Heat exchangers
  - Effective but expensive
- Water storage
  - Security and flexibility

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Session 3

Water quality parameters and testing

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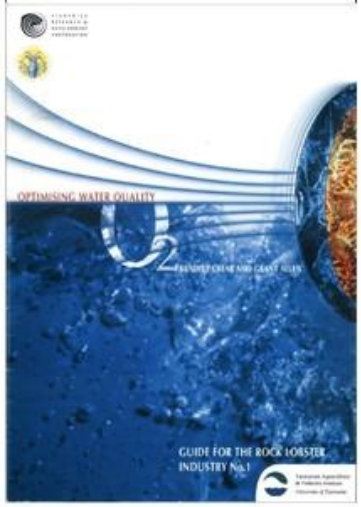
Introduction

- Aims
  - To facilitate evidence based approaches for water quality monitoring

- Methods
  - Evaluate a range of water quality testing approaches for ease, value and accuracy
  - Parameters: pH, ammonia, nitrite, nitrate, phosphate, alkalinity

- Recommended maximum limits
  - Clean Green & Crear FRDC report
  - Most recommendations are not well studied in SRL
  - No recommendations consider chronic effects



Methods

- Test kits
  - API 5 in 1 test strips
  - API water testing kits
  - YSI colourimetric kits
  - Hach spectrophotometric kits
- Standards prepared according to published EPA methods
- Hach kits verified against results from Analytical Services Tasmania lab results ($200 per analysis)

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Introduction

- General pros and cons of each method

	Pros	Cons
API 5 in 1 strips	• Easy to use • Test multiple parameters at once	• Interpreting colour results can be difficult • Expensive compared to test kits • No flexibility in parameters • One parameter (GH) is useless
API test kits	• Economical • Easy to get • Easy to use	• Interpreting colour result can be difficult
YSI test kits	• Precise • Include data storage and management options	• Significant capital investment, meter = $2000
Hach test kits	• Precise • Include data storage and management options	• Significant capital investment, spectrophotometer = $5000, meter = $1700 • Most reagents classified as dangerous goods • Require accurate measurement of sample (e.g. pipette)

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pH

- Seawater pH is generally around 8.05-8.15
- Clean Green standards: 7.80-8.20
- FRDC Optimising Water Quality recommendation: 7.80-8.40
- In facilities, pH ranges from 6.79 – 7.88
  - Low pH can disrupt acid-base balance in blood
  - Affects ammonia toxicity, alkalinity, other parameters

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pH comparison

	$	Tests	$/test	Time	Pros	Cons
5 in 1	$40 / $8	25	$1.60 / $0.32	30 s		• Least precise • Colours not easy to interpret
API kit	$8	250	$0.03	10 s	• Quick results	• Least precise • Colours not easy to interpret
YSI	$145	250	$0.58	15-20 s	• Moderately precise	• Lowest high-end range
Hach Rugged pH	$1300	Infinite	N/A	5-10 s	• N/A	• N/A
Testo 205	$600	Infinite, though probe ($300) must be replaced occasionally	N/A	~60 s	• Most precise • Adjusts for temperature	• Requires frequent calibration • Longest test

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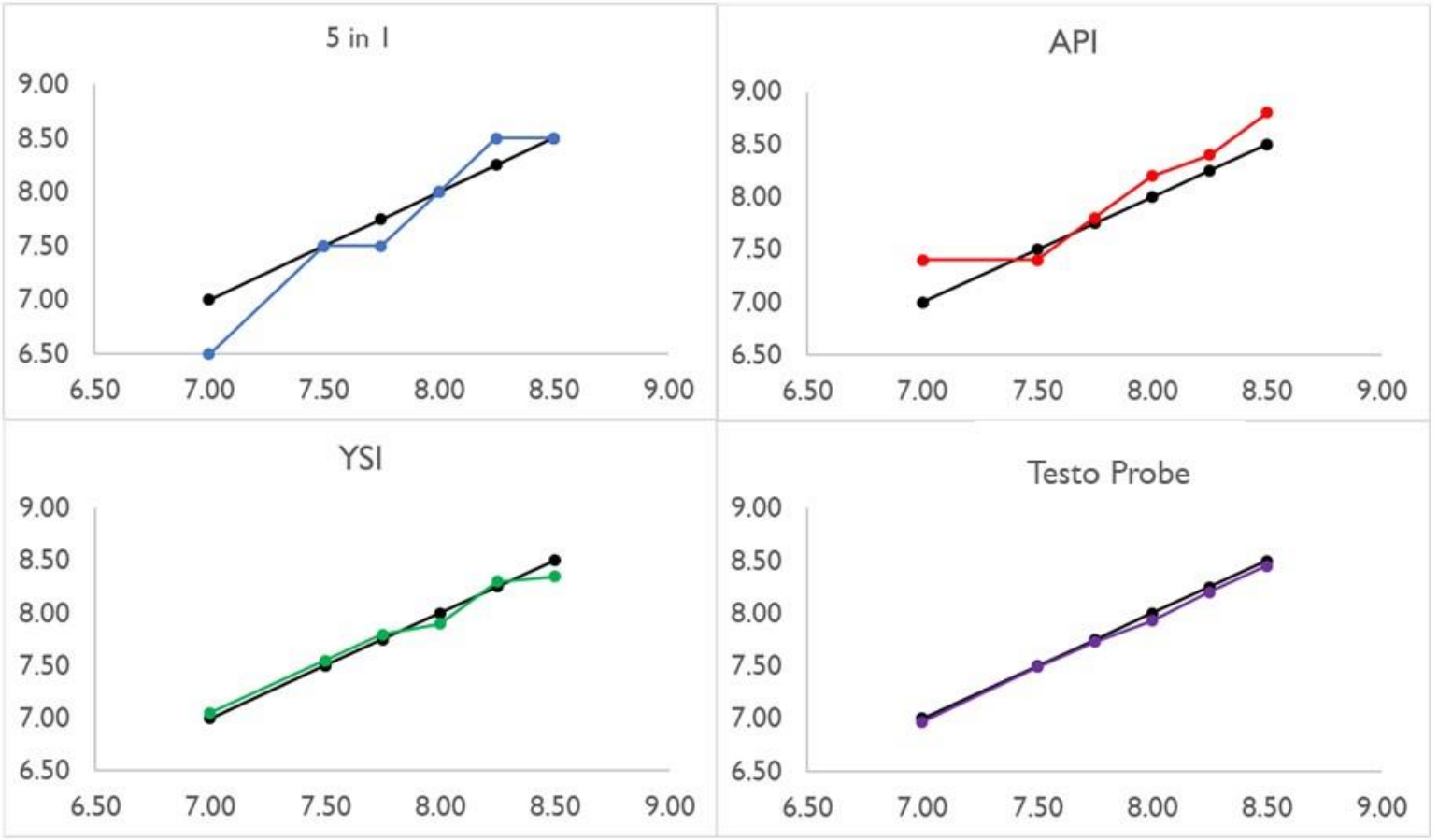
pH Results



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pH Results

- 5 in 1 strips and API test colour scale hard to read, some results were between two colours requiring a judgement call
- YSI test performed well but have potential for error as "pills" dissolved well but occasionally test needed to be run more than once to allow debris to settle
- Testo probe took longest of each test but offered best accuracy
- Temperature correction of electronic probes an important factor for accuracy
- Hach rugged probe not evaluated, but represents a robust, precise, and accurate option that complements investment in other meters

- What factors are driving the low pHs recorded in facilities?
  - CO_2? Acidic waste products?
- What methods are available for better buffered water?
- Does chronic exposure to lower pH have any impact on health?

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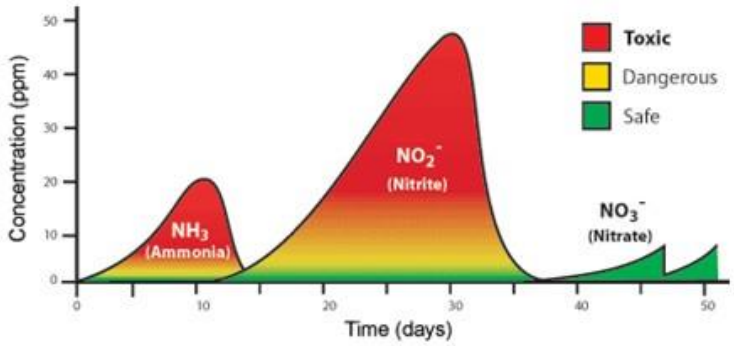
Nitrogen cycle



- Ammonia (NH_3/NH_4; toxic) is introduced via lobster waste
- Nitrifying bacteria convert ammonia to nitrite (NO_2; somewhat less toxic)
- Nitrite is then converted to nitrate (NO_3; much less toxic)
- Nitrate removed via water exchange
- Biological filtration capacity reduces peaks/duration
- Any time biological filtration capacity is exceeded, ammonia can spike

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Ammonia

- High concentrations of ammonia damage gills and negatively affect acid-base balance of blood
- In clean seawater, no ammonia should be detected
  - Agricultural, industrial and urban sources can contaminate coastal water
- Lethal levels = 20-30 mg/L (Battaglene et al. 2004)
  - LC50, does not account for sublethal effects
- Clean Green standard: <0.5 mg/L
- FRDC Optimising Water Quality recommendation: <2 mg/L
- In facilities, ammonia levels ranged from 0.0-0.55 mg/L

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Ammonia and pH

- Ammonia comes in two forms:
  - Toxic NH_3
  - Less toxic NH_4
- Test kits convert both forms and report the sum (i.e. they don't tell you how much of toxic vs less toxic)

Percent toxic unionised ammonia (NH_3) in seawater by temperature and pH

pH	Temperature (°C)		
	8	12	16
7.0	0.2	0.2	0.3
8.0	1.6	2.1	2.9
8.2	2.5	3.3	4.5
8.4	3.9	5.2	6.9
8.6	6.0	7.9	10.6
8.8	9.2	12.0	15.8
9.0	13.8	17.8	22.9

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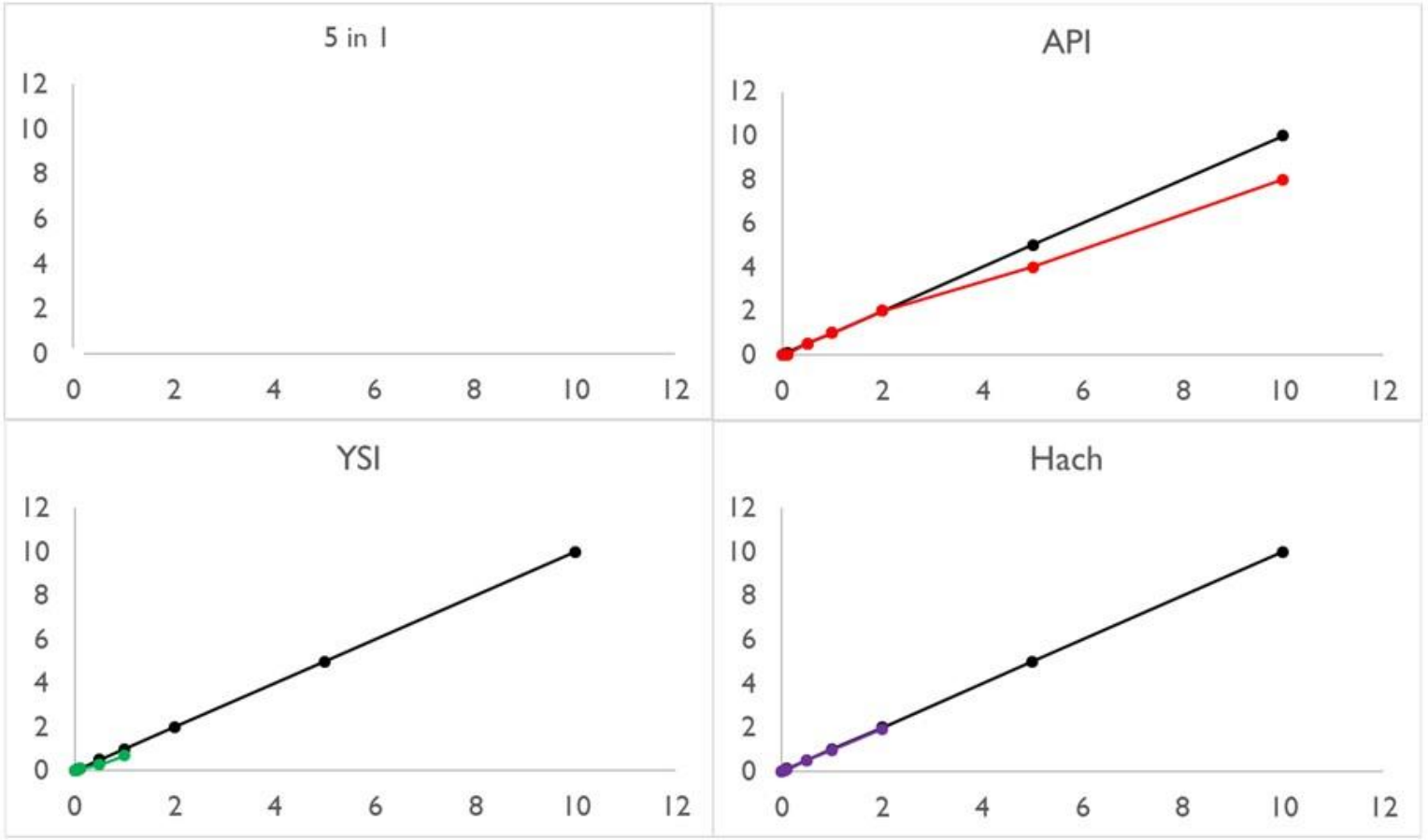
Ammonia comparison

	$	Tests	$/test	Time	Pros	Cons
5 in 1						• Does not test ammonia
API kit	$8	250	$0.12	5 m	• Easy	• Colours not easy to interpret; Imprecise results
YSI	$329	250	$1.32	10 m	• Precise results	• Limited range, requires dilution; • Pills difficult to crush in tube
Hach	$183	25	$7.32	20 m	• Precise results	• Requires accurate measurement of sample • Reagents classified as dangerous goods

Ammonia results



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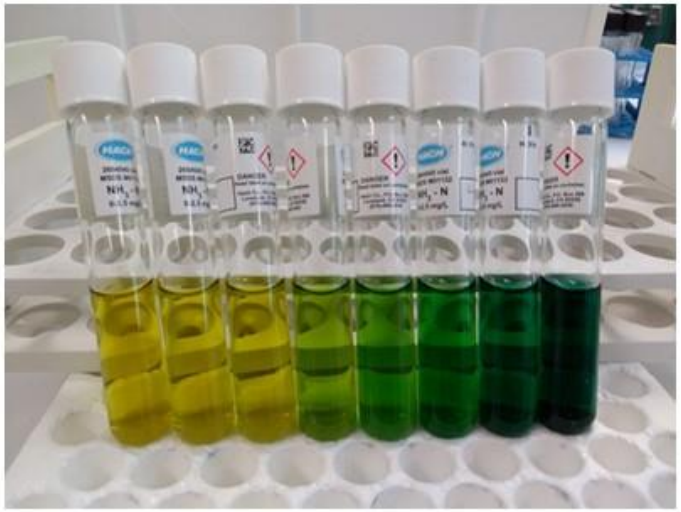
Ammonia results

- API test performed well with the greatest range
  - Test benefited from standard concentrations aligning with Colour Card
- Hach test performed well and was most precise, can be diluted for greater range



- Are there sublethal effects we should be concerned about?
  - Stress, health
- We need a better understanding of ammonia levels in holding facilities
  - Peaks after adding new stock – how high? how long?

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Nitrite

- Nitrite toxicity reduces ability of blood to carry O_2
  - Stimulates ventilation and ammonia secretion, creating feedback loop
- In clean seawater, no nitrite should be detected
- Toxicity limits of nitrite not well studied in decapods
  - 10 mg/L used as sub-lethal exposure experimentally
  - Toxicity may increase with reduced salinity levels
  - Chronic sub-lethal exposure not sufficiently studied
- Clean Green standard: <1.0 mg/L
- FRDC Optimising Water Quality recommendation: <5 mg/L
- In facilities, nitrite levels ranged from 0.0-0.58 mg/L

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Nitrite comparison

	$	Tests	$/test	Time	Pros	Cons
5 in 1	$40 / $8	25	$1.60 / $0.32	30 s		• Difficult to differentiate colours
API kit	$11	180	$0.06	5 m	• Good colour spread	
YSI	$85	250	$0.34	10 m	• Accurate and precise results	• Limited range
Hach	$77	50	$1.54	20 m	• Accurate and precise results	• Limited range • Requires careful measurement of sample • Reagents classified as dangerous goods

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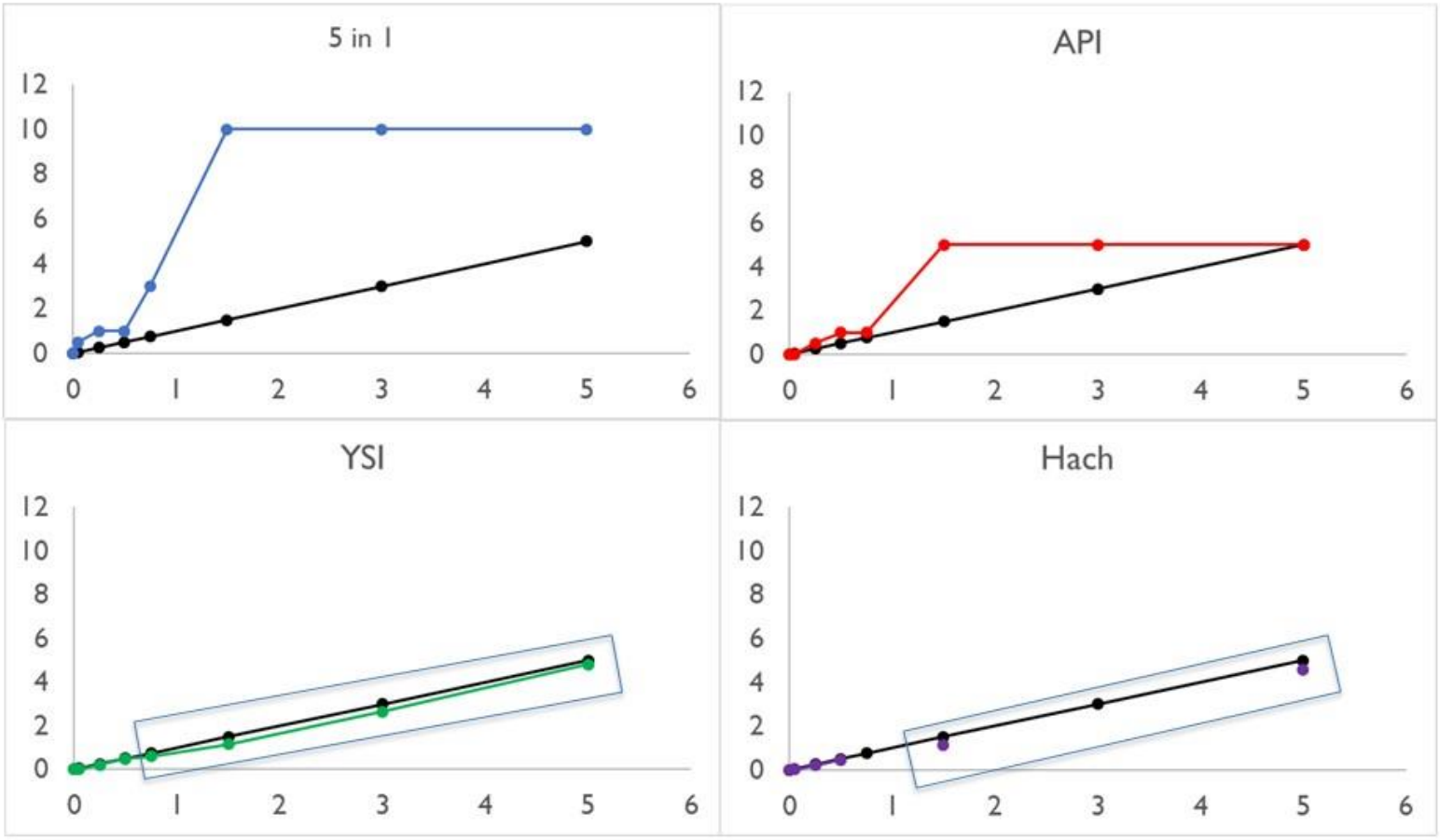
Nitrite results



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Nitrite results

- 5 in 1 strips difficult to differentiate colours
- API test over reported levels, potentially due to colour card interpretation
- YSI and Hach tests returned accurate results across range, but limited range required dilution

- Similar to ammonia, sublethal effects need better understanding
- Nitrite levels require monitoring for accurate characterisation

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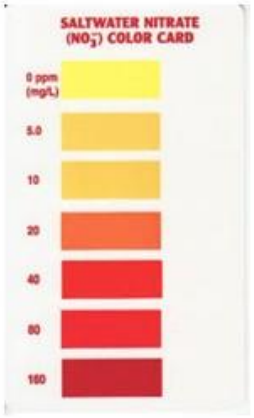
Nitrate

- Nitrate is the final step in the nitrogen cycle
  - No further conversion
  - Builds up in recirc systems
  - Controlled via water changes
- Toxicity levels very poorly studied
  - In prawns, toxic above 2,200 mg/L
- Clean Green standard: 100-140 mg/L
- FRDC Optimising Water Quality recommendation: <100 mg/L
- In facilities, nitrate levels ranged from 0.036 – 13.0 mg/L



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Nitrate comparison

	$	Tests	$/test	Time	Pros	Cons
5 in 1	$40 / $8	25	$1.60 / $0.32	30 s	• Easy	• Difficult to differentiate colours
API kit	$14	90	$0.16	7 m	• Easy	• Colours difficult to differentiate in high range
YSI	$206	200	$1.03	15 m		• One of the most complex tests
Hach	$86	100	$0.86	6 m		• Cadmium waste • Reagent classified as dangerous good

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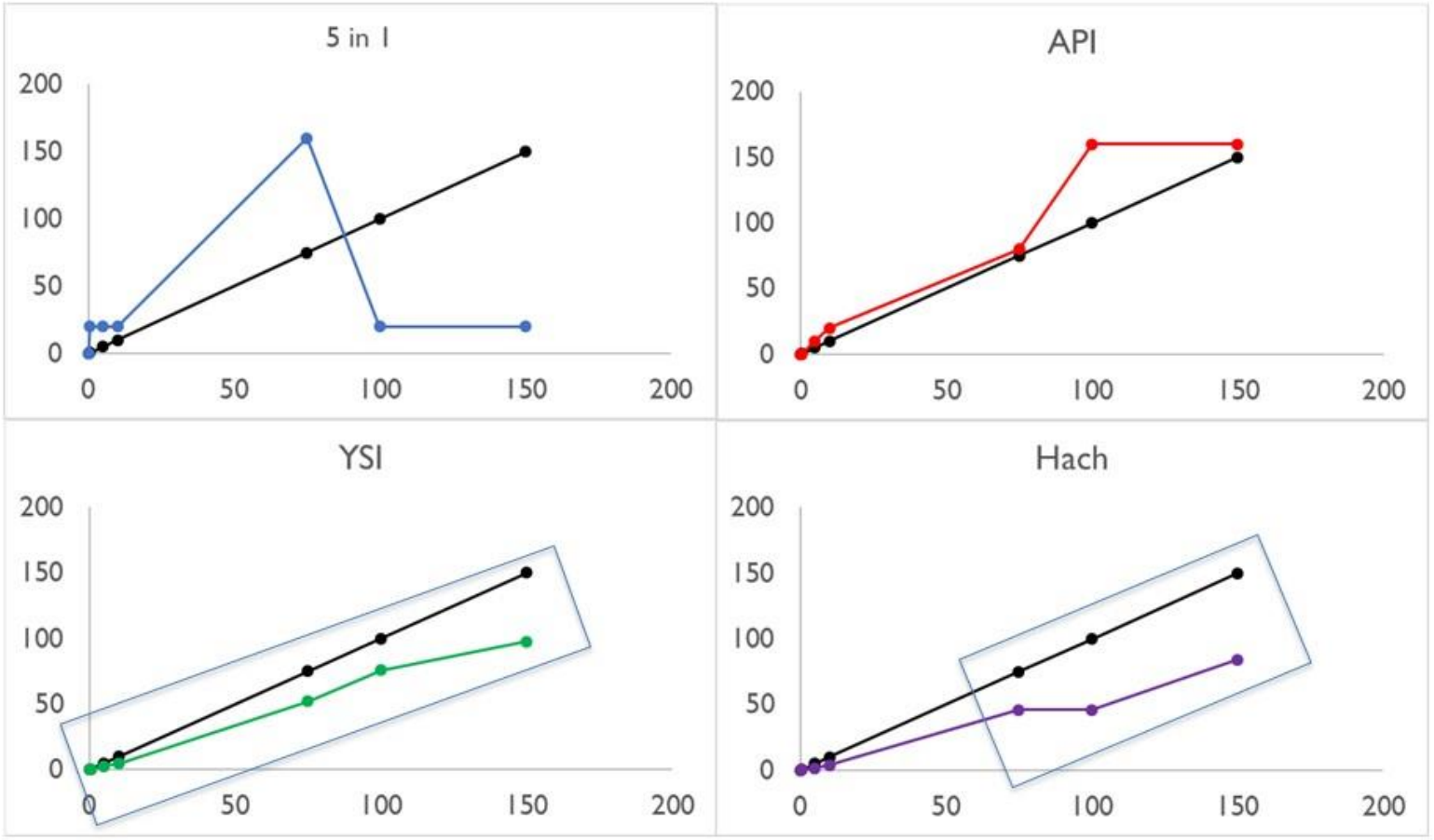
Nitrate results



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Nitrate results

- No test performed particularly well
- API kit performed well for its price
  - Over reported
  - Potential bias: known standard concentration
- Hach tests performed well in undiluted range
  - User error?

- What are the best ways to measure nitrate levels?

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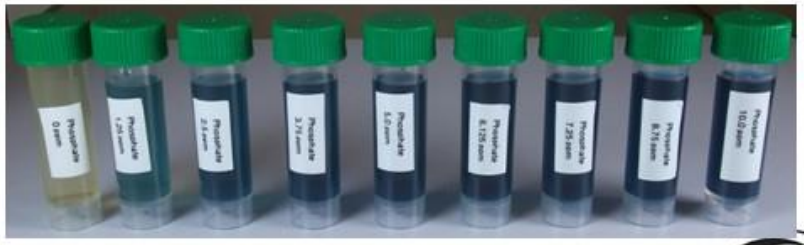
Phosphorus

- Phosphorus can enter coastal waters from wastewater treatment plants, agricultural run-off (e.g. fertiliser or animal manure) or in facilities from the use of detergents
- Not likely to be harmful to lobsters except at very high levels
  - But, is a limiting nutrient for algal growth
- Most facilities had no detectible phosphorus
  - Where detected, phosphorous was found at concentrations of 10-20 mg/L
  - Most likely a result of exposed concrete blocks/mortar leaching into seawater
- Currently there is no Clean Green standard or FRDC Optimising Water Quality recommendation for phosphorus



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Phosphorus comparison

	$	Tests	$/test	Time	Pros	Cons
5 in 1						• Does not test
API kit	$18	150	$0.12	3 m	• Easy	
YSI	$134	250	$0.54	10-15 m	• Offers high and low range	• Low range lacks sensitivity • Requires dilution at high levels
Hach	$73	25	$2.92	10 m	• Easy compared to other Hach tests	• Requires accurate measurement of sample • Reagent classified as dangerous good

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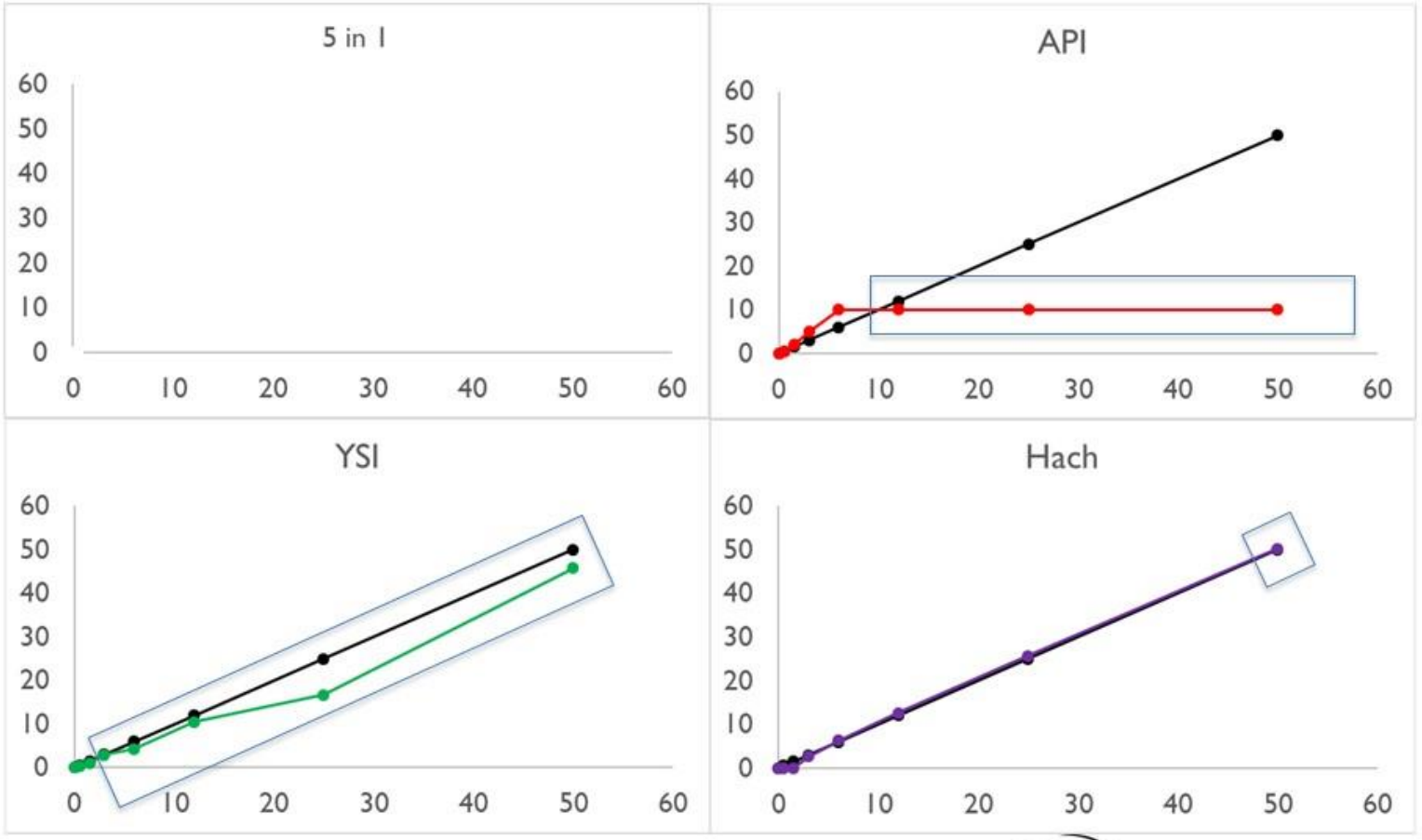
Phosphorus results



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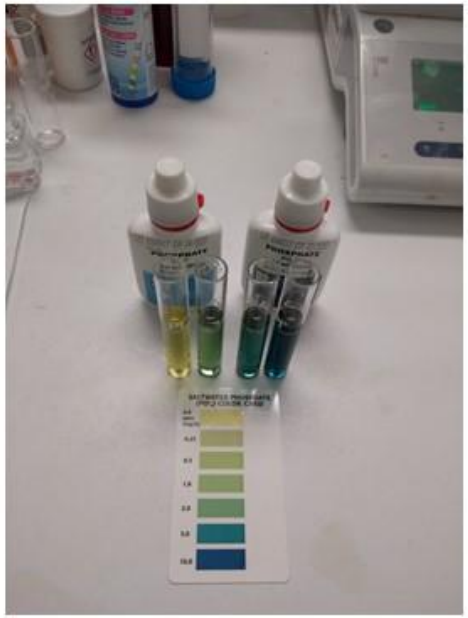
Phosphorus results

- Hach most accurate at high range, not sensitive at low range
  - Low range most applicable to facilities
- API sensitive and accurate at low range, in high range even diluted samples read as maximum
- Given low toxicity, all performed reliably



- Is phosphorus from exposed tank materials or from seawater contamination
  - Depending on source, there may be other contaminants

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Alkalinity

- Measure of buffering capacity in water = stability of pH when acids are added (e.g. CO_2, nitrogenous waste)
  - Low alkalinity means water will decrease in pH more rapidly due to acidic metabolites; rapid changes in pH can be harmful
  - Improved by addition of shell grit, bicarbonate
- Seawater alkalinity = around 116 mg/L $CaCO_3$
- We currently do not have sufficient data on alkalinity in facilities (2 facilities: 68 and 164 mg/L $CaCO_3$)
- Clean Green standard: 100-200 mg/L $CaCO_3$
- FRDC Optimising Water Quality recommendation: 100-200
- Alkalinity frequently referred to as "carbonate hardness" (KH or dKH) in aquaria trade

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Alkalinity comparison

	$	Tests	$/test	Time	Pros	Cons
5 in 1	$40 / $8	25	$1.60 / $0.32	0 s	• Very easy	• Results given as degrees carbonate hardness (dKH) rather than mg/L $CaCO_3$
API kit	$11	180	$0.06	0 s – 1 m	• Easy	• Results given as degrees carbonate hardness (dKH) rather than mg/L $CaCO_3$
YSI	$85	250	$0.34	1 m	• Easy assay	
Hach	$77	50	$1.54	2 – 5 m	• Accurate and precise titration method	• Requires additional equipment • Technically difficult titration • Requires complex calculations • Reagent classified as dangerous good

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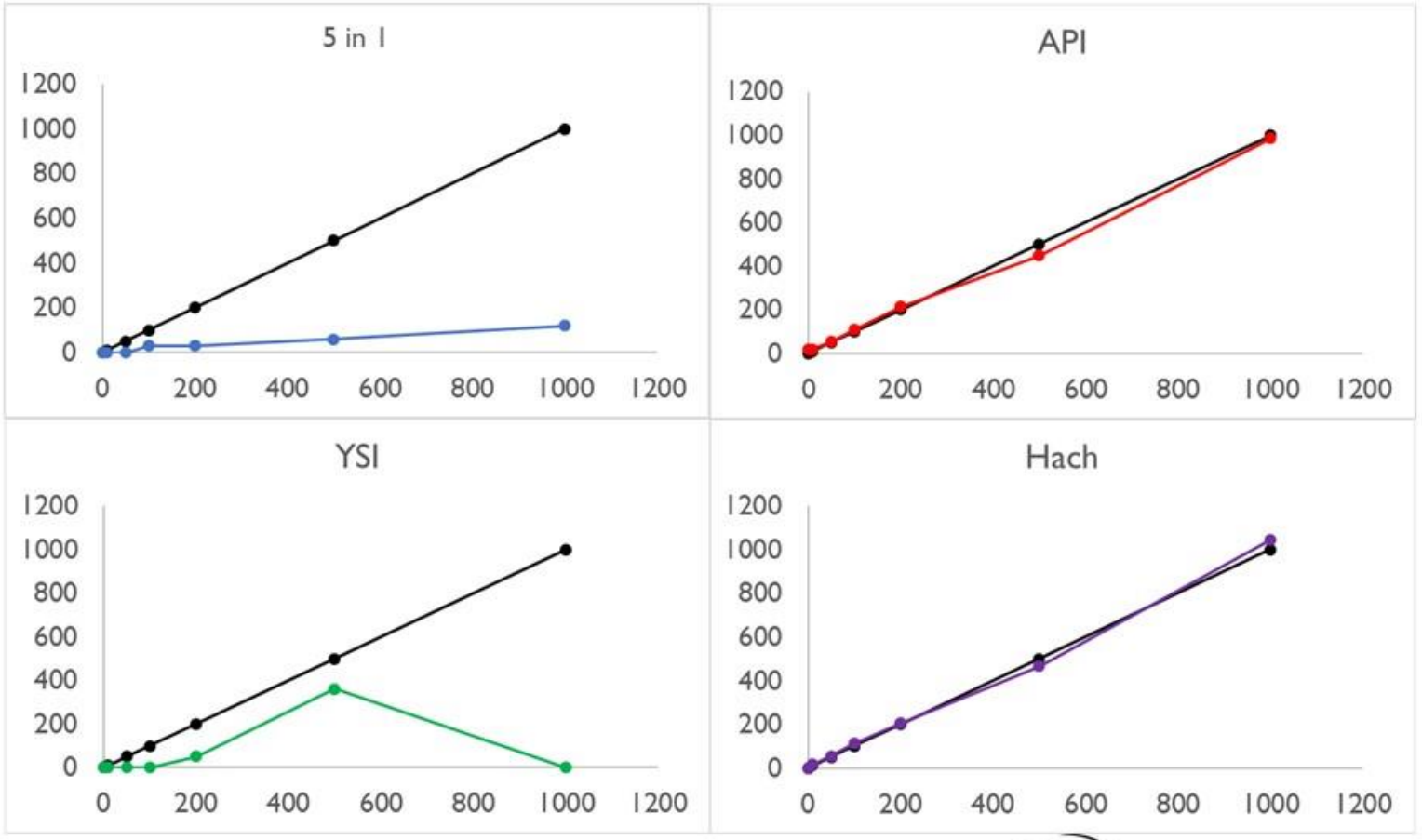
Alkalinity results



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Alkalinity results

- Hach test most accurate, but difficult to perform and calculate results
- API test was easy to conduct and performed nearly as well
  - Results easy to interpret
- 5 in 1 strips and YSI test performed poorly

- Collecting alkalinity data and better understanding buffering capacity in facilities should be a priority

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Salinity

- Cannot test salinity with kit, other equipment required
  - Hydrometer ($)
  - Refractometer ($$)
  - Salinity meter ($$$)
- Evaporation (and topping up with seawater) drive salinity up
- Seawater salinity = around 35 ppt
- Clean Green standard: 30-38 ppt, 35-36 ppt optimum
- FRDC Optimising Water Quality recommendation: 30-38
- In facilities, salinity ranged from 33.3-36.6

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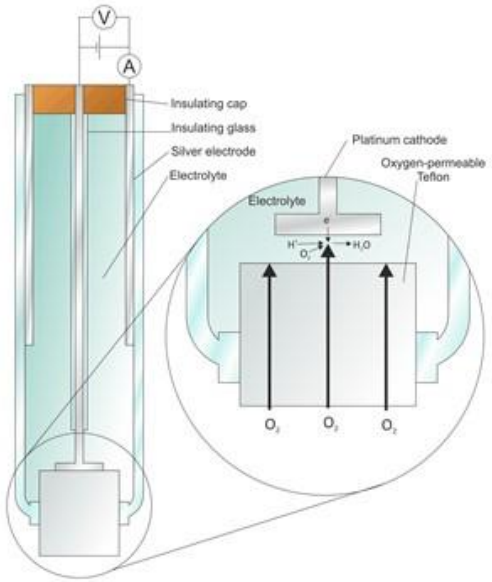
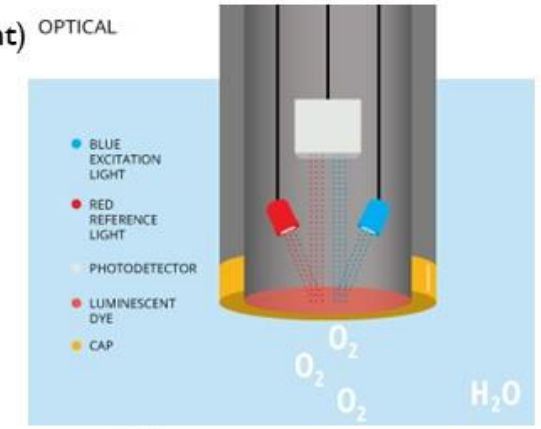
Dissolved oxygen

- Addition of stock to system will cause reduction of dissolved oxygen
  - Lobster metabolism, especially after emersion
  - Proliferation of nitrification bacteria after nutrients added
- Low oxygen → Anaerobic metabolism → Build up of ammonia → Waste in water
- Good aeration, water flow important, especially when heavily stocked
  - Can go over 100%
- Ways of measuring oxygen
  - Clark electrode = cheaper, more upkeep (membrane replacement)
  - Optical probe = more expensive, more foolproof
- Clean Green standards: >70%
- FRDC Optimising Water Quality recommendation: minimum 70%, preferably >80%
- In facilities, DO measurements ranged from 79.4%-100%





Lactate

- Lactate is a metabolic by-product that indicates anaerobic conditions
  - Emersion, heavy muscle use
- Used in European lobster (Bakke and Woll 2014) and American freshwater crayfish (Bonvillain et al. 2013) as a stress indicator
- Laboratory assay is expensive and technically difficult
- Handheld meters are made for human blood
  - Quick and easy to use
  - Use in lobsters requires validation

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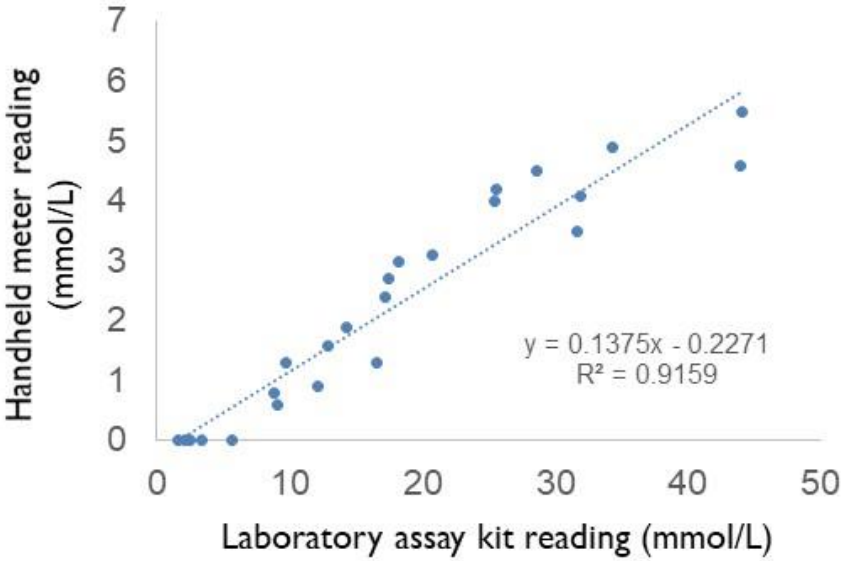
Lactate methods and results

- 5 lobsters transported to IMAS
  - Held over night
- Removed from tank
- Blood sampled
  - 0, 1, 2, 4, 6 h
- Blood tested using handheld meter
- Blood tested using laboratory assay
- Results compared



- Next step
  - Confirming validation on a large sample of lobsters
  - Development of methods for incorporating lactate measurements in processing
  - Evaluate lactate levels as a predictor for mortality

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In conclusion

- Best practice:
  - Most important parameters to monitor: pH, ammonia, nitrite, alkalinity (KH), DO & salinity
  - Daily testing during routine holding times
  - As much as hourly testing during purge/heavy stocking/mortality events
- No nitrate test offered suitable results
- 5 in 1 strips not recommended for use
  - Not cost effective, not accurate
- API tests kits are an economical option for day to day monitoring, Hach kits offer the best performance
- Quality DO and salinity probes are recommended (e.g. Hach HQ40d meter) for both performance and lifespan
  - Traceability project working to develop low cost sensor
- A better understanding of the effect of water quality on lobsters is needed to make the most out of investment ($ and time)
- Lactate results offer a validated tool for measuring stress with potential to be developed into a powerful indicator of survivability

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Appendix: Equipment and test kits

Kit	Product number
API 5 in 1 Test Strips	
API Ammonia	
API Nitrite	
API Nitrate	
API pH	
API Phosphate	
API Carbonate Hardness	
YSI 9500 Photometer	YPT950
YSI Palintest Ammonia Test Kit	YAP 152
YSI Palintest Ammonia Conditioner	YAT1707
YSI Palintest Nitrite Test Kit (Nitricol)	YAP109
YSI Palintest Nitrate Test Kit	YAP163
YSI Palintest pH Test Kit (pH Phenol Red)	YAP130
YSI Palintest Alkalinity Test Kit (Alkaphot)	YAP188
YSI Palintest Phosphate Low Range Test Kit	YAP177
YSI Palintest Phosphate High Range Test Kit	YAP114
Hach DR1900 Portable Spectrophotometer	DR1900-01H
Hach Nitrogen-Ammonia TNT AmVer, Low Range	2604545
Hach NitriVer 3 TNT, Low Range	2608345
Hach NitraVer 5 Nitrate Reagent Powder Pillows	2106169
Hach Sample Cell 1" Square Glass 10 ml matched pair	2495402
Hach Phosphorus (Reactive) TNTplus Vial	TNT846
Hach Digital Titrator	1690001
Hach Alkalinity Reagent Set	2271900
Hach HQ40D Portable Multi Meter (pH, DO, Conductivity/Salinity)	HQ40D53000000
Hach Intellical LDO101 Field Dissolved Oxygen Sensor	LDO10105
Hach Intellical CDC401 Field Conductivity Cell	CDC40105
Hach Intellical PHC101 Field pH Electrode	PHC10105
Testo pH Meter	0563 2051

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Session 4

Biosecurity

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Enterprise Biosecurity – What can we do?

Dr Kandarp Patel BVSc&AH, PGDipVPH, PhD (Epidemiology)

Dr Charles Caraguel DVM, MSc, PhD, MANZCVS (Epidemiology)

School of Animal & Veterinary Sciences, The University of Adelaide, South Australia

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What is biosecurity.....?

Definition – 'barriers' to stop the movement of an infectious agent from one 'group' of the population to another

Protect stock from infectious agent only!

Shared responsibility

- Between Industry, Government, and community



How high do you rank biosecurity in your business' priorities?

A. Very important
B. Important
C. Somehow important
D. Not important
E. Not important at all

Please answer using the clicker

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Why we need biosecurity……?

- Protect fish health and welfare
- Protect fish value
  - Moribund fish: ↓ $/Kg
- Prevent stock losses
- Protect market access and trade
- Protect product reputation & social license

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Biosecurity Process

1) **Hazard identification**—identify a disease of concern that could impact your farm's sustainability

2) **Risk assessment**—for each route of spread, estimate the levels of **likelihood** and **consequences** of this disease entering your farm

RISK = **LIKELIHOOD Of OCCURENCE** x **IMPACT**

- *Animal heath and welfare*
- *Market access & reputation*
- ***ECONOMICS $$$***

3) **Risk management**— identify **Biosecurity measures** to reduce the identified risks to an **acceptable level**

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Biosecurity Principles

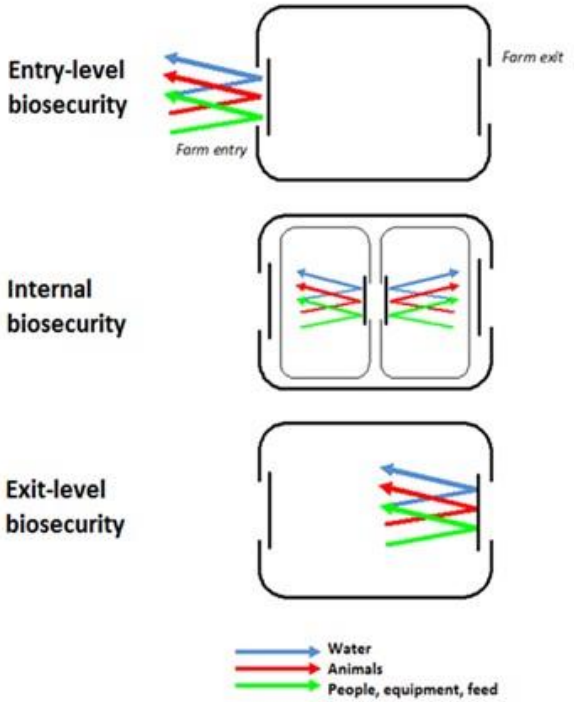
Purposes

1) Mitigation of the risk of diseases being introduced into your farm (entry-level biosecurity)

2) Mitigation of the risk of diseases transmission within your farm (internal biosecurity)

3) Mitigation of the risk of diseases escaping from your farm (exit-level biosecurity)

4) have emergency response protocols in place for serious disease outbreaks (all three levels of biosecurity): contingency plan



Source: Ellard K (2015) Disease control recommendations to support aquatic animal health? In, proceedings of 3rd OIE Global Conference on Aquatic Animal Health 'Riding the wave to the future'. Ho Chi Minh City (Vietnam), 22 January 2015.

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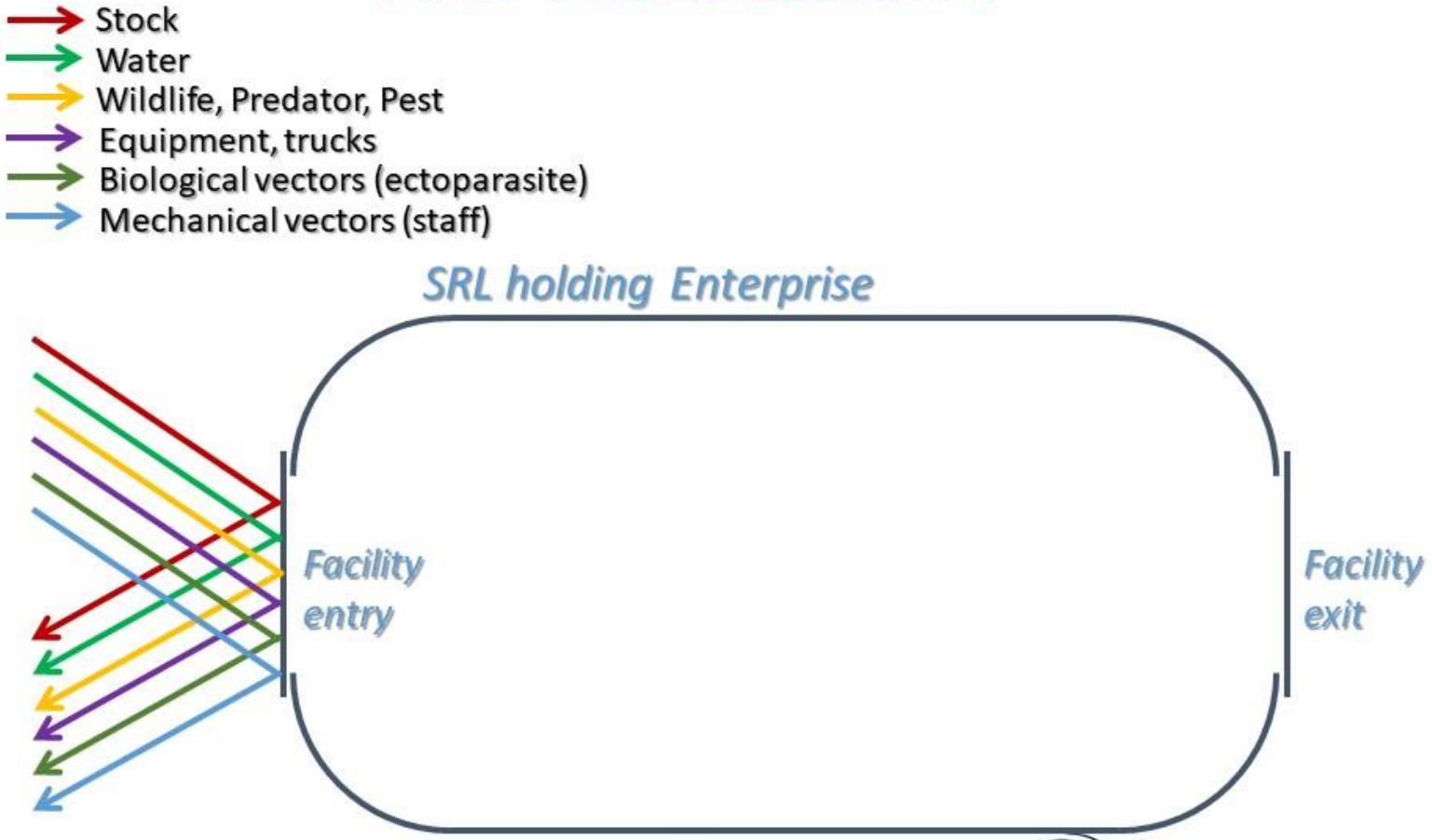
Entry-level biosecurity:
BIO-EXCLUSION



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Which pathway is the most likely for point of entry?

A. Stock movements

B. Water movements

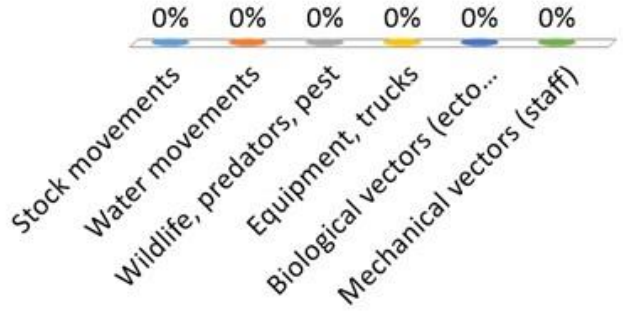
C. Wildlife, predators, pest

D. Equipment, trucks

E. Biological vectors (ectoparasite)

F. Mechanical vectors (staff)

Please answer using the clicker



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ROUTES OF ENTRY

Movements of disease into a disease free facility

Routes	Disease introduction prevention measures
Direct route Stock entry	Limit stocks from - multiple sources (landing port) - mixed sources (other facilities)
Indirect routes Water	- 'Clean' source - Pre-use treatment
Inanimate vehicle (not free of movement) Shared equipment e.g. crates	- Don't share equipment (water tests) - Clean/disinfect transport equipment (truck/crates)
Animate vehicle (VECTOR): (free of movement)	
Mechanical vectors - Staff	- Not shared between facility - Separate work clothing (e.g. footwear) - Basic hygiene @ start of the day (hand washing)
- Visitors (not necessarily strangers)	- Limit numbers - Separate visiting clothing (boot) - Visitor risk assessment (visits in last 48h) - No direct contact with tank/water/stock

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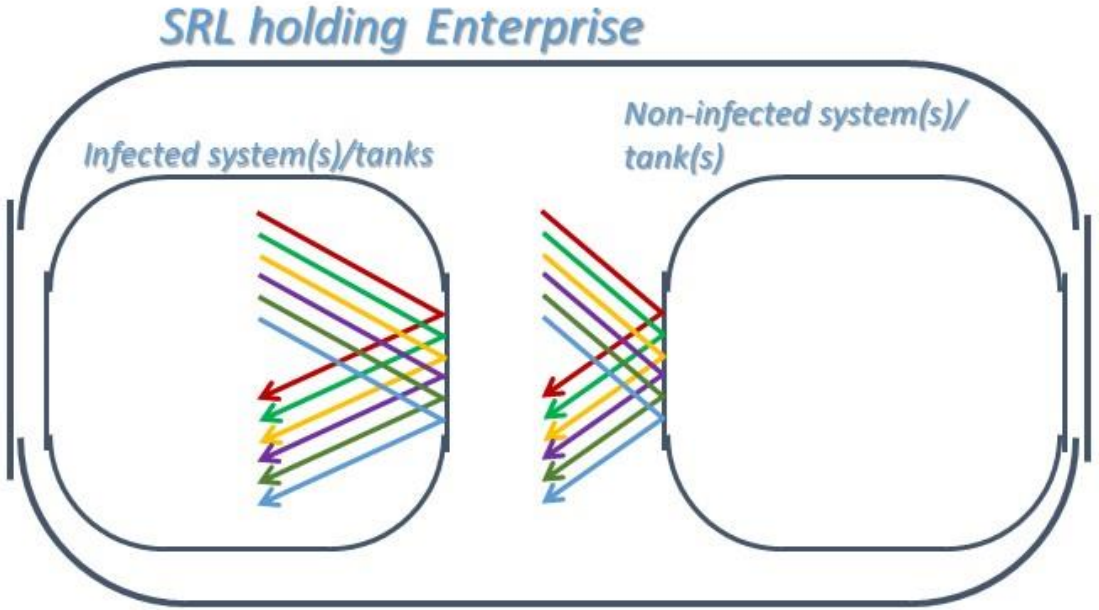
Internal biosecurity:

BIO-CONTAINMENT & EXCLUSION

- Stock mixing: healthy and affected
- Water: shared between system/tanks, waste
- Wildlife, Predator, Pest
- Equipment: e.g. water testing probes
- Biological vectors (ectoparasite)
- Mechanical vectors (staff)



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Which pathway is the most likely of route of disease spread?

A. Stock movements

B. Water movements

C. Wildlife, predators, pest

D. Equipment, trucks

E. Biological vectors (ectoparasite)

F. Mechanical vectors (staff)

Please answer using the clicker

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ROUTES OF SPREAD

Movements of disease within a facility

Routes	Disease spread prevention measures
Direct route **Stock contact**	- Don't move stock between tank system - Don't mix stock from multiple sources - Removing moribund/dead stock asap (no sick bay within tank)
Indirect routes **Water**	Separate independent water systems, purging and stocking tanks
Inanimate vehicle (not free of movement) Shared equipment e.g. nets	 Don't share equipment (water tests!) Clean/disinfect equipment regularly
Animate vehicle (VECTOR): (free of movement)	
Mechanical vectors - Staff	Separate work clothing (eg. Footwear) Basic hygiene at exit (hand/boot washing)
- Visitors	Limit numbers Separate visiting clothing (boot) No direct contact with tank/water/stock

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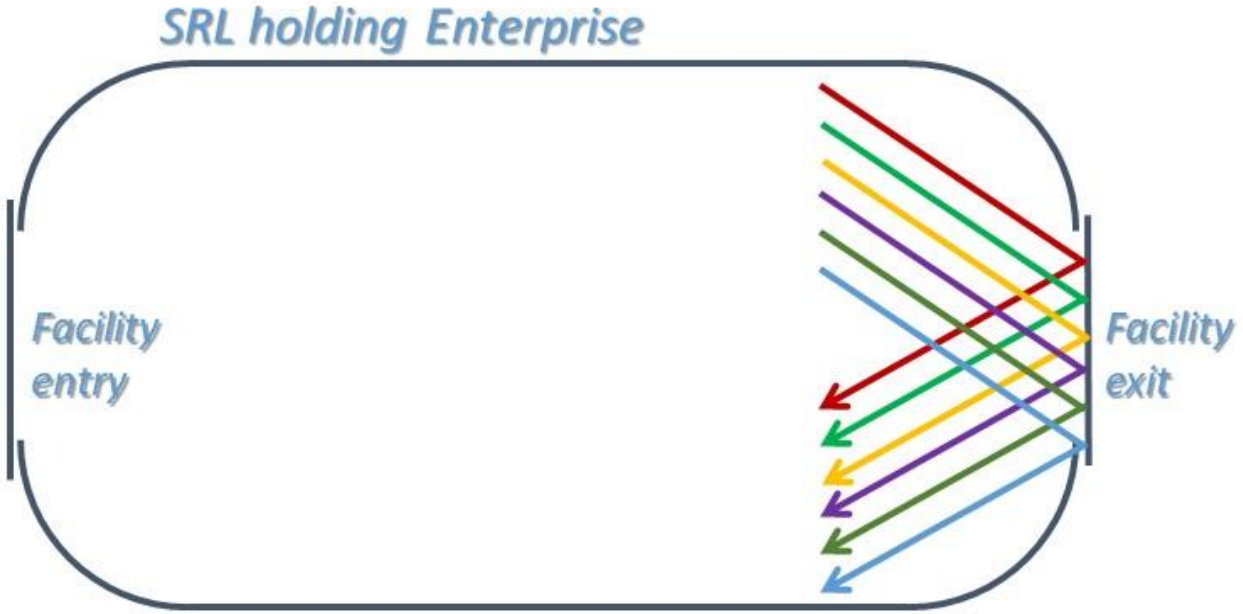
Exit-level biosecurity:
BIO-CONTAINMENT

- Stock (incl. dead)
- Water, waste
- Wildlife, Predator, Pest
- Equipment
- Biological vectors (ectoparasite)
- Mechanical vectors (staff)



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Which pathway is the most likely of route of disease exit?

A. Stock movements

B. Water movements

C. Wildlife, predators, pest

D. Equipment, trucks

E. Biological vectors (ectoparasite)

F. Mechanical vectors (staff)

Please answer using the clicker

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ROUTES OF EXIT

Movements of disease out from a facility

Routes	Disease exit prevention measures
Direct route - Stock movement - Dead stock disposal - Escapes	Avoid stocks: - sending to other facilities - Separate disposal of shells (not in water waste or as bait!)
Indirect routes Water	- Post-use treatment - Water disposal away from waterways (not in sewage!)
Inanimate vehicle (not free of movement) Shared equipment e.g. crates	- Don't share equipment (e.g. water test probe/kits) - Clean/disinfect transport equipment (truck/crates)
Animate vehicle (VECTOR): (free of movement)	
Mechanical vectors - Staff	- Not shared with other facilities - Basic hygiene regularly/between system (hand/boot washing)
- Visitors	- Limit numbers - Separate visitor clothing (e.g. boot) - Visitor risk assessment (visits in last 48h) - No direct contact with tank/water/stock

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Which level of biosecurity is worth focusing on?

Entry level biosecurity (reduce risk of disease introduction)

Incoming stocks pose highest risk of disease

Internal biosecurity (reduce risk of disease transmission)

- SRL to SRL
- System to system (Tank to tank)

Exit level biosecurity

Reduce the risk of disease exiting your facility

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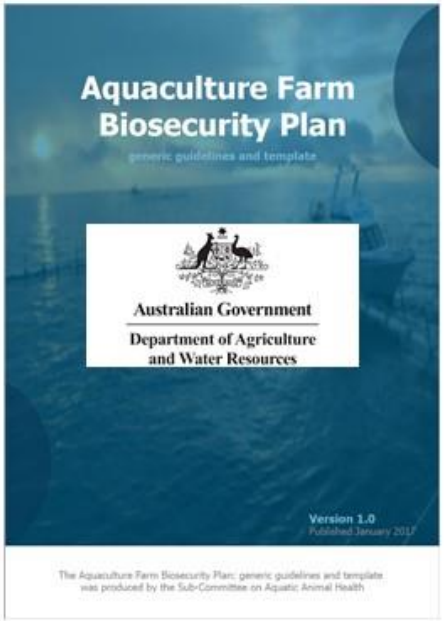
More information on devising a customised biosecurity plan

AQUAVETPLAN

http://www.agriculture.gov.au/fisheries/aquaculture/farm-biosecurity-plan



https://www.researchgate.net/publication/281719252_A_Standardized_Approach_for_Meeting_National_and_International_Aquaculture_Biosecurity_Requirements_for_Preventing_Controlling_and_Eradicating_Infectious_Diseases

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Section 5

Lobster pathology – **The Lobster-centric analysis**

Overview

- Pathology is bad
- Summary of the pathology Component
- What we have learnt from this project
- Two examples of steps in better and easier health surveillance options.
  - Blood biochemistry
  - Understanding organ function – Antennal Gland.

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Pathology is NOT something you really want to see

More of this ……than …

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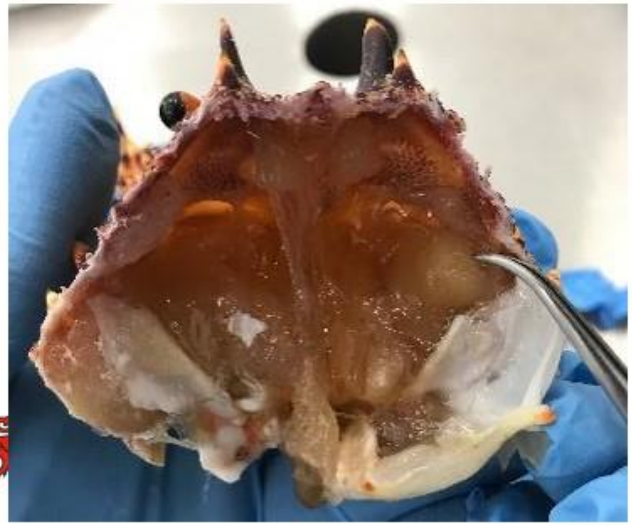
This…



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But what does all this change actually mean?

From a diagnostic sense this is a **very important** question.

How do you understand abnormal when there isn't enough information on normal?

A national survey of wild Southern Rock Lobster discovered a range of changes (from what the perception of normal is) and they were documented.

Challenge is how do these changes, when they appear, tell us anything about SRL wild caught and held in captivity for market??

So in an attempt to answer this question during the investigation under the project we documented the pathologies found from reports of low survivability to see if there was an underlying cause common to all…and we have observed a range of pathologies seen and unseen previously.

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Summary of the activities of the Pathology research component

1. Gross and histopathological analysis of SRL samples from low survivability events 2016, 2017 and early 2018. Animals sourced SA, TAS and VIC.
2. Microbiological analysis including isolation, identification and genetic analysis of significant isolates associated with morbid animals.
3. Physiological sample collection from SRLs during 2017 and 2018.
4. Preliminary virological assessment and sample collection for 2017-18 samples.
5. Correlation of 2016 and 2017-18 diagnostic sample results from SA and TAS investigations.

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Pathology research component

Gross and histopathological findings summary

Generally gross pathological findings of external lesions (carapace, telson, uropods) were consistent with traumatic cause with subsequent chronic active healing.

Significant histopathology findings are limited to a number of organ systems that showed consistent pathology throughout all accessions
(i.e: gills, hepatopancreas, nerves, antennal gland and reserve cell tissue)

Pathology research component

It should be noted that pathology was not consistent across all accessions for each year class and the magnitude of changes were also variable.

For instance;_

The significant histopathology findings for the **2016** cohort:

- gill biofouling
- hepatopancreatic changes consistent with poor food intake and organ atrophy
- nerve ganglia incidental changes included vacuolisation of neurons and mild degenerative changes (mild neuritis and neural degeneration)
- antennal gland ranging from mild atrophy to necrosis and acute inflammation (green polarising deposits)

Summary findings

The significant histopathology findings for the **2017** cohort:

- gill biofouling with occasional ectocomensal unidentified metazoans and mild multifocal melanising branchitis.
- hepatopancreas were consistent with those seen in 2016 with the addition of granulomatous hepatopancreatitis consistent with bacterial infection and one animal of the 61 animals processed for 2017 exhibited amorphous pink eosinophilic inclusions within the interstitial septa of the hepatopancreas.
- Nerve ganglia -vacuolated and mild degenerative changes were occasionally seen in the nerves of some fish with occasional melanised peri-ganglionic haemocyte aggregations also being observed.
- antennal glands - eosinophilic cytoplasmic inclusions were noted in many of the animals examined and in many cases being extensively associated with apparently normal antennal gland tissue. A range of degenerative and inflammatory lesions were noted in antennal glands with some fish having extensive destruction of the antennal gland tissue.

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Microbiological examinations – bacteria and viruses

Microbiological analysis including isolation, identification and preliminary genetic analysis of samples from moribund animals within the study.

- Haemolymph from SRL submitted for examination was routinely taken for microbiological culture and identification. Despite the origin of the fish a consistent finding across accessions of 2016 and 2017 was the culture of Vibrio spp. including *Vibrio tapetis.*

(Stress association or primary pathogen??) – results suggest opportunistic infections and some bacteria can be amplified in systems and become a biota present for infection if animals stressed.

- Virological assessment of tissues from animals displaying signs consistent with viral inclusions were negative for WSSV infection by PCR (skewed sample)

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So lots of data....and so what does it mean?

Because many of the findings are non-specific and inconsistent across all submissions.....well then the interpretation is that we have no one single pathogen or insult (or do we)*

* There may be one insult -- which produces a stress in the animals which manifests in a number of ways.

Many of the pathologies are consistent with stressed fish in poor environments (i.e. gill biofouling, hepatopancreatic atrophy, low reserve cell activity).

(Sample numbers were low compared to anecdotal information – but still a useful outcome)

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What have we learned from the project?

Fire Brigade vs **Surveillance and monitoring**

We were looking to rule in AND rule out a potential primary pathogen or cause of reduced survivability.

Better prepared for disease outbreak with improved methods of surveillance and monitoring.

As diagnosticians our role is to work with our clients to solve problems.

- Better understand the physiology → Interpret Changes → Solve Problems.

I see a change but what does it mean???

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Let me give you two examples – *work that was undertaken within the project that wasn't just bug catching.*

Haemolymph biochemistry.

Antennule gland pathology - (aka structure and function).

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Haemolymph biochemistry related to pathology and reference range development.

Sodium	Creatinine Kinase
Potassium	Cholesterol
Chloride	Amylase
Bicarbonate	Lipase
Glucose	Total Bile Acids
Urea	Magnesium
Creatinine	GGT
Calcium	Iron
Inorganic Phosphate	Triglyceride
Albumin	GLDH
Total Protein	D-3 Hydroxybutyrate
Total Bilirubin C	Uric Acid
Alk Phos	Lactate
AST	Globulin
ALT	Na:K ratio
	Anion Gap

Haemolymph samples was collected from all live fish submitted and kept on ice to avoid clotting and analysed immediately. Samples were analysed for the following parameters utilising a Beckman Coulter AU480 machine within veterinary diagnostic laboratory of the University of Adelaide;

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Could we do it in country and develop a clinical pathology capacity – external lab versus in house.

- Inter laboratory comparison of samples – i.e. analyse the same samples in two different labs
- Intra laboratory comparison – fresh vs frozen – clotting issues
- Individual organ levels of metabolites – helps differentiate what organs are damaged simply by taking a haemolymph sample.

AND that's the whole purpose of diagnostic haemolymph biochemistry. However if you don't know what each organ contains its hard to make an interpretation of variability in the haemolymph from an individual animal.

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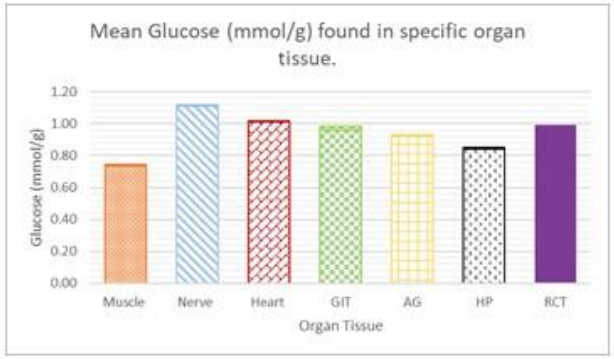
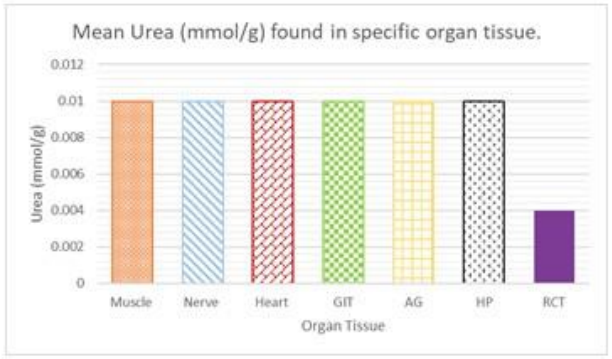
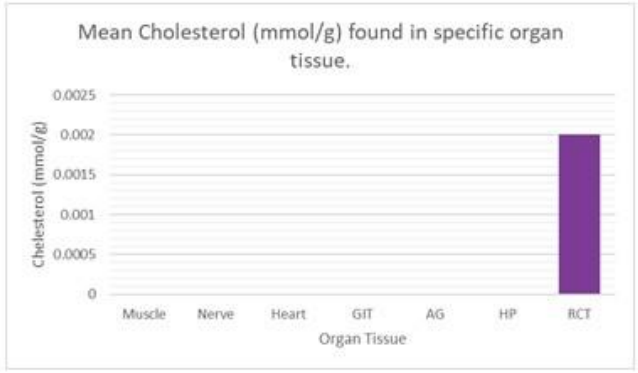
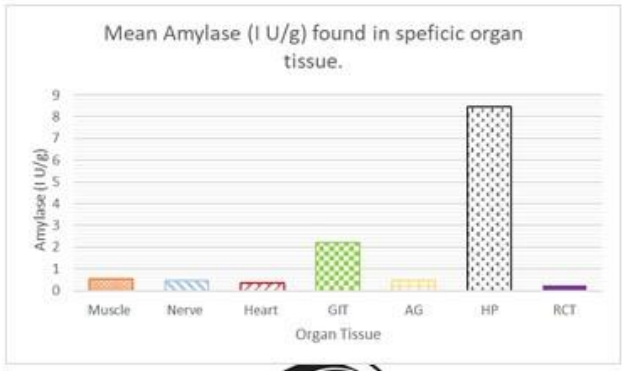
Example organ levels









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With further work this can be a powerful diagnostic tool for ongoing daily monitoring of stock.
Routine haemolymph sampling.

Requires a transitional plan and protocol development for industry use.

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Antennal gland Anatomy

- Located in the anterior cephalothrorax (near the base of the antennae)[4]
- Three main parts
  - End Sac (Coelomosac)
  - Labyrinth (Glandular plexus)
  - Bladder
    - Duct (Ureter)

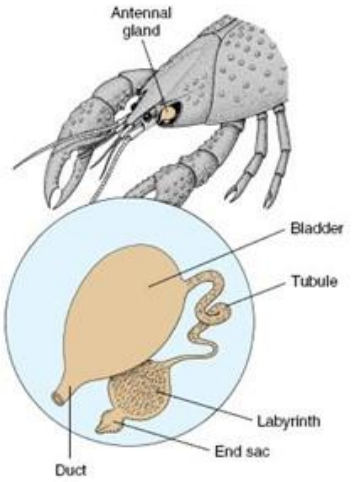

Figure 4: Crustacean Antennal Gland location and anatomy[5]

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Antennal Gland anatomy

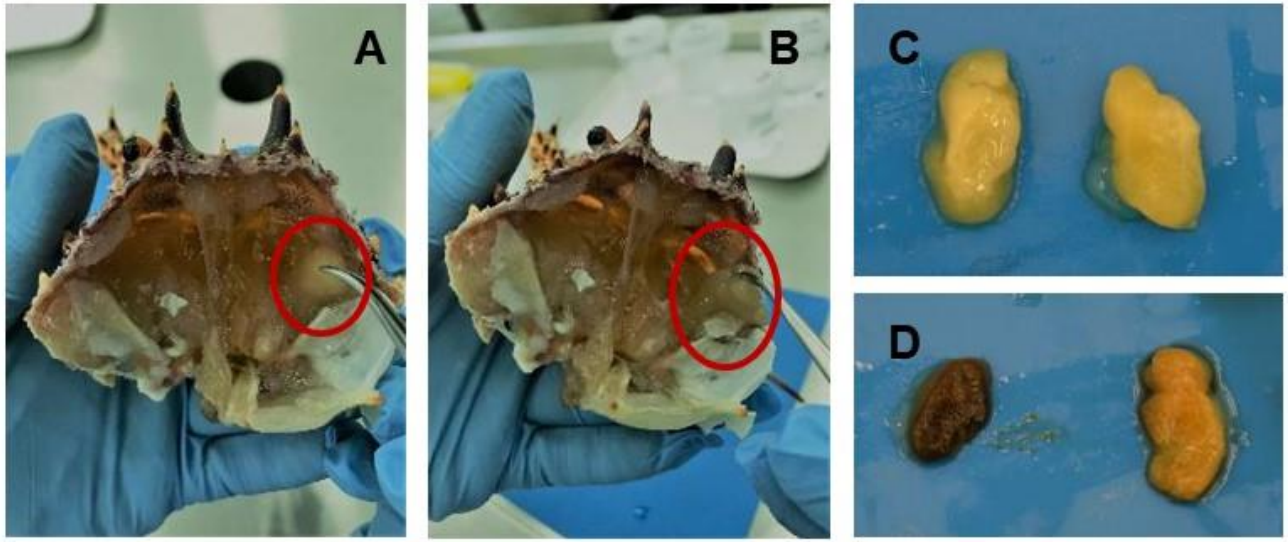

Figure 5: A: Antennal Gland located in the anterior cephalothorax. B: Removal of antennal gland. C: Healthy Antennal Gland. D: Necrotic abnormal antennal gland.

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Antennal Gland Function

- Ion regulation[4]
- Osmoregulation[4]
- Excretory organ[5]
  - Elimination of nitrogenous end-products

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Normal Histology

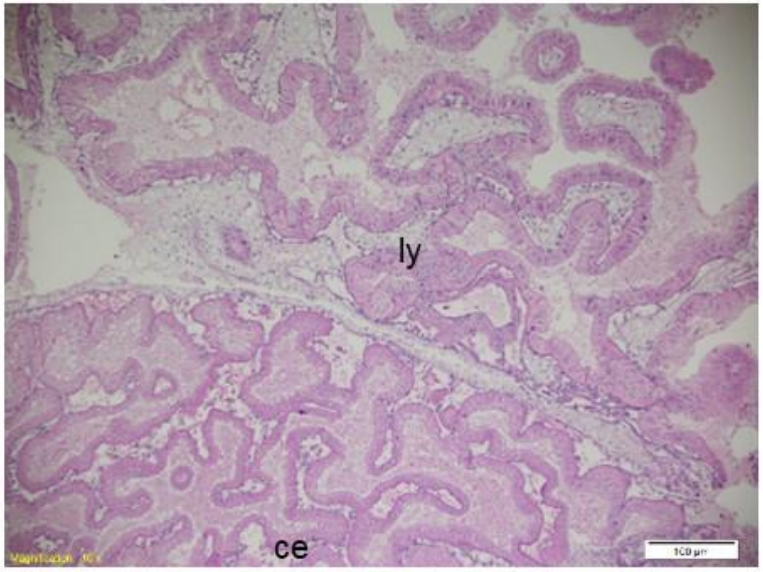

Figure 6: Histological regions of the antennal gland. [ce:coelomosac; ly: labyrinth

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Mineralized inclusions

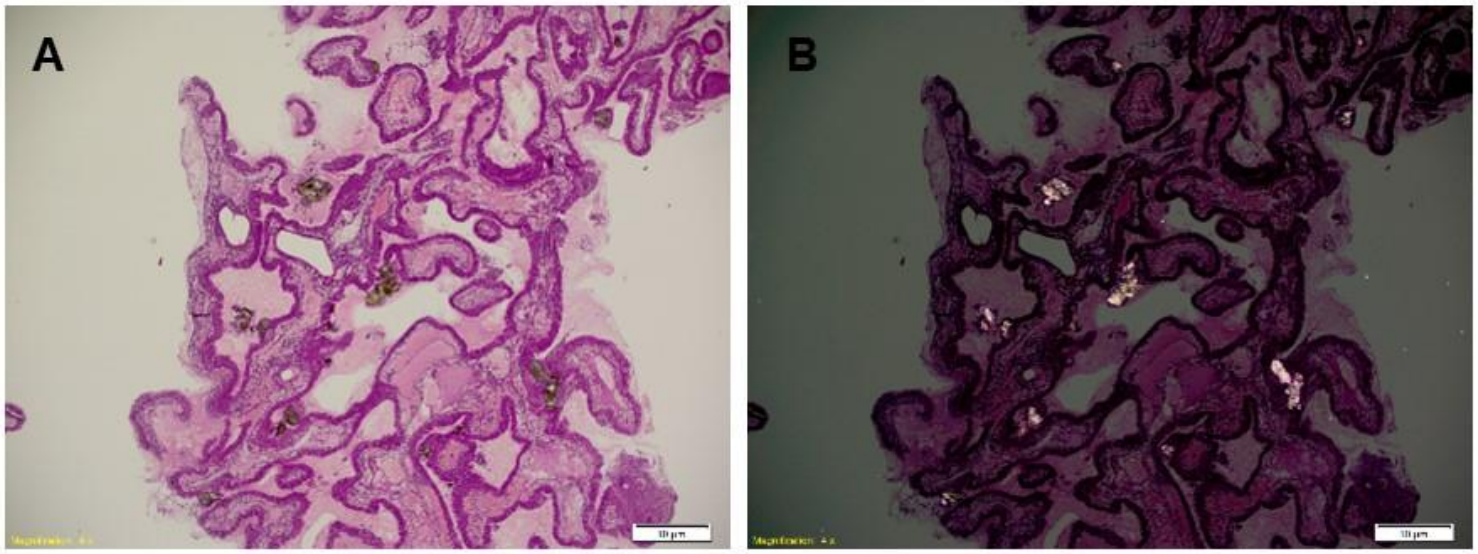

Figure 7: Yellow to green polarising birefringent crystalline deposits within the antennal gland. [A :Normal image B: Reduced light pronouncing birefringence]

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Mineralised inclusions

- Etiology remains undetermined
- Observed in 2 fish.
- Moribund Animals
- Discoloured Antennal Glands
  - Pale
  - Generalised Brown discolouration. (Melanisation?)

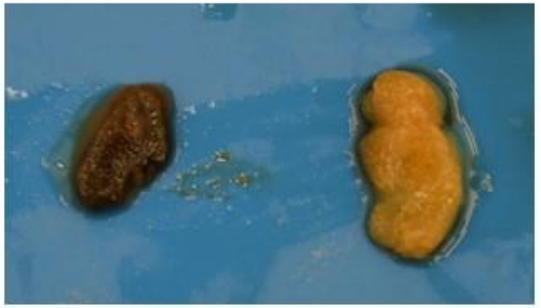

Figure 8: Gross pathology of a necrotic abnormal antennal gland

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Mineralized inclusions

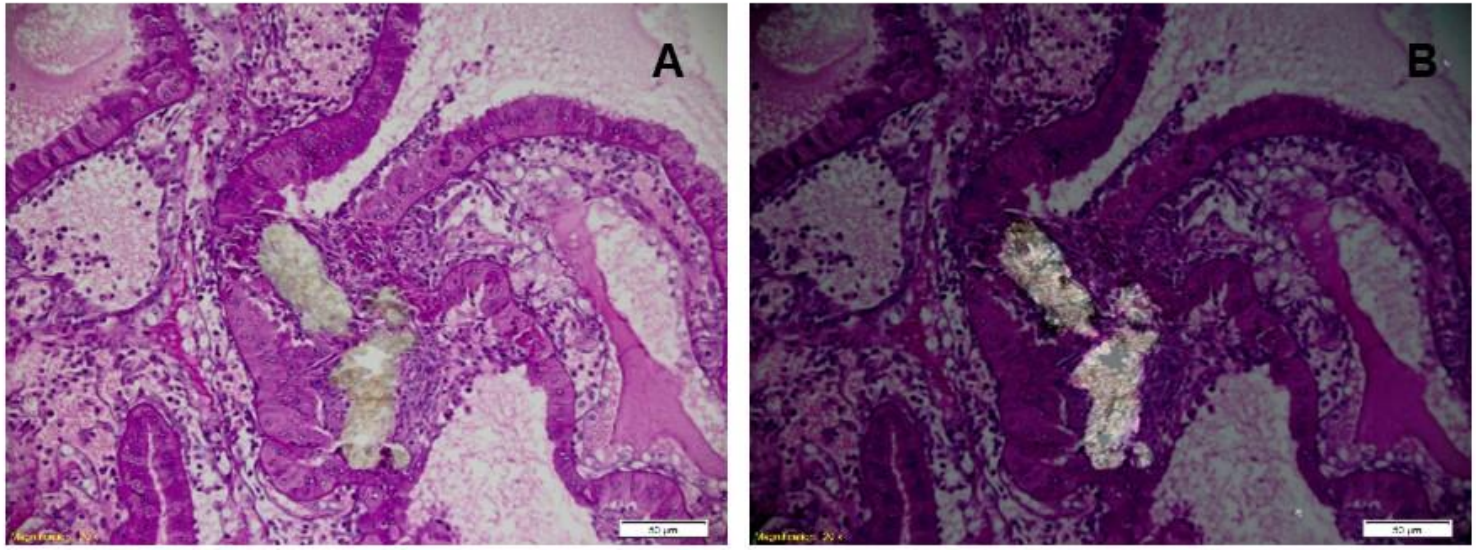

Figure 9: Gold to brown polarising birefringent crystalline deposits within the antennal gland. [A :Normal image B: Reduced light pronouncing birefringence]

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Mineralized inclusions

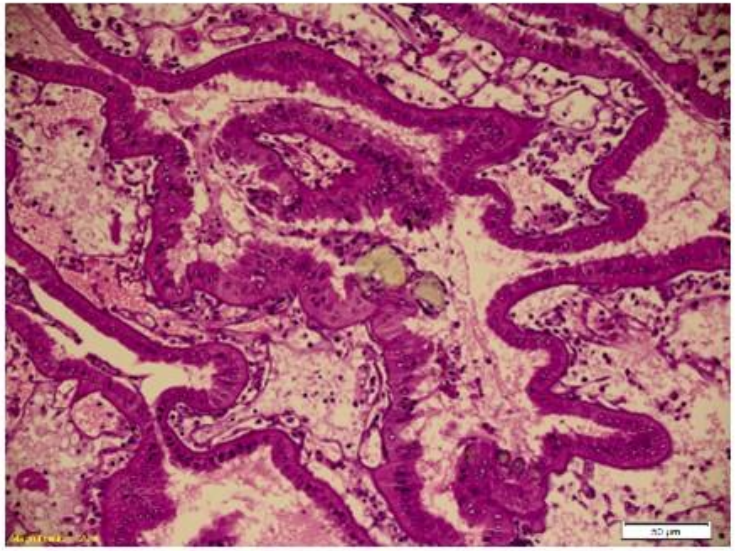

Figure 10: Gold to brown polarising birefringent crystalline deposits within the antennal gland.

- No clinical identification performed.
- Golden brown colouration
- Birefringence under polarising light
- Grossly resemble:
  - Uric Acid[8]
  - Amorphous urates[8]
  - Sodium urate[8]

- Aquatic crustaceans are **ammonotelic**[8]
  - Ammonia is the main nitrogenous waste product with only minimal amounts of urea and uric acid produced under normal conditions.
- Nitrogen waste cycle – Ammonia =72% of total nitrogen excreted by SRL[5]
  - Urine nitrogen = 11.6% of total nitrogen loss
  - Urine production rate is 4.8% of body weight per day

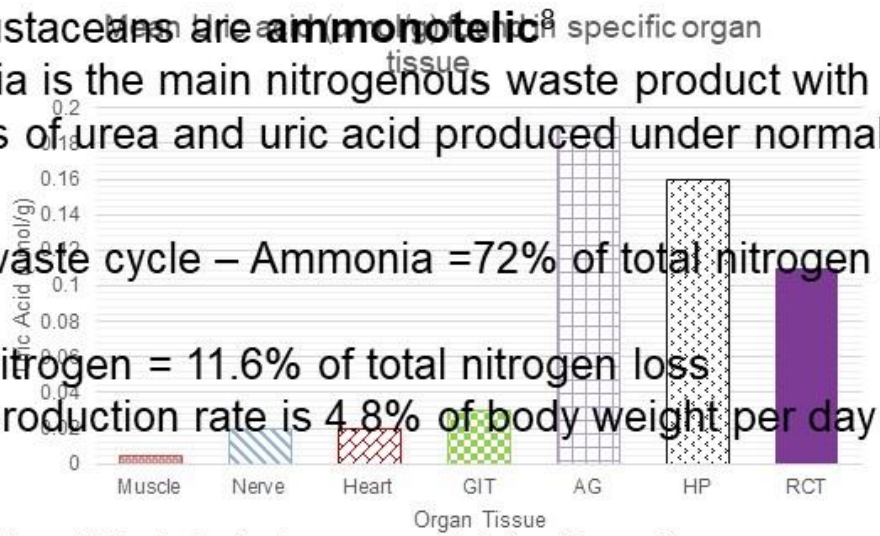

Figure 12: Graph showing the mean amount of uric acid present in seven separate organs of a healthy Southern rock lobster.

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Possible Etiology

- Increased uric acid (intake / production)
  - Environment
- Abnormal uric acid catabolism
  - Increased stressed
- Decreased uric acid excretion
  - Blocked excretory pore

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VIRAL CONUNDRUM
Basophilic (Intranuclear) inclusions

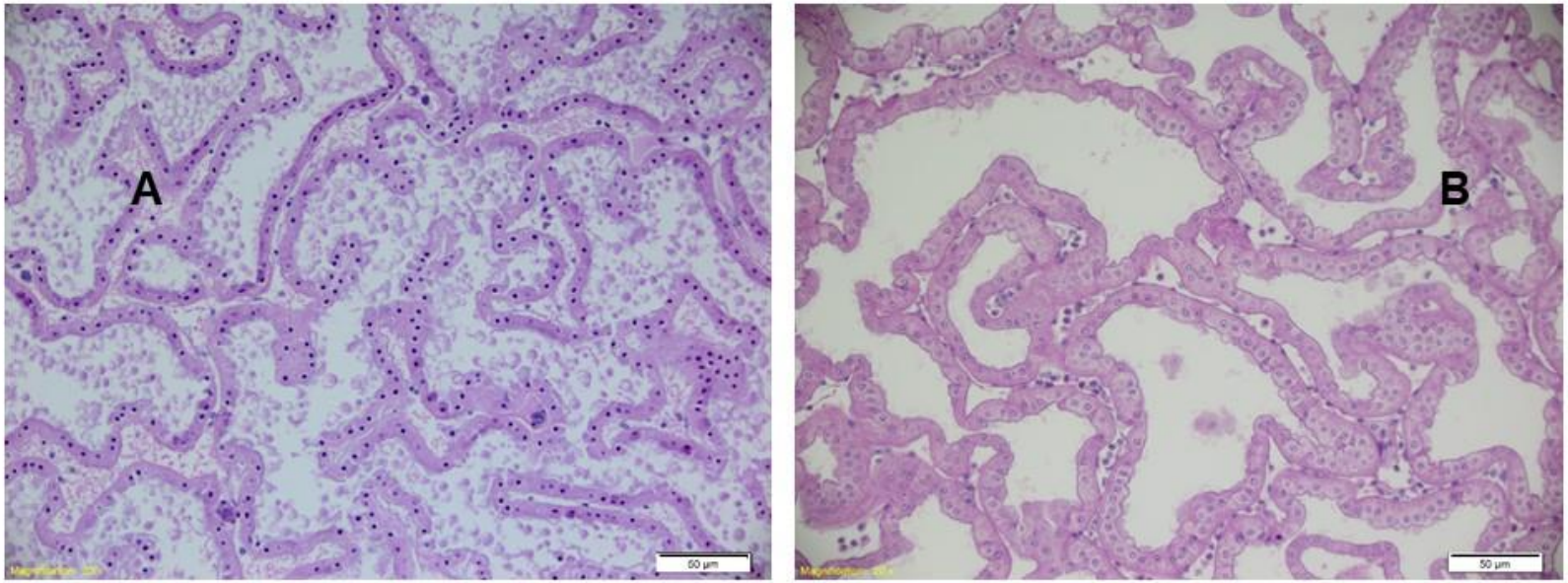

Figure 13: Comparison of normal and abnormal antennal gland tissue. [A: Multifocal basophilic intra-cytoplasmic inclusions; B: Healthy antennal gland tissue]

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Basophilic Inclusions

- **Aetiology remains undetermined**
- Observed in 4 fish.
- 3 Dead on Arrival
- 1 Moribund Animal
- White discolouration to aspects of the antennal gland.

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Basophilic inclusions

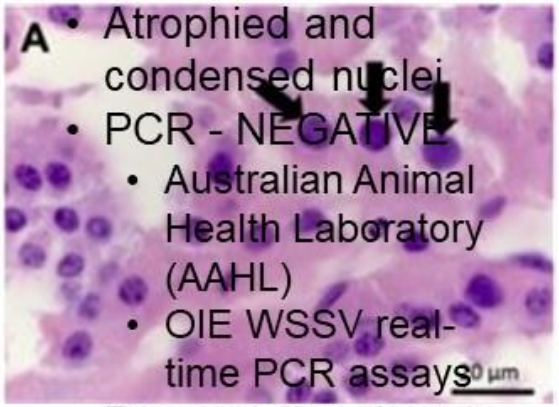

Figure 15: *Homarus americanus* antennal gland tissue infected with WSSV. [Arrows showing hypertrophied nuclei with inclusions][11]

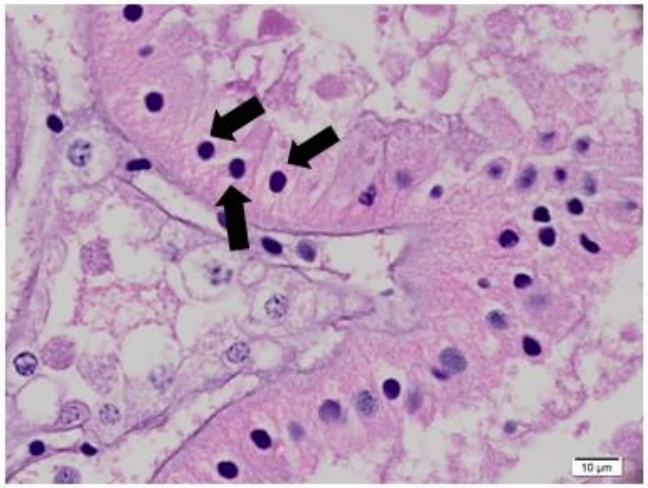

Figure 16: Basophilic inclusion bodies antennal gland tissue of *Jasus Edwardsii*. [Arrows showing inclusions]

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Cytoplasmic Eosinophilic inclusions

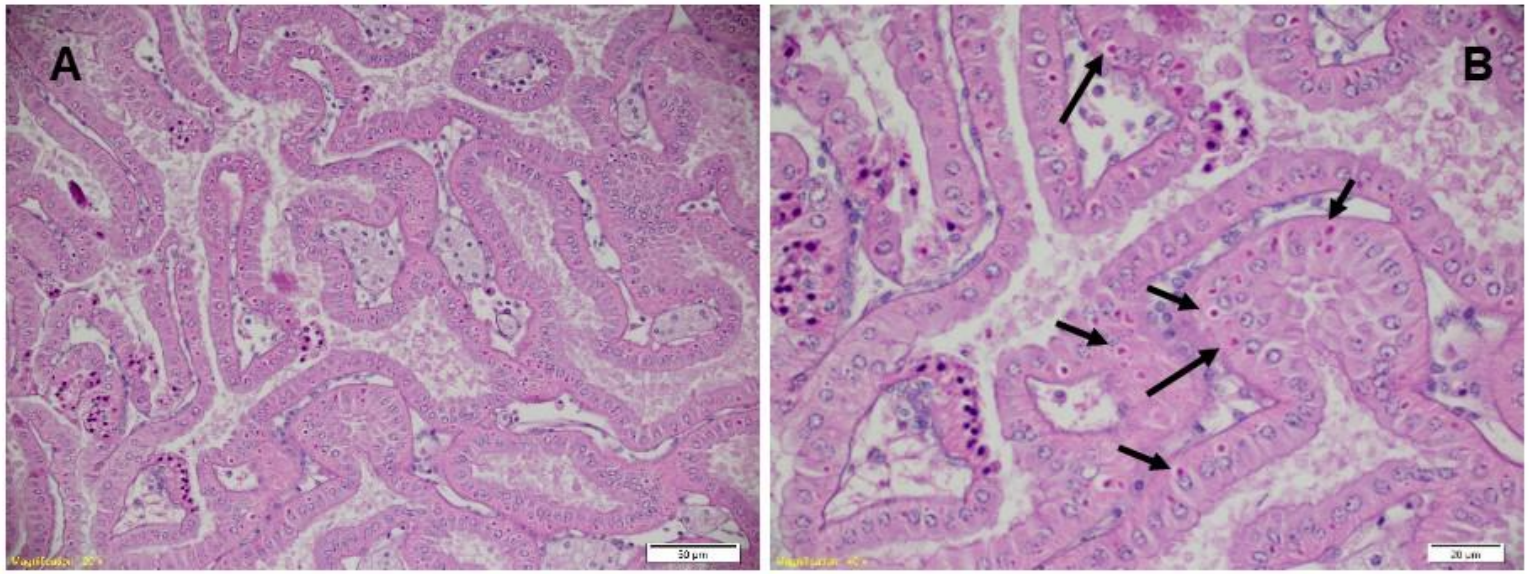

Figure 17:Intracytoplasmic eosinophilic inclusions within SRL antennal gland tissue. [A: Image at 20x magnification; B: Image at 40x magnification]

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Eosinophilic inclusions

- Aetiology remains undetermined
- Prevalent 55% lobsters assessed in 2017
- Association with moult stages?
- No obvious and consistent gross pathology associated

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Eosinophilic inclusions

Figure 18:Intracytoplasmic eosinophilic inclusions within SRL antennal gland tissue of FRDC Project No. 2001/094[12].

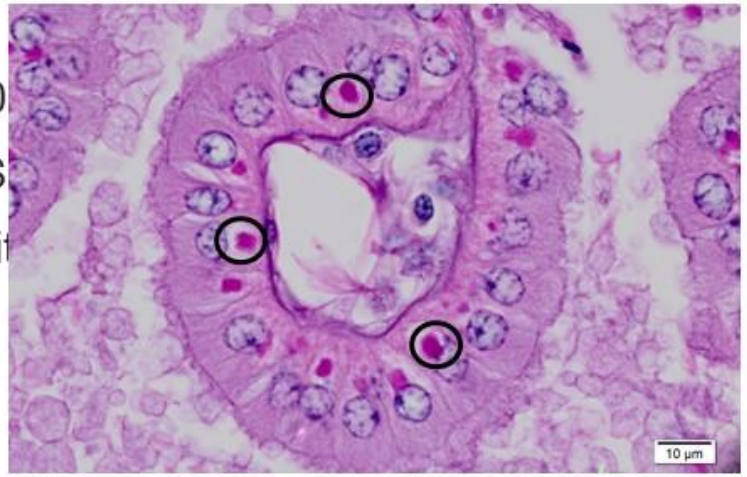

Figure 19: Intracytoplasmic eosinophilic inclusions within SRL antennal gland tissue.

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Eosinophilic inclusions

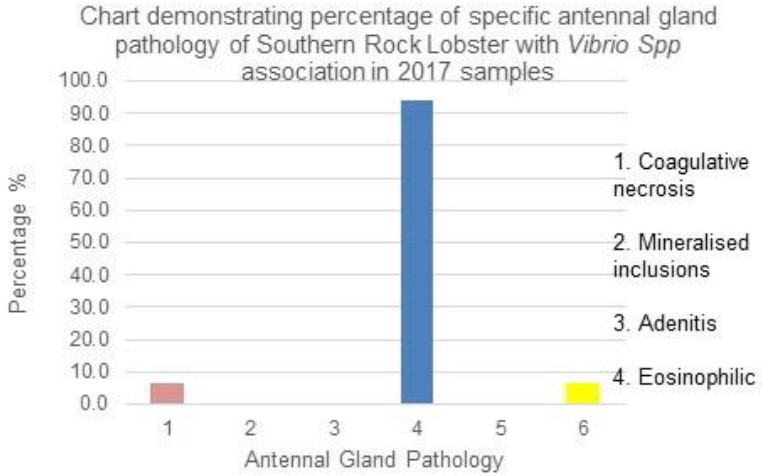

Figure 20: Table graph demonstrating percentage of antennal gland pathology of SRL with *Vibrio spp* associated.

- 55% samples in 2017 → Eosinophilic inclusions
- 93% were associated with *Vibrio spp* isolated in haemolymph.
- Could they be related to moult stage
- Pathogenesis currently undetermined

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Summary

These two aspects of the work undertaken within the project highlights that the more we know, the greater the indication of what we don't know about these animals.

The end is not purely academic it is about applying the knowledge to allow industry transition in practices and understanding so that profitability remains in the industry.

AND when issues occur there are 'knowns for the diagnosticians to find instead of many many unknowns that have attached little understanding for interpretation.

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Session 6

Traceability

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Traceability Systems for Wild Caught Lobster

FRDC Project No 2016/228

Survivability and Traceability Workshop, Hobart 11 October 2018

A/Prof Laurie Bonnie, A/Prof Paul Turner, Prof Caleb Gardener, Prof Quinn Fitzgibbon, Dr Ryan Day, Dr Luke Mirowski

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Background

- **Emerging risks** from Chinese trade barriers,
  - **Consumer buying habits**
  - **Information about the product**
  - Foreign competition
  - **Domestic risks** (e.g. Algal blooms)
  - Free trade agreement

Project Objectives

- Introducing **low cost, low impact traceability** framework to suit variety of different business models
- Supporting implementation of technologies with **'best practises' guidelines** for companies in the supply chain

ARC Pathways to Market

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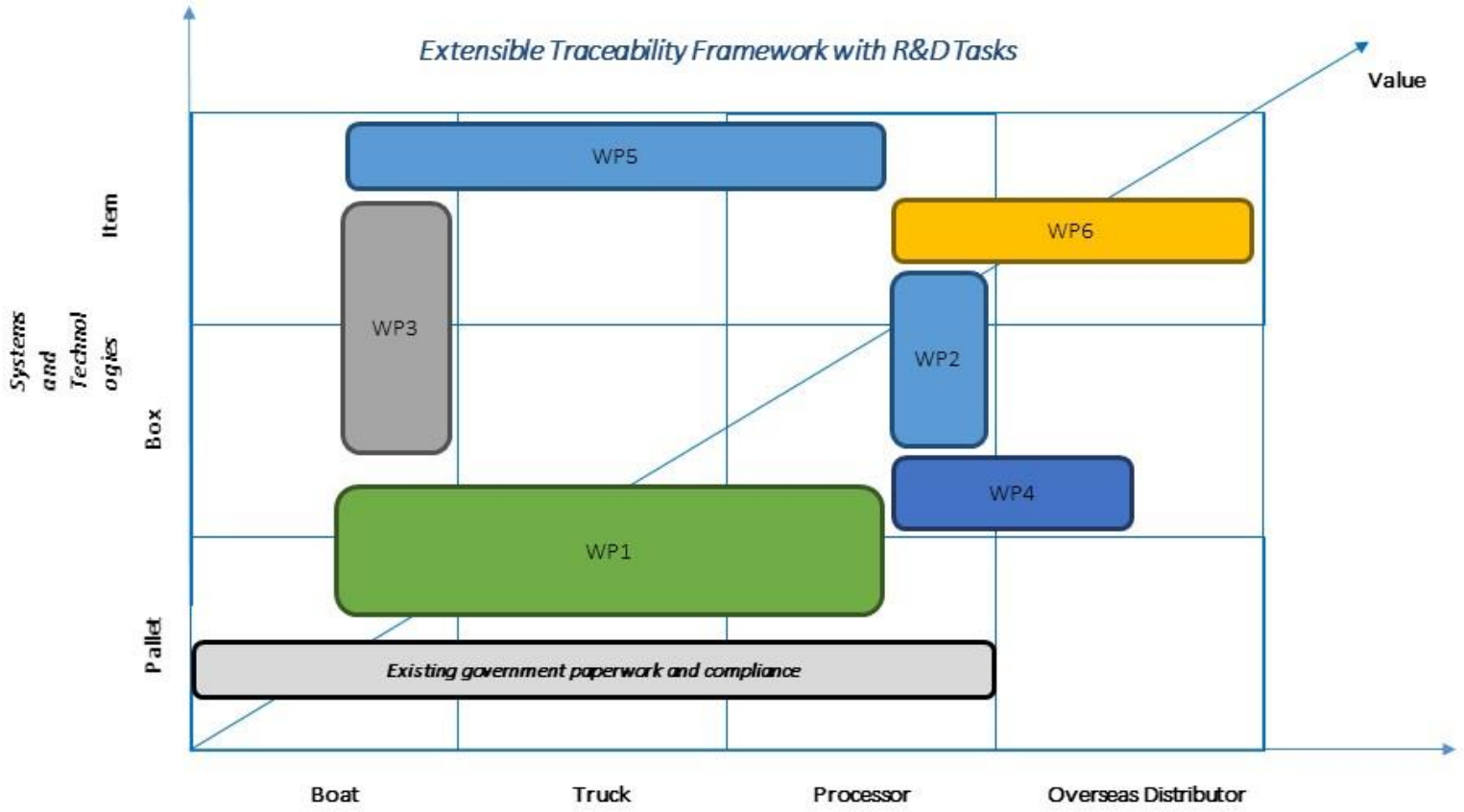
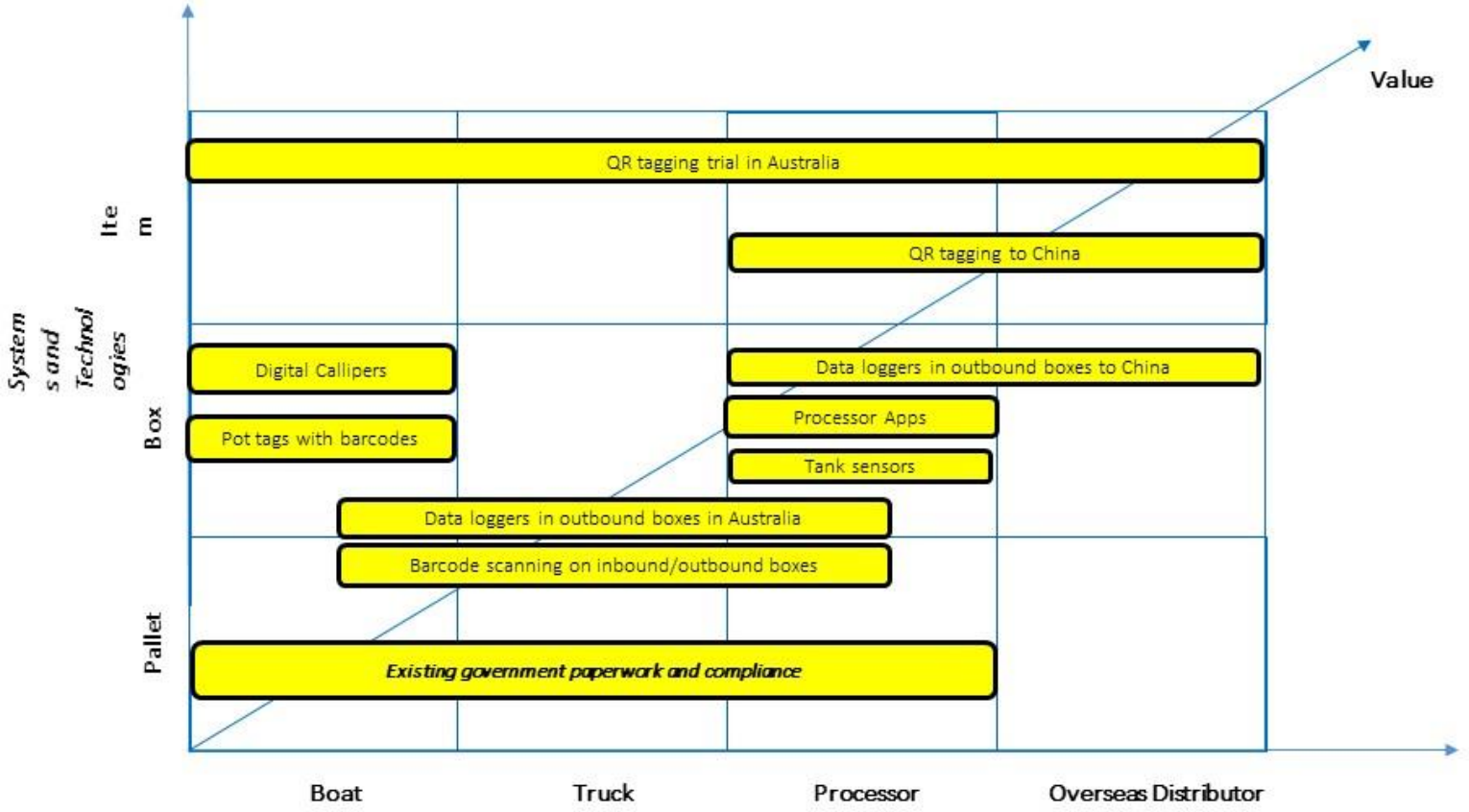
SRL Traceability Model



SRL Traceability Trials



Stage 1 Trials – Nov to Jan

Boat/Truck/Processor

1. Data loggers in plastic crates
2. Scanning of barcode on plastic crates inbound/outbound

Processor

1. Use of (6) Processor apps for inventory management
2. QR tagging trial in Australia
3. Tank sensors
4. QR & Barcode Box Label to China
5. Data loggers in poly boxes to China
6. QR tagging trial from Australia to China

Boat

1. Pot tag with barcode scanning
2. Digital Calipers with Bluetooth
3. QR tagging trial Australia or China

Trial: Boat Pot Tag & Digital Calipers

Trial: Boat-Processor Barcoding & Data Loggers

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ARC Pathways to Market

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Trial: Processor Water Sensors & Inventory Apps

Trial: China Carton Data Loggers and Labelling

12345678

175

Trial: Australia and China Tagging

Trial Period

Stage 1 trial period November to January 2019

1. Baseline evaluation of existing traceability practices
2. Installation of equipment and training
3. Data collection 4-8 weeks
4. Follow up evaluation with company looking at impacts

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Key future research questions

Please score in order of priority the suggested further research questions for the SRL holding industry

Name; **Company;**

Future research question	Priority score (High/Medium/Low)	Comments/suggestions
Optimum temperature and acclimation rates for SRL in holding and transport?		
Chronic tolerance and optimal levels for most water and environmental parameters, NH_4, NO_2, NO_3, Salinity, Alkalinity?		
Better methods to determine moult cycle, relationship between Brix index and moult cycle?		
Benefits of socking or banding to reduce tail flipping and negative physical interactions?		
Maximum starvation time and the effect of moult/nutritional stage and temperature?		
The optimal lighting regime during SRL holding?		
pH/alklinity threshold range and optimal pH dosing practices for SRL holding?		
Optimal and threshold levels of ozonation in SRL holding?		
Optimum dry transport methods and transport time thresholds?		
Effect of a changing environment on lobster physiology (nutritional condition/moult cycles) across the fishery range?		
Improved tools and validation of condition/vitality assessments and relationship to survivability during holding, brix, lactate, reflex responses?		
Effect of holding industry practices, purging, grading, crating etc. on lobster performance?		
Biosecurity quality control of lobster stocks at the fishery source		
Continued over page		

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Key future research questions

Please score in order of priority the suggested further research questions for the SRL holding industry

Name; **Company;**

Future research question	Priority score (High/Medium/Low)	Comments/suggestions
Preparedness for response to disease outbreaks		
Improved understanding of seawater chemistry during holding and more accurate and efficient tools for measurement		
Lobster stock monitoring tools and data collection systems during holding		
Establish haemolymph analysis capacity in country (based at Roseworthy VDL) for access by all of industry		
Develop and undertake a diagnostic sample collection workshop for industry for on farm health assessments and sample submission for diagnostics.		
Continued facilitation by the SRL industry for investigations of morbidity and mortality in animals		
Please add more if required		

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Further comments/suggestions

Please provide any further comments or suggestion about the workshop presentation or suggested other future research priorities

Name; **Company;**

Comments/suggestions about the workshop presentation
Comments/suggestions about other future research priorities

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Appendix 11: ‘Atlas of Selected Normal Histology & Histopathology of Southern Rock Lobster (*Jasus Edwardsii)*’

Atlas of Selected Normal Histology & Histopathology of Southern Rock Lobster

(*Jasus edwardsii*)

J. Mahadevan & S. Pyecroft

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SUB CRUCE LUMEN
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TABLE OF CONTENTS

INTRODUCTION

This manual resulted from an opportunity to undertake diagnostic investigations on a range of Southern Rock Lobsters (SRL), *Jasus edwardsii* and is the first such manual produced for this species.

It is a collection of selected images of normal histology and histopathology of various organs systems of the SRL as they were available and is a starting point for researchers and scientists working with this species to allow a greater understanding of structure and function of this species.

Continued work with SRL will allow the addition of further images to subsequent versions of this manual.

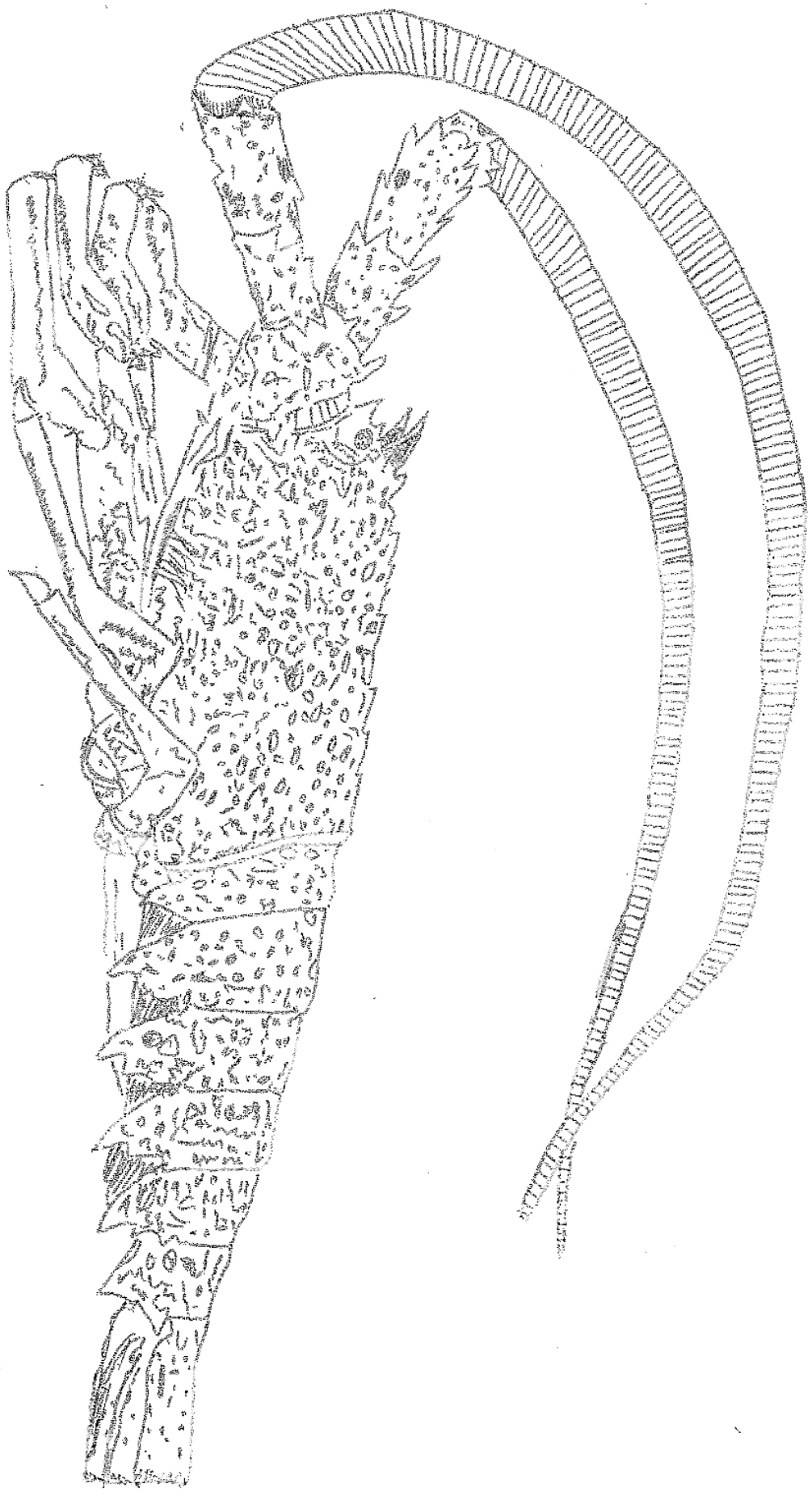


Figure 1: Left lateral external overview of a southern rock lobster (SRL)

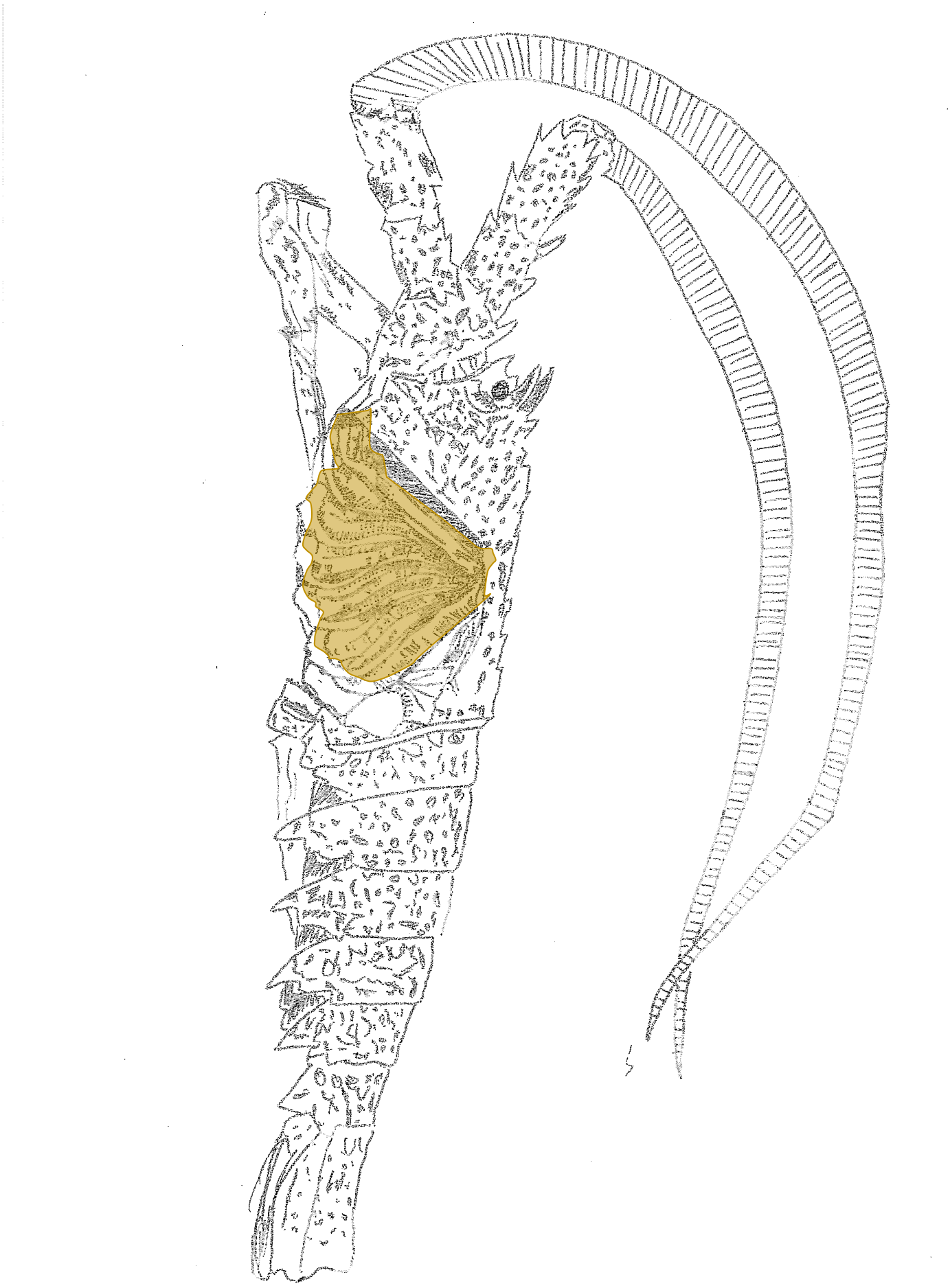


Figure 2: Left lateral view of SRL where external carapace layer is removed providing visualisation of gills as well as their positioning (Yellow)

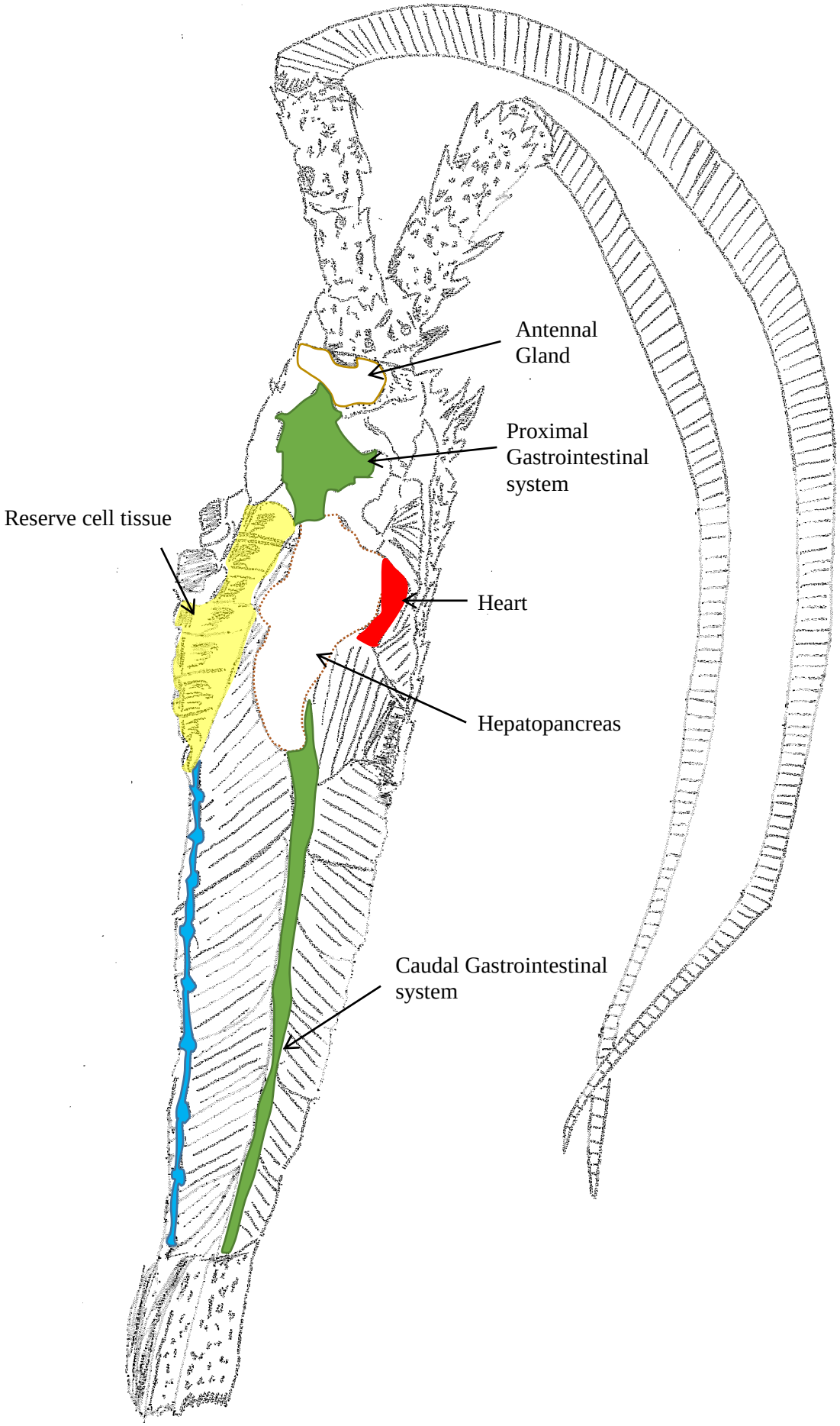


Figure 3: Left lateral view of SRL depicting the position and location of the internal organs.

COMPOUND EYE

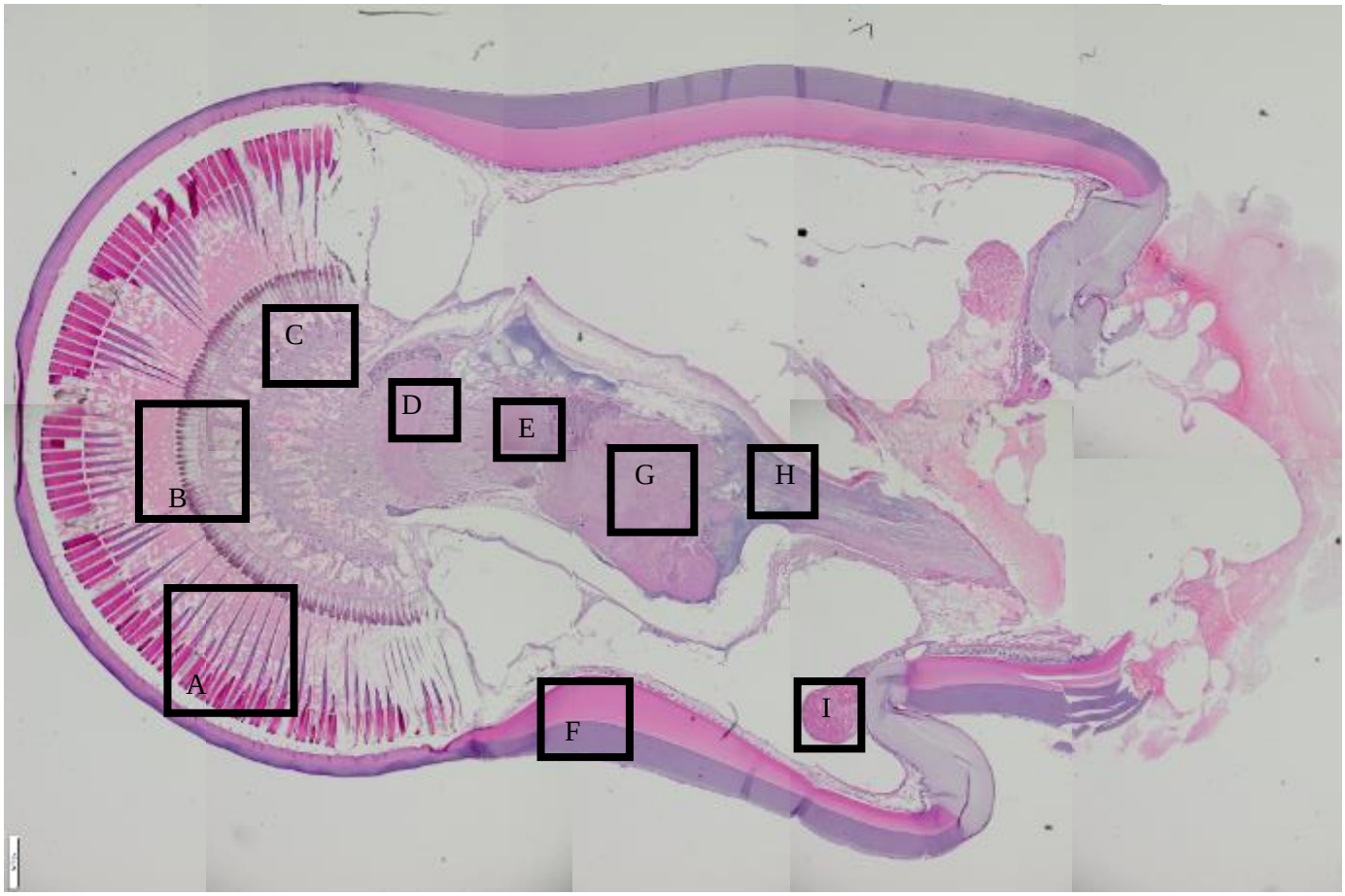
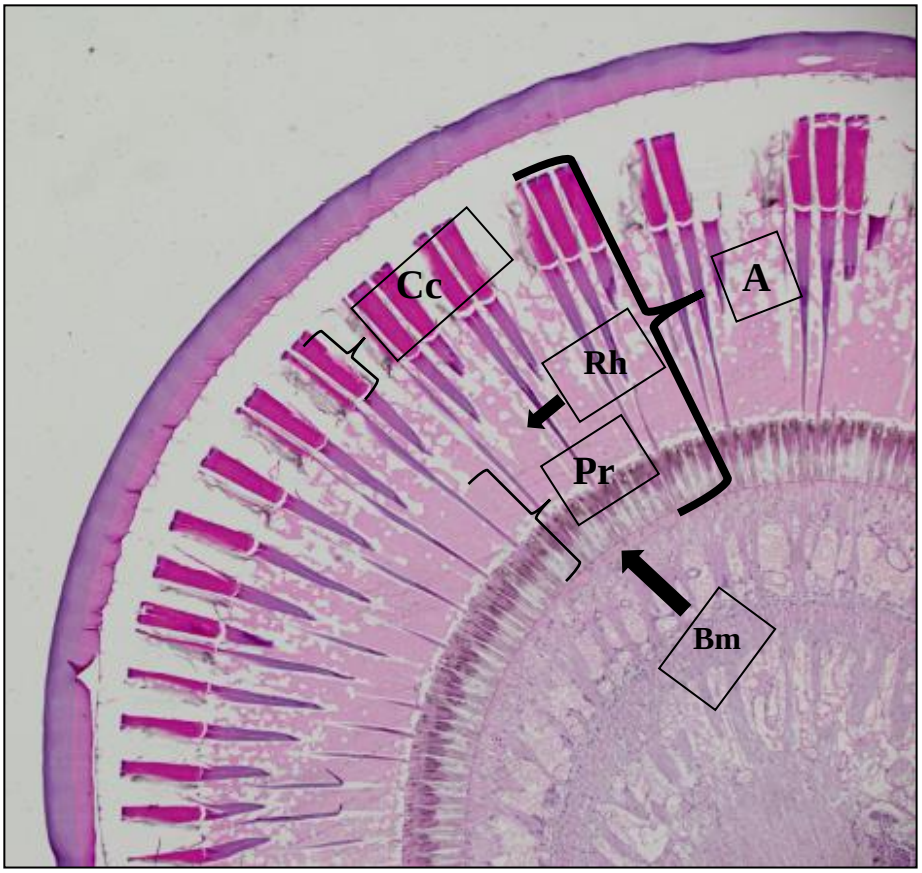


Figure 4: Overall view of the compound eye. [A: Ommatidia, B: Basement membrane (Bm) of the ommatidial region, C: Intermediate membrane, D: Medulla externa (Me), E: Medulla interna (Mi), F: Cuticle (Cut), G: Medulla terminalis (Mt), H: Optic nerve attachment, I: Ocular muscle]

Overall view of the compound eye. The eye contains three ganglia that are interconnected by nerve fibres and connective tissue: the medulla externa (Me), the medulla interna (Mi) and the medulla terminalis (Mt). The most rostral section of the eye is termed as the ommatidia which is made up by: the outer cuticle (Cut), the crystalline cones (Cc), the rhabdoms (Rh), the proximal retiluna (Pr) and the basement membrane (Bm). The intermediate membrane and lamina ganglionaris (Lg) lie between the ommatidia and the medulla externa.



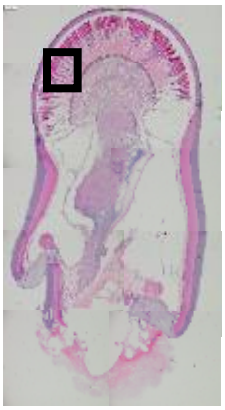


Figure 5: View of the ommatidia. Note the space between the ommatidia and cuticle is an artefact of fixation. [A: ommatidia, Cc: crystalline cone, Bm: basement membrane, Rh: rhabdom, Pr: proximal retiluna,]

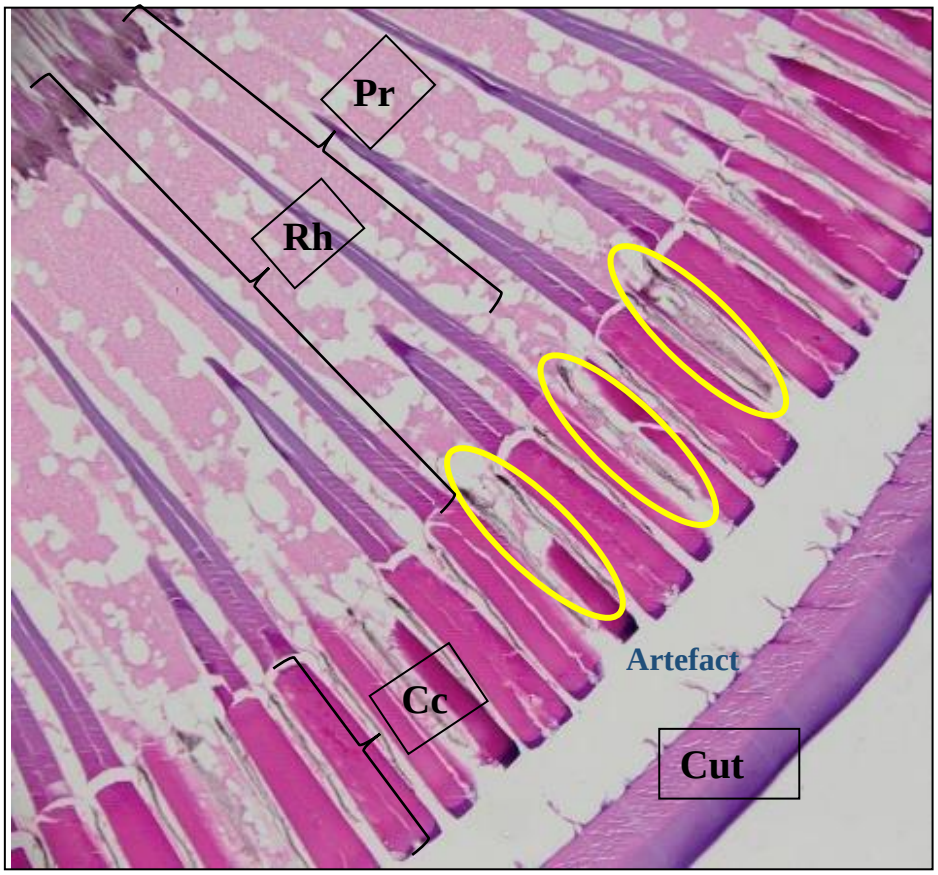
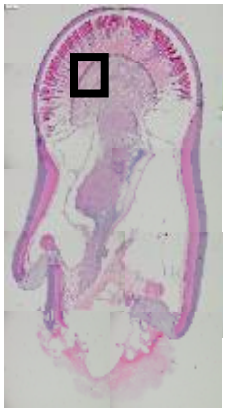


Figure 6: View of the ommatidia. The screening pigments of the accessory pigment cells present around the crystalline cone are circled (yellow). [Cc: crystalline cone, Rh: rhabdom, Pr: proximal retiluna, Cut: cuticle]



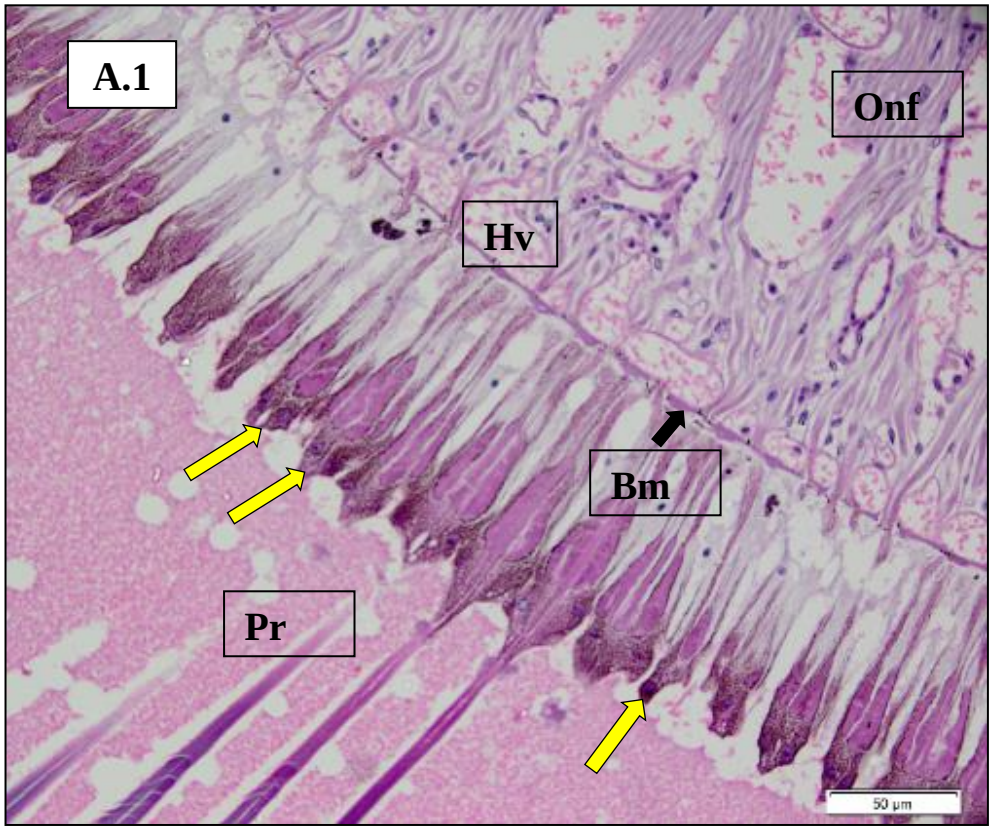


Figure 7: Image A.1 shows the transition of the ommatidia to the intermediate membrane. The yellow arrows are directed toward screening pigments that surround the proximal sections of the rhabdom. The black arrow is focused toward the basement membrane which separates the ommatidia from the intermediate membrane. [Pr: proximal retiluna, Bm: basement membrane, Hv: haemolymph vasculature, Onf: optic

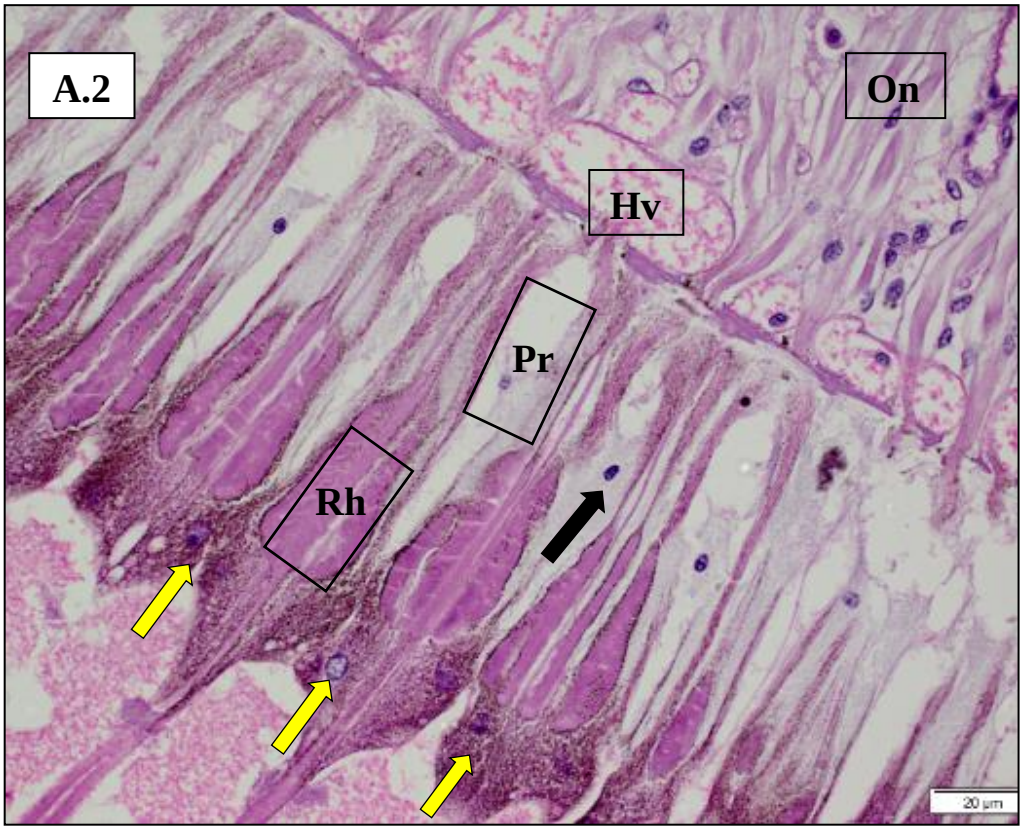
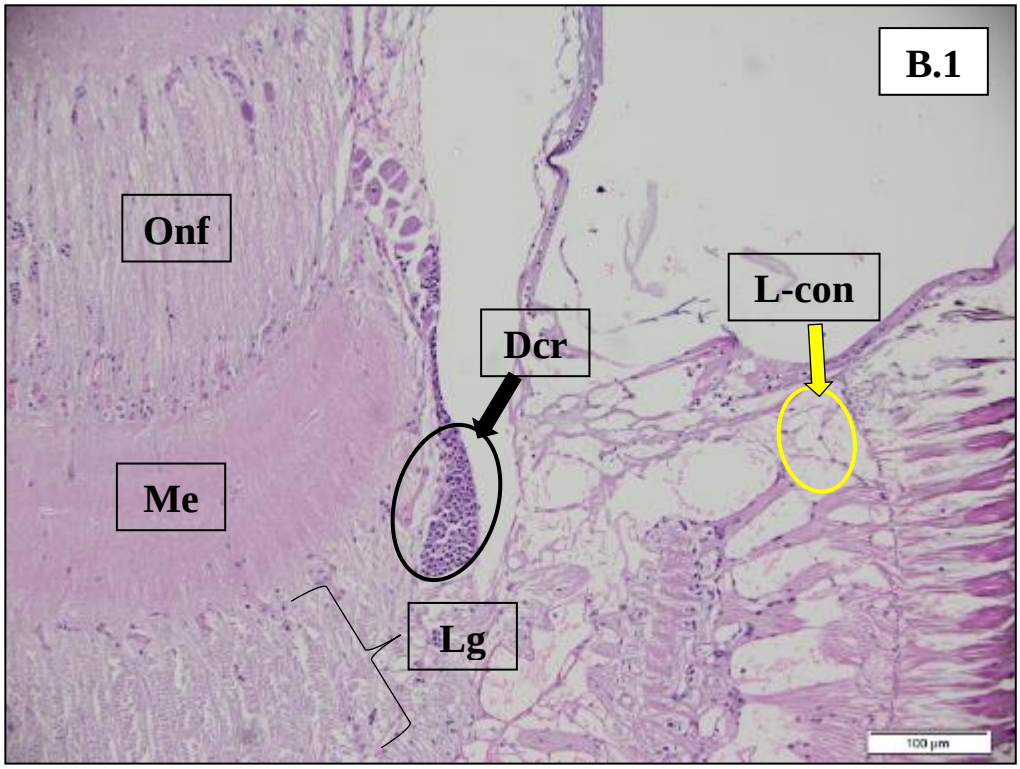


Figure 8: Image A.2 is a magnified section of image A.1. The locations of the proximal rhabdom with reference to screening pigments (yellow arrows) are displayed. The black arrow is pointed toward a nucleus of a pigment cell. [Rh: rhabdom, Pr: proximal retiluna, Hv: haemolymoh vasculature, Onf: optic nerve fibres]



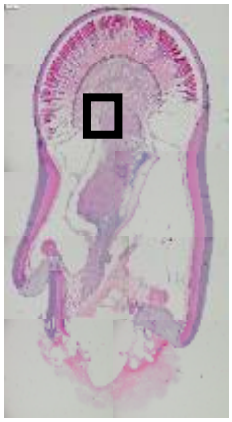


Figure 9: Image B1 represents an enlarged view of the lamina ganglionaris (Lg) which lies between the intermediate membrane and the medulla externa (Me). Note that due to the dissecting angle the distal cell rind (Dcr) is only captured on either side of the Me when in actual fact it should form a complete ring across the entire rostral portion of the medulla externa. [Onf: optic nerver fibres, Me: medulla externalis. Lg: lamina ganglionaris, L-con: loose connective tissue, Dcr: Distal cell rind]

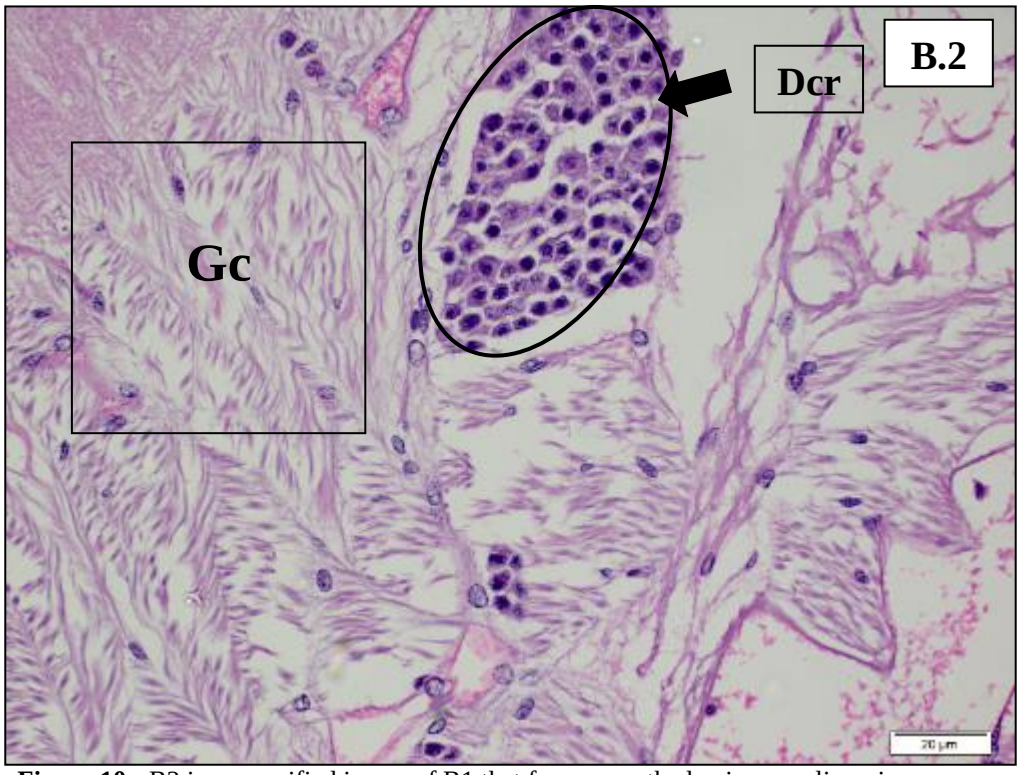
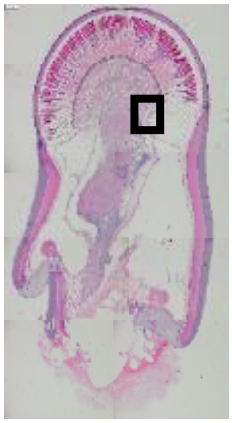


Figure 10: B2 is a magnified image of B1 that focuses on the lamina ganglionaris interface. The majority of this region is composed of nerve and glial cells (Gc). As previously mentioned the cell rind (Dcr) should extend over the whole rostral portion of the interface. [Dcr: distal cell rind, Gc: glial cells



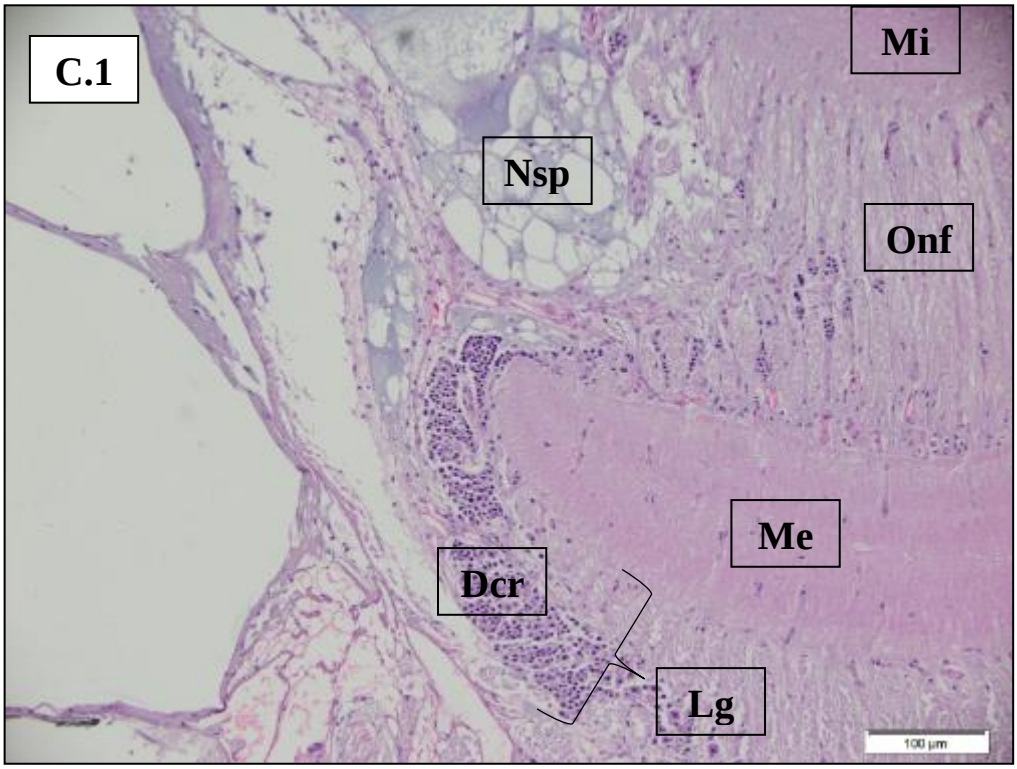


Figure 11: Image C1 represents an enlarged view of the lamina ganglionaris (Lg) as well as the medulla externa. As mentioned in the figures 6 and 7 the distal cell rind (Dcr) should extend over the entire rostral portion of the medulla externa as a ring. [Mi: medulla interna, Nsp: neuro secretory product, Onf: optic nerve fibres, Me: medulla externa, Dcr: distal cell rind, Lg: lamina ganglionaris.

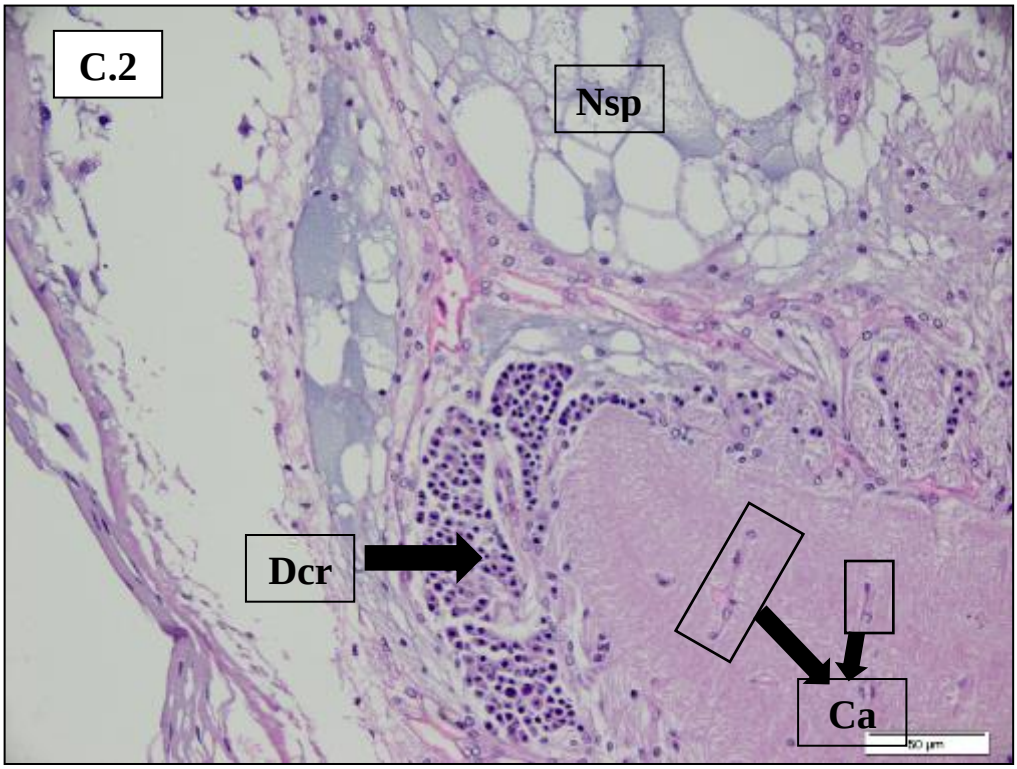
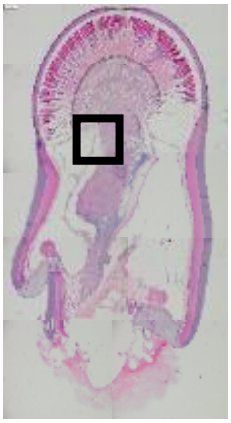


Figure 12: C2 is a magnified image of C1 focusing on the medulla externa (Me). Capillaries (Cap) found in the Me are composed of simple squamous endothelium. [Nsp: neuro secretory product, Dcr: distal cell rind, Cap: Capillaries]



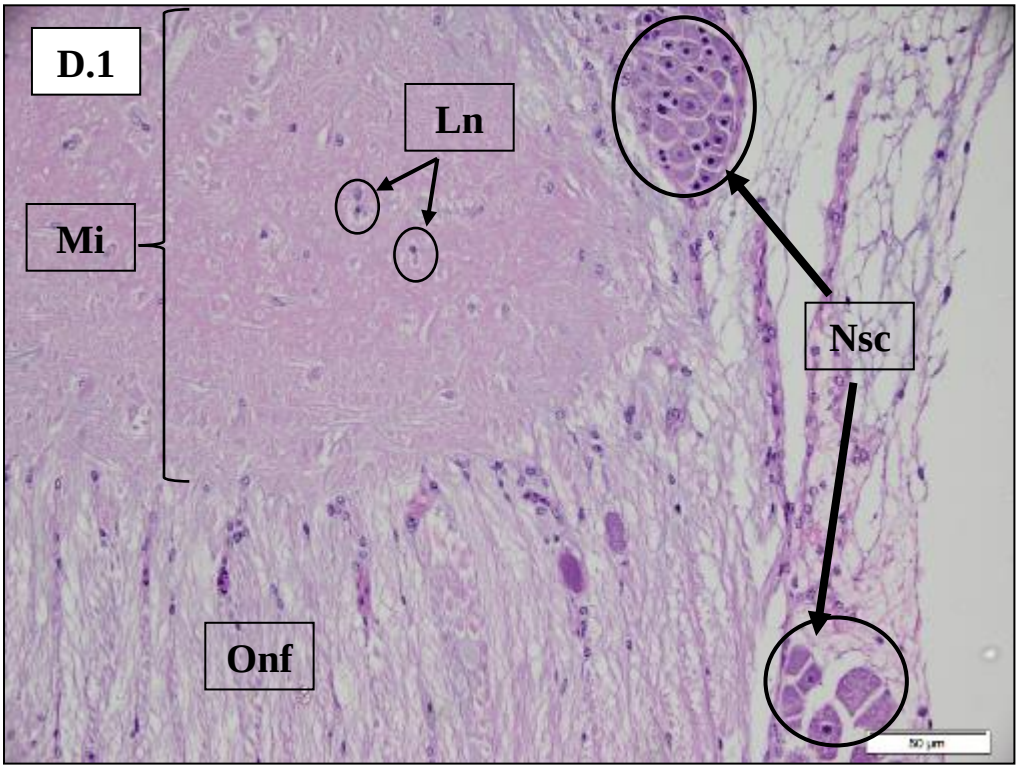


Figure 13: Image D1 represents an enlarged view of the medulla interna (Mi). The medulla interna is similar in structure to the medulla externa (Me), including the interspersed capillaries (Cap). Neurosecretory cells occur loosely around the Mi which will also be seen around the medulla terminalis (Mt). [Mi: medulla interna, Onf: optic nerve fibres, Cap: Capillaries, Nsc: Neuro-secretory cells]

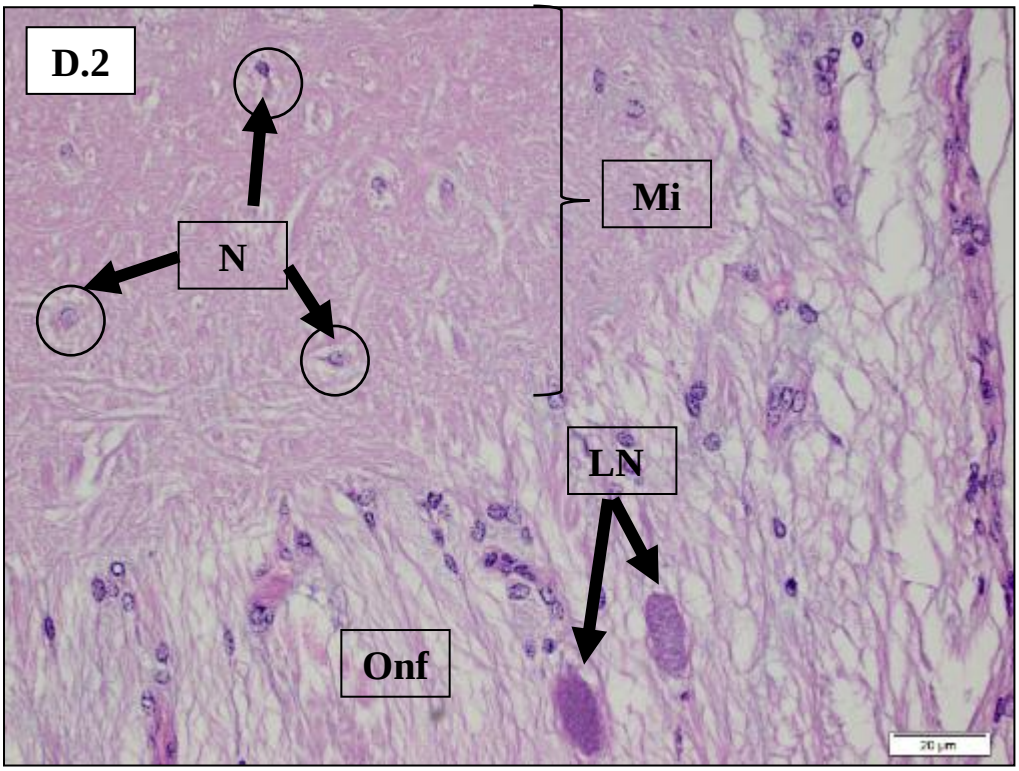
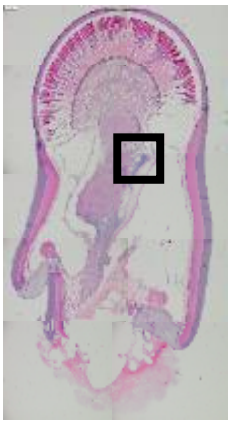


Figure 14: D2 is a magnification of image D1 of the medulla interna (Mi). Further detail is provided of the optic nerve fibres (Onf) that transition into the Mi where the large neuron nucleus's (LNn) are visualised. [Mi: medulla interna, Onf: optic nerve fibres, N: Neuron, LNn: large neuron nucleus]



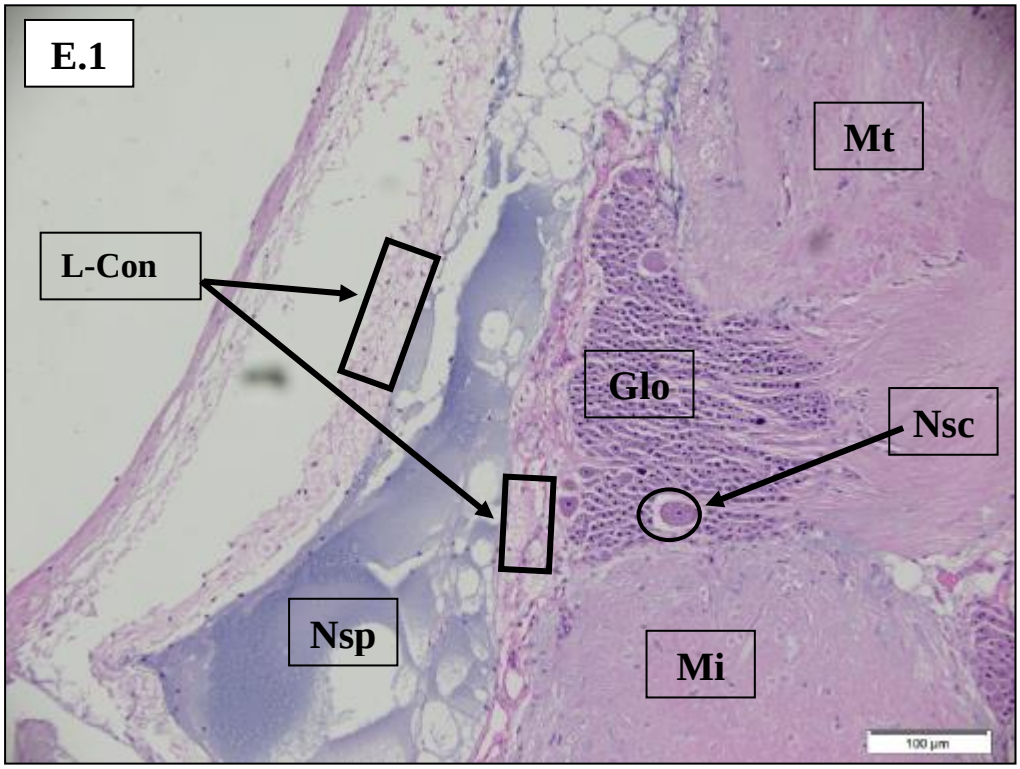


Figure 15: The image above shows the transition between the medullar interna (Mi) and the medulla terminalis (Mt). Located between these ganglia are globuli cells (Glo) of the hemielliopsoid body. [L-Con: loose connective tissue, Nsp: neuro-secretory product, Glo: globuli cells, Mi: medulla interna, Nsc: neuro-secretory cells, Mt: medulla terminalis]

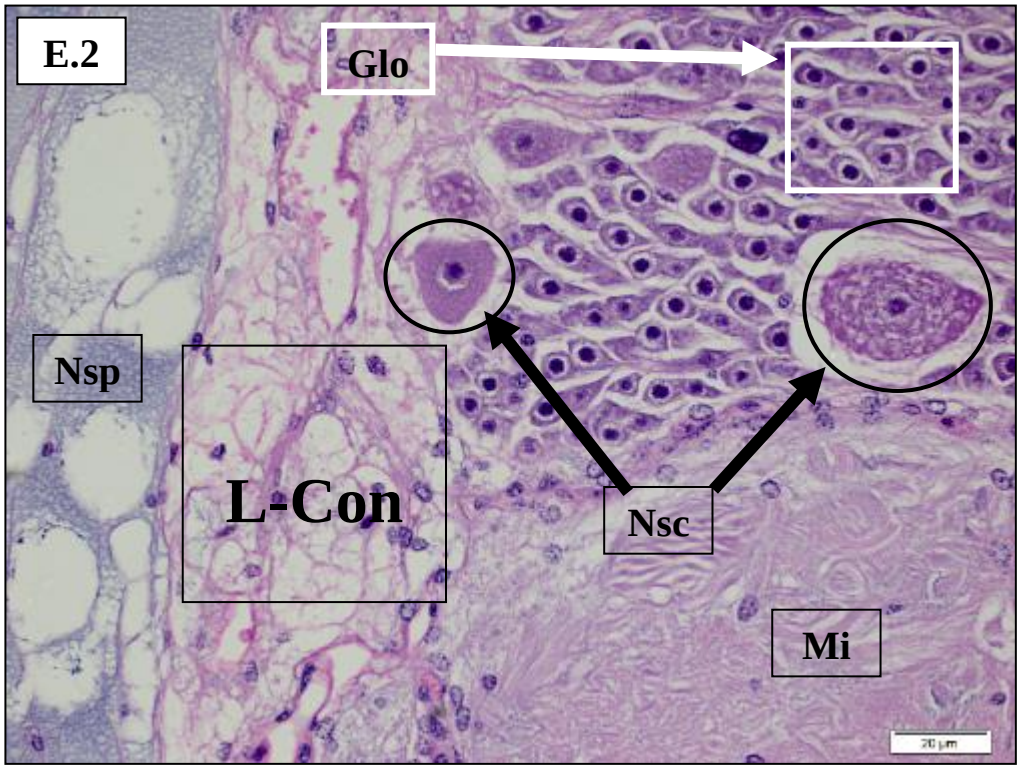
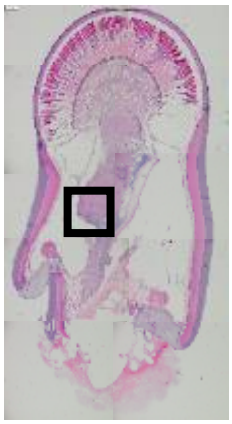


Figure 16: E2 is a magnification of E1 that shows the detail of the globuli cells (Glo) as well as the neuro-secretory cells (Nsc) located between the medullar interna (Mi) and medulla terminalis (Mt). [Nsp: Neuro-secretory product, L-Con: loose connective tissue, Glo: Globuli cells, Nsc: Neuro-secretory cells, Mi: medulla interna]



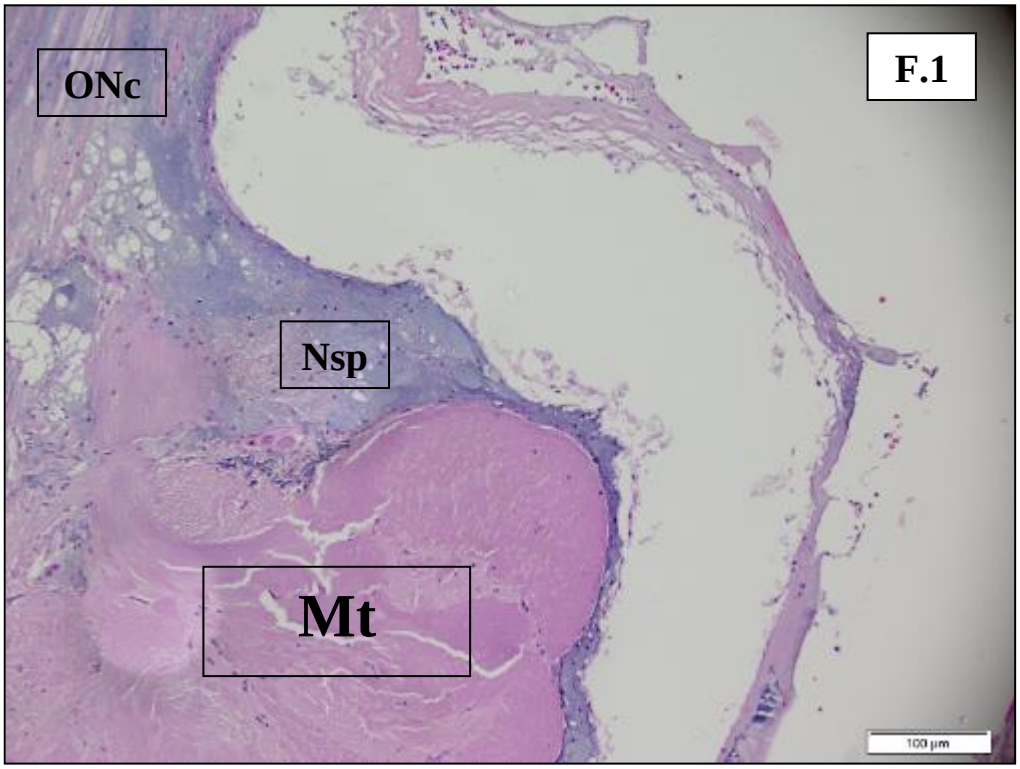


Figure 17: Image F1 represents the caudal portion of the medulla terminalis (Mt). Fibres from it directly form the optic nerve cord (Onc) that continues from here to the central ganglia. [Mt: Medulla terminalis, Nsp: neuro-secretoy product, ONc: optic nerve cord]

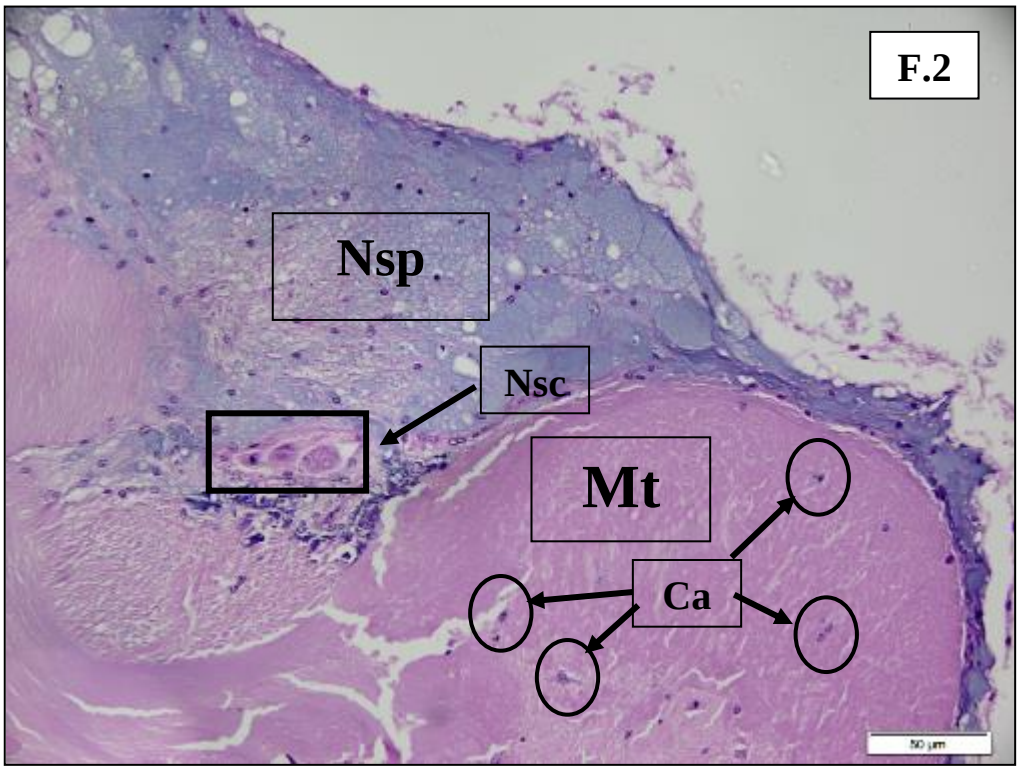
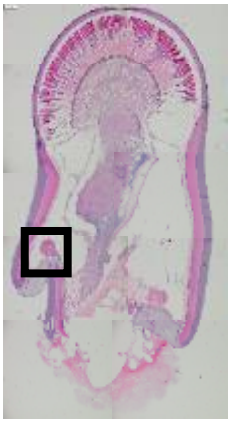


Figure 18: F2 is a magnification of image F1 providing further visual detail of the medulla terminalis (Mt). As noted in other ganglia the medulla terliminalis (Mt) contains a matrix of capillaries (Cap). [Mt: Medulla terminalis, Nsp: neuro-secretoy product, Nsc: neuro-secretory cells]



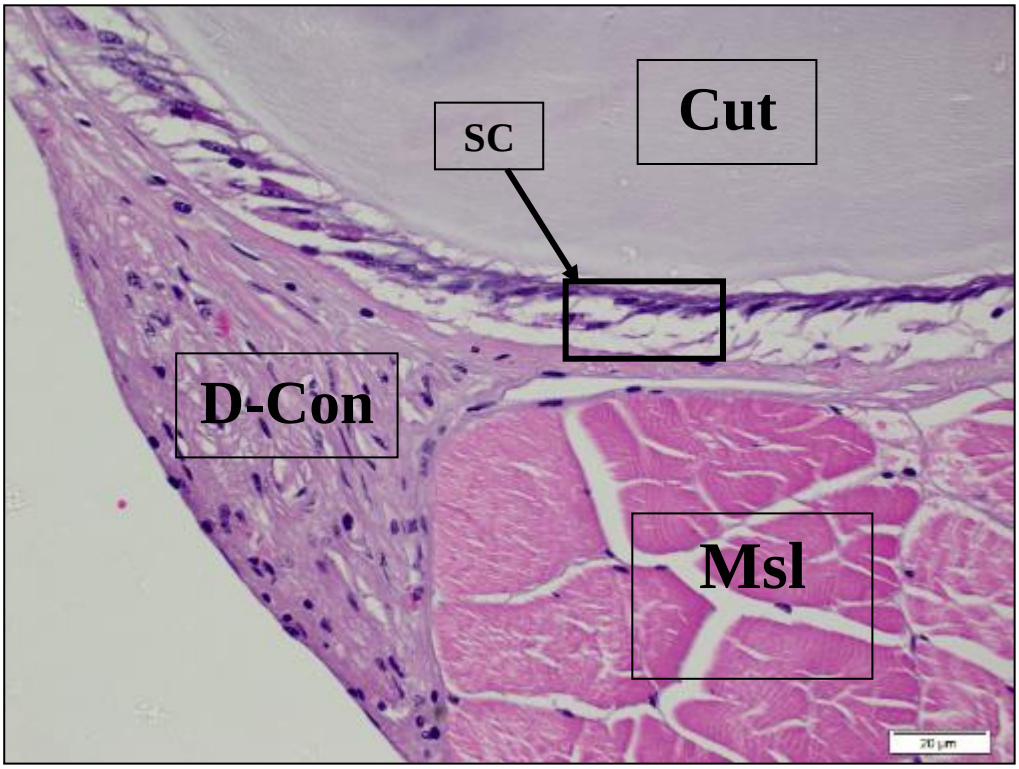
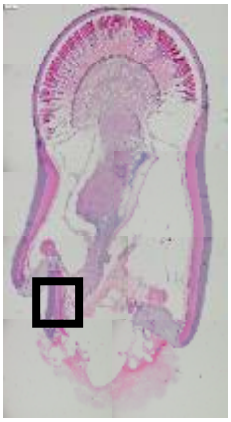


Figure 19: Image of one muscle attachment observed within the eye. The muscle (Msl) is connected and surrounded by dense collagen rich connective tissue (D-Con) which underlies the cuticle (Cut) and simple columnar epithelium (SCe). [Msl: muscle, D-Con: dense connective tissue, SCe: simple columnar epithelum, Cut: cuticle]



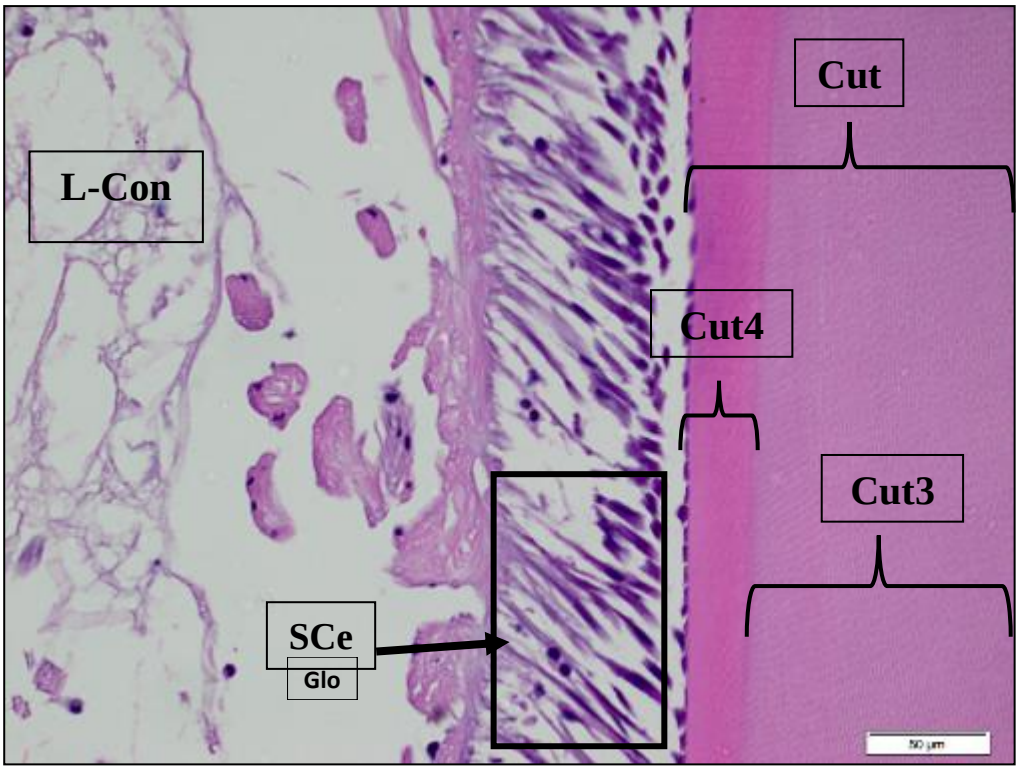


Figure 20: A magnified image of cuticle (Cut) attachment. Note the sporadic appearance of the dense connective tissue that has occurred as artefact during the fixation process. The cuticle layers is discussed in further detail in the musculoskeletal section of the atlas. [L-Con: loose connective tissue, D-Con: dense connective tissue, SCe: simple columnar epithelium, Cut4: inner most layer of the cuticle, Cut3: endocuticle, Cut: cuticle]

NORMAL GILL HISTOLOGY

The gill lamella (Lam) are made up of primary filaments that branch out of the branchial septum (BrancS). Each primary filament then further divides into secondary non branching filaments. These filaments contain basally located vessels (efferent and afferent) that carry haemolymph and empty into the branchial septum.

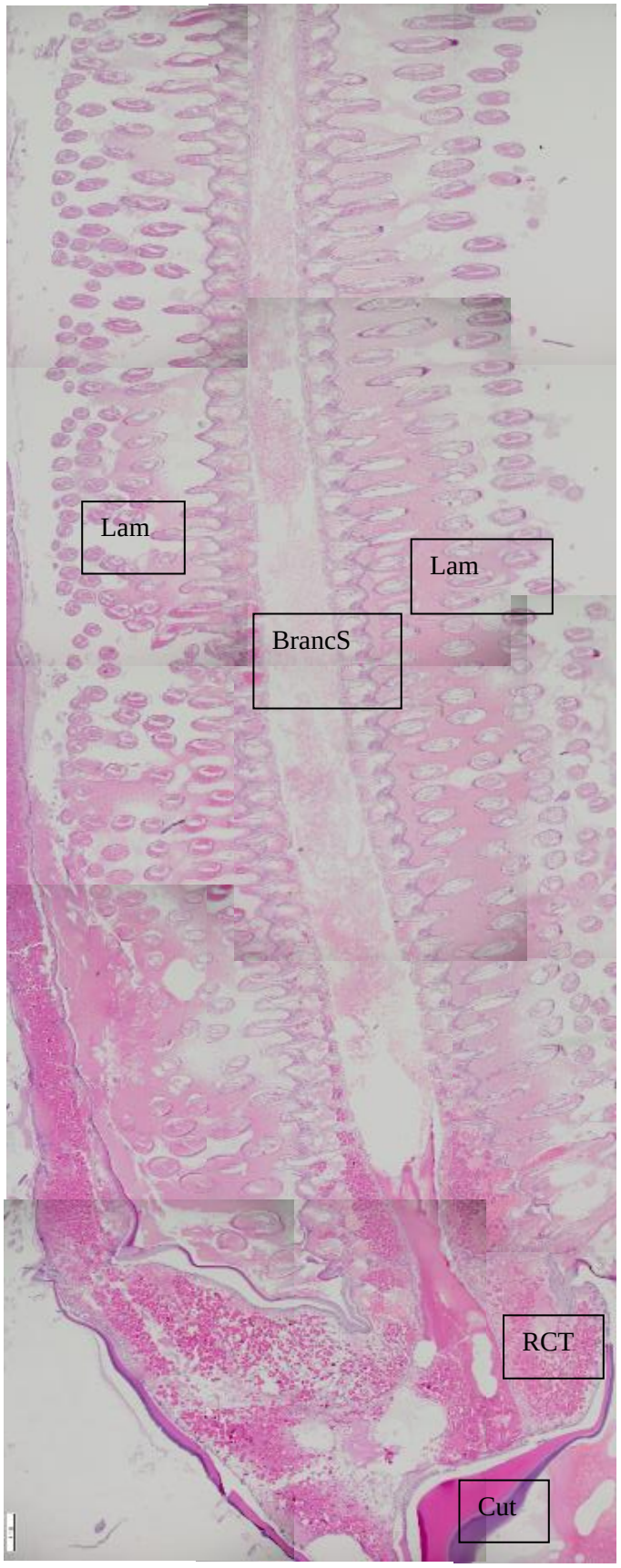


Figure 21: Longitudinal section representing an overview of the SRL gill. [RCT: reserve cell tissue, Cut: Cuticle, Lam: lamella, BrancS: branchial septum]

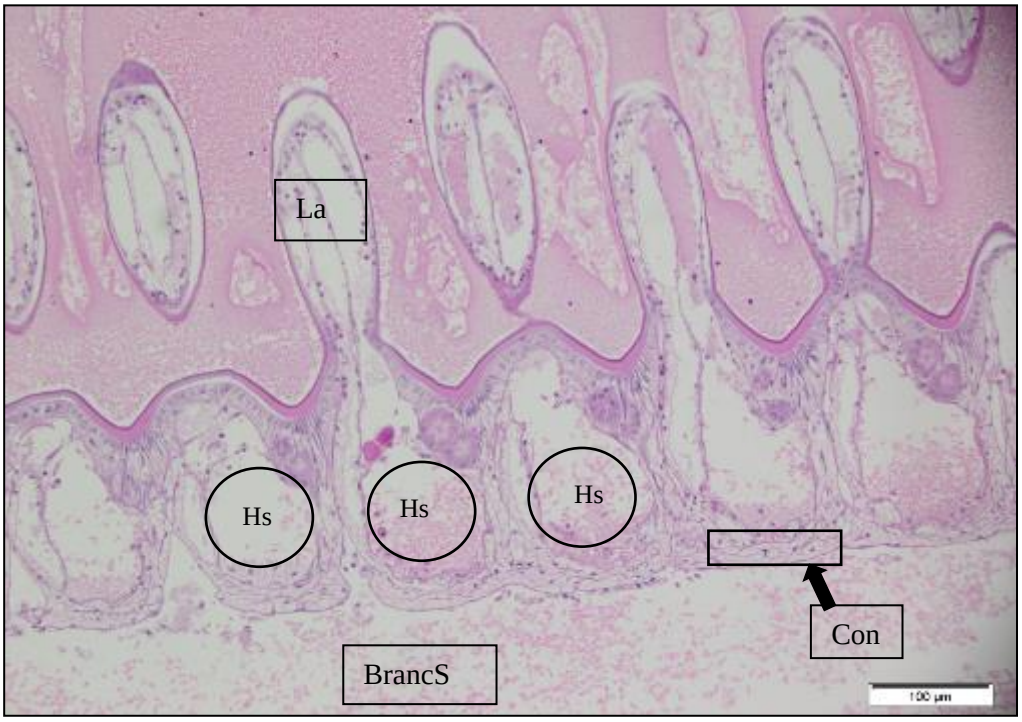


Figure 22: Longitudinal section showing lamellae (Lam) attachment to the branchial stem (BrancS). [Lam: Lamellae, Hs: Haemal sinus, Con: Connective tissue, BrancS: Branchial septum]

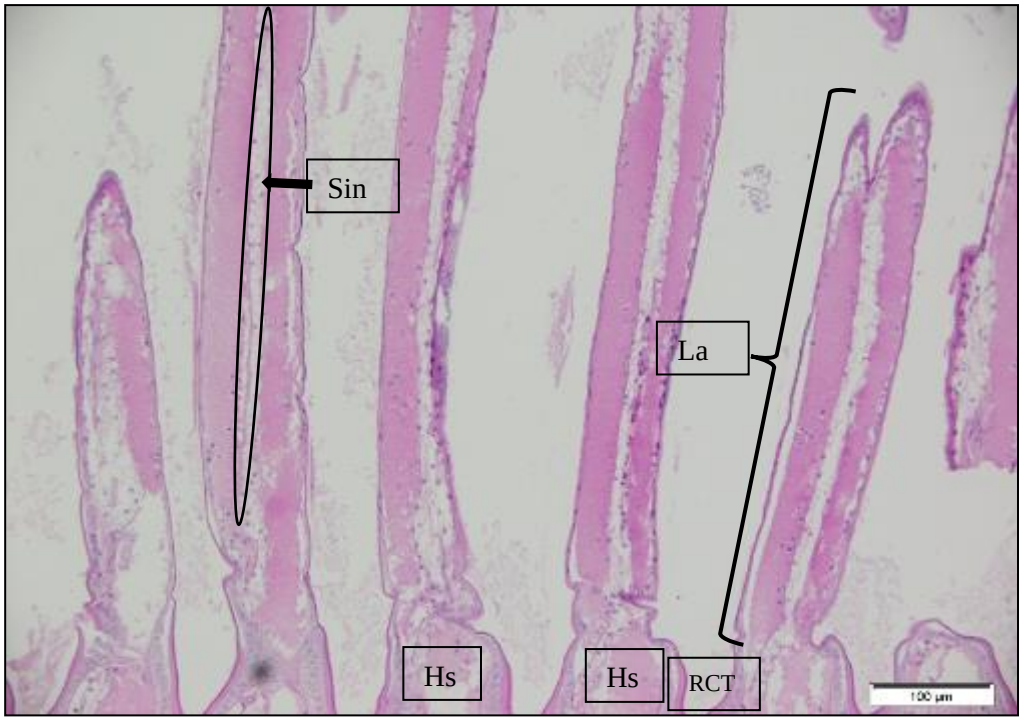


Figure 23: Longitudinal section of gill demonstrating further detail of gill lamellae. Note the sinus (Sin) located along the midline of each lamellae (Lam). These empty into the afferent and efferent channels located in the haemal sinus (Hs).[Sin: sinus, Lam: lamella, Hs: Haemal sinus]

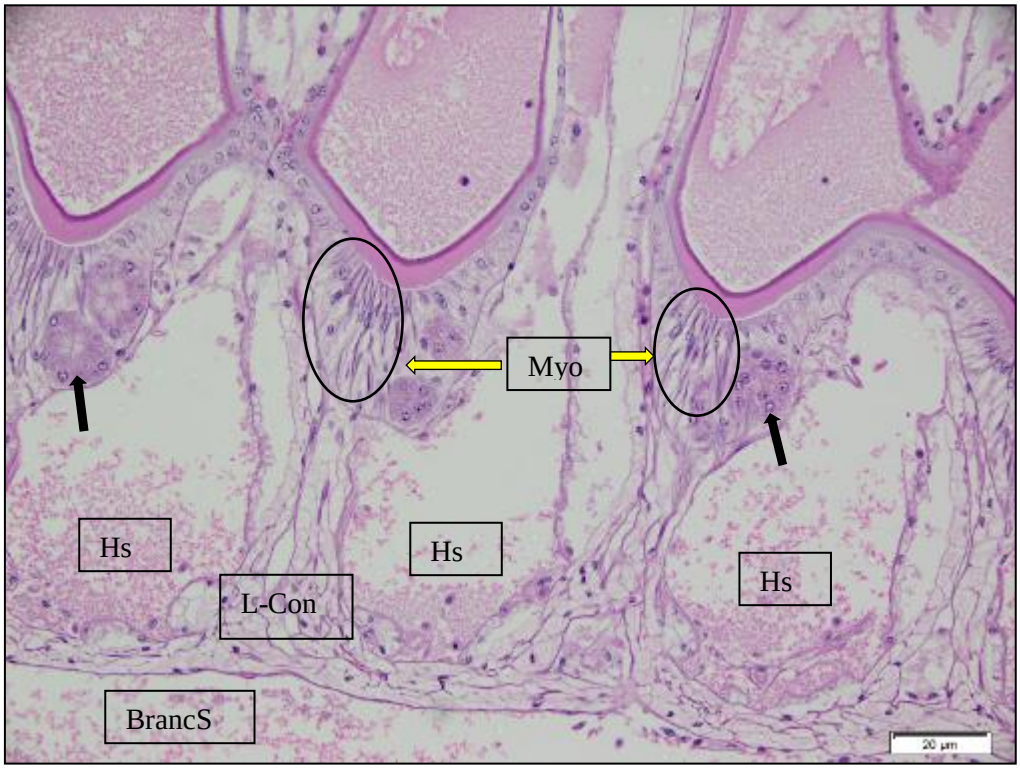


Figure 24: Longitudinal section providing further detail to the haemal sinuses. Image shows the myofibrils (Myo) located at the base of the lamellae as well as the tegmental lobed glands (black arrows). Further detail of tegmental gland architecture is shown in figure 25. [Myo: Myofibrils, Hs: haemal sinus, L-Con: loose connective tissue, BrancS: branchial stem.

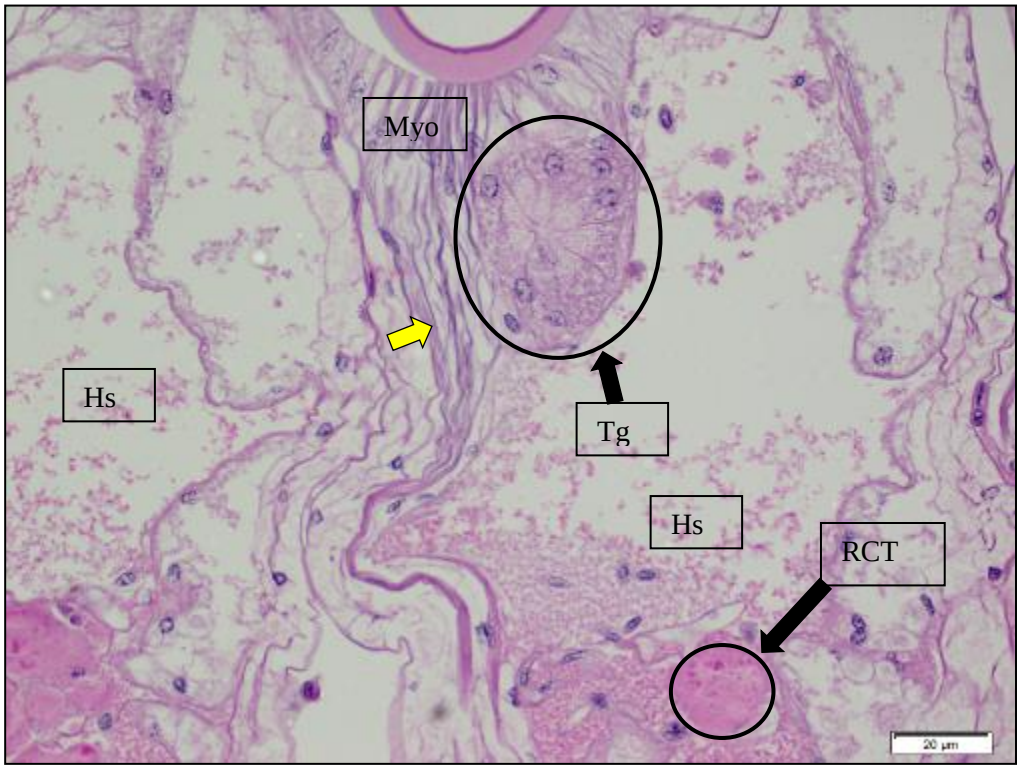


Figure 25: This is a magnification of figure 24 above of the gill channel. As previously mentioned the myofibrils are located at the base of the lamella as well as the tonofibrillae (yellow arrow). The tegmental lobed glands are located in the same area. The nucleus cells of the glands are located on the peripheral portion of the cell with the collecting duct positioned in its centre. [Hs: haemal sinus, Myo: myofibrils, Tg: tegumental glands, L-Con: loose connective tissue, RCT: reserve cell tissue]

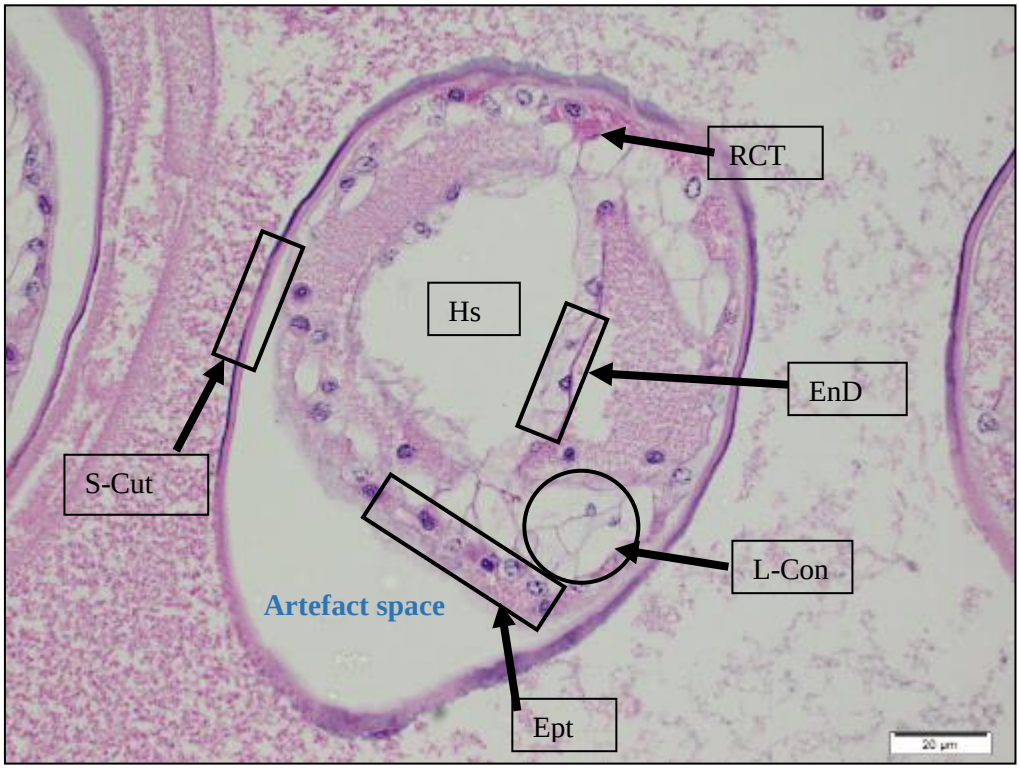


Figure 26: Cross section of a gill lamellae. Chief cells make up the majority of the epithelia (Ept) while the loose connective tissue (L-Con) (also referred to as trabecular cells) are supportive cells providing structural framework contributing to efficient haemolymph flow. [RCT: reserve cell tissue, Hs: haemal sinus, S-Cut: subcuticle, Ept: epithelium, L-Con: loose connective tissue, EnD: endothelium.

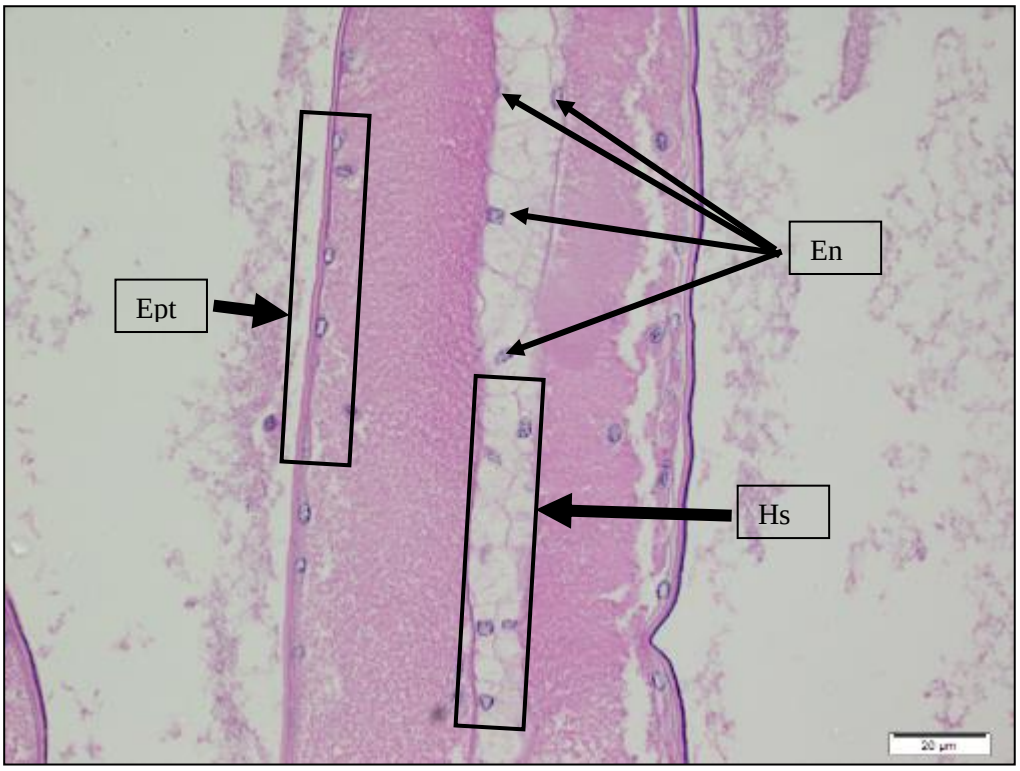


Figure 27: Longitudinal section of a gill lamellae. Note the presence of endothelial (EnD) cells along the lateral edges of the haemal sinus (Hs). [Ept: epithelium, Hs: Haemal sinus, EnD: endothelial cells]

GILL HISTOPATHOLOGY

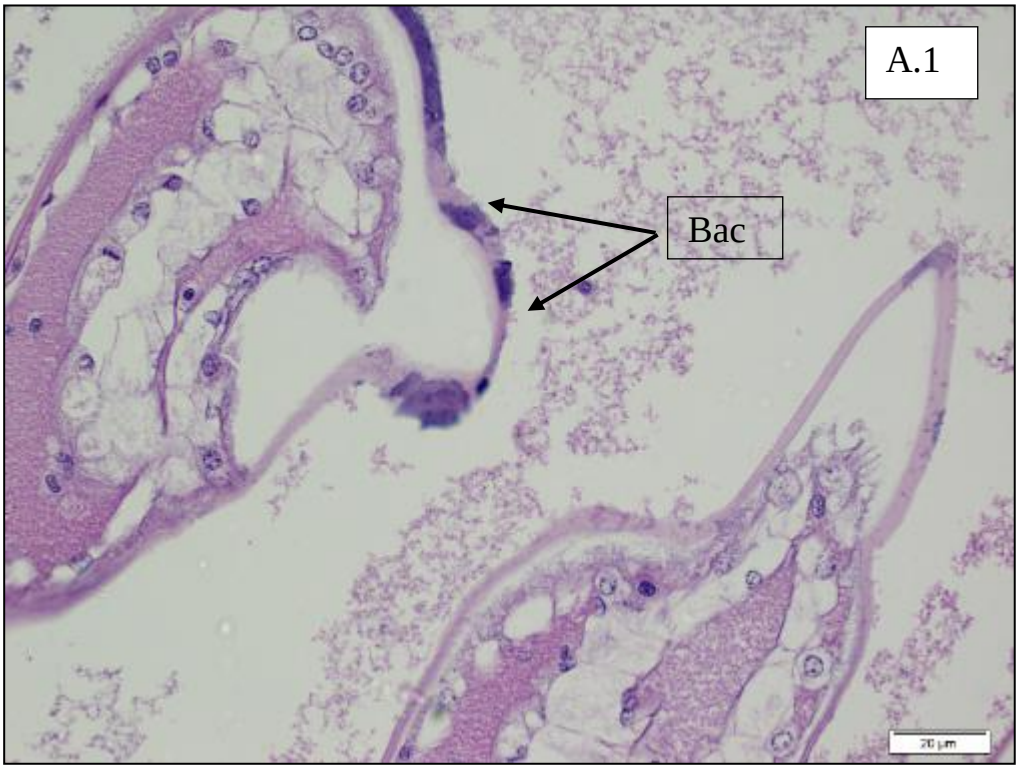


Figure 28: Grade 1 biofouling. This image shows bacterial biofouling with chitin erosion present. Note there is a mixed bacterial population present. [Bac: bacteria]

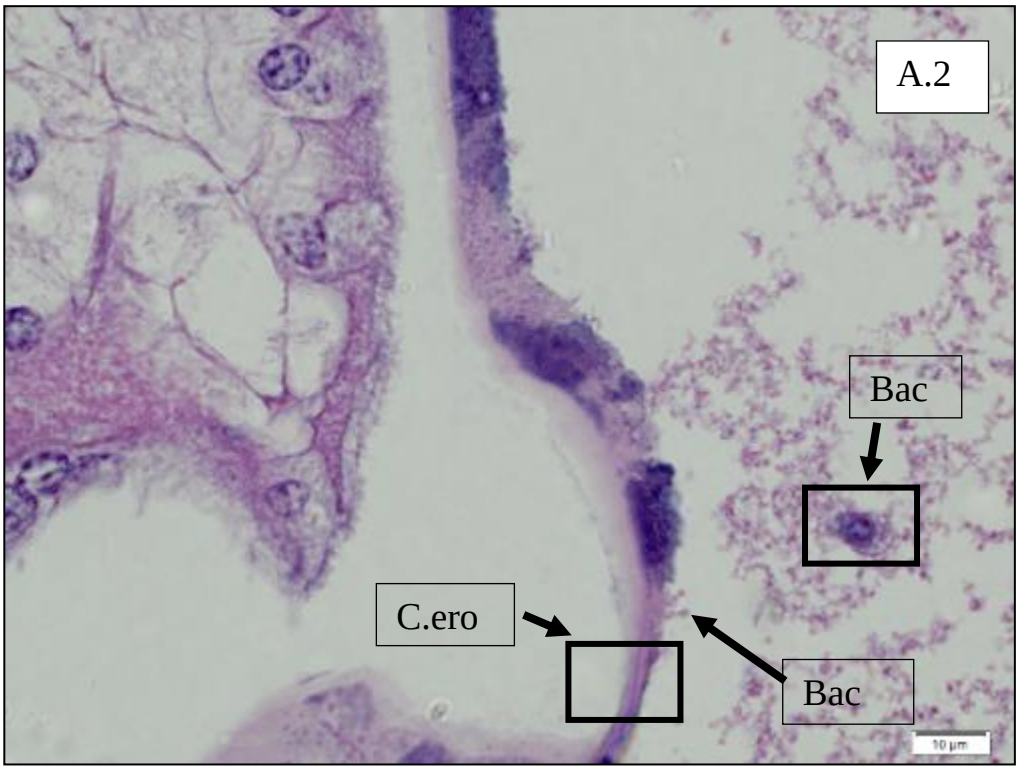


Figure 29: A2 is a magnification of image A1 showing grade 1 biofouling. This image provides further detail to chitin erosion (C.ero), and mixed bacterial (Bac) presence. [C.ero: chitin erosion, Bac: bacteria]

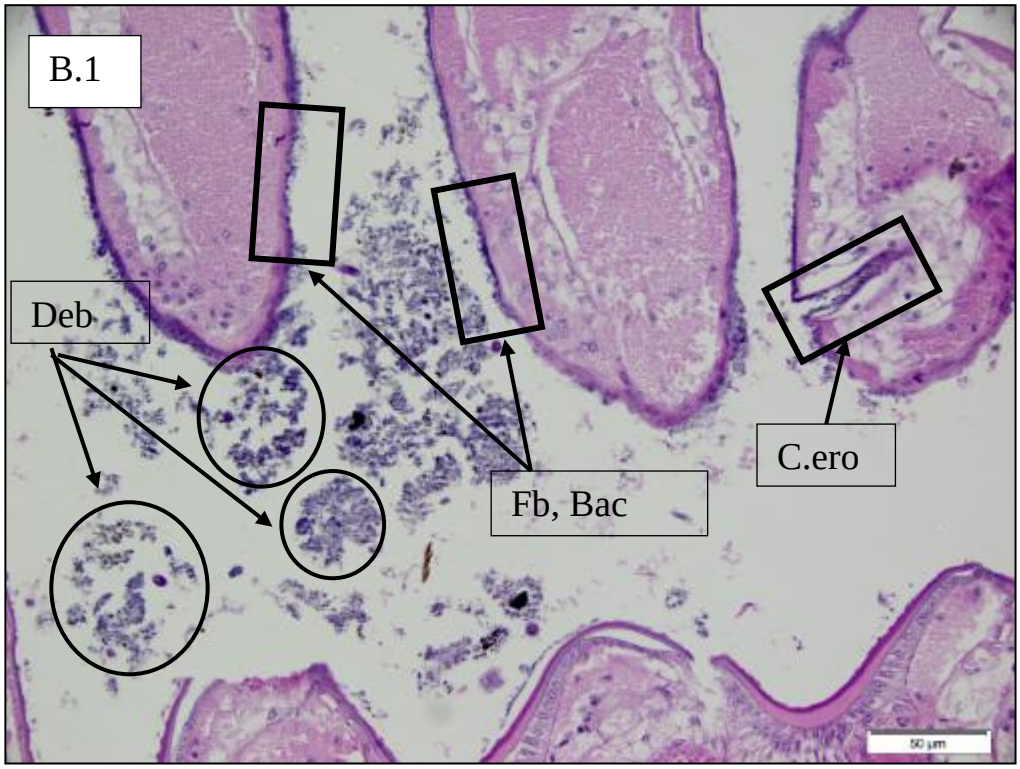


Figure 30: Grade 2 biofouling. This image shows a high level of bacterial biofouling consisting of mixed (Bac) and filamentous bacteria (Fb). There are also areas of chitinoclastic destruction and erosion (C.ero) within these gills with marked amounts of debri (Deb) in the inter-lamellae spaces. [Deb: debris, Fb: filamentous bacteria, Bac: bacteria, C.ero: chitin erosion]

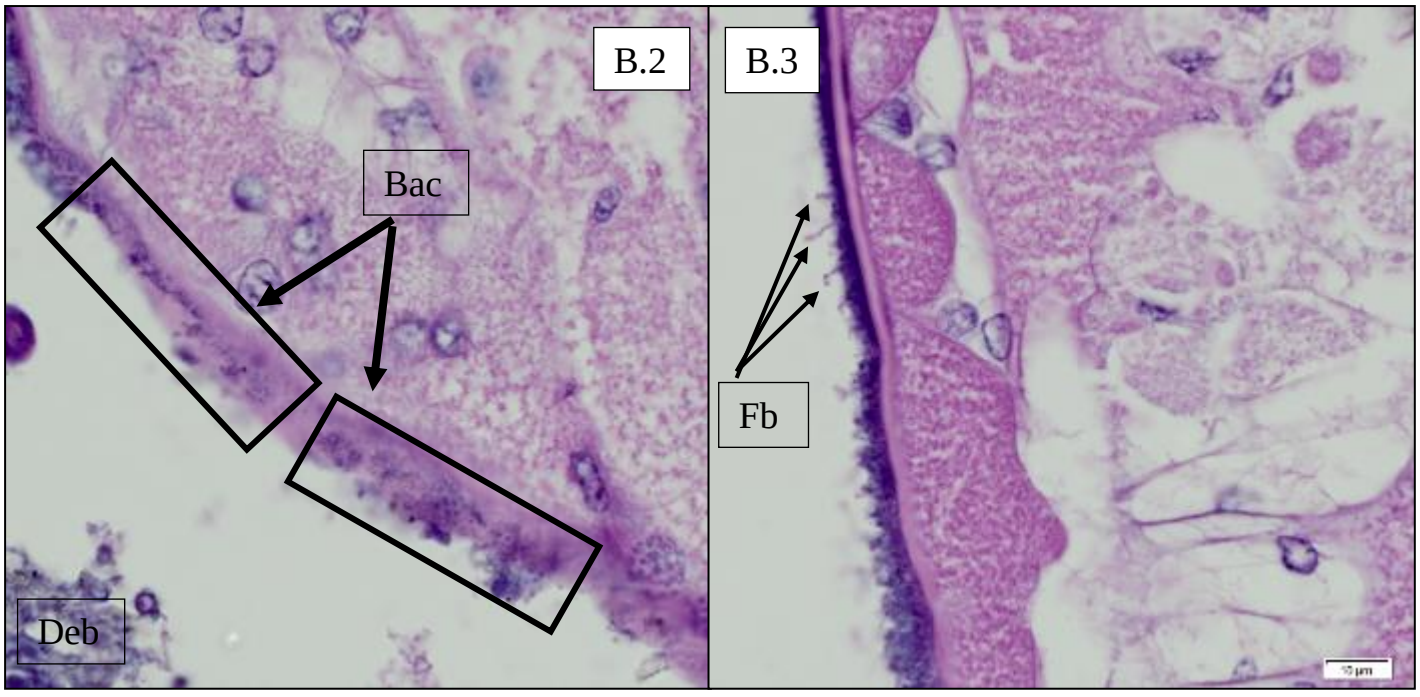


Figure 31: Image B2 and B3 are magnified views showing the detail of mixed bacteria and filamentous bacteria observed in gills for grade 2 biofouling. Note the focal cocci nature of the bacteria in image B2 and the elongated nature of the filamentous bacteria in image B3. [Deb: debris, Bac: bacteria, Fb: filamentous bacteria]

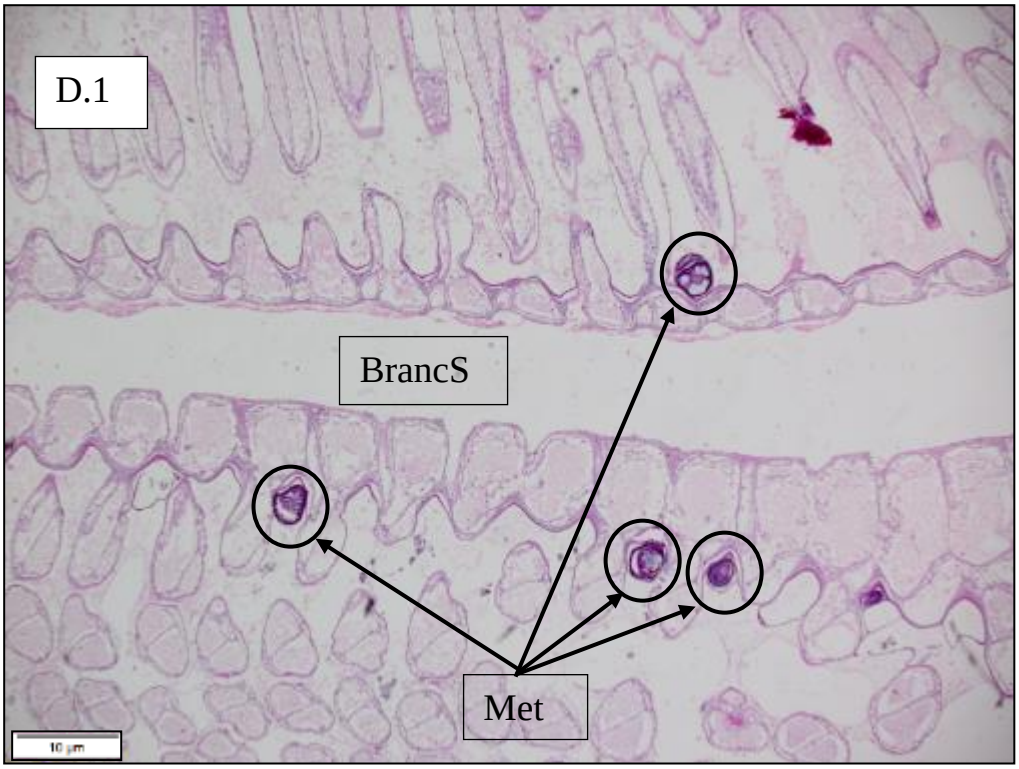


Figure 32: Cross sections of metazoans at the base of the primary lamellae. [BrancS: branchial septum, Met: metazoans]

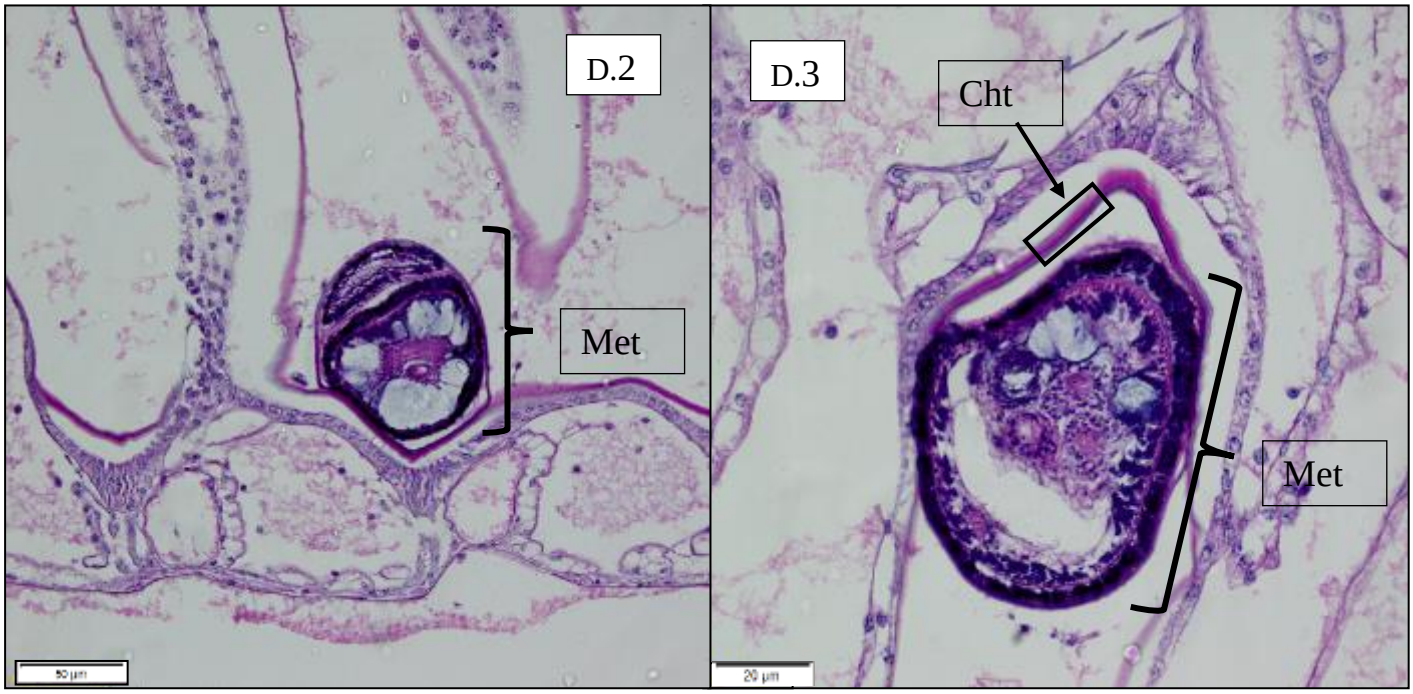


Figure 33: Magnification of image D1 providing a detailed appearance of metazoans (Met) observed in the gills. Note the presence of a host reaction where the metazoans on both D2 and D3 are walled off by chitin (Cht). [Met: metazoan, Cht: Chitin]

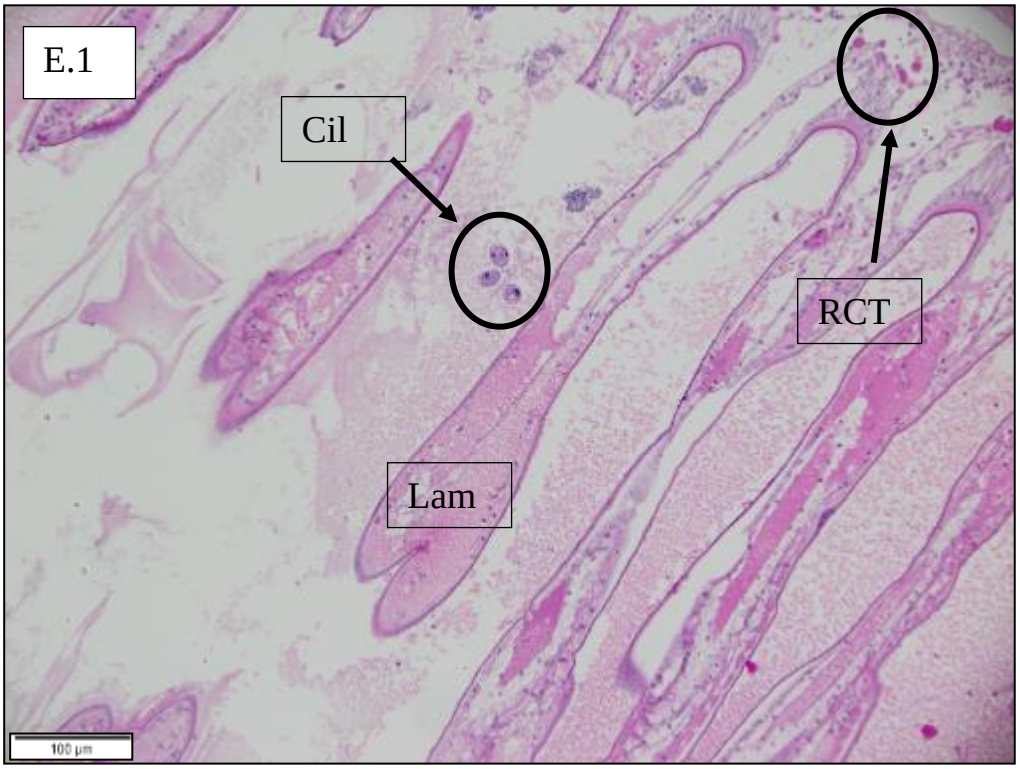


Figure 34: Cross sections of ciliates (Clt) present in the inter-lamellae spaces of the primary lamellae (Lam). Note these is no host response associated with protozoa presence. [Lam: lamellae, RCT: reserve cell tissue, Cil: Ciliates]

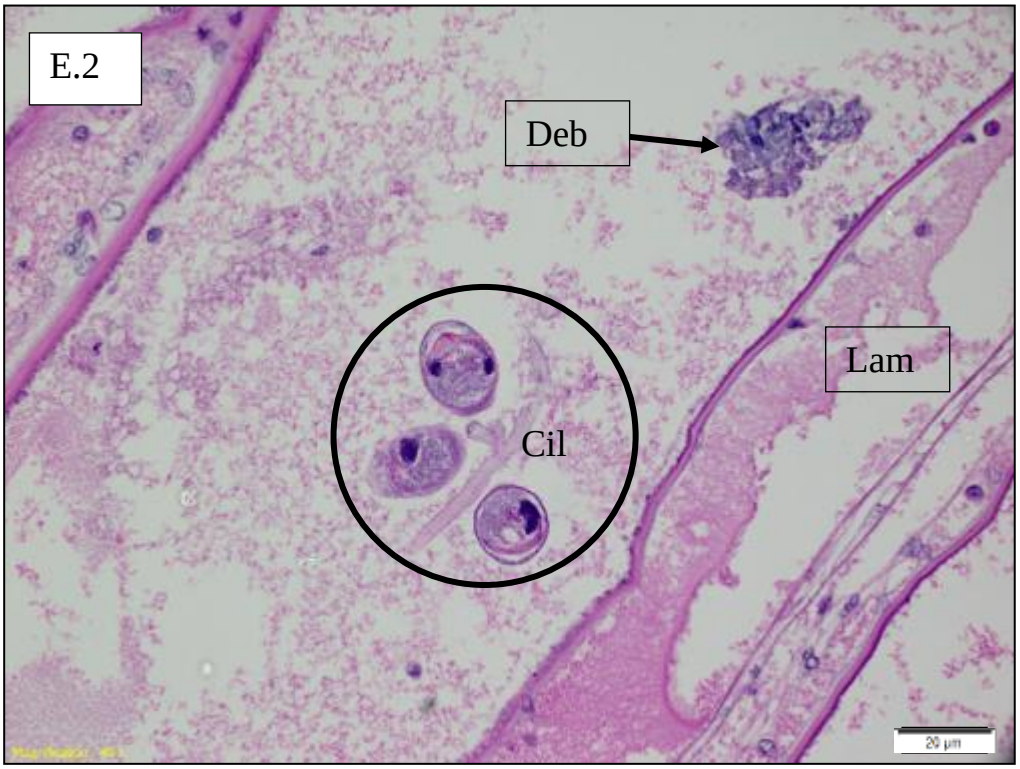


Figure 35: Magnification of image E1 of ciliates (Cil) cross sections in the inter-lamellae spaces of the primary lamellae (Lam) with no associated host response. [Cil: Ciliates, Lam: lamellae, Deb: debris]

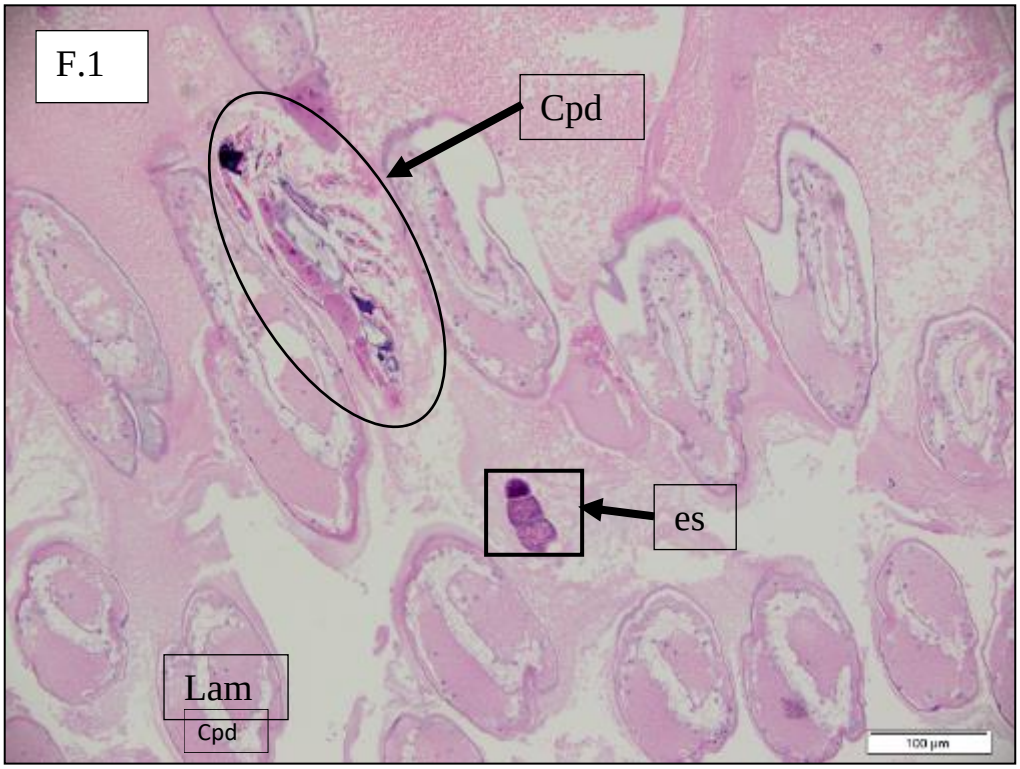


Figure 36: Cross section of copepod (Cpd) present in the inter lamellae space of the gills. Note the presence of the copepod egg sac (es). [Lam: lamella, Cpd: copepod, es: egg sac.]

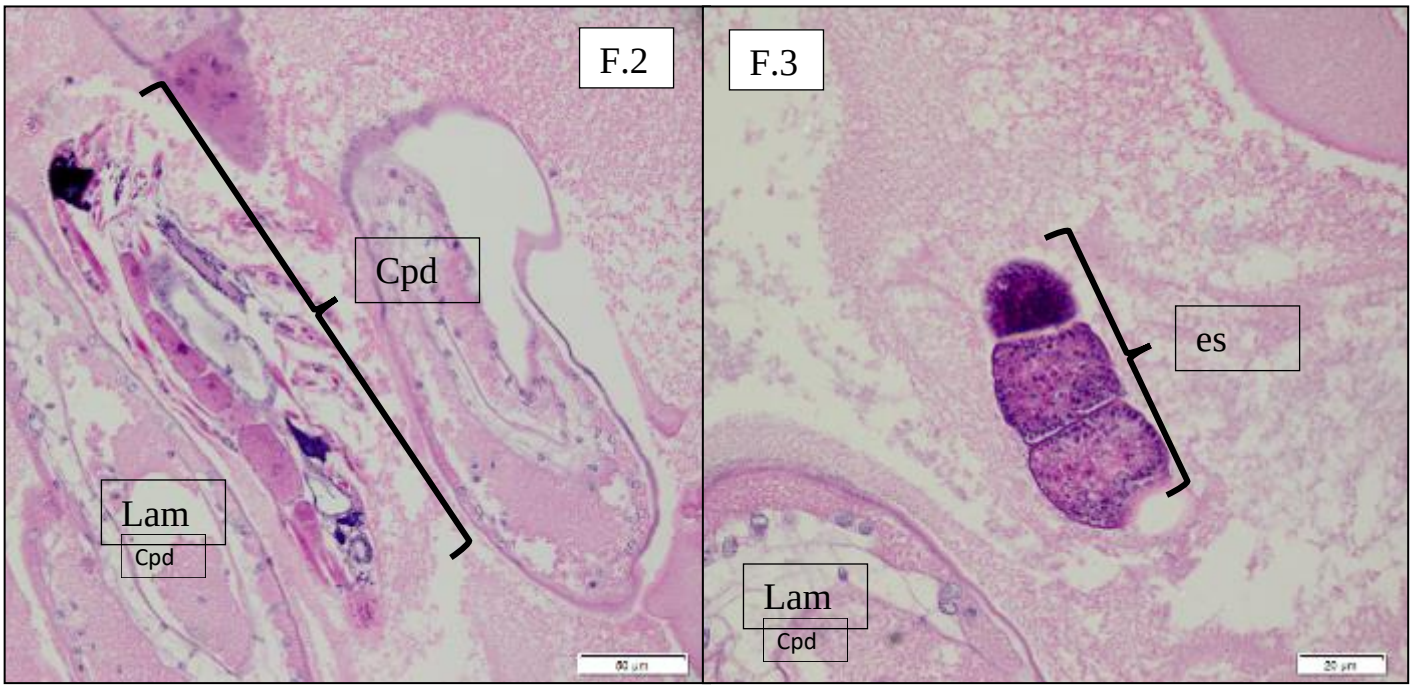


Figure 37: Magnification of image F1 where image F2 provides a detailed view of a copepod structure and anatomy while image F3 is a magnified view of the egg sac. [Cpd: copepod, es: egg sac, Lam: lamellae]

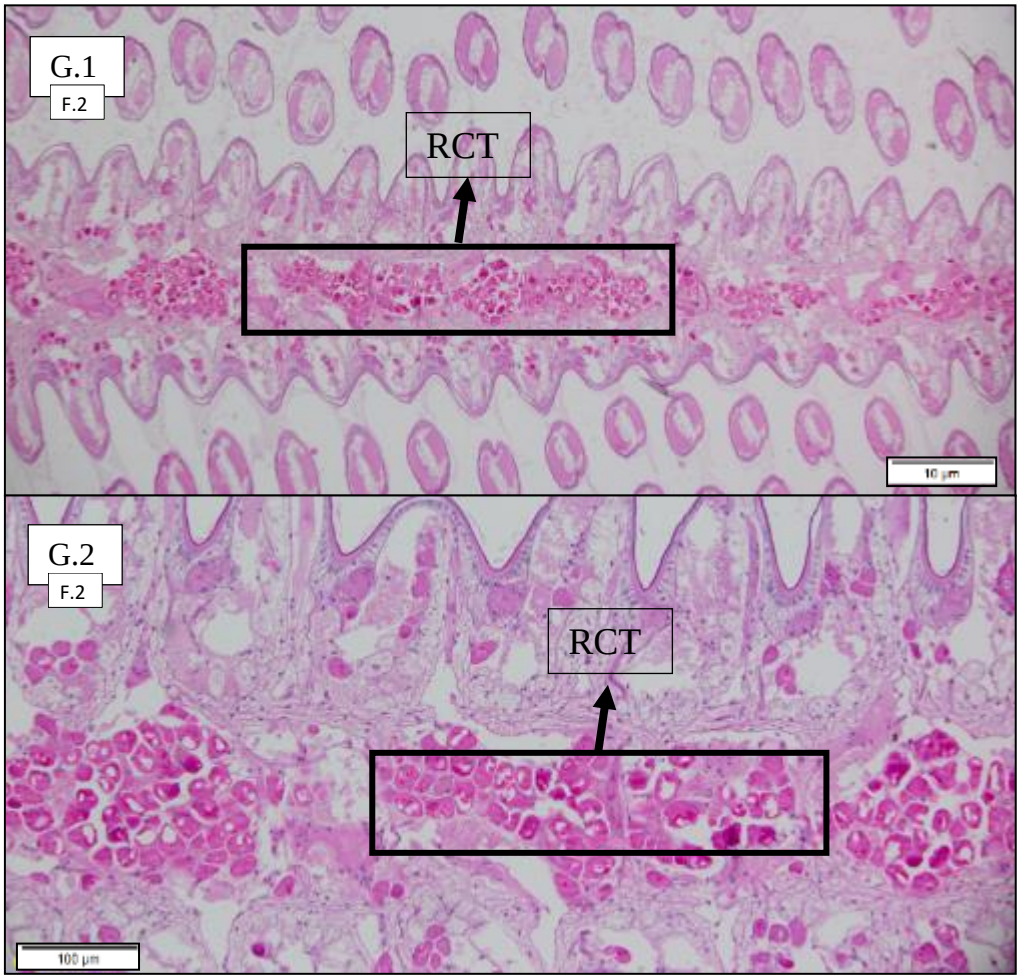


Figure 38: Ample reserve cell tissue (RCT) within the gill tissue. Note that this is a normal finding as reserve tissue is associated with crustaceans in good condition. Image G2 is a magnification of image G1. Mild vacuolisation appears as a common artefact. [RCT: reserve cell tissue]

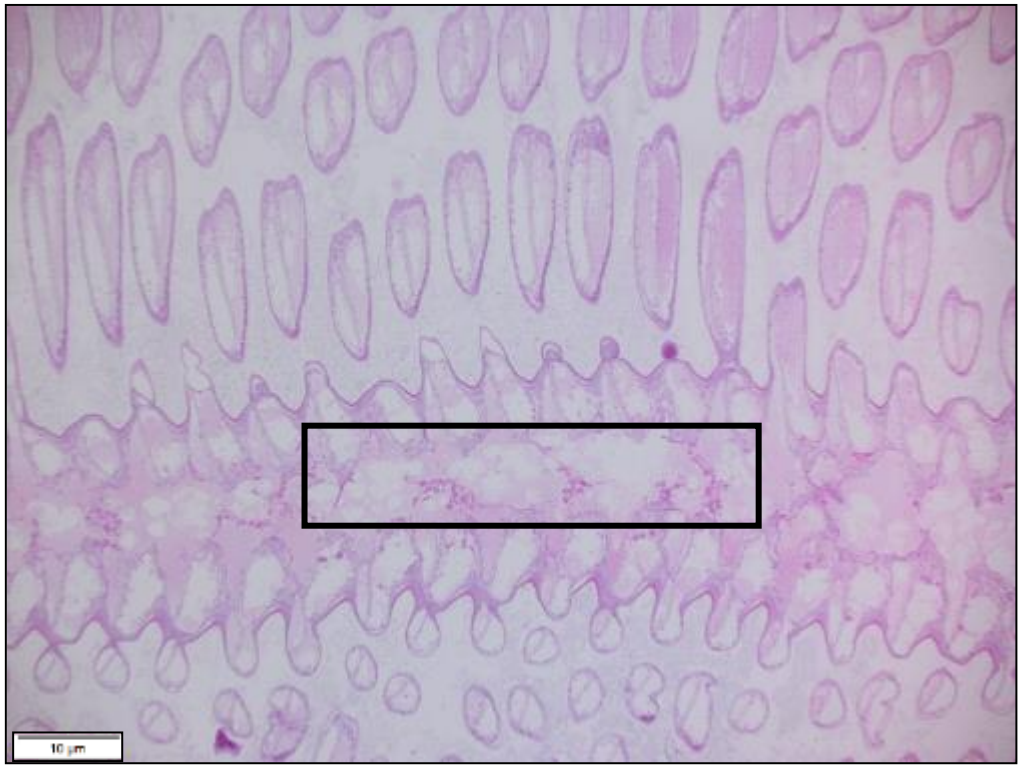


Figure 39: Gill tissue showing abnormal finding where there is a marked reduction in reserve cell tissue (RCT). The black square demarcates the location in which healthy gills should be filled with normal reserve tissue however this image shows a marked reduction and vacuolisation of cells in the area.

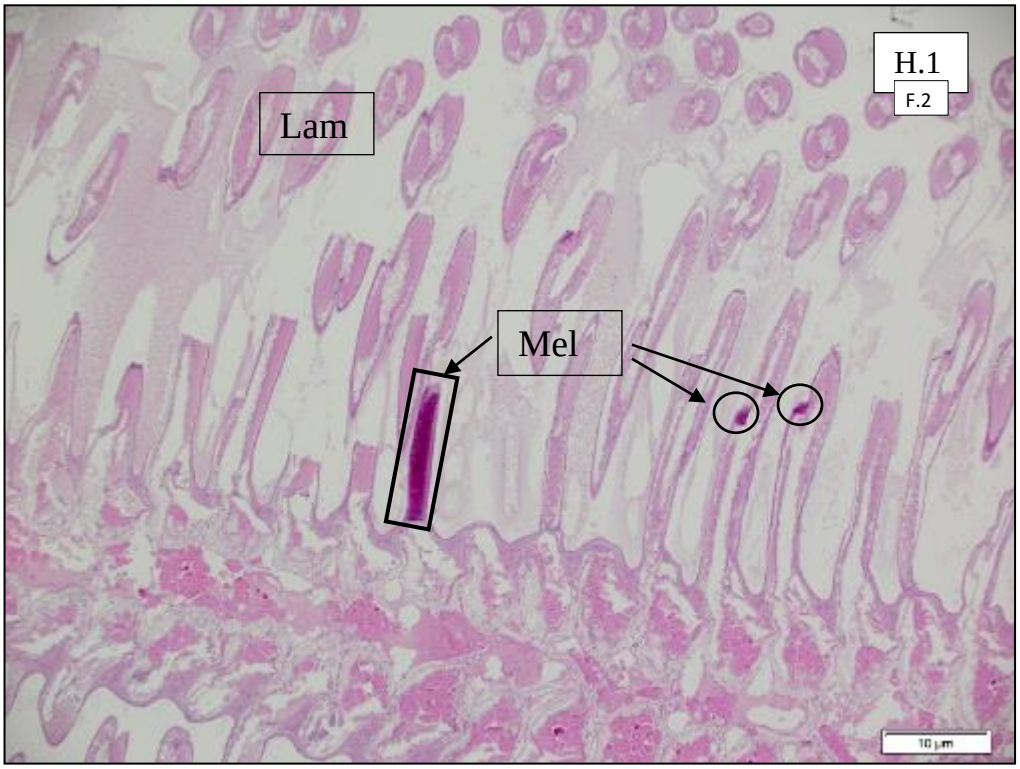


Figure 40: Cross section of gill section showing multifocal melanisation gill filaments. [Lam: lamella, Mel: melanisation]

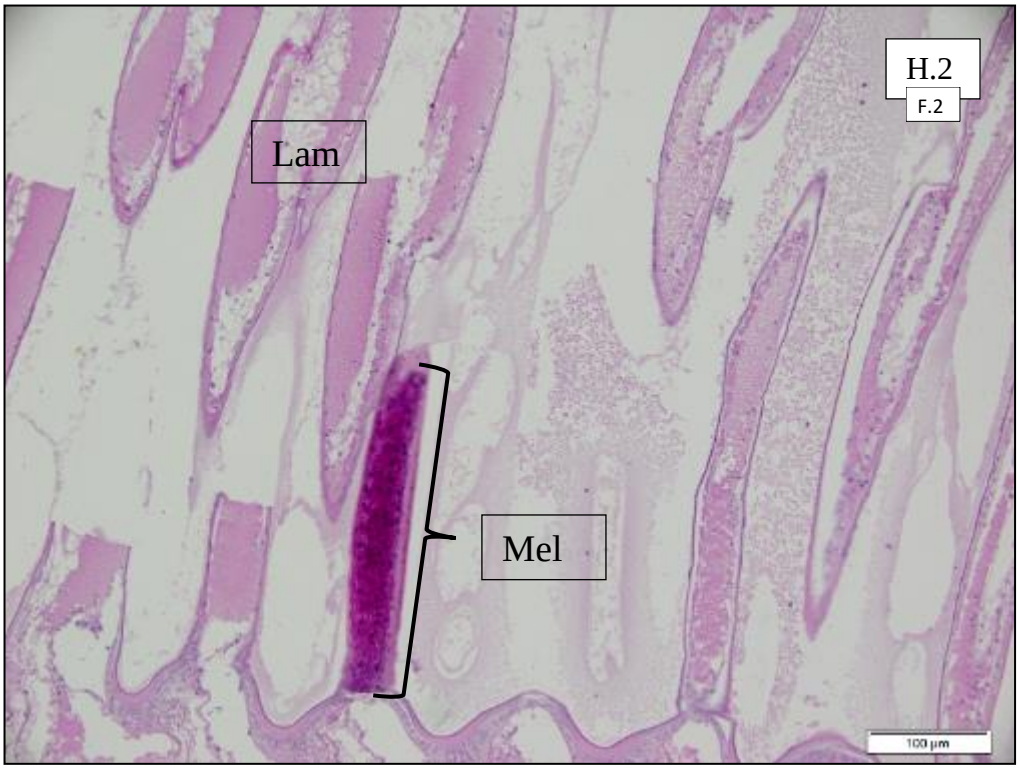


Figure 41: Magnification of image H1 section of a melanised gill filament inflammation. [Lam: lamella, Mel: melanisation]

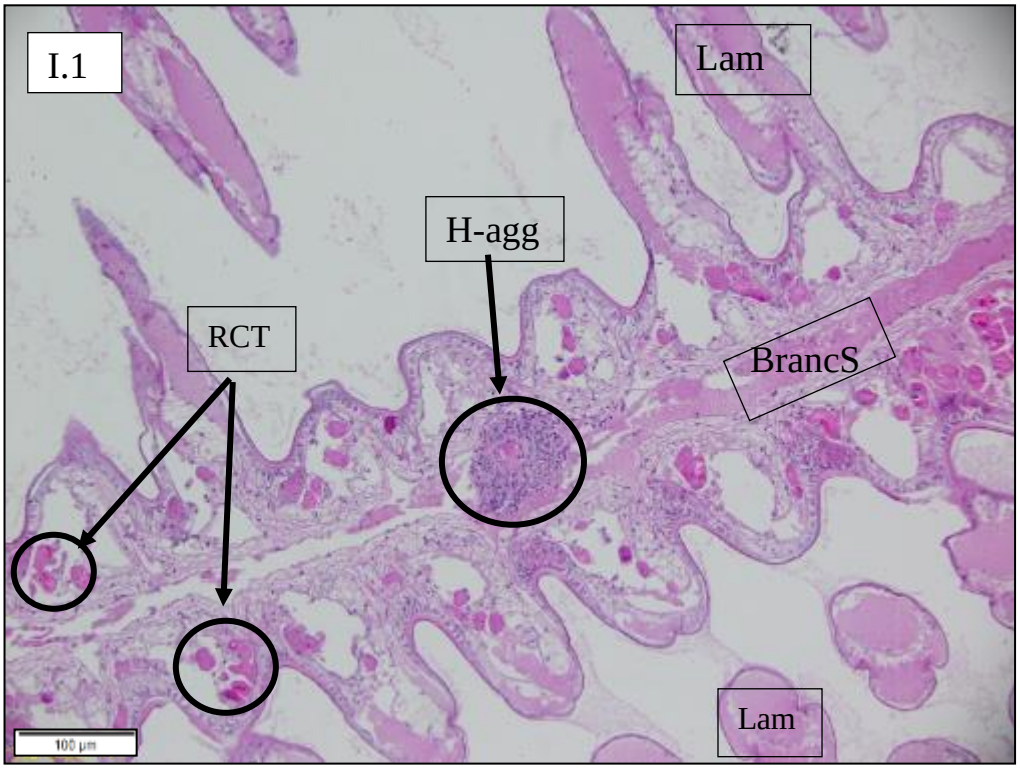


Figure 42: Cross section of gill section showing an area of reactive haemocyte aggregations within the central sinus / branchial septum. Note the depleted reserve cells presence observed. [H-agg: Haemocyte aggregations, RCT: reserve cell tissue, Lam: lamellae, BrancS: branchial sinus]

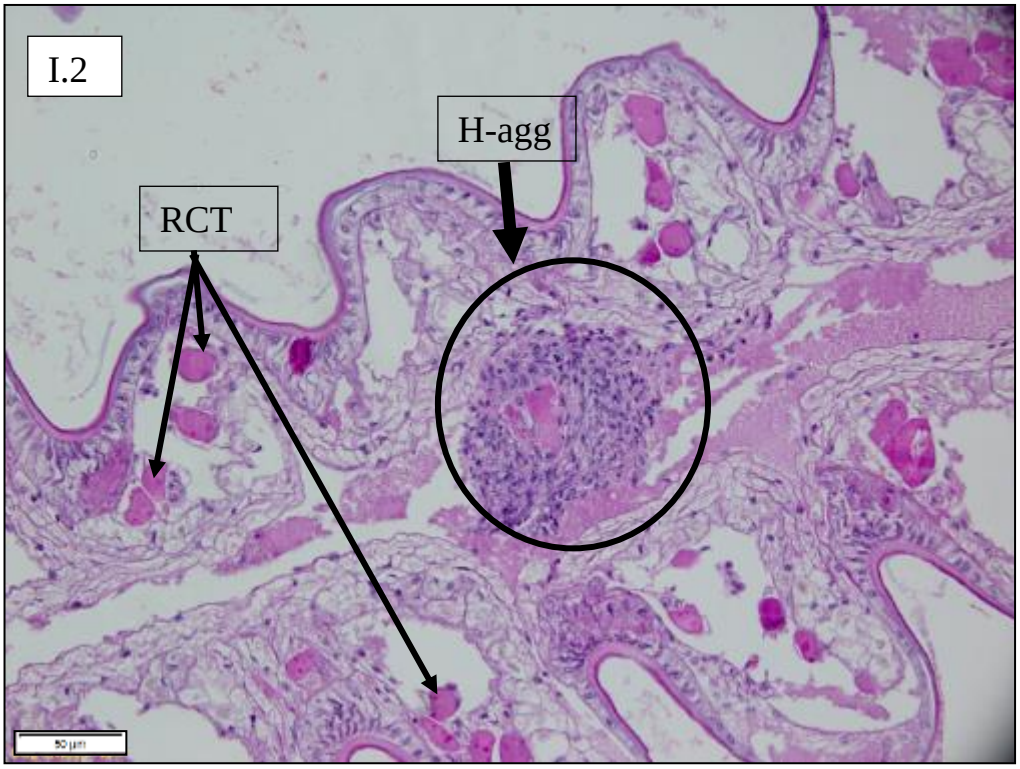


Figure 43: Magnification of image I1 focusing on the detail of the reactive haemocyte aggregation present in the central sinus/ branchial septum of the gill sample. [H-agg: haemocyte aggregation, RCT: reserve cell tissue]

ANTENNAL GLAND NORMAL HISTOLOGY

The adult SRL has a pair of antennal glands located in the anterior cephalothorax near the base of the antennae. There are three main parts for this gland. The first is a blindly ending coelomosac (end sac) which is well perfused with haemolyph as ultrafiltration occurs across this tissue wall. This then opens to the second main section of the gland called the labyrinth (grandular plexus). The labyrinth is made up of a network of tubules leading to the third section the bladder where urine is held before disposal through an opening termed the excretory pore also located at the base of the antennae.

In this atlas the histological focus is placed on the coelomosac and the labyrinth.

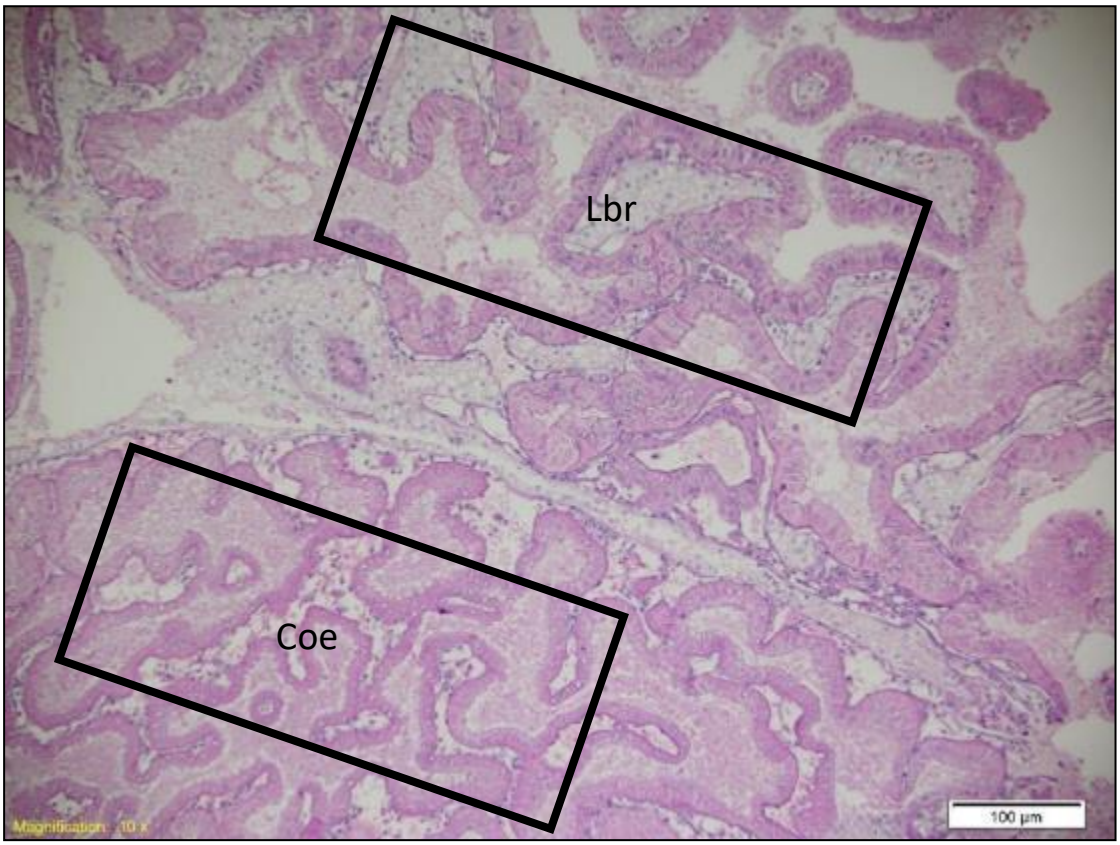


Figure 44: Tissue structure of coelomosac and labyrinth observed in a normal antennal gland. Note the coelomosac tissue is arranged in a more compact manner compared to the labyrinth tissue arrangement that appears more open [Coe: coelomosac, Lbr: Labyrinth]

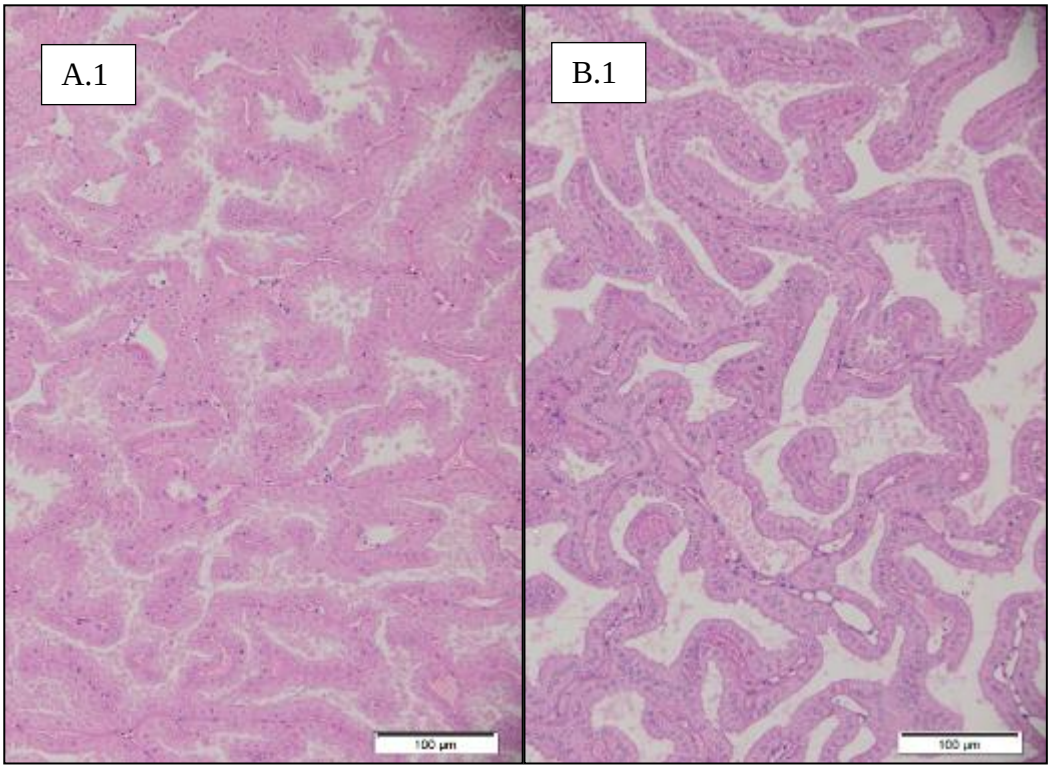


Figure 45: Image A1 is of the coelomosac zone while image B1 represents the labyrinth zone of the antennal gland. Note the compact nature of the coelomosac compared to the more open tissue arrangement of the labyrinth.

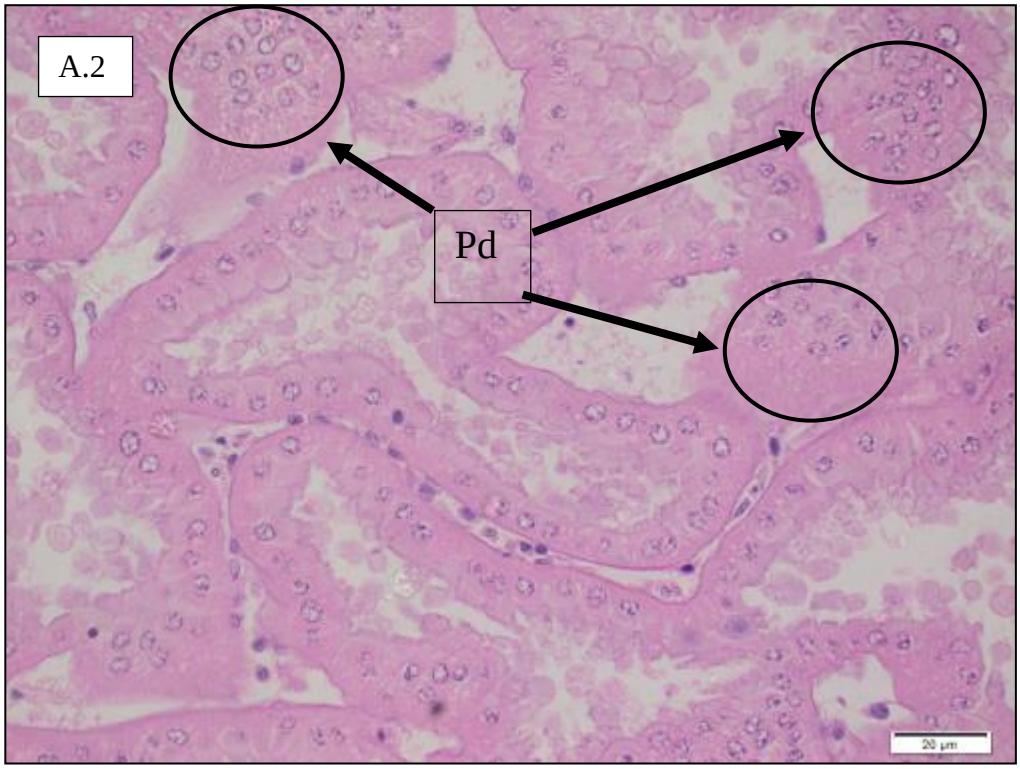


Figure 46: Image A2 is a magnification of image A1 of the coelomosac. This zone of the antennal gland is composed of podocytes and functions similar to that of the vertebrate Bowmans capsule. [Pd: podocytes]

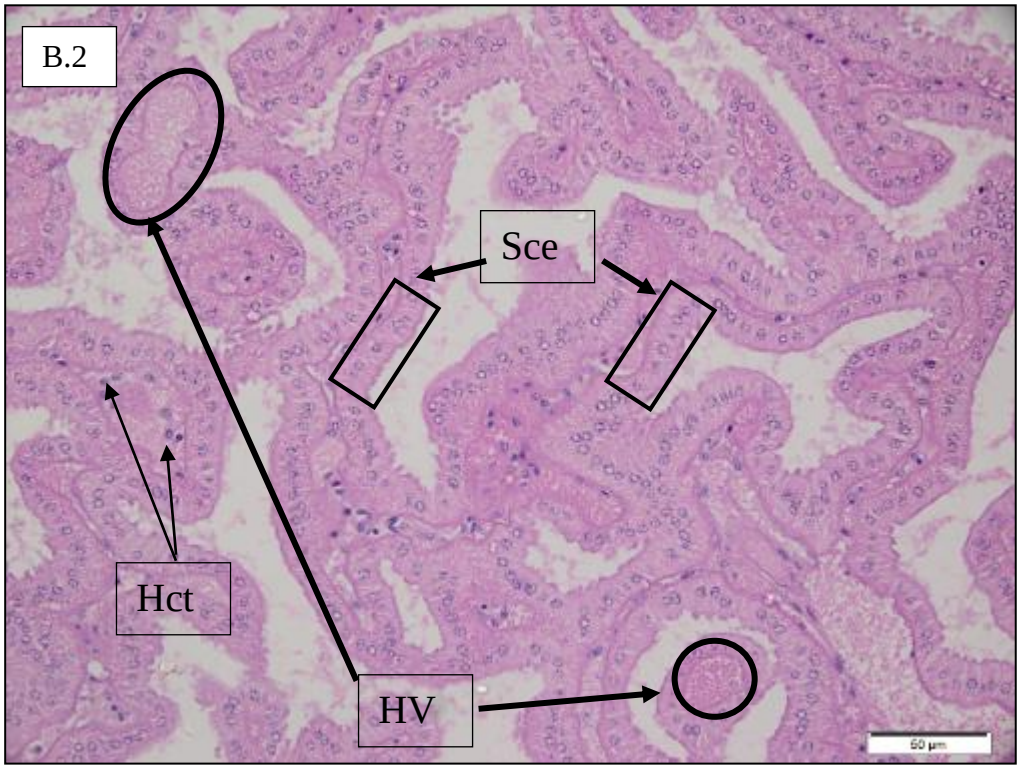


Figure 47: Image B2 is a magnification of image B1 of the labyrinth. The labyrinth is composed of a network of epithelial cells that can appear cuboidal when inactive and columnar in appearance when active. Both cell types typically have a centrally located nucleus. [SCe: simple columnar epithelium, HV: haemal vasculature, Hct: haemocytes]

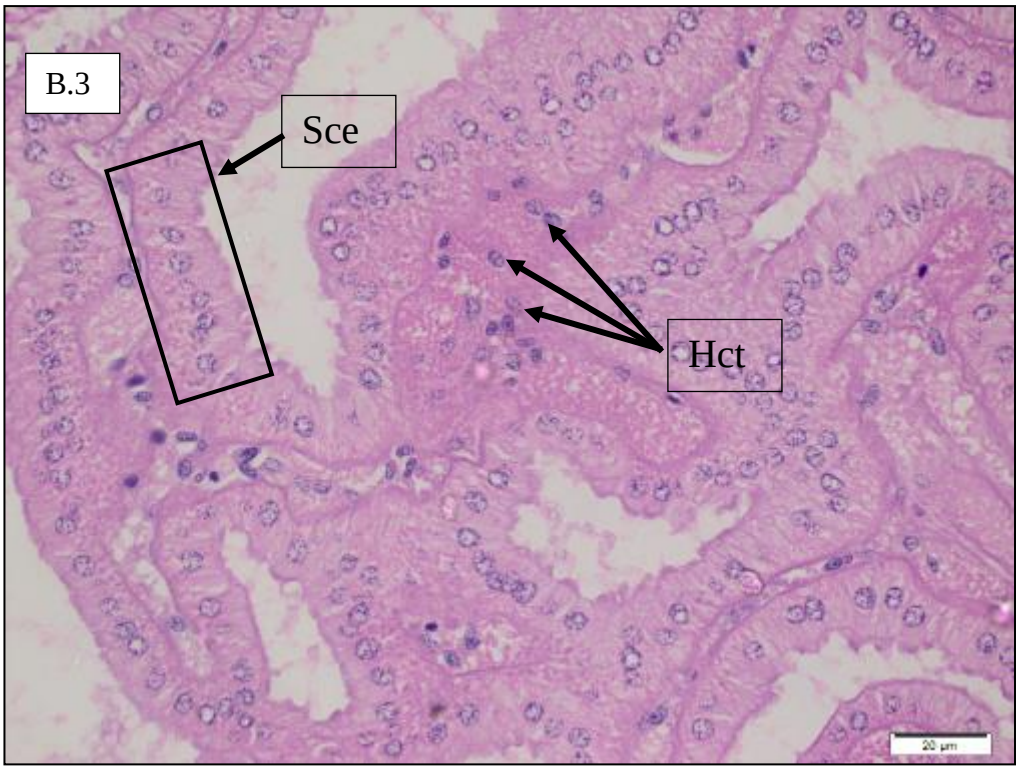


Figure 48: Image B3 is a further magnification of image B2 providing further detail to the structural alignment of image the labyrinth [SCe: simple columnar epithelium, Hct: haemocytes]

ANTENNAL GLAND HISTOPATHOLOGY

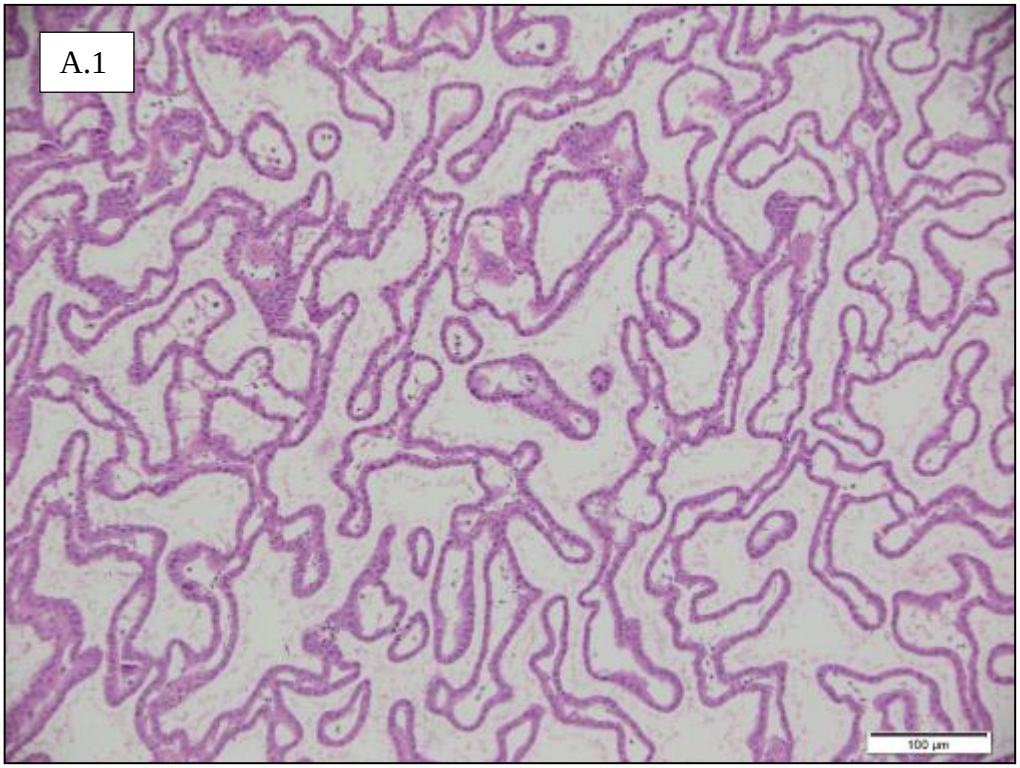


Figure 49: The figure above is of an atrophic antennal gland. The tissue may be reactive and has an open appearance which can be mistaken for oedema.

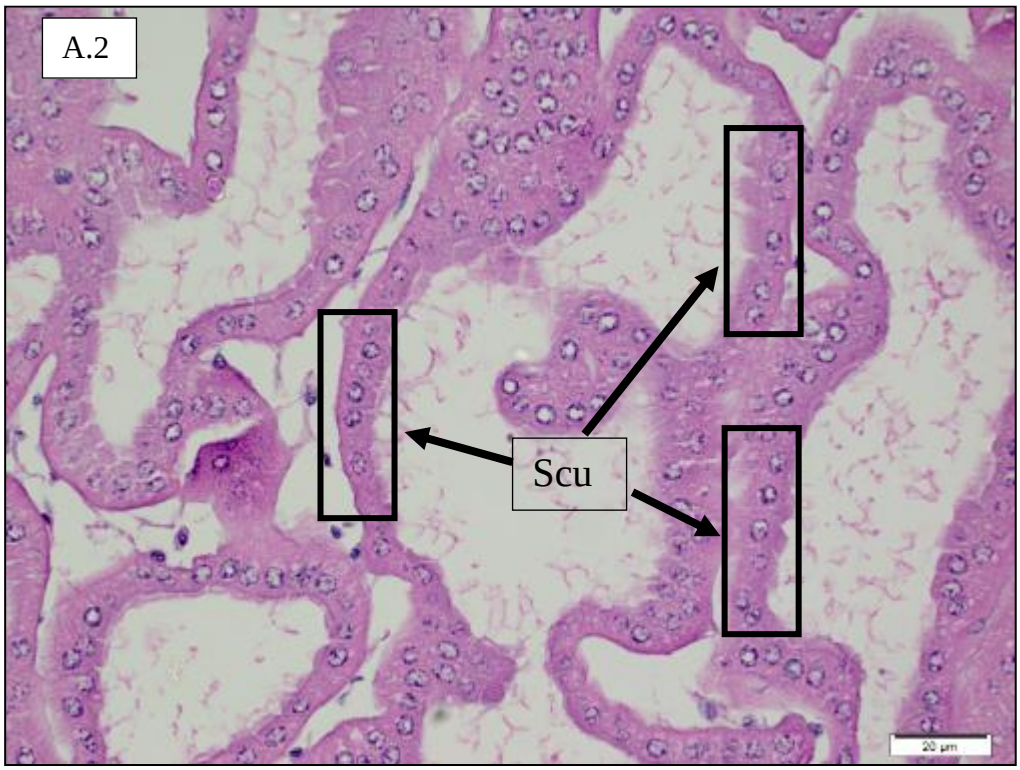


Figure 50: Magnification of image A1 demonstrating the structural difference in an inactive antennal gland. Note the epithelial cells are cuboidal in nature compared to columnar epithelial that is observed in active glands. [Scu: simple cuboidal epithelium]

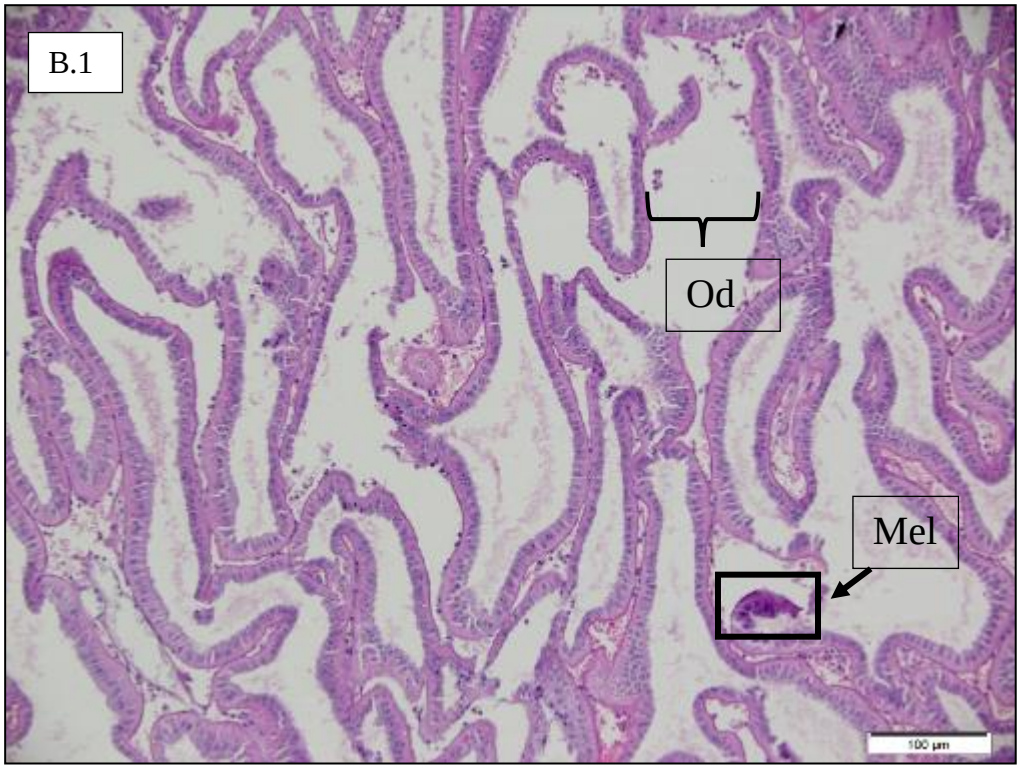


Figure 51: True oedema in the antennal gland with generalised eosinophilic inclusions present. The whole gland appears washed out and degenerative with focal areas of melanisation present. Note the tissue is not well organised with large vacuoles surrounding the eosinophilic inclusions as well as oedema within the epithelia of the gland. [Mel: melanisation, Od: oedema]

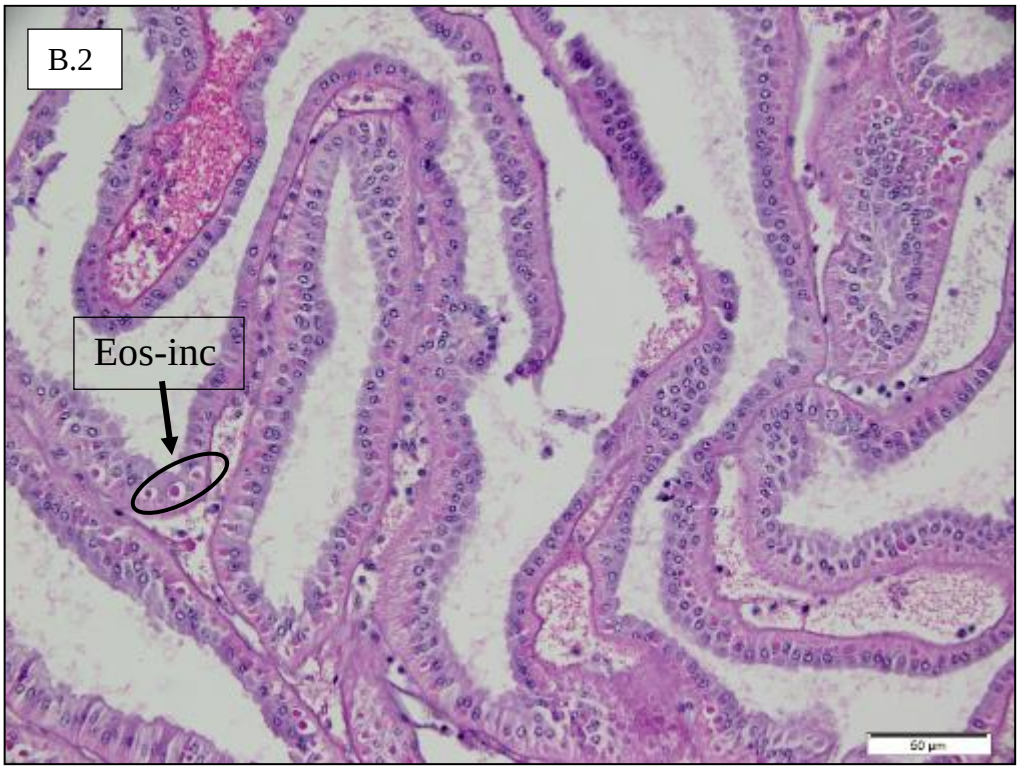


Figure 52: Magnification of image B1 showing degeneration of antennal gland tissue with loss of structure to tissue arrangement. Note the generalised eosinophilic inclusions that are surrounded by large vacuoles. [Eos-inc: eosinophilic inclusion bodies,]

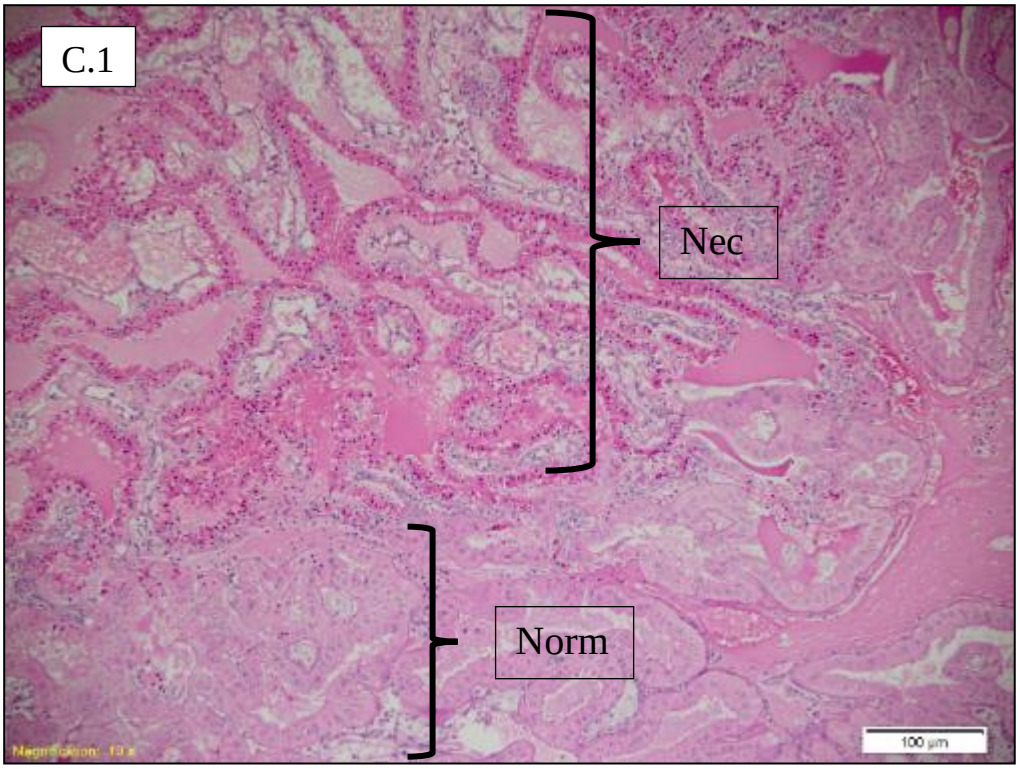


Figure 53: Focally extensive necrosis of antennal gland epithelium tissue in the coelosomac region. [Nec: necrosis, Norm: normal tissue]

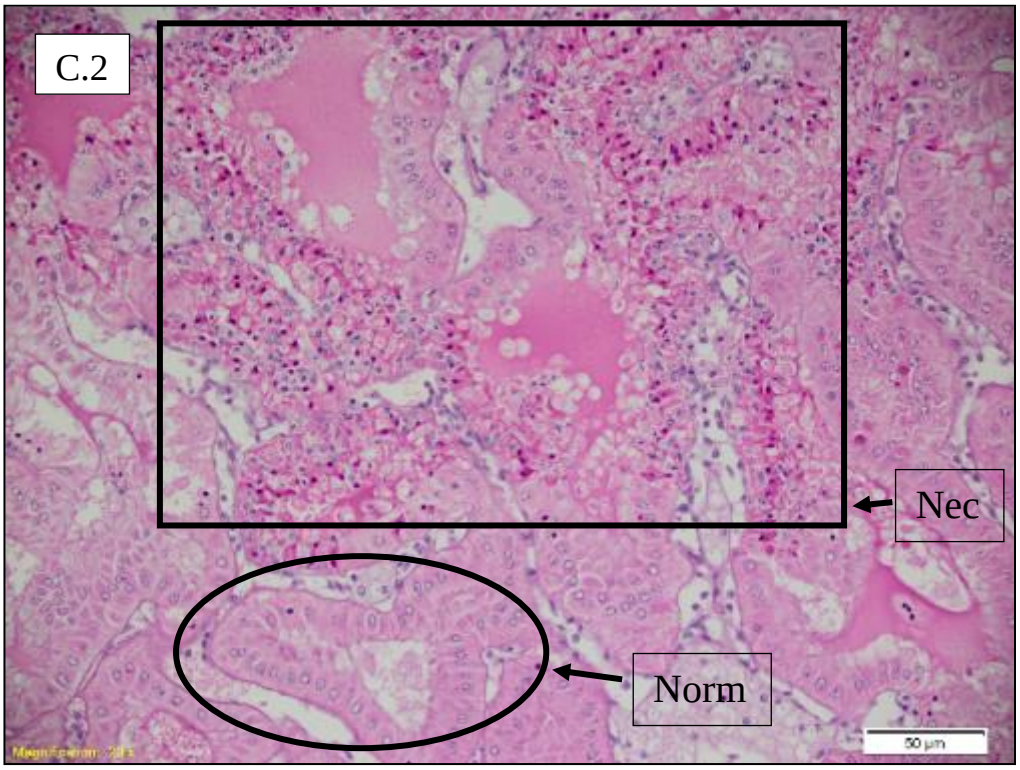


Figure 54: Magnification of image C1 demonstrating focally extensive necrosis of antennal gland epithelium tissue in the coelosomac region. Characterised by nuclear pyknosi and karyhexis [Nec: necrosis, Norm: normal tissue].

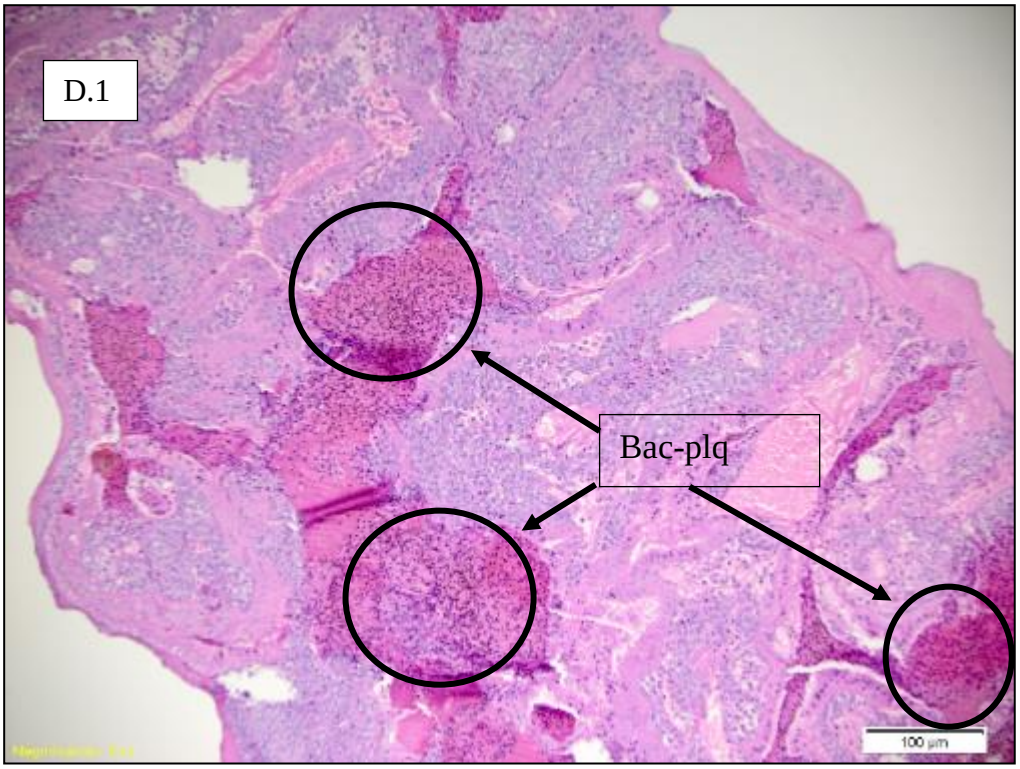


Figure 55: Overview showing focally extensive areas of antennal gland necrosis with bacterial plaque aggregations within the interstitium and melanisation [bac-plq: bacterial plaques].

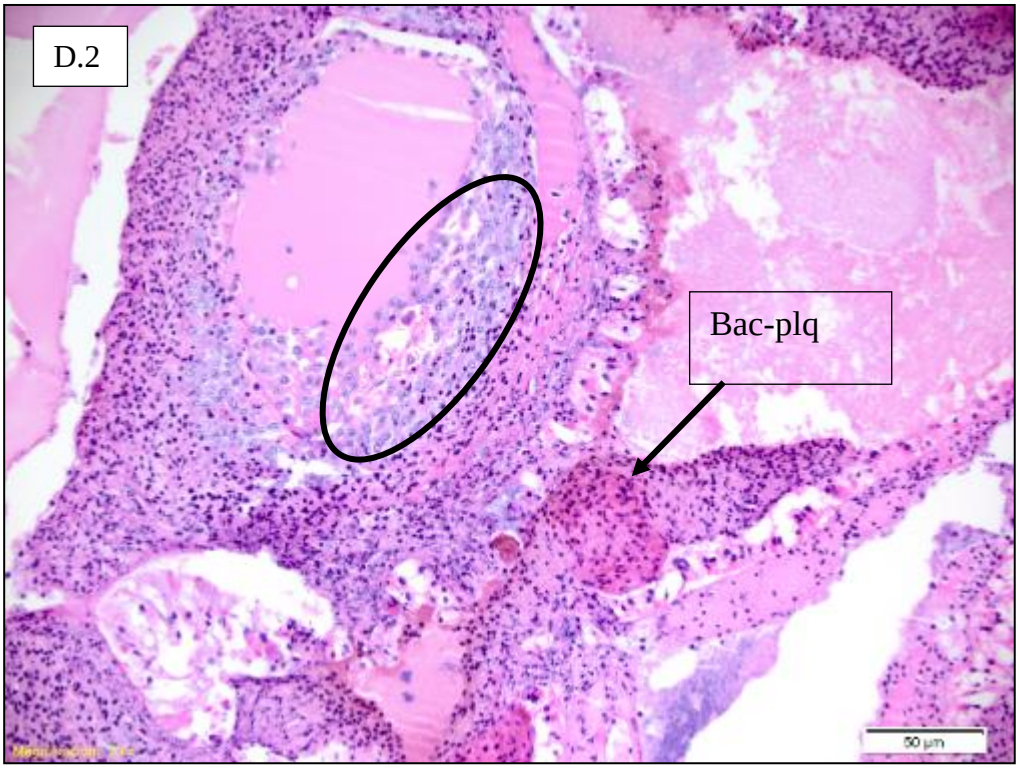


Figure 56: Magnification of image D1 showing a focally extensive area of antennal gland necrosis with bacterial plaque aggregations within the interstitium and melanisation. Note the loss of epithelial architecture (black circle) [bac-plq: bacterial plaques]

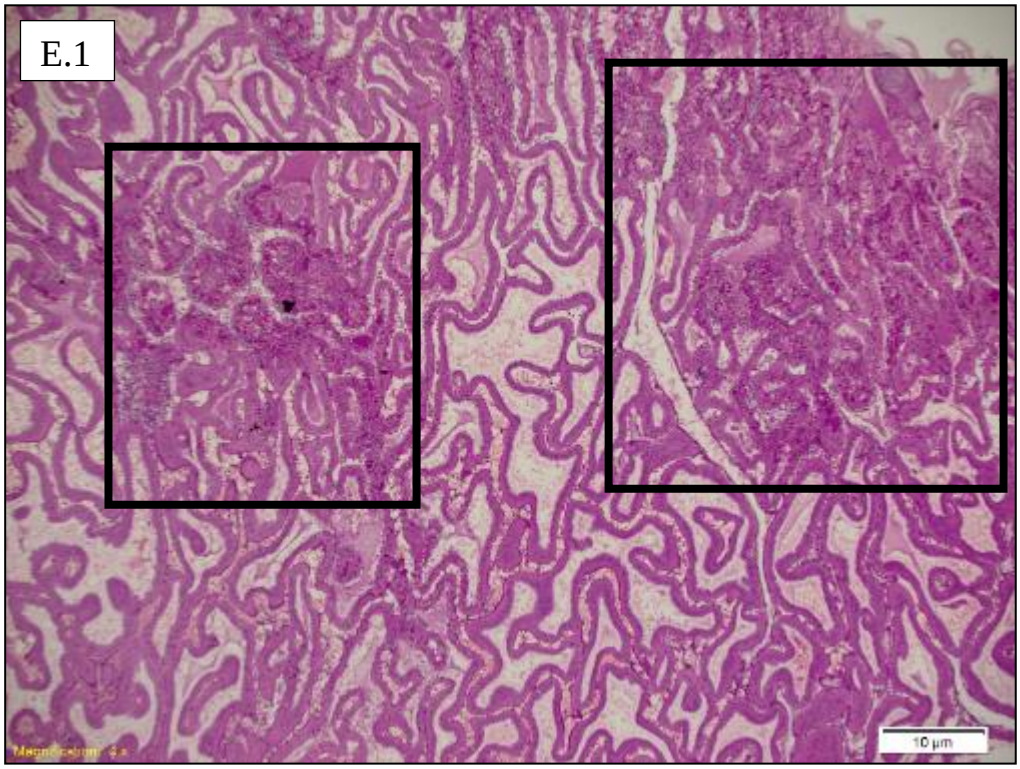


Figure 57: Overview of abnormal antennal gland tissue where areas of coagulative necrosis is observed (black squares) with haemocyte aggregations and melanisation present.

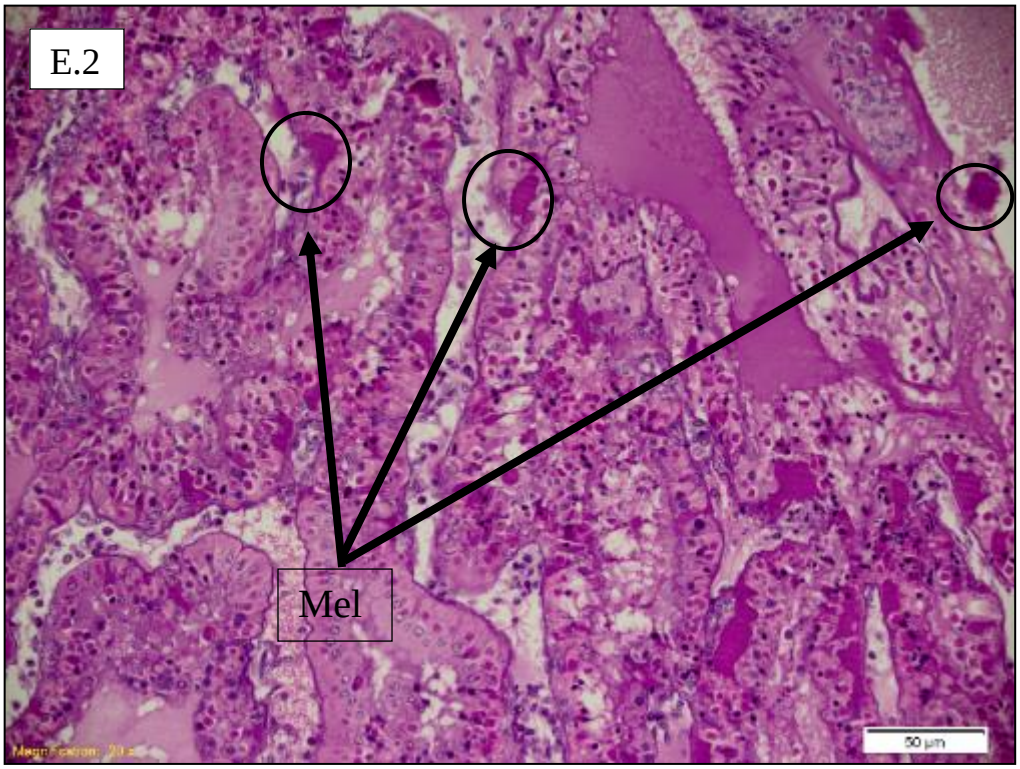


Figure 58: Magnification of area with coagulative necrosis as shown in image E1. Note the loss of tissue architecture and multiple zones of melanised tissue appearance. [Mel: melanisation]

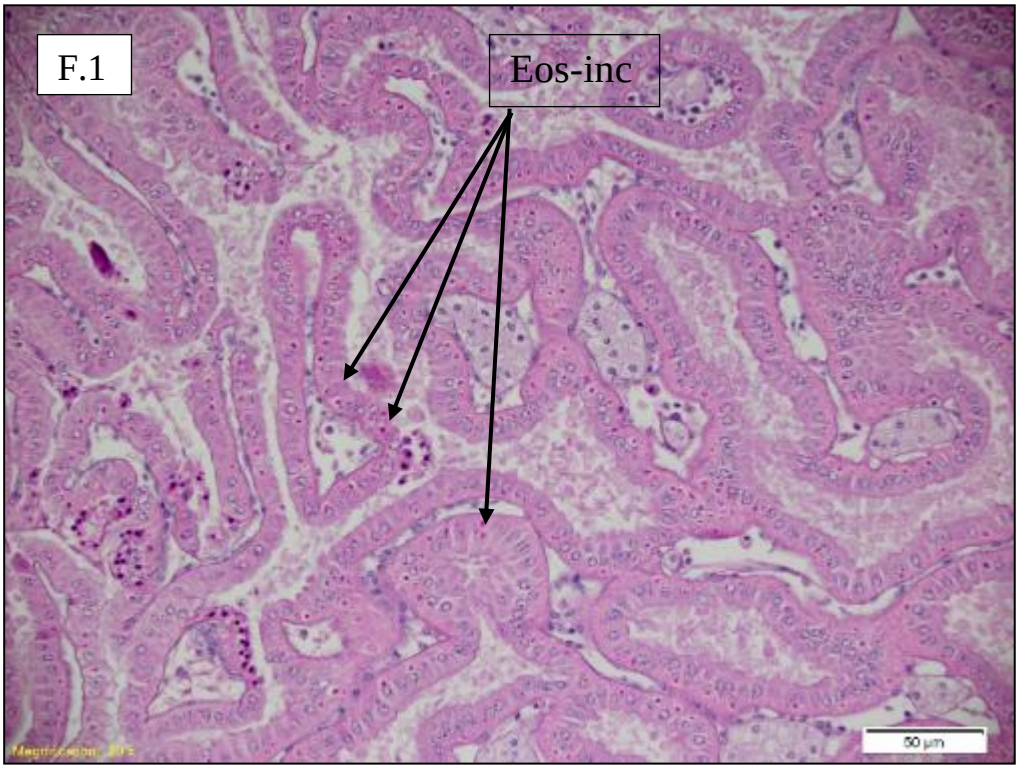


Figure 59: Section of antennal gland showing generalised cytoplasmic eosinophilic inclusion bodies that are surrounded by vacuoles. Note the tissue architecture is undisturbed. Aetiology for these inclusion bodies remain uncertain. [Eos-inc: eosinophilic inclusion bodies]

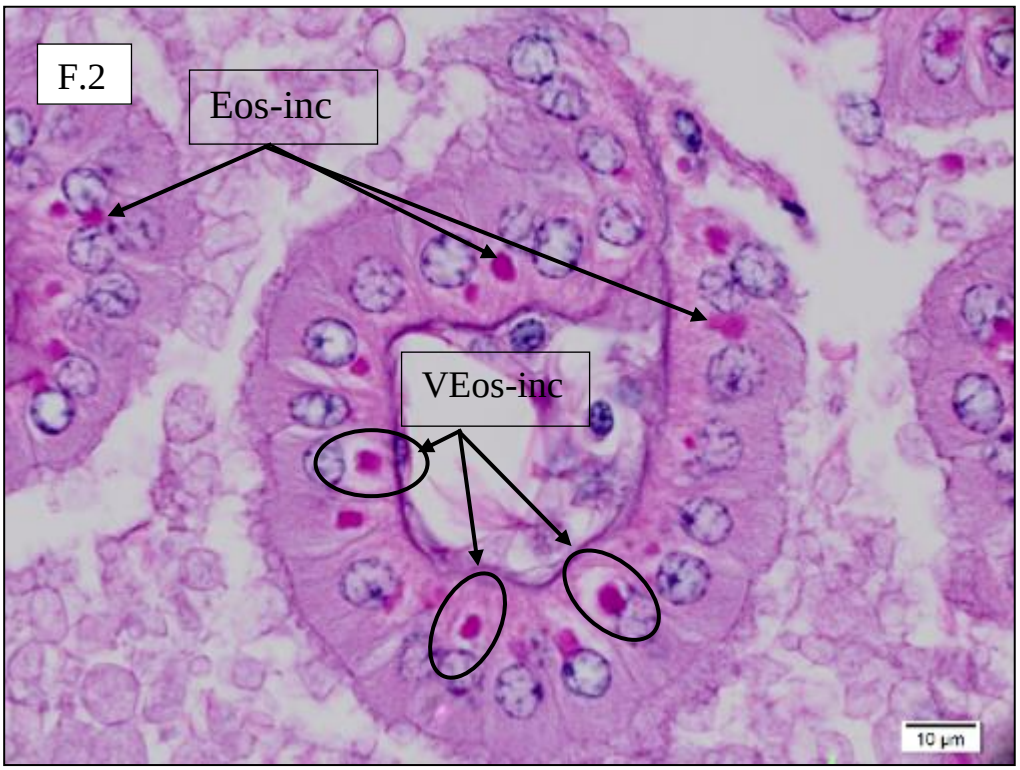


Figure 60: Magnification of eosinophilic inclusion bodies observed in image F1. Note some inclusions are surrounded by vacuoles whilst some are not. [VEos-inc: vacuolated eosinophilic inclusion bodies, Eos-inc: eosinophilic inclusion bodies]

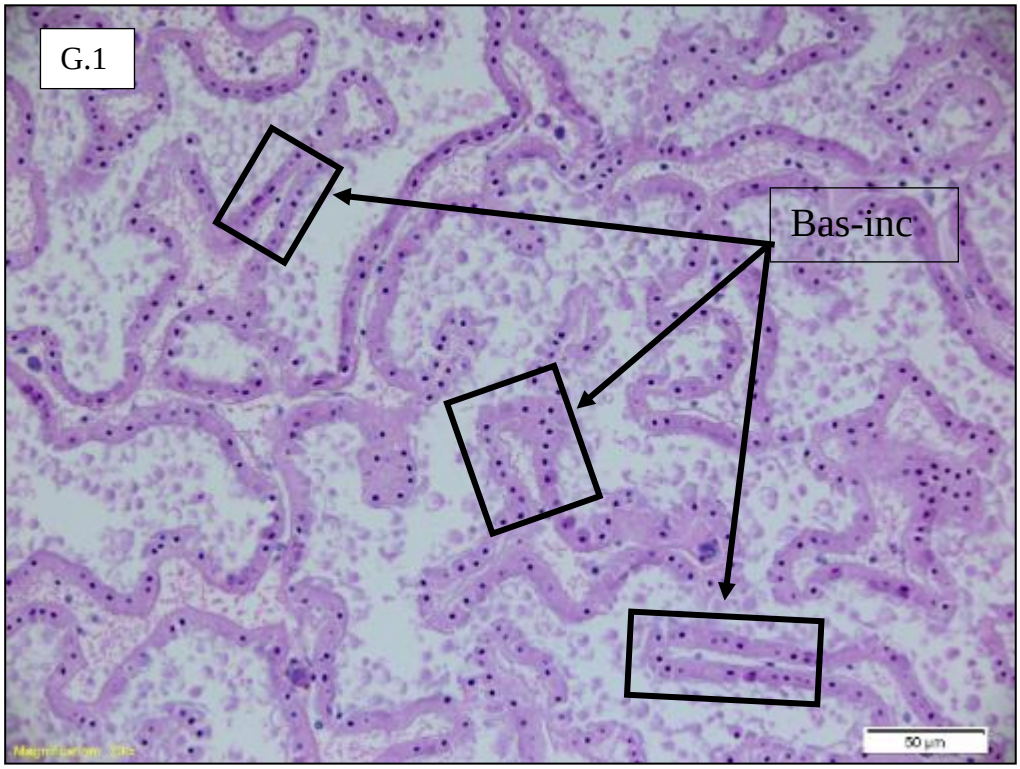


Figure 61: Cross section of an area showing multifocal intra-lesional bacterial rods as well as generalised intra-nuclear basophilic inclusion bodies. Note there is chromatin marginalisation in areas with normal tissue. [Bas-inc: basophilic inclusion bodies]

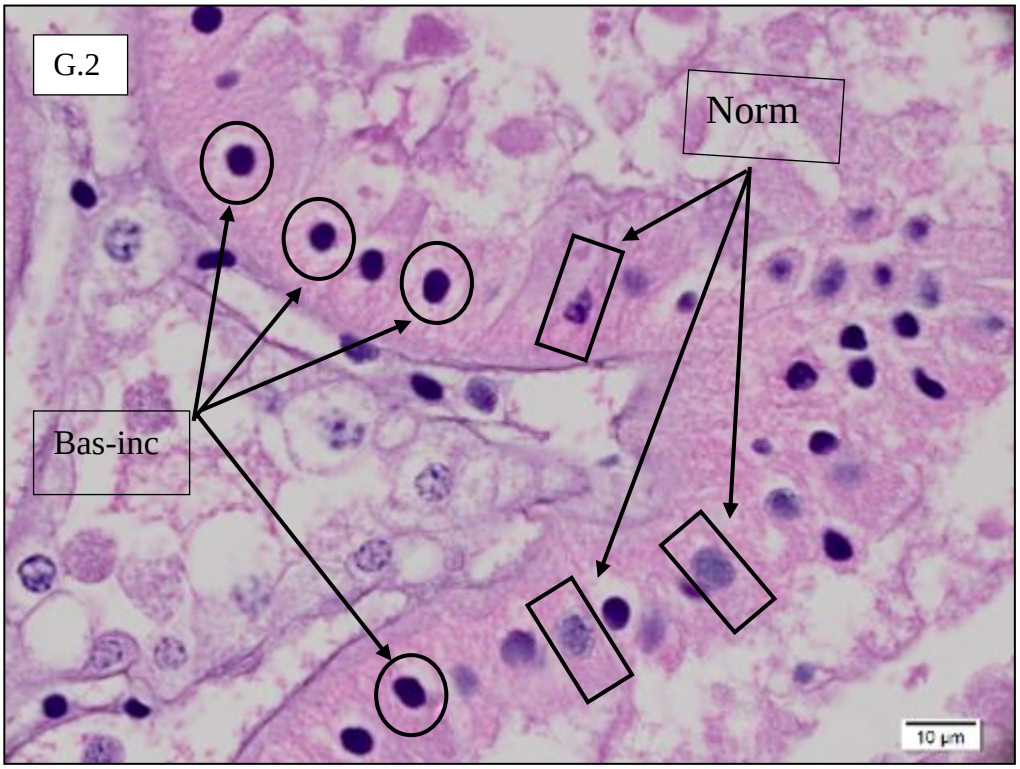


Figure 62: Magnification providing detail of the basophilic intranuclear inclusion bodies. Note the condensed chromatin and shrinkage of the basophilic cell compared to the normal cell. These inclusions have been attributed to apoptosis. [Bas-inc: basophilic inclusion bodies, Norm: normal tissue]

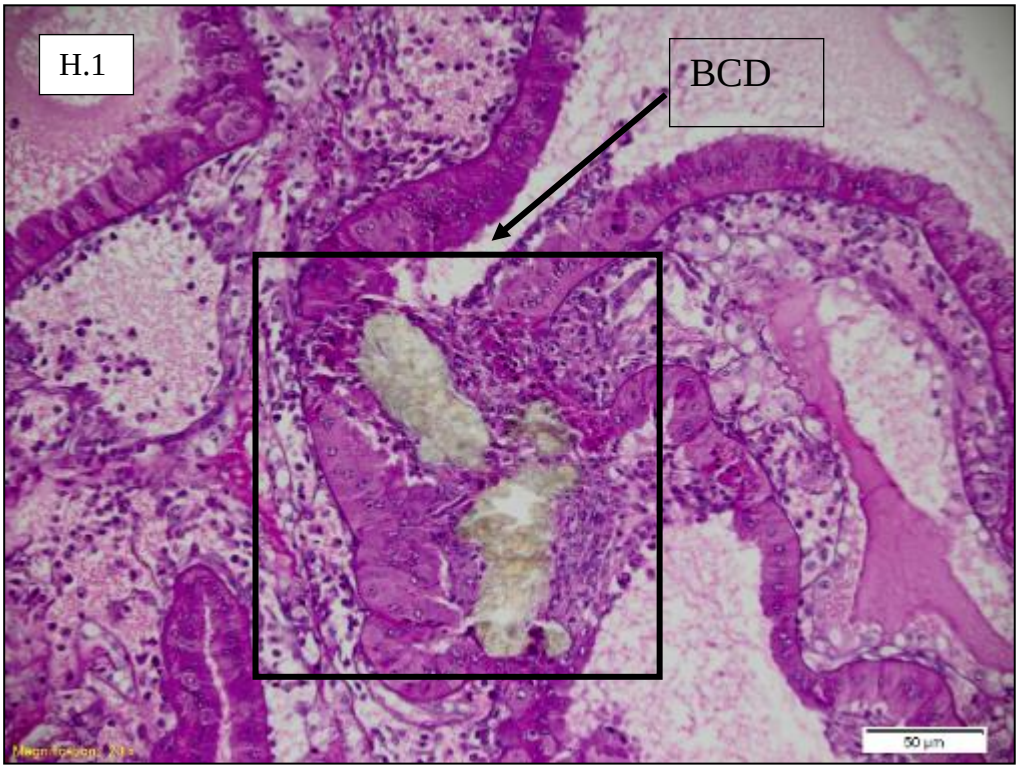


Figure 63: Focal area of birefringent crystalline deposits in the lumen of antennal gland tubules associated with erosion and ulceration with occasional metaplasia of the lining cells. The deposits are composed of pigmented granulated aggregates that are not birefringent and are also encapsulated. [BCD: birefringent crystalline deposit]

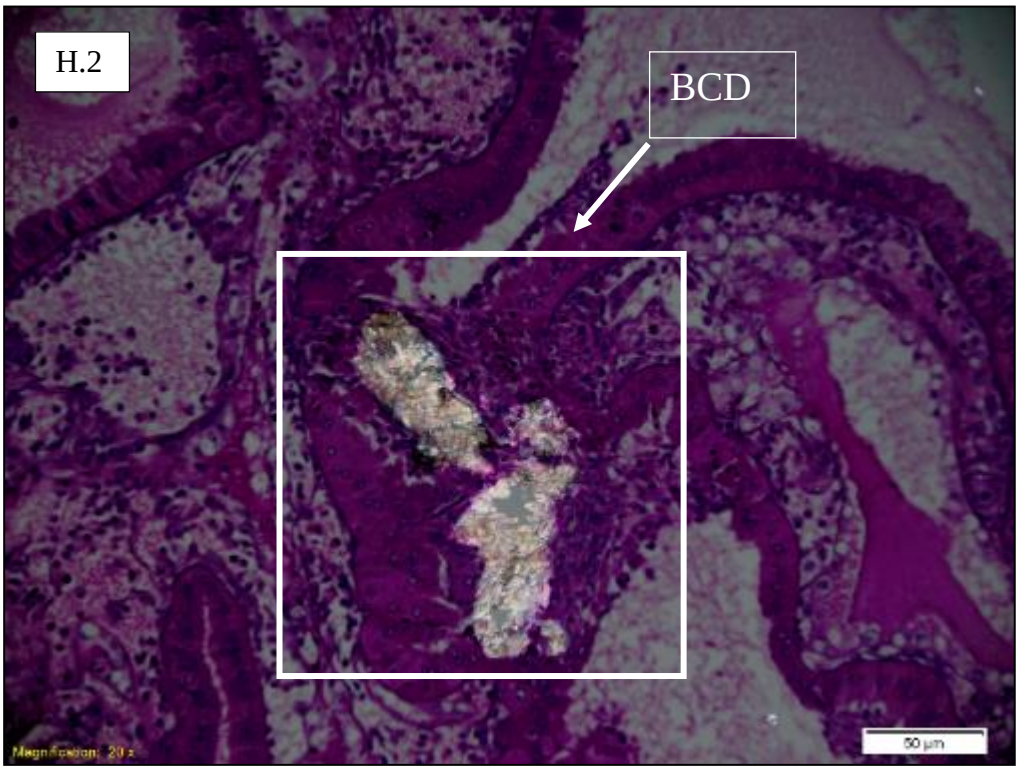


Figure 64: This image represents image H1 under polarised lighting showing the positive birefringence of the crystalline deposit. [BCD: birefringent crystalline deposit]

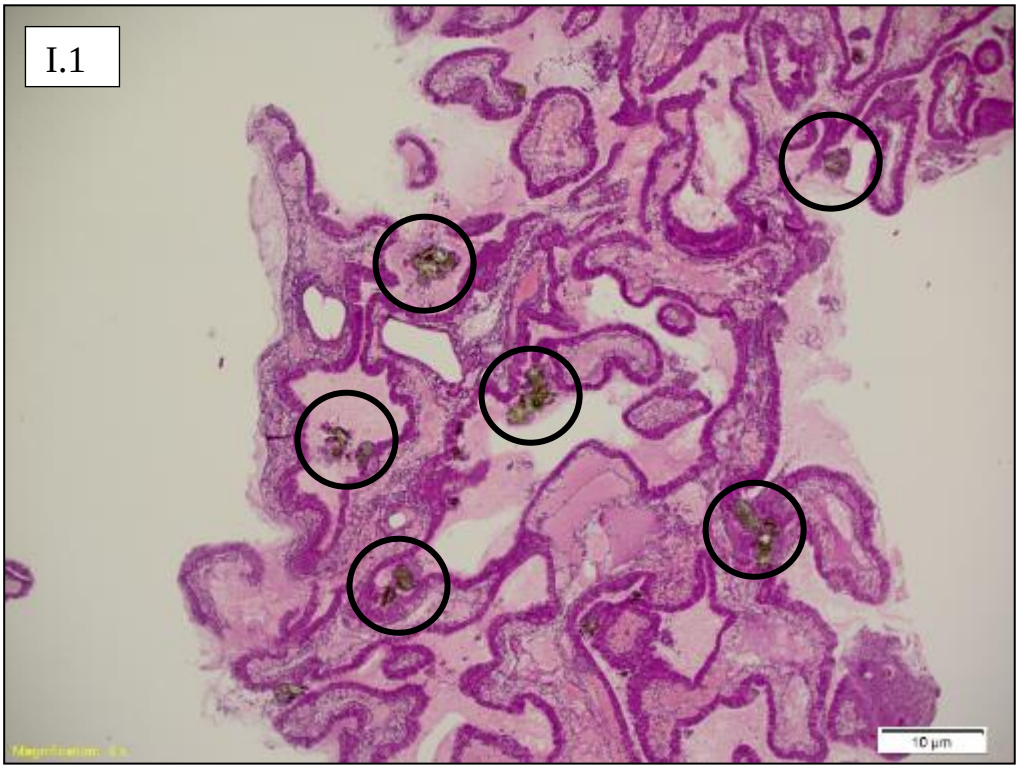


Figure 65: Multifocal areas of birefringent crystalline deposits (black circles) in the lumen of antennal gland tubules. The deposits are brown to yellow in colouring and are composed of pigmented granulated aggregates that when not exposed to polarised light and are also encapsulated by inflammatory tissue

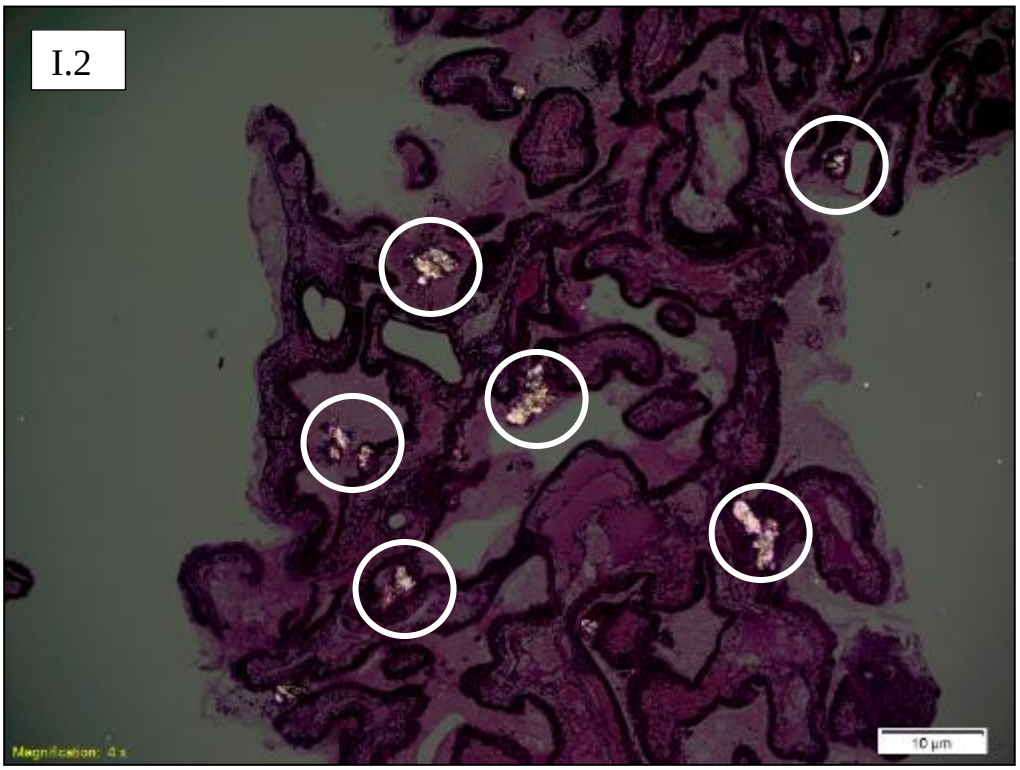


Figure 66: This image represents image I1 under polarised lighting showing the positive birefringence of the crystalline deposits (white circles).

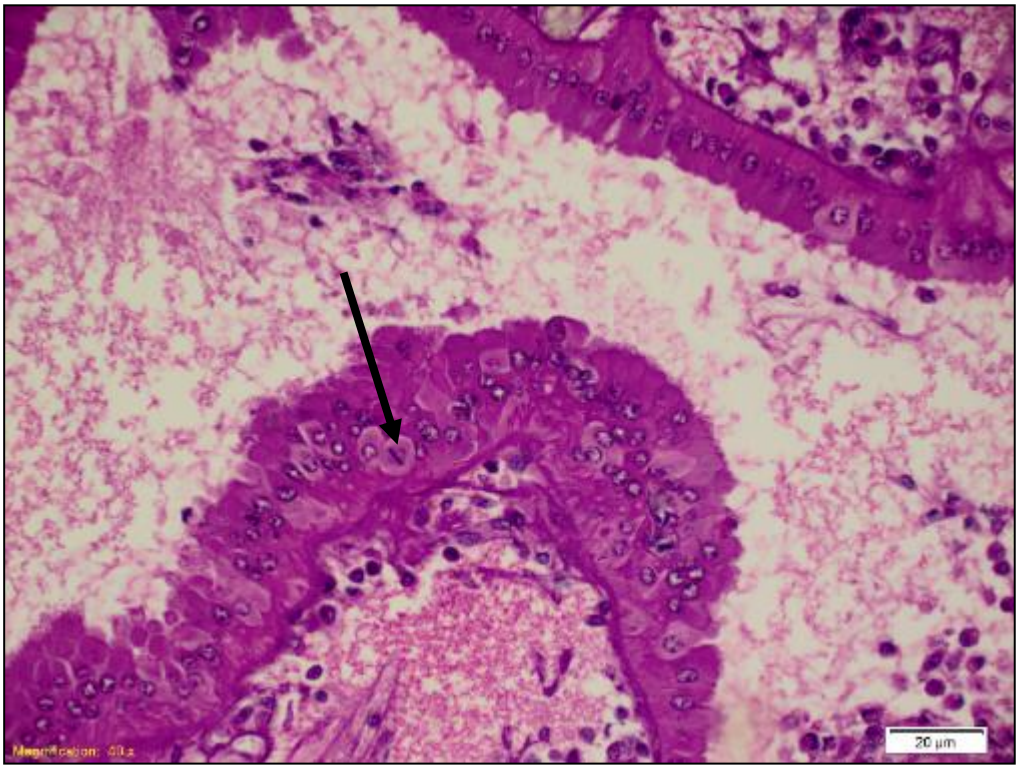


Figure 67: Section of antennal gland tissue showing melanised blebbing of the epithelium as well as refractory inclusion bodies. Note the presence of a mitotic cell where the chromosal alignment can be visualised in the middle of the cell (black arrow)

CARDIAC NORMAL HISTOLOGY

The heart is located dorsally in southern rock lobster (SRL) in the posterior region of the cephalothorax. It is surrounded by a pericardial sac and is similar in cellular composition to a vertebrate heart.

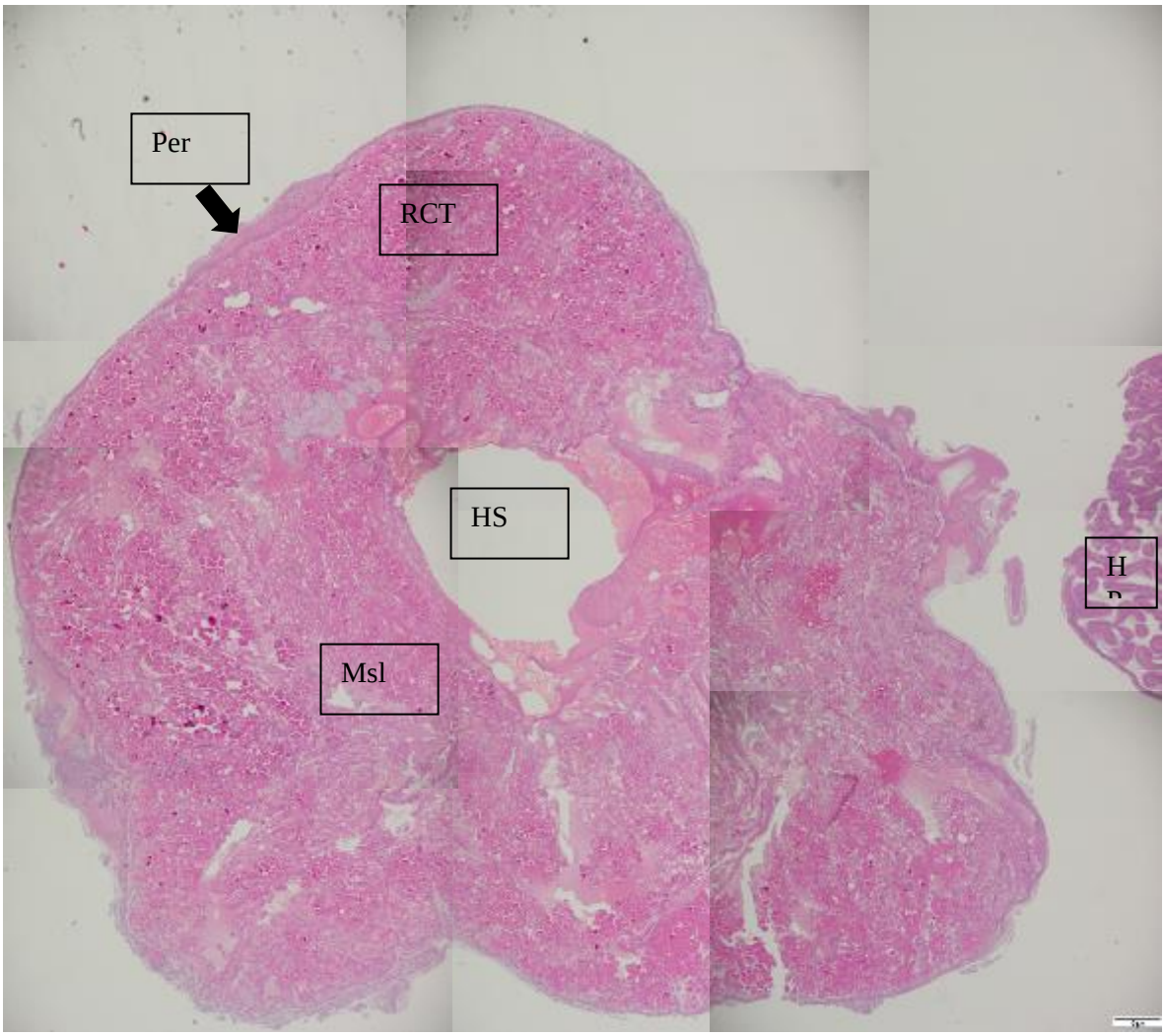


Figure 68: Cross sectional overview of the SRL myocardium. [Msl: muscle, HS: haemal sinus, Per: pericardium, RCT: reserve cell tissue, HP: hepatopancreas]

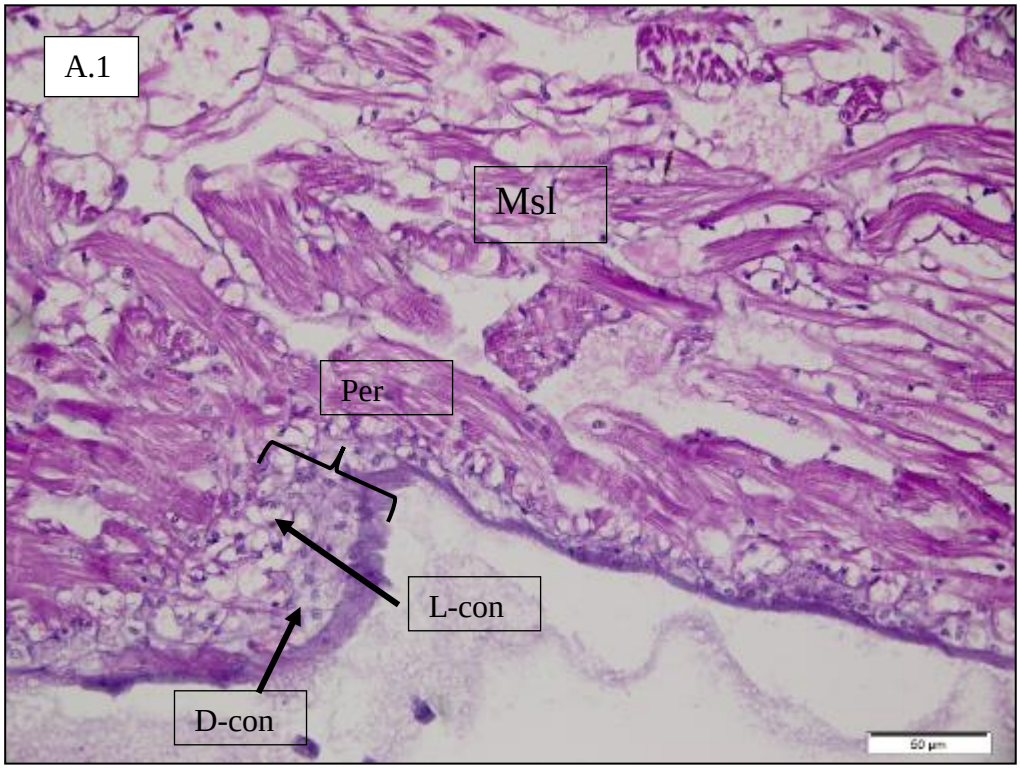


Figure 69: Image showing the pericardial layer surrounding the heart as well as the transition between the pericardium and heart muscle tissue. The outer layer of dense connective tissue serves to support the loose tissues of the pericardium. [Per: pericardium, D-con: fibrous connective tissue, L-con: loose connective tissue, Msl: muscle]

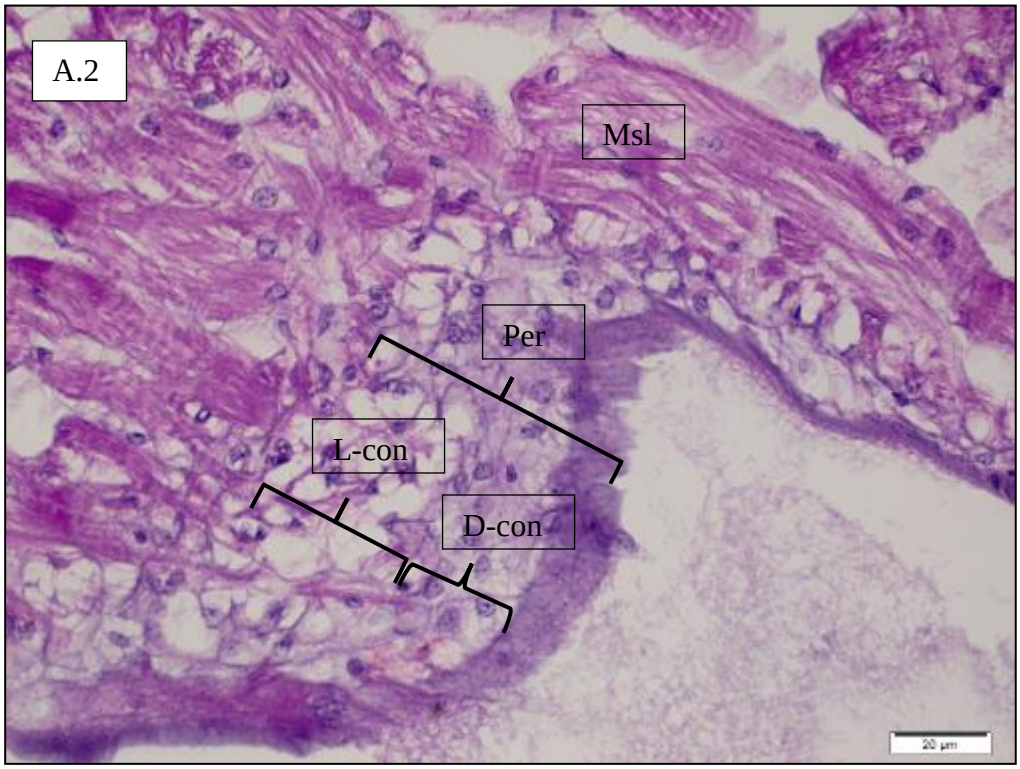


Figure 70: Magnification of image A1 providing detail to the pericardial layer. Note the muscle attachments along the pericardium at the transitional point of the tissues. [D-con: dense connective tissue, L-con: loose connective tissue, Per: pericardium, Msl: muscle]

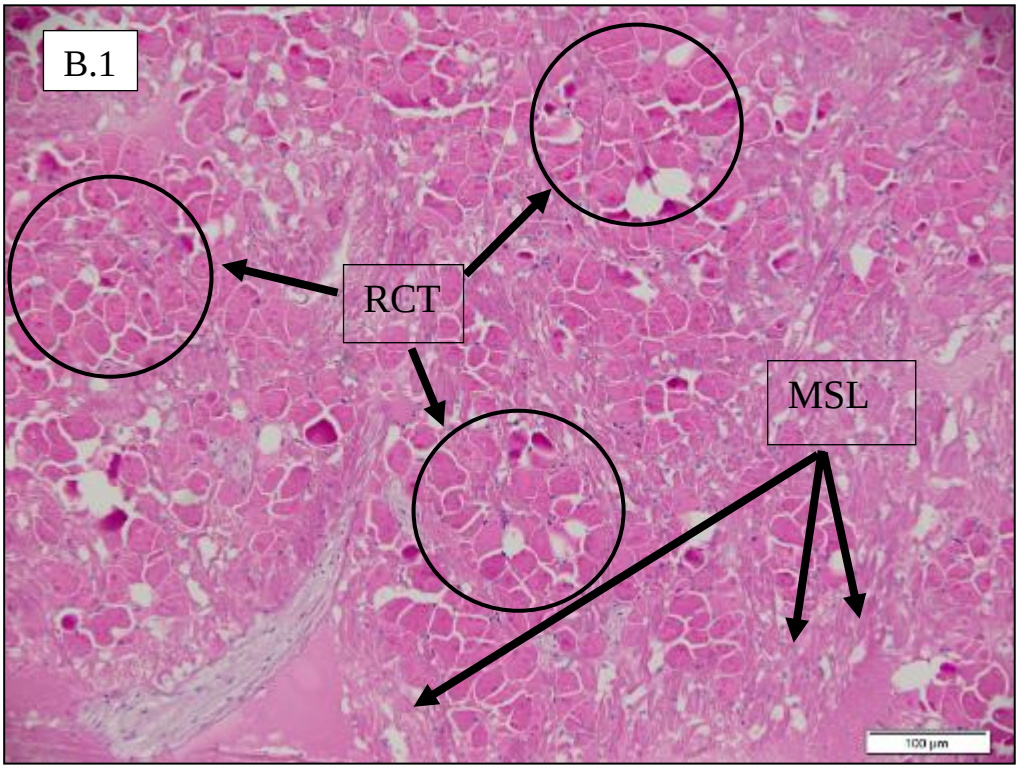


Figure 71: Cross section of myocardial tissue showing ample reserve cell tissue situation between the muscle fibres. [Msl: muscle, RCT: reserve cell tissue]

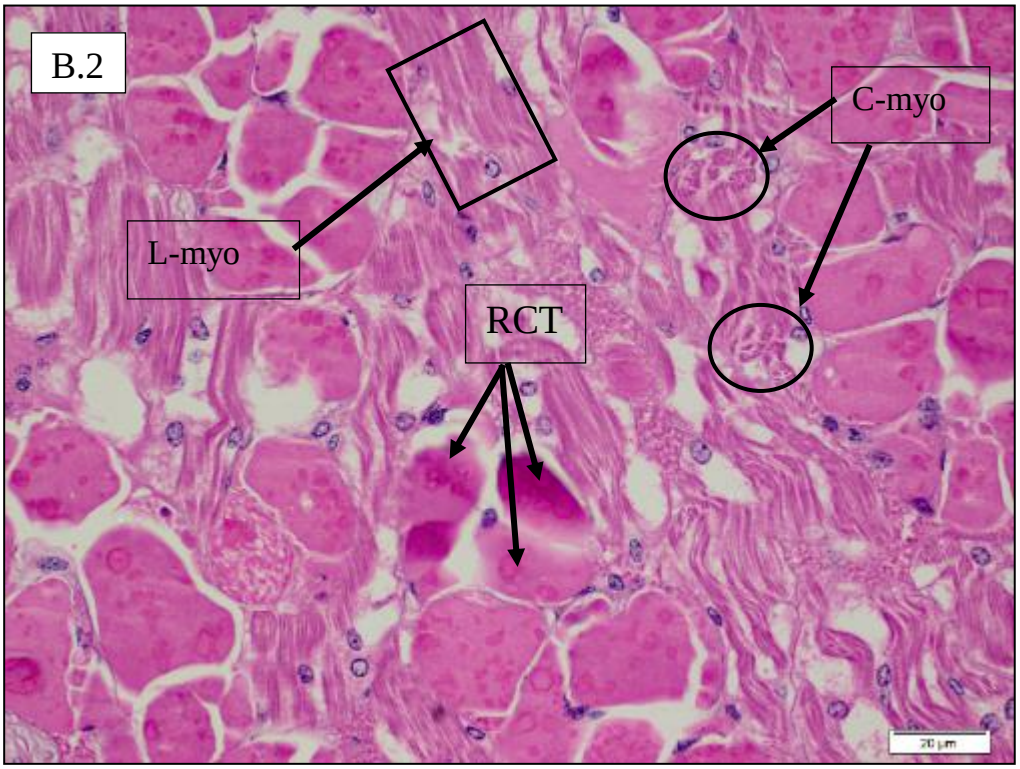


Figure 72: Magnification of image B2 providing structural detail of the myocardium. Note the intercalated nature of the myofibrils as muscles appear to be sectioned in multiple section planes (longitudinal and cross sectional). [RCT: reserve cell tissue, L-myo: longitudinal muscle myofibres, C-myo: cross sectional muscle myofibres]

CARDIAC HISTOPATHOLOGY

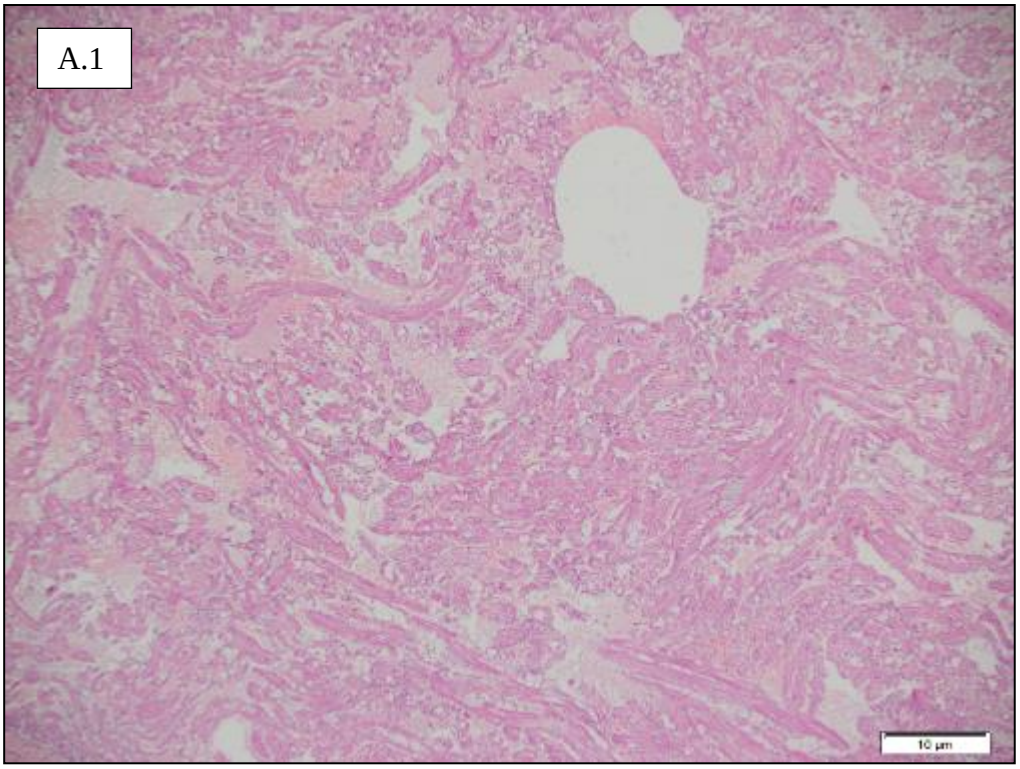


Figure 73: Cross section of abnormal myocardium that appears spongiform in nature with no reserve tissue present.

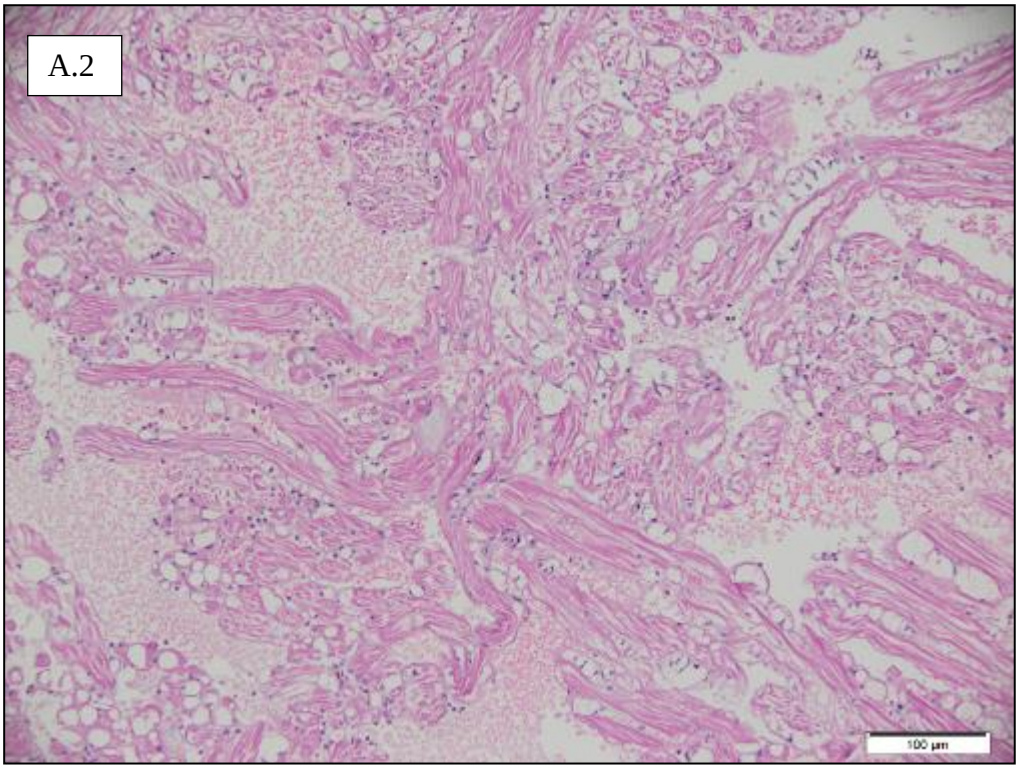


Figure 74: Magnification of image A1 showing cardiac tissue with spongiform appearance. Note the areas of reduced reserve cell tissue accumulation appear vacuolated.

NORMAL GASTROINTESTINAL HISTOLOGY

The gastrointestinal system of southern rock lobsters is composed of a grinding stomach, as well as a foregut, midgut and hindgut. Cuticular structures can be observed in the grinding stomach which functions to sieve masticated food. There are thick layers of muscle surrounding the stomach with loose connective tissue occupying most regions. The foregut is located dorsally in the cephalothorax and will not be focused on in this atlas. Midgut tissue is endodermaly derived and exhibits a prominent microvillous brush boarder which defers to that of the ectodermally derived hindgut that is lined with chitin. However, the most striking feature that differentiates the hindgut from the midgut is the presence of cuticle glands that are only observed in the distal sections of the intestinal system.

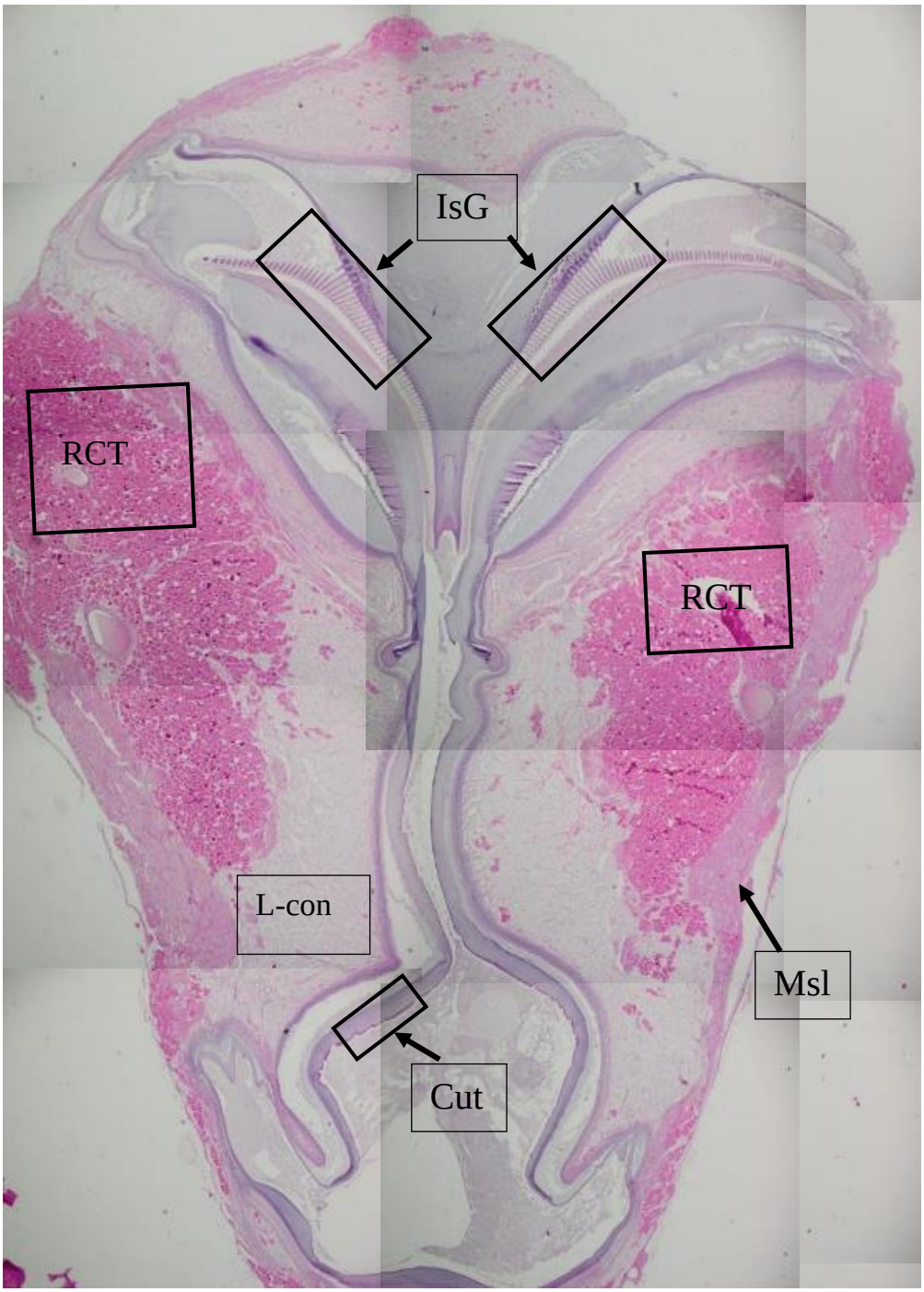


Figure 75: Overview of the grinding stomach. [Cut: cuticle, RCT: reserve cell tissue, L-con: loose connective tissue, IsG: inter-setal grooves. Msl: muscle]

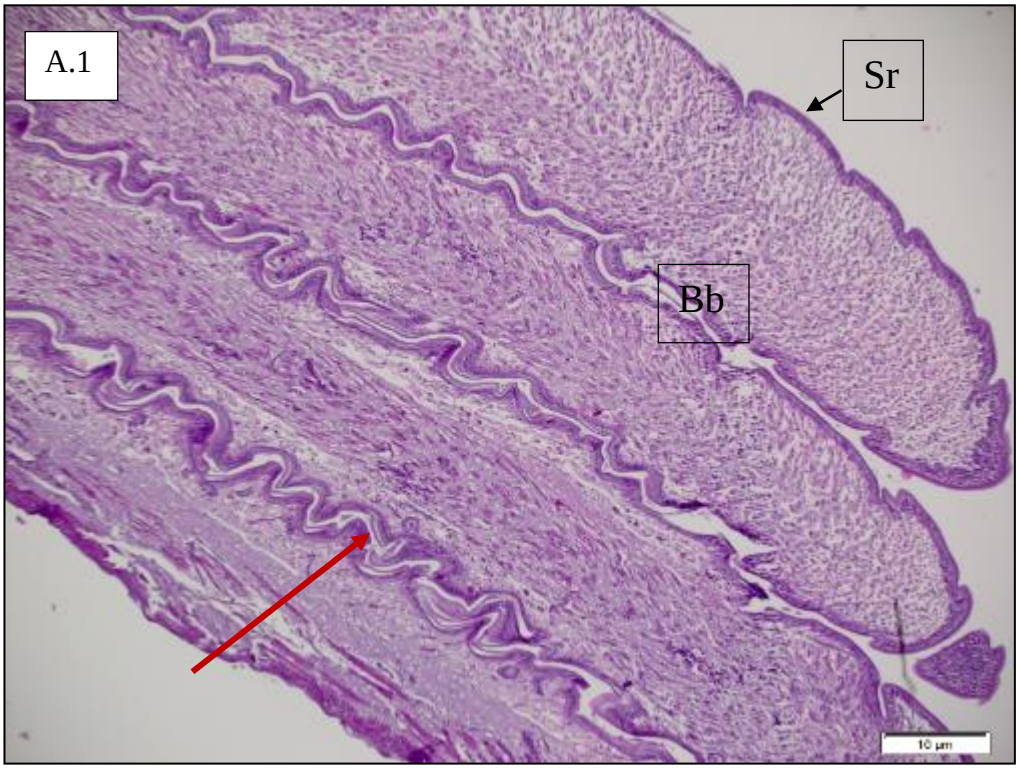


Figure 76: Overview of a longitudinal section of midgut. Note there is debri present within the luminal spaces (red arrow). [Sr: serosa, Bb: brush border]

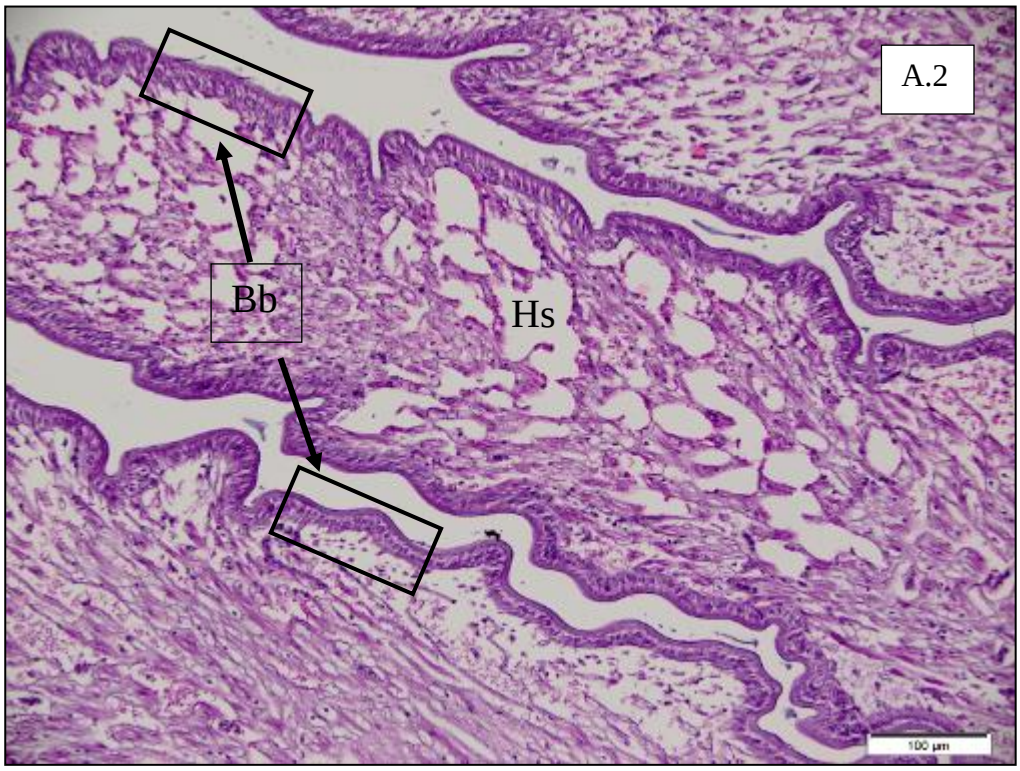


Figure 77: Magnification of image A1 of midgut tissue providing further detail to the microvillous brush border and presence of sinus's within the tissue. [Hs: haemal sinus, Bb: brush border]

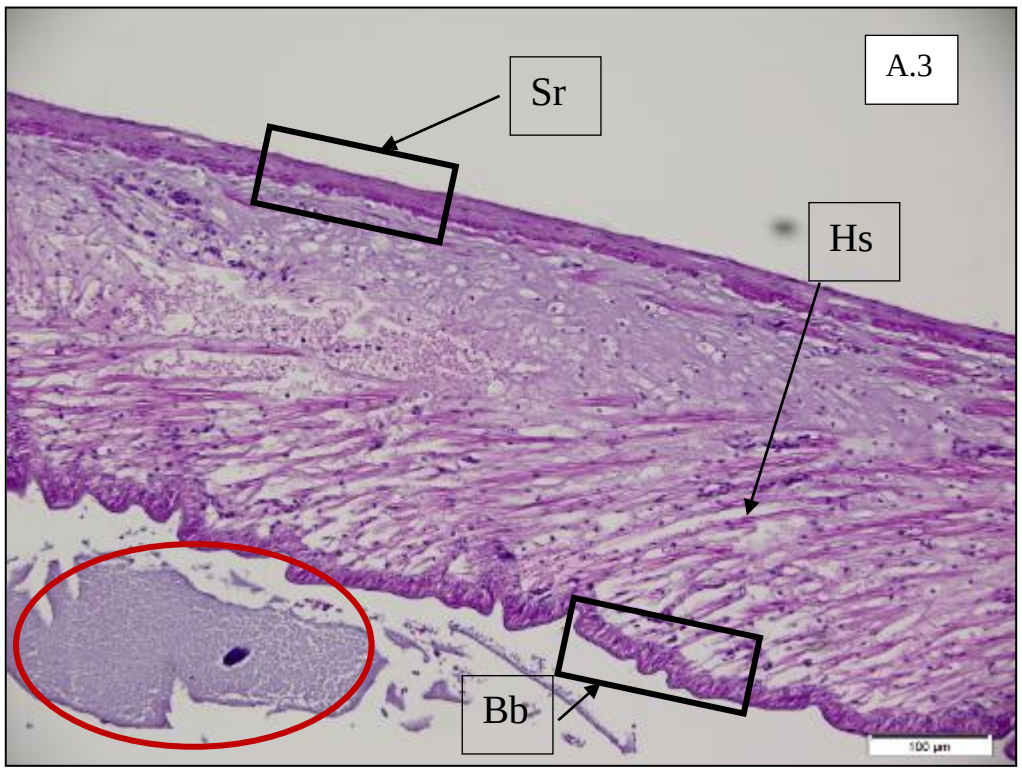


Figure 78: Magnification of image A1 of midgut tissue providing further detail to outter serosal layer. Note the presence of a bacterial plaque within the luminal space (red circle) [Sr: serosa, Bb: brush border, Hs: haemal sinus]

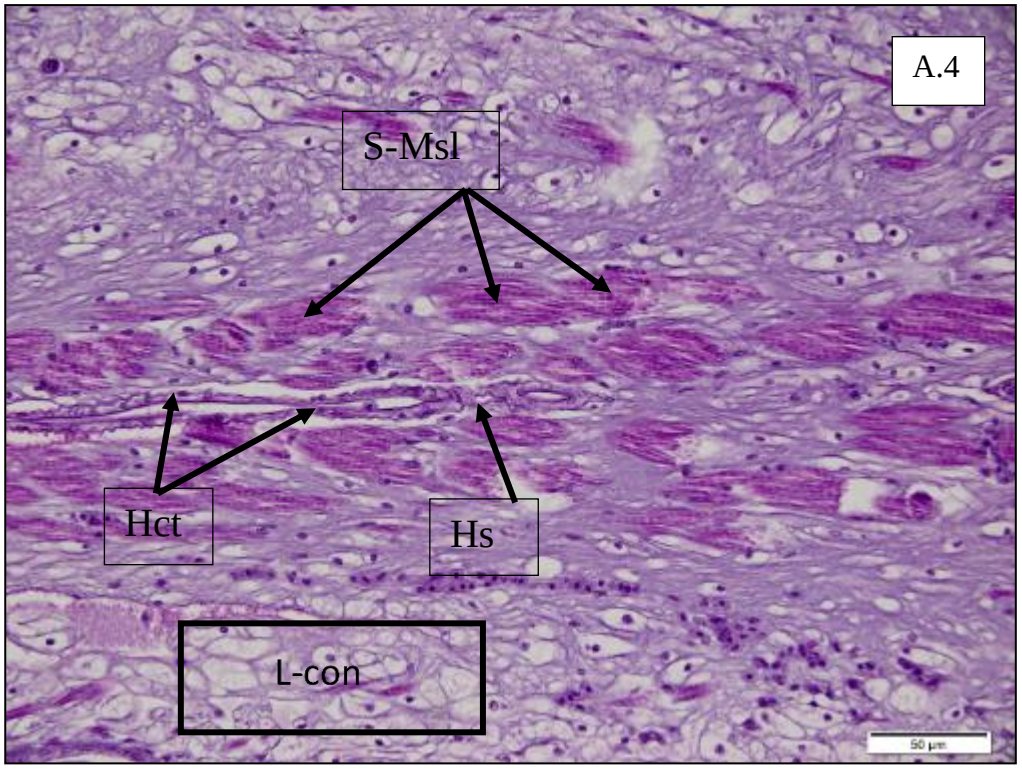


Figure 79: Magnification of image A1 of midgut tissue providing further detail of the muscular layers present. [S-Msl: smooth muscle, Hct: haemocyte, Hs: haemal sinus, L-con: loose connective tissue]

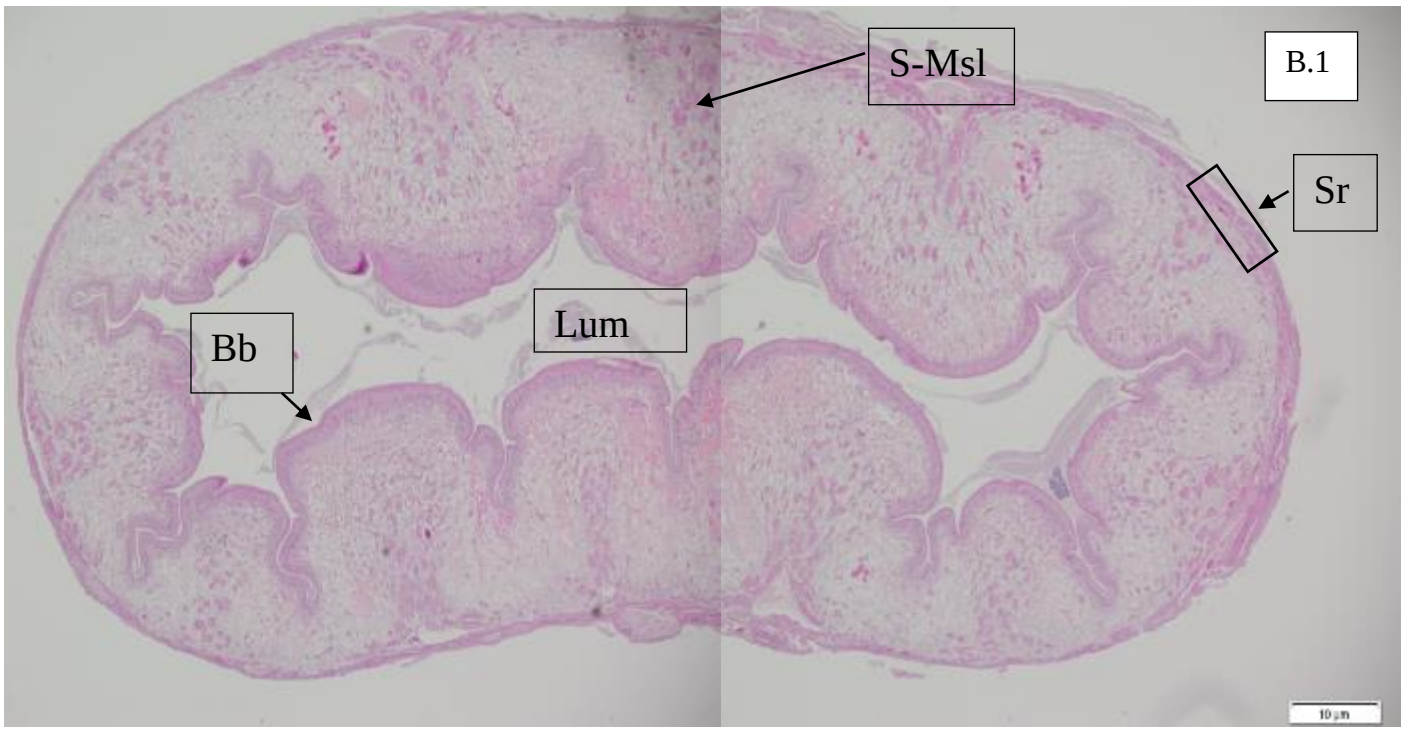


Figure 80: Overview of a cross sectional section of the midgut. [Sr: serosa, Lum: lumen, Bb: brush border, S-Msl: smooth muscle]

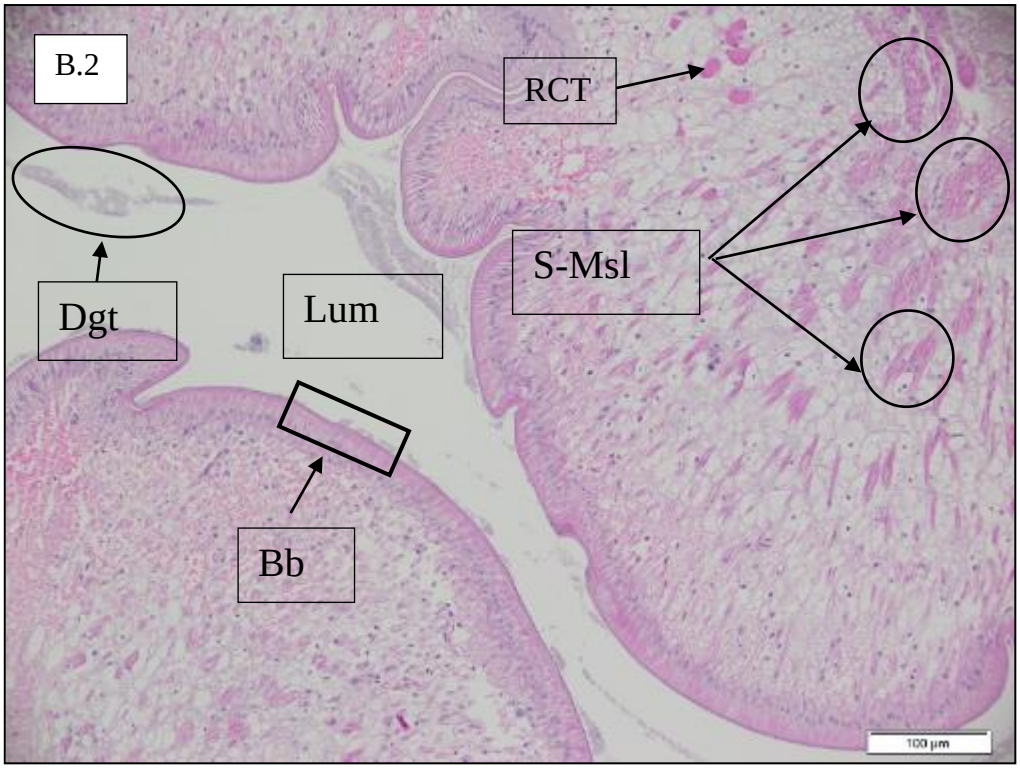


Figure 81: Magnification of image B1 depicting a cross section of the midgut tissue. [Dgt: digesta, Bb: brush border, Lum: lumen, RCT: reserve cell tissue, S-Msl: smooth muscle]

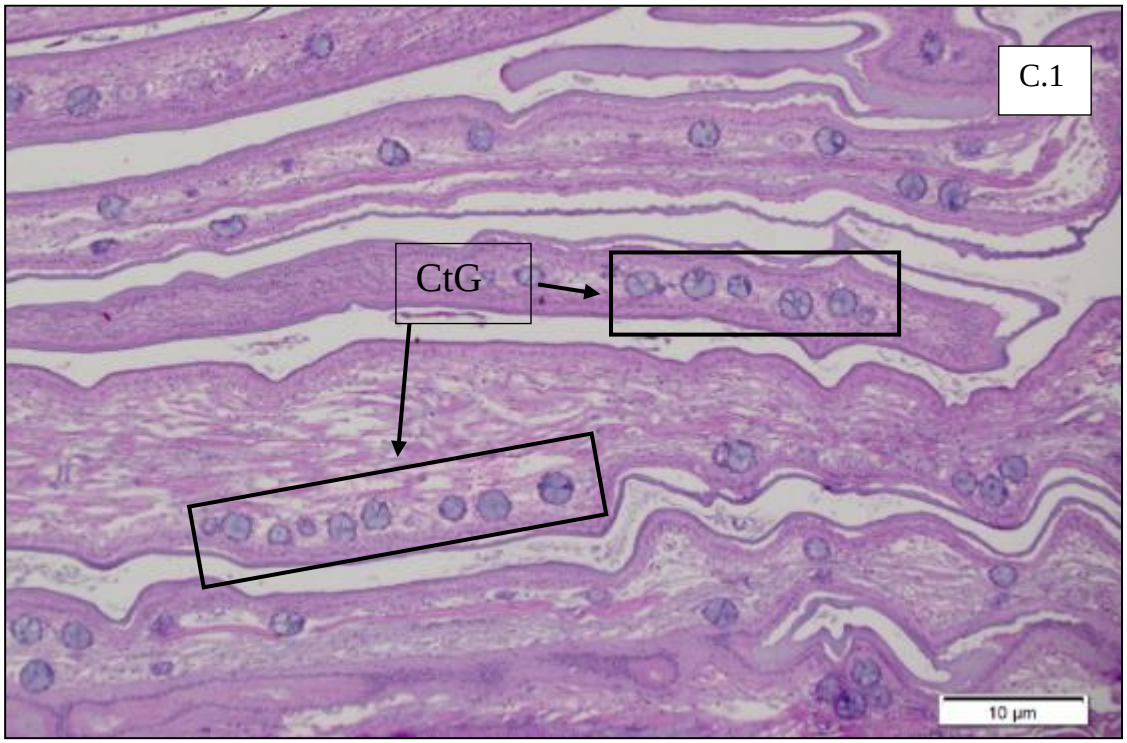


Figure 82: Overview of a longitudinal section of hindgut/caudal gut. Note the presence of cuticle glands that are not present in the midgut. [CtG: cuticle gland]

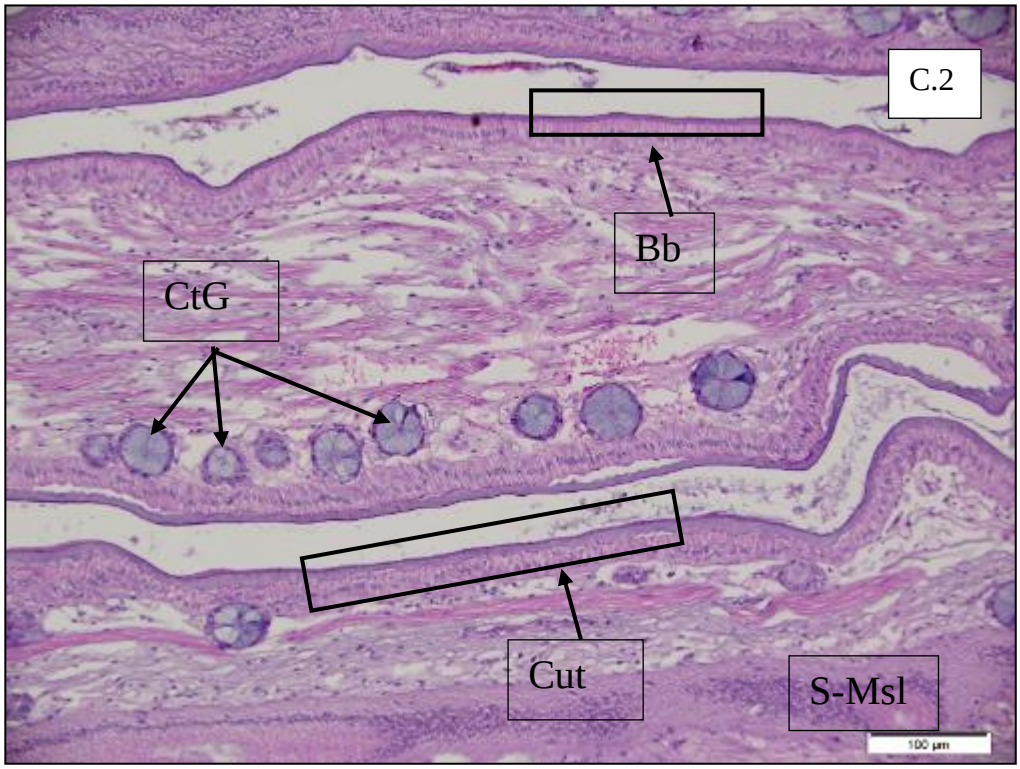


Figure 83: Magnification of the longitudinal section of image C1. The presence of both a brush border and cuticle is present in the hindgut. This may be a possible interface from midgut to hindgut. [Bb: brush border, Cut: cuticle, CtG; cuticle gland, S-Msl: smooth muscle]

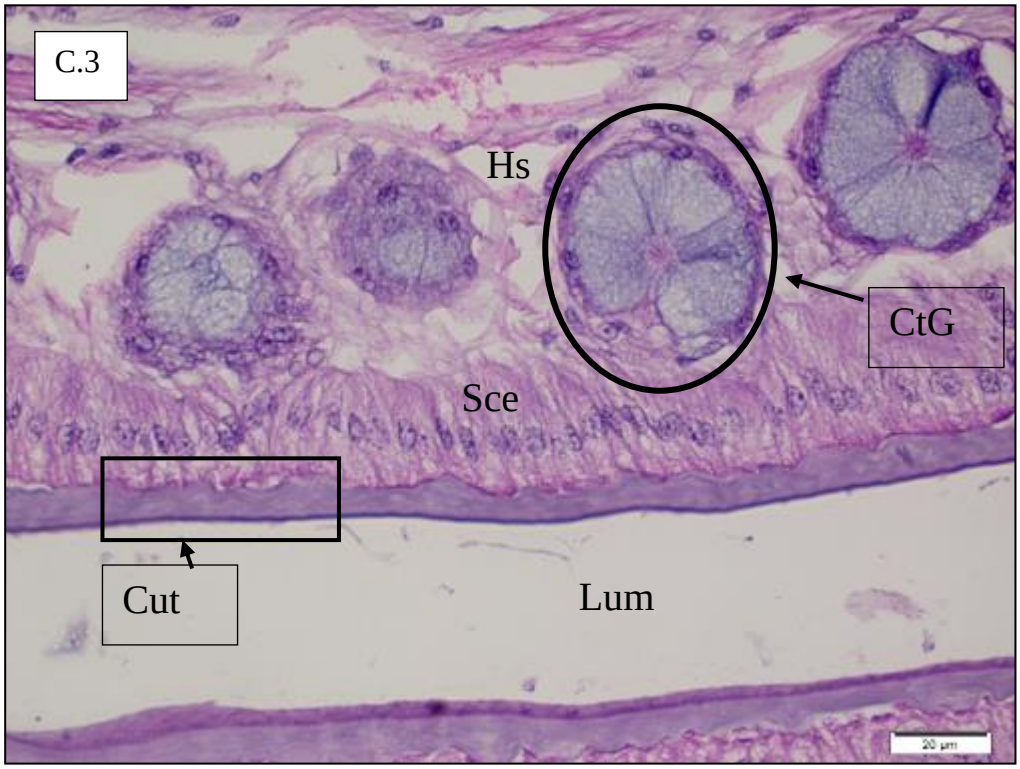


Figure 84: Magnification of the longitudinal section of image C1 focusing on the cuticle layer and the cuticle gland. [CtG: cuticle gland, Cut: cuticle. L-con: loose connective tissue, Sce: simple columnar epithelium, Lum: lumen, Hs: haemal sinus]

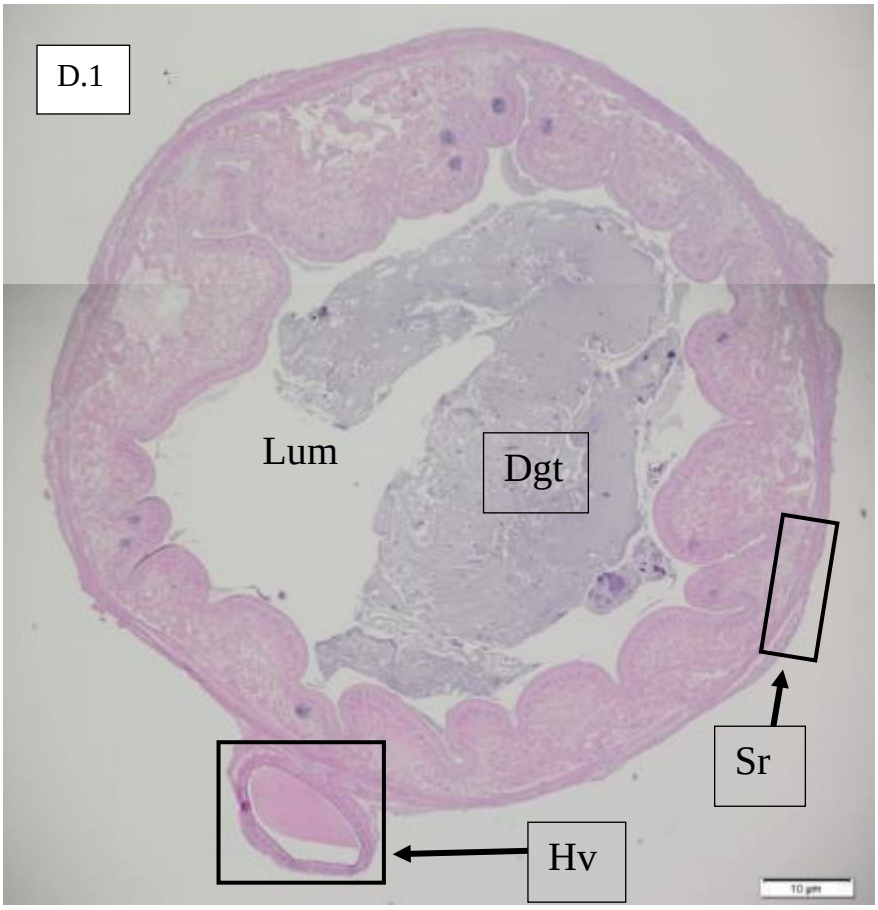


Figure 85: Image D1 is an overview of a cross sectional segment of hindgut/ caudal gut. [Hv: haemolymph vessel, Sr: serosa, Dgt: digesta, Lum: lumen]

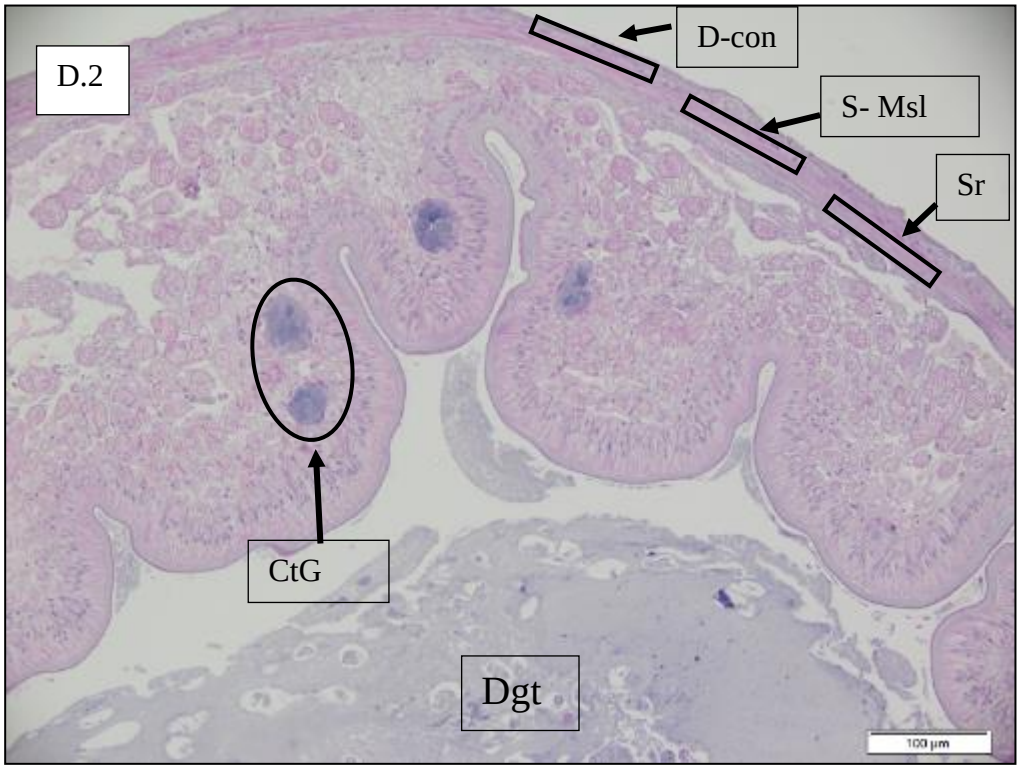


Figure 86: Magnified cross section of the hindgut focussing on the outer external layers. [D-con: dense connective tissue, S-Msl: smooth muscle, Sr: serosa, CtG: cuticle gland, Dgt: digesta]

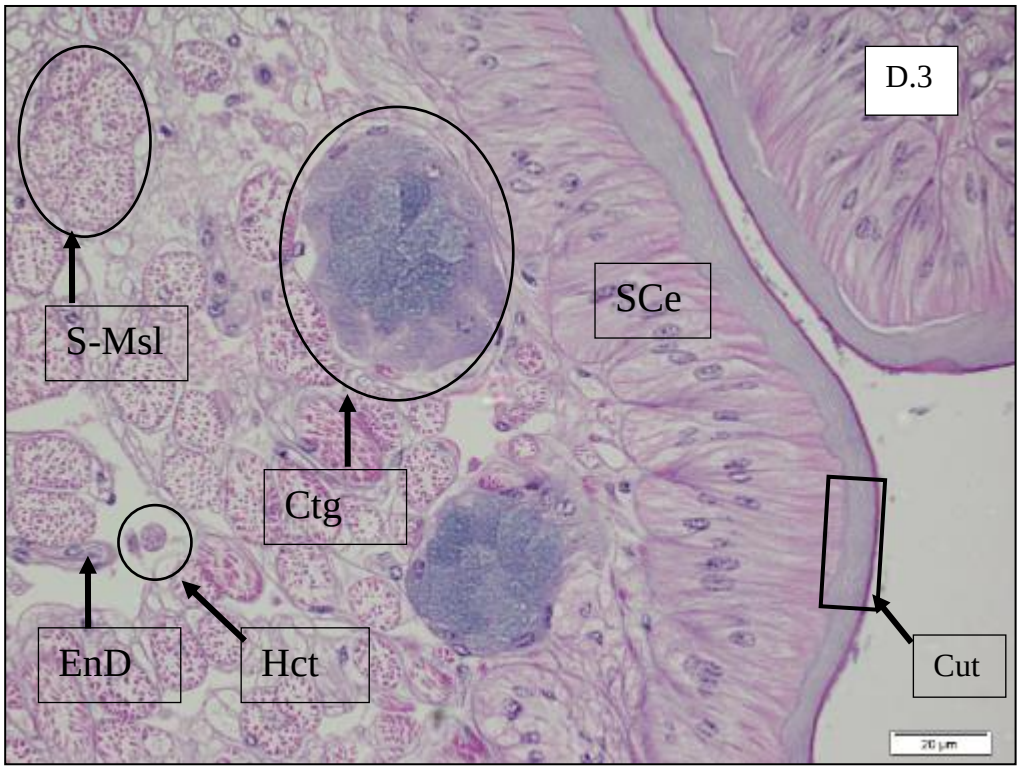


Figure 87: Magnified cross section of the hindgut focussing on the inner medial layers. Note the smooth muscle is arranged in bundles.[Cut: cuticle, CtG: cuticle gland, S-Msl: smooth muscle, EnD: endothelium, Hct: haemocytes, SCe: simple columnar epithelium]

GASTROINTESTINAL HISTOPATHOLOGY

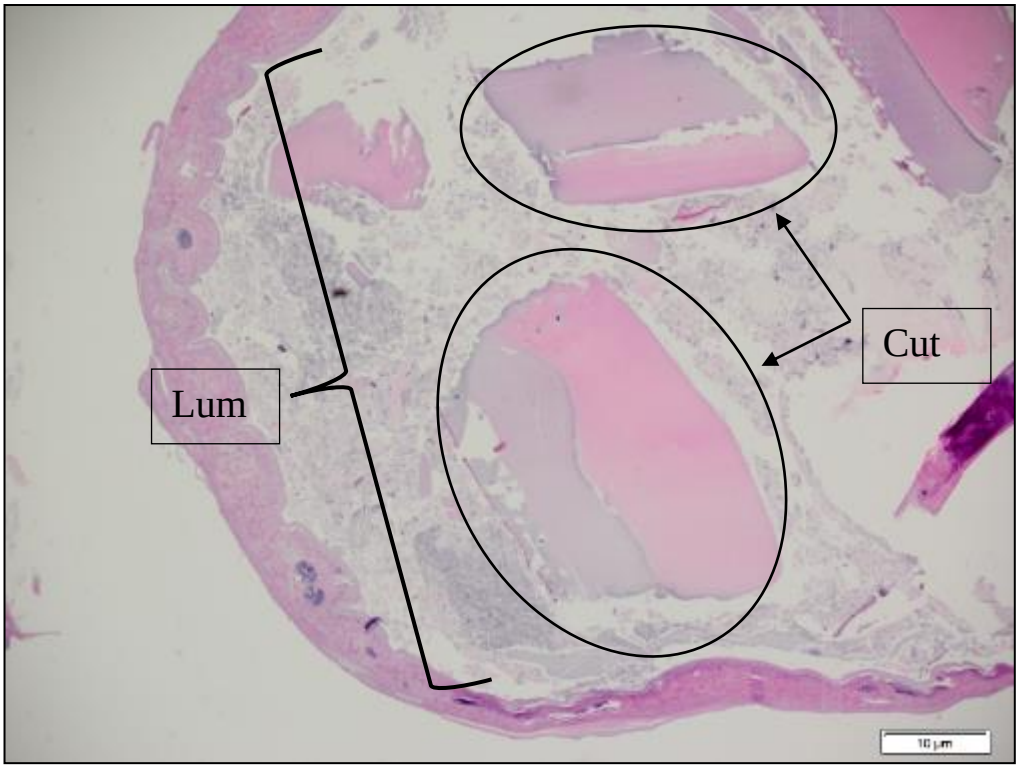


Figure 88: Cross sectional overview of the hindgut where there is evidence of cannibalism within the luminal space. Note the presence of cuticle within the lumen. [Cut: cuticle, Lum: lumen]

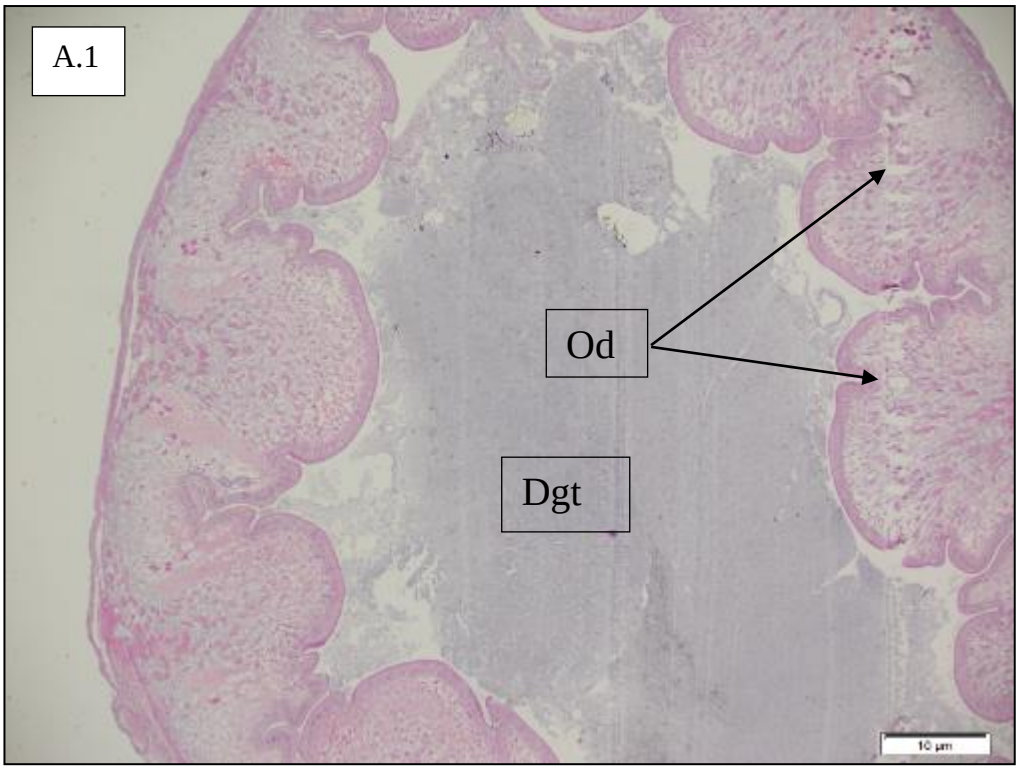


Figure 89: Cross sectional view of the midgut where is vacuolisation and oedema present. Note the lumen enlarged and full of digesta. [Dgt: digesta, Od: oedema]

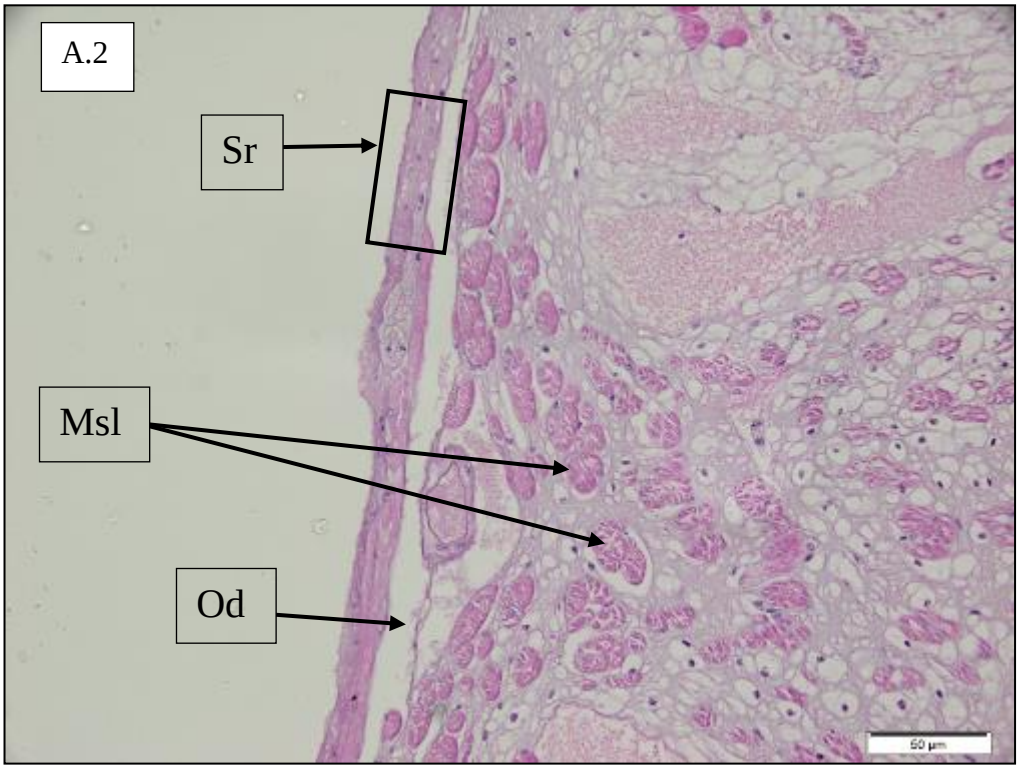


Figure 90: Maginification of image A1 showing oedema and vacuolisation of the midgut. [Sr: serosa, Od: oedema, Msl: muscle]

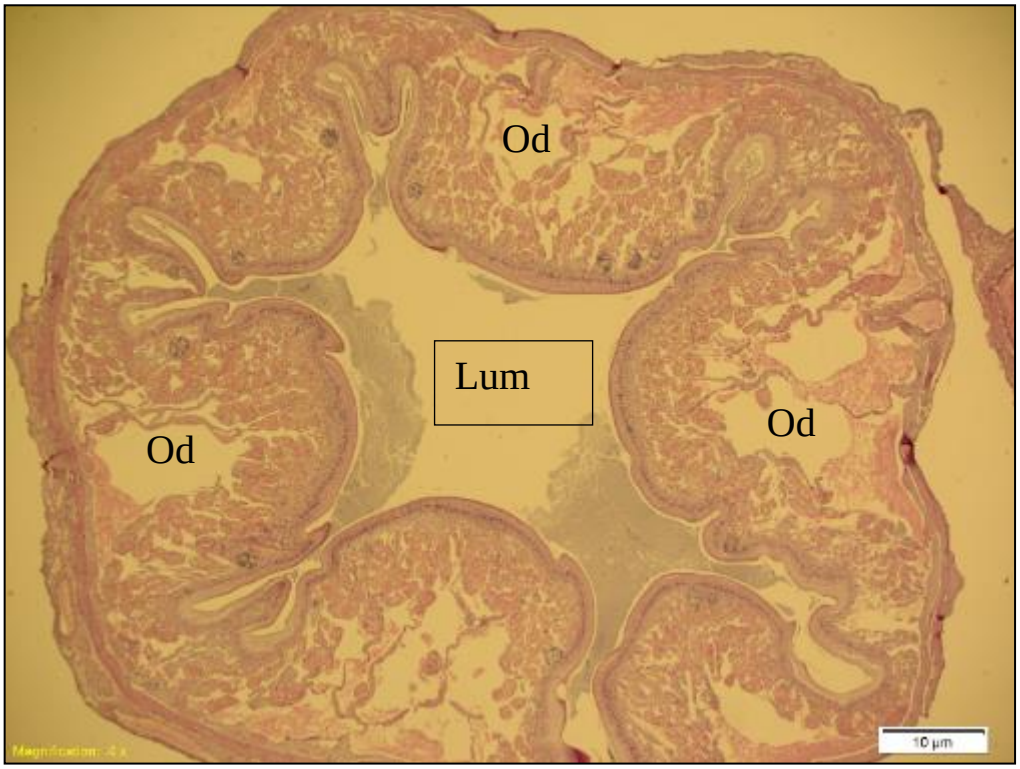


Figure 91: Cross sectional overview of oedela within the hindgut/ caudal gut. [Lum: lumen, Od: oedema]

HEPATOPANCREAS NORMAL HISTOLOGY

The hepatopancreas (digestive gland) is an organ comprised of a mass of individual tubules. These tubules possess functions in food absorption, transport, nutrient storage and secretion of digestive enzymes. They are made up of four main basic cell types: Enbryionic cells (E-cells), Restzellen cells (R-cells), Fribrillar cells (F-cells) and Blister cells (B-cells).

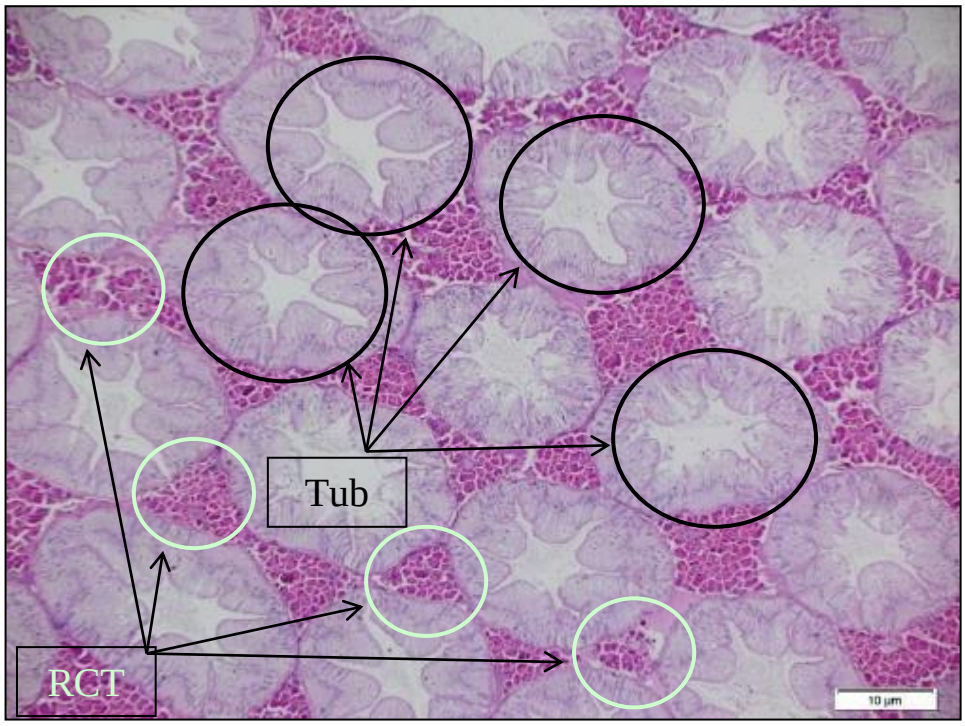


Figure 92: Overview of a cross sectional view of the hepatopancreas(HP). Note the appearance of individual tubules (black circles) that are surrounded by dense amounts of reserve tissue (white circles) in the inter-tubular spaces. [Tub: tubule, RCT: reserve cell tissue]

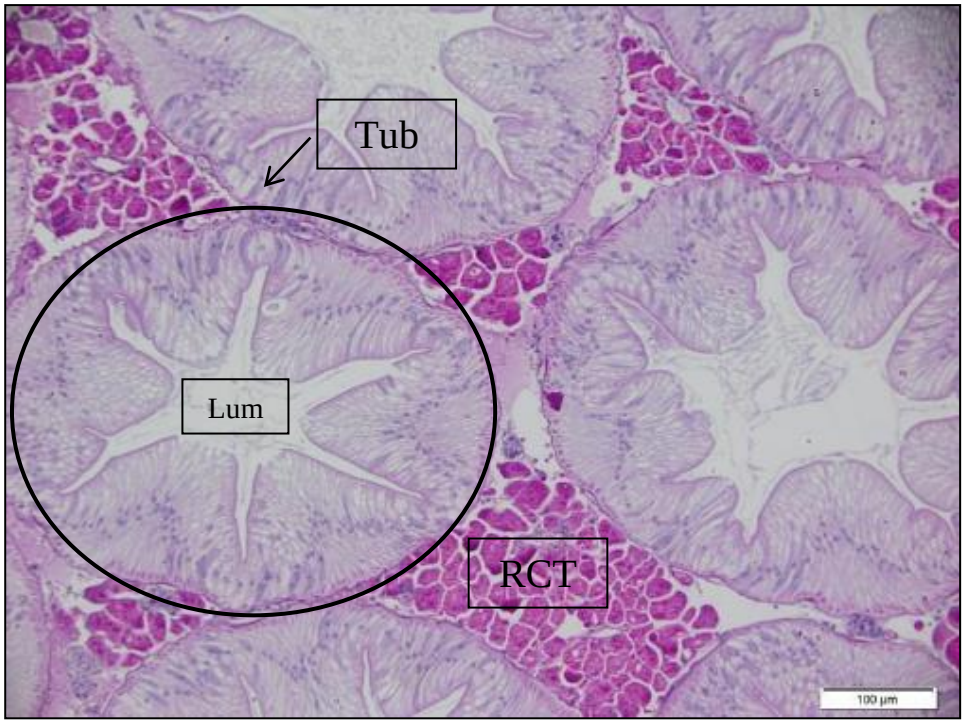


Figure 93: Magnified cross sectional view of the hepatopancreas(HP). [Tub: tubule, RCT: reserve cell tissue, Lum: lumen]

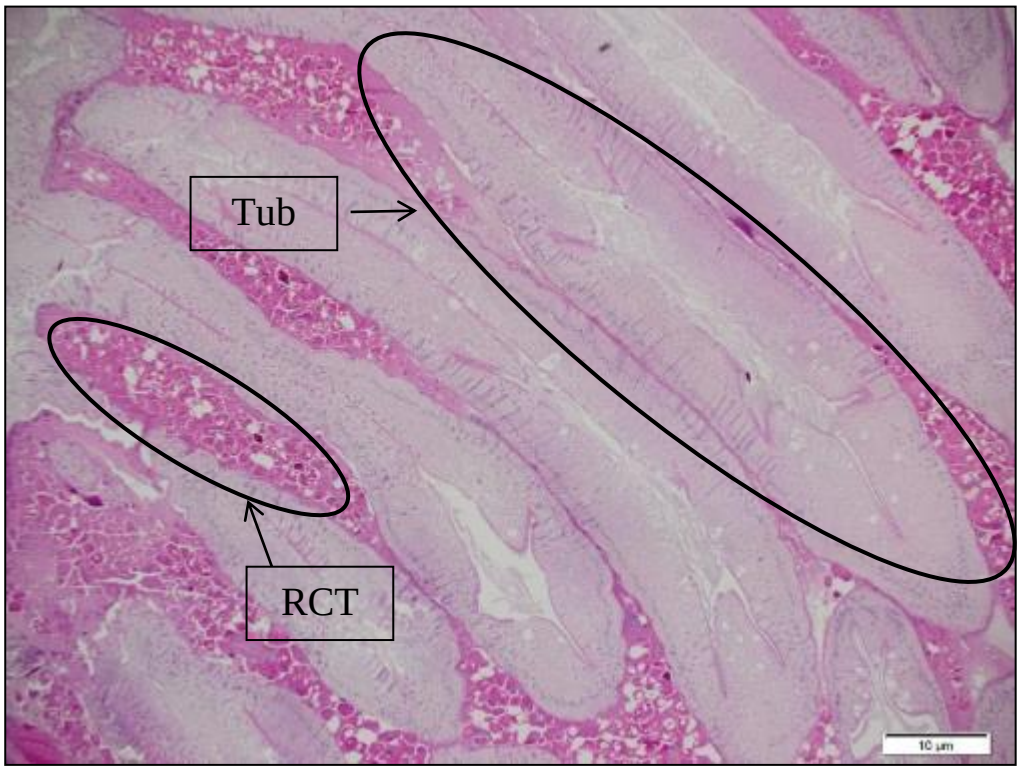


Figure 94: Overview of a longitudinal section of the hepatopancreas(HP). Note the appearance of individual tubules (black circles) that are surrounded by dense amounts of reserve tissue (white circles) in the inter-tubular spaces. [Tub: tubule, RCT: reserve cell tissue]

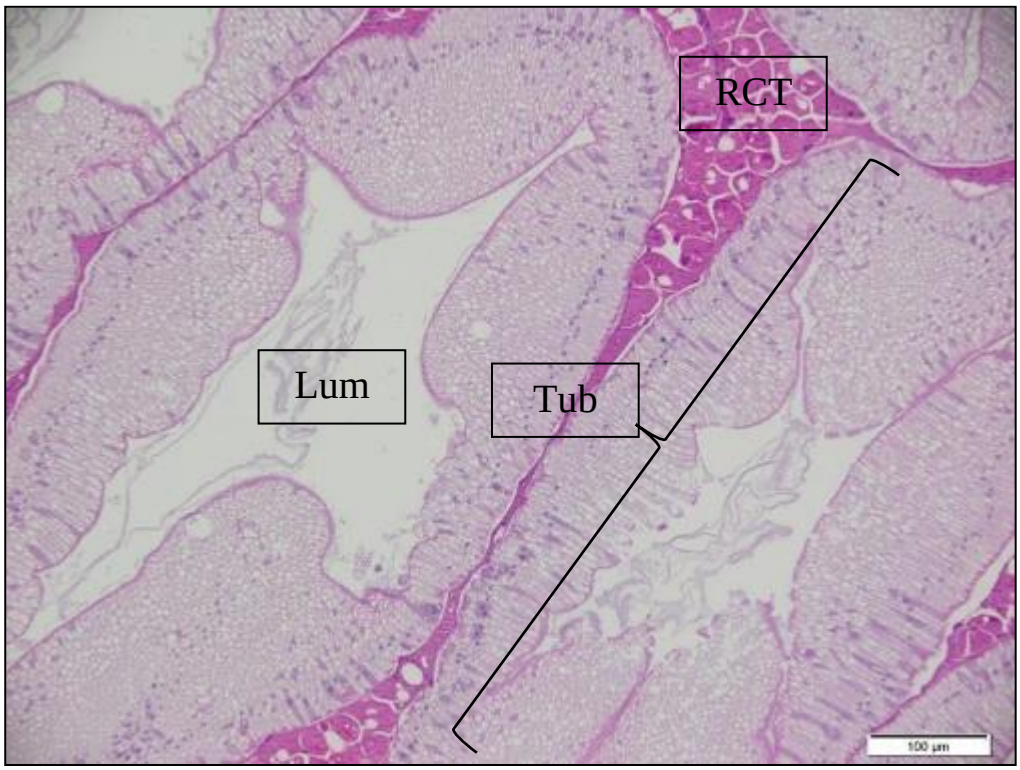


Figure 95: Magnified longitudinal section of the hepatopancreas(HP).. [Tub: tubule, RCT: reserve cell tissue, Lum: lumen]

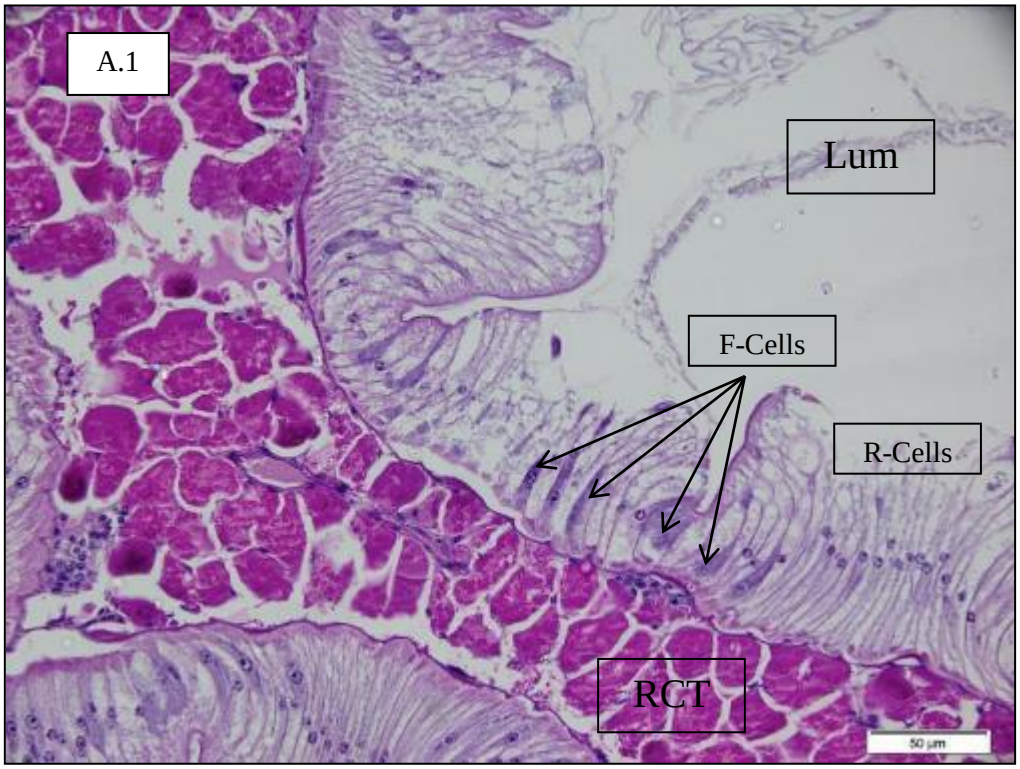


Figure 96: Cross section of the hepatopancreatic tubule. Note the fibrillar cells (F-cells) have a basally located nucleus and a full cytoplasm giving it a stained appearance. The R-cells are tall columnar cells and are the most numerous cell type of the hepatopanceas (HP). [Lum: lumen, RCT: reserve cell tissue, F-cells: fibrillar cells, R-cell: restzellen cells]

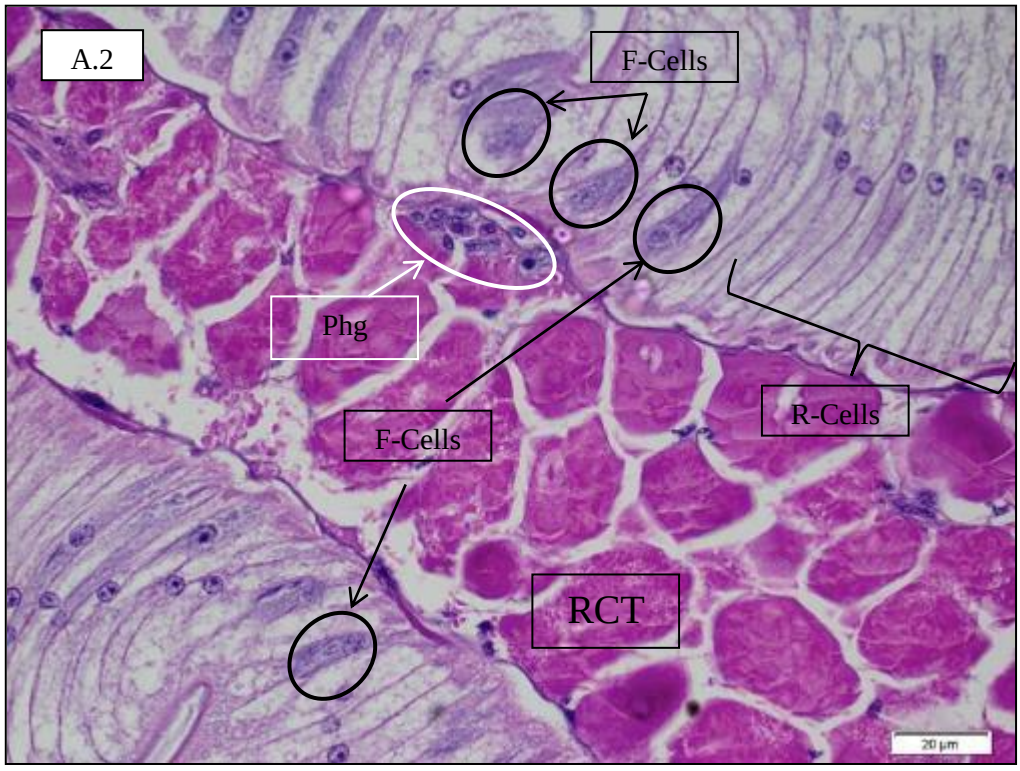


Figure 97: Magnification of image A1 providing further detail to the appearance and structure to F-cells and R-cells. [F-cells: fibrillar cells. R-cells: restzellen cells, Phg: Phagocytes, RCT: reserve cell tissue]

HEPATOPANCREAS HISTOPATHOLOGY

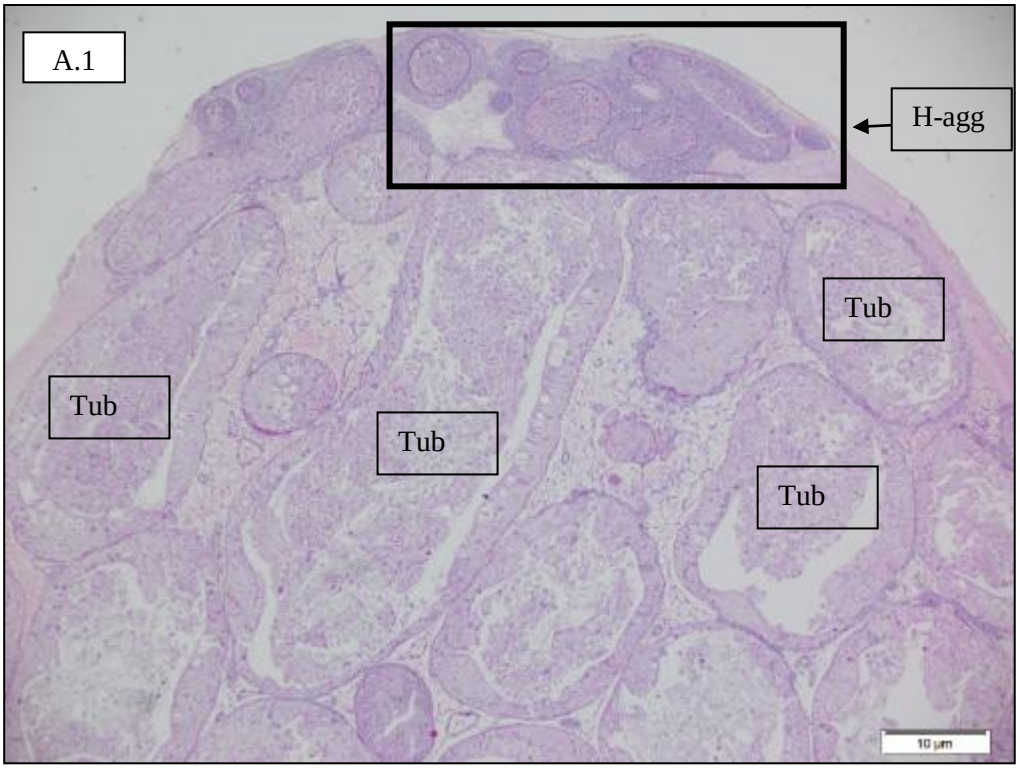


Figure 98: Cross sectional overview of an abnormal hepatopancreas where severe dilation of tubules is observed with autolysis and necrosis present. Focal areas of haemocyte infiltration can also be seen in image A1 above (Black square). [Tub: tubules, H-agg: haemocyte aggregation]

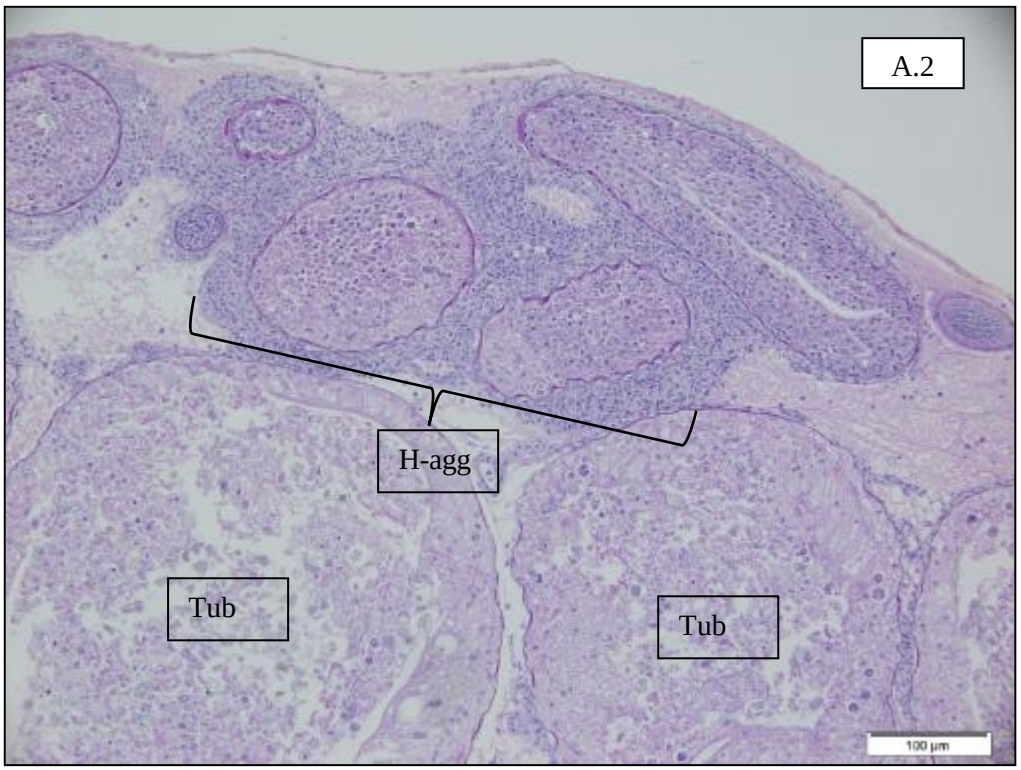


Figure 99: Magnification of image A1 focusing on the focal zone of haemocyte infiltration. Note the loss of structure to tubules with atrophy and degredation of the lining cells. [H-agg: haemocyte aggregation, Tub: tubule]

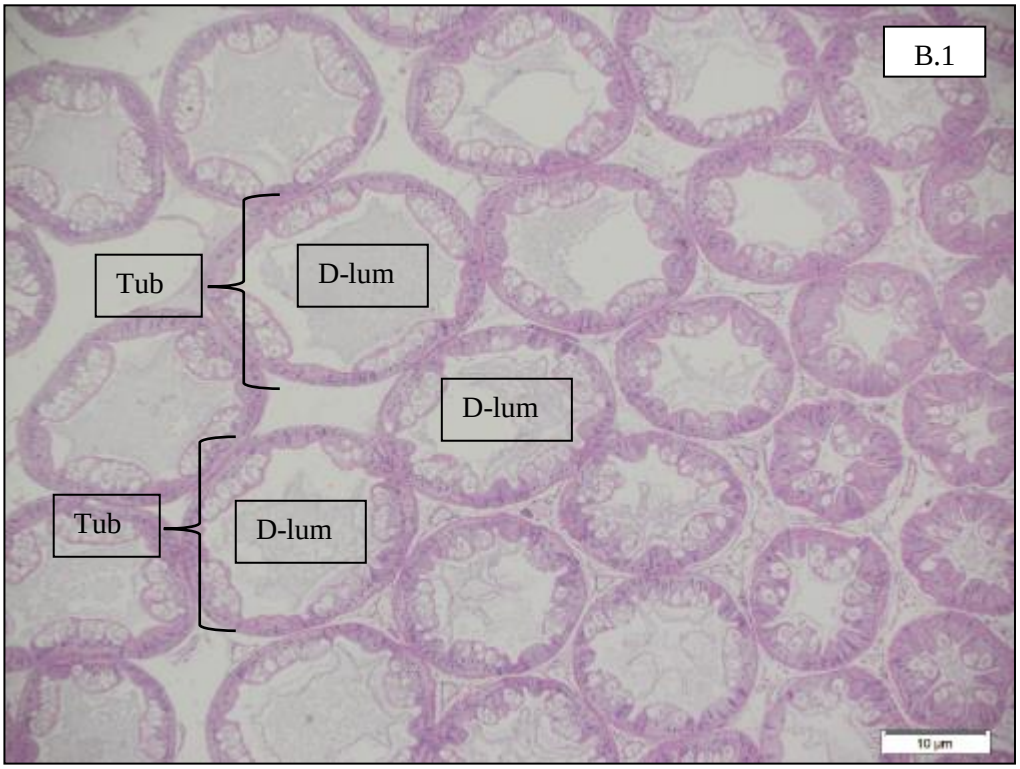


Figure 100: Cross section of abnormal hepatopancreas where there is marked dilation of the luminal areas of the tubules with generalised thinning of the tubular lining. Note the minimal to no reserve tissue observed in the intertubular spaces. [D-lum: dilated lumen, Tub: tubule]

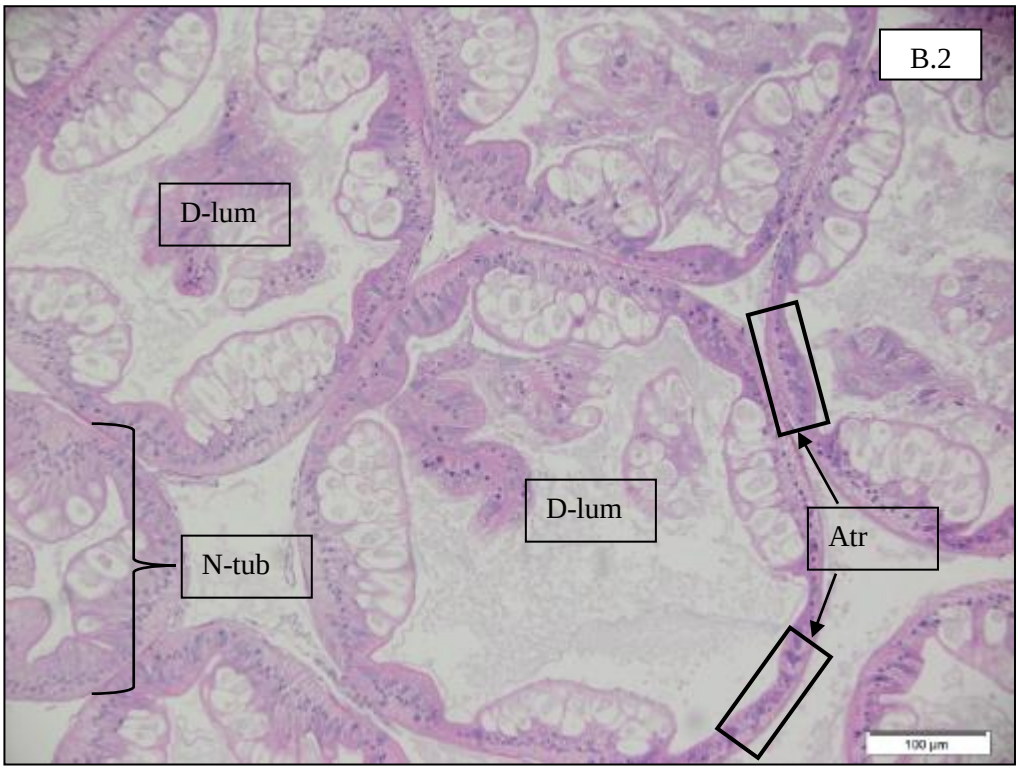


Figure 101: Magnification of image B1 focusing on the abnormal luminal dilation and the focal atrophy of the tubular lining. [D-lum, Atr: atrophy, N-tub:normal tubule]

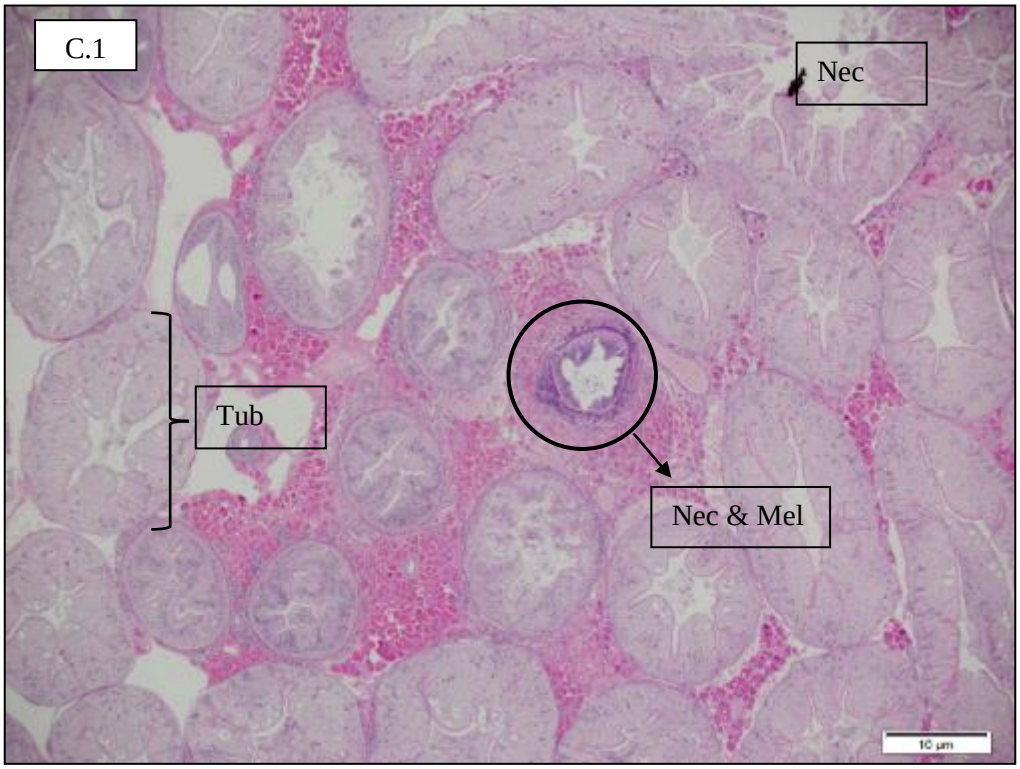


Figure 102: Cross section of abnormal hepatopancreas demonstrating multifocal areas of tubular necrosis with haemocyte and melanisation reactions. [Nec: necrosis, Tub: tubules, Mel: melanisation]

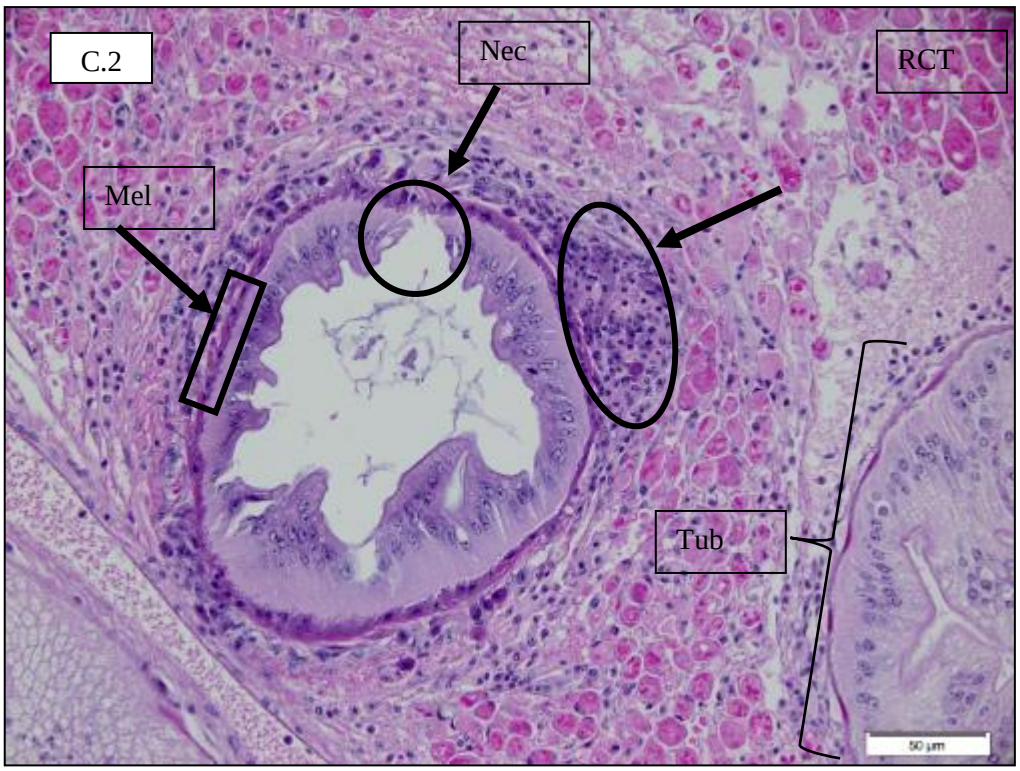


Figure 103: Magnified section of image C1 focusing on a necrotic melanised tubule that is surrounded by aggregating haemocytes. [Mel: melanisation, Nec: necrotic, H-agg: haemocyte aggregation, RCT: reserve cell tissue, Tub: tubule]

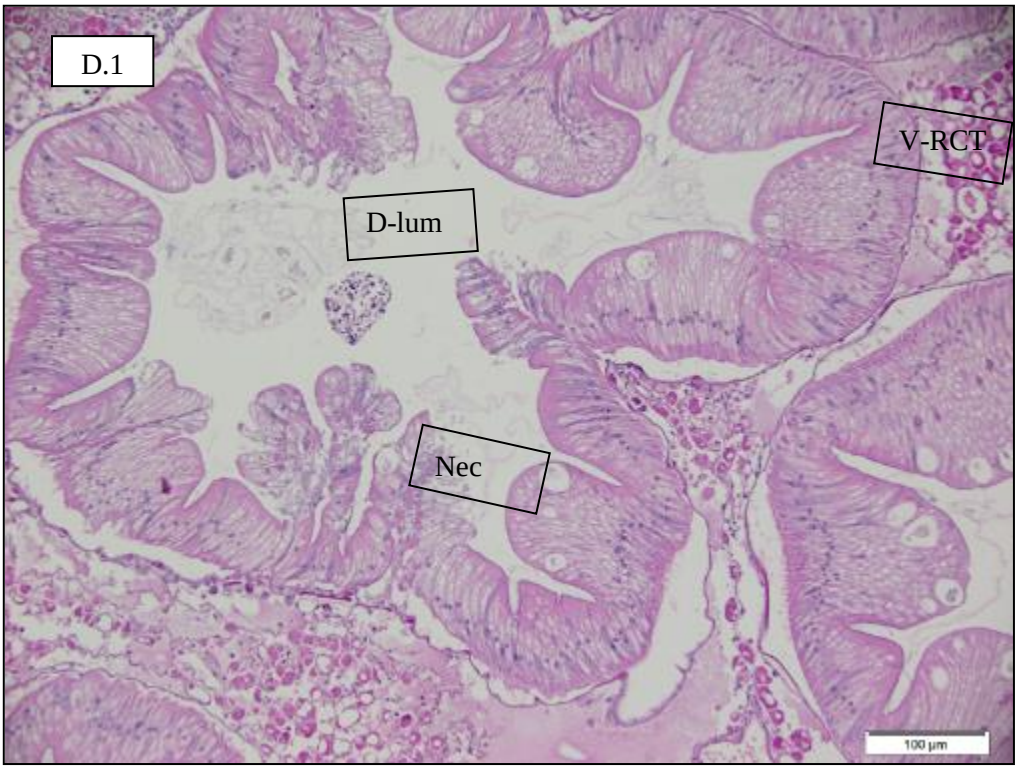


Figure 104: Cross section of abnormal hepatopancreas with moderately dilated luminal spaces as well as necrotic epithelial tissue. Note the presence of abnormal reserve cell tissue that appears vacuolated and degenerative. (Nec: necrosis, D-lum: dilated lumen, V-Rct: Vacuolated reserve cell tissue]

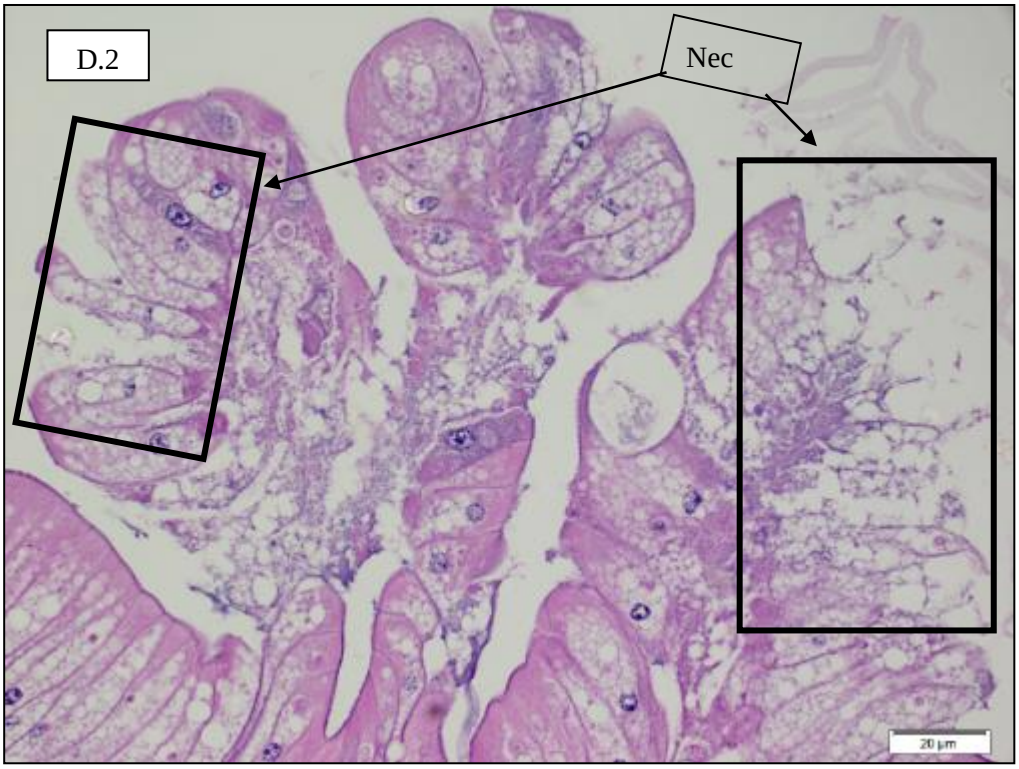


Figure 105: Magnified section of image D1 focusing on a necrotic epithelial tubular tissue. Note the loss of structure to the columnar epithelial cells. [Nec: necrosis]

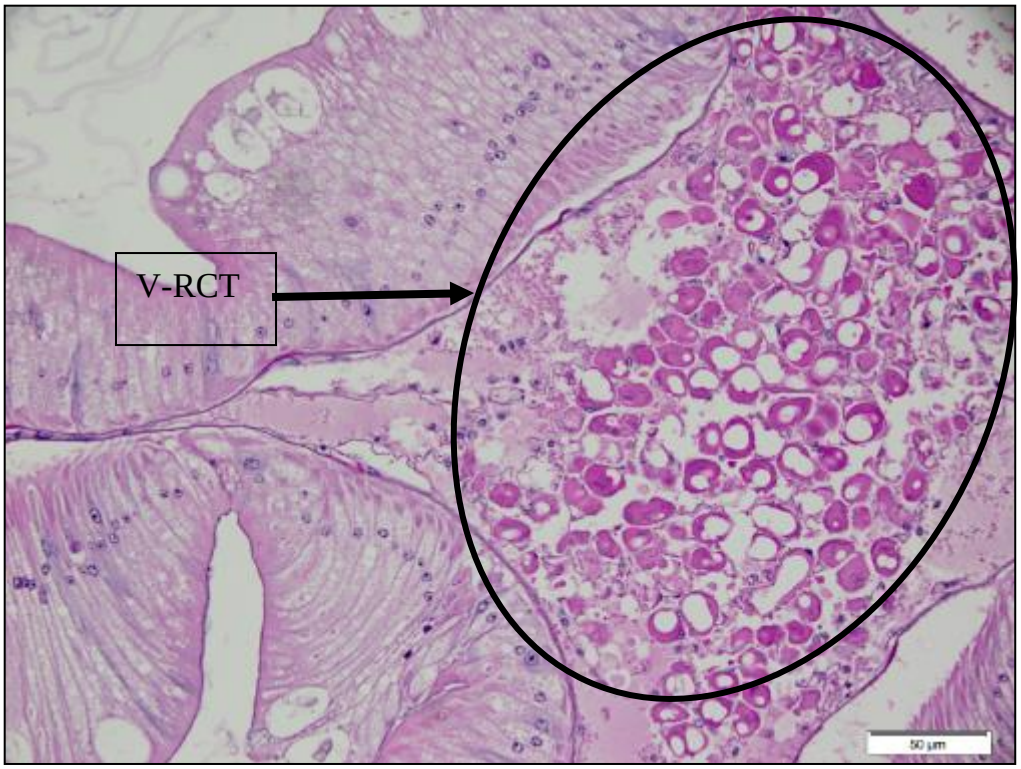


Figure 106: Magnified cross section of abnormal hepatopancreas reserve tissue that appears vacuolated. [V-RCT: vacuolated reserve cell tissue]

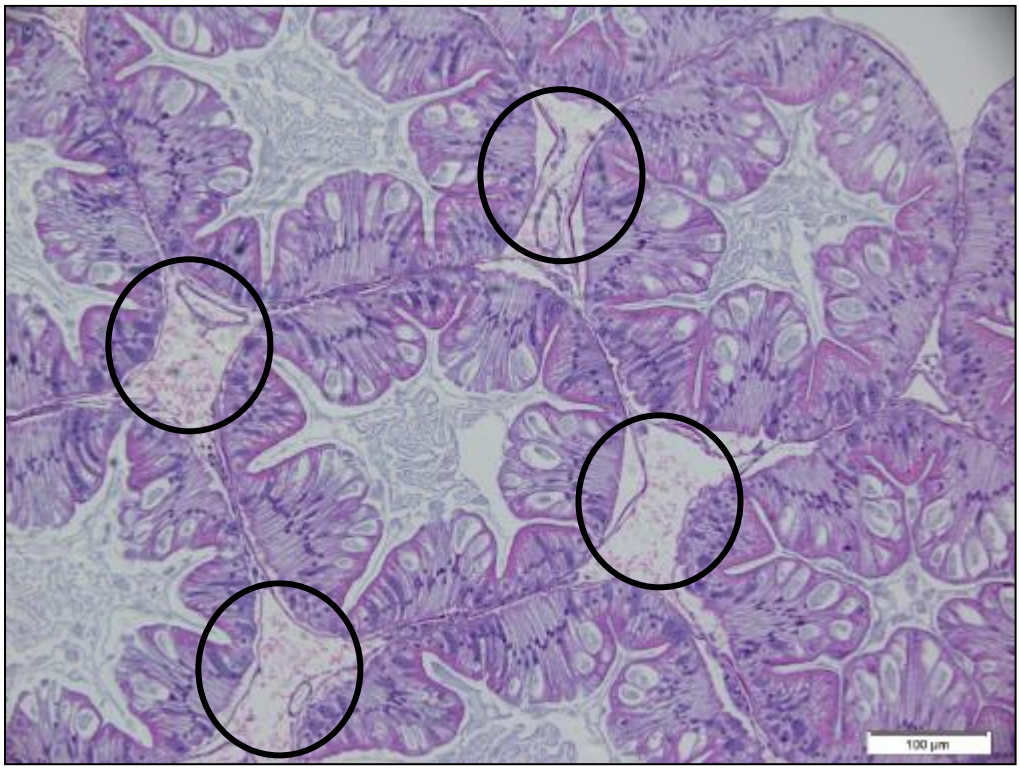


Figure 107: Magnified cross section of the hapatopancreas showing a marked reduction in reserve cell tissue in the intertubular spaces (black circles).

MALE REPRODUCTIVE ORGAN NORMAL HISTOLOGY

The male reproductive organ in crustaceans consists of the testes, the vas deferens, the ejaculatory duct and the spermatozoa. Due to the sampling site at necropsy this atlas will focus only on the vas deferens. The vas deferens are paired structures that function to transport the sperm from the testis to the gonadopores. In addition to this it is also responsible for compartmentalising sperm into spermatophores.

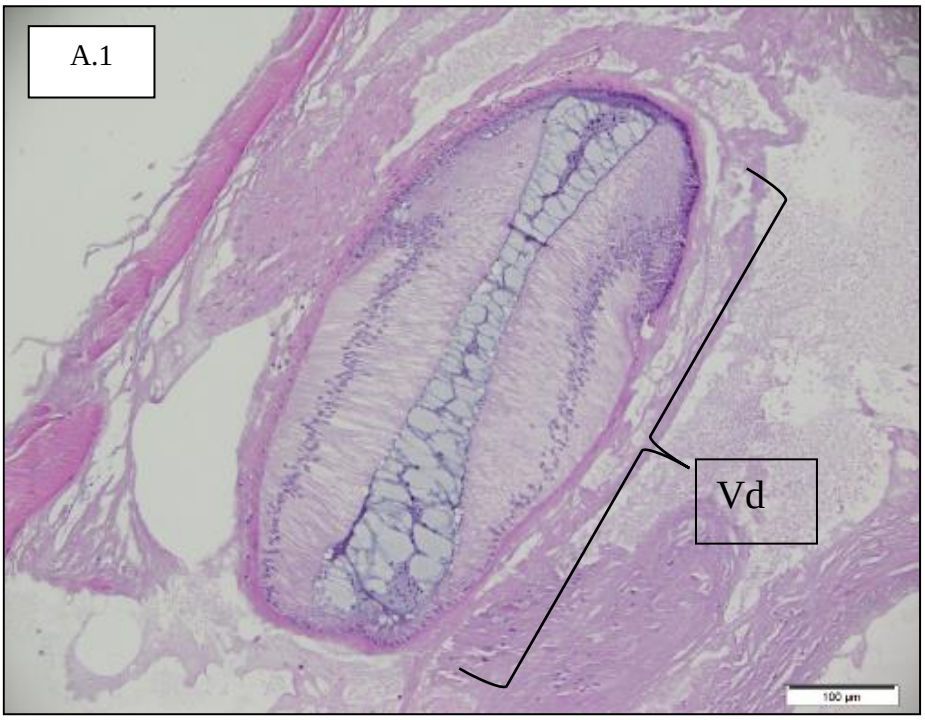


Figure 108: Overview of a longitudinal section of the vas deferens. Note the peripheral sheath that envelopes the vas deferens comprises of fibrous and muscular components [Vd: vas deferens, PS: peripheral sheath]

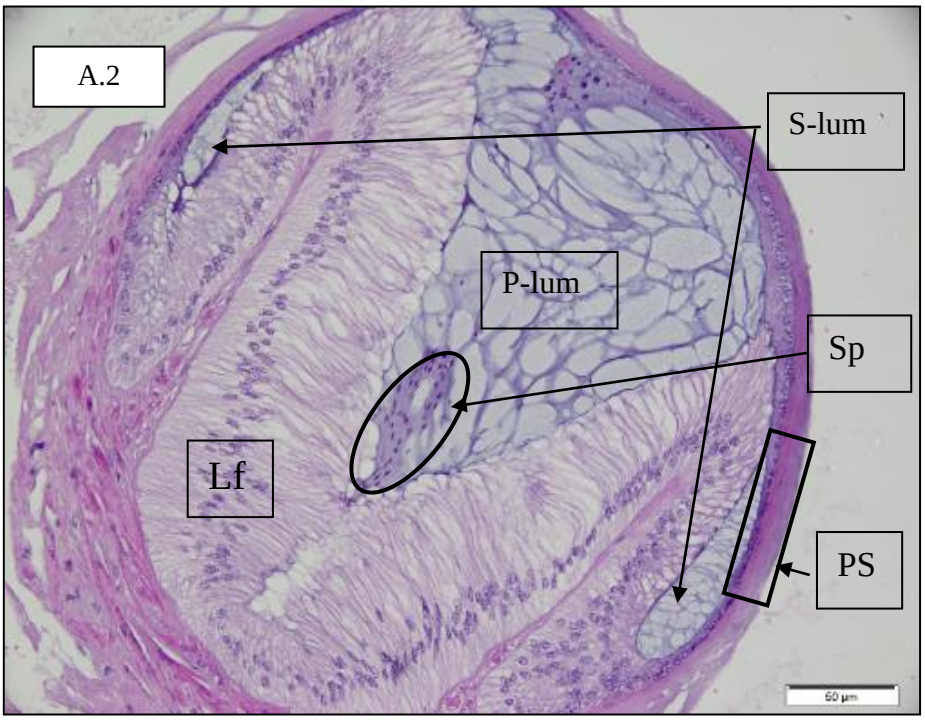


Figure 109: Magnification of image A1 providing further structural detail of the vas deferens. The longitudinal fold are composed of elongated epithelia with dense staining nuclei. [Lf: longitudinal folds, PS: peripheral sheath, P-lum: primary lumen, S-lum: secondary lumen, Spm: spermatozoa]

FEMALE REPRODUCTIVE ORGAN NORMAL HISTOLOGY

The ovary is a paired organ that lies dorsal to the hepatopancreas and ventral to the heart. Age and reproductive maturity are factors that influence the size of the ovary in each individual crustacea.

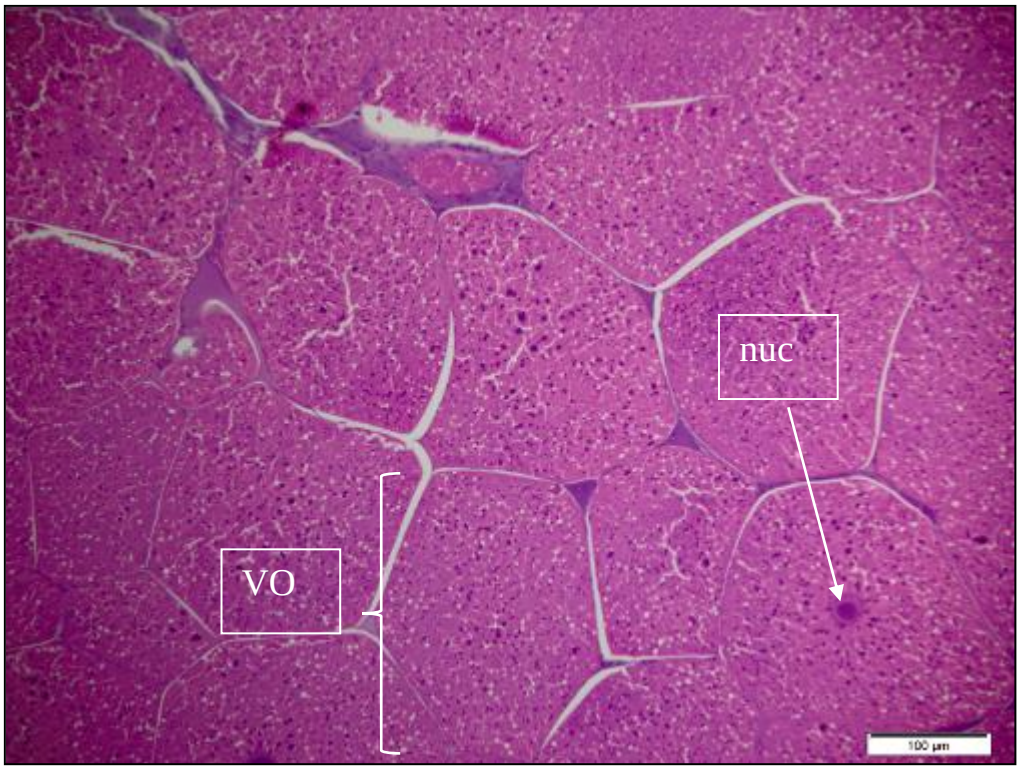


Figure 110: Detail of mature ovarian lobules. [VO: vitellogenic oocyte, nuc: oocyte nucleus]

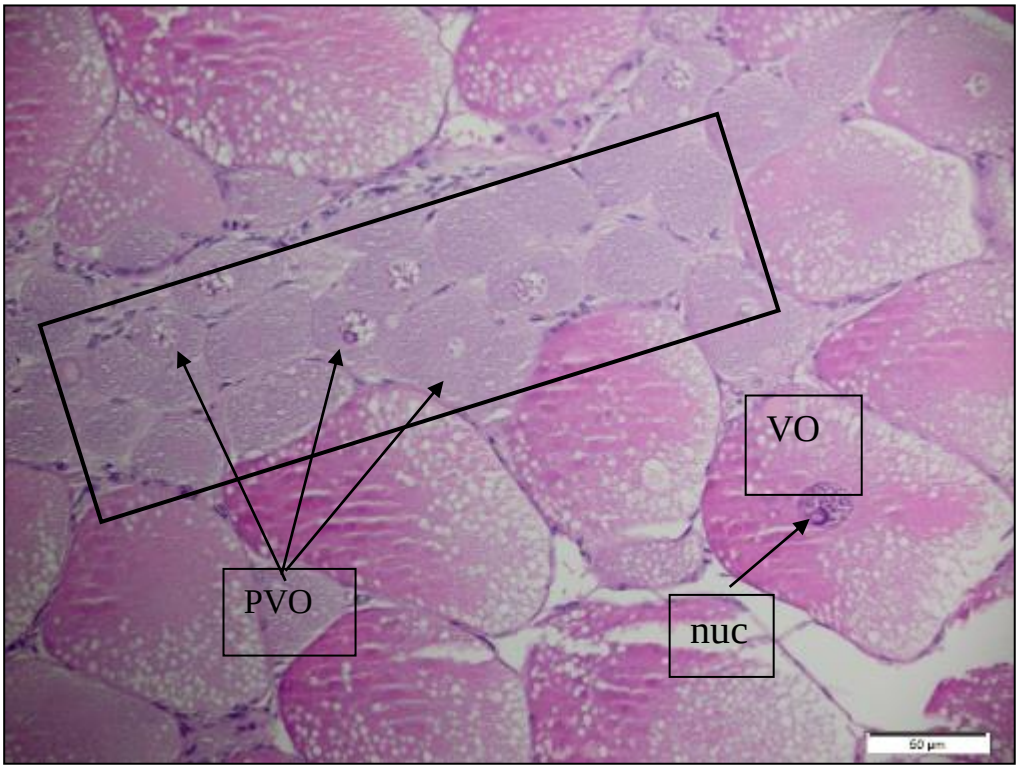


Figure 111: Detail of immature ovarian lobules (Black Square). Note the aggregations and vacuoles observed are artefact from the fixation process [VO: vitellogenic oocyte, nuc: oocyte nucleus, PVO: previtelllogenic oocyte]

FEMALE REPRODUCTIVE ORGAN HISTOPATHOLOGY

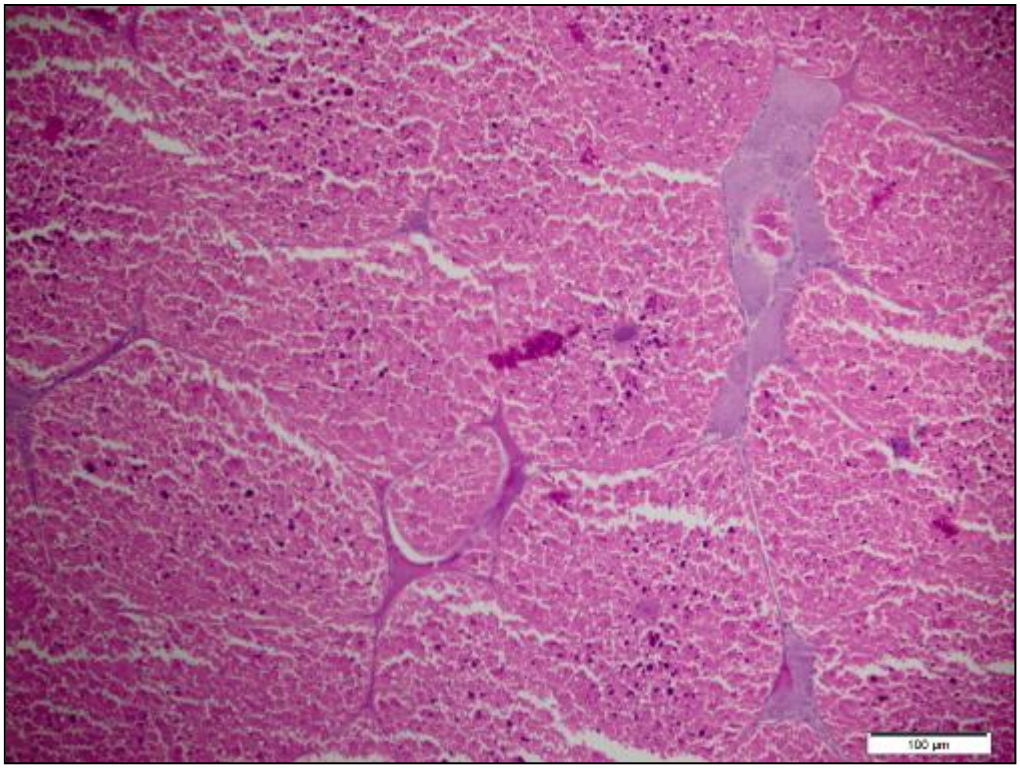


Figure 112: Degeneration of mature ovarian tissue. Note the loss of demarcation between individual oocytes.

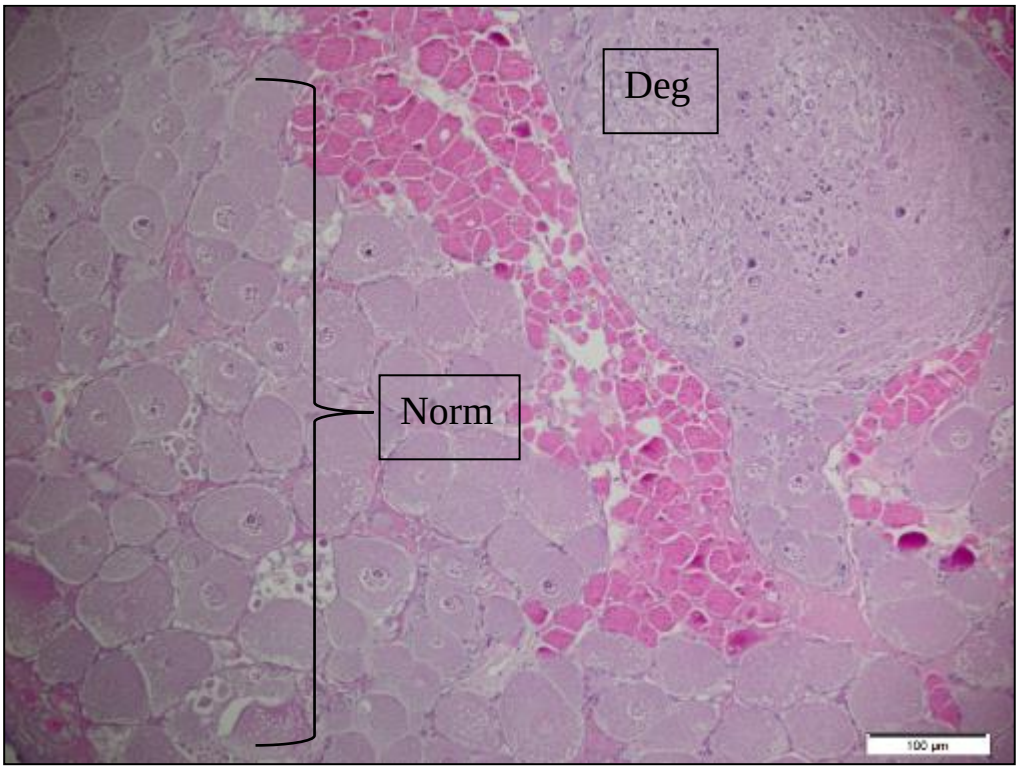


Figure 113: Degeneration of ovarian tissue. Note the difference between the normal and degenerated tissue where individual oocytes cells are no longer identifiable. [Norm: normal tissue, Deg: degenerated tissue, RCT: reserve cell tissue]

PERIPHERAL NERVE NORMAL HISTOLOGY

The nervous system of a decapod crustacean comprises of a dorsal brain that is connected to a ventral longitudinal nerve cord. The ventral cord is located along the ventral aspect of the ventral tail muscle below the caudal gastrointestinal tract. This atlas will focus on the ventral nerve cord that is commonly sampled during a necropsy procedure.

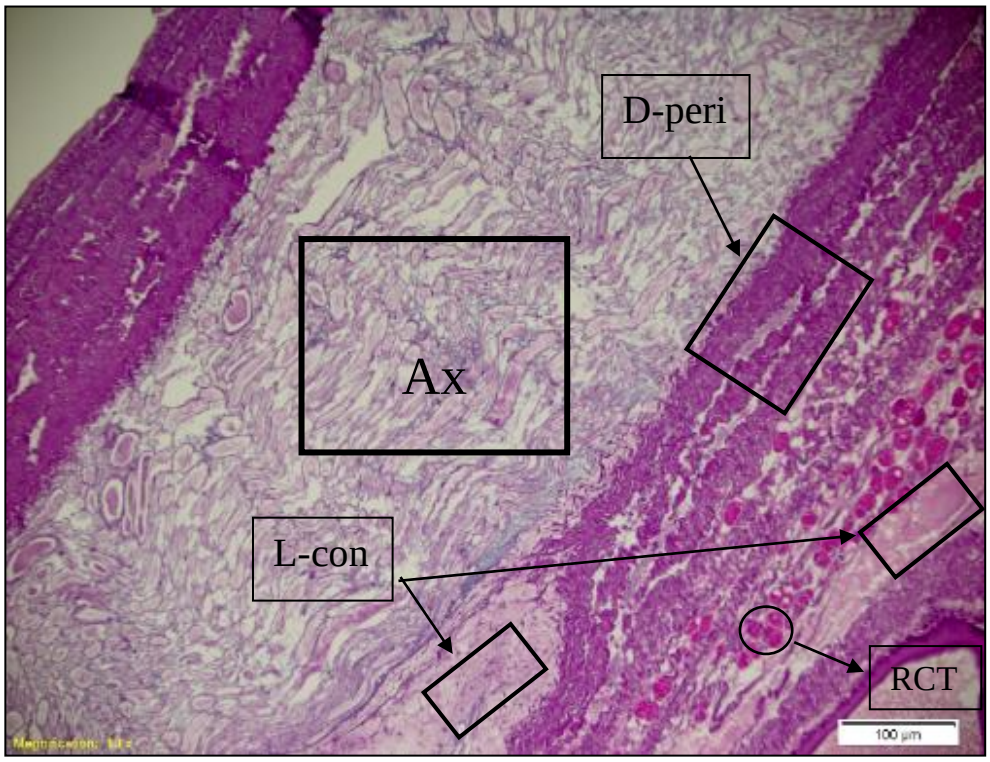


Figure 114: Overview of the peripheral nerve tissue. [L-con: loose connective tissue, RCT: reserve cell tissue, D-peri: dense perineurium, Ax: axons/nerve bodies]

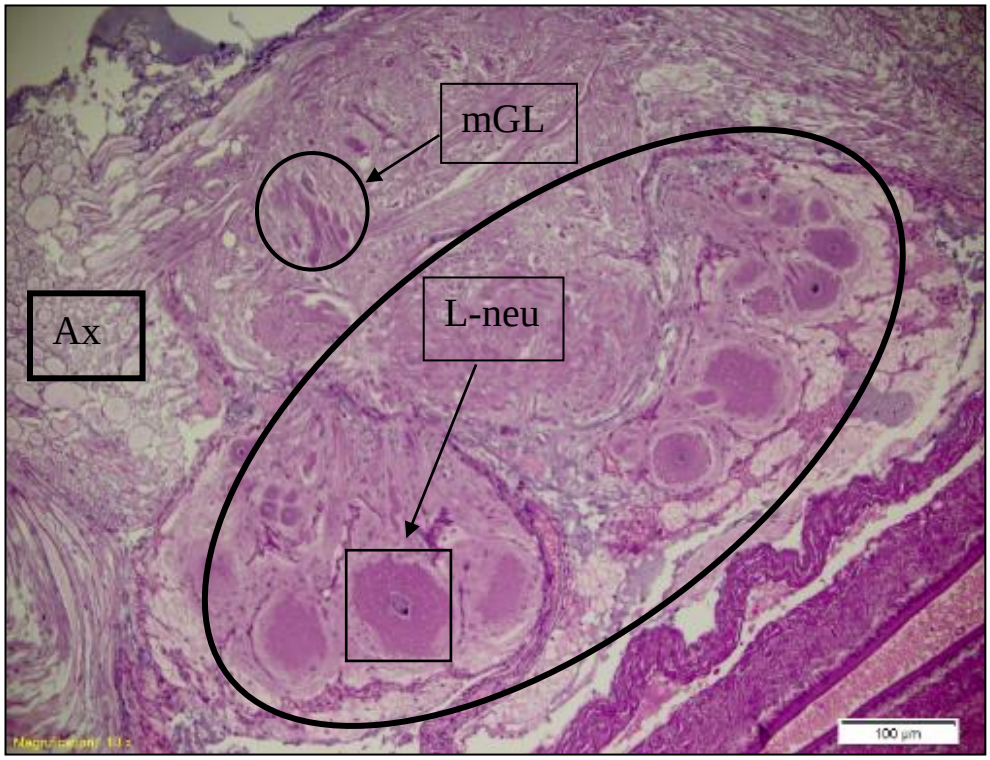


Figure 115: Overview of the peripheral nerve tissue with paired ganglionic bodies (black circle) [mGL: microglia, L-neu: large neuron, Ax: axons/ nerve bodies]

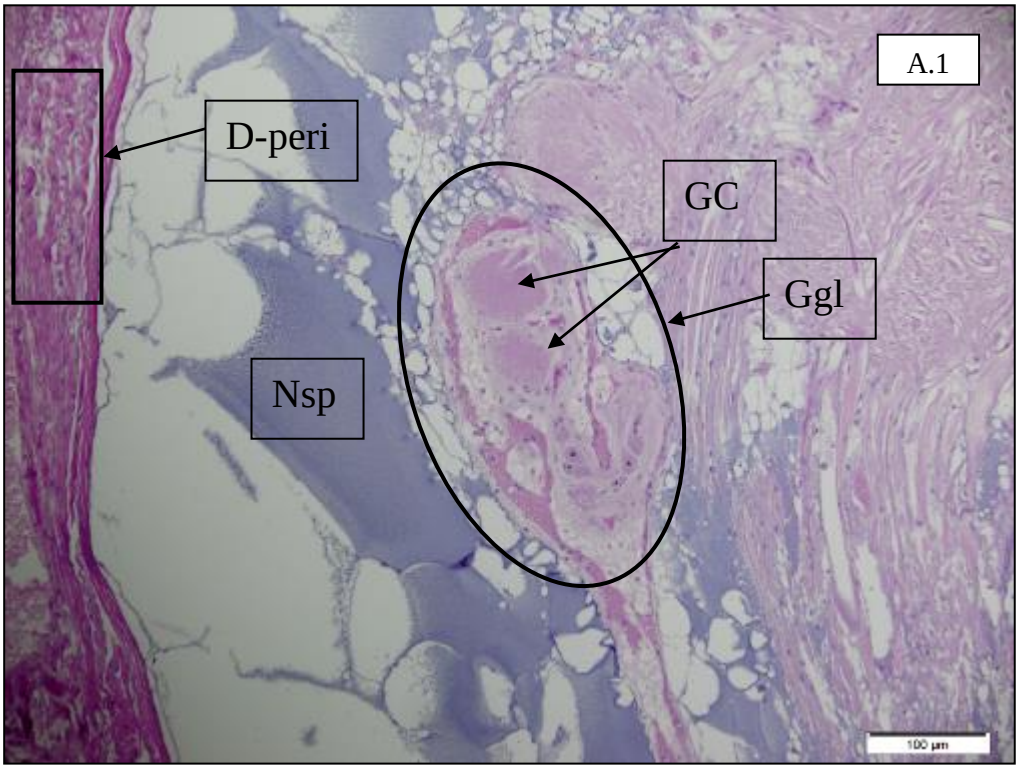


Figure 116: Overview of the peripheral nerve tissue.[D-peri: dense perineurium, Ggl: ganglia, Nsp: neuro-secretory product, GC: giant cell]

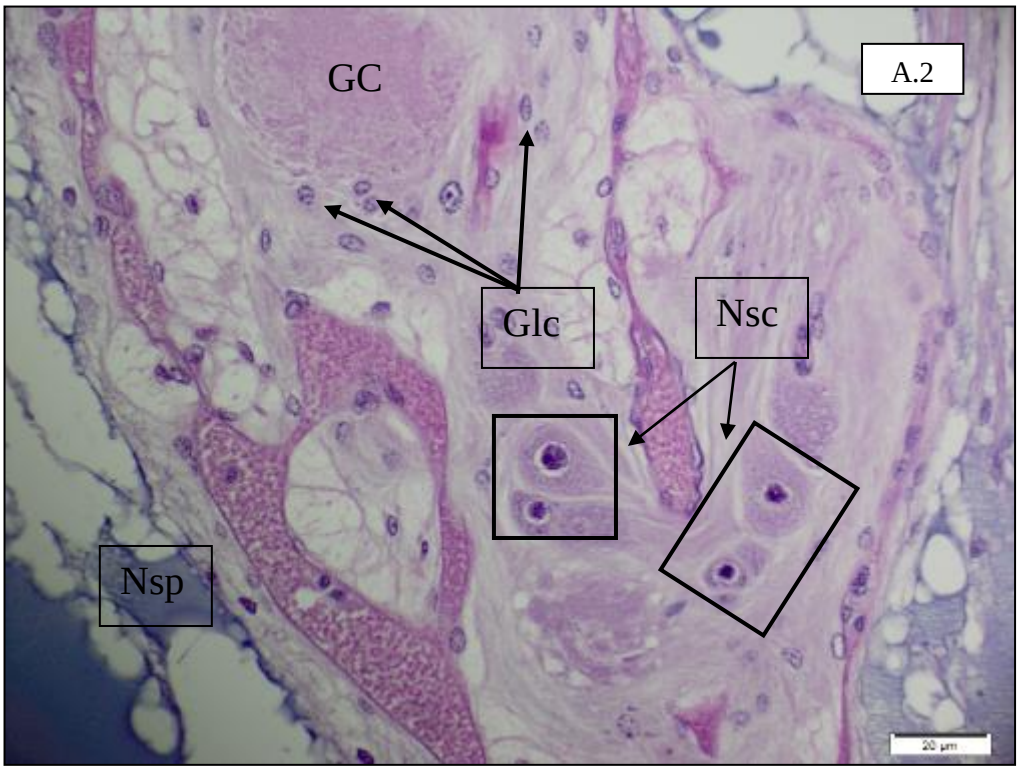


Figure 117: Magnification of image A1 providing further detail to the ganglion. Neurosecretory cells are separated from the giant cells cortical glia. Note cells that surround the giant cells are glia cells. [Nsc: neuro-secretory cells, Glc: glial cells, GC: giant cells, Nsp: neuro-secretory product]

PERIPHERAL NERVE HISTOPATHOLOGY

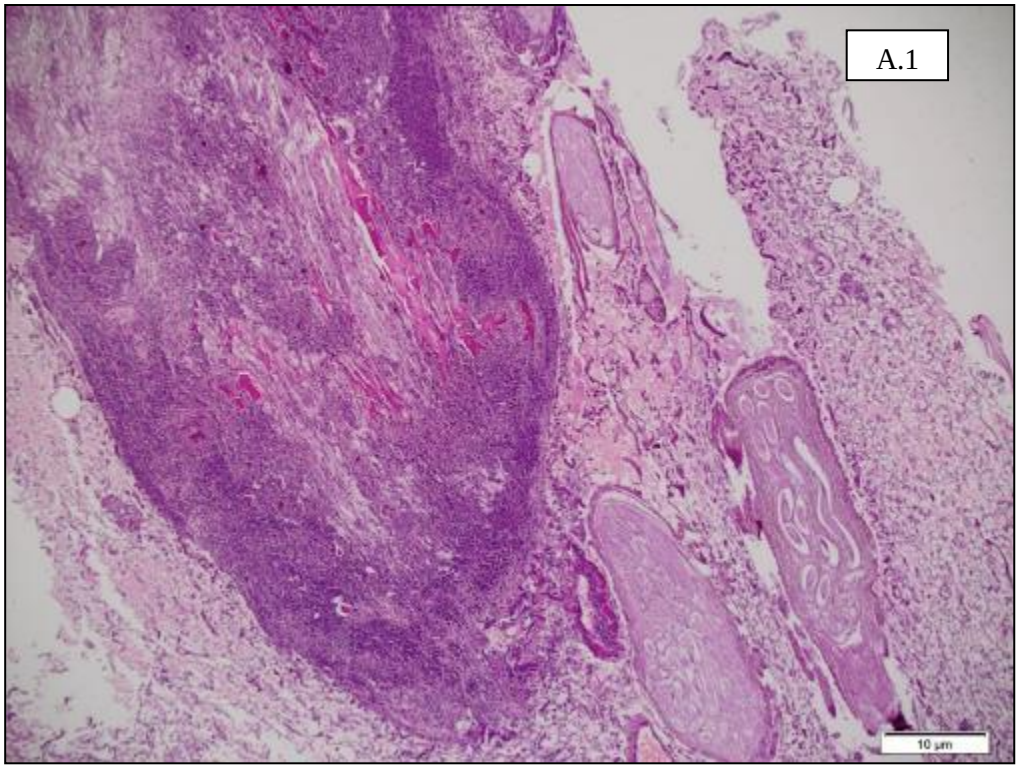


Figure 118: Extensive focal necrotising neuritis of the peripheral nerve.

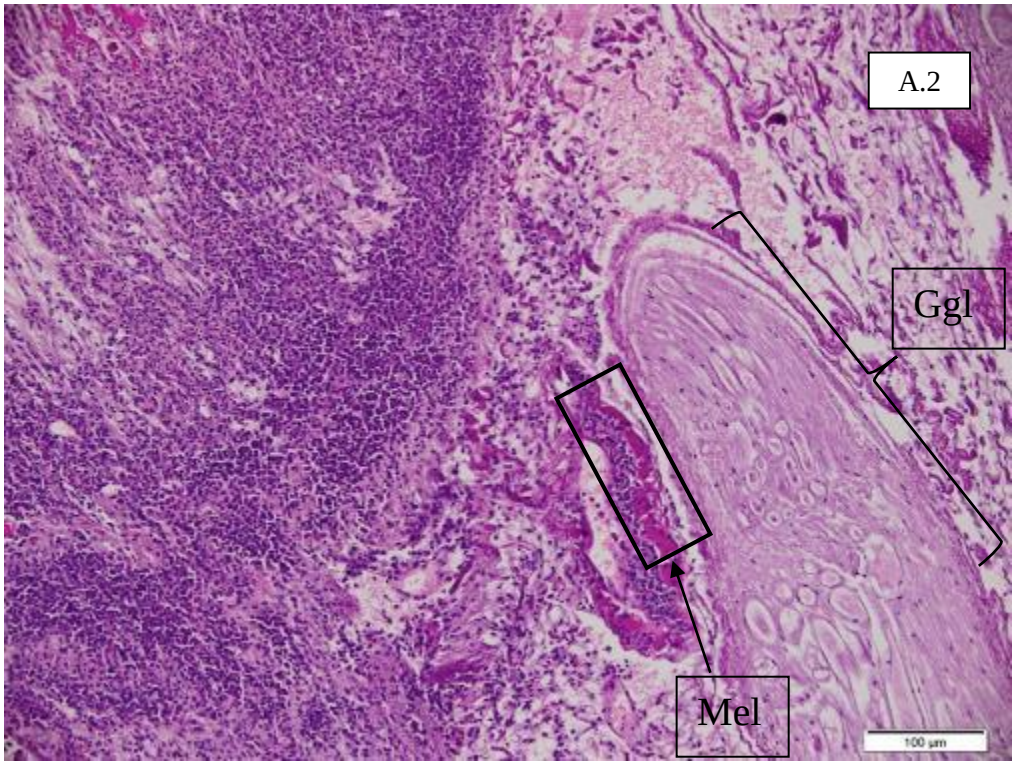


Figure 119: Magnification of image A1 showing focal necrotising neuritis of the peripheral nerve. Note the loss of architecture to the ganglion with vacuolisation present within. Random focal zones of melanisation can also be observed. [Ggl: ganglia, Mel: melanisation]

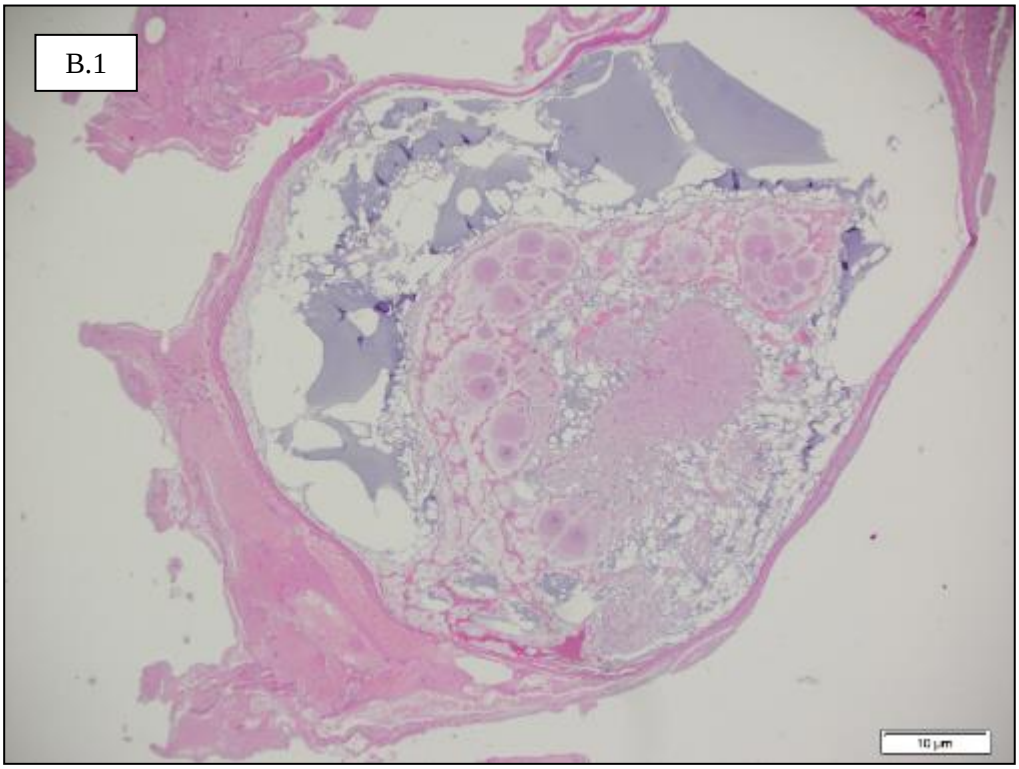


Figure 120: Excessive vacuolisation in the matrix surrounding the nerve

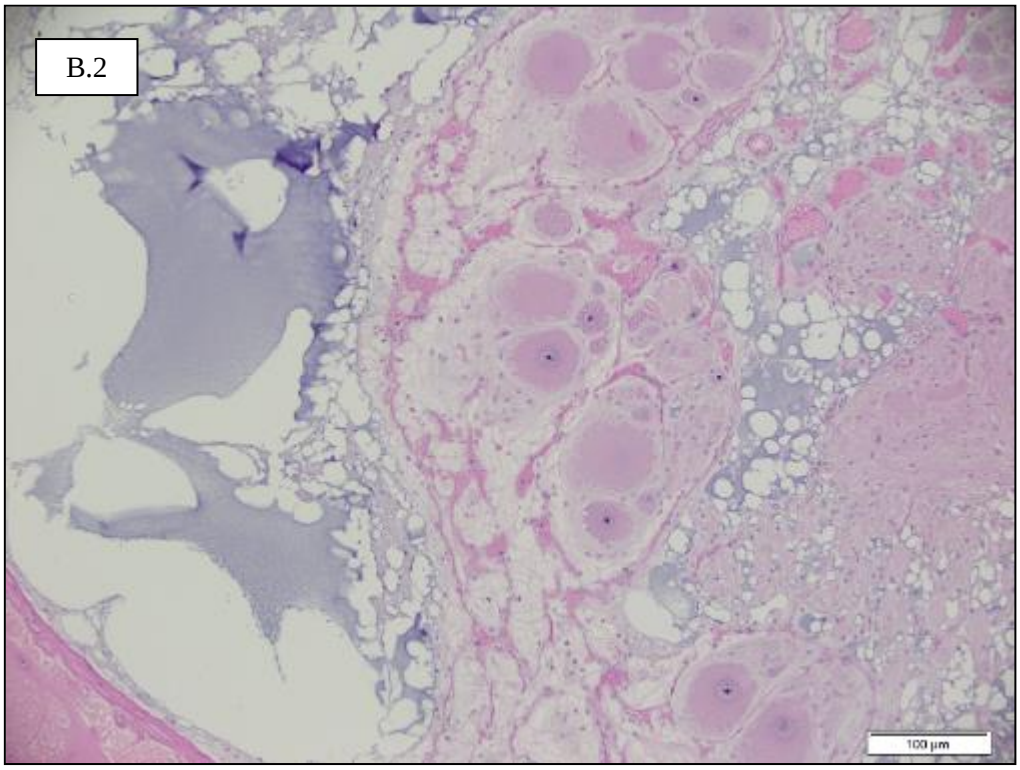


Figure 121: Magnification of image B1 showing excessive vacuolisation in the matrix surrounding the nerve

MUSKULOSKELETAL NORMAL HISTOLOGY

The exoskeletal cuticle comprises of four layers as well as an underlying epidermis. The outmost layer is called the epicuticle (Cut1) followed by the exocuticle layer (Cut2). The third layer is termed the endocuticle (Cut3), and the fourth innermost layer is the membranous / uncalcified layer. Skeletal muscle tissue of crustaceans resemble that of other vertebrates with tightly packed longitudinal muscle fibres organised in a compact arrangement.

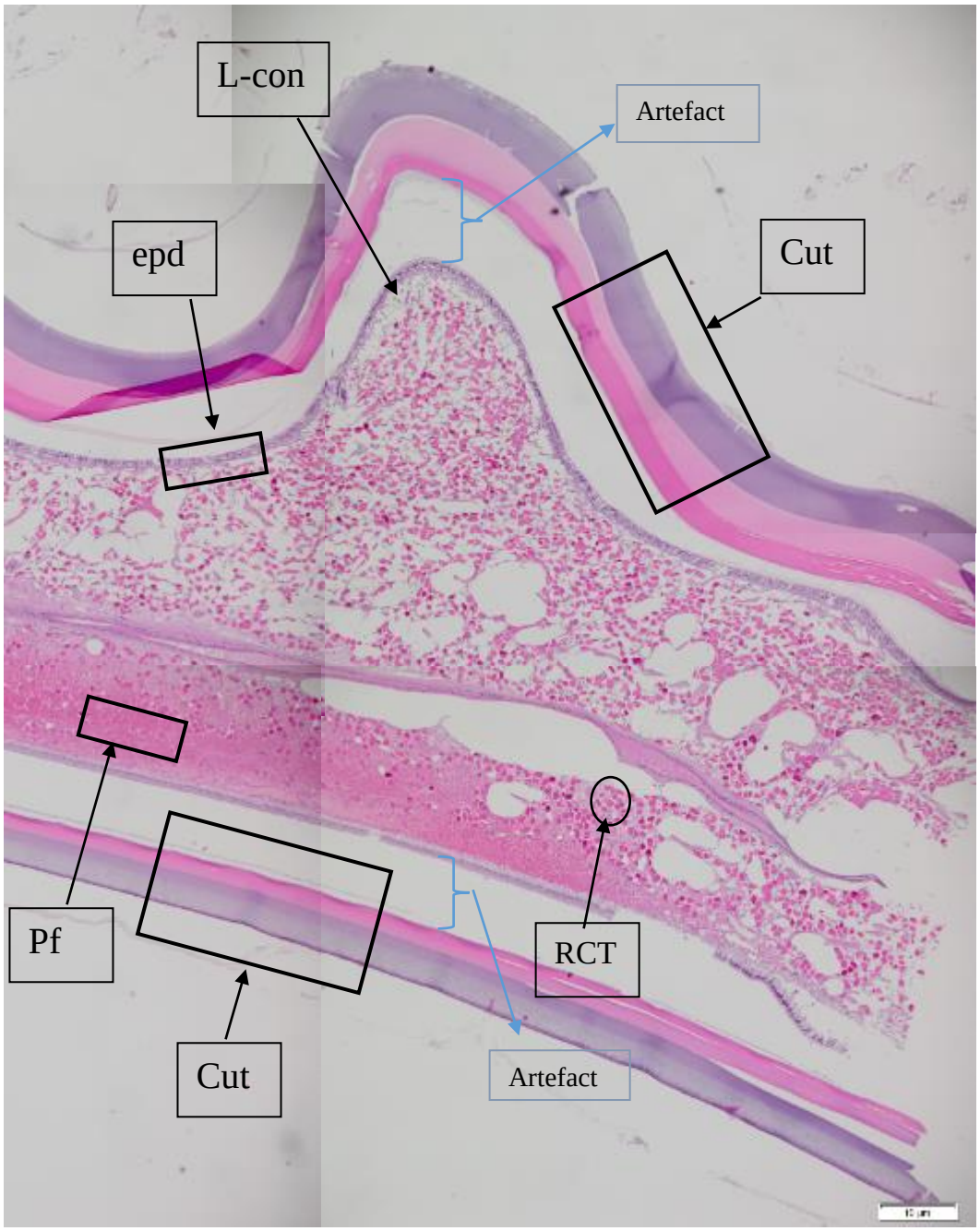


Figure 122: Overview of a longitudinal section of carapace sampled from the dorsal carapace of a SRL. Note the space between the cuticle and epidermis is artefact from the fixation process. [Cut: cuticle, epd: epidermis, RCT: reserve cell tissue, Pf: proteinacious fluid, L-con: loose connective tissue]

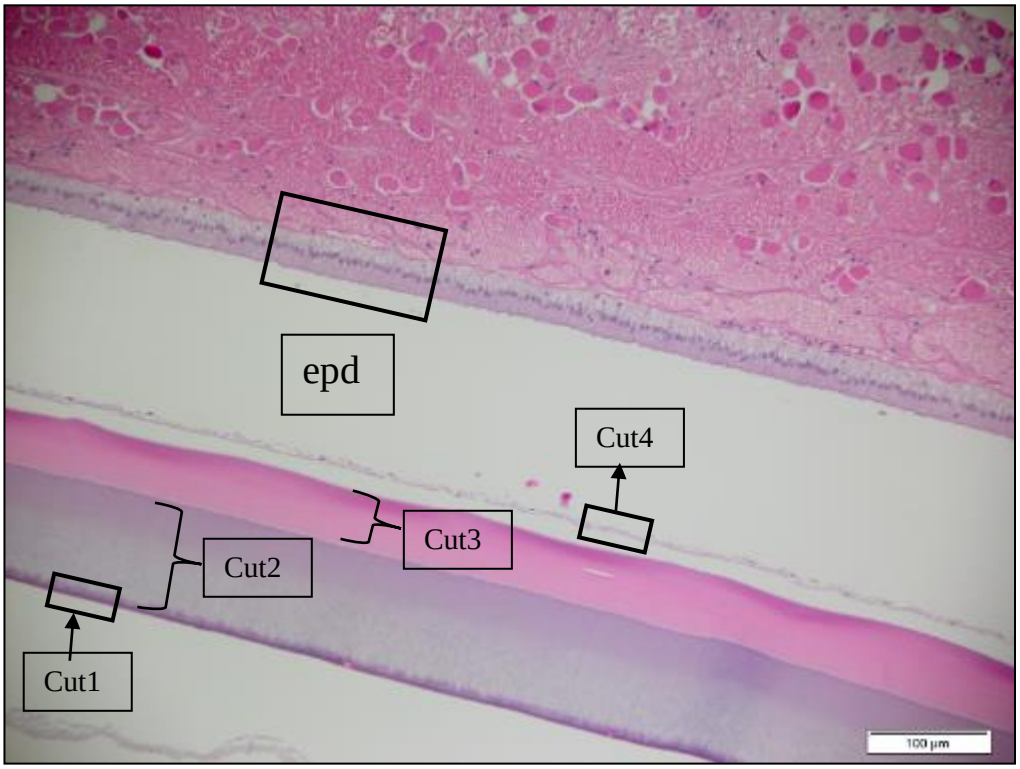


Figure 123: Overview of cuticle layers. Note the space between the epidermis and cuticle as well as between the endocuticle and membranous layer is artefact from the fixation process. [Cut1: epicuticle, Cut2: exocuticle, Cut3: endocuticle, Cut4: membranous layer, epd: epidermis]

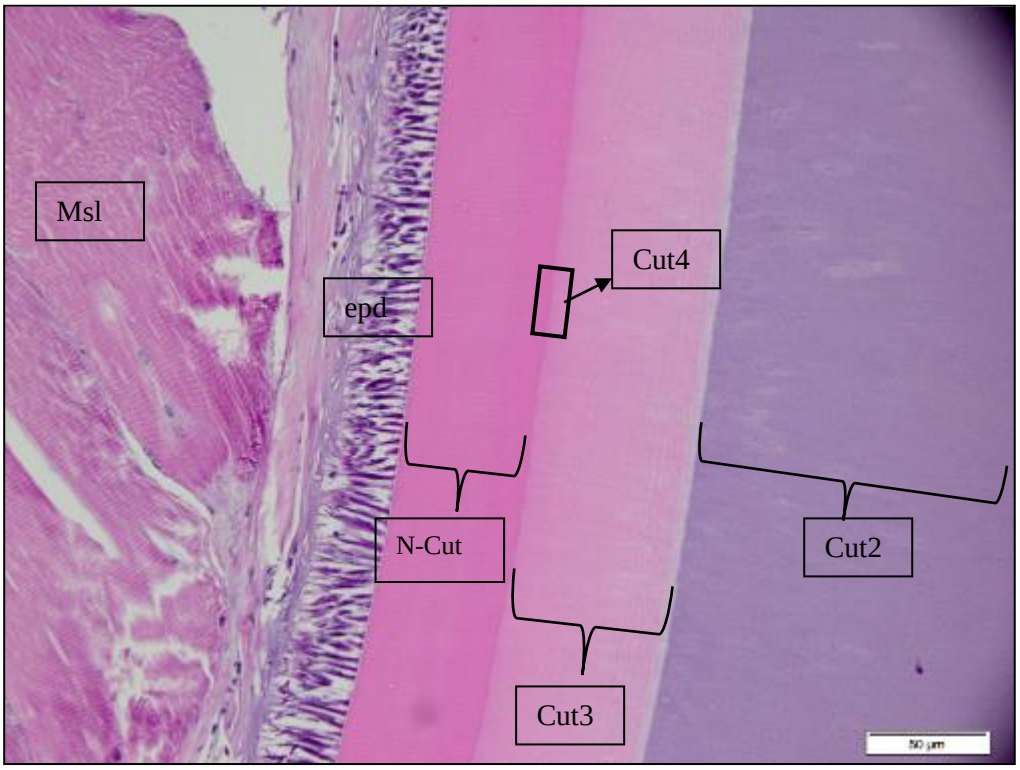


Figure 124: Magnification focusing on cuticle of animal in a moult stage. The new cuticle lies beneath the old. [Cut2: exocuticle, Cut3: endocuticle, Cut4: membranous layer, N-cut: new cuticle epd: epidermis, Msl: muscle]

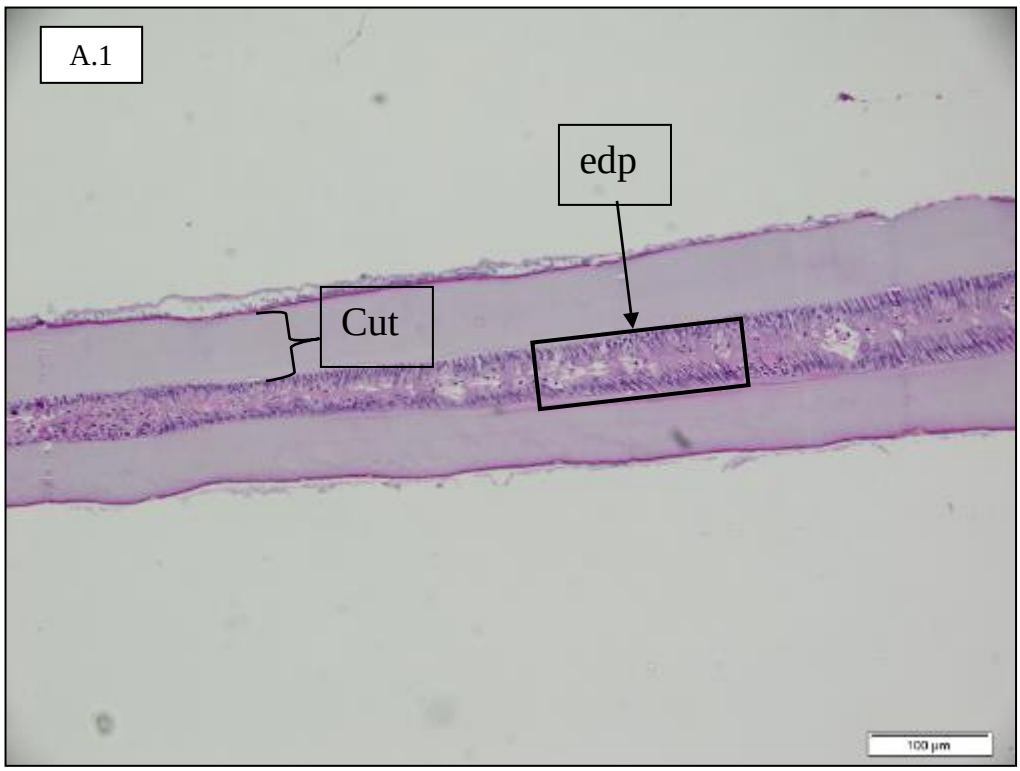


Figure 125: Overview of a longitudinal section of uropod. [Cut: cuticle. epd: epidermis]

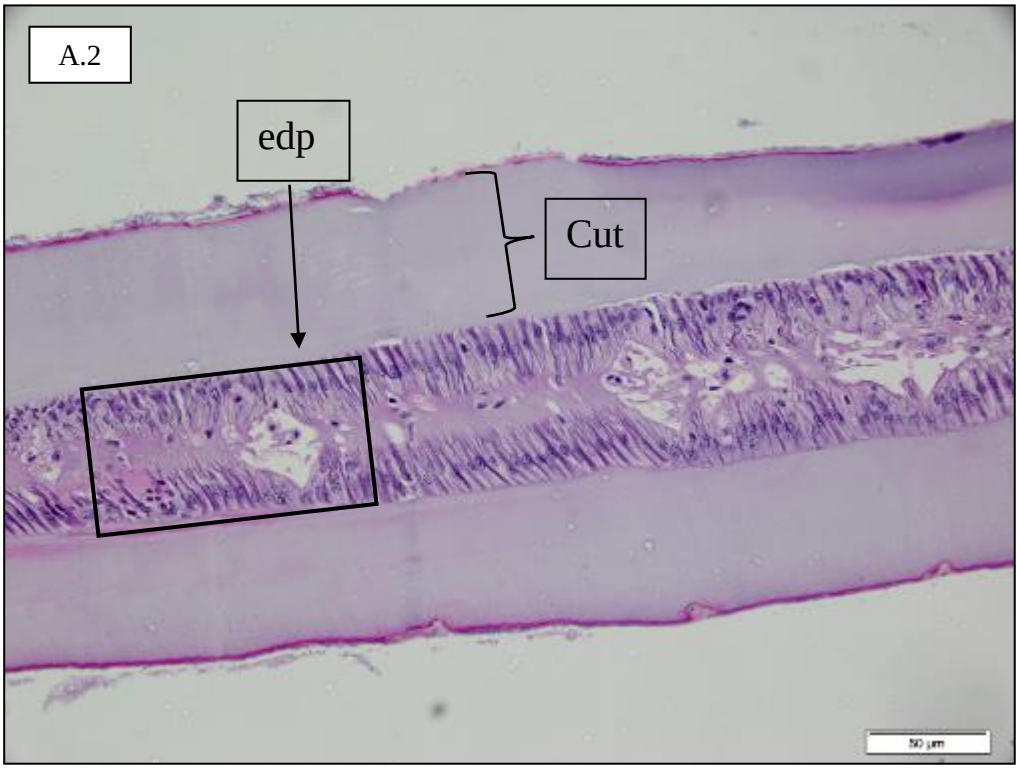


Figure 126: Magnified view of image A1 showing the structure of a normal uropod. [Cut: cuticle, epd: epidermis]

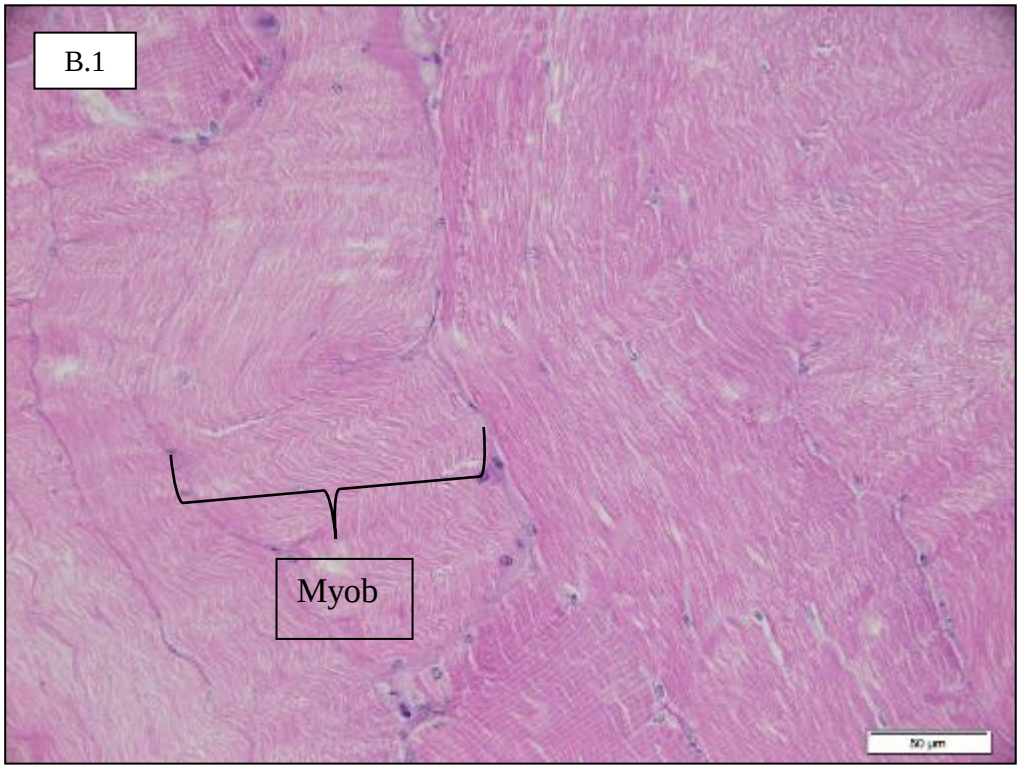


Figure 127: Overview of skeletal muscle tissue of SRL. Note the myofibres are arranged in myobundles. [Myob: myobundles]

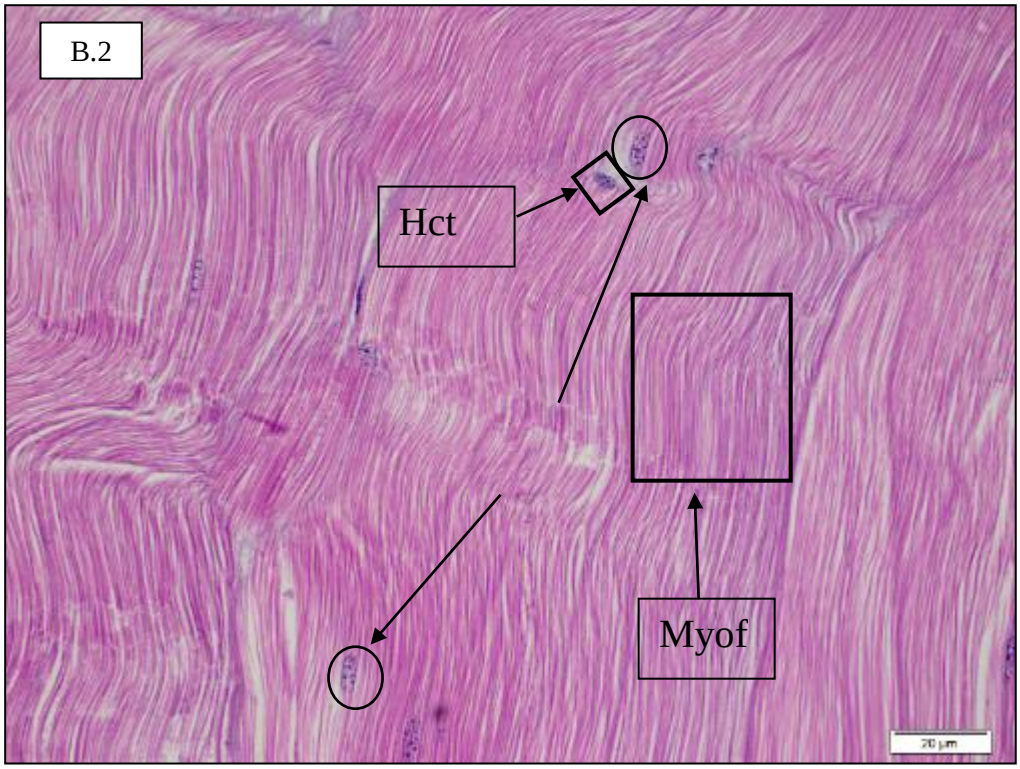


Figure 128: Magnified view of image B1 showing the structural arrangement of skeletal muscle myofibres. [Myof: myofibres, Vmn: vesicular muscle nucleus, Hct: haemocytes]

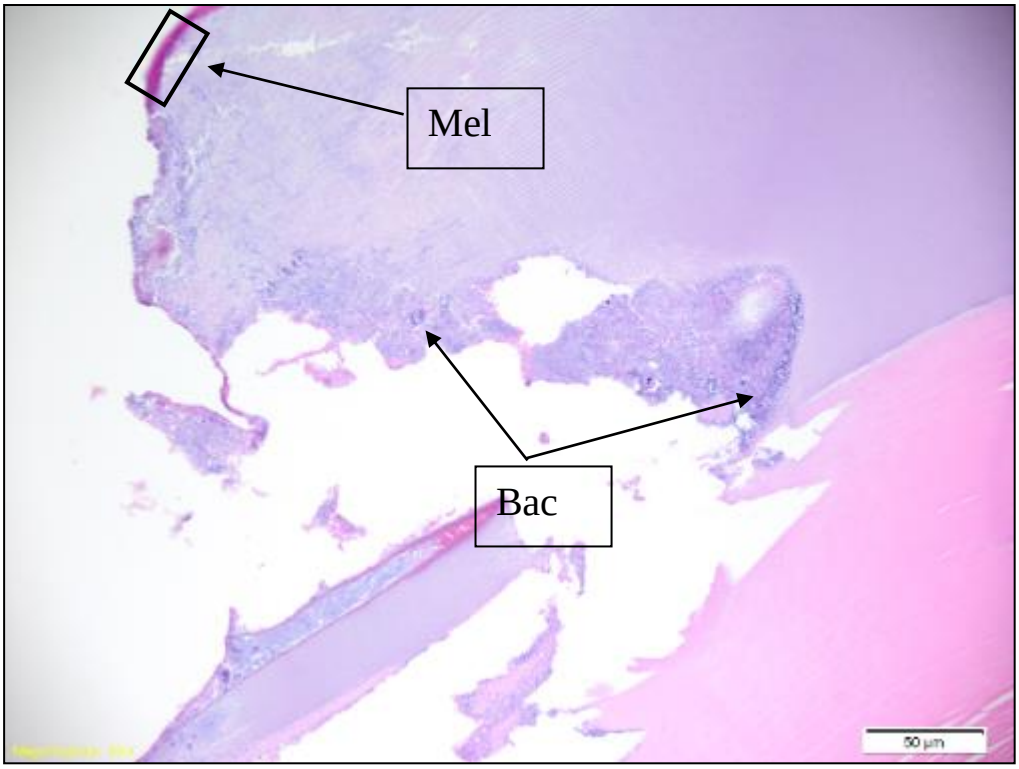


Figure 129: Overview of a melanising erosive lesion with adhered plaques of bacteria found on the carapace. Note the presence of bacterial plaques along the eroded edges. [Bac: bacteria, Mel: melanisation]

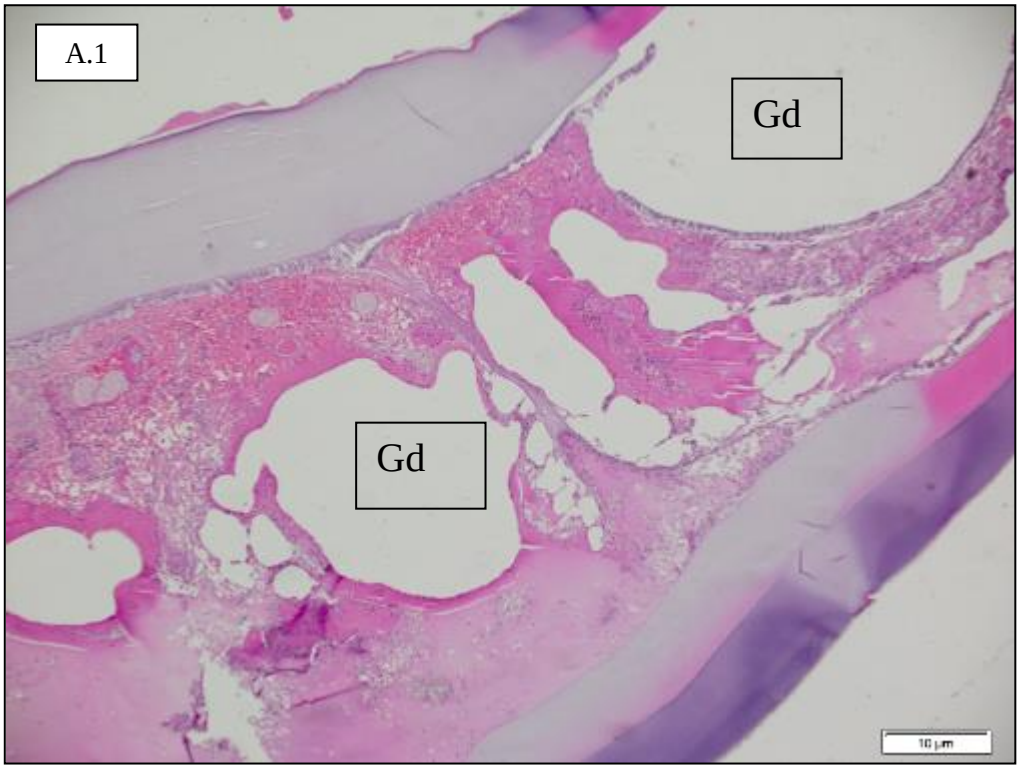


Figure 130: Acute organising haemolymphotoma. Note the large areas of circular gas dilation. [Gd: gas dilation]

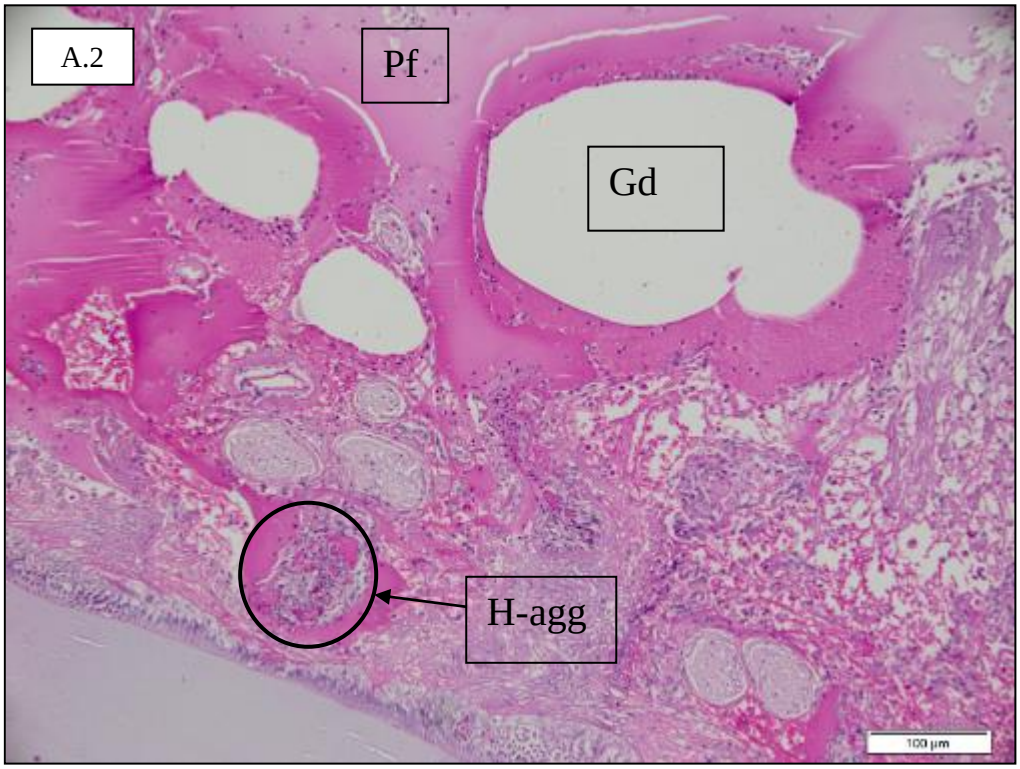


Figure 131: Magnified view of an acute organising haemolymphotoma. Note the large areas of circular gas dilation. [Gd: gas dilation, H-agg: haemocyte aggregations, Pf: proteinacious fluid]

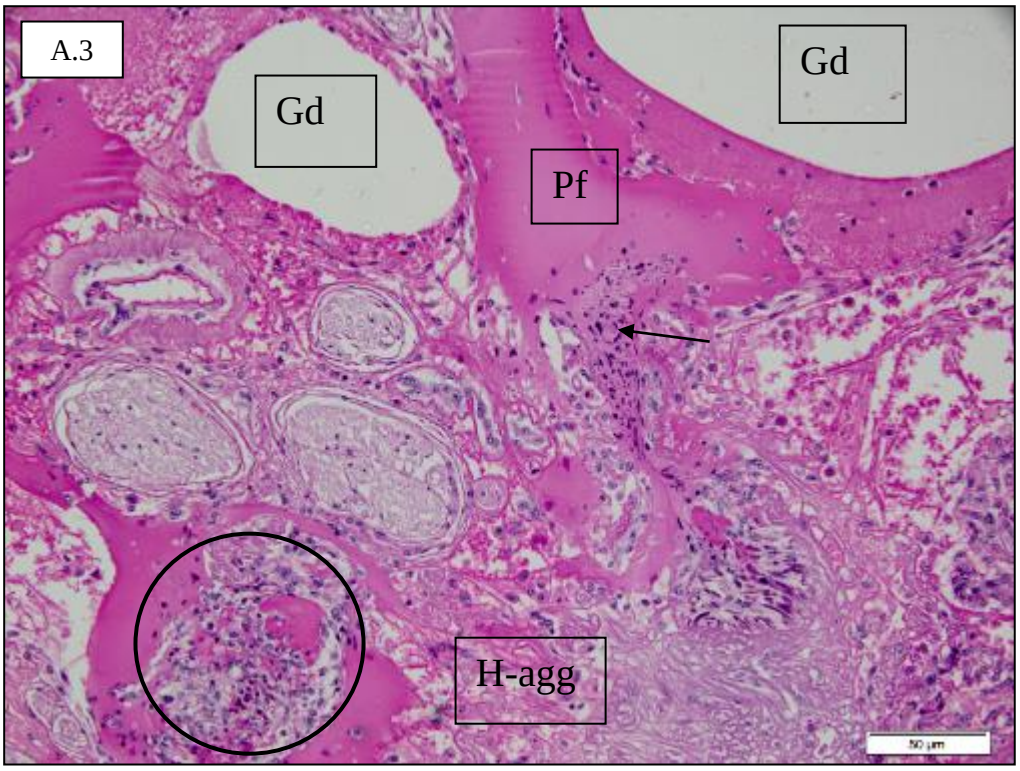


Figure 132: Magnification of image A2 displaying the structure of an acute organising haemolymphotoma. ;Gd: gas dilation, H-agg: haemocyte aggregates, Bac: bacteria, Pf: proteinacious fluid]

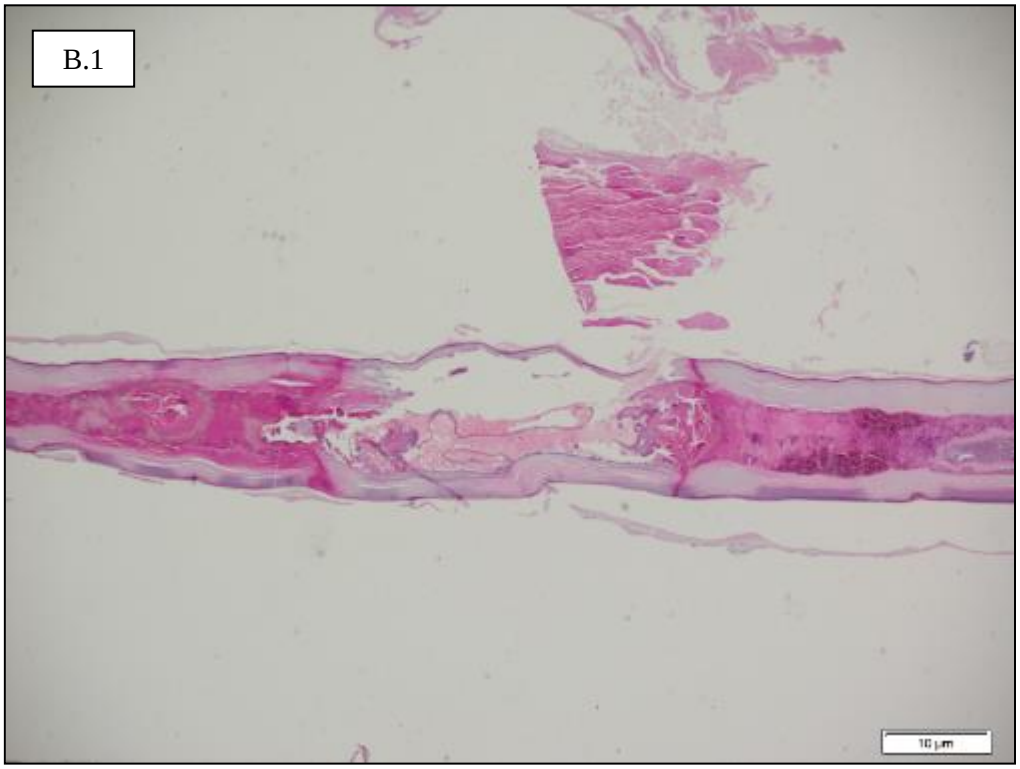


Figure 133: Overview of a melanising ulcerative haemolymphotoma of the uropod.

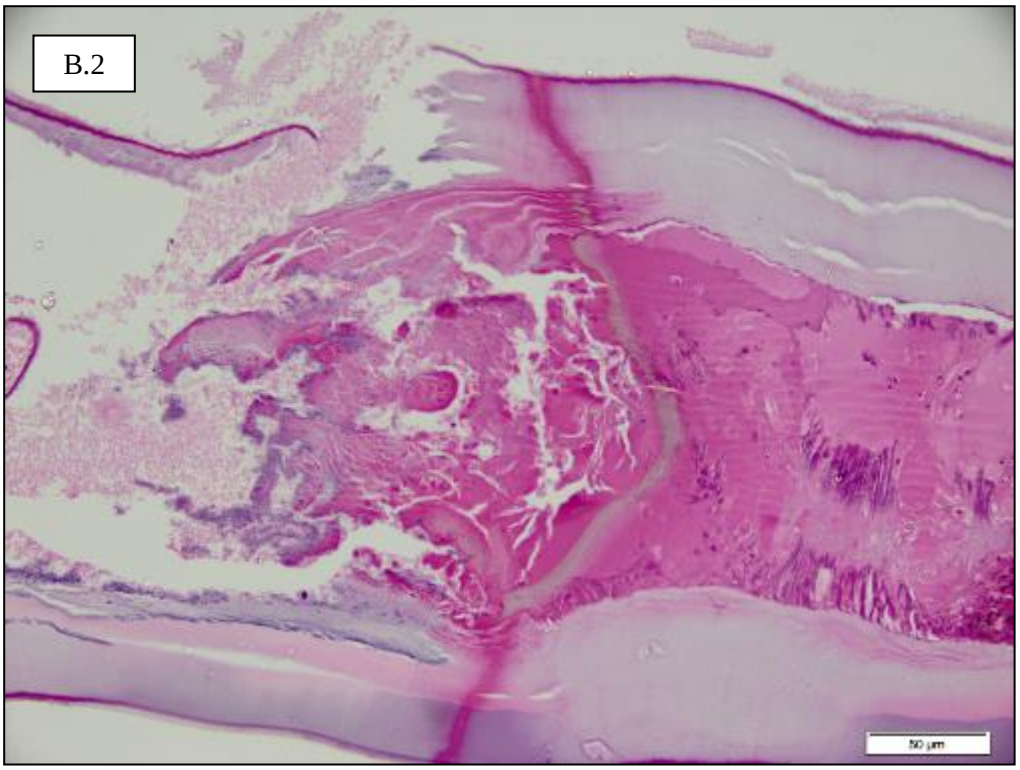


Figure 134: Magnification of lesion displayed on image B1 focusing on the erosion of the cuticle with bacteria observed at the eroded edges. Note the loss of epidermal structure.

Figure 135: Magnification of lesion displayed on image B1 focusing on the melanisation observed in the uropod. Note the loss of epidermal architecture and structure. [Mel: melanisation]

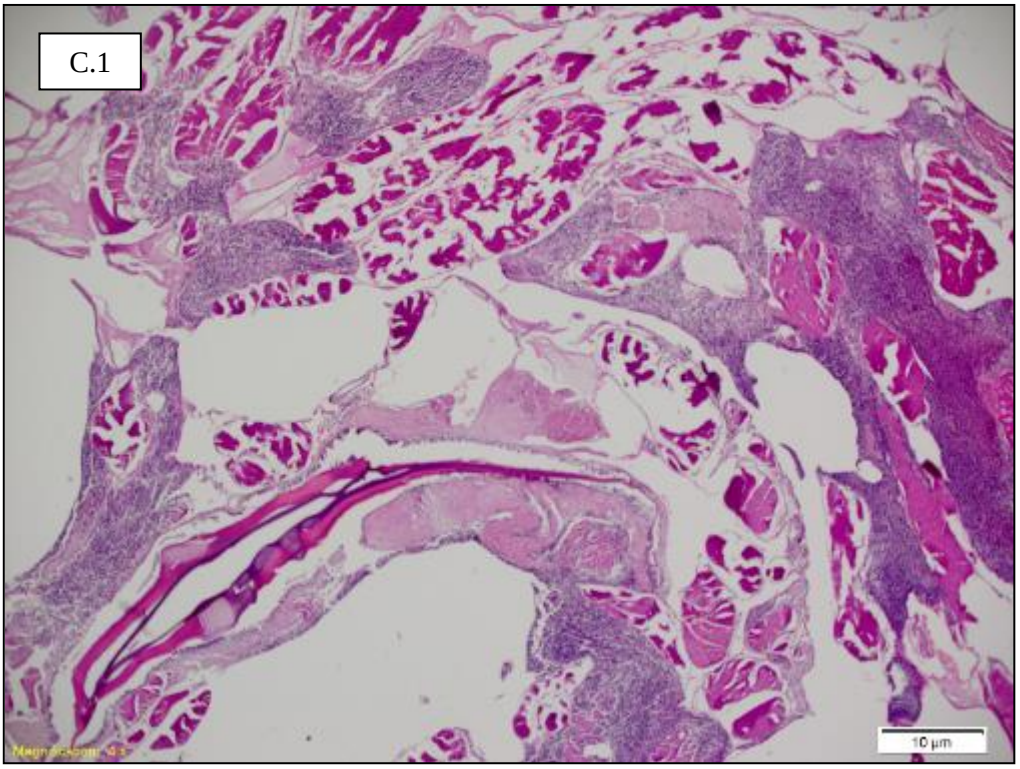


Figure 136: Overview of muscle necrosis. There is liquefaction of the muscles within the section and loss of muscle mass. Marked myositis is present with areas of coagulative necrosis, melanisation and haemolymph clots.

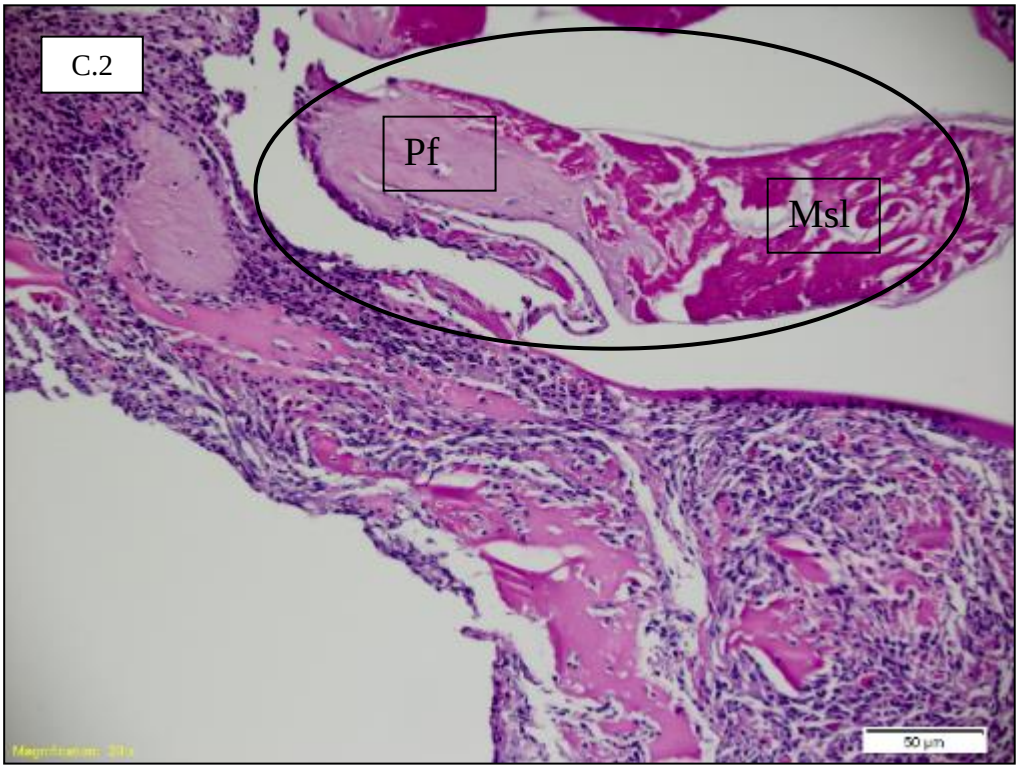


Figure 137: Magnification of image C1 depicting liquefactive necrosis. Note the transition of normal tissue to a proteinacious fluid (black circle). [Msl: muscle, Pf: proteinacious fluid]

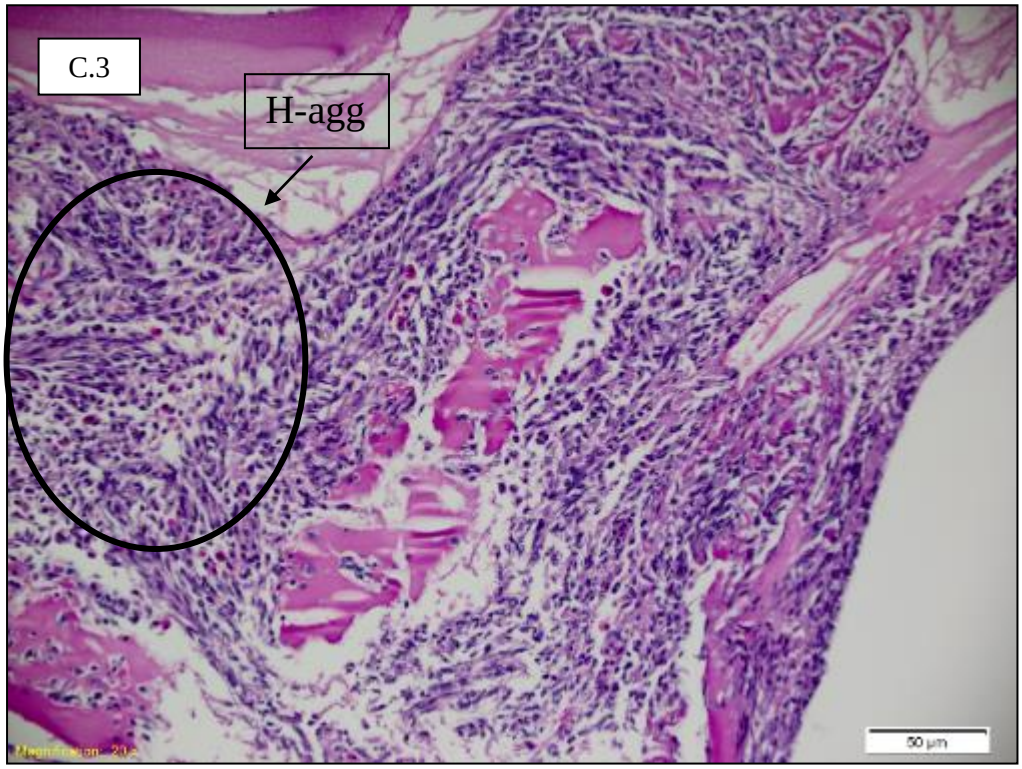


Figure 138: Magnification of image C1 depicting liquefactive necrosis. Normal muscle tissue is no longer visible and appears infiltrated by aggregating haemocytes. [H-agg: haemocyte aggregations]

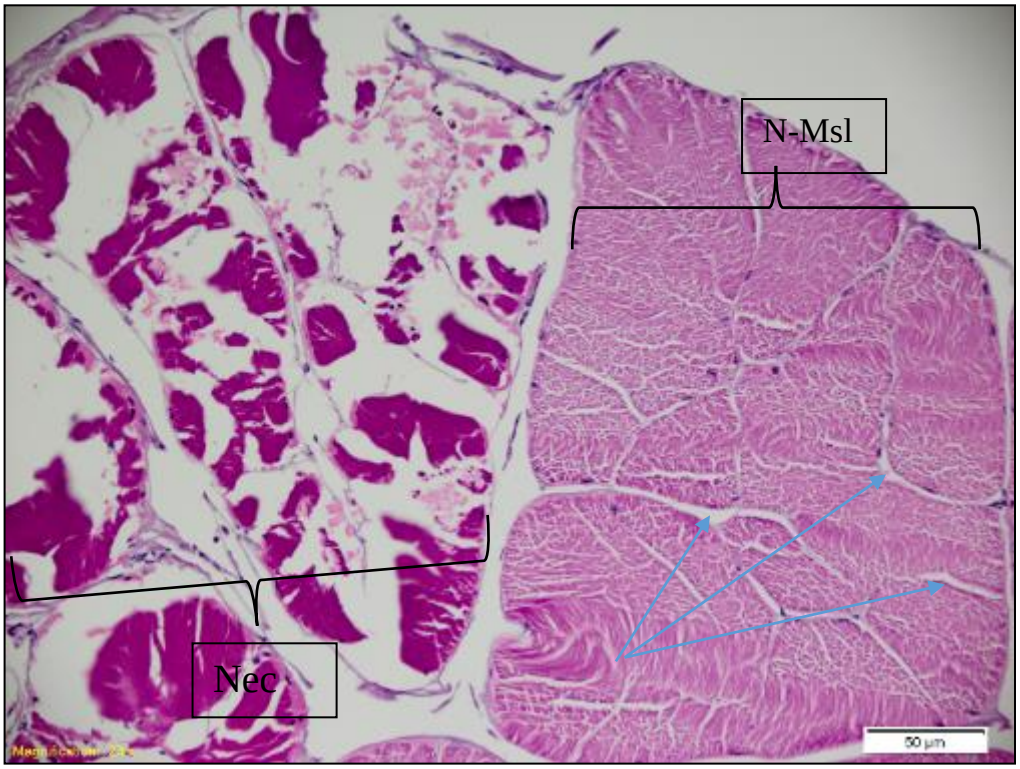


Figure 139: Overview of muscle necrosis compared to normal skeletal muscle. Note the spaces observed in the normal skeletal tissue is blade artefact that occurred during the fixation process (blue arrows). [N-msl: normal muscle, Nec: necrosis]

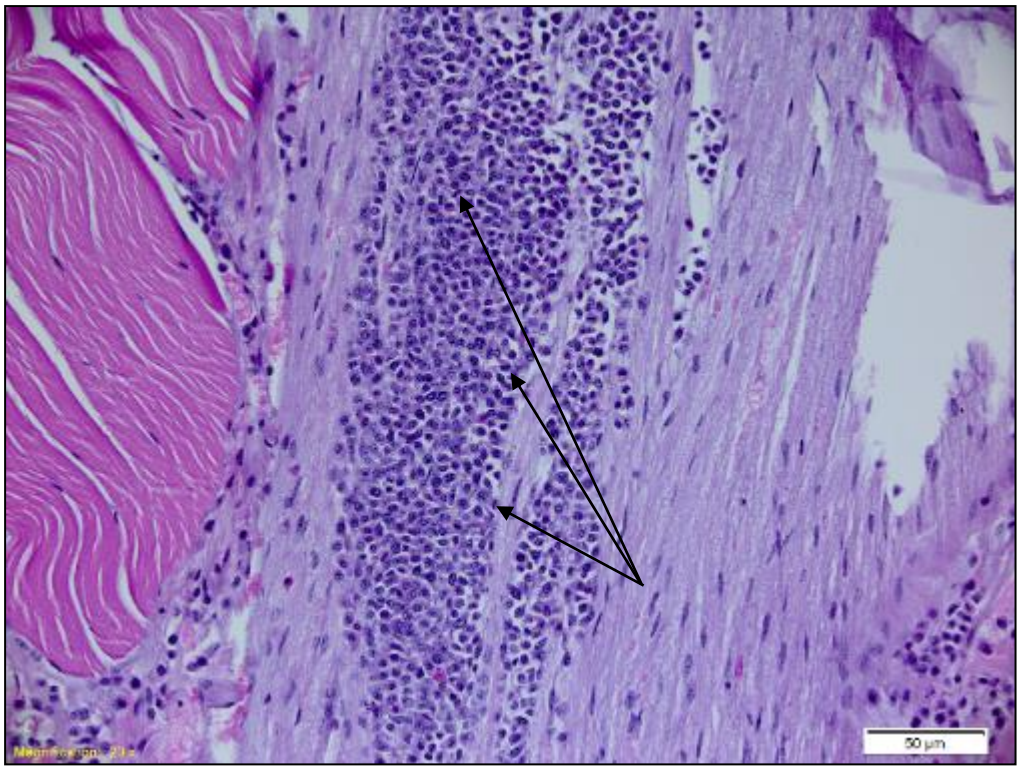


Figure 140: Haemocyte aggregations showing signs of mitosis where chromosal alignment can be seen within the muscle tissue (black arrows) suggestive of a neoplastic change.

References:

1. Bell, T.H., Lightner, D.V. 1988, A handbook of normal penaeid shrimp histology:Aquaculture development program, Department of Land and Natural Resources, State of Hawaii, The Work Aquaculture Society, Baton Rouge, Louisiana.
2. Factor, J., 1995. Biology of the Lobster *Homarus americanus*. 1st ed. Academic Press: ELSELVIER
3. Felgenhauer, B.E, 1992, Internal anatomy of the Decapoda: An overview. In: Humes, A.G. (ed) Decapod Crustacea. Microscopic Anatomy of Invertebrates. Harrison, F.W (series ed) Vol.10. Wiley-Liss , Inc. Pg 45-75
4. Handlinger, J., *et al*. (2006). "Rock Lobster Enhancement and Aquaculture Sub-program; Health Assurance of Southern Rock Lobster." Australian Government Fisheries Research and Development Corporation. FRDC Project No. 2001.094(73)
5. Shields, J.D., R.Boyd, 2014, Atlas of Lobster Anatomy and Histology, Virginia Institute of Marine Science.

FRDC FINAL REPORT CHECKLIST

Project Title:	Improving post-harvest survivability of southern rock lobster in a changing environment		
Principal Investigators:	Quinn P. Fitzgibbon, Charles Caraguel, Stephen B. Pyecroft, Caleb Gardner, Ryan Day, Kandarp Patel, Johanna J. Mahadevan		
Project Number:	2016/235		
Description:	Here we present a 2-year study which focused on characterising the mortality conditions in SRL holding and the development of mitigating strategies to minimise its impact on the lobster exporting and fisheries industries. This project used the disciplines of epidemiology, physiology and pathology along with providing recommendations for "best" industry practices and the initial validation of industry lobster assessment tools.		
Published Date:		**Year:**	
ISBN:		**ISSN:**	
Key Words:	Southern Rock Lobster, Jasus edwardsii, post-harvest, processors and exporting sector, physiology, pathology, health, epidemiology, aquaculture systems.		

Please use this checklist to self-assess your report before submitting to FRDC. Checklist should accompany the report.

	Is it included (Y/N)	**Comments**
Foreword (optional)	N	
Acknowledgments	Y	
Abbreviations	N	
Executive Summary		
– **What the report is about**	Y	
– **Background – why project was undertaken**	Y	
– **Aims/objectives – what you wanted to achieve at the beginning**	Y	
– **Methodology – outline how you did the project**	Y	
– **Results/key findings – this should outline what you found or key results**	Y	
– **Implications for relevant stakeholders**	Y	
– **Recommendations**	Y	
Introduction	Y	
Objectives	Y	
Methodology	Y	Brief as each results section presents specific methologies
Results	Y	Structured as individual scientific manuscripts be submitted to separate journals or as activity reports
Discussion	N	Provided in individual results chapters
Conclusion	Y	
Implications	Y	
Recommendations	Y	
Further development	Y	

Extension and Adoption	Y	
Project coverage	Y	
Glossary	N	
Project materials developed	Y	
Appendices	Y	