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Fish Health Indicators for the Gladstone Harbour Report Card

Final Project Report
Project ISP016c-2018

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The authors would like to take this opportunity to respectfully acknowledge the Traditional Owners of the land on which we live, work and learn, and pay our respects to the Elders, past, present and future for they hold the memories, the traditions, the culture and hopes of Indigenous Australia. In particular we pay our respects to the peoples on whose Country this research was carried out.

Version history

Version Number	Purpose/Changes	Authors	Date
1.1	Initial draft of interim report minus October data – to GHHP	Flint, Anastasi, Jackson, Irving, De Valck, Chua	22/08/2018
1.2	Comments from GHHP incorporated	Flint, Anastasi, Jackson, Irving, De Valck, Chua	30/08/2018
1.3	Initial draft of final report addition of October data, combining Milestones 1, 2 and 3 reports	Flint, Jackson, Anastasi, Irving, De Valck, Chua, Rose, French	06/12/2018
1.4	Final report incorporating comments from ISP	Flint, Jackson, Anastasi, Irving, De Valck, Chua, Rose, French	15/02/2019
1.5	Final report incorporating comments from MC	Flint, Jackson, Anastasi, Irving, De Valck, Chua, Rose, French	22/07/2019

Contents

Executive Summary

As the link between land and sea environments, estuaries are complex ecosystems vulnerable to human impacts, which directly and indirectly affect plants and animals, including fish. Fish are key biological indicators of environmental contamination, as they are water breathers, common in aquatic ecosystems, play a variety of important ecological roles, are readily identified and have high importance to the community.

Various waste water sources, of industrial, agricultural and domestic origins, can pollute downstream waterways. When fish are exposed to contaminated water, they are affected at the population level (numbers and diversity of fish species) down to biochemical impacts on single cells within individual fish. Fish health indicators range from relatively low to high cost and complexity. For this project, preference was given to testing and developing low to medium cost and complexity fish health indicators such as external measurements, pathological changes that can be seen with the naked eye, parasite count, the application of an existing health assessment index, and histopathological analysis (analysis of tissue condition using a microscope by an aquatic veterinarian).

Ultimately, the results of this project will be considered for incorporation into the Gladstone Harbour Report Card, providing stakeholders and the community with accessible information about the condition of Gladstone Harbour, with potential for application to other Northern Australian ports and estuaries. The main outcome is an improved understanding of fish health in Gladstone Harbour (and beyond) leading to the potential for improved environmental and fisheries management practices, marketability of fisheries products and enhanced sustainability of fisheries resources. The objectives of the research project were:

1. To review and identify suitable methods to monitor fish health in Gladstone Harbour.
2. To develop and implement a data collection approach to monitor fish health in Gladstone Harbour that is both cost-effective and suitable for a fish health indicator.
3. To evaluate the potential to adapt and transfer the methods and indicators developed to monitor fish health in other estuaries and ports in Northern Australia (a separate report addressing this objective is provided as Attachment B to this report).
4. To develop fish health indicator(s) based on the data collected.

The key steps considered in the development of fish health indicators are: indicator selection, species selection, site selection, sample size and temporal replication. The project was informed by data collected in April 2018 (Autumn, post-wet season) and September/October 2018 (Spring, pre-wet season); these dates were selected to allow for any seasonal effects on fish health.

Three fish taxa were initially targeted for condition assessments, based on the recommendations of previous GHHP projects, and of the GHHP Independent Science Panel. The three taxa sampled in Autumn 2018 were: barramundi (*Lates calcarifer*), bream (pikey bream *Acanthopagrus pacificus* and yellowfin bream *A. australis*), and large mullet (sea mullet *Mugil cephalus* and diamondscale mullet *Liza vaigiensis*). In Spring 2018, an additional target species, barred javelin (*Pomadasys kaakan*) was added. An important consideration in selection of target species is their relative mobility, or how far they may travel. Fish catch and recapture tagging data was provided from the SunTag program by InfoFish Australia and was valuable in assessing the relative mobility of possible target species. Barramundi in particular are highly transient and the condition of a fish caught in a particular area may have been previously influenced by conditions many hundreds of kilometres away.

Fish were sampled at 12 Gladstone Harbour zones and two reference sites (Stanage Bay and Baffle Creek). Reference sites were selected based on a series of selection criteria relating to their geographical location, human impacts on the local environment, accessibility, availability of habitat suitable for the target species, and their use in previous fish health studies relating to Gladstone Harbour.

Options for suitable fish health monitoring approaches for Gladstone Harbour were identified using researcher knowledge and a range of relevant scientific literature and reports. The review of methods provided several suitable approaches to be tested in Gladstone Harbour, using an adaptive sampling technique. These included Fulton's condition factor (K; a ratio of body weight and length), hepatosomatic index (HSI, a ratio of liver weight to body weight), gonadosomatic index (GSI, a ratio of gonad weight to body weight), health assessment index (HAI, which individually scores damage to seven organs), fluctuating asymmetry of eye diameter (differences in size of left and right eyes), prevalence of parasites and pathogens (diseases, etc), and histopathological analysis of selected fish tissues (checking organs for cell damage using a microscope). Each of these indicators has positive and negative qualities and selection depends on many factors including data availability, resource/ cost constraints, availability of expertise and equipment required for analysis, and the possible environmental impacts occurring at the study site.

In Autumn 2018 a total of 249 fish from 33 species were caught at 12 Gladstone Harbour zones and two reference sites. The species that were caught at the most sites were: barramundi (8 Gladstone zones and 1 reference sites); blue catfish *Neoarius graffei* (8 Gladstone zones); blue threadfin *Eleutheronema tetradactylum* (7 Gladstone zones); barred javelin (6 Gladstone zones); diamondscale mullet (5 Gladstone zones and 1 reference site); and giant queenfish *Scomberoides commersonnianus* (5 Gladstone zones and 1 reference site).

During the Spring 2018 sampling event a total of 291 fish from 33 species were caught at 11 Gladstone Harbour zones sampled and the two reference sites. The species that were caught at the most sites were: barred javelin (9 Gladstone zones, 1 reference site); blue catfish (9 Gladstone zones, 1 reference site); diamondscale mullet (7 Gladstone zones, 1 reference site); blue threadfin (8 Gladstone zones); barramundi (5 Gladstone zones, 1 reference site) and sea mullet (5 Gladstone zones).

All fish were measured, weighed, checked for abnormalities and released, with the exception of target species which were humanely killed for further analysis, up to a maximum of five specimens per site. The gears and methods used were chosen to select for the target species, however additional fish species were also captured, and while most of these were released alive, any fish that died during capture were kept and returned to the laboratory for dissection and future analysis.

Using the results obtained from sampling in 2018, several preliminary fish health measures that are particularly promising for possible inclusion in the fish health indicator for the Gladstone Harbour Report Card have been identified. The two measures that appear most useful for the Gladstone Harbour Report Card in the short term are:

1. **Health Assessment Index (HAI)**: requires a gross pathological analysis during dissection, and produces a score based on the condition of several organs and tissues. The index scores add together to reflect the acute and chronic stressors that are present in the fish's environment. A fish with a high HAI score is less healthy than a fish with a very low score, and individual fish scores can be averaged to give a total HAI for an area.

2. **An index of relative histopathological condition:** requires microscopic study of the changes to tissues caused by disease. A draft metric is in development to compile data across four organ types, this is currently being further tested using organs collected in Spring 2018. The organs selected for this pilot project included gills, liver and a skin/muscle block. Histopathology is a useful indicator as it provides data on the medium-term responses of fish to a wide range of environmental issues. Using the draft metric, a fish with a score of 1.0 is normal, and scores below 1.0 suggest poorer health.

Based on the results of the 2018 pilot sampling year, eight recommendations have been provided for GHHP's consideration.

Recommendation 1: GHHP continues to monitor HAI and histopathology in Autumn 2019, in order to calculate scores for a pilot fish health indicator using Spring 2018 and Autumn 2019 data.

Recommendation 2: GHHP considers whether to provide a wider range of fish tissues for histopathological analysis, to increase the comprehensiveness of fish health assessments.

Recommendation 3: GHHP continues to monitor Fulton's K, HSI, GSI and fluctuating asymmetry of eye diameter to collate a dataset which may in future be used to inform the fish health indicator.

Recommendation 4: GHHP considers testing for bioaccumulation of metals and other toxicants in collected fish tissue samples.

Recommendation 5: GHHP considers including a hook and line fishing component in 2019 to capture more bream.

Recommendation 6: GHHP considers adding barred javelin and blue catfish as target species in 2019.

Recommendation 7: GHHP considers targeting fish sampling at a reduced number of zones in Gladstone Harbour.

Recommendation 8: GHHP considers continuing to sample at reference sites at least once a year.

Introduction

Australia's oceanic borders mean that shipping and port facilities are vital infrastructure enabling international trade. In the state of Queensland there are 20 major ports (DTMR, 2018). Twelve of these operate either within or adjacent to the Great Barrier Reef World Heritage Area (GBRMPA, 2013), one of the seven natural wonders of the world. In recent years renewed attention has been given to the water quality issues that accompany port activities in Queensland. Port areas are like any other coastal area, in that water quality is affected by terrestrial activities as well as marine activities. The activities that take place in ports, including shipping, port-side industries, construction and maintenance, can also have environmental impacts. These impacts can be more pronounced in ports that are situated in estuaries, which themselves may be heavily influenced by discharge from modified, industrialised or agriculturally-impacted river catchments (Flint, Jackson, Wilson, Verlis, & Rolfe, 2015).

Estuaries, as the link between terrestrial and aquatic environments, are particularly complex ecosystems. The development of many human activities close to estuaries leave these transitional systems vulnerable to impacts, which can directly and indirectly affect biota including both resident and migratory fish species (Whitfield & Elliott, 2002). Northern Australian estuaries can mainly be classified as tide-dominated tropical estuaries, with semi-diurnal tides and summer rainfall (Boyd, Dalrymple, & Zaitlin, 1992).

As decision makers and the public call for robust information on the condition of aquatic ecosystems, report cards have become increasingly popular as a tool for communicating relative environmental performance and trends (Flint, Rolfe, et al., 2017). Report cards facilitate the transformation of suites of relevant ecological indicators into management tools. The pilot report card for Gladstone Harbour was released in 2014 and was the first report card scoring environmental health of a port in northern Australia.

In order to be more than a useful means of documenting decline, environmental reporting needs to adaptive, scientifically current, linked to clear objectives, responsive to changing values and suitable for informing management actions (Bunn et al., 2010). One of the most important steps in ensuring these needs are met is selecting environmental indicators to be monitored and reported. Ward, Butler, and Hill (1998) describe environmental indicators as *"physical, chemical, biological or socio-economic measures that best represent the key elements of a complex ecosystem or environmental issue. An indicator is embedded in a well-developed interpretative framework and has meaning beyond the measure it represents"*. Indicators are used to evaluate the fundamental condition of the environment without capturing all of the complexity associated with an ecosystem (Whitfield & Elliott, 2002).

Fish are key biological indicators of environmental contamination, as they are continuously exposed, ubiquitous in aquatic ecosystems, and play a variety of important ecological roles including as prey, predators and habitat modifiers (Van der Oost, Beyer, & Vermeulen, 2003). Another advantage over other aquatic organisms, such as invertebrates, is that there are relatively few fish species and most can be quickly identified in the field (Pidgeon, 2004). As a component of human diets, fish are also useful indicators in situations where there are contaminants of concern to human health. When employing fish as indicators of ecosystem health, most Australian report cards consider population and community-level fish health indicators (FPRH, 2016; Healthy Land & Water, 2017; HRRP, 2017). While this approach is well-tested and generally doesn't require any laboratory analysis following field sampling, its limitations are that extensive field work can be required to ensure the monitoring program is both spatially and temporally representative, avoids sampling gear bias and guarantees sufficient statistical power to detect change. In circumstances where it is possible to establish a

representative sampling regime, population and community-level indicators, such as probability of encounter, are useful tools for reporting on a site-specific level. Previous studies have developed such fish assemblage indicators for estuaries, including in Northern Australia (Sheaves, Johnston, & Connolly, 2012).

An alternative approach to population and community-level indicators is to measure indicators of individual fish health, such as morphometry, gross pathology, histopathology (Bernet, Schmidt, Meier, Burkhardt-Holm, & Wahli, 1999; Mishra & Mohanty, 2009), fish parasite load and diversity (Sasal, Mouillot, Fichez, Chifflet, & Kulbicki, 2007) or chromosomal mutations (Pak, Moiseenko, Sergienko, & Chitaeva, 2012). This report focuses on these individual fish health indicators; however we recognise that population and community-level indicators are useful measures of environmental condition and may be the most appropriate option in some situations.

For the purposes of this report, individual fish health is defined as structural and morphological health and functioning in terms of the physiology of the organism (Whitfield & Elliott, 2002). If a broader population-level sampling regime is being established, individual fish health indicators can also be incorporated for particular species or areas of interest. Fish are useful biological indicators of conditions in estuaries for several reasons, as described by Schlacher, Mondon, and Connolly (2007): 1. measuring physicochemical parameters does not always provide information on ecological responses to pollution, while monitoring fish response provides a direct measure of the ecological consequences of human impacts on the environment; 2. because many fish are relatively long-lived the impacts of pollution are measured over longer periods than physicochemical variables, which are typically highly variable in time; 3. the important trophic role of fish allows for the measurement of higher order pollution effects than can be gained from other biota, such as aquatic plants; 4. fish allow for a multiple-lines-of-evidence approach to data collection through the use of several species and different endpoints; and 5. the high public profile and socio-economic importance of many estuarine fish species results in a more positive public response to environmental management.

As well as describing the benefits of using fish as indicators of biological integrity, Whitfield and Elliott (2002) provide a summary of some of the issues associated with fish indicators, as follows: 1. sampling gears are selective for certain habitats, sizes and species; 2. fish are mobile on seasonal and diel time scales, which can lead to sampling bias; 3. fish may be less susceptible to toxicants than other biota; 4. because fish are mobile, they can move away from localised pollutant inputs; and 5. even estuarine environments that have been modified by human activities still contain diverse fish assemblages. The authors emphasise that these negative aspects are outweighed by the many advantages of using fish for biological monitoring of the aquatic environment, especially given that some of the negative points apply equally to other aquatic biota. However, it pays to be cognisant of these potential issues when selecting fish indicators for biological monitoring and environmental reporting programs.

The aim of this research project was to develop a fish sampling program and fish health indicators for the Gladstone Harbour Report Card. The project builds upon previous studies commissioned by GHHP (Cowled, 2016; Kroon, Streten, & Harries, 2017). A first round of sampling was undertaken during April 2018 (Autumn), and a second trip was completed in September October 2018 (Spring); the dates were selected to allow for any seasonal effects. For this project, preference has been given to testing and developing low to medium cost and complexity fish health indicators such as external morphometry, gross pathology, parasite count and the application of a health assessment index (Cowled, 2016). Histopathological analysis of three organs has also been included in the project as a potentially useful indicator of chronic exposure to stressors.

Ultimately, the results of this project will be considered for incorporation into the Gladstone Harbour Report Card, providing stakeholders and the community with accessible information about the condition of Gladstone Harbour, with potential for application to other Northern Australian ports. The primary outcome is an improved understanding of fish health in Gladstone Harbour (and beyond) leading to the potential for improved environmental and fisheries management practices, marketability of fisheries products and enhanced resource sustainability. Specifically, the objectives of the research project were:

1. To review and identify suitable methods to monitor fish health in Gladstone Harbour.
2. To develop and implement a data collection approach to monitor fish health in Gladstone Harbour that is both cost-effective and suitable for a fish health indicator.
3. To evaluate the potential to adapt and transfer the methods and indicators developed to monitor fish health in other estuaries and ports in Northern Australia.
4. To develop fish health indicator(s) based on the data collected and apply them to the Gladstone Harbour Report Card.

Methods

Permits and approvals

The following permits and approvals are in place for this research:

- General Fisheries Permit (Queensland Department of Agriculture and Fisheries; Permit Number 196040)
- Animal Ethics Approval (CQUniversity Animal Ethics Committee; Approval Number 20969)
- Authorisation for research in the Great Barrier Reef Marine Park (Approval Number G18/03-029)
- Field Work Risk Assessment (CQUniversity OHS Unit)

Sampling design

Site selection

Gladstone Harbour Sites

All 13 of the GHHP Gladstone Harbour water quality zones were considered as potential monitoring sites for fish health indicators (Figure 1). For consistency in reporting, attention was given to sampling sites used for other indicators in the Gladstone Harbour Report Card (e.g. the mud crab indicator; Flint, Anastasi, et al. (2017)) and previous fish health studies in Gladstone Harbour including Wesche, Lucas, Mayer, Waltisbuhl, and Quinn (2013) and Dennis et al. (2016).

The selection criteria and scoring system used to assess long term monitoring sites are described in Box 1. All 13 of the GHHP Gladstone Harbour water quality zones were considered as potential monitoring sites for fish health indicators. Twelve of the sites were sampled in Autumn 2018, the only exception being Zone 4: Boat Creek, which was not sampled because the gill nets required for fish surveys are too large to allow for adequate sampling in this small estuary. Sites were resampled in Spring 2018 to account for potential seasonal variation in species catchability and health, and prior to a final assessment of site suitability. During the Spring sampling event, in addition to excluding Boat Creek due to the size of the creek, the Outer Harbour zone was excluded due to poor catches of suitable indicator species at these sites in the earlier Autumn sampling.

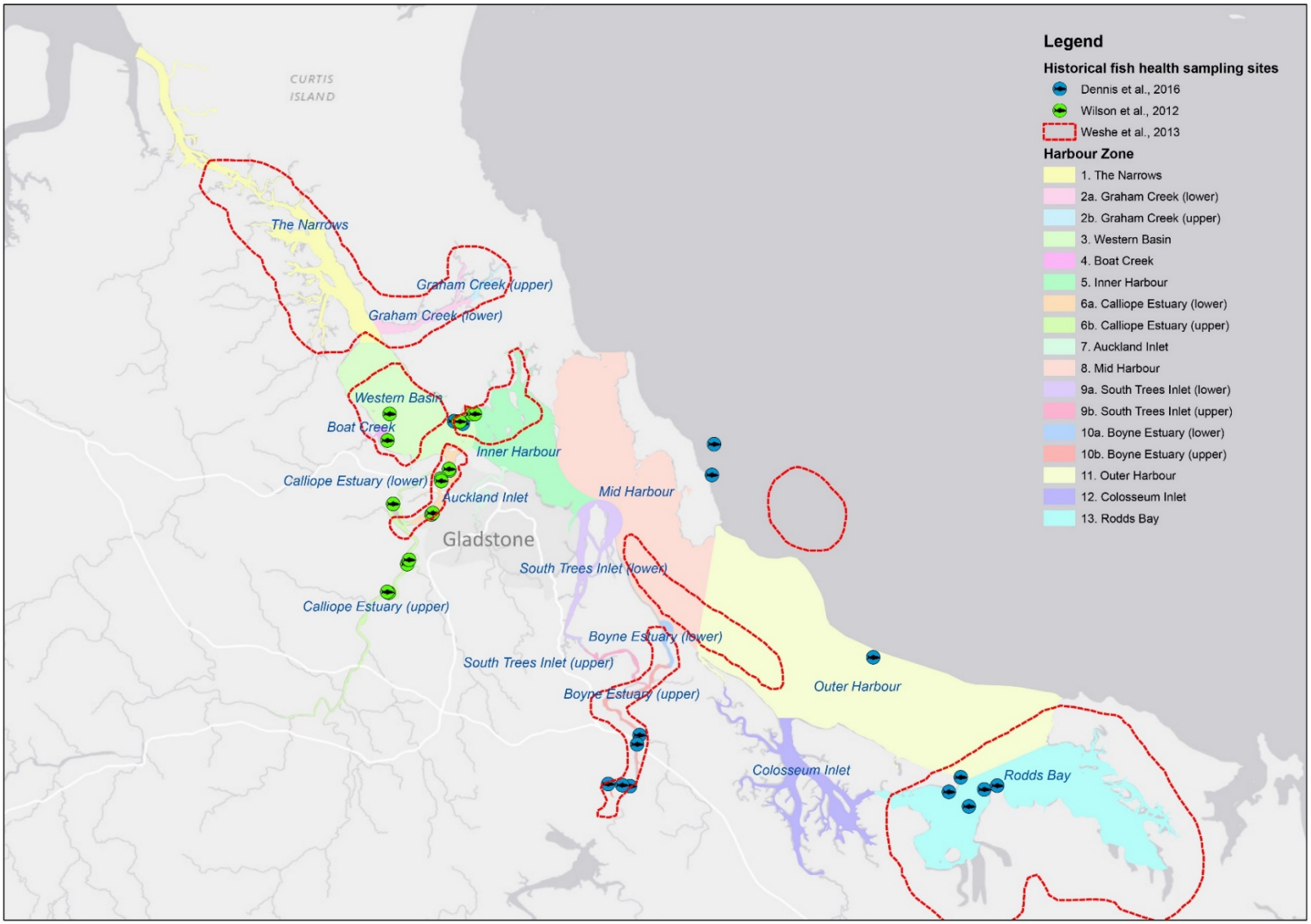


Figure 1: The Gladstone Harbour zones and previous fish sampling sites

Box 1: Long term monitoring site selection criteria for Gladstone Harbour

Preliminary long term fish monitoring site selection criteria:

1. Accessibility – present accessibility, considering tidal restrictions and vessel travel time
2. Likely to remain accessible through time
3. Appropriate fish habitat and ability to catch target species
4. Proximity to other GHHP monitoring sites, particularly for other Fish & Crabs indicators
5. Historical fish health monitoring locations in Gladstone Harbour

The primary aim of sampling was to collect the three target fish taxa identified by GHHP as priorities for further analysis: barramundi (*Lates calcarifer*), bream (pikey bream *Acanthopagrus pacificus* and yellowfin bream *A. australis*), and large mullet (sea mullet *Mugil cephalus* and diamondscale mullet *Liza vaigiensis*).The ability to catch target fish species in each zone is a key criterion for selecting long term monitoring sites. Some of the GHHP zones yielded few of the target species during the first sampling event in Autumn, so an additional species was targeted in Spring: barred javelin (*Pomadasys kaakan*).

Assessments of long-term monitoring sites were based on sampling in Autumn and Spring. Each area was scored for each selection criterion based following the methods outlined in the Milestone 2 report for this project (Attachment A). Based on this assessment, all zones with the exception of Boat Creek and Outer Harbour contain appropriate long-term sampling sites going forwards. However, operational and cost efficiencies could be gained by sampling in fewer zones, particularly the inshore zones, as is the case for the mud crab indicator which samples at seven zones (Flint, Anastasi, et al., 2017).

Reference Sites

Reference sites are sites that are considered to be pristine. In reality, few pristine estuarine environments remain in Central Queensland, therefore comparatively undeveloped regions with similar environmental conditions but outside of Gladstone Harbour were scoped as reference sites.

Eight sites were considered as possible reference sites outside of Gladstone Harbour, as follows:

Estuarine areas north of the Fitzroy River

To the north of the Fitzroy Basin, small coastal catchments drain via a series of sandy rivers and creeks to the Great Barrier Reef lagoon.

1. **Stanage Bay** in the Shoalwater Catchment is 250km north of Gladstone, has minimal anthropogenic pollutant loads, agricultural land use in the western part of the catchment and conservation/military training areas in the eastern part of the catchment. Stanage Bay was used as a reference site by Dennis et al. (2016).
2. **Corio Bay** drains Waterpark Creek in the Waterpark Catchment, 170km north of Gladstone, large areas of conservation land use with some grazing.

Estuarine tributaries feeding into the Fitzroy River delta

The Fitzroy River delta is directly north of GHHP Zone 1 (Narrows). The Fitzroy Basin, the largest eastward-draining catchment in Australia, has significant inland industrial activity, urban areas and

vast agricultural land holdings (primarily cattle grazing) (Flint et al., 2015). The Fitzroy River delta was used as a control site by Wesche et al. (2013). Three tributaries within the Fitzroy delta were considered as potential reference sites for this study:

3. **Casuarina Creek**
4. **Inkerman Creek**
5. **Connor Creek**

Estuaries south of Gladstone and north of Bundaberg

Small-medium agricultural catchments are located along the coast between Gladstone and Bundaberg, in varying states of environmental condition (Meynecke, Bunce, & Einoder, 2008). Rivers in the Bundaberg region were used as control sites by Wesche et al. (2013). Three estuaries were considered as potential reference sites for this study:

6. **Baffle Creek**
7. **Eurimbula Creek**

   Baffle Creek and Eurimbula Creek are both located in the Baffle Catchment, 100-120km south of Gladstone, have limited industrial impact, and are dominated by agricultural and nature conservation land uses. The Baffle Catchment also includes Rodds Bay (GHHP Zone 13).

8. **Kolan River** is a large river that drains the Kolan Catchment, situated directly south of the Baffle Catchment. The Kolan's upper waters are contained in Lake Monduran by the Fred Haigh Dam, and land uses are mostly grazing and horticultural, with small conservation and forestry areas.

Scoping trips were undertaken to determine the suitability of the proposed reference sites from 26-29 March 2018. Each area was scored for each of five selection criteria described in Box 2 (results were provided in the Milestone 2 report; Attachment A). Based on this assessment, Stanage Bay and Baffle Creek were selected as appropriate reference sites for this research project. Casuarina Creek and the Kolan River would also be useful control sites for comparison to Gladstone, but due to upstream impacts are not suitable as reference sites.

Box 2: Reference site selection criteria

Reference fish monitoring site selection criteria:

1. Location within Central Queensland – proximity to Gladstone and to other reference sites
2. Minimal anthropogenic impact to provide background conditions
3. Accessibility – considering tidal restrictions and vessel travel time
4. Appropriate fish habitat (mangrove estuary) and ability to catch target species
5. Historical fish health control/reference sites used for other Gladstone Harbour studies

Target fish species selection

Several fish taxa were highlighted by GHHP as being of particular interest: barramundi, pikey and yellowfin bream and mullet. The most likely mullet species to be targeted was identified as sea mullet, a species which is common in coastal areas across Northern Australia, but other locally-relevant large mullet species were also retained for analysis.

Kroon, Streten, and Harries (2016) recommended three of these species as suitable for biomarker studies (barramundi, yellowfin bream and sea mullet). Other suitable species recommended by Kroon et al. (2016) included blue threadfin (*Eleutheronema tetradactylum*) and school mackerel (*Scomberomorus queenslandicus*). Cowled (2016) also recommended bream, barramundi and mullet (as well as flathead, grunter, cod and snapper) as candidates for biomonitoring on the basis that they are: present and abundant, commercially or recreationally fished, and spend time low in the water column. Demersal or benthic species are in closer contact with pollutants accumulated in sediments and as a result are more likely than pelagic species to present with abnormalities (Cowled, 2016).

Based on the recommendations of Kroon et al. (2017), Cowled (2016) and the GHHP ISP, the following fish species were targeted for retention and laboratory analysis in Autumn 2018:

1. Barramundi (*Lates calcarifer*)
2. Pikey bream and yellowfin bream (*Acanthopagrus pacificus* and *A. australis*, respectively)
3. Diamondscale mullet and sea mullet (*Liza vaigiensis* and *Mugil cephalus* respectively)

In the second sampling event in Spring 2018, two additional species were retained to increase sample size numbers:

4. Barred javelin (*Pomadasys kaakan*)
5. Dusky flathead (*Platycephalus fuscus*)

Both of the two additional species spend time low in the water column and were also recommended by Kroon et al. (2017) and Cowled (2016). Gears and methods employed were chosen to select for these species, however additional fish species were also captured, and any fish that died during capture were retained and returned to the laboratory for dissection.

Fish mobility

Understanding the mobility of fish in Gladstone Harbour was an important consideration for scoring fish health indicators. Inshore and estuarine fish tagging studies have shown that estuarine species including barramundi may travel long distances between capture and recapture events (e.g. Moore and Reynold (1982); Russell and Garrett (1988)). However some fish, including barramundi, may also stay resident in an area for prolonged periods (e.g. Russell and Garrett (1988); Meynecke, Poole, Werry, and Lee (2008)). While the preference is to produce scores for each of the GHHP Gladstone Harbour zones, in the case of mobile, adult fish, it may be more biologically relevant to pool data by broader zones or even across the harbour.

Fish release and recapture tagging data for Stanage Bay, Gladstone and Baffle Creek were provided to CQUni by Infofish Australia to assess the adult home ranges of potential target fish species for bioindicator selection. A species with a life time home range within the location being monitored would provide a more relevant bioindicator than one which migrates large distances or is found in different locations at different life history stages. Whilst fish tagging data does not provide the data on spatial distribution of all life history stages it can provide useful information on the adult home

range. The methods and results of an assessment of adult ranges of the target species in the areas of interest are presented in Appendix 1.

For the majority of species examined, many tagged individuals were re-captured at their original tagging location. Fish tagged in large numbers but showing smaller ranges included yellowfin bream, pikey bream and goldspotted rock cod (*Epinephelus coioides*). The average distance moved for most species examined was less than 10 km from the original tagging location. Blue threadfin (average 28.67 km) and king threadfin (*Polydactylus macrochir*; average 55.28 km) were notable exceptions, although the large variation among samples of king threadfin reduces certainty about the distance moved. Barramundi show the largest ranges (mean 8.42 km, max 704 km), which may negatively confound their use as a bioindicator at smaller geographical scales. Fish with small home ranges and sufficient tagging records include: goldspotted rockcod, pikey bream, yellowfin bream, black jewfish (*Protonibia diacanthus*), blackspotted rockcod (*Epinephelus malabaricus*), estuary cod (most likely a mix of *E. coioides* and *E. malabaricus*). Whilst these fish may show smaller home ranges, they still exceed the spatial scale of water quality monitoring zonation within the Gladstone Harbour, but would be suitable for the assessment of Gladstone Harbour as a whole.

Fish Health Indicator selection and analysis

Options for suitable fish health monitoring approaches for Gladstone Harbour were identified using researcher knowledge, information from reviews including Cowled (2016) and a range of relevant scientific and grey literature. Full details of this review are presented in the Milestone 3 report from this project (Attachment B). The review of methods provided several suitable approaches to be tested, using adaptive sampling techniques, to select one or more for future use. These indicators are summarised below.

Fish species and abundance

When employing fish as indicators of ecosystem health, most Australian report cards consider population and community-level fish health indicators (FPRH, 2016; Healthy Land & Water, 2017; HRRP, 2017). While this approach is well-tested and generally doesn't require any laboratory analysis following field sampling, its limitations are that extensive field work can be required to ensure the monitoring program is both spatially and temporally representative, avoids sampling gear bias and guarantees sufficient statistical power to detect change. In circumstances where it is possible to establish a representative sampling regime, population and community-level indicators, such as probability of encounter, are useful tools for reporting on a site-specific level. Previous studies have developed such fish assemblage indicators for estuaries, including in Northern Australia (Sheaves et al., 2012).

This project focused primarily on individual-level fish health indicators, a range of which have been applied in previous studies and are described in below. Further details are provided in the Milestone 3 report (Attachment B).

Fulton's condition factor

Fulton's condition factor, *K*, aims to indicate an individual fish's health based on the relationship between its standard weight (W) divided by the cube of its length (L) (Nash, Valencia, & Geffen, 2006). Practically, *K* expects that if a fish has doubled its length, then its weight should have increased by a factor of eight (i.e. isometric growth). The calculation is multiplied by 100 to scale the factor close to a value of 1 for comparative purposes.

Hepatosomatic Index

The Hepatosomatic Index (HSI) is calculated as the ratio of the fish's liver weight to its body weight and is generally considered an indicator of the status of energy reserves within a fish (larger HSI equating to greater energy reserves). Research has shown a series of correlations between HSI and environmental conditions. Calculation of HSI requires lethal sampling methods, but is relatively cheap and easy to obtain in comparison to the more complex fish health indicators such as biomarkers and parasite analysis (Cowled, 2016). Due to the need to kill and dissect fish, HSI is often calculated in conjunction with the Gonadosomatic Index (Nunes, Silva, & Soares, 2011; Sadekarpawar & Parikh, 2013).

Gonadosomatic Index

The Gonadosomatic index (GSI) is calculated as the ratio of the gonad weight to the fish's body weight. Similar to the HSI, it is used as an estimate of the reproductive condition of the fish, with a larger GSI indicative of greater reproductive potential. The GSI of fish can be altered (typically negatively) by pollutants that affect sexual development, such as endocrine disruptors (Scholz & Klüver, 2009), and thus may be useful as an indirect indicator of environmental condition.

Health Assessment Index

The fish Health Assessment Index (HAI) is a composite metric that integrates observer evaluations of the condition of multiple organs and tissues, including skin, eyes, fins, gills, spleen, kidney, hindgut, and liver. Developed by Adams, Brown, and Goede (1993), its premise is that scores will cumulatively reflect the acute and chronic stressors present in the fish's environment, with poorer anatomical condition resulting in higher HAI scores, indicative of a more stressful environment.

Fluctuating asymmetry

Fluctuating asymmetries (FA) are small random deviations in size or shape from, an assumed, perfect symmetry which are observed between certain bilaterally paired structures. Such structures include (but not limited to) fin rays, eyes, barbels, and feathers (Van Valen, 1962). The observed deviations in symmetry are considered to be reflective of levels of endogenic genetic and exogenic environmental stress which the individuals or populations experienced during development. The standard approach to using FA is to measure the right minus the left value of one or a number of selected bilaterally paired traits and compare the means of the unsigned differences between sides (Tomkins & Kotiaho, 2001). Fluctuating asymmetries were assessed in the current study based on eye diameter.

Parasites and Pathogens

Disease in fish has repeatedly been linked to environmental stress, with physical factors such as temperature, and chemical composition of the water, being key factors influencing predisposition to disease (Roberts, 2012). Diseases in fish are caused by infectious agents including viruses, parasites, bacteria, fungi, as well as non-infectious diseases and deformities associated with environmental exposures (Roberts, 2012).

Disease conditions caused by environmental stress provide a more relevant indicator in the context of long-term monitoring of the general ecological condition of a waterway (discussed further in section 2.5, below) than the prevalence of infectious diseases. While infectious disease prevalence can provide information on the condition of a fish population and other species that rely upon it, catastrophic infectious disease is normally episodic and species-specific. A single infectious disease outbreak may not be useful as an indicator of overall ecosystem health, however repeated episodes would require close environmental monitoring. One infectious disease condition that has been

previously suggested as a useful bioindicator is parasitism in fish (Vidal-Martínez, Pech, Sures, Purucker, & Poulin, 2010).

There is conflicting evidence in the literature regarding the link between fish parasitism and ecological degradation (Sures, 2008). Previously, aquatic pollution has been linked to the abundance of parasites, with a general rationale that higher proportions of diseased fish would reflect more severe habitat degradation (Vidal-Martínez et al., 2010). It would be difficult to develop appropriate fish parasite indicators for Gladstone Harbour until parasite abundance and assemblages local to the area are better understood. An understanding of the biology and lifecycle of the parasite being studied, its host/s, their relationship to each other, and the impacts of environmental degradation on both, are required for ecological indicator applications (Palm, 2011). At this time even the range of parasite species that may be present in the area is not well documented, and still less is known about their impacts on host species. Despite these constraints with parasite indicators, monitoring for known problematic macroparasites (such as the parasitic flatworm, *Neobenedenia* sp., which was identified on some fish from Gladstone Harbour in 2010-11; Wesche et al. (2013)) is a valuable component of general fish health assessments during necropsy.

Histopathological analysis

Cumulative and chronic environmental stress causes physiological changes to the body systems of fish, particularly soft tissue systems including the integumentary, respiratory, cardiovascular, muscular and digestive systems. Organs such as gills, liver and kidney have functional roles defending organisms from environmental toxicity (Zhang et al., 2018). Gills and skin of fishes are also directly exposed to environmental conditions. Morphological alterations to these and other fish organs can indicate impacts of either acute or chronic exposure to toxicants such as metals and metalloids, pesticides, hydrocarbons and complex mixtures (Gerber, Wagenaar, Smith, Ikenaka, & Smit, 2017; Santana et al., 2018). Histopathological analysis microscopically studies the changes to tissues caused by disease. This analysis has been frequently applied to study the condition of wild and cultured fish around the world, and provides an assessment of tissue changes induced by environmental stressors such as water pollution (Bernet et al., 1999).

Histopathology is a useful indicator due to its attention to an intermediate level of biological organisation – providing data on medium term responses of fish to a wide variety of sublethal stressors (Bernet et al., 1999). As the natural baseline for histopathology is 'no pathological change', deviations from this can be determined in impacted sites, relative to the natural prevalence of various pathologies in unimpacted sites. While histopathology is within the higher cost category of fish health indicators (Cowled, 2016), it has many advantages as a monitoring tool, including the clear link between in situ exposure to pollution and structural alterations to organs that present a direct measure of fish health. Some histological changes are non-specific, and can be caused by multiple stressors, while other changes can be linked to specific causes. Long term and seasonal comparative data are very useful for assessing fish health at a study site and provide a baseline for future impacts and toxicity studies.

Fish health indicator selection criteria

Selection criteria for fish health indicators are described in the Milestone 3 report (Attachment B). These criteria are proposed for programs where the results are to be scored against a benchmark and then communicated to the community (for example through a waterway health report card) but may be reduced or modified to suit the specific situations or objectives of individual programs. Where there are currently insufficient data from Gladstone Harbour to assess the indicators, this is noted in the results.

Trialling long term monitoring sites

Field sampling methods

Field collections of fish were undertaken with 3 x 50m long gill nets with stretched mesh sizes 4.5”, 6” and 8”. A fourth gill / ring net of 110m length, 2.13” stretched mesh size was used at some sites to supplement catch. As described above, the primary aim of sampling was to collect the three target fish taxa for further analysis: barramundi, bream and large mullet. Barred javelin and dusky flathead were added as target species in the second sampling event in Spring 2018. Gears were deployed in areas and at times when the chances of catching these species were maximised.

Field sampling was undertaken at the 12 GHHP Zones and two reference sites during Autumn 2018 and 11 GHHP Zones and two reference sites in Spring 2018 (Figures 2-4, Tables 1-2). Depending on travel times, either one or two sites were sampled each day (full details of sampling time and positions are provided in Appendix 2).

Step by step details of sampling procedures are provided in the Milestone 2 report (Attachment A). In summary, at each sampling location nets were deployed, details of deployment were recorded (Appendix 2) and physicochemical measurements (including temperature (°C), dissolved oxygen (% and mg/L), electrical conductivity (µS/cm), pH, turbidity (NTU), total dissolved solids (TDS; mg/L), oxidation reduction potential (ORP; mV) and salinity (ppt)) were recorded (Appendix 3). Nets were soaked for approximately 30 minutes during each deployment, and several deployments of nets occurred at each site over a maximum of five hours. Captured fish were assigned a unique identifier code and either processed immediately or placed into an aerated swim tank to be kept alive until on-board processing. Time of catch was recorded and each captured fish photographed. Teleost fish were measured and weighed, and the skin, fins and eyes were examined for abnormalities, parasites, lesions or erosion. Cartilagenous fishes (sharks and rays) were recorded and photographed but were not handled except to ensure their safe removal from the net and live release. Non-target fish were released, while up to five of each target species were retained at each site and euthanized for laboratory analysis. Immediately following euthanasia gill arch samples were collected and fixed in 10% formalin. Non-target fish that died during capture were also retained for laboratory analysis. All retained fish were individually bagged with the unique identifier tag and placed in an ice slurry for return to the laboratory as soon as possible on the same day.

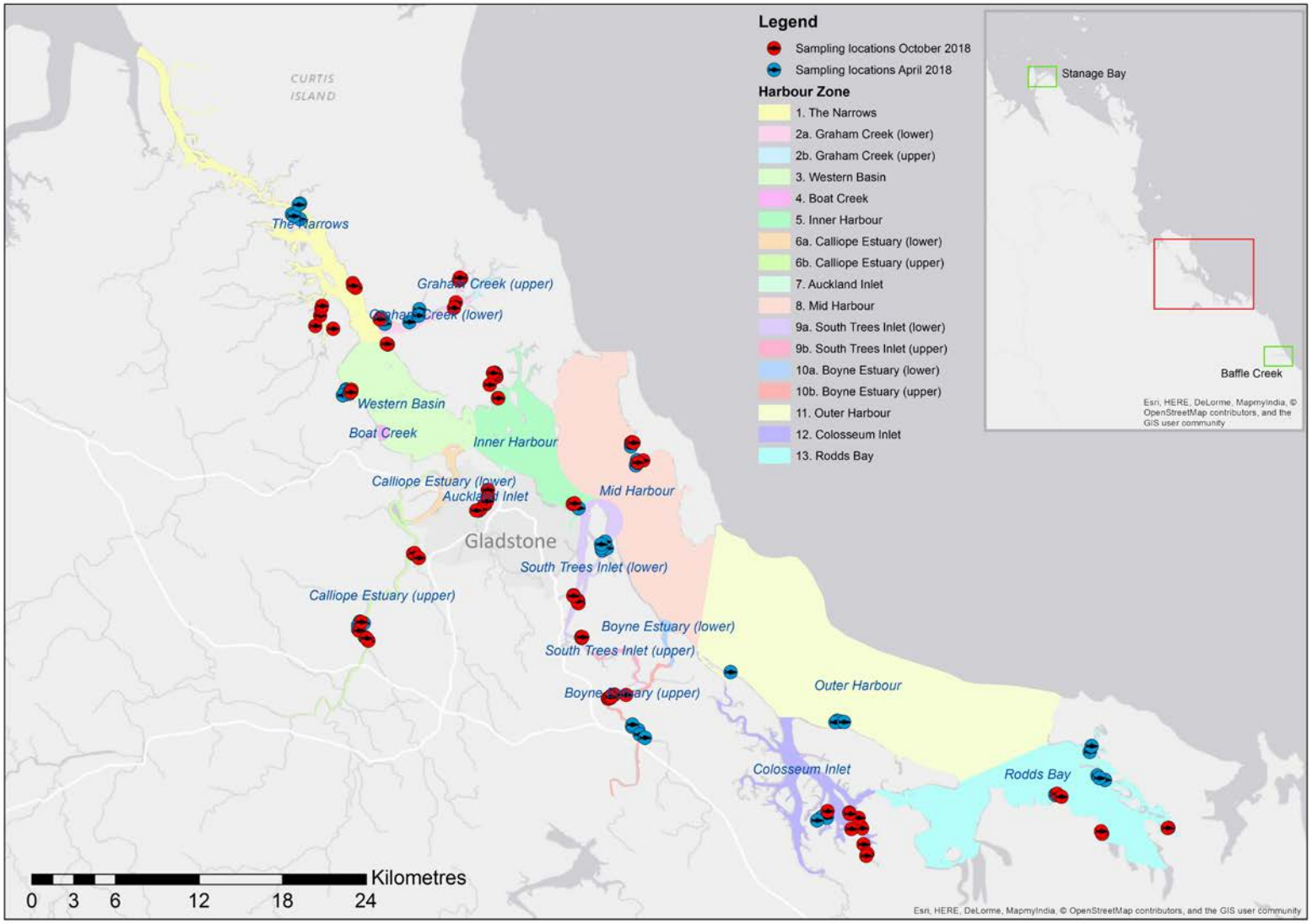


Figure 2: Autumn 2018 (blue) and Spring 2018 (red) sampling locations within the Gladstone Harbour zones.

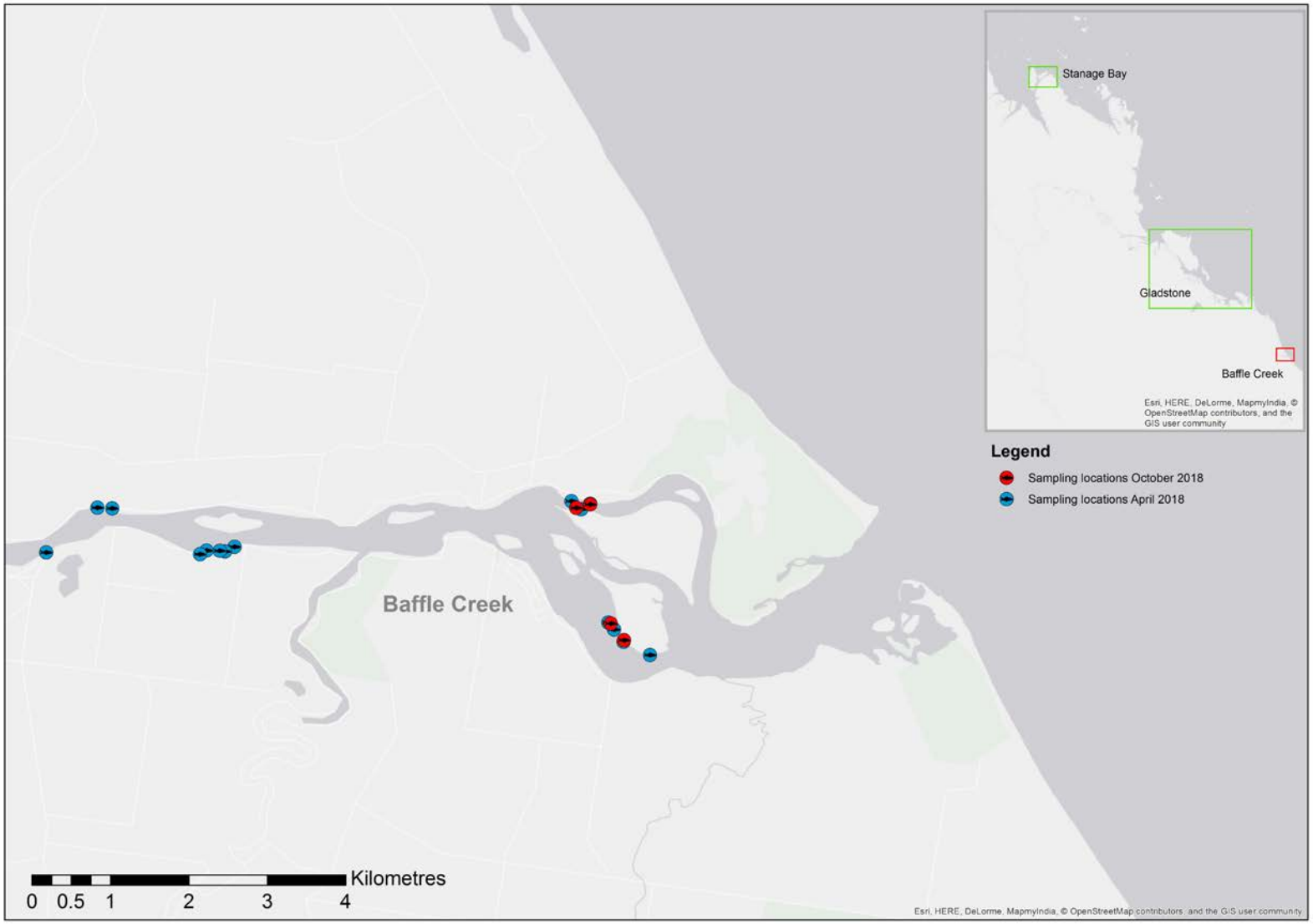


Figure 3: Autumn 2018 (blue) and Spring 2018 (red) sampling locations within the Baffle Creek reference site.

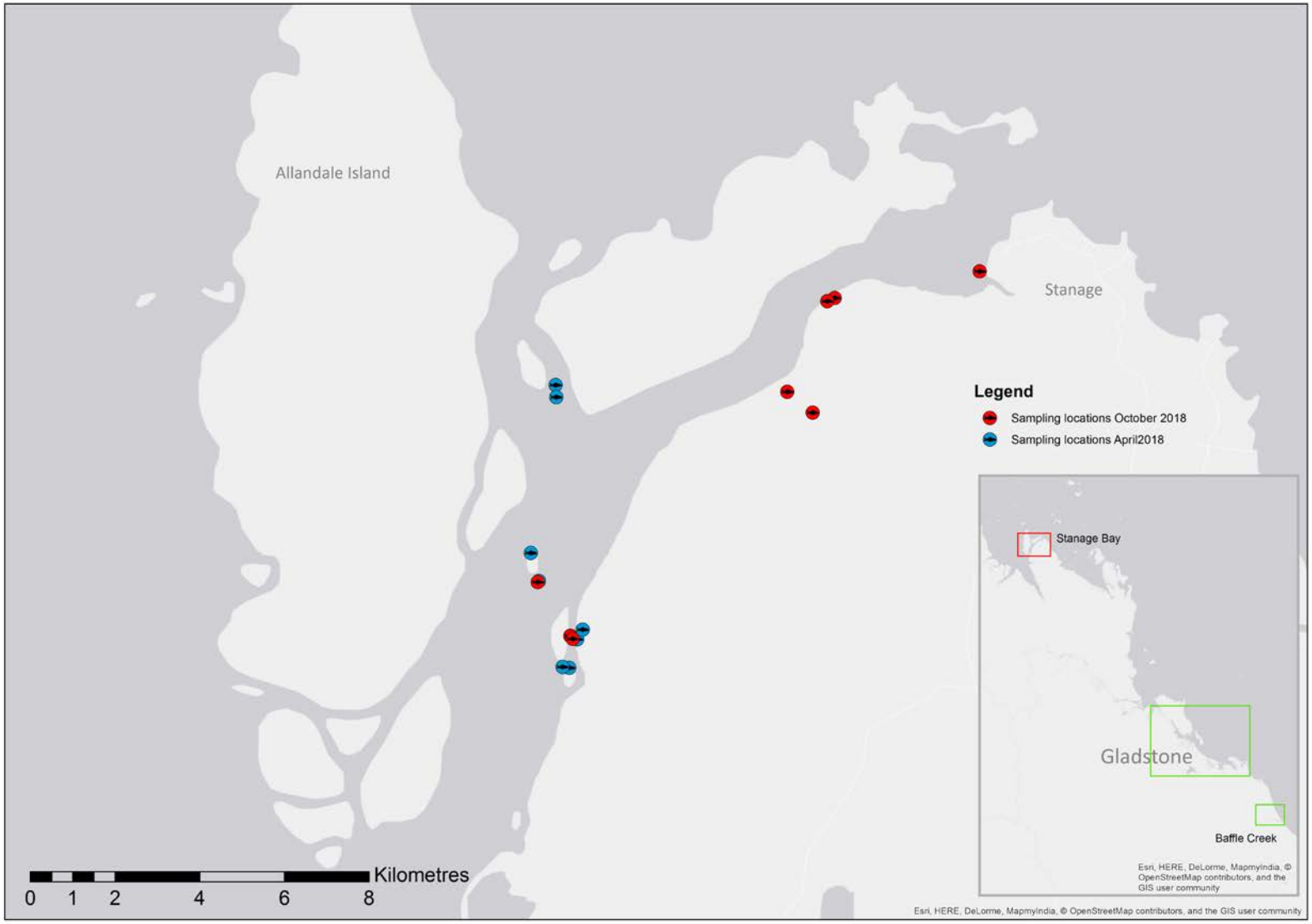


Figure 4: Autumn 2018 (blue) and Spring 2018 (red) sampling locations within the Stanage Bay reference site.

Laboratory methods

Fish dissection

Retained fish from all sites except Stanage Bay were returned to the lab at CQUniversity's Gladstone Marina Campus, and fish from Stanage Bay were returned to the closer North Rockhampton Campus, for same day mid-level pathological examination as described by Cowled (2016). Pathological examination also included the dissection of organs and fixation in 10% formalin for further histopathological analysis. The fish dissection methods are described in the Milestone 2 report for the project (Attachment A, pages 7-8).

Rapid parasite analysis

Parasite collection was performed following the methods of Cribb and Bray (2010) and Justine, Briand, and Bray (2012) during the Autumn 2018 sampling event. Where available, bag waters (not sampled for all fish), gills, and the pyloric caecae and intestines were examined for macroscopic parasites (e.g. crustaceans, monogeneans, trematodes, cestodes or nematodes). Suspected cysts or lesions from the fish were also examined, and some live cysts were unencysted (using forceps) and the parasites isolated and collected. The rapid parasite analysis methods used are described in in the Milestone 2 report for the project (Attachment A, page 8).

Parasite analysis was not repeated in Spring 2018, but the collection has been retained for possible later analysis.

Histopathological analysis

Fish samples destined for histopathological analysis were transported by road freight to the NATA-accredited Queensland Government Biosecurity Sciences Laboratory (BSL) in Brisbane. Samples included gill, liver and skin/muscle tissue. Aquatic pathologists from BSL processed the prepared fish organs according to standard histological protocols. Diagnostic pathology reports for each sample were provided to CQUniversity by Senior Veterinary Pathologist (Aquatic Health) Dr Roger Chong. Details of the NATA-accredited histology methods used to prepare tissues are provided in Appendix 7 (Fish Histopathology Summary Report, pages 1-2).

Results of histopathological analysis of fish collected in Autumn 2018 are provided in the results section, and at the time of writing the analysis of fish collected in Spring 2018 was still underway.

Calculating fish condition measures

The five established fish condition measures were calculated based on the gross pathological data collected during fish dissections. The measures include: Fulton's condition factor (K), Hepatosomatic index (HSI), Gonadosomatic index (GSI), the Health Assessment Index (HAI, following a similar approach to the original method of Adams et al. (1993); also used by Wesche et al. (2013)), and fluctuating asymmetry (FA) of eye diameter as a measure of developmental instability. Calculations used were as follows:

Fulton's condition factor:

$$K = 100*(W/L^3)$$

where: W = wet body weight (g); L = total length (cm)

Hepatosomatic index:

HSI = 100*(H/W)

where: H = wet liver weight (g); W = wet body weight (g)

Gonadosomatic index:

GSI = 100*(G/W)

Where: G = wet gonad weight (g); W = wet body weight (g)

Health assessment index:

HAI was calculated as the average of condition scores given to skin, eyes, fins, gills, spleen, kidney, hindgut and liver during dissections

Fluctuating asymmetry of eye diameter:

FA = DL – DR

Where: DL = diameter of left eye (mm); DR = diameter of right eye (mm)

Statistical analytical methods

Formal statistical tests to compare fish health indices among zones during Autumn sampling were conducted based on the criterion of a minimum replication of two sampled fish per zone. Due to uneven replication among zones, analyses were done using PERMANOVA (Permutational Analysis of Variance, conducted in PRIMER 7 + PERMANOVA software package) as a statistical method robust to departures from even replication and non-normality of data.

This resulted in the following possible statistical tests for Autumn 2018 sampling event:

- Barramundi: Comparison of all five indices among Inner Harbour (zone 5), Auckland Inlet (zone 7), and Stanage Bay (zone R1).
- Diamondscale mullet: Comparison of all five indices among the Narrows (zone 1), Auckland Inlet (zone 7), Middle Harbour (zone 8), and Boyne Estuary (zone 10).
- Sea mullet: Comparison of all five indices between Calliope Estuary (zone 6) and Baffle Creek (zone R2).
- Bream: No comparisons possible due to low capture rates (two fish caught at two different zones).

Formal statistical tests to compare fish health indices among zones and sampling times (i.e. 2-way PERMANOVA) were based on the criteria of having a minimum replication of two sampled fish per zone during each sampling period (April and September). This resulted in the following possible statistical tests:

- Barramundi: Comparison of all five indices between Auckland creek (zone 7) and Stanage Bay (zone R1).

Due to uneven replication among zones at and times, no further 2-way analyses were possible for bream, diamondscale mullet, or sea mullet.

Data were then pooled across both sampling times to determine statistical comparisons among zones independent of sampling time (i.e. 1-way PERMANOVA). This resulted in the following possible statistical tests:

- Barramundi: Comparison of all five indices between the Narrows (zone 1), Inner Harbour (zone 5), Auckland creek (zone 7), Middle harbour (zone 8), South Trees Inlet (zone 9), Colosseum Inlet (zone 12) and reference site Stanage Bay (zone R1).
- Barred javelin: Comparison of all five indices between the reference site Baffle Creek (zone R2), and the Gladstone Harbour zones Graham Creek (zone 2), Mid Harbour (zone 8), Auckland Creek (zone 7), Inner Harbour (zone 5).
- Bream: Comparison of all five indices between Graham Creek (zone 2) and Boyne Estuary (zone 10).
- Diamondscale Mullet: Comparison of all five indices between the Narrows (zone 1), Graham Creek (zone 2), Western Basin (zone 3), Inner Harbour (zone 5), Auckland creek (zone 7), Middle harbour (zone 8), South Trees Inlet (zone 9), Boyne estuary (zone 10) and Stanage Bay (zone R1).
- Sea Mullet: Comparison of all five indices between the Narrows (zone 1), Western Basin (zone 3), Calliope Estuary (zone 6), Middle harbour (zone 8), South Trees Inlet (zone 9), and Baffle Creek (Zone R2).

Results

Autumn 2018 sampling

In April 2018 a total of 249 fish from 33 species were caught at 12 Gladstone Harbour zones and two reference sites (Table 1). The species that were caught at the most sites were: barramundi (8 Gladstone zones and 1 reference sites); blue catfish *Neoarius graffei* (8 Gladstone zones); blue threadfin (7 Gladstone zones); barred javelin (6 Gladstone zones); diamondscale mullet (5 Gladstone zones and 1 reference site); and giant queenfish *Scomberoides commersonnianus* (5 Gladstone zones and 1 reference site).

Table 1: Fish species (listed by common name) and abundance at Gladstone Harbour zones (1-3, 5-13) and two reference sites (R1, R2) in Autumn 2018. White = 0; blue = 1-5; orange = 6-10; green = 10+ specimens. Common names of target species retained for further analysis are shaded grey. Species names provided in Appendix 4. Site R1 = Baffle Creek; R2 = Stanage Bay.

	Zone / site													
Fish species	**1**	**2**	**3**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**R1**	**R2**
Australian Giant Herring	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Bartailed flathead	0	0	0	0	0	0	0	1	0	0	0	1	0	0
Barramundi	1	1	0	4	0	4	1	1	1	0	0	1	6	0
Barred Javelin	3	8	1	13	0	0	4	0	0	0	0	9	0	0
Beach Salmon	0	2	2	3	0	0	2	0	0	0	0	0	0	0
Blubber-lip Bream	1	0	0	0	0	0	0	1	1	0	0	0	0	1
Blue Catfish	2	1	0	3	2	6	0	1	7	0	11	0	0	0
Blue Threadfin	1	2	0	2	1	1	0	1	0	0	0	1	0	0
Bull Shark	0	1	0	0	2	0	0	0	2	0	2	0	0	0
Common Ponyfish	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Common Silverbiddy	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Diamondscale mullet	2	1	0	0	0	3	3	0	2	0	0	0	1	0
Giant Queenfish	0	0	0	0	4	2	1	14	1	0	0	0	0	1
Giant Shovelnose Ray	1	1	0	0	0	0	1	0	0	0	0	0	1	0
Giant Trevally	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Golden trevally	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Goldlined Rabbitfish	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Hairback Herring	0	0	0	0	0	0	0	0	0	0	0	0	0	13
King Threadfin	0	0	0	3	0	0	0	0	0	0	0	0	1	0
Lemon Shark	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Pikey Bream	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Sand whiting	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Sea Mullet	0	0	0	0	8	1	0	0	0	0	0	0	0	6
Sicklefish	0	0	0	0	0	0	0	2	3	0	0	2	0	6
Shovelnose Ray	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Silver Javelin	0	0	0	0	0	0	0	0	0	0	0	0	0	6
Silver Jewfish	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Sliteye Shark	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Striped Scat	0	0	0	0	0	1	0	0	0	0	0	8	0	0
Swallow-tailed dart	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Threadfin Silverbiddy	0	0	0	0	0	0	0	0	0	0	0	0	0	9
Tripletail	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Yellowfin Bream	0	0	0	0	1	0	0	0	0	0	0	0	0	0

Fish condition measures

Fulton's condition factor (K) of barramundi varied slightly by zone, with the lowest average factor recorded at Zone 5, Inner Harbour (n = 5). The two species of bream (n = 1 of each) recorded had different condition factors. There was little variation in Fulton's condition factor of either sea mullet or diamondscale mullet (Figure 5).

Hepatosomatic index (HSI) was highest in barramundi from Zone 5 (Inner Harbour); sea mullet from Zone 7 (Auckland Inlet); and diamondscale mullet from Zones 7 and 10 (Boyne Estuary). The two species of bream recorded different HSIs (Figure 6).

Barramundi gender was unclear in many of the recorded specimens, and may have been transitional between male and female, so GSI was not considered for this species. The two species of bream caught were of different genders (the yellowfin bream was female and the gender of the pikey bream was unclear) so GSI has not been considered. Only one male sea mullet was caught, four sea mullet had unclear gender and seven were clearly female. Of these seven females, six were caught in Zone 6 (Calliope Estuary) and one in Zone 7 (Auckland Inlet); GSI of the fish from Auckland Inlet was higher than from Calliope Estuary. Of the 12 diamondscale mullet caught, two were male and 10 were female – these have been plotted separately. Females were caught in five Gladstone Harbour zones, while males were caught in one zone and one reference site (Stanage Bay) (Figure 7).

Health assessment index (HAI) scores of barramundi varied from the best possible score of 0 (Zones 1, 8 and 9, and reference site R1) up to 60 (Zone 10). Most of the abnormalities scored were found in the kidney, liver, occasionally in the skin, and in the case of the barramundi caught in Zone 10 (Boyne Estuary) in one eye. The pikey bream caught in Zone 3 (Western Basin) scored a 0 while the yellowfin bream caught in Zone 6 (Calliope Estuary) scored 30 for a liver abnormality. The single sea mullet caught in Zone 7 (Auckland Inlet) scored a 0; while scores for sea mullet in Zone 6 (Calliope Estuary) ranged from 0 to 40; and in reference site R2 (Baffle Creek) ranged from 0 to 30. Diamondscale mullet scores also varied between zones. The single specimen caught at reference site R2 (Stanage Bay) scored 0. Four diamondscale mullet from Gladstone Harbour zones also scored 0, and the maximum score was 60 for a specimen caught in Zone 7 (Auckland Inlet) (Figure 8).

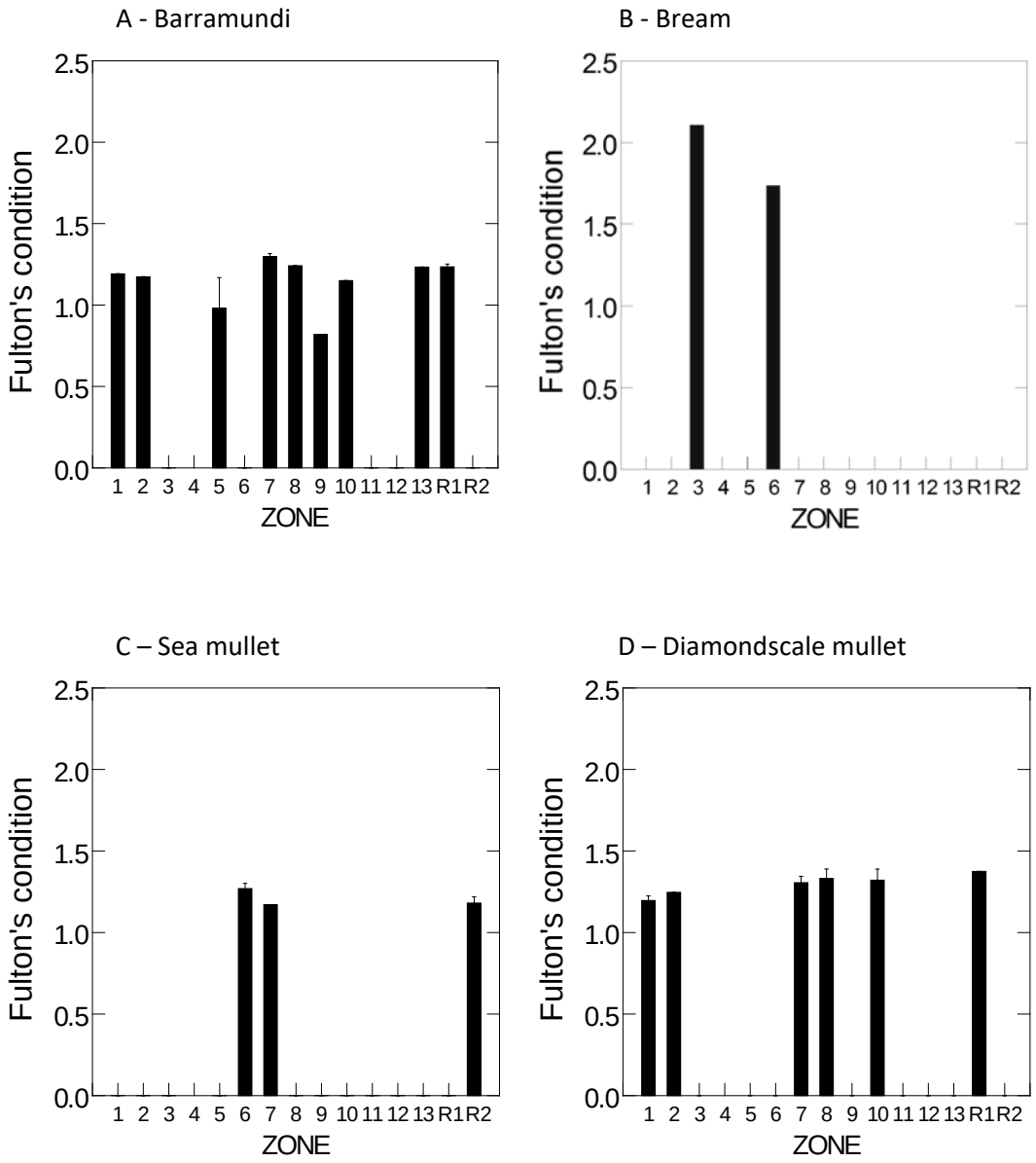


*Figure 5: Fulton's condition factor by zone of A – barramundi (*L. calcarifer*, n = 19); B – bream (*A. australis *and* A. pacificus*, n = 2); C –sea mullet (*M. cephalus*, n = 12); and D – diamondscale mullet (*L. vaigiensis*, n = 12).*

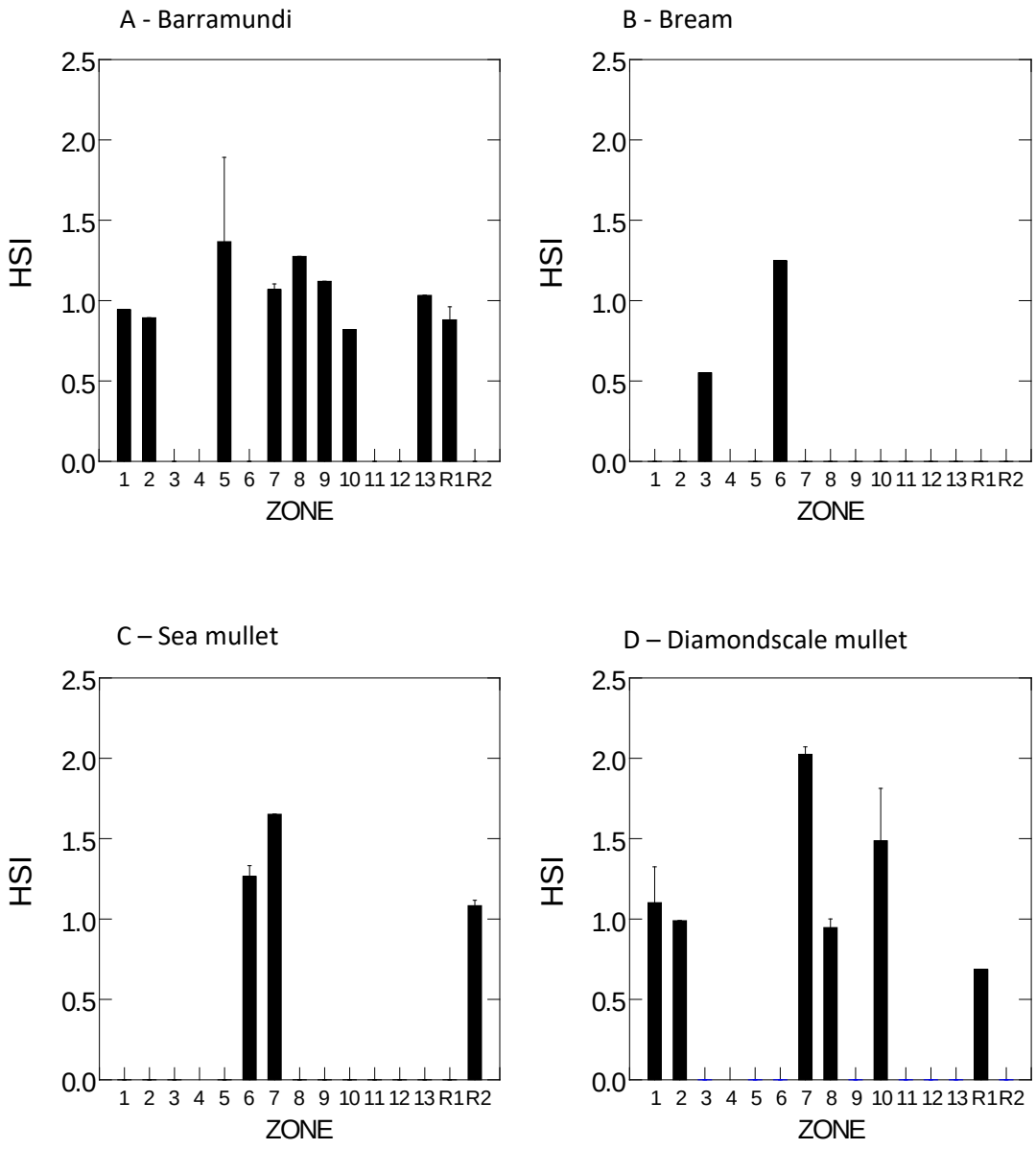


*Figure 6: Hepatosomatic index (HSI) by zone of A – barramundi (*L. calcarifer*, n = 19); B – bream (*A. australis *and* A. pacificus*, n = 2); C –sea mullet (*M. cephalus*, n = 12); and D – diamondscale mullet (*L. vaigiensis*, n = 12).*

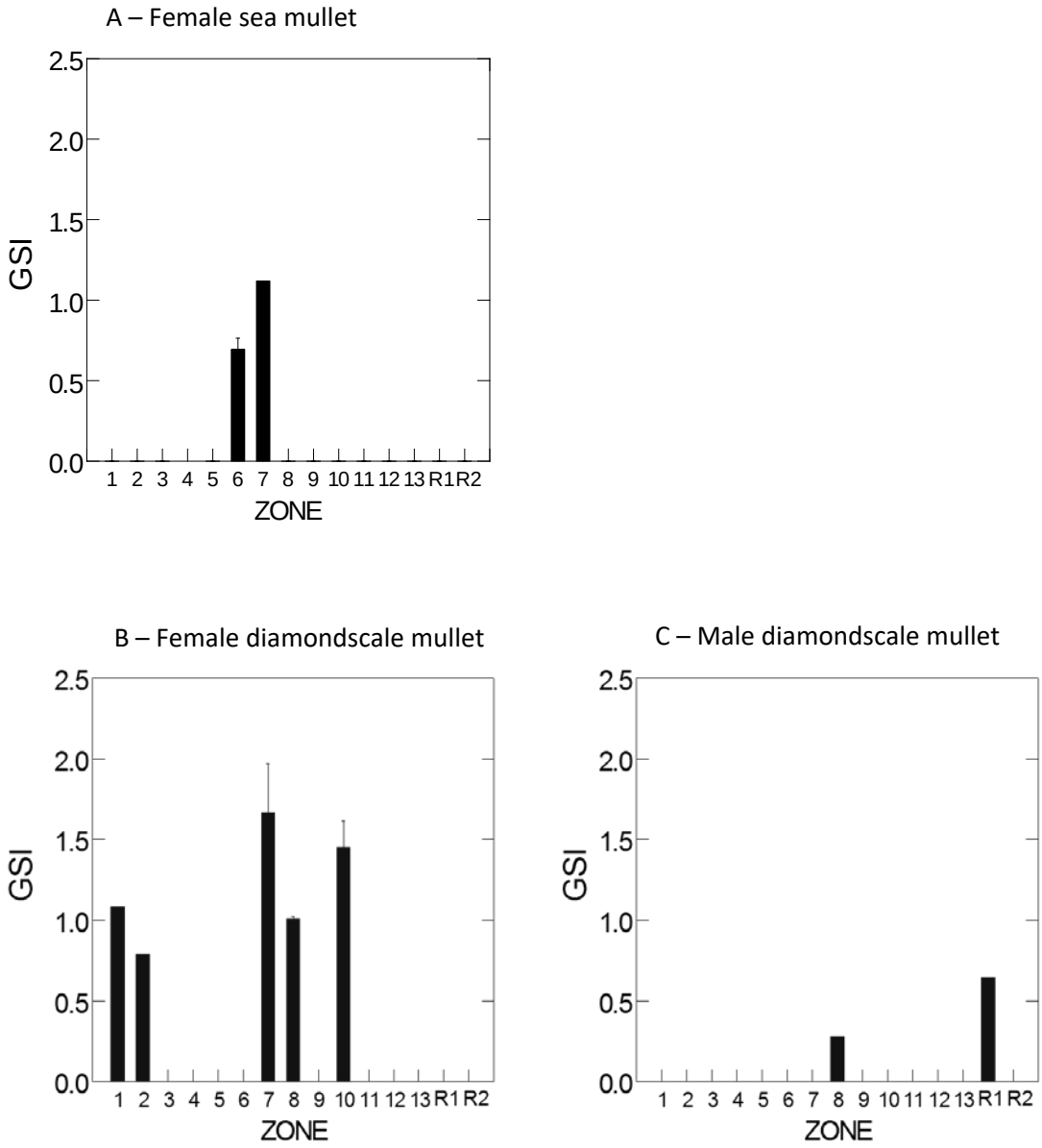


*Figure 7: Gonadosomatic index (GSI) by zone of A – female sea mullet (*M. cephalus*, n = 7); B – female diamondscale mullet (*L. vaigiensis*, n = 10), and C – male diamondscale mullet (n = 2).*

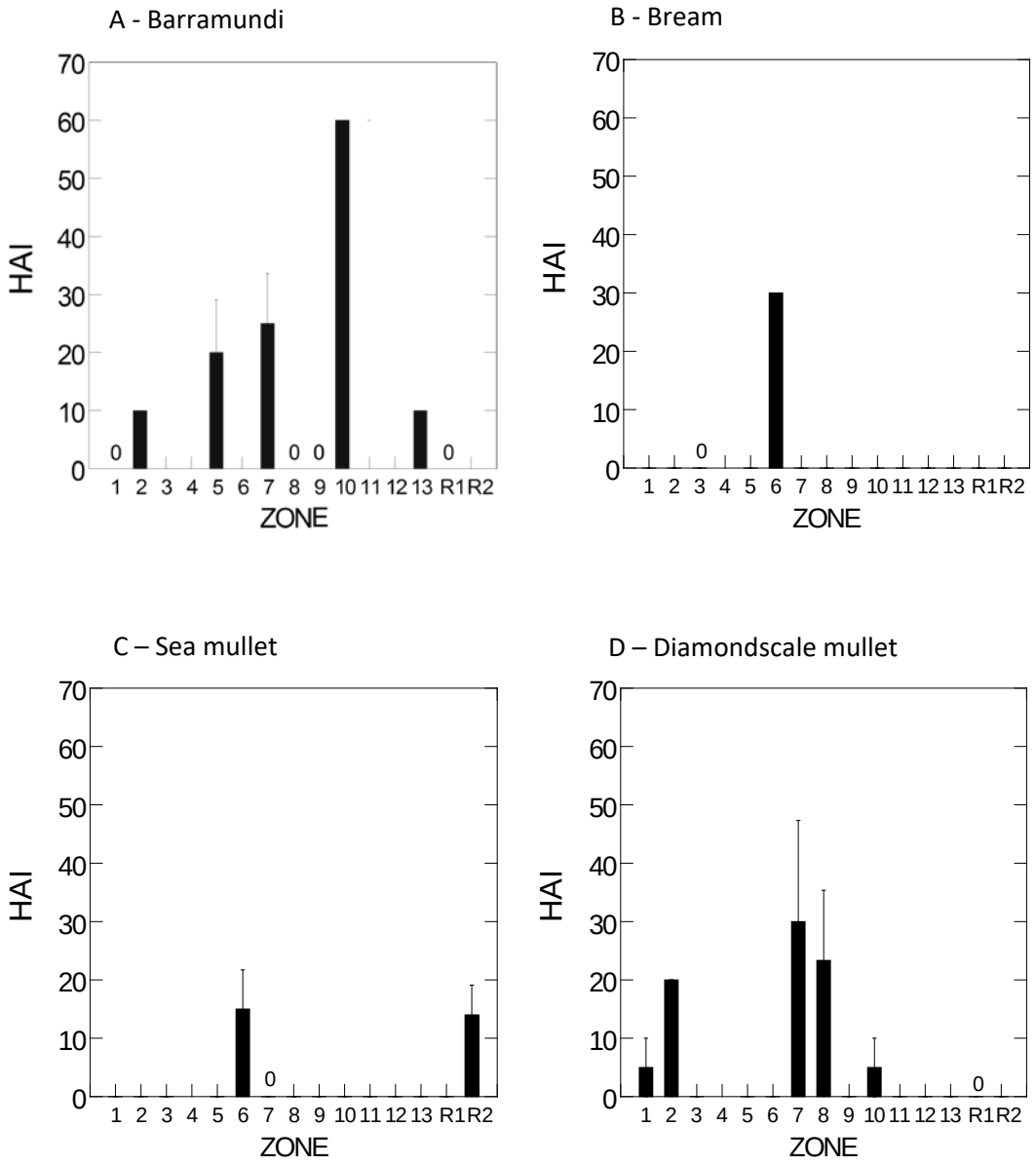


*Figure 8: Health Assessment Index (developed by Adams et al., 1993) by zone of A – barramundi (*L. calcarifer*, n = 19); B – bream (*A. australis *and* A. pacificus*, n = 2); C –sea mullet (*M. cephalus*, n = 12); and D – diamondscale mullet (L. vaigiensis, n = 12).*

PERMANOVA detected a significant difference among zones for both GSI and Fulton's condition factor (Fulton's K) of barramundi. Barramundi sampled in Inner Harbour had a higher GSI value than barramundi sampled in either Auckland Inlet or Stanage Bay. Barramundi sampled in Auckland Inlet had higher values of Fulton's K than those sampled in Stanage Bay, which in turn had greater values than barramundi sampled in Inner Harbour. Barramundi HSI, Eye asymmetry, and HAI were all similar among the three zones compared.

A significant difference among zones was also detected for HSI of diamondscale mullet, but low replication prevented an unambiguous test of the location of differences using post-hoc tests. Visually, however, it appears that diamondscale mullet sampled from Auckland Inlet had the highest HSI values. Diamondscale mullet GSI, Fulton's K, Eye asymmetry, and HAI were all similar among the four zones compared.

No differences were detected for any indices measured between sea mullet sampled in Calliope Estuary and Baffle Creek, and comparisons were not possible for bream due to low capture rates. Full details of the PERMANOVA analysis are provided in Appendix 5.

Rapid parasite analysis

Rapid parasite analysis of the intestinal tract and gills of dissected fish, as well as the water contents of fish storage bags, identified a range of fish parasites. Parasites that were identified included: sea lice and other externally parasitic crustaceans from water in fish storage bags; parasitic copepods and monogeneans on fish gills; cestodes in the visceral cavity; and a variety of trematodes, cestodes, digeneans, nematodes and acanthocephalans in the intestinal tract. Some fish had no parasites while others had large numbers, with no clear patterns. Most could not be identified to species level as parasite assemblages of Australian inshore fish are not currently well known. The detailed results are provided in Appendix 6, and the collection has been retained for further study.

Histopathological analysis

Histopathology lesions recorded from the fish collected in Autumn 2018 included inflammation, degeneration, pigment accumulation, hyperplasia, granulomas, necrosis, metaplasia and neoplasia (see Appendix 7). Some changes were caused by parasites, but most did not appear to have been caused by infection. A semi-quantitative assessment method and scoring system (Relative Fish Health Index) was developed by Dr Roger Chong, Aquatic Pathologist at BSL, providing a rate of lesions across the organ types analysed, to compare the severity of histopathology between individual fish, fish species, and sample locations, including the fish sampled from the reference sites. Over time, the Index can be used as a tool to infer trends in fish histological health for the locations studied.

The results for this index are available for the Autumn 2018 sampling event (Figure 9). RFHI ranges from 0 – worst to 1 – best, and was lowest for barramundi at Zone 7, Auckland Inlet (n = 4). Zone 7 was the only zone that scored an average of less than 0.8. None of the barramundi sampled in Gladstone Harbour or at reference site 1 (Stanage Bay) scored a perfect, normal histopathological score of 1.0. Bream and sea mullet scored higher than barramundi in the RFHI, with averages between 0.9 and 1.0 at all sampling sites. Diamondscale mullet scored lowest (worst) at Zone 2, Graham Creek, with an average of less than 0.8 (n = 1).

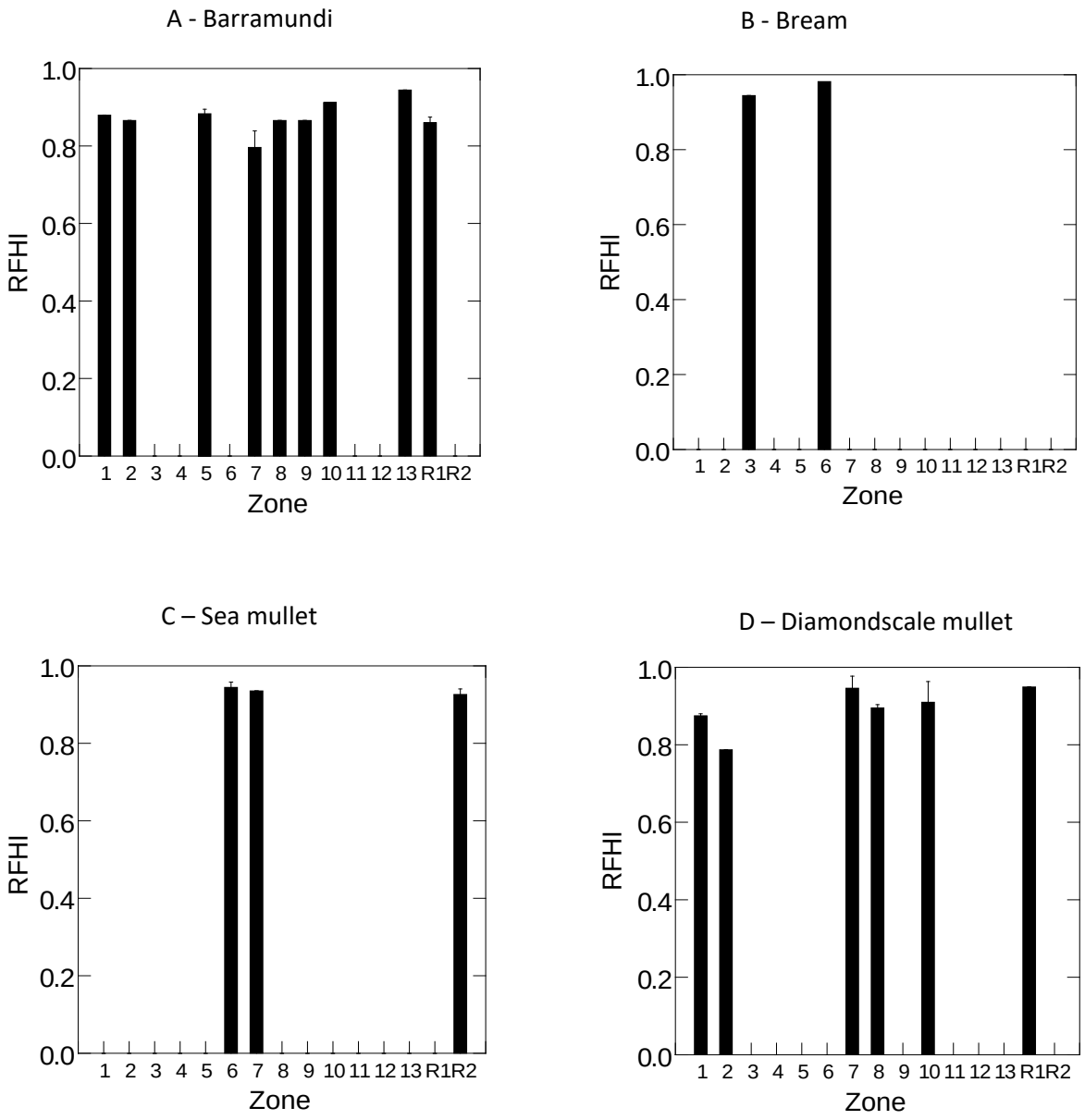


*Figure 9: Relative Fish Health Index (in development, Appendix 7) by zone of A – barramundi (*L. calcarifer*, n = 19); B – bream (*A. australis *and* A. pacificus*, n = 2); C –sea mullet (*M. cephalus*, n = 12); and D – diamondscale mullet (L. vaigiensis, n = 12).*

Spring 2018 sampling

During the Spring 2018 sampling event a total of 291 fish from 33 species were caught at the 11 Gladstone Harbour zones sampled and the two reference sites. The species that were caught at the most sites were: barred javelin (9 Gladstone zones, 1 reference site); blue catfish (9 Gladstone zones, 1 reference site); diamondscale mullet (7 Gladstone zones, 1 reference site); blue threadfin (8 Gladstone zones); barramundi (5 Gladstone zones, 1 reference site) and sea mullet (5 Gladstone zones). Two species of bream were captured during Spring, five pikey bream and one yellowfin bream. Most of the bream were caught by handlining while nets were soaking.

Table 2: Fish species (listed by common name) and abundance at Gladstone Harbour zones and two reference sites sampled in Spring 2018. White = 0; blue = 1-5; orange = 6-10; green = 10+ specimens. Common names of target species retained for further analysis are shaded grey. Species names provided in Appendix 4. Site R1 = Baffle Creek; R2 = Stanage Bay.

	Zone / site												
Fish species	**1**	**2**	**3**	**5**	**6**	**7**	**8**	**9**	**10**	**12**	**13**	**R1**	**R2**
Barramundi	2					3	1	1		6			6
Barred Javelin	1	14	2	13	1	9	9	1			1	4	
Beach Salmon		2	6										1
Blubber lip bream						3							1
Blue Catfish	5	1		1	4	2		5	1	10	6		26
Blue Threadfin	2	1	5	3		3	9			2	11		
Blue tuskfish													1
Bony bream											1		
Common Silverbiddy										1			
Diamondscale Mullet		4	3	7	1	1	1	3					1
Dusky flathead				1				1				1	
Giant queenfish						2	1	1		1			
Giant Shovelnose Ray												1	
Goldlined Rabbitfish											1		
Goldspotted rockcod					1								
Green backed mullet		1											
Grey mackerel					1								
Hairback Herring						3							
King Threadfin				1									
Mangrove Jack													1
Moses snapper									2				
Mulloway				1		1							
Pikey Bream		2		1					2				
Popeye Mullet			4										
Sand whiting			1										
Sea mullet	3		19				3	3		1			
Shovelnose Ray			1				1						
Sicklefish					1	4							
Sliteye Shark										1			
Snub-nosed dart					1								
Spotted Scat						6							
Whitespotted Eagle Ray									1				
Yellowfin Bream									1				

Fish condition measures

Fulton's condition factor (K) of barramundi varied only very slightly by zone, with the lowest average Fulton's *K* recorded at Zone 7, Auckland Inlet (n = 3). The lowest average Fulton's *K* for barred javelin was recorded at Zone 5, Inner Harbour (n = 5). The pikey bream recorded across three sites had different condition factors, with the lowest identified at Zone 10, Boyne Estuary (n=2) and the highest at Zone 2, Graham Creek (n=2). There was large variation in Fulton's *K* for diamondscale mullet with the lowest condition factor recorded at Zone 7, Auckland Inlet (n=1). Sea mullet showed only slight variation in Fulton's *K* across five zones, with fish in Zone 3, Western Basin having the highest average (n=3) and Zone 9, South Trees Inlet the lowest (n = 3) (Figure 10).

Average HSI was highest in barramundi from Zones 8, Mid Harbour and 9, South Trees Inlet; barred javelin from Zone 5, Inner Harbour; sea mullet from Zone 1, The Narrows; and diamondscale mullet from Zone 5, Inner Harbour. The pikey bream from Zone 10, Boyne Estuary, had a higher HSI than those caught in Zone 2, Grahams Creek, and Zone 5, Inner Harbour (Figure 11).

Barramundi gender was distinct in more of the recorded specimens in the Spring 2018 sampling event than in Autumn 2018, and GSI could be assessed for male fish across five Gladstone zones and one reference site. Average GSI of barramundi was highest in Zone 1 (The Narrows, n = 2). Four out of five pikey bream were female, and female GSI was highest in fish collected from Zone 10, Boyne Estuary. Both male (n = 4) and female (n = 23) barred javelin were caught at various Gladstone Harbour zones and females at a reference site, Baffle Creek (n = 4). The highest male GSI was highest in Zone 7, Auckland Inlet, and the highest female GSI at Zone 13, Rodds Bay. Of the 18 mature diamondscale mullet caught, 15 were female. Females were caught in seven Gladstone Harbour zones and at one reference site (Stanage Bay) and average GSI was highest at Stanage Bay. All but one of the 13 mature sea mullet caught were female. Of these twelve females the fish from Zone 8, Mid Harbour had the highest average GSI (Figure 12).

The HAI score is scored as a subtractive measure, such that a score of 0 is ideal (all assessed organs appear normal) and higher scores equating to more abnormalities (up to a maximum score of 210). Barramundi caught in Zone 1, the Narrows (n = 2), Zone 8, Mid Harbour (n = 1) and Zone 9, South Trees Inlet (n = 1), had no abnormalities recorded. The best possible score of 0 was also recorded in barred javelin from Zone 13, Rodds Bay (n = 1), diamondscale mullet from Zone 3, Western Basin (n = 3), Zone 6, Calliope Estuary (n = 1), Zone 7, Auckland Inlet (n = 1), Zone 9, South Trees Inlet (n = 3) and reference site 1, Stanage Bay (n = 1), and sea mullet from Zone 9, South Trees Inlet (n = 3) and Zone 12, Colosseum Inlet (n = 1). None of the six bream captured scored a 0 for HAI (Figure 13).

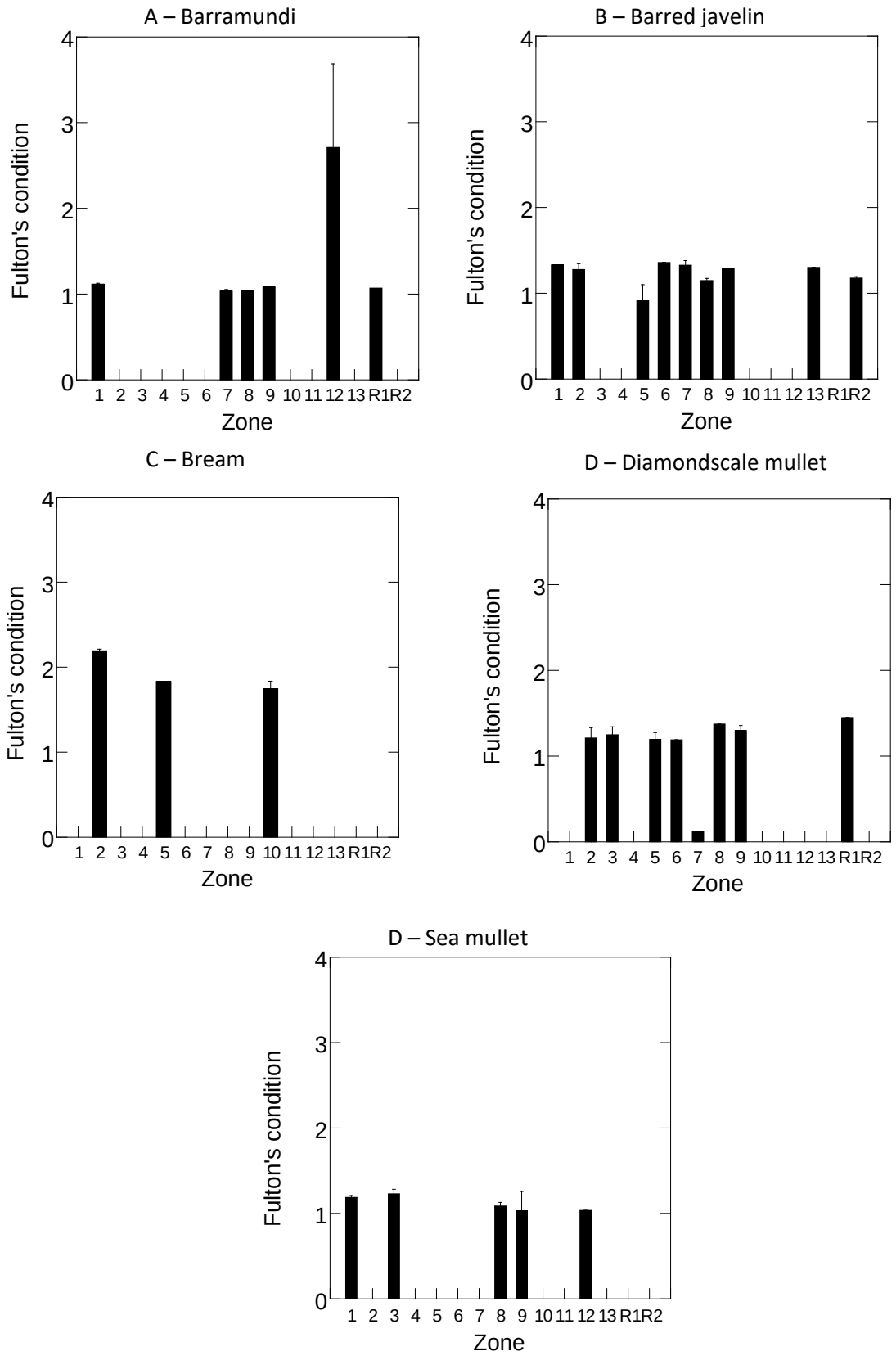


*Figure 10: Fulton's condition factor by zone of A – barramundi (*L. calcarifer*, n = 15); B – barred javelin (*P. kaakan, *n = 31); C – bream (*A. australis *and* A. pacificus*, n = 6); C – diamondscale mullet (*L. vaigiensis*, n = 18); and D – sea mullet (*M. cephalus*, n = 13).*

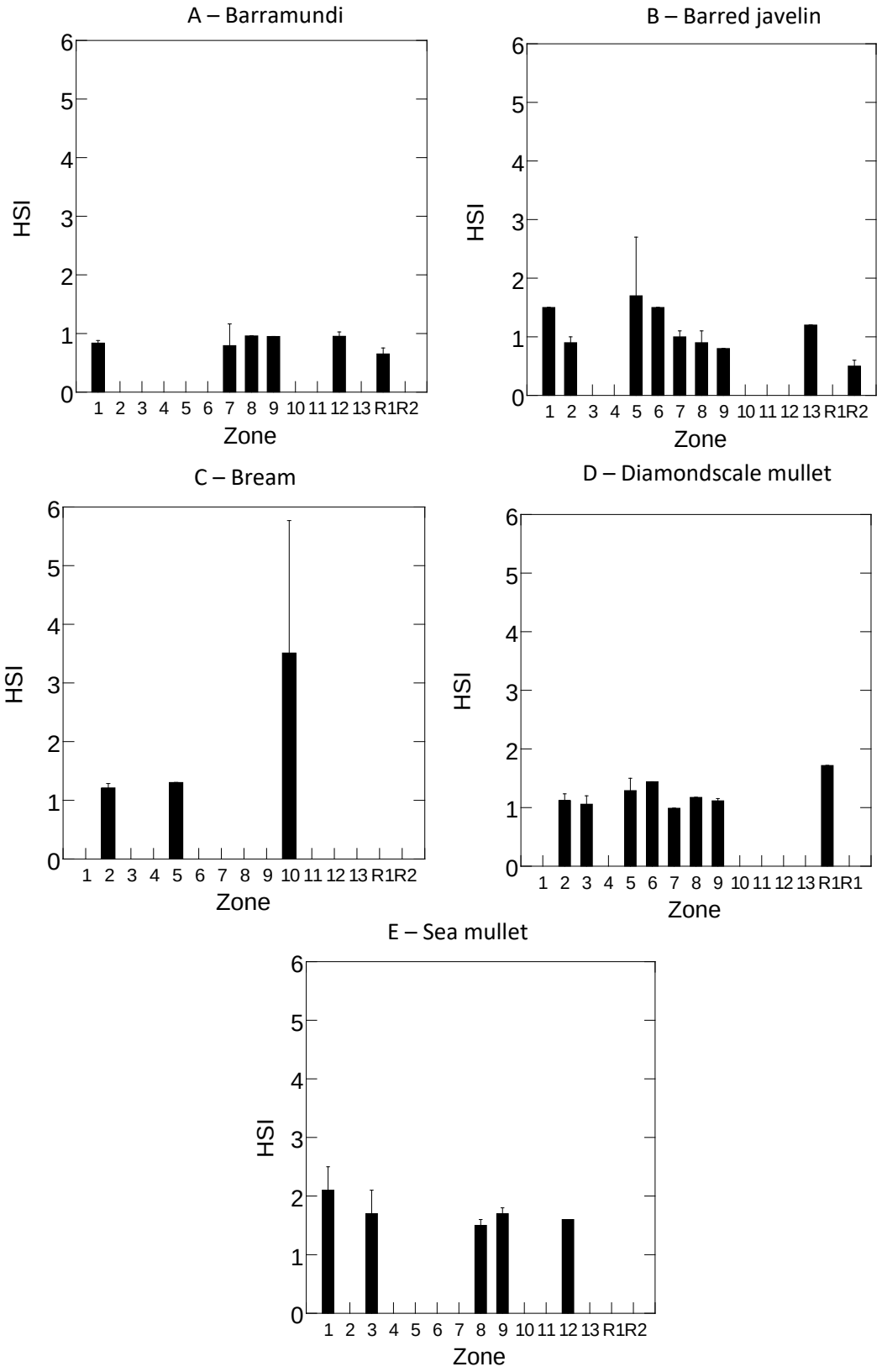
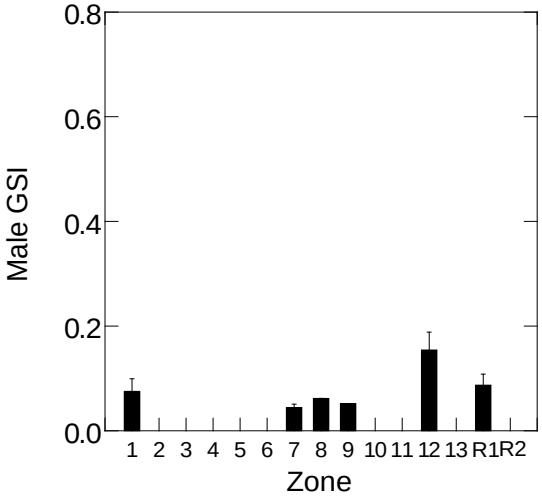
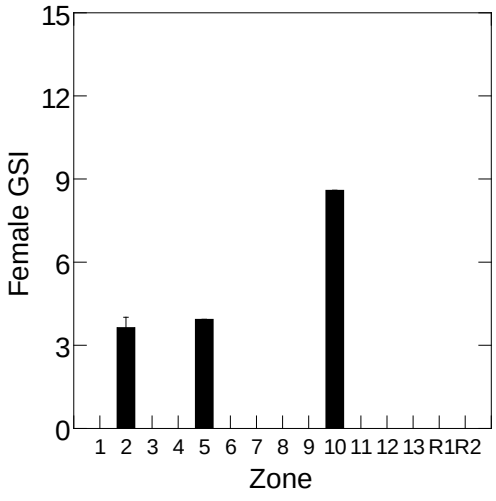


*Figure 11: Hepatosomatic index (HSI) by zone of A – barramundi (*L. calcarifer*, n = 15); B – barred javelin (*P. kaakan*, n = 31); C – bream (*A. australis *and* A. pacificus*, n = 6); D – diamondscale mullet (*L. vaigiensis*, n = 18); and E – sea mullet (*M. cephalus*, n = 13).*

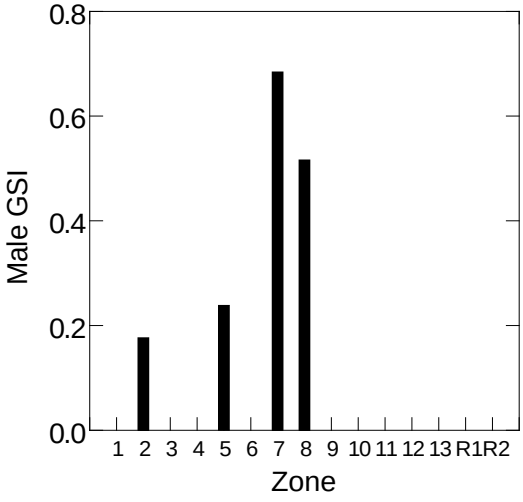
A – Male barramundi



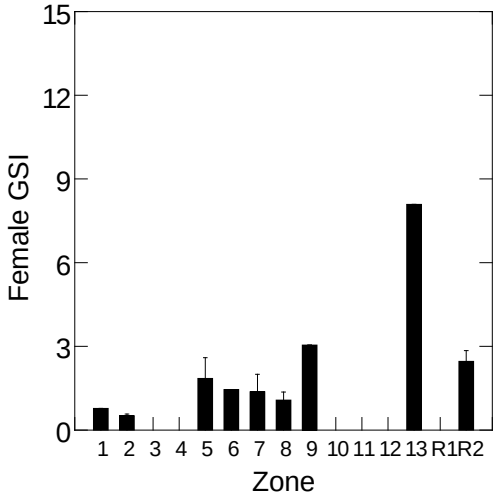
B – Female pikey bream



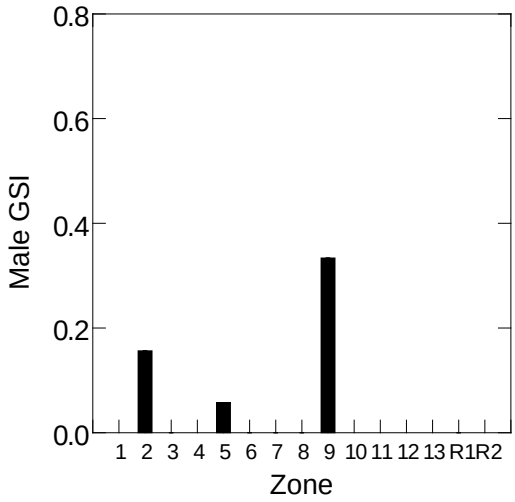
C – Male barred javelin



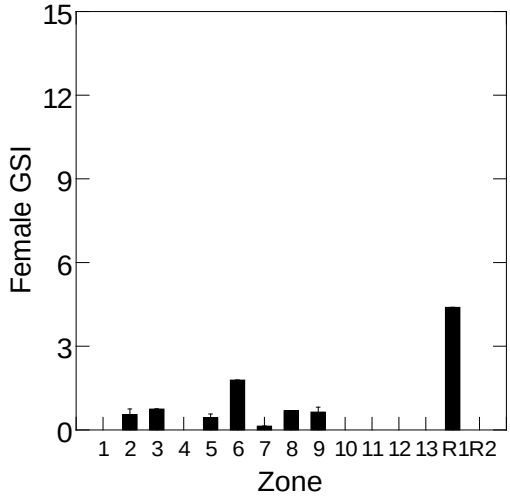
D – Female barred javelin



E – Male diamondscale mullet



F – Female diamondscale mullet



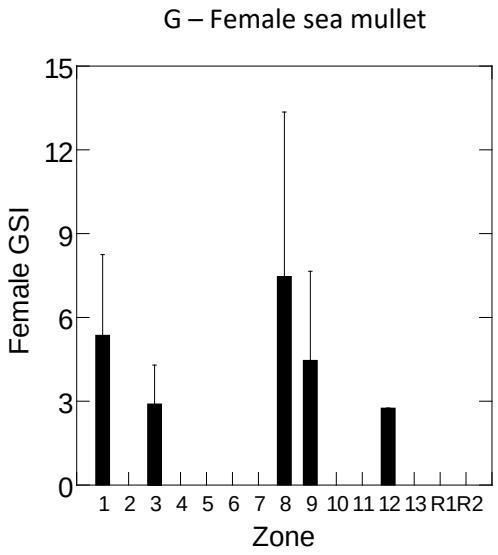


*Figure 12: Gonadosomatic index (GSI) by zone of A – male barramundi (*L. calcarifer*, n = 14); B – female pikey bream (*A. pacificus*, n = 4); C – male barred javelin (*P. kaakan*, n = 4); D – female barred javelin (n = 27); E – male diamondscale mullet (*L. vaigiensis*, n = 3); F – female diamondscale mullet (n = 15); and G – female sea mullet (*M. cephalus*, n = 12).*

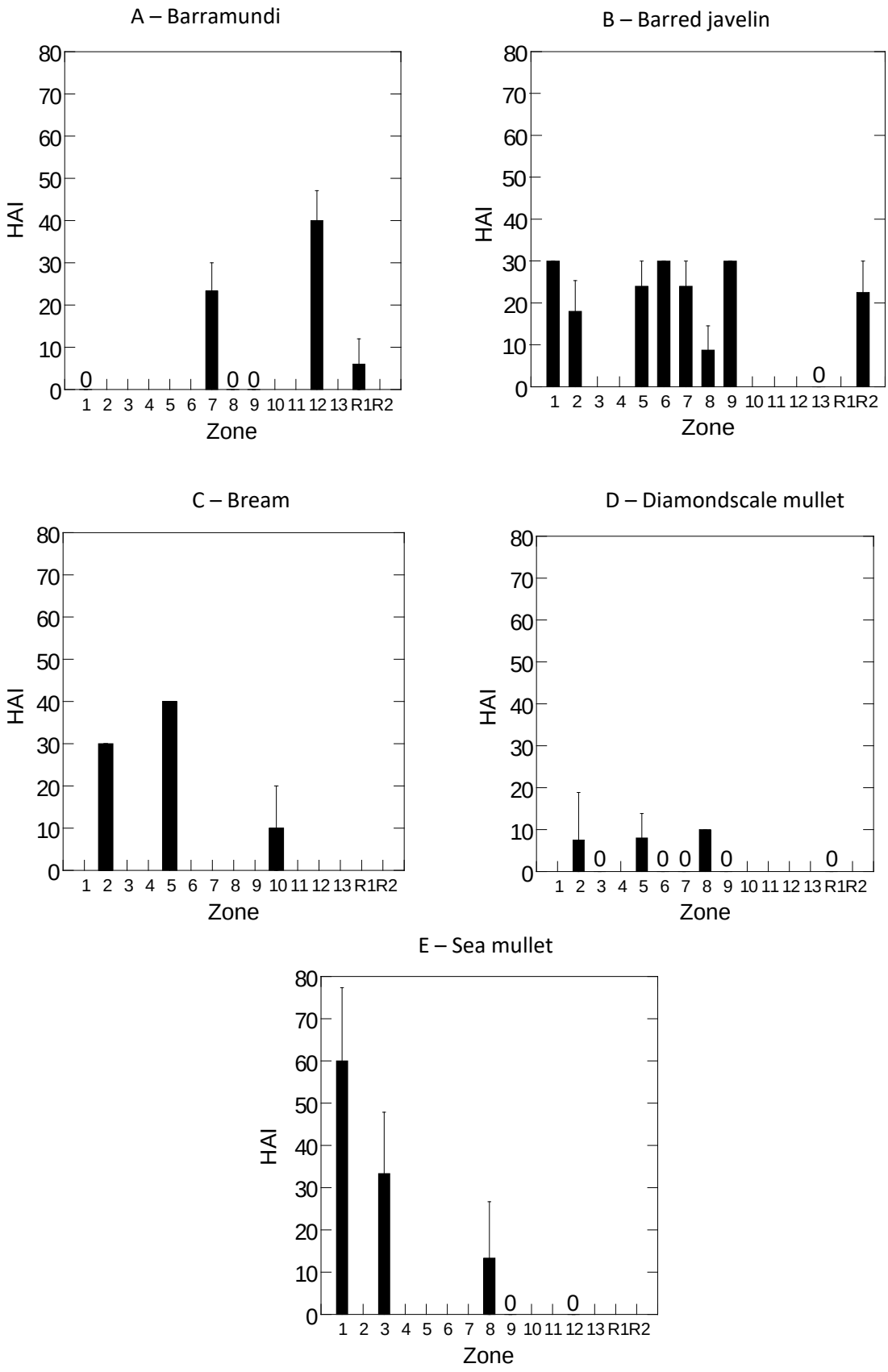


*Figure 13: Health Assessment Index by zone of A – barramundi (*L. calcarifer*, n = 15); B – barred javelin (*P. kaakan*, n = 31); C – bream (*A. australis *and* A. pacificus*, n = 6); C – diamondscale mullet (*L. vaigiensis*, n = 18); and D – sea mullet (*M. cephalus*, n = 13).*

Using 1-way PERMANOVA a significant difference was detected among zones for HAI of barramundi. Barramundi from Zone 12, Colosseum Inlet scored higher (worse) for HAI than most other zones, while fish from the reference site at Stanage Bay (site RF1) had a lower (better) HAI score than most other zones. Barramundi GSI, HSI, Fulton's condition index, and Eye asymmetry were all similar among the six zones compared.

Using 2-way PERMANOVA a significant 2-way interaction was detected between sampling time and zone for Fulton's condition factor (K). Barramundi sampled in April at Zone 7, Auckland Inlet had a greater Fulton's K than fish sampled in April at Stanage Bay, with fish sampled in April at both zones having greater values than fish sampled in September at either zone. An effect of zone was detected for HAI, with barramundi in Zone 7, Auckland Inlet, having higher (worse) HAI scores than barramundi from Stanage Bay.

1-way PERMANOVA detected a significant difference among zones for Spring 2018 barred javelin, in both GSI and Fulton's K. GSI of barred javelin from the reference site Baffle Creek (site RF2) was higher than in fish from both Zone 2, Graham Creek and Zone 8, Mid Harbour. Fulton's K of barred javelin from Zone 2, Graham Creek and Zone 7, Auckland Inlet was higher than in fish from Zone 5, Inner Harbour and Zone 8, Mid Harbour. HSI, eye asymmetry, and HAI of barred javelin were all similar among the five zones compared.

For bream caught in Spring 2018, no differences were detected for any of the five metrics between Zone 2, Graham Creek and Zone 10, Boyne Estuary. This result should be interpreted cautiously due to the overall low replication of Bream in the data set analysed (total of five fish).

1-way PERMANOVA detected a significant difference among zones for HIS of sea mullet. Sea mullet sampled at the reference site Baffle Creek (RF2) had lower HSI values than most other zones, while fish sampled from Zone 6, Calliope Estuary also exhibited lower HSI than most other zones. PERMANOVA also detected a significant difference among zones for HAI of sea mullet. Sea mullet sampled from Zone 1, the Narrows exhibited higher (worse) HAI values than fish sampled from Zone 6, Calliope Estuary and the reference site Baffle Creek.

Preliminary Fish Health Indicators for Gladstone Harbour

Using the results obtained from sampling in 2018, several preliminary fish health measures that are particularly promising for possible inclusion in the fish health indicator for the Gladstone Harbour Report Card have been identified. The two measures that appear most useful for the Gladstone Harbour Report Card are:

3. **Health Assessment Index (HAI)**: requires a gross pathological analysis during dissection and produces a composite metric that integrates evaluations of the condition of multiple organs and tissues. The premise of the index is that scores will cumulatively reflect the acute and chronic stressors present in the fish's environment, with poorer anatomical condition resulting in higher HAI scores and thus indicative of a more stressful environment. The version of the HAI used in this study was used by Wesche et al. (2013) during the fish health investigation in Gladstone Harbour in 2011-2012.

4. **An index of relative histopathological condition:** requires microscopic study of the changes to tissues caused by disease. A draft metric (Relative Fish Health Index) is in development by Dr Roger Chong (Aquatic Pathologist, BSL) to compile data across four organ types, this is currently being further tested using organs from the Spring 2018 sampling event.

Histopathological analysis has been frequently applied to study the condition of wild and cultured fish around the world, and provides an assessment of tissue changes induced by environmental stressors such as water pollution (Bernet et al., 1999). The organs selected for this project included gills, liver and a skin/muscle block. Histopathology is a useful indicator due to its attention to an intermediate level of biological organisation – providing data on medium term responses of fish to a wide variety of sublethal stressors.

Preliminary indicator baselines and worked examples

1. Health Assessment Index

A Health Assessment Index was calculated for all dissected fish in Autumn and Spring 2018, by scoring and summing gross pathology scores for the following organs: skin, eyes, fins, gills, spleen, kidney, hindgut and liver. Parasite score was not included in 2018 calculations as parasites were assessed separately this year, however it is recommended that this component is added to the scoring system (note: parasites were recorded in 2018 and can easily be incorporated for the purposes of 2019 reporting). The best possible score for each organ, and in total, is 0. Any increase from a score of 0 indicates the identification of gross pathologies visible during a routine necropsy dissection.

HAI is designed to be a used as a summed average for a sample population (Adams et al., 1993). Using this method, the Gladstone Harbour-wide HAI results (seven organs) have been determined, by species, using Autumn and Spring 2018 data (Table 3). Reference site data have been excluded from these calculations and are provided by site in Table 4 for comparison. Average HAI scores for reference sites in the present study ranged from 0 to 23, while scores for Gladstone Harbour ranged from 5 to 28.

Benchmark: The natural individual fish benchmark for HAI is 0 – no observable pathologies. In this study, a score of 0 was achieved by a total of 23 of the 57 fish dissected in Autumn 2018, and 51 of the 108 fish dissection in Spring 2018 (across all species including non-target).

Worst Case Scenario: Using the HAI method applied in 2018 (scoring seven organs), the maximum total score for an individual fish is 210. When parasites are added to this total in 2019 the maximum score will be 240. The level of deviation from normal (0) would constitute a biological tipping point beyond which a fish population is severely diseased must be derived from other studies.

During the 2011-2012 fish health investigation in Gladstone Harbour, the highest HAI score was recorded in the upper Boyne Estuary (Wesche et al. (2013), provided in Table 5, below). This score was for all of the fish species collected, the report did not detail HAI scores for all individual fish species, although barramundi were reported separately. Again for all fish species considered, adjusted means of HAI score identified by Wesche et al. (2013) were 18.3 for fish without a clear disease diagnosis during field assessment, vs. 31.0 for fish with a field diagnosis of diseased, a difference that was significant at the $p < 0.01$ level (Wesche et al. (2013), Appendix B, pages 108-109). For barramundi only, the adjusted mean HAI score was 16.6 for fish without a clear disease diagnosis during field assessment, vs. 32.6 for fish with a field diagnosis of diseased, a difference that was again significant at the $p < 0.01$ level (Wesche et al. (2013), Appendix B, page 116). The locations with the highest HAI scores for barramundi were the Upper Boyne Estuary (Trip 1 47.7 and Trip 2 48.5) and the Lower Boyne Estuary (Trip 1 41.8 and Trip 2 30.0) (Wesche et al. (2013), provided in Table 6, below). Differences in mean barramundi HAI between locations were significantly different at the $p < 0.01$ level (Wesche et al. (2013), Appendix B, page 117). Based on the results of Wesche et al. (2013), a possible WCS for average HAI is 40.0.

Table 3: HAI calculations for fish species caught across all Gladstone Harbour zones. ND = no data. HAI scores are in bold.

		Autumn 2018		Spring 2018	
Common name	**Species name**	**Number of fish (n)**	**HAI**	**Number of fish (n)**	**HAI**
Bream (pikey and yellowfin)	*Acanthopagrus* spp.	2	**15**	6	**22**
Barramundi	*Lates calcarifer*	14	**19**	12	**28**
Diamondscale mullet	*Liza vaigiensis*	11	**18**	17	**5**
Sea mullet	*Mugil cephalus*	7	**13**	13	**25**
Barred javelin	*Pomadasys kaakan*	ND	**ND**	27	**18**

Table 4: HAI calculations for fish species caught at the two reference sites. ND = no data. HAI scores are in bold.

			Autumn 2018		Spring 2018	
Reference site	**Common name**	**Species name**	**Number of fish (n)**	**HAI**	**Number of fish (n)**	**HAI**
Stanage Bay	Barramundi	*Lates calcarifer*	5	**0**	5	**6**
Stanage Bay	Diamondscale mullet	*Liza vaigiensis*	1	**0**	1	**0**
Baffle Creek	Sea mullet	*Mugil cephalus*	5	**14**	ND	**ND**
Baffle Creek	Barred javelin	*Pomadasys kaakan*	ND	**ND**	4	**23**

Table 5: Response variables tested by Wesche et al. (2013) during the Gladstone fish health investigation in 2011-2012, all fish species combined.

Table 3.6a. Effects of locations and trips on the response variables.

Location	Trip	Hepat. index	Condition factor	HAI score	%# diseased	%# skin cond. > 0
Fitzroy	1	0.82	1.34	11.8	7.6	7.8
Bundy	1	1.74	1.46	32.8	7.9	6.6
Hamilton	1	1.17	1.22	9.3	7.4	8.5
Calliope	1	2.00	1.36	18.7	3.6	0.0
Harbour	1				4.0	4.9
Spoil	1	1.57	1.28	3.6	0.0	4.8
UpBoyne	1	1.26	1.31	30.9	10.0	3.1
LwBoyne	1	1.31	1.29	32.7	13.6	3.8
Rodds	1	1.66	1.36	21.6	5.6	4.9
Lake Awoonga	1	1.66	1.41	3.7	0.0	0.0
Fitzroy	2	1.56	1.25	19.8	10.0	9.0
Bundy	2	1.49	1.42	24.6	3.5	3.6
Hamilton	2	1.49	1.23	19.9	12.4	13.6
Calliope	2	1.79	1.39	22.2	13.4	11.6
Harbour	2				7.8	7.3
UpBoyne	2	1.80	1.24	38.8	7.6	5.2
LwBoyne	2	2.08	1.36	28.2	10.8	10.2
Rodds	2	1.57	1.35	14.9	5.7	4.4
Sig. of -	Locations	**	**	**	*	*
	Trips	**				**
	Interaction	**			*	**

#as assessed in the field; ** $P < 0.01$; * $P < 0.05$

Table 6: Response variables tested by Wesche et al. (2013) during the Gladstone fish health investigation in 2011-2012, barramundi (Lates calcarifer) only.

Table 4.2a. Effects of locations and trips on the response variables.

Location	Trip	Hepat. index	Condition factor	HAI score	%# diseased	%# skin cond. > 0
Fitzroy	1	1.05	1.36	16.5	16.6	14.4
Bundy	1	1.24	1.36	22.1	21.5	22.8
Hamilton	1	0.84	1.11	10.6	21.7	24.2
Calliope	1	1.08	1.15	15.0	6.6	0.0
Harbour	1				12.6	13.4
UpBoyne	1	1.17	1.27	47.7	56.0	18.1
LwBoyne	1	1.12	1.18	41.8	63.3	3.6
Rodds	1	1.25	1.16	12.8	42.2	42.6
Lake Awoonga	1	1.25	1.42	29.1	0.1	0.0
Fitzroy	2	1.19	1.32	27.2	42.4	29.4
Bundy	2	1.37	1.45	21.4	0.0	0.0
Hamilton	2	1.08	1.12	20.2	44.8	47.2
Calliope	2	0.98	1.30	13.1	32.9	38.8
UpBoyne	2	1.43	1.32	48.5	63.0	39.4
LwBoyne	2	1.18	1.17	30.0	42.1	41.6
Rodds @	2	1.06	0.93	19.5	99.9	100.0
Sig. of -	Locations	*	**	**	**	
	Trips				*	**
	Interaction				*	*

#as assessed in the field; @ based on two (diseased) fish; ** $P < 0.01$; * $P < 0.05$

Using a preliminary benchmark score of an average HAI of 0, and a preliminary WCS score of an average HAI of 40, example HAI scores and grades can be calculated using a distance from the benchmark method, as is used for similar ecological indicators including South East Queensland Report Card (Healthy Land & Water, 2017), the Fitzroy Basin Report Card (Flint, Rolfe, et al., 2017) and for GHHP's Mud Crab Indicator (Flint, Anastasi, et al., 2017). These example scores and grades have been calculated in Table 7 using data from August 2018 and Spring 2018. The distance from the benchmark function used is as follows:

Calculated score = 1-((x-B)/(WCS-B))

Where:

x = recorded value

B = benchmark

WCS = worst case scenario

Table 7: Worked examples of HAI scores and grades for Gladstone Harbour using data from Autumn 2018 and Spring 2018. ND = no data. NOTE: Scores and grades are examples only and not for incorporation into the report card.

Species	Average HAI Autumn 2018	Benchmark	WCS	Example calculated score	Example GHHP Grade
Bream	15	0	40	0.625	C
Barramundi	19	0	40	0.525	C
Diamondscale mullet	18	0	40	0.55	C
Sea mullet	13	0	40	0.675	B
Barred javelin	ND	0	40	ND	ND

Species	Average HAI Spring 2018	Benchmark	WCS	Example calculated score	Example GHHP Grade
Bream	22	0	40	0.45	D
Barramundi	28	0	40	0.3	D
Diamondscale mullet	5	0	40	0.875	A
Sea mullet	25	0	40	0.375	D
Barred javelin	18	0	40	0.55	C

2. Relative Fish Health Index (gills, liver, skin, muscle):

The use of a benchmarking approach to scoring the Relative Fish Health Index may be less biologically relevant than tracking and reporting changing trends in this Index through time. The primary reason for this is that no pathologies should be observed in fish that live in a pristine environment. The value of the RFHI is in providing a reproducible method to measure relative fish health status in a semi-quantitative way over temporal and spatial scales (Chong, BSL, 2018, Appendix 7).

However, the natural baseline for histopathology is 'no pathological change', scored in the RFHI as 1.0 – no reduction in score as a result of pathologies. A RFHI of 1.0 is expected for a pristine habitat, any decrease means a loss of health, an increasing response to stressors, and an increased risk of approaching a grossly observable tipping-point in fish condition. The RFHI provides a semi-quantitative assessment of histopathological changes based on a categorisation of lesions as: incidental, low severity, low to moderate severity, moderate severity, moderate to high severity, or high severity (Chong, BSL, 2018, Appendix 7).

The approach has some limitations including: scoring is dependent on the subjective assessment of the fish pathologist (as such, a more experienced pathologist will make a more reliable assessment), potential for bias towards knowledge of the fish species as species that are less familiar to the pathologist may not be assessed as accurately, lesion significance depends on a variety of environmental and non-environmental factors which can potentially lead to under- or over-interpretation (Chong, BSL, 2018, Appendix 7). The senior aquatic pathologist analysing samples from this project (Dr Roger Chong, BSL) recommends some measures to minimise potential for error in lesion scoring, including: sampling a high number of fish over an extended period of time to help exclude incidental findings, ensure high quality of fish tissue by fixing tissues immediately after fish death, include as many tissue types as possible in the histopathology assessment, and include information about the environment (water chemistry and quality) and clinical history of the fish in the final lesion analysis (Chong, 2018, Appendix 7).

A score of 1.0 was achieved by 1 of the 46 fish that were analysed in Autumn 2018, a diamondscale mullet caught in Zone 7, Auckland Inlet. The lowest score was 0.67, for a barramundi also caught in Auckland Inlet (Figure 14). Averaging of the RFHI across multiple fish obscures these results, as can be seen in Tables 8 and 9.

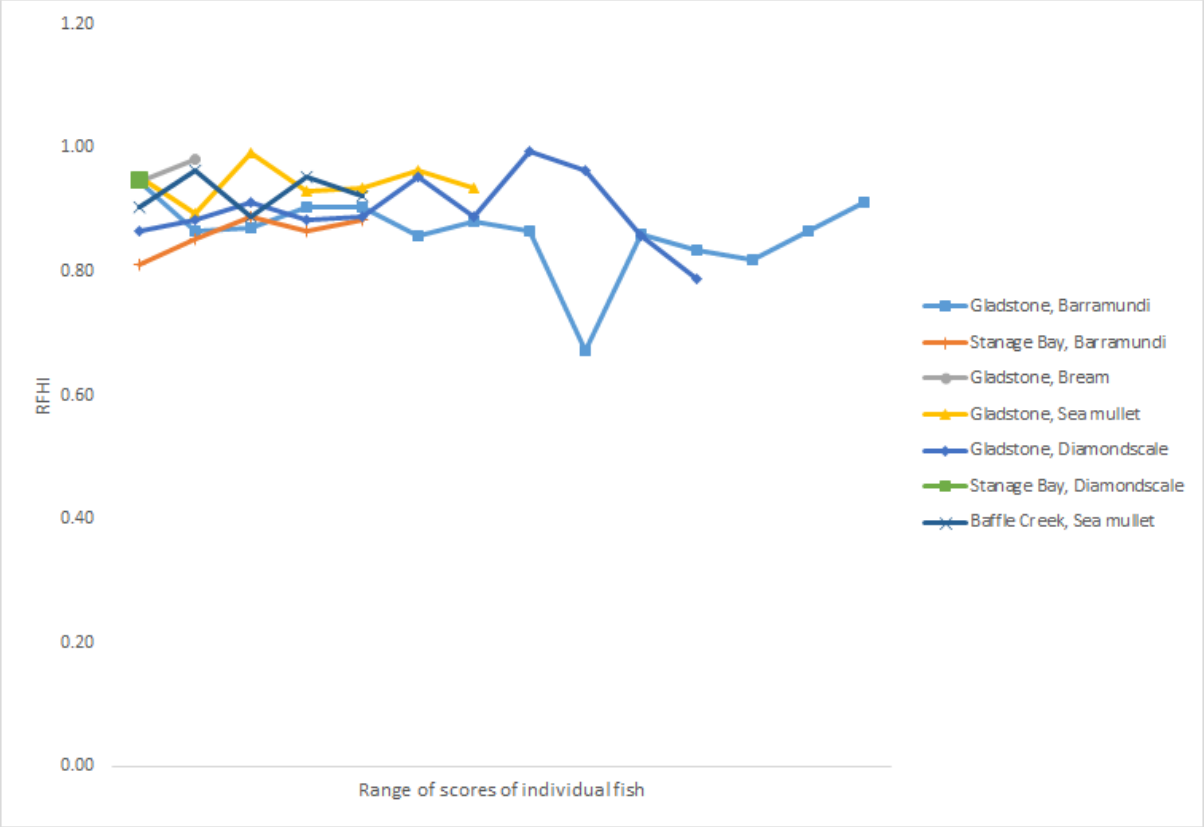


Figure 14: RFHI scores for all fish caught in Gladstone Harbour and the two reference sites, by species

Deviations from a benchmark could be determined in impacted sites, relative to the prevalence of background pathologies in unimpacted sites. However in Central Queensland, few truly unimpacted sites remain. Dennis et al. (2016) found much lower prevalence of histopathological abnormalities at Stanage Bay during the fish health investigations of 2011-2012, as described further below. In contrast, the present study found little difference in averages between Gladstone Harbour (Table 8) and reference sites (Table 9) during Autumn 2018, and sea mullet scored slightly lower at the reference sites. This result suggests that the current reference sites are not sufficiently unimpacted, as an ideal reference site would have fish organ lesion rates that are equal to or very close to zero. However, if a 'best available' approach is taken, the sampling period could potentially be considered as a baseline condition for Gladstone Harbour, that is, a time when lesion prevalence is similar to a 'best available' (though not pristine) reference site. Spring 2018 samples are currently being processed at BSL and will further inform the development of this measure as a component of the Fish Health Indicator.

There is some existing information that may help inform the setting of baselines. During the fish health investigations in 2011-2012, Dennis et al. (2016) observed significant histopathological abnormalities at high prevalence in fish sampled from Gladstone Harbour (34 of 36, prevalence = 94.4%) but few abnormalities in fish sampled from the reference site (3 of 23, prevalence = 13.0%; $p < 0.0001$). The most common abnormality that Dennis et al. (2016) observed was inflammatory disease associated with significant parasitism, which was identified in 27 of 36 fish sampled from Gladstone Harbour (prevalence = 75.0%), but in only 3 of 23 (13.0%) of fish from the reference site

(Stanage Bay). Dermal lesions were present in 22 of 32 (prevalence = 68.8%) fish sampled from Gladstone Harbour, and in 0 of 22 fish from the reference site (Dennis et al., 2016).

Table 8: Average RFHI calculated using data from all Gladstone Harbour zones sampled in Autumn 2018

Common name	Species name	Number of fish (n)	RFHI
Bream	*Acanthopagrus* spp.	2	0.96
Barramundi	*Lates calcarifer*	14	0.86
Diamondscale mullet	*Liza vaigiensis*	11	0.90
Sea mullet	*Mugil cephalus*	7	0.94

Table 9: Average RFHI calculated using data from reference sites sampled in Autumn 2018. No bream were caught at reference sites in Autumn 2018.

Reference site	Common name	Species name	Number of fish (n)	RFHI
Stanage Bay	Barramundi	*Lates calcarifer*	5	0.86
Stanage Bay	Diamondscale mullet	*Liza vaigiensis*	1	0.95
Baffle Creek	Sea mullet	*Mugil cephalus*	5	0.93

A worked example of RFHI scores and grades is not provided, as the Spring 2018 fish samples are currently being processed at BSL and will provide much greater replication to inform the development of appropriate methods for this measure. Combining the two sampling periods will also assist in statistical testing of the RFHI (comparing Gladstone Harbour zones to reference sites).

A further consideration in the application of the RFHI is that in a situation where a specific impact has occurred (e.g. introduction of a toxicant, disease outbreak or a fish kill), categorisation and separate assessment of lesions into acute non-reversible lesions vs. chronic reversible lesions will provide a more accurate assessment. That is, the effect of acute lesions can be diluted by assessment of chronic lesions and provide a false-negative result, i.e. a relatively good RFHI score when a fish kill has occurred. In a fish kill event, the RFHI-acute should be very low for affected fish and the RFHI-chronic should be higher for surviving fish (Chong, BSL, 2018, Appendix 7).

Discussion of results and preliminary recommendations

Preliminary fish health indicators

The 2018 results of the fish health indicator sampling and analysis have identified several fish health indicators that are particularly promising for further analysis and possible inclusion in the Gladstone Harbour Report Card. A full review of the use of different fish health indicators is provided in the Milestone 3 report, Fish health indicators for ports and estuaries in Northern Australia (Attachment B). The two indicators that appear most useful for the Gladstone Harbour Report Card are HAI and RFHI.

HAI can be modified in 2019 to include a score for parasites (which were examined separately in 2018) and the comprehensiveness of the RFHI can potentially be increased by including more fish tissues in the analysis. During this pilot study in 2018, fish gill, liver and skin/muscle tissue were

provided to BSL for histopathological analysis, but kidney, spleen, heart and gonad tissues were also collected and fixed during dissection and could be provided for analysis subject to funding.

Recommendation 1: GHHP continues to monitor HAI and RFHI in Autumn 2019, in order to calculate scores for a pilot fish health indicator using Spring 2018 and Autumn 2019 data.

Recommendation 2: GHHP considers whether to provide a wider range of fish tissues for histopathological analysis, to increase the comprehensiveness of fish health assessments.

The condition measures Fulton's K, HSI and GSI are extremely biologically variable which would make the establishment of a baselines difficult in the short term. For example, all three measures are affected by reproductive status of the fish. A much larger dataset spanning many biological cycles (including seasons) is required to allow for the effective development of these indicators for Australian inshore fish species. Fluctuating asymmetry of eye diameter is also currently problematic due to a lack of information on 'normal' levels of asymmetry in Australian inshore species.

Despite these difficulties, all four of these condition metrics can be rapidly measured during dissections, so while they may not yet be useful indicators for the Report Card, it is worthwhile continuing to collect data from future samples to establish a long time series, which may eventually provide the information required to develop suitable metrics. Following the Gladstone fish health investigation in 2011-2012, Wesche et al. (2013) reported significantly lower condition factors of barramundi from Gladstone harbour than from reference sites (at the $p < 0.05$ level), and barramundi from Gladstone also had significantly higher proportions of sunken abdomens and lower levels of mesentery fat. During events such as that experienced in 2011-2012, noticeable changes in condition measures are more likely.

Recommendation 3: GHHP continues to monitor Fulton's K, HSI, GSI and fluctuating asymmetry of eye diameter to collate a dataset which may in future be used to inform the fish health indicator.

Another metric which could possibly be considered for monitoring and reporting is the bioaccumulation of toxicants in fish tissues. While bioaccumulation only becomes an indicator of fish health at levels that cause the initiation of detoxification mechanisms and tissue damage (Whitfield & Elliott, 2002), it also provides information on the bioavailability of toxicants in the environment and is an important consideration for fish that are consumed by people. Bioaccumulation is regarded as an integrative measure and an indicator of exposure of organisms to toxicants in polluted ecosystems. Metals are not metabolised by organisms, and therefore, bioaccumulation of metals and metalloids is of particular value (Luoma & Rainbow, 2005). Relationships between bioaccumulated toxins and adverse effects are complex and interpretation must take into account comparisons across different species, for different toxins, and environmental factors. Generally there are no standardised methods for investigating the accumulation of organic trace pollutants in aquatic organisms. Since the concentrations of these contaminants in the water column are often too low for reliable quantification, it can be difficult to measure bioaccumulation in wild caught fish (Van der Oost et al., 2003).

As part of an environmental risk assessment in Port Curtis, tissues of sea mullet and barramundi have previously been analysed for metal concentrations (Jones et al., 2005). Concentrations of metals measured in the barramundi were thought to be likely to reflect values for the overall region,

rather than the harbour specifically, based on local tagging knowledge of fish movements (Jones et al., 2005). Bioaccumulation is normally measured in gill, liver and muscle tissues with other tissues such as brain, gut and gonads used less frequently. Many studies have examined bioaccumulation in muscle tissue as a measure of human consumption risk. However, particularly in the case of metals, bioaccumulation may not directly reflect environmental concentrations. Bioaccumulation of metals can vary with many factors, including fish species, tissue type, life stage, exposure pathway, season and bioavailability of the metal in question. Given these difficulties, trials of bioaccumulation analysis should be conducted before deciding whether or not to include it in a monitoring regime.

During the 2011-2012 Gladstone fish health investigation, concentrations of iron, cadmium, arsenic and zinc were at times found to be higher in the livers of barramundi captured in Gladstone than in reference sites, although small sample sizes mean these results should be interpreted with caution (Wesche et al., 2013). A recent pilot sampling program undertaken by Gladstone Ports Corporation (GPC) at multiple locations identified per- and poly-fluoroalkyl substances (PFAS) in mullet and bream caught at one location in Ship Creek, at concentrations above the Food Standards Australia New Zealand guideline values (GPC, 2018).

Recommendation 4: GHHP considers testing for bioaccumulation of metals and other toxicants in collected fish tissue samples.

Fish species for monitoring

In Autumn 2018, target species were barramundi, bream (including pikey bream and yellowfin bream) and large bodied mullet (including diamondscale mullet and sea mullet). Small numbers of bream were caught (n = 2). As a contingency, barred javelin were also retained during sampling in Spring 2018. Slightly more bream were caught in this round (n = 6, most by handlining). Barred javelin proved to be more prolific and were caught in higher numbers than the other target species (n = 55 in Spring 2018). An option to be considered for 2019 is to include targeted hook and line fishing for bream in the monitoring program.

Recommendation 5: GHHP considers including a hook and line fishing component in 2019 to capture more bream **(Acanthopagrus spp.).**

The fish movement analysis (Appendix 1) provides information on both the range and the average movements of a variety of recreationally caught inshore and estuarine fish species, using tag-recapture data provided by the SunTag recreational fishing tagging program. Barramundi are a wide-ranging fish species and can move many hundreds of kilometres between tagging and recapture. This tendency renders the interpretation of identified health issues difficult, as it will often be impossible to know whether the fish is resident in the area or has moved from another area with different environmental conditions. However, barramundi are a species of interest in Gladstone Harbour following a fish health incident that occurred in 2012 (Wesche et al., 2013) so monitoring is likely to continue.

Yellowfin and pikey bream and barred javelin are all more resident, and don't tend to move large distances between tagging and recapture, so inclusion of these taxa is recommended. Large mullet can also travel long distances but haven't been included in the fish movement analysis as there are no tagging records for these species, which aren't normally targeted by recreational fishers.

During the Autumn and Spring 2018 sampling events, a range of other inshore and estuarine fish species were captured incidentally. The species caught across the most sites were: barramundi; blue catfish; blue threadfin; barred javelin; diamondscale mullet; and giant queenfish (23 fish from 5 Gladstone sites and 1 reference site). Of these, barramundi, blue catfish, barred javelin, diamondcale mullet are demersal or benthic species that are likely to be in closer contact with pollutants accumulated in sediments, making them useful indicator species (Cowled, 2016). Other demersal and benthic species caught included: bartailed flathead, silver javelin and goldspotted rockcod, but these species were all caught in much smaller numbers.

One option for future years might be to target barramundi and bream, but also retain a mix of demersal species depending on the catch at each site. This would increase the replication at each site and potentially provide some interesting comparisons in regard to the overall health of the demersal fish assemblages in Gladstone Harbour, although variation between species will need to be accounted for. This possibility was raised at the ISP workshop in Brisbane in August 2018 and will be considered for 2019. Based on 2018 sampling, the most suitable additional demersal fish species to include are barred javelin and blue catfish. Both were caught in reasonable numbers across the Gladstone Harbour zones.

Recommendation 6: GHHP considers adding barred javelin (*Pomadasys kaakan*) and blue catfish* (Neoarius graffei) *as target species in 2019.

Sampling sites

The fish movement analysis also detected high transience of fish between different areas within Gladstone Harbour. As such, it would likely be more appropriate to report scores of fish health at the harbour-wide scale than at the zonal scale used for water quality reporting. This will be discussed further in the final report. In Autumn 2018 no target fish species were caught in the Outer Harbour site, which is more open than the other zones and has less habitat for the target species. At the ISP workshop in Brisbane, it was decided that Outer Harbour would be removed from the sampling regime in Spring 2018 and attention focused elsewhere in the harbour.

As the fish health indicator scores are most likely to be reported on a harbour-wide scale rather than a zonal scale, it may be appropriate to focus sampling on fewer harbour zones. One suggestion for discussion with the ISP is to focus on six mainland zones along the coast of Gladstone Harbour, from north to south: Zone 1, the Narrows; Zone 6, Calliope Estuary; Zone 7, Auckland Inlet; Zone 9, South Trees Inlet; Zone 10, Boyne Estuary; and Zone 13 Rodds Bay. These zones all had high catches of target species and are located in close proximity to boat ramps. The zones are naturally stratified along the coastline and could be sampled for an entire day each, rather than splitting time between two different sites.

An advantage of the proximity of these sites to boat ramps is that it may be possible to conduct fish dissections on shore at the sites being sampled. This would minimise the time between fish death and dissection, solving the issue of autolysis in some fish tissue samples that occurred during 2018. It is a recommendation of the senior aquatic pathologist that the issue of time to dissection needs to be resolved in order to achieve the most comprehensive assessment of fish health during histopathological analysis (Chong, BSL, 2018, Appendix 7). Targeted sampling at mainland zones of Gladstone Harbour, where on shore dissections can be undertaken, would have this effect. Another option is to also retain key sites such as Zone 5, Inner Harbour, Zone 8, Mid Harbour and Zone 3, Western Basin, and on days when those sites are sampled use a larger research vessel which can retain live fish for longer periods of time.

Recommendation 7: GHHP considers targeting fish sampling at a reduced number of zones in Gladstone Harbour.

In 2018, two reference sites were monitored to assist with the development of baselines for fish health measures, Stanage Bay and Baffle Creek. It should be noted that barramundi from Stanage Bay did not appear to be in pristine condition, which may reflect either local environmental effects in the reference area, or a situation in which environmental effects that have occurred elsewhere, and compromised barramundi have later moved into the reference area. If the latter is true, it will be difficult to remedy for this issue for a highly transient fish species such as barramundi. Regardless, in order to continue to assess the condition of fish in Gladstone Harbour in a relative way, it may be beneficial for GHHP to continue to sample at these sites (although once a year may be sufficient), as a precaution against misinterpreting more widespread changes as localised impacts.

Recommendation 8: GHHP considers continuing to sample at reference sites at least once a year.

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Appendix 1: Fish Movement Analysis

SunTag fish tag and recapture data from Stanage Bay, Gladstone and Baffle Creek were provided to CQUniversity by Bill Sawynok, Infofish Australia, to assess the adult home ranges of potential target fish species for indicator selection. Species that remain resident within the location being monitored would provide a more relevant localised indicator than species that migrate large distances or are found in different locations at different life history stages.

Analysis methods

Original recapture data spreadsheets for the three regions were combined, and start and end latitude and longitude attribute columns added. Preference was given to demersal and benthic inshore and estuarine species. As such, species with a pelagic or commonly offshore habit were removed from the dataset, including: Australian tarpon, Barcheek coral trout, Bigeye trevally, Blue tuskfish, Coral trout, Crimson snapper, Giant trevally, Golden snapper, Golden trevally, Grass emperor, Mangrove jack, Moses snapper, Queenfish, Red emperor, Saddletail snapper, Snapper, Spangled emperor, Spanish mackerel, Spotted mackerel, Whaler. All locations with the "Movement" attribute labelled either "RECAPTURED SAME AREA", "FOUND SAME AREA", "FOUND DEAD SAME AREA", "FOUND SAME AREA", "RECAPTIURED SAME AREA", "RECAPTURED SAMAE AREA", "RECAPTURED SAME ARA", "RECAPTURED SAME ARE", "RECAPTURED SANE AREA", "RECAPTURED SMAE AREA", "RECAPTURES SAME AREA", but with either (but not both) the start or end positions in latitude and longitude missing were assigned the same latitude and longitude as the available data. All locations with no recapture position but with the same map reference grid code as tag location, were assigned the same recapture position.

Finally all duplicate tag recaptures (i.e. a fish recaptured more than once) were updated to give subsequent recaptures, using the last capture as a start point. Data where uploaded to ARC GIS v10 as a point shapefile. The point to line tool was used to calculate the direct distance between start and recapture positions. The new distance data were amalgamated with existing original distance data for statistical analysis of the species of interest, as a proxy for the distance travelled by the fish. Data mining was undertaken to examine the mean and variance of distances travelled. Descriptive statistics are presented here to inform discussions on species selection.

Results

Figure 1 illustrates the straight line distances between tagging and recapture. Of the species considered, barramundi (*Lates calcarifer*) show the largest ranges (mean 8.42km, max 704km, Figure 2). Fish tagged in large numbers but showing smaller ranges included yellowfin bream (*Acanthopagrus australis*; Figure 3), pikey bream (*A. pacificus*; Figure 4), goldspotted rockcod (*Epinephelus coioides*; Figure 5), and blackspotted rockcod (*E. malabaricus*; Figure 6). Dusky flathead (*Platycephalus fuscus*) moved between Gladstone Harbour and Baffle Creek (Figure 7).

For the majority of species examined, many tagged individuals were recaptured at their original tagging location (indicated by the large columns on the "0" category on the frequency histograms, Figure 8). Concomitantly, the frequency at which individual fish moved away from their original tagging location typically declined with increasing distance (e.g. barramundi). Obvious exceptions to this pattern occurred for blue threadfin (*Eleutheronema tetradactylum*) and king threadfin (*Polydactylus macrochir),* for which frequency of movement was more even over distance. Seven fish species had more than 50 fish tagged across the three locations. Several species exhibited low numbers of tag-recapture records (e.g. ≤ 3 records for fringe-eye flathead, long-fin rockcod, northern whiting, sand flathead, and sand whiting), making any generalisations about their movement patterns in Central Queensland tenuous until further data become available.

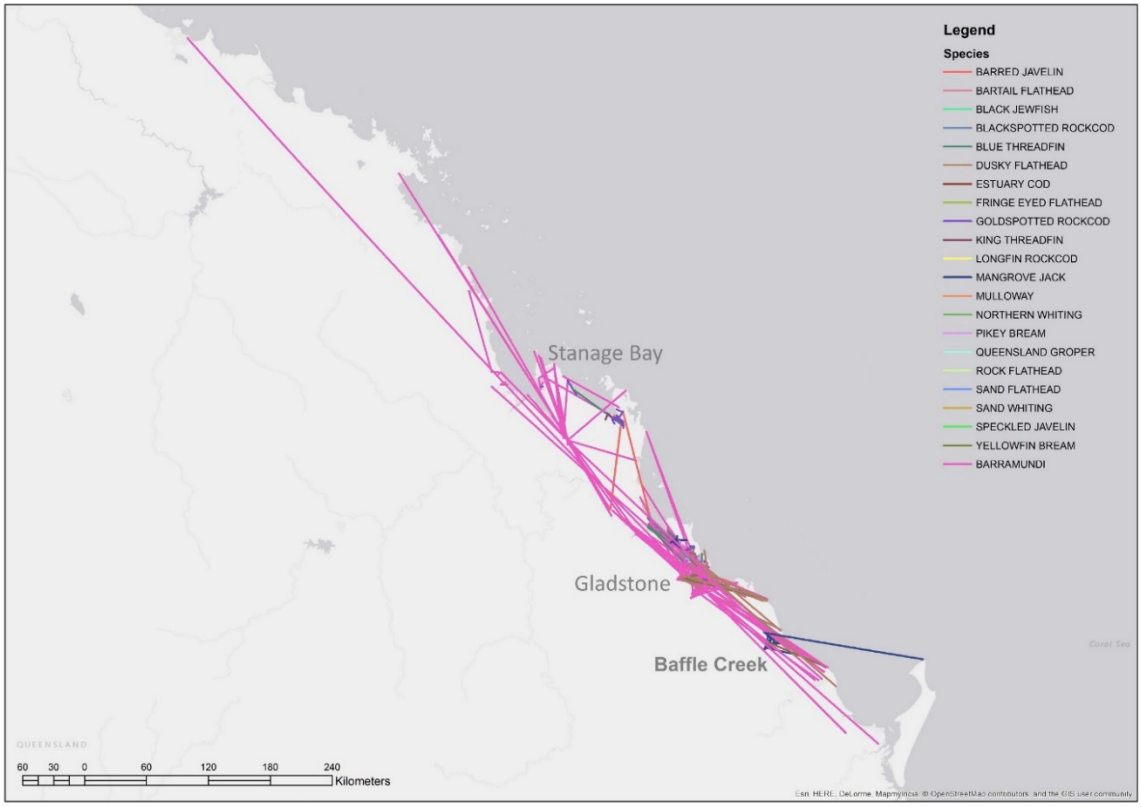


Figure 1 Straight line distance travelled by recaptured tagged fish from Stanage Bay, Gladstone and Baffle Creek (Data source: SunTag).

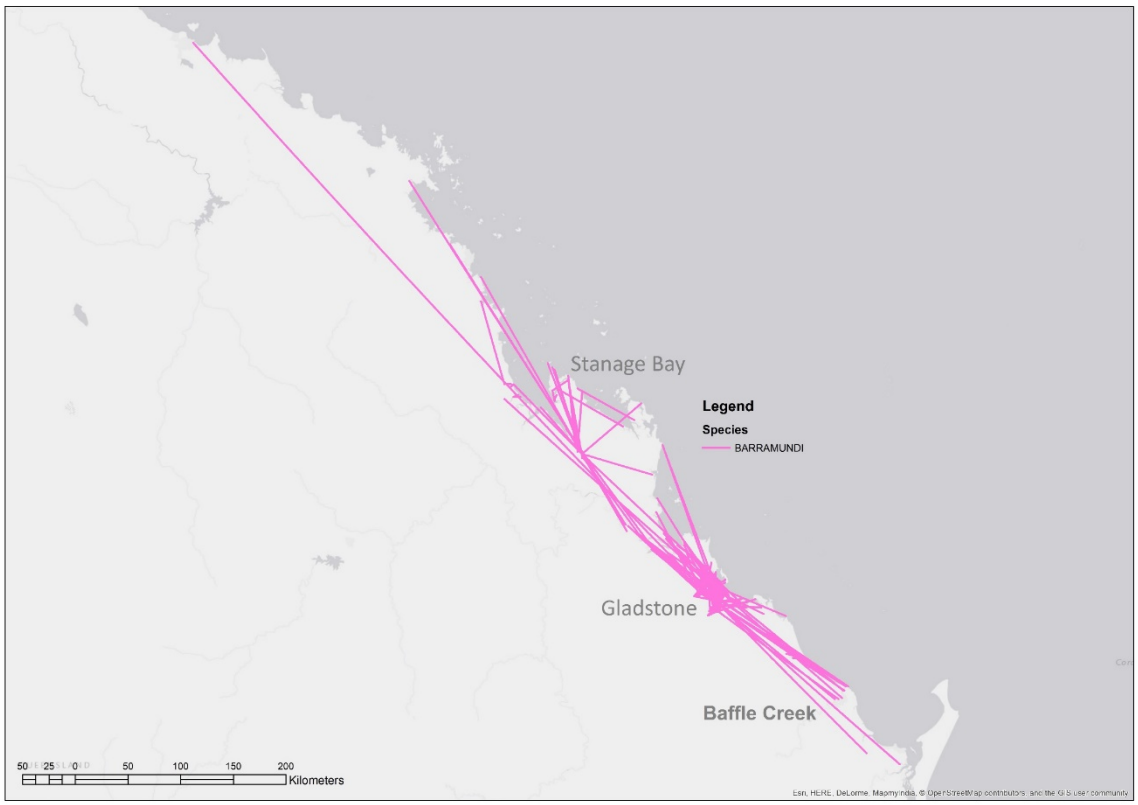


Figure 2 Straight line distance travelled by recaptured barramundi from Stanage Bay, Gladstone and Baffle Creek, n = 1817. (Data source: SunTag).

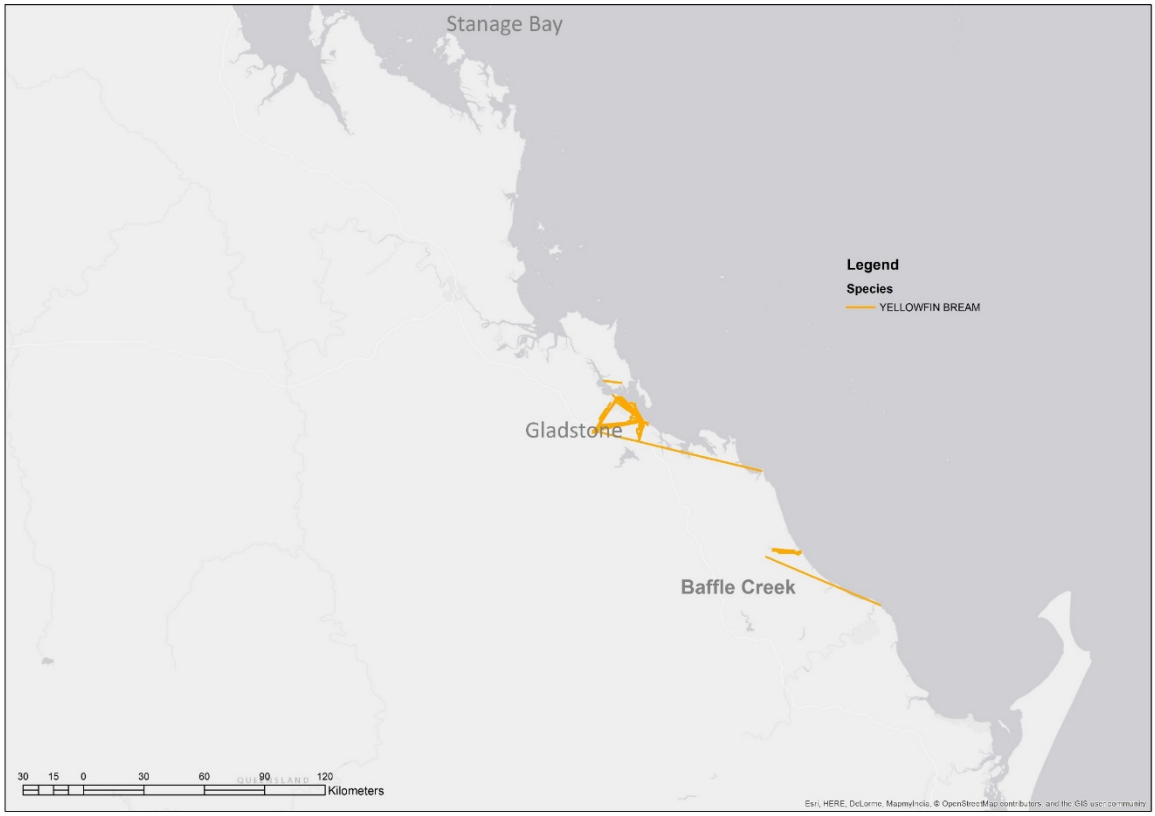


Figure 3 Straight line distance travelled by recaptured yellowfin bream from Stanage Bay, Gladstone and Baffle Creek, n = 245 (Data source: SunTag).

Figure 4 Straight line distance travelled by recaptured pikey bream from Stanage Bay, Gladstone and Baffle Creek, n = 184. (Data source: SunTag).

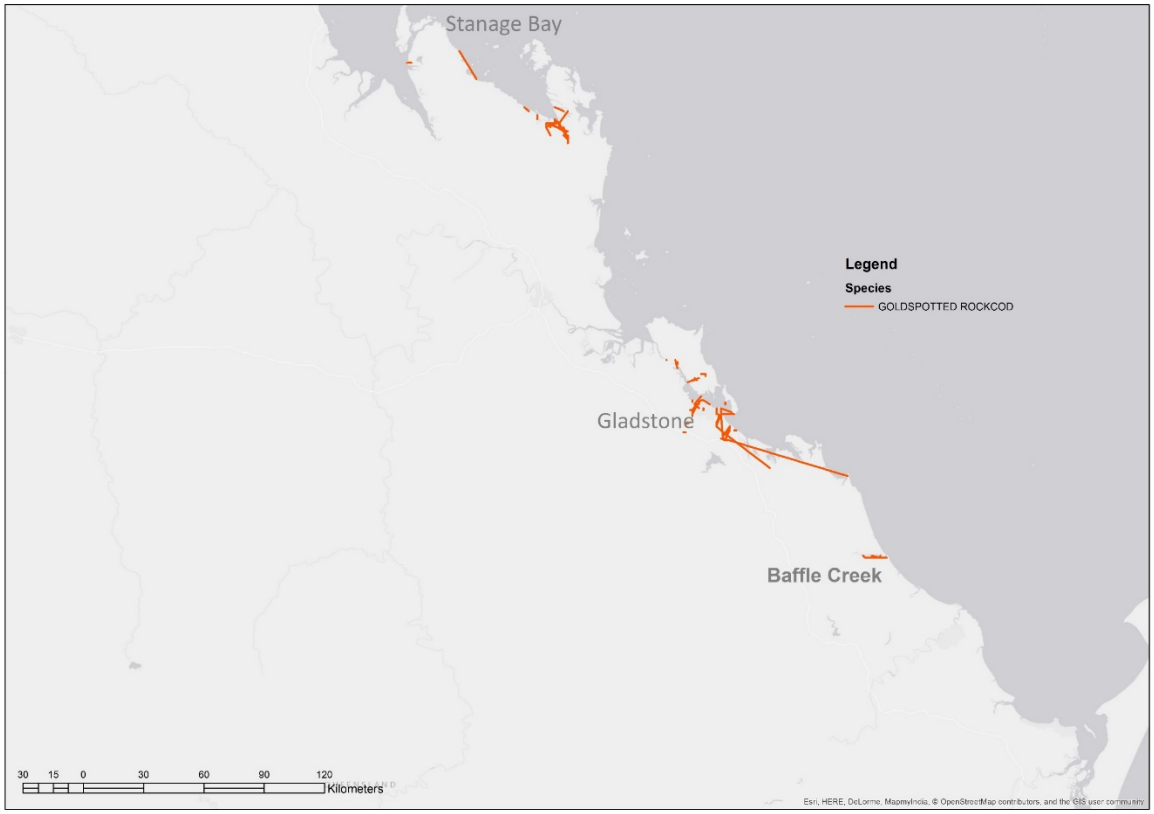


Figure 5 Straight line distance travelled by recaptured goldspotted rockcod from Stanage Bay, Gladstone and Baffle Creek, n = 547. (Data source: SunTag).

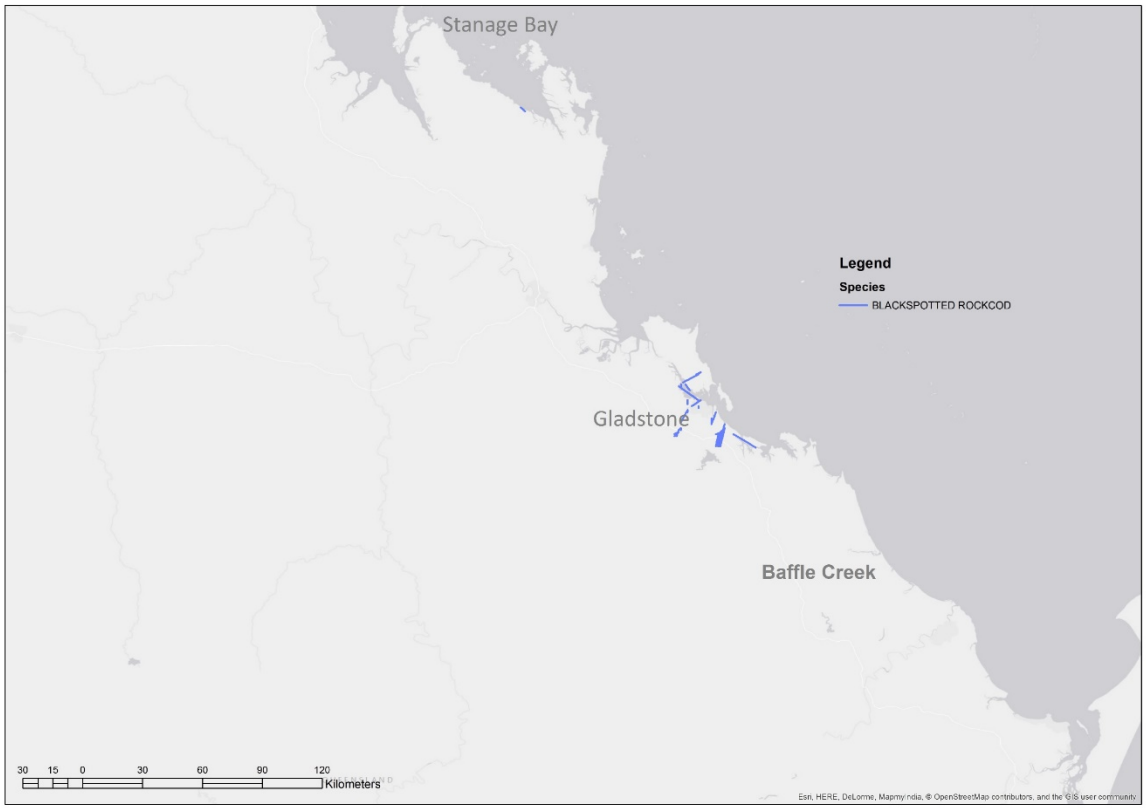


Figure 6 Straight line distance travelled by recaptured blackspotted rockcod from Stanage Bay, Gladstone and Baffle Creek, n = 200. (Data source: SunTag).

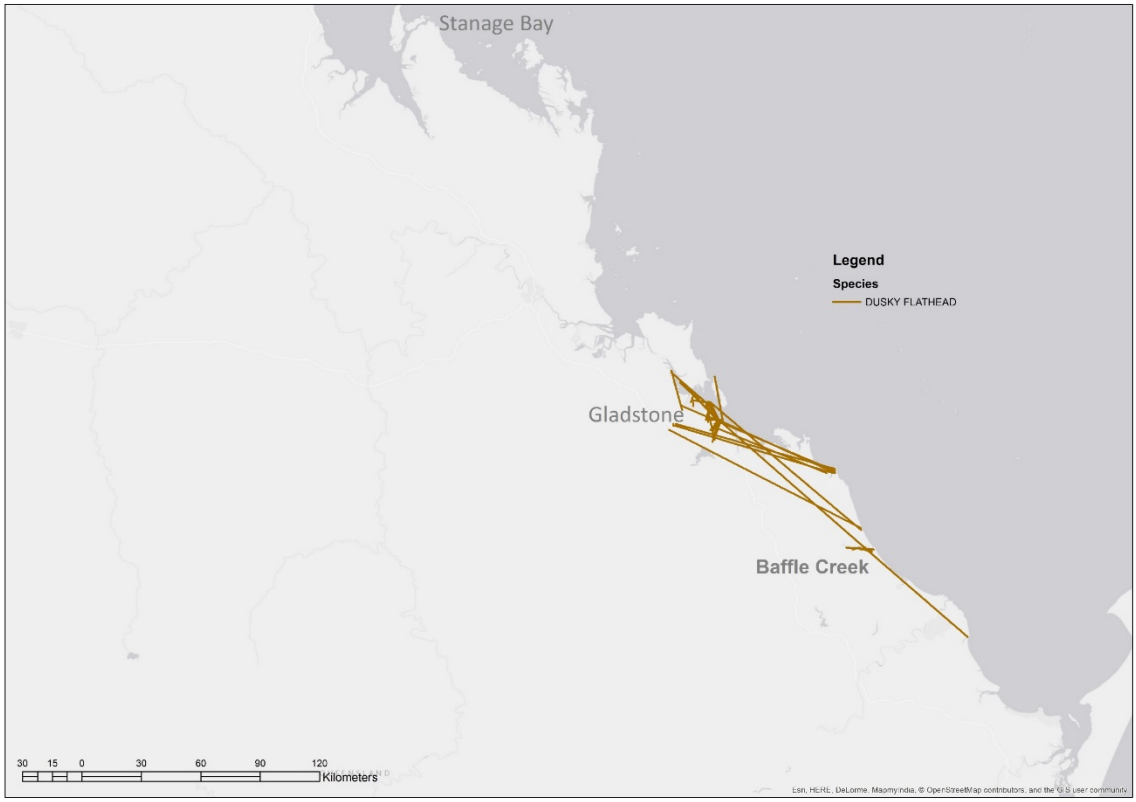
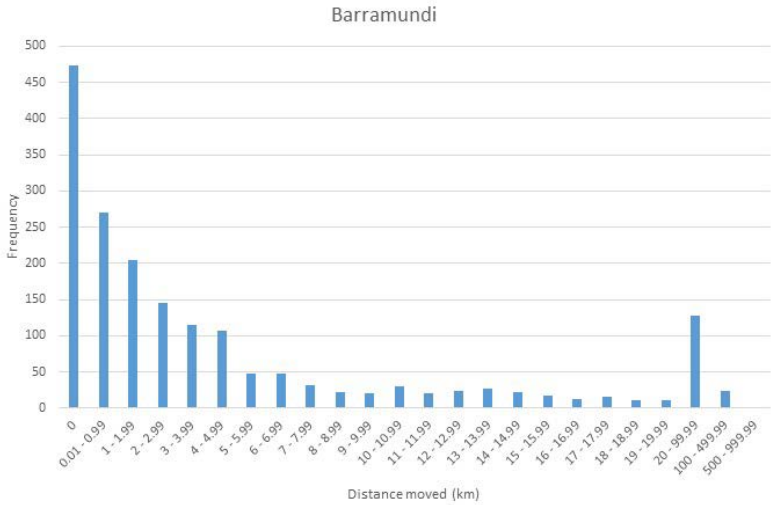
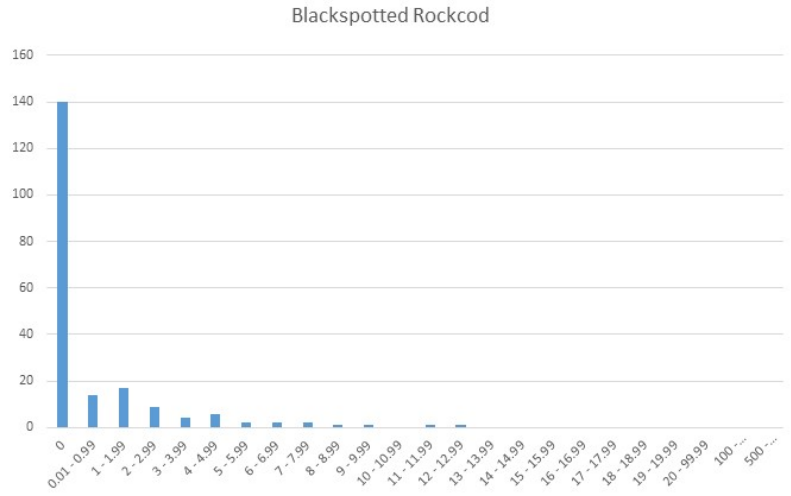
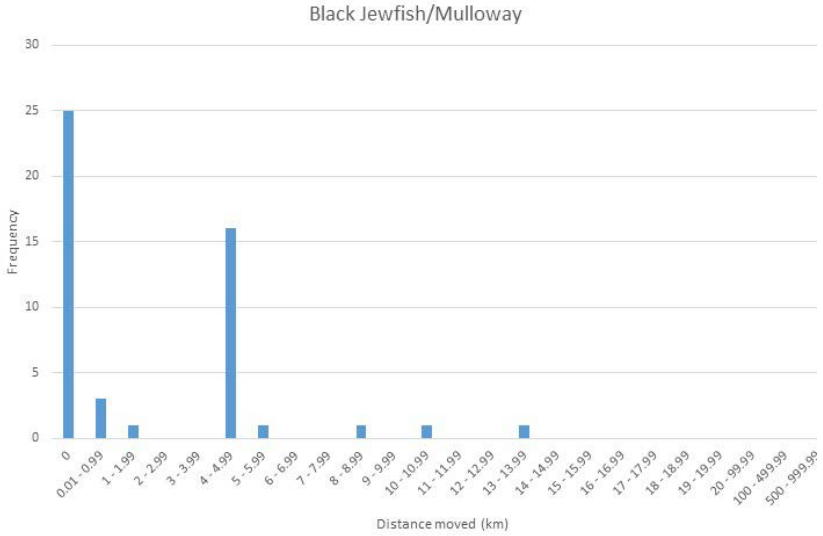


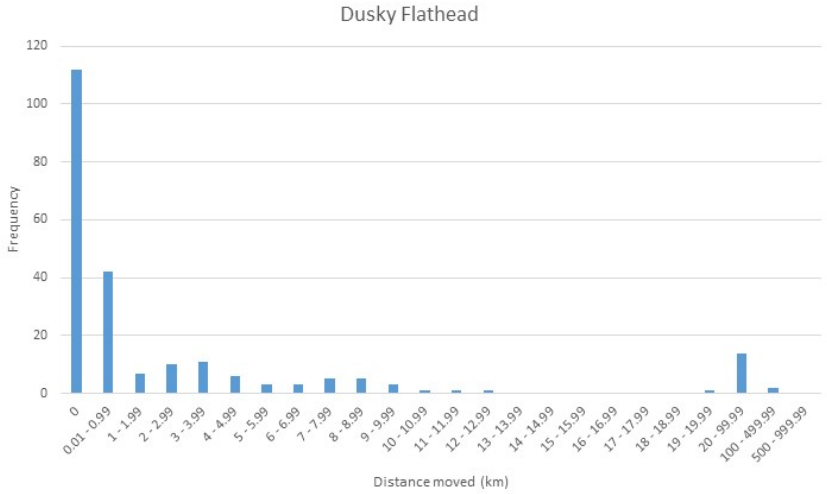
Figure 7 Straight line distance travelled by recaptured dusky flathead from Stanage Bay, Gladstone and Baffle Creek, n = 227. (Data source: SunTag).

The average distance moved for most fish species examined was ≤ 10 km from the original tagging location (Figures 8 and 9). Blue threadfin (average 28.67 km) and king threadfin (average 55.28 km) were notable exceptions, although the large variation among samples for king threadfin reduces certainty about this calculated average distance moved.


Barramundi
Frequency
Distance moved (km)


Blackspotted Rockcod


Black Jewfish/Mulloway
Frequency
Distance moved (km)


Dusky Flathead
Frequency
Distance moved (km)

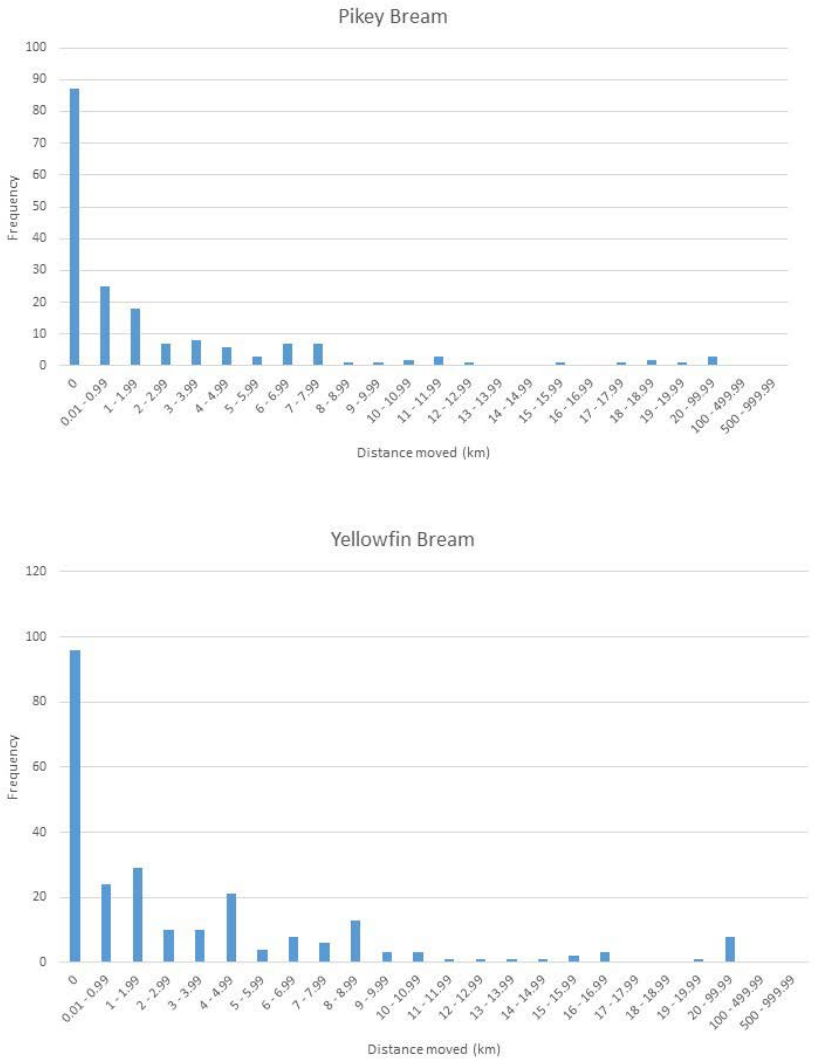


Figure 8 Frequency histograms of straight line distance travelled for fish with greater than 50 individuals tagged and recaptured.

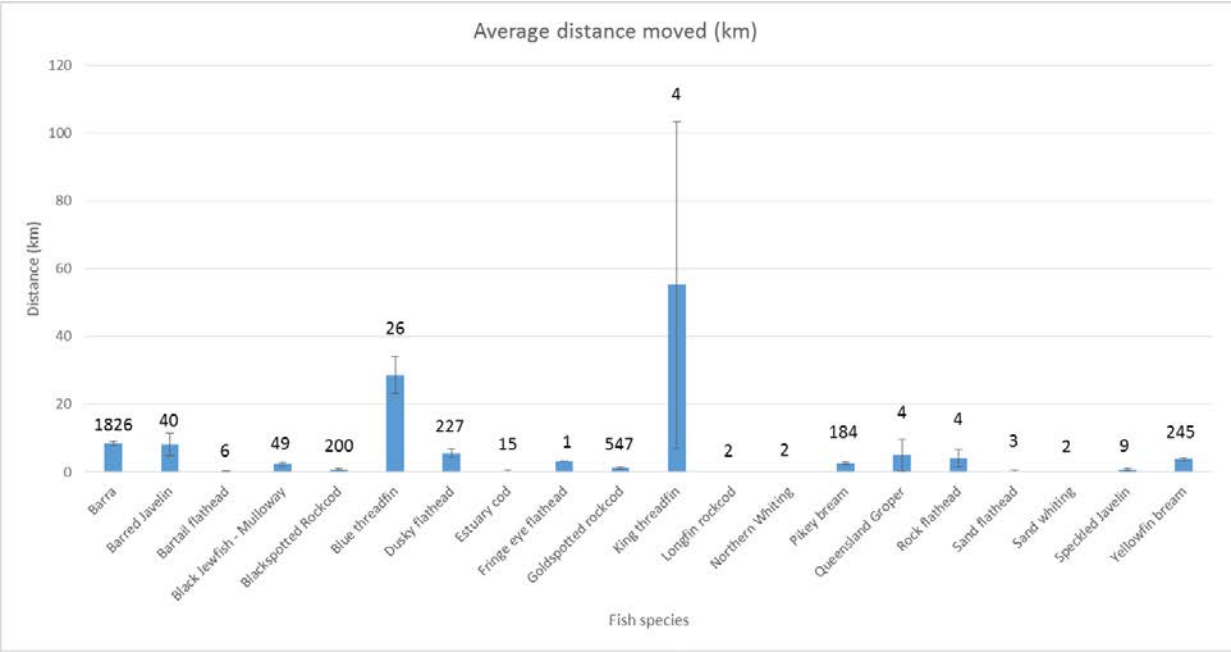


Figure 9 Average distance moved by species (error bars represent standard error around the mean, SEM, and numbers above the bar show the number of records). Barra = barramundi.

Conclusion

Of the seven fish species tagged in large enough numbers to be confident in the patterns observed (set at n=50 for this enquiry), barramundi show by far the greatest ranges, which may negatively confound their use for fish health indicator analysis at smaller geographical scales. Fish species with smaller home ranges: black jewfish (*Protonibea diacanthus),* goldspotted rockcod (*E. coioides*), blackspotted rockcod (*E. malabaricus*), pikey bream (*A. pacificus*), and yellowfin bream (*A. australis*). Whilst these fish show smaller home ranges, their movements are still larger than the spatial scale of the 13 water quality monitoring zones within Gladstone Harbour.

Further investigation of the data by tag location (not presented here) identified that a large proportion of the zero-distance data in the case of barramundi was due either to short recapture times, or tagging took place in Awoonga Dam where the movement of fish is physically restricted. Further analysis of the data is possible to control for recapture time period, restricted movement and calculating the shortest movement over water.

Appendix 2: Details of all sampling locations and times

GHHP Zone Number	Location	Survey	Gill net mesh size (inches)	Date	Deploy time	Soak time (h:mm)	Latitude	Longitude
1	Narrows	Apr-2018	6	11/04/2018	8:07	0:30	-23.6697	151.120123
1	Narrows	Apr-2018	6	11/04/2018	8:38	0:37	-23.6697	151.120123
1	Narrows	Apr-2018	6	11/04/2018	9:20	0:34	-23.6697	151.120123
1	Narrows	Apr-2018	8	11/04/2018	8:19	0:33	-23.6665	151.115062
1	Narrows	Apr-2018	8	11/04/2018	8:54	0:35	-23.6665	151.115062
1	Narrows	Apr-2018	4.5	11/04/2018	8:27	0:31	-23.6676	151.116316
1	Narrows	Apr-2018	4.5	11/04/2018	9:03	0:36	-23.6676	151.116316
1	Narrows	Apr-2018	4.5	11/04/2018	9:40	0:35	-23.6676	151.116316
1	Narrows	Apr-2018	4.5	11/04/2018	10:40	0:35	-23.6596	151.120376
1	Narrows	Apr-2018	6	11/04/2018	10:50	0:37	-23.6603	151.119464
2	Graham Creek	Apr-2018	8	10/04/2018	7:45	0:38	-23.7373	151.175057
2	Graham Creek	Apr-2018	8	10/04/2018	8:30	0:30	-23.7373	151.175057
2	Graham Creek	Apr-2018	6	10/04/2018	7:55	0:40	-23.7344	151.173353
2	Graham Creek	Apr-2018	6	10/04/2018	8:37	0:53	-23.7344	151.173353
2	Graham Creek	Apr-2018	4.5	10/04/2018	8:02	0:38	-23.7332	151.172646
2	Graham Creek	Apr-2018	4.5	10/04/2018	8:50	0:30	-23.7332	151.172646
2	Graham Creek	Apr-2018	6	10/04/2018	9:56	0:31	-23.7362	151.190914
2	Graham Creek	Apr-2018	4.5	10/04/2018	10:10	0:36	-23.7279	151.197328
2	Graham Creek	Apr-2018	6	10/04/2018	10:40	0:46	-23.732	151.196839
2	Graham Creek	Apr-2018	4.5	10/04/2018	11:10	0:34	-23.732	151.196974
3	Western Basin	Apr-2018	8	13/04/2018	6:29	0:36	-23.7798	151.150074
3	Western Basin	Apr-2018	8	13/04/2018	7:09	0:33	-23.7798	151.150074
3	Western Basin	Apr-2018	8	13/04/2018	7:48	0:44	-23.7798	151.150074
3	Western Basin	Apr-2018	8	13/04/2018	8:36	0:34	-23.7798	151.150074
3	Western Basin	Apr-2018	8	13/04/2018	9:12	0:39	-23.7798	151.150074
3	Western Basin	Apr-2018	6	13/04/2018	6:36	0:44	-23.7807	151.149664
3	Western Basin	Apr-2018	6	13/04/2018	7:25	0:30	-23.7807	151.149664
3	Western Basin	Apr-2018	6	13/04/2018	7:59	0:41	-23.7807	151.149664
3	Western Basin	Apr-2018	6	13/04/2018	8:42	0:33	-23.7807	151.149664
3	Western Basin	Apr-2018	4.5	13/04/2018	6:50	0:43	-23.7827	151.150293
3	Western Basin	Apr-2018	4.5	13/04/2018	7:36	0:29	-23.7827	151.150293
3	Western Basin	Apr-2018	4.5	13/04/2018	8:10	0:38	-23.7827	151.150293
3	Western Basin	Apr-2018	4.5	13/04/2018	8:53	0:11	-23.7827	151.150293
3	Western Basin	Apr-2018	4.5	13/04/2018	9:07	0:33	-23.7834	151.147996
3	Western Basin	Apr-2018	4.5	13/04/2018	9:45	0:43	-23.7834	151.147996
3	Western Basin	Apr-2018	6	13/04/2018	9:25	0:33	-23.7824	151.15217
3	Western Basin	Apr-2018	6	13/04/2018	10:01	0:35	-23.7824	151.15217
5	Inner Harbour	Apr-2018	4.5	10/04/2018	13:50	0:30	-23.769	151.246356
5	Inner Harbour	Apr-2018	4.5	10/04/2018	14:20	0:35	-23.769	151.246356

GHHP Zone Number	Location	Survey	Gill net mesh size (inches)	Date	Deploy time	Soak time (h:mm)	Latitude	Longitude
5	Inner Harbour	Apr-2018	6	10/04/2018	14:00	0:35	-23.7693	151.245362
5	Inner Harbour	Apr-2018	4.5	10/04/2018	15:03	0:31	-23.7717	151.247196
5	Inner Harbour	Apr-2018	6	10/04/2018	15:16	0:48	-23.7706	151.245652
6	Calliope River	Apr-2018	4.5	11/04/2018	13:56	0:32	-23.9308	151.161531
6	Calliope River	Apr-2018	6	11/04/2018	14:08	0:34	-23.9298	151.159017
6	Calliope River	Apr-2018	6	11/04/2018	14:45	0:42	-23.9298	151.159017
6	Calliope River	Apr-2018	8	11/04/2018	14:20	0:34	-23.9322	151.157926
6	Calliope River	Apr-2018	8	11/04/2018	14:57	0:43	-23.9322	151.157926
6	Calliope River	Apr-2018	4.5	11/04/2018	14:53	0:17	-23.9348	151.157899
6	Calliope River	Apr-2018	6	11/04/2018	16:11	0:09	-23.9399	151.162232
7	Auckland Creek	Apr-2018	4.5	12/04/2018	13:32	0:28	-23.8447	151.241658
7	Auckland Creek	Apr-2018	6	12/04/2018	13:47	0:28	-23.8494	151.241507
7	Auckland Creek	Apr-2018	6	12/04/2018	15:21	0:29	-23.8541	151.240071
7	Auckland Creek	Apr-2018	4.5	12/04/2018	15:31	0:34	-23.8573	151.236449
7	Auckland Creek	Apr-2018	4.5	12/04/2018	16:11	0:36	-23.8573	151.236449
7	Auckland Creek	Apr-2018	6	12/04/2018	15:54	0:42	-23.8582	151.235676
8	Mid Harbour	Apr-2018	8	12/04/2018	8:00	0:40	-23.8288	151.337315
8	Mid Harbour	Apr-2018	8	12/04/2018	8:43	0:37	-23.8288	151.337315
8	Mid Harbour	Apr-2018	8	12/04/2018	9:25	0:25	-23.8288	151.337315
8	Mid Harbour	Apr-2018	6	12/04/2018	8:07	0:38	-23.8272	151.338465
8	Mid Harbour	Apr-2018	6	12/04/2018	8:49	0:41	-23.8272	151.338465
8	Mid Harbour	Apr-2018	6	12/04/2018	9:35	0:30	-23.8272	151.338465
8	Mid Harbour	Apr-2018	4.5	12/04/2018	8:19	0:34	-23.8255	151.338033
8	Mid Harbour	Apr-2018	4.5	12/04/2018	8:59	0:42	-23.8255	151.338033
8	Mid Harbour	Apr-2018	6	12/04/2018	10:15	0:30	-23.8165	151.334056
8	Mid Harbour	Apr-2018	6	12/04/2018	10:46	0:34	-23.8165	151.334056
8	Mid Harbour	Apr-2018	8	12/04/2018	10:24	0:31	-23.8142	151.334055
8	Mid Harbour	Apr-2018	8	12/04/2018	10:56	0:41	-23.8142	151.334055
8	Mid Harbour	Apr-2018	4.5	12/04/2018	10:30	0:35	-23.8142	151.334696
9	South Trees Inlet	Apr-2018	4.5	17/04/2018	4:46	0:18	-23.8564	151.300243
9	South Trees Inlet	Apr-2018	6	17/04/2018	5:21	0:38	-23.8823	151.315152
9	South Trees Inlet	Apr-2018	6	17/04/2018	6:01	0:49	-23.8823	151.315152
9	South Trees Inlet	Apr-2018	8	17/04/2018	5:27	0:35	-23.8838	151.315614
9	South Trees Inlet	Apr-2018	8	17/04/2018	6:04	0:59	-23.8838	151.315614
9	South Trees Inlet	Apr-2018	4.5	17/04/2018	5:36	0:35	-23.8824	151.31845
9	South Trees Inlet	Apr-2018	4.5	17/04/2018	6:13	0:15	-23.8824	151.31845
9	South Trees Inlet	Apr-2018	6	17/04/2018	7:26	0:44	-23.882	151.315175

GHHP Zone Number	Location	Survey	Gill net mesh size (inches)	Date	Deploy time	Soak time (h:mm)	Latitude	Longitude
9	South Trees Inlet	Apr-2018	4.5	17/04/2018	7:36	0:26	-23.8799	151.314794
10	Boyne River	Apr-2018	8	16/04/2018	4:53	0:37	-23.9998	151.338812
10	Boyne River	Apr-2018	8	16/04/2018	5:35	0:43	-23.9998	151.338812
10	Boyne River	Apr-2018	8	16/04/2018	6:25	0:33	-23.9998	151.338812
10	Boyne River	Apr-2018	8	16/04/2018	7:00	0:35	-23.9998	151.338812
10	Boyne River	Apr-2018	8	16/04/2018	7:37	0:26	-23.9998	151.338812
10	Boyne River	Apr-2018	6	16/04/2018	5:12	0:38	-23.9976	151.334924
10	Boyne River	Apr-2018	6	16/04/2018	5:55	0:37	-23.9976	151.334924
10	Boyne River	Apr-2018	6	16/04/2018	6:37	0:26	-23.9976	151.334924
10	Boyne River	Apr-2018	4.5	16/04/2018	5:25	0:35	-23.9963	151.335049
10	Boyne River	Apr-2018	4.5	16/04/2018	6:05	0:40	-23.9963	151.335049
10	Boyne River	Apr-2018	4.5	16/04/2018	6:55	0:43	-23.9963	151.335049
10	Boyne River	Apr-2018	6	16/04/2018	7:25	0:42	-24.0027	151.339784
10	Boyne River	Apr-2018	6	16/04/2018	8:09	0:18	-24.0027	151.339784
10	Boyne River	Apr-2018	4.5	16/04/2018	8:15	0:25	-24.0049	151.343027
11	Outer Harbour	Apr-2018	6	16/04/2018	11:10	0:33	-23.9946	151.469791
11	Outer Harbour	Apr-2018	6	16/04/2018	11:46	0:11	-23.9946	151.469791
11	Outer Harbour	Apr-2018	8	16/04/2018	11:26	0:51	-23.9938	151.467448
11	Outer Harbour	Apr-2018	8	16/04/2018	12:20	0:26	-23.9938	151.467448
11	Outer Harbour	Apr-2018	4.5	16/04/2018	11:38	0:34	-23.9948	151.466162
11	Outer Harbour	Apr-2018	4.5	16/04/2018	12:15	0:30	-23.9948	151.466162
11	Outer Harbour	Apr-2018	4.5	16/04/2018	12:48	0:34	-23.9948	151.466162
11	Outer Harbour	Apr-2018	6	16/04/2018	12:06	0:31	-23.9946	151.471769
11	Outer Harbour	Apr-2018	6	16/04/2018	12:39	0:29	-23.9946	151.471769
11	Outer Harbour	Apr-2018	4.5	16/04/2018	13:46	0:39	-23.9624	151.398396
12	Colosseum Inlet	Apr-2018	8	17/04/2018	9:42	0:30	-24.056	151.457425
12	Colosseum Inlet	Apr-2018	8	17/04/2018	10:18	0:22	-24.056	151.457425
12	Colosseum Inlet	Apr-2018	8	17/04/2018	10:45	0:27	-24.056	151.457425
12	Colosseum Inlet	Apr-2018	8	17/04/2018	11:15	0:31	-24.056	151.457425
12	Colosseum Inlet	Apr-2018	6	17/04/2018	9:54	0:33	-24.0565	151.460695
12	Colosseum Inlet	Apr-2018	6	17/04/2018	10:30	0:28	-24.0565	151.460695
12	Colosseum Inlet	Apr-2018	6	17/04/2018	11:02	0:20	-24.0565	151.460695
12	Colosseum Inlet	Apr-2018	6	17/04/2018	11:26	0:31	-24.0565	151.460695
12	Colosseum Inlet	Apr-2018	6	17/04/2018	11:58	0:22	-24.0565	151.460695
12	Colosseum Inlet	Apr-2018	4.5	17/04/2018	10:05	0:29	-24.0522	151.461052
12	Colosseum Inlet	Apr-2018	4.5	17/04/2018	10:37	0:29	-24.0522	151.461052
12	Colosseum Inlet	Apr-2018	4.5	17/04/2018	11:08	0:23	-24.0522	151.461052
12	Colosseum Inlet	Apr-2018	4.5	17/04/2018	11:33	0:26	-24.0522	151.461052
12	Colosseum Inlet	Apr-2018	4.5	17/04/2018	12:00	0:13	-24.0522	151.461052
12	Colosseum Inlet	Apr-2018	4.5	17/04/2018	12:30	0:34	-24.0582	151.454311
13	Rodds Bay	Apr-2018	6	9/04/2018	8:42	0:49	-24.0293	151.635435

GHHP Zone Number	Location	Survey	Gill net mesh size (inches)	Date	Deploy time	Soak time (h:mm)	Latitude	Longitude
13	Rodds Bay	Apr-2018	6	9/04/2018	9:40	0:31	-24.0293	151.635435
13	Rodds Bay	Apr-2018	8	9/04/2018	8:14	0:34	-24.0321	151.640291
13	Rodds Bay	Apr-2018	8	9/04/2018	8:50	0:25	-24.0321	151.640291
13	Rodds Bay	Apr-2018	8	9/04/2018	9:22	0:33	-24.0309	151.636808
13	Rodds Bay	Apr-2018	6	9/04/2018	10:33	0:31	-24.014	151.630523
13	Rodds Bay	Apr-2018	4.5	9/04/2018	10:42	0:33	-24.0103	151.63166
13	Rodds Bay	Apr-2018	6	9/04/2018	12:03	0:43	-24.0419	151.610159
13	Rodds Bay	Apr-2018	4.5	9/04/2018	12:11	1:04	-24.0419	151.608657
	Baffle Creek	Apr-2018	8	19/04/2018	5:48	0:50	-24.5275	152.02836
	Baffle Creek	Apr-2018	8	19/04/2018	6:39	0:23	-24.5275	152.02836
	Baffle Creek	Apr-2018	6	19/04/2018	5:55	0:46	-24.5261	152.027277
	Baffle Creek	Apr-2018	6	19/04/2018	6:42	0:04	-24.5261	152.027277
	Baffle Creek	Apr-2018	4.5	19/04/2018	6:04	0:40	-24.5253	152.026607
	Baffle Creek	Apr-2018	4.5	19/04/2018	6:45	0:14	-24.5253	152.026607
	Baffle Creek	Apr-2018	2	19/04/2018	6:19	0:09	-24.529	152.031369
	Baffle Creek	Apr-2018	8	19/04/2018	7:28	0:37	-24.5166	151.983755
	Baffle Creek	Apr-2018	8	19/04/2018	8:06	0:34	-24.5166	151.983755
	Baffle Creek	Apr-2018	4.5	19/04/2018	7:36	0:36	-24.5172	151.982678
	Baffle Creek	Apr-2018	4.5	19/04/2018	8:14	0:41	-24.5172	151.982678
	Baffle Creek	Apr-2018	6	19/04/2018	7:45	0:41	-24.5171	151.980542
	Baffle Creek	Apr-2018	6	19/04/2018	8:28	0:40	-24.5171	151.980542
	Baffle Creek	Apr-2018	2	19/04/2018	7:53	0:07	-24.5171	151.98205
	Baffle Creek	Apr-2018	2	19/04/2018	8:30	0:04	-24.5175	151.979798
	Baffle Creek	Apr-2018	6	19/04/2018	9:19	0:35	-24.5122	151.969731
	Baffle Creek	Apr-2018	6	19/04/2018	9:56	0:38	-24.5122	151.969731
	Baffle Creek	Apr-2018	6	19/04/2018	10:36	0:25	-24.5122	151.969731
	Baffle Creek	Apr-2018	6	19/04/2018	11:02	0:31	-24.5122	151.969731
	Baffle Creek	Apr-2018	4.5	19/04/2018	9:25	0:32	-24.5122	151.968022
	Baffle Creek	Apr-2018	4.5	19/04/2018	9:58	0:40	-24.5122	151.968022
	Baffle Creek	Apr-2018	4.5	19/04/2018	10:41	0:24	-24.5122	151.968022
	Baffle Creek	Apr-2018	4.5	19/04/2018	11:07	0:18	-24.5122	151.968022
	Baffle Creek	Apr-2018	8	19/04/2018	9:38	0:32	-24.5173	151.962157
	Baffle Creek	Apr-2018	8	19/04/2018	10:12	0:30	-24.5173	151.962157
	Baffle Creek	Apr-2018	8	19/04/2018	10:45	0:25	-24.5173	151.962157
	Baffle Creek	Apr-2018	8	19/04/2018	11:12	0:04	-24.5173	151.962157
	Baffle Creek	Apr-2018	6	19/04/2018	12:14	0:31	-24.5123	152.023484
	Baffle Creek	Apr-2018	6	19/04/2018	12:46	0:30	-24.5123	152.023484
	Baffle Creek	Apr-2018	4.5	19/04/2018	12:19	0:29	-24.5117	152.024529
	Baffle Creek	Apr-2018	4.5	19/04/2018	12:51	0:34	-24.5117	152.024529
	Baffle Creek	Apr-2018	2	19/04/2018	12:27	0:09	-24.5114	152.022366
	Stanage Bay	Apr-2018	4.5	23/04/2018	7:46	0:28	-22.2257	149.940709

GHHP Zone Number	Location	Survey	Gill net mesh size (inches)	Date	Deploy time	Soak time (h:mm)	Latitude	Longitude
	Stanage Bay	Apr-2018	4.5	23/04/2018	8:16	0:44	-22.2257	149.940709
	Stanage Bay	Apr-2018	6	23/04/2018	8:00	0:34	-22.2255	149.939269
	Stanage Bay	Apr-2018	8	23/04/2018	8:25	0:45	-22.2189	149.940914
	Stanage Bay	Apr-2018	8	23/04/2018	9:12	0:41	-22.2189	149.940914
	Stanage Bay	Apr-2018	8	23/04/2018	9:57	0:23	-22.2189	149.940914
	Stanage Bay	Apr-2018	6	23/04/2018	8:50	0:56	-22.2196	149.942341
	Stanage Bay	Apr-2018	6	23/04/2018	9:47	0:29	-22.2196	149.942341
	Stanage Bay	Apr-2018	6	23/04/2018	10:18	0:16	-22.2196	149.942341
	Stanage Bay	Apr-2018	4.5	23/04/2018	9:31	0:34	-22.2176	149.943521
	Stanage Bay	Apr-2018	6	23/04/2018	10:49	0:25	-22.2073	149.934223
	Stanage Bay	Apr-2018	4.5	23/04/2018	11:31	0:26	-22.2013	149.932506
	Stanage Bay	Apr-2018	4.5	23/04/2018	12:19	0:18	-22.1656	149.937772
	Stanage Bay	Apr-2018	2	23/04/2018	12:25	0:07	-22.1681	149.937948
1	Narrows	Sep-2018	4.5	19/09/2018	5:45	0:35	-23.7141	151.156203
1	Narrows	Sep-2018	4.5	19/09/2018	6:21	0:49	-23.7141	151.156203
1	Narrows	Sep-2018	4.5	19/09/2018	7:11	0:40	-23.7141	151.156203
1	Narrows	Sep-2018	6	19/09/2018	5:57	0:38	-23.711	151.15445
1	Narrows	Sep-2018	6	19/09/2018	6:37	0:26	-23.711	151.15445
1	Narrows	Sep-2018	6	19/09/2018	7:04	0:31	-23.711	151.15445
1	Narrows	Sep-2018	8	19/09/2018	6:12	0:35	-23.7128	151.154593
1	Narrows	Sep-2018	8	19/09/2018	6:49	0:25	-23.7128	151.154593
1	Narrows	Sep-2018	8	19/09/2018	7:15	0:10	-23.7128	151.154593
1	Narrows	Sep-2018	8	19/09/2018	8:04	0:33	-23.732	151.13328
1	Narrows	Sep-2018	8	19/09/2018	8:39	1:05	-23.732	151.13328
1	Narrows	Sep-2018	4.5	19/09/2018	8:15	0:37	-23.7387	151.130262
1	Narrows	Sep-2018	4.5	19/09/2018	8:54	0:33	-23.7387	151.130262
1	Narrows	Sep-2018	6	19/09/2018	8:29	0:17	-23.7405	151.141738
1	Narrows	Sep-2018	2	19/09/2018	8:59	0:11	-23.7274	151.134107
1	Narrows	Sep-2018	2	19/09/2018	9:16	0:07	-23.7257	151.134556
2	Graham Creek	Sep-2018	8	18/09/2018	5:50	0:40	-23.7234	151.221056
2	Graham Creek	Sep-2018	8	18/09/2018	6:32	0:38	-23.7234	151.221056
2	Graham Creek	Sep-2018	8	18/09/2018	7:12	1:31	-23.7234	151.221056
2	Graham Creek	Sep-2018	4.5	18/09/2018	6:00	0:41	-23.7271	151.219848
2	Graham Creek	Sep-2018	4.5	18/09/2018	6:42	0:24	-23.7271	151.219848
2	Graham Creek	Sep-2018	6	18/09/2018	6:20	0:30	-23.7092	151.222903
2	Graham Creek	Sep-2018	6	18/09/2018	6:51	0:36	-23.7092	151.222903
2	Graham Creek	Sep-2018	6	18/09/2018	7:28	0:37	-23.7092	151.222903
2	Graham Creek	Sep-2018	6	18/09/2018	8:06	0:34	-23.7092	151.222903
2	Graham Creek	Sep-2018	4.5	18/09/2018	7:22	0:18	-23.7081	151.224461
2	Graham Creek	Sep-2018	4.5	18/09/2018	7:50	0:21	-23.7075	151.223527
2	Graham Creek	Sep-2018	4.5	18/09/2018	9:02	0:22	-23.7345	151.171835

GHHP Zone Number	Location	Survey	Gill net mesh size (inches)	Date	Deploy time	Soak time (h:mm)	Latitude	Longitude
3	Western Basin	Sep-2018	6	17/09/2018	6:00	0:30	-23.7798	151.15353
3	Western Basin	Sep-2018	6	17/09/2018	7:06	0:18	-23.7798	151.15353
3	Western Basin	Sep-2018	4.5	17/09/2018	6:09	0:26	-23.7811	151.153484
3	Western Basin	Sep-2018	4.5	17/09/2018	6:50	0:25	-23.7811	151.153484
3	Western Basin	Sep-2018	2	17/09/2018	6:19	0:26	-23.7815	151.152893
3	Western Basin	Sep-2018	6	17/09/2018	7:59	0:45	-23.7508	151.17698
3	Western Basin	Sep-2018	4.5	17/09/2018	8:04	0:46	-23.7503	151.176325
5	Inner Harbour	Sep-2018	4.5	19/09/2018	10:49	0:39	-23.7853	151.248258
5	Inner Harbour	Sep-2018	6	19/09/2018	10:53	0:30	-23.7855	151.248331
5	Inner Harbour	Sep-2018	4.5	19/09/2018	11:59	0:10	-23.7717	151.246523
5	Inner Harbour	Sep-2018	6	19/09/2018	12:01	0:15	-23.7716	151.247072
5	Inner Harbour	Sep-2018	6	19/09/2018	12:22	0:17	-23.7701	151.2468
5	Inner Harbour	Sep-2018	4.5	19/09/2018	12:26	0:17	-23.7699	151.246881
5	Inner Harbour	Sep-2018	4.5	19/09/2018	12:50	0:45	-23.7692	151.245318
5	Inner Harbour	Sep-2018	6	19/09/2018	12:52	0:46	-23.7691	151.244874
5	Inner Harbour	Sep-2018	6	19/09/2018	14:09	0:19	-23.7768	151.242899
6	Calliope River	Sep-2018	4.5	17/09/2018	9:58	0:45	-23.8859	151.193351
6	Calliope River	Sep-2018	6	17/09/2018	10:06	0:13	-23.8856	151.194227
6	Calliope River	Sep-2018	6	17/09/2018	10:27	0:23	-23.8886	151.197135
6	Calliope River	Sep-2018	4.5	17/09/2018	11:15	0:32	-23.9356	151.15851
6	Calliope River	Sep-2018	4.5	17/09/2018	11:48	0:39	-23.9356	151.15851
6	Calliope River	Sep-2018	4.5	17/09/2018	12:28	0:12	-23.9356	151.15851
6	Calliope River	Sep-2018	8	17/09/2018	11:23	0:31	-23.9422	151.164402
6	Calliope River	Sep-2018	8	17/09/2018	11:55	0:37	-23.9422	151.164402
6	Calliope River	Sep-2018	8	17/09/2018	12:33	0:33	-23.9422	151.164402
6	Calliope River	Sep-2018	6	17/09/2018	11:37	0:20	-23.9406	151.163221
6	Calliope River	Sep-2018	6	17/09/2018	11:58	0:36	-23.9406	151.163221
6	Calliope River	Sep-2018	6	17/09/2018	12:35	0:25	-23.9406	151.163221
6	Calliope River	Sep-2018	4.5	17/09/2018	12:53	0:36	-23.9301	151.159856
7	Auckland Creek	Sep-2018	4.5	18/09/2018	10:44	0:23	-23.8446	151.241689
7	Auckland Creek	Sep-2018	6	18/09/2018	10:54	0:33	-23.851	151.241271
7	Auckland Creek	Sep-2018	6	18/09/2018	11:28	0:12	-23.851	151.241271
7	Auckland Creek	Sep-2018	4.5	18/09/2018	11:11	0:20	-23.85	151.24151
7	Auckland Creek	Sep-2018	4.5	18/09/2018	11:32	0:37	-23.85	151.24151
7	Auckland Creek	Sep-2018	6	18/09/2018	11:55	0:35	-23.8543	151.239478
7	Auckland Creek	Sep-2018	6	18/09/2018	12:31	0:27	-23.8543	151.239478
7	Auckland Creek	Sep-2018	6	18/09/2018	12:59	0:26	-23.8543	151.239478
7	Auckland Creek	Sep-2018	6	18/09/2018	13:27	0:28	-23.8543	151.239478
7	Auckland Creek	Sep-2018	6	18/09/2018	13:56	0:33	-23.8543	151.239478
7	Auckland Creek	Sep-2018	4.5	18/09/2018	12:15	0:25	-23.852	151.241073
7	Auckland Creek	Sep-2018	4.5	18/09/2018	12:41	0:32	-23.852	151.241073

GHHP Zone Number	Location	Survey	Gill net mesh size (inches)	Date	Deploy time	Soak time (h:mm)	Latitude	Longitude
7	Auckland Creek	Sep-2018	4.5	18/09/2018	13:29	0:12	-23.8572	151.236792
7	Auckland Creek	Sep-2018	4.5	18/09/2018	14:00	0:20	-23.858	151.234179
8	Mid Harbour	Sep-2018	6	20/09/2018	5:42	0:28	-23.8256	151.34201
8	Mid Harbour	Sep-2018	4.5	20/09/2018	5:53	0:29	-23.827	151.338466
8	Mid Harbour	Sep-2018	4.5	20/09/2018	6:42	0:35	-23.8143	151.335491
8	Mid Harbour	Sep-2018	4.5	20/09/2018	7:20	0:30	-23.8143	151.335491
8	Mid Harbour	Sep-2018	4.5	20/09/2018	7:51	0:24	-23.8143	151.335491
8	Mid Harbour	Sep-2018	4.5	20/09/2018	8:16	0:54	-23.8143	151.335491
8	Mid Harbour	Sep-2018	6	20/09/2018	6:47	0:37	-23.8139	151.334647
8	Mid Harbour	Sep-2018	6	20/09/2018	7:25	0:30	-23.8139	151.334647
8	Mid Harbour	Sep-2018	6	20/09/2018	7:57	0:21	-23.8139	151.334647
8	Mid Harbour	Sep-2018	6	20/09/2018	8:19	0:45	-23.8139	151.334647
8	Mid Harbour	Sep-2018	2	20/09/2018	8:26	0:09	-23.8141	151.335764
9	South Trees Inlet	Sep-2018	4.5	20/09/2018	9:57	0:28	-23.8538	151.296601
9	South Trees Inlet	Sep-2018	6	20/09/2018	10:06	0:31	-23.8533	151.29757
9	South Trees Inlet	Sep-2018	6	20/09/2018	11:32	0:13	-23.9172	151.299994
9	South Trees Inlet	Sep-2018	4.5	20/09/2018	11:37	0:16	-23.918	151.300125
9	South Trees Inlet	Sep-2018	4.5	20/09/2018	11:57	0:20	-23.9162	151.299973
9	South Trees Inlet	Sep-2018	6	20/09/2018	12:04	0:07	-23.9166	151.299562
9	South Trees Inlet	Sep-2018	4.5	20/09/2018	12:22	0:44	-23.9127	151.296961
9	South Trees Inlet	Sep-2018	6	20/09/2018	12:29	0:32	-23.9132	151.29684
9	South Trees Inlet	Sep-2018	4.5	20/09/2018	13:36	0:33	-23.9397	151.302121
9	South Trees Inlet	Sep-2018	6	20/09/2018	13:40	0:35	-23.9404	151.3022
9	South Trees Inlet	Sep-2018	2	20/09/2018	13:48	0:01	-23.9398	151.302757
10	Boyne River	Sep-2018	4.5	3/10/2018	5:39	0:37	-23.9772	151.330924
10	Boyne River	Sep-2018	4.5	3/10/2018	6:17	0:47	-23.9772	151.330924
10	Boyne River	Sep-2018	6	3/10/2018	5:51	0:47	-23.9768	151.323476
10	Boyne River	Sep-2018	8	3/10/2018	6:06	0:39	-23.9794	151.319165
10	Boyne River	Sep-2018	8	3/10/2018	6:46	0:39	-23.9794	151.319165
10	Boyne River	Sep-2018	8	3/10/2018	7:26	0:27	-23.9794	151.319165
10	Boyne River	Sep-2018	8	3/10/2018	7:54	0:40	-23.9794	151.319165
10	Boyne River	Sep-2018	8	3/10/2018	8:35	0:28	-23.9794	151.319165
10	Boyne River	Sep-2018	6	3/10/2018	6:50	0:40	-23.9796	151.319861
10	Boyne River	Sep-2018	4.5	3/10/2018	7:20	0:38	-23.9791	151.320867
10	Boyne River	Sep-2018	4.5	3/10/2018	7:59	0:41	-23.9791	151.320867

GHHP Zone Number	Location	Survey	Gill net mesh size (inches)	Date	Deploy time	Soak time (h:mm)	Latitude	Longitude
10	Boyne River	Sep-2018	4.5	3/10/2018	8:41	0:27	-23.9791	151.320867
10	Boyne River	Sep-2018	8	3/10/2018	9:04	0:22	-23.9791	151.320867
10	Boyne River	Sep-2018	8	3/10/2018	9:27	0:33	-23.9791	151.320867
10	Boyne River	Sep-2018	8	3/10/2018	10:01	0:35	-23.9791	151.320867
10	Boyne River	Sep-2018	6	3/10/2018	7:47	0:33	-23.9778	151.32123
10	Boyne River	Sep-2018	6	3/10/2018	8:22	0:36	-23.9778	151.32123
10	Boyne River	Sep-2018	6	3/10/2018	8:59	0:31	-23.9778	151.32123
10	Boyne River	Sep-2018	6	3/10/2018	9:31	0:17	-23.9778	151.32123
10	Boyne River	Sep-2018	6	3/10/2018	9:49	0:36	-23.9778	151.32123
10	Boyne River	Sep-2018	6	3/10/2018	10:26	0:23	-23.9778	151.32123
10	Boyne River	Sep-2018	4.5	3/10/2018	9:20	0:31	-23.9784	151.320912
10	Boyne River	Sep-2018	4.5	3/10/2018	9:52	0:28	-23.9784	151.320912
10	Boyne River	Sep-2018	4.5	3/10/2018	10:21	0:35	-23.9784	151.320912
12	Colosseum Inlet	Sep-2018	6	21/09/2018	7:51	0:40	-24.0627	151.483019
12	Colosseum Inlet	Sep-2018	6	21/09/2018	8:32	0:38	-24.0627	151.483019
12	Colosseum Inlet	Sep-2018	6	21/09/2018	9:11	0:21	-24.0627	151.483019
12	Colosseum Inlet	Sep-2018	4.5	21/09/2018	7:59	0:35	-24.0634	151.483419
12	Colosseum Inlet	Sep-2018	4.5	21/09/2018	8:35	0:38	-24.0634	151.483419
12	Colosseum Inlet	Sep-2018	4.5	21/09/2018	9:14	0:26	-24.0634	151.483419
12	Colosseum Inlet	Sep-2018	2	21/09/2018	8:18	0:09	-24.0566	151.481177
12	Colosseum Inlet	Sep-2018	2	21/09/2018	8:58	0:09	-24.0523	151.461117
12	Colosseum Inlet	Sep-2018	4.5	21/09/2018	9:51	0:59	-24.0736	151.484303
12	Colosseum Inlet	Sep-2018	6	21/09/2018	10:03	0:42	-24.0796	151.486894
12	Colosseum Inlet	Sep-2018	6	21/09/2018	10:46	0:26	-24.0796	151.486894
12	Colosseum Inlet	Sep-2018	2	21/09/2018	10:30	0:12	-24.0812	151.486048
12	Colosseum Inlet	Sep-2018	6	21/09/2018	11:26	0:37	-24.0532	151.475174
12	Colosseum Inlet	Sep-2018	4.5	21/09/2018	11:33	0:57	-24.054	151.475459
13	Rodds Bay	Sep-2018	6	5/10/2018	6:22	0:39	-24.0631	151.681281
13	Rodds Bay	Sep-2018	6	5/10/2018	7:02	0:32	-24.0631	151.681281
13	Rodds Bay	Sep-2018	6	5/10/2018	7:35	0:37	-24.0631	151.681281
13	Rodds Bay	Sep-2018	4.5	5/10/2018	6:28	0:36	-24.0633	151.6808
13	Rodds Bay	Sep-2018	4.5	5/10/2018	7:05	0:32	-24.0633	151.6808
13	Rodds Bay	Sep-2018	4.5	5/10/2018	7:38	0:41	-24.0633	151.6808
13	Rodds Bay	Sep-2018	6	5/10/2018	9:11	0:27	-24.0669	151.638595
13	Rodds Bay	Sep-2018	6	5/10/2018	9:39	0:30	-24.0669	151.638595
13	Rodds Bay	Sep-2018	6	5/10/2018	10:10	0:25	-24.0669	151.638595
13	Rodds Bay	Sep-2018	4.5	5/10/2018	9:29	0:38	-24.0653	151.63775
13	Rodds Bay	Sep-2018	6	5/10/2018	10:56	0:27	-24.0411	151.609374
13	Rodds Bay	Sep-2018	6	5/10/2018	11:24	0:28	-24.0411	151.609374
13	Rodds Bay	Sep-2018	4.5	5/10/2018	11:03	0:12	-24.043	151.6122
13	Rodds Bay	Sep-2018	4.5	5/10/2018	11:16	0:15	-24.043	151.6122

GHHP Zone Number	Location	Survey	Gill net mesh size (inches)	Date	Deploy time	Soak time (h:mm)	Latitude	Longitude
	Baffle Creek	Sep-2018	4.5	4/10/2018	7:02	0:30	-24.5273	152.028412
	Baffle Creek	Sep-2018	4.6	4/10/2018	7:33	0:31	-24.5273	152.028412
	Baffle Creek	Sep-2018	4.5	4/10/2018	8:05	0:29	-24.5273	152.028412
	Baffle Creek	Sep-2018	4.5	4/10/2018	8:35	0:25	-24.5273	152.028412
	Baffle Creek	Sep-2018	4.5	4/10/2018	9:01	0:31	-24.5273	152.028412
	Baffle Creek	Sep-2018	4.5	4/10/2018	9:33	0:28	-24.5273	152.028412
	Baffle Creek	Sep-2018	4.5	4/10/2018	10:02	0:29	-24.5273	152.028412
	Baffle Creek	Sep-2018	4.5	4/10/2018	10:32	0:25	-24.5273	152.028412
	Baffle Creek	Sep-2018	6	4/10/2018	7:10	0:31	-24.5254	152.026882
	Baffle Creek	Sep-2018	6	4/10/2018	7:42	0:31	-24.5254	152.026882
	Baffle Creek	Sep-2018	6	4/10/2018	8:14	0:31	-24.5254	152.026882
	Baffle Creek	Sep-2018	6	4/10/2018	8:46	0:28	-24.5254	152.026882
	Baffle Creek	Sep-2018	6	4/10/2018	9:15	0:32	-24.5254	152.026882
	Baffle Creek	Sep-2018	6	4/10/2018	9:48	0:34	-24.5254	152.026882
	Baffle Creek	Sep-2018	6	4/10/2018	11:49	0:35	-24.5118	152.024517
	Baffle Creek	Sep-2018	6	4/10/2018	12:25	0:20	-24.5118	152.024517
	Baffle Creek	Sep-2018	4.5	4/10/2018	11:58	0:31	-24.5122	152.022942
	Stanage Bay	Sep-2018	2	1/11/2018	7:35	0:12	-22.1411	150.028201
	Stanage Bay	Sep-2018	6	1/11/2018	8:02	0:31	-22.1468	149.997358
	Stanage Bay	Sep-2018	4.5	1/11/2018	8:20	0:28	-22.1475	149.995807
	Stanage Bay	Sep-2018	4.5	1/11/2018	9:16	0:38	-22.1712	149.992689
	Stanage Bay	Sep-2018	6	1/11/2018	9:25	1:00	-22.1667	149.987293
	Stanage Bay	Sep-2018	6	1/11/2018	11:01	0:29	-22.2072	149.934349
	Stanage Bay	Sep-2018	6	1/11/2018	11:31	0:18	-22.2072	149.934349
	Stanage Bay	Sep-2018	6	1/11/2018	11:50	1:04	-22.2072	149.934349
	Stanage Bay	Sep-2018	4.5	1/11/2018	11:56	0:35	-22.2187	149.941211
	Stanage Bay	Sep-2018	2	1/11/2018	12:08	0:20	-22.2193	149.941655

Appendix 3: Site physicochemical data

Autumn 2018

Site	Zone	GHHP Zone Number	Date/Time	Temp (°C)	DO (%)	DO (mg/L)	EC (µs/cm)	pH	Turbidity (NTU)	TDS	ORP	Salinity (ppt)
Rodds Bay	RB	13	09/04/2018 11:29	24.1	95.1	6.54	52413	8.07	12.7	34684	-103.0	35.24
Graham Creek	GC	2	10/04/2018 8:12	24.1	91.4	6.28	52068	7.83	8.1	34419	24.2	34.94
Inner Harbour	IH	5	10/04/2018 16:20	26.5	101.4	6.70	53368	8.00	10.2	34689	-75.4	35.19
Narrows	NW	1	11/04/2018 10:59	25.0	77.2	5.20	53713	7.55	20.1	34906	-84.3	35.47
Calliope River	CR	6	11/04/2018 16:15	29.4	99.3	6.26	52215	7.83	6.8	33951	-105.9	34.24
Mid Harbour	MH	8	12/04/2018 8:36	23.7	93.8	6.49	53264	7.93	10.8	34627	-81.9	35.18
Auckland Creek	AC	7	12/04/2018 16:34	26.5	89.1	5.86	52156	7.68	30.6	33915	-113.1	34.30
Western Basin	WB	3	13/04/2018 8:24	23.7	94.9	6.57	53092	7.92	8.6	34517	-100.6	35.06
Boyne River	BR	10	16/04/2018 8:45	26.2	81.1	5.79	34835	7.52	3.3	22667	-92.7	21.92
Outer Harbour	OH	11	16/04/2018 12:29	27.2	99.2	6.47	53711	8.27	4.5	34914	-135.1	35.43
South Trees	STI	9	17/04/2018 7:54	25.3	85.7	5.75	53596	8.01	10.0	34839	-126.1	35.40
Colosseum	RCI	12	17/04/2018 11:34	26.1	77.3	5.12	54661	7.95	12.6	35529	-148.3	36.16
Baffle Creek	BC		19/04/2018 13:33	26.2	93.9	6.37	47708	8.26	10.5	30996	-143.9	31.00
Stanage Bay	SB		23/04/2018 11:48	25.6	95.4	6.35	55401	8.20	18.7	35575	-128.8	36.22

Spring 2018

Site	Zone	GHHP Zone Number	Date/Time	Temp (°C)	DO (%)	DO (mg/L)	EC (µs/cm)	pH	Turbidity (NTU)	TDS	ORP	Salinity (ppt)
Western Basin	WB	3	17/09/2018 8:36	22.2	94.7	6.66	55890	8.10	5.1	36336	-182.9	37.17
Calliope River	CR	6	17/09/2018 13:23	23.1	98.9	7.05	48739	8.00	7.0	31684	-179.0	31.88
Graham Creek	GC	2	18/09/2018 9:25	22.5	91.5	6.39	56092	7.80	3.3	36463	-190.1	37.31
Auckland Creek	AC	7	18/09/2018 14:46	23.1	96.6	6.69	55699	7.70	10.0	36209	-200.4	37.01
Narrows	NW	1	19/09/2018 9:51	22.2	83.4	5.85	56958	7.60	3.4	37027	-188.1	37.96
Inner Harbour	IH	5	19/09/2018 14:24	25.3	99.4	6.63	55759	7.80	14.4	36241	-197.2	37.01
Mid Harbour	MH	8	20/09/2018 9:39	22.3	94.5	6.66	54673	8.00	7.4	35623	-181.7	36.27
South Trees	STI	9	20/09/2018 14:26	23.8	66.1	4.44	56304	7.80	6.1	36592	-221.9	37.43
Colosseum	RCI	12	21/09/2018 8:42	22.0	83.9	5.89	56437	7.90	4.2	36887	-195.4	37.79
Boyne River	BR	10	03/10/2018 11:03	23.8	87.4	6.12	49964	7.80	3.3	32487	-165.5	32.76
Baffle Creek	BC		04/10/2018 12:48	23.8	105.5	7.32	52145	8.00	2.1	33921	-168.4	34.35
Rodds Bay	RB	13	05/10/2018 11:55	23.5	100.1	6.89	55172	8.10	3.8	35801	-181.4	36.61
Stanage Bay	SB		01/11/2018 13:00	ND	ND	ND	ND	ND	ND	ND	ND	ND

Appendix 4: Species (scientific and common name) catch by zone and season

		Autumn															Spring													
Common name	**Scientific name**	**1**	**2**	**3**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**R1**	**R2**	**Total**	**1**	**2**	**3**	**5**	**6**	**7**	**8**	**9**	**10**	**12**	**13**	**R1**	**R2**	**Total**
Australian Giant Herring	*Elops machnata*	1														1														
Barramundi	*Lates calcarifer*	1	1		4		4	1	1	1			1		6	20	2					3	1	1		6			6	19
Barred Javelin	*Pomadasys kaakan*	3	8	1	13			4					9			38	1	14	2	13	1	9	9	1			1	4		55
Bartailed flathead	*Platycephalus indicus*								1				1			2														
Beach Salmon	*Leptobrama muelleri*		2	2	3			2								9		2	6										1	9
Blubber lip bream (Brown Sweetlips)	*Plectorhinchus gibbosus*	1							1	1				1		4						3							1	4
Blue Catfish	*Neoarius graeffei*	2	1		3	2	6		1	7		11				33	5	1		1	4	2		5	1	10	6		26	61
Blue Threadfin	*Eleutheronema tetradactylum*	1	2		2	1	1		1				1			9	2	1	5	3		3	9			2	11			36
Blue tuskfish	*Choerodon cyanodus*																												1	1
Bony bream	*Carcharhinus leucas*																										1			1
Bull Shark	*Nematalosa erebi*		1			2				2		2				7														
Common Ponyfish	*Leiognathus equulus*													1		1														
Common Silverbiddy	*Gerres subfasciatus*													1		1										1				1
Diamond Scale Mullet	*Liza vaigiensis*	2	1				3	3		2					1	12		4	3	7	1	1	1	3					1	21
Dusky flathead	*Platycephalus fuscus*																			1				1				1		3
Giant queenfish	*Scomberoides commersonnianus*					4	2	1	14	1				1		23						2	1	1		1				5
Giant Shovelnose Ray	*Glaucostegus typus*	1	1					1							1	4												1		1
Giant Trevally	*Caranx ignobilis*						1									1														
Golden Trevally	*Gnathanodon speciosus*									1						1														
Goldlined Rabbitfish	*Siganus lineatus*									1						1											1			1
Goldspotted rockcod	*Epinephelus coioides*																				1									1

Green backed mullet	*Lisa subviridis*																1														1
Grey mackerel	*Scomberomorus semifasciatus*																		1												1
Hairback Herring	*Nematalosa come*													13			13				3										3
King Threadfin	*Polydactyus macrochir*				3								1			4				1											1
Lemon Shark	*Negaprion acutidens*							1								1															
Mangrove Jack	*Lutjanus argentimaculatus*																													1	1
Moses snapper (Moses perch)	*Lutjanus russelli*																									2					2
Mulloway	*Argyrosomus japonicus*																			1		1									2
Pikey Bream	*Acanthopagrus pacificus*			1												1		2		1						2					5
Popeye Mullet	*Rhinomugil nasutus*																		4												4
Sand whiting	*Sillago ciliata*													3		3			1												1
Sea mullet	*Mugil cephalus*					8	1							6		15	3		19				3	3		1				29
Shovelnose Ray (Whitespotted guitarfish)	*Rhynchobatus australiae*	1						1								2			1				1								2
Sicklefish	*Drepane punctata*								2	3			2	6		13					1	4									5
Silver Javelin	*Loxodon macrorhinus*													6		6															
Silver jewfish	*Pomadasys argenteus*													1		1															
Sliteye Shark	*Nibea soldado*			1				1								2											1				1
Snub-nosed dart	*Trachinotus blochii*																				1										1
Spotted Scat	*Scatophagus argus*																					6									6
Striped Scat	*Selenotoca multifasciata*						1						8			9															
Swallow-tailed dart	*Trachinotus coppingeri*										1					1															
Threadfin Silverbiddy	*Gerres filamentosus*													9		9															
Tripletail	*Lobotes surinamensis*							1								1															
Whitespotted Eagle Ray	*Aetobatus ocellatus*																									1					1
Yellowfin Bream	*Acanthopagrus australis*					1										1										1					1
Grand Total		13	17	5	28	18	19	16	21	19	1	13	22	48	9	249	13	25	41	28	10	37	25	15	7	22	20	6	37		286

Appendix 5: Permanova analysis results

Autumn 2018

Barramundi

PERMANOVA detected a significant difference among zones for both GSI and Fulton's condition factor (Fulton's K). Barramundi sampled in Inner Harbour had a higher GSI value than barramundi sampled in either Auckland Inlet or Stanage Bay.

Barramundi sampled in Auckland Inlet had higher values of Fulton's K than those sampled in Stanage Bay, which in turn had greater values than barramundi sampled in Inner Harbour.

Barramundi HSI, Eye asymmetry, and HAI were all similar among the three zones compared.

Comparison: Inner Harbour (IH) vs Auckland Inlet (AI) vs Stanage Bay (SB)				
Index	df	*F*	*P*	Outcome of *post-hoc* tests
GSI	2,8	10.778	**0.002**	IH > AI = SB
HSI	2,8	0.9678	0.493	IH = AI = SB
Fulton's K	2,8	2.8830	**0.001**	AI > SB > IH
Eye asymmetry	2,8	0.5140	0.610	IH = AI = SB
HAI	2,8	3.4099	0.123	IH = AI = SB

Diamondscale mullet

PERMANOVA detected a significant difference among zones only for HSI but the low replication prevented an unambiguous test of the location of differences using post-hoc tests. Visually, however, it appears that Diamondscale mullet sampled from Auckland Inlet had the highest HSI values.

Diamondscale mullet GSI, Fulton's K, Eye asymmetry, and HAI were all similar among the four zones compared.

Comparison: the Narrows (N) vs Auckland Inlet (AI) vs Mid Harbour (MH) vs Boyne Estuary (BE)				
Index	df	*F*	*P*	Outcome of *post-hoc* tests
GSI	3,5	2.2365	0.124	N = AI = MH = BE
HSI	3,5	7.7284	**0.039**	Ambiguous due to low replication
Fulton's K	3,5	1.0709	0.406	N = AI = MH = BE
Eye asymmetry	3,5	0.9176	0.521	N = AI = MH = BE
HAI	3,5	2.5686	0.194	N = AI = MH = BE

Sea Mullet

No differences were detected for any indices measured between sea mullet sampled in Calliope Estuary and Baffle Creek.

Comparison: Calliope Estuary (CE) vs Baffle Creek (BC)				
Index	df	*F*	*P*	Outcome of *post-hoc* tests
GSI	1,7	1.7672	0.245	CE = BC
HSI	1,7	3.5237	0.093	CE = BC
Fulton's	1,7	3.8767	0.078	CE = BC
Eye asymmetry	1,7	0.2500	0.736	CE = BC
HAI	1,7	0.6867	0.378	CE = BC

Diamondscale Mullet

No significant differences were detected among the four zones compared for any of the five metrics tested.

Comparison: Graham Creek (GC) vs Western Basin (WB) vs Inner Harbour (IH) vs South Trees Inlet (STI)				
Index	df	*F*	*P*	Outcome of *post-hoc* tests
GSI	3,10	0.3369	0.972	GC = WB = IH = STI
HSI	3,10	0.6198	0.656	GC = WB = IH = STI
Fulton's	3,10	0.9402	0.449	GC = WB = IH = STI
Eye asymmetry	3,10	0.3479	0.829	GC = WB = IH = STI
HAI	3,10	0.5269	0.993	GC = WB = IH = STI

Sea Mullet

For all condition indices, no differences were detected for Sea Mullet sampled across all four zones analysed.

Comparison: the Narrows (NW) vs Western Basin (WB) vs Mid Harbour (MH) vs South Trees Inlet (STI)				
Index	df	*F*	*P*	Outcome of *post-hoc* tests
GSI	3,8	0.1878	0.885	NW = WB = MH = STI
HSI	3,8	0.9128	0.426	NW = WB = MH = STI
Fulton's	3,8	0.5958	0.661	NW = WB = MH = STI
Eye asymmetry	3,8	0.7115	0.625	NW = WB = MH = STI
HAI	3,8	3.9570	0.068	NW = WB = MH = STI

Autumn 2018 and Spring 2018 combined

Formal statistical tests to compare fish health indices among zones and sampling times (i.e. 2-way PERMANOVA) were based on the criteria of having a minimum replication of two sampled fish per zone during each sampling period (April and September). This resulted in the following possible statistical tests:

Barramundi: Comparison of all five indices between Auckland creek (zone 7) and Stanage Bay (zone R1).

Due to uneven replication among zones at and times, no further 2-way analyses were possible for Bream, Diamond-scale Mullet, or Sea Mullet.

Data were then pooled across both sampling times to determine statistical comparisons among zones independent of sampling time (i.e. 1-way PERMANOVA). This resulted in the following possible statistical tests:

Barramundi: Comparison of all five indices between the Narrows (zone 1), Inner Harbour (zone 5), Auckland creek (zone 7), Middle harbour (zone 8), South Trees Inlet (zone 9), Colosseum Inlet (zone 12) and reference site Stanage Bay (zone R1).

Barred javelin: Comparison of all five indices between the reference site Baffle Creek (zone R2), and the Gladstone Harbour zones Graham Creek (zone 2), Mid Harbour (zone 8), Auckland Creek (zone 7), Inner Harbour (zone 5).

Bream: Comparison of all five indices between Graham Creek (zone 2) and Boyne Estuary (zone 10).

Diamond Scale Mullet: Comparison of all five indices between the Narrows (zone 1), Graham Creek (zone 2), Western Basin (zone 3), Inner Harbour (zone 5), Auckland creek (zone 7), Middle harbour (zone 8), South Trees Inlet (zone 9), Boyne estuary (zone 10) and Stanage Bay (zone R1).

Sea Mullet: Comparison of all five indices between the Narrows (zone 1), Western Basin (zone 3), Calliope Estuary (zone 6), Middle harbour (zone 8), South Trees Inlet (zone 9), and Baffle Creek (Zone R2).

2-way PERMANOVA: Barramundi

PERMANOVA detected a significant 2-way interactions between sampling time and zone for Fulton's condition index. Here, Barramundi sampled in April at Auckland creek had a greater index that fish sampled in April at Stanage Bay, with fish sampled in April at both zones having greater values than fish sampled in September at either zone (see post-hoc test in table below).

An effect of zone was detected for HAI, with fish in Auckland creek having larger values than those from Stanage bay.

Comparison: Auckland Creek (AC) vs Stanage Bay (SB)					
Index	Source	df	*F*	*P*	Outcome of *post-hoc* tests
GSI	Time	1	2.0904	0.163	Apr = Sep
	Zone	1	1.8635	0.204	AC = SB
	Time x Zone	1	3.3642	0.107	Apr AC = Apr SB = Sep AC = Sep SB
	Residual	13			
HSI	Time	1	2.8761	0.114	Apr = Sep
	Zone	1	1.2398	0.340	AC = SB
	Time x Zone	1	0.0230	0.900	Apr AC = Apr SB = Sep AC = Sep SB
	Residual	13			
Fulton's	Time	1	104.7000	**0.001**	Apr > Sep
	Zone	1	0.4217	0.521	AC = SB
	Time x Zone	1	5.5987	**0.039**	Apr AC > Apr SB > Sep AC = Sep SB
	Residual	13			
Eye asymmetry	Time	1	0.1366	0.812	Apr = Sep
	Zone	1	0.9678	0.502	AC = SB
	Time x Zone	1	0.1366	0.817	Apr AC = Apr SB = Sep AC = Sep SB
	Residual	13			
HAI	Time	1	0.1316	0.740	Apr = Sep
	Zone	1	12.5580	**0.006**	AC > SB
	Time x Zone	1	0.4119	0.529	Apr AC = Apr SB = Sep AC = Sep SB
	Residual	13			

1-Way PERMANOVA: Barramundi

PERMANOVA detected a significant difference among zones for HAI. Barramundi from Colosseum Inlet (zone 12) exhibited a greater HAI than most other zones, while fish from Stanage Bay (zone 14) had a lower HAI than most other zones (see post-hoc tests below).

Barramundi GSI, HSI, Fulton's condition index, and Eye asymmetry were all similar among the six zones compared.

Comparison: Narrows (NW), Inner Harbour (IH), Auckland creek (AC), Middle harbour (MH), South Trees Inlet (STI), Colosseum Inlet (RCI) and Stanage Bay (SB)				
Index	df	*F*	*P*	Outcome of *post-hoc* tests
GSI	6,26	2.2797	0.117	NW = IH = AC = MH = STI = RCI = SB
HSI	6,26	0.9850	0.438	NW = IH = AC = MH = STI = RCI = SB
Fulton's	6,26	2.0877	0.106	NW = IH = AC = MH = STI = RCI = SB
Eye asymmetry	6,26	0.4160	0.856	NW = IH = AC = MH = STI = RCI = SB
HAI	6,26	6.4883	**0.006**	RCI > AC > SB = NW = IH = MH = STI

Post-hoc tests:

Zones	t	P(perm)	perms
1, 5	1.8516	0.249	7
1, 7	2.9084	0.04	10
1, 8	Denominator is 0		
1, 9	Denominator is 0		
1, 12	2.694	0.028	16
1, 14	0.53109	1	2
5, 7	0.4402	0.717	12
5, 8	1.4606	0.285	6
5, 9	1.4606	0.281	6
5, 12	1.8049	0.124	19
5, 14	2.3397	0.04	9
7, 8	2.3414	0.095	7
7, 9	2.3414	0.077	7
7, 12	2.1271	0.037	19
7, 14	3.7581	0.001	16
8, 9	Denominator is 0		
8, 12	2.1466	0.095	11
8, 14	0.43033	1	2
9, 12	2.1466	0.076	11
9, 14	0.43033	1	2
12, 14	4.5985	0.001	13

1-Way PERMANOVA: Barred javelin

PERMANOVA detected a significant difference among zones for GSI and Fulton's condition index. For GSI, Barred Javelin from Baffle Creek (zone 15) exhibited greater GSI that fish from both Graham Creek (zone 2) and Mid Harbour (zone 8). For Fulton's condition index, fish from Graham creek (zone 2) and Auckland Creek (zone 7) exhibited a greater value than fish from Inner Harbour (zone 5) and Mid Harbour (zone 8).

Barred Javelin HSI, Eye asymmetry, and HAI were all similar among the five zones compared.

Comparison: Graham Creek (GC) vs Inner Harbour (IH) vs Auckland Creek (AC) vs Mid Harbour (MH) vs Baffle creek (BC)				
Index	df	*F*	*P*	Outcome of *post-hoc* tests
GSI	4,22	3.5985	**0.030**	GC = IH = AC = MH < BC
HSI	4,22	0.7979	0.628	GC = IH = AC = MH = BC

Fulton's	4,22	3.1783	**0.006**	GC = AC > IH = MH = BC
Eye asymmetry	4,22	2.4433	0.075	GC = IH = AC = MH = BC
HAI	4,22	1.2173	0.336	GC = IH = AC = MH = BC

1-way PERMANOVA: Bream

No differences were detected for any of the five metrics tested between Graham Creek (zone 2) and Boyne Estuary (zone 10). This result should be interpreted cautiously due to the overall low replication of Bream in the data set analysed (total of five fish).

Comparison: Graham Creek (GC) vs Boyne Estuary (BR)				
Index	df	*F*	*P*	Outcome of tests
GSI	1,3	73.0670	0.121	GC = BR
HSI	1,3	0.6211	0.596	GC = BR
Fulton's	1,3	15.361	0.102	GC = BR
Eye asymmetry	1,3	0.5436	0.474	GC = BR
HAI	1,3	2.4651	0.190	GC = BR

1-Way PERMANOVA: Diamondscale Mullet

No significant differences were detected among the nine zones compared for any of the five metrics tested.

Comparison: the Narrows (NW), Graham Creek (GC), Western Basin (WB), Inner Harbour (IH), Auckland creek (AC), Middle harbour (MH), South Trees Inlet (STI), Boyne estuary (BC) and Stanage Bay (SB).				
Index	df	*F*	*P*	Outcome of *post-hoc* tests
GSI	8,20	2.1685	0.086	NW = GC = WB = IH = AC = MH = STI = BC = SB
HSI	8,20	1.9341	0.114	NW = GC = WB = IH = AC = MH = STI = BC = SB
Fulton's	8,20	0.9563	0.434	NW = GC = WB = IH = AC = MH = STI = BC = SB
Eye asymmetry	8,20	0.3178	0.943	NW = GC = WB = IH = AC = MH = STI = BC = SB
HAI	8,20	1.3590	0.235	NW = GC = WB = IH = AC = MH = STI = BC = SB

1-Way PERMANOVA: Sea Mullet

PERMANOVA detected a significant difference among zones for HSI. Sea mullet sampled at Baffle creek had lower HSI values than most other zones, while fish sampled from the Calliope Estuary also exhibited lower HSI than most other zones (see post hoc tests below).

PERMANOVA also detected a significant difference among zones for HAI. Sea mullet sampled from the Narrows (zone 1) exhibited greater HAI values than fish sampled from the Calliope estuary (zone 6) and Baffle creek (zone 15) (see post hoc tests below).

Comparison: the Narrows (NW), Western Basin (WB), Calliope Estuary (CR), Middle harbour (MH), South Trees Inlet (STI), and Baffle Creek (BC).				
Index	df	*F*	*P*	Outcome of *post-hoc* tests
GSI	5,17	1.1485	0.387	NW = WB = CR = MH = STI = BC
HSI	5,17	4.6430	**0.011**	NW = WB = MH = STI > CR > BC
Fulton's	5,17	1.2777	0.335	NW = WB = CR = MH = STI = BC
Eye asymmetry	5,17	1.0779	0.383	NW = WB = CR = MH = STI = BC
HAI	5,17	3.9527	**0.016**	WB = MH = STI = NW > CR = BC

Post-hoc tests for HSI

Zones	t	P(perm)	perms
1, 3	0.77857	0.505	10
1, 6	3.3265	0.012	84
1, 8	1.6864	0.189	10
1, 9	0.97612	0.485	10
1, 15	3.8841	0.015	56
3, 6	1.6188	0.117	84
3, 8	0.54767	0.891	10
3, 9	0.11479	0.898	10
3, 15	2.1918	0.035	56
6, 8	1.8817	0.092	84
6, 9	4.0738	0.015	84
6, 15	2.3089	0.027	410
8, 9	1.8765	0.216	10
8, 15	4.8081	0.018	56
9, 15	7.683	0.021	56

Post-hoc tests for HAI

Zones	t	P(perm)	perms
1, 3	1.1795	0.466	4
1, 6	3	0.044	11
1, 8	2.135	0.203	6
1, 9	3.4641	0.105	4
1, 15	3.2032	0.036	17
3, 6	1.341	0.291	13
3, 8	1.0142	0.479	6
3, 9	2.2942	0.095	4
3, 15	1.5341	0.209	11
6, 8	0.12685	1	11
6, 9	1.5275	0.219	6
6, 15	0.11457	1	12
8, 9	1	1	1
8, 15	0.056136	1	10
9, 15	2.0592	0.109	7

Appendix 6: Rapid Parasite Analysis

A total of 57 individual fish were analysed, of which 48 were found to contain parasites. It is difficult to identify parasites to species level without genetic testing, therefore for the purpose of analysis, parasites were classified into eight major taxa groupings (Table 1 and Figure 1). Parasites of the class Trematoda (714 individuals) were the most commonly recorded, followed by Monogenea (295 individuals) and Cestoda (235 individuals). Each site sampled presented varying taxa of parasites with not all taxa found at all sites (Figure 2). Parasite diversity varied with each site (Figure 2), with Trematoda found in fish at the most sites (*n* = 11 sites), whereas Acanthocephala were only found at two sites (Auckland Creek and Baffle Creek) (Figure 2). Similarly, the percentage prevalence of Trematoda dominated most sites (Figure 3).

Table 1. Total number of parasite individuals by taxa in all fish analysed

Taxa	Total number of individuals
Trematoda	714
Monogenea	295
Cestoda	235
Crustacea (copepoda)	57
Nematoda	27
Crustacea (other)	14
Acanthocephala	7
Other	60

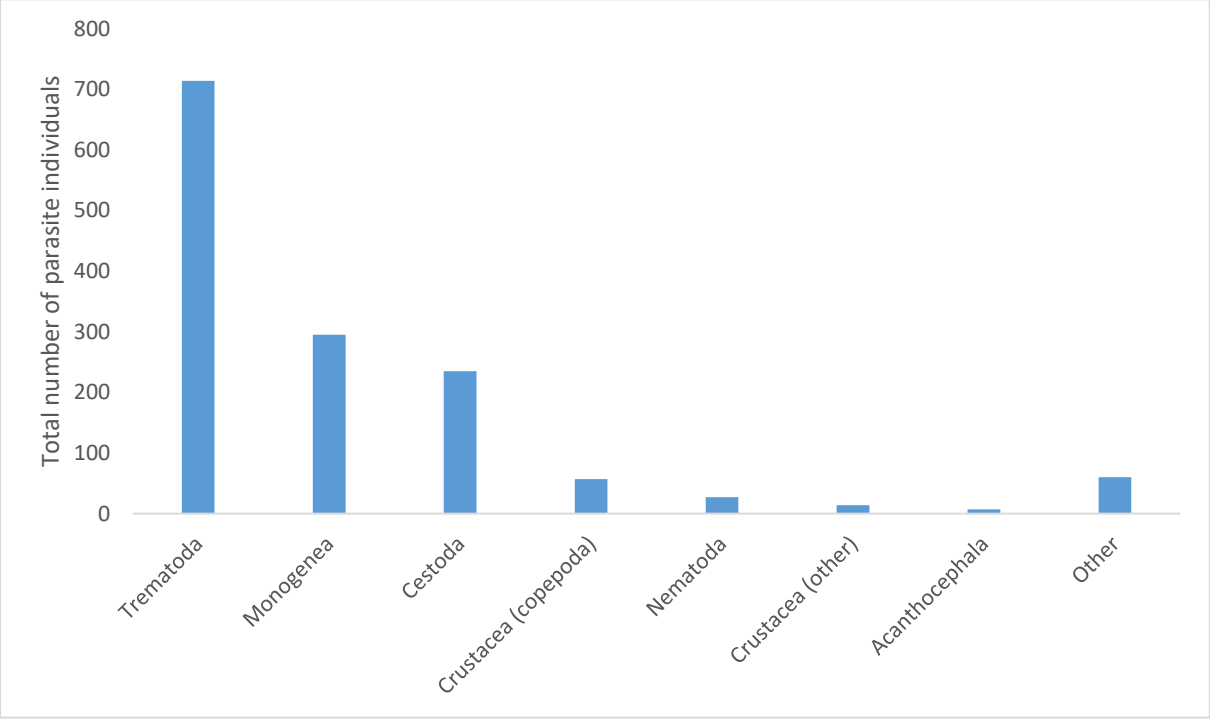
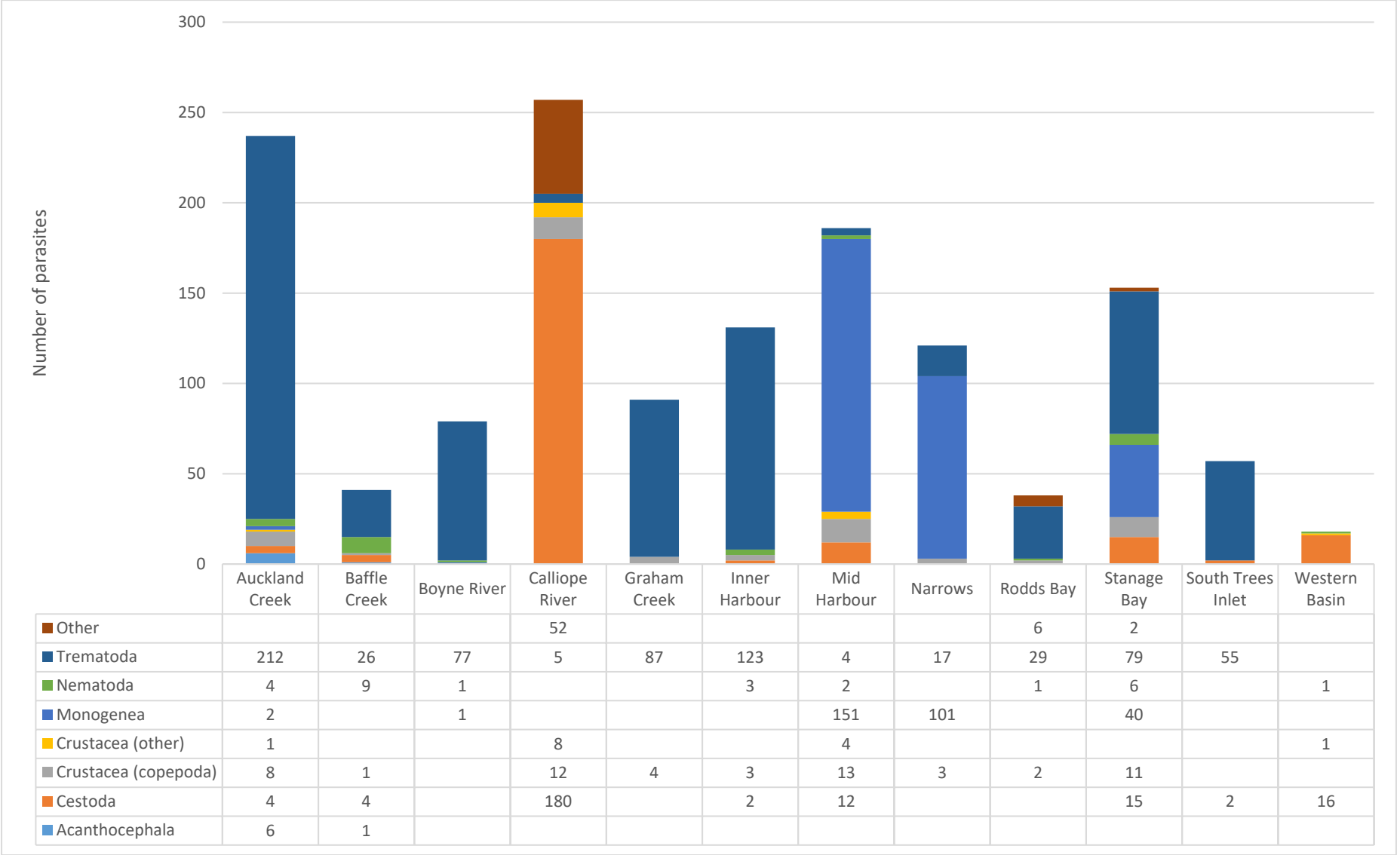


Figure 1. Number of parasite individuals counted in all fish analysed



	Auckland Creek	Baffle Creek	Boyne River	Calliope River	Graham Creek	Inner Harbour	Mid Harbour	Narrows	Rodds Bay	Stanage Bay	South Trees Inlet	Western Basin
Other				52					6	2		
Trematoda	212	26	77	5	87	123	4	17	29	79	55	
Nematoda	4	9	1			3	2		1	6		1
Monogenea	2		1				151	101		40		
Crustacea (other)	1			8			4					1
Crustacea (copepoda)	8	1		12	4	3	13	3	2	11		
Cestoda	4	4		180		2	12			15	2	16
Acanthocephala	6	1										

Figure 2. Number of parasites categorised by taxa found in fish at each site

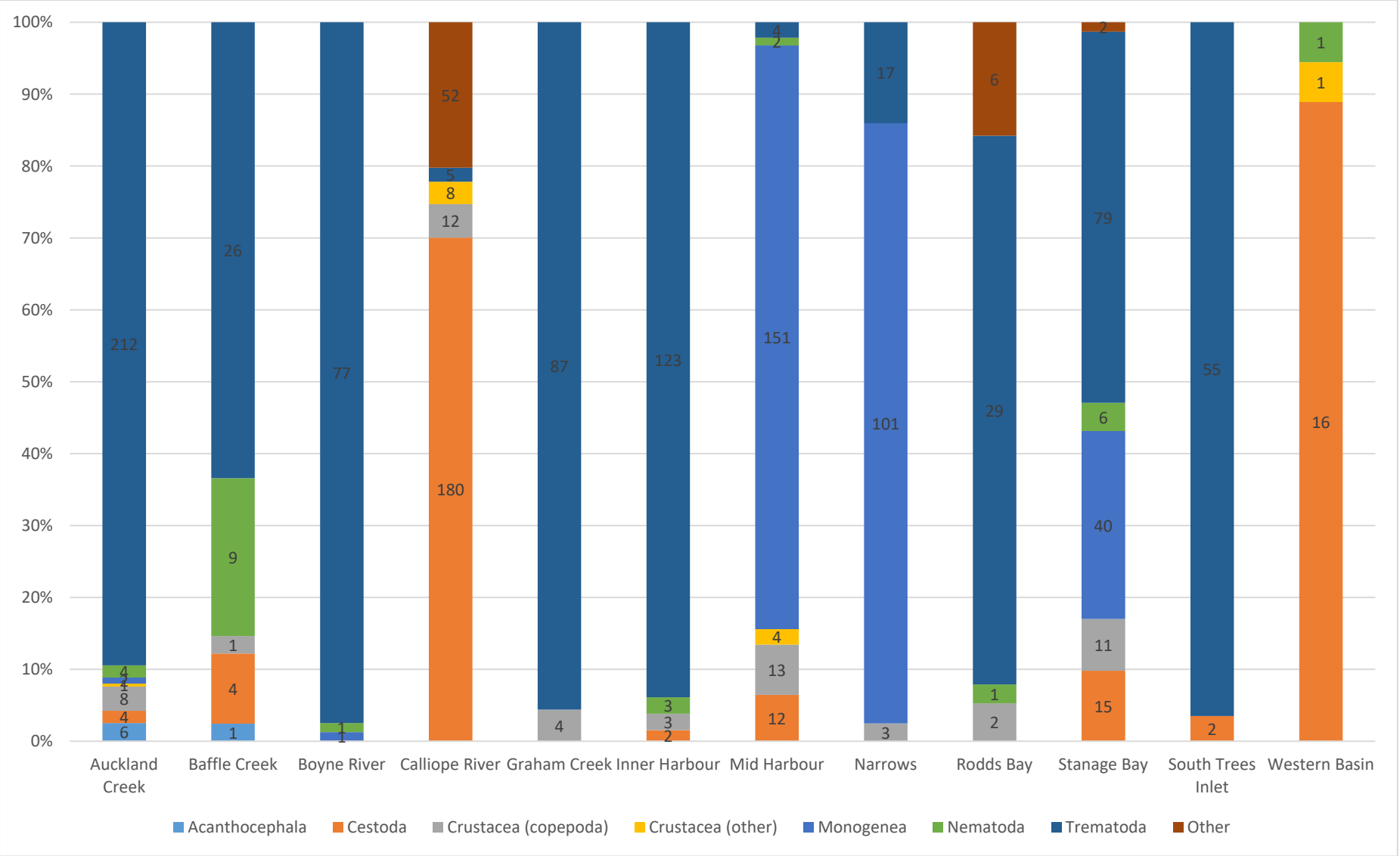


Figure 3. Percentage of parasite taxa per site

Zone 1 (the Narrows) had the highest average number of parasites per fish (40.3 ± 12.7; *n* = 3), whilst Reference site (Baffle Creek) had the lowest incidence of parasites (5.13 ± 2.58; *n* = 8) (Figure 4). All other sites were found to have varying incidence of parasites in fish (Figure 4).

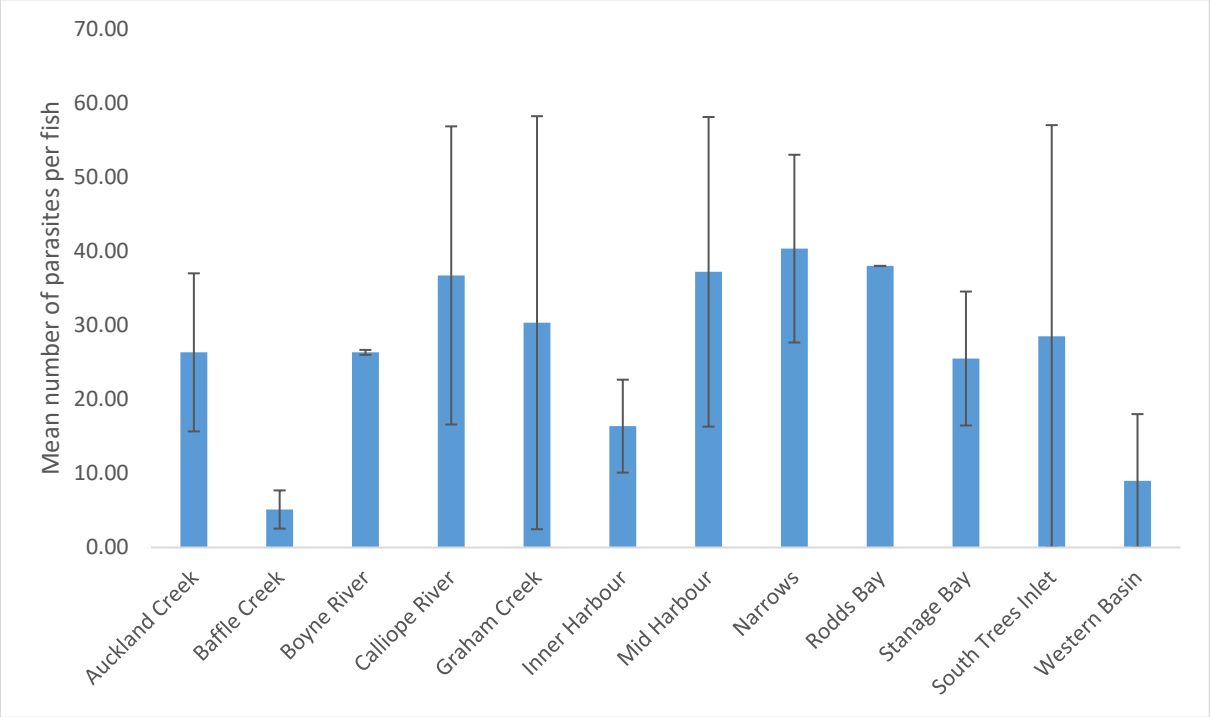


Figure 4. Mean number of parasites per fish at each of the sampled sites (error bars show ± standard error).

Of the four target fish taxa (barramundi *Lates calcarifer,* sea mullet *Mugil cephalus,* diamondscale mullet *Liza vaigiensis* and bream *Acanthopagrus* spp.), barramundi was caught at the most sampling sites and also found to contain parasites in the highest number of sites (n = 9; Figure 5). Of those nine sites, on average, Zone 2 (Graham Creek) had the highest number of parasites per barramundi (86.0 ± 0.00; *n* = 1 fish), whilst the Mid Harbour site had the lowest incidence of parasites per fish (1.00 ± 0.00; *n* = 1 fish) (Figure 5).

Sea mullet were caught at three sites, with Zone 6 (Calliope Estuary) recording the highest number of parasites per sea mullet (40.2 ± 23.5; *n* = 6 fish), whilst Reference site 2 (Baffle Creek) had the lowest (3.50 ± 1.44; *n* = 4 fish) (Figure 6).

Diamondscale mullet were caught at six sites. Of the six sites, on average, Zone 8 (Mid Harbour) had the highest number of parasites per mullet (57.3 ± 30.6; *n* = 3 fish), whilst Zone 2 (Graham Creek) had the lowest (5.00 ± 0.00; *n* = 1 fish) (Figure 7).

Bream were caught at two sites. No parasites were detected in the one pikey bream (*A. pacificus*) collected from Zone 3 (Western Basin), whilst 16 parasites were detected in the one yellowfin bream (*A. australis*) collected from Zone 6 (Calliope Estuary) (Figure 8).

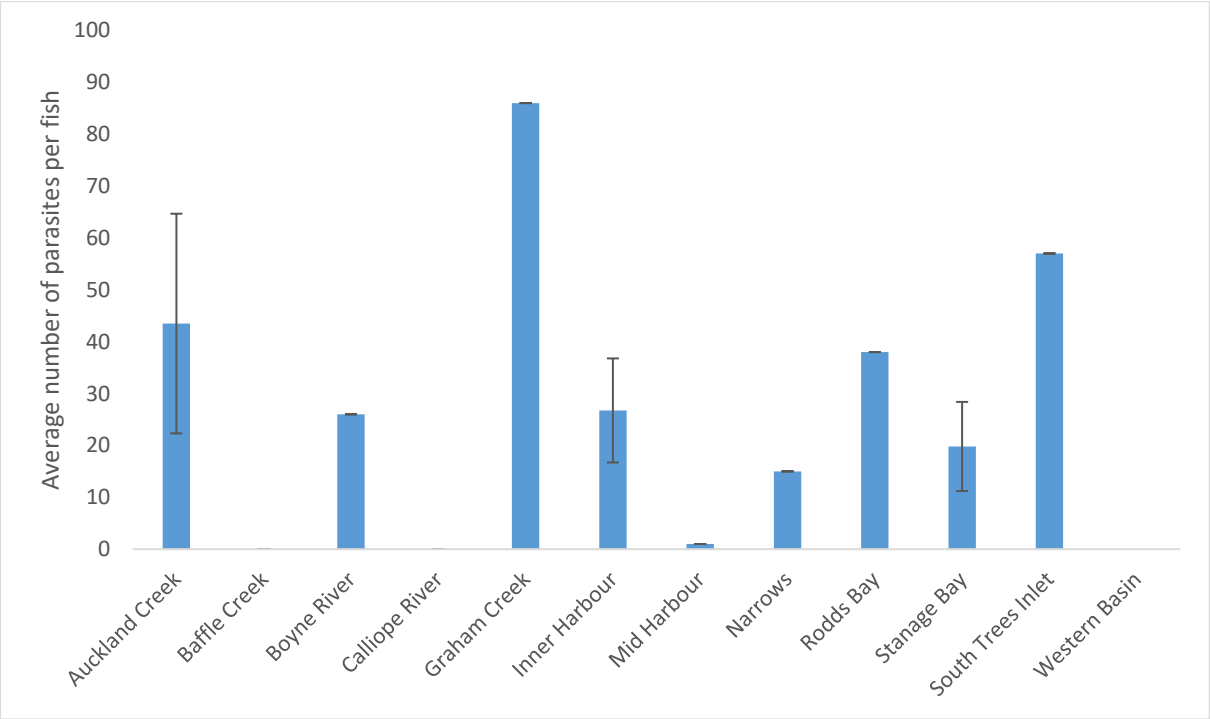


Figure 5. Average no of parasites per individual fish by site for *Lates calcarifer* (error bars show ± standard error).

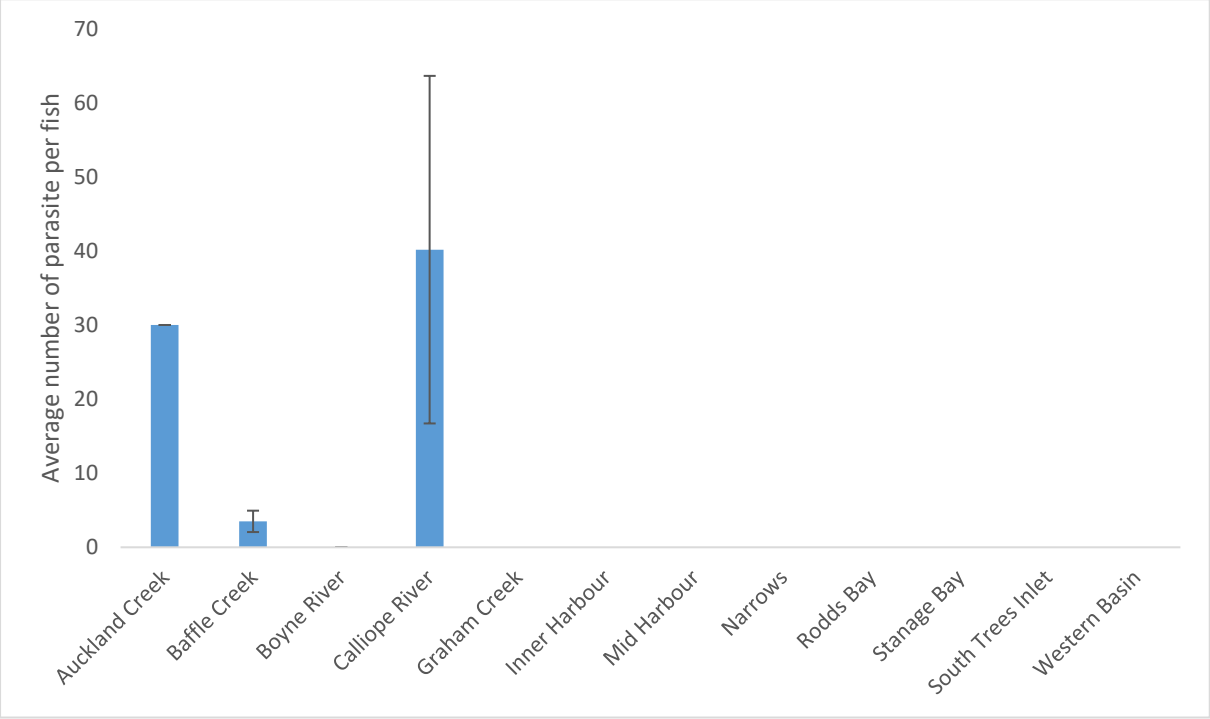


Figure 6. Average no of parasites per individual fish by site for *Mugil cephalus* (error bars show ± standard error).

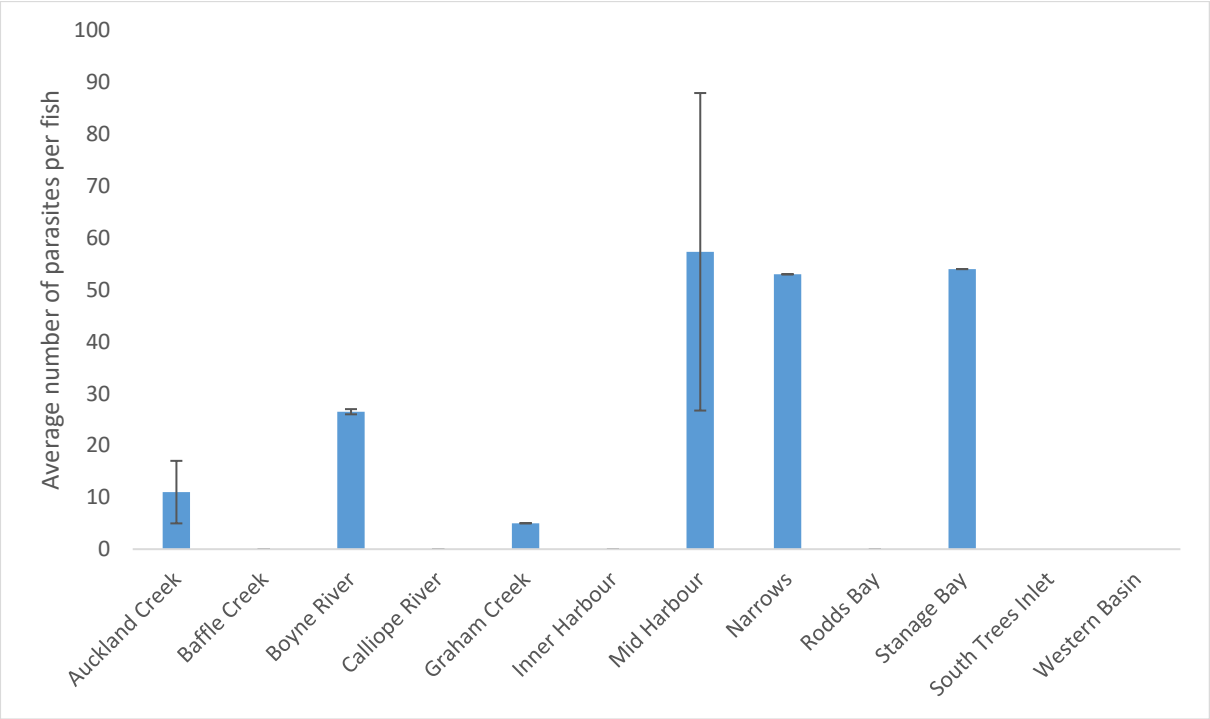


Figure 7. Average no of parasites per individual fish by site for *Liza vaigiensis* (error bars show ± standard error).

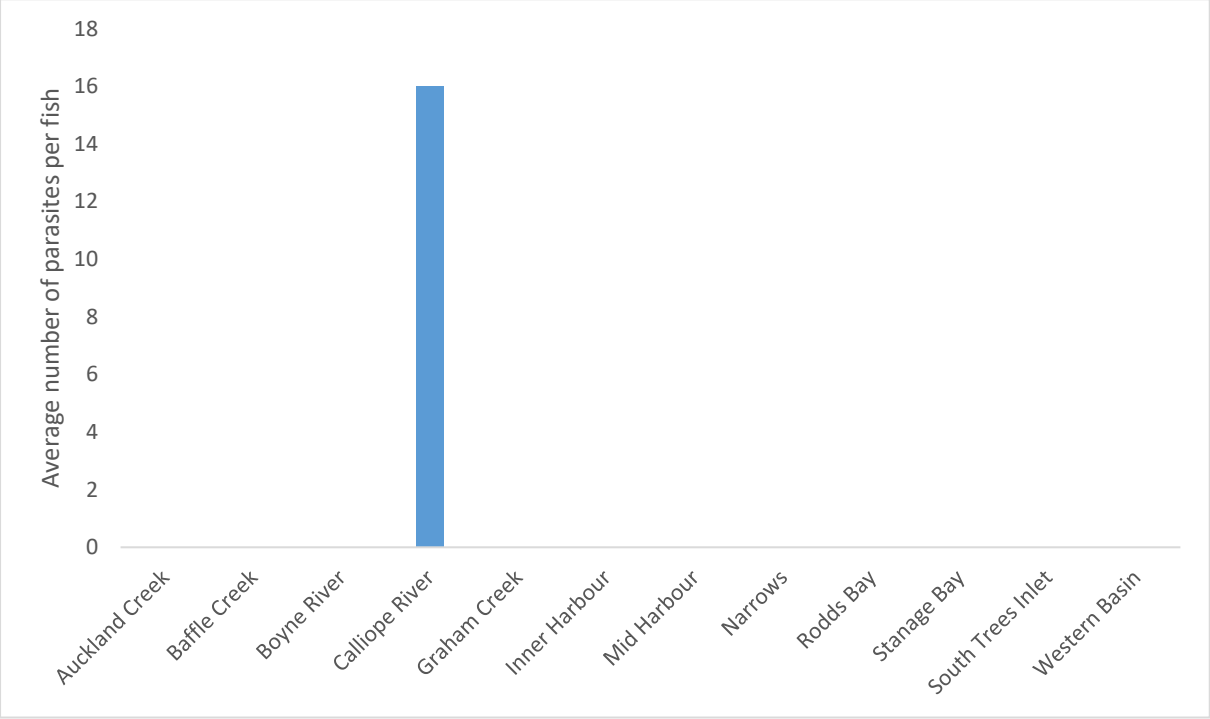


Figure 8. Average no of parasites per individual fish by site for *Acanthopagrus* spp. (error bars show ± standard error). No parasites were found in the *A. pacificus* caught at Mid Harbour.

Cestoda were detected in barramundi and sea mullet, but not in the other target fish taxa. Cestoda were detected in seven barramundi collected from five sites and in seven sea mullet from three sites (Table 2).

Table 2. Cestoda presence/absence in barramundi (*L. calcarifer*) and sea mullet (*M. cephalus*) by site

	Cestoda present/absent:	
Site:	***Lates calcarifer***	***Mugil cephalus***
Auckland Inlet	**Present**	**Present**
Baffle Creek	Absent	**Present**
Boyne Estuary	Absent	Absent
Calliope Estuary	Absent	**Present**
Graham Creek	Absent	Absent
Inner Harbour	**Present**	Absent
Mid Harbour	**Present**	Absent
The Narrows	Absent	Absent
Rodds Bay	Absent	Absent
Stanage Bay	**Present**	Absent
South Trees Inlet	**Present**	Absent
Western Basin	Absent	Absent

Overall parasite diversity varied between species and sites (Figures 9 – 12). Bream were found to contain two taxa of parasite (Figure 9), while barramundi and the two mullet species contained a maximum of four taxa of parasites (Figures 10 – 12).

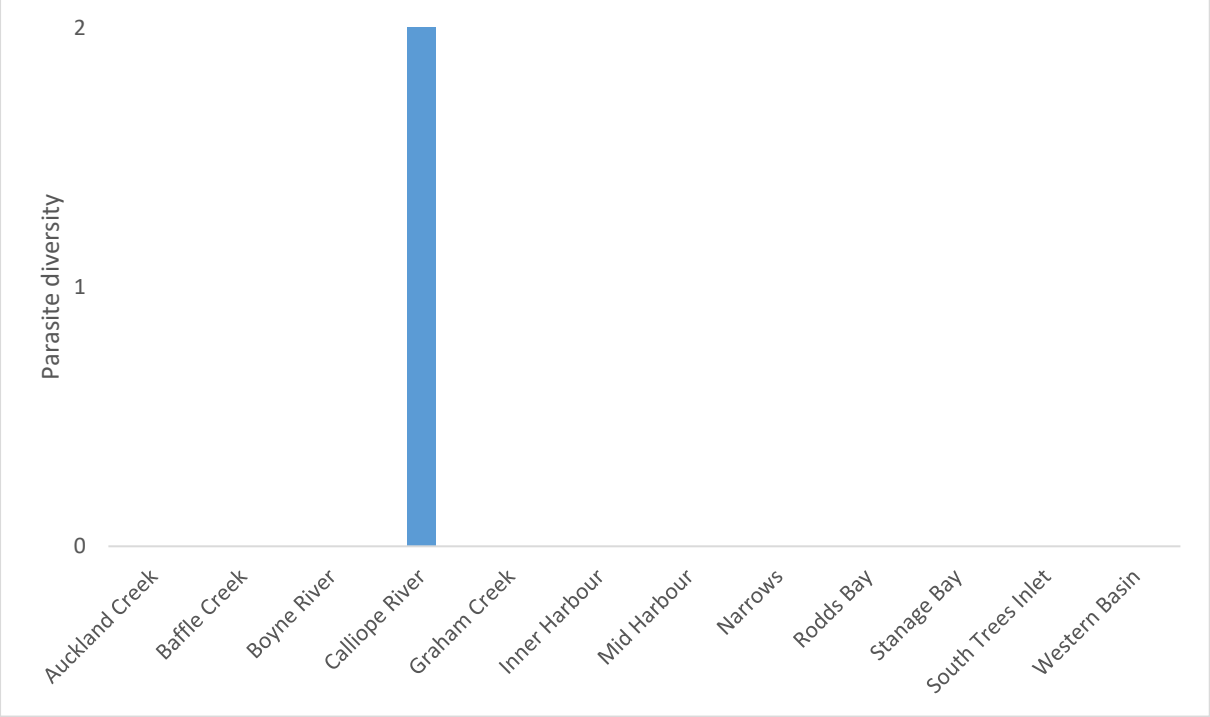


Figure 9. Parasite diversity in *Acanthopagrus* spp. Bar size represents the total parasite taxa types found per site. No parasites were found in the *A. pacificus* caught at Mid Harbour.

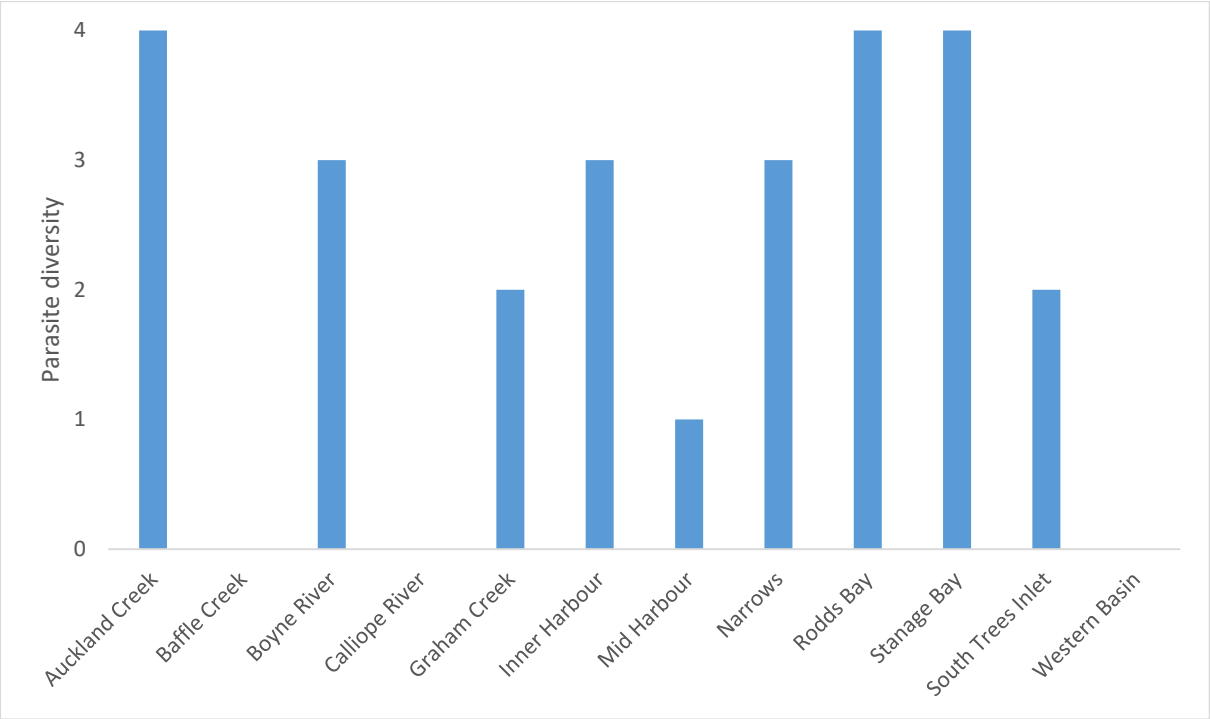


Figure 10. Parasite diversity in *L. calcarifer*. Bar size represents the total parasite taxa types found per site.

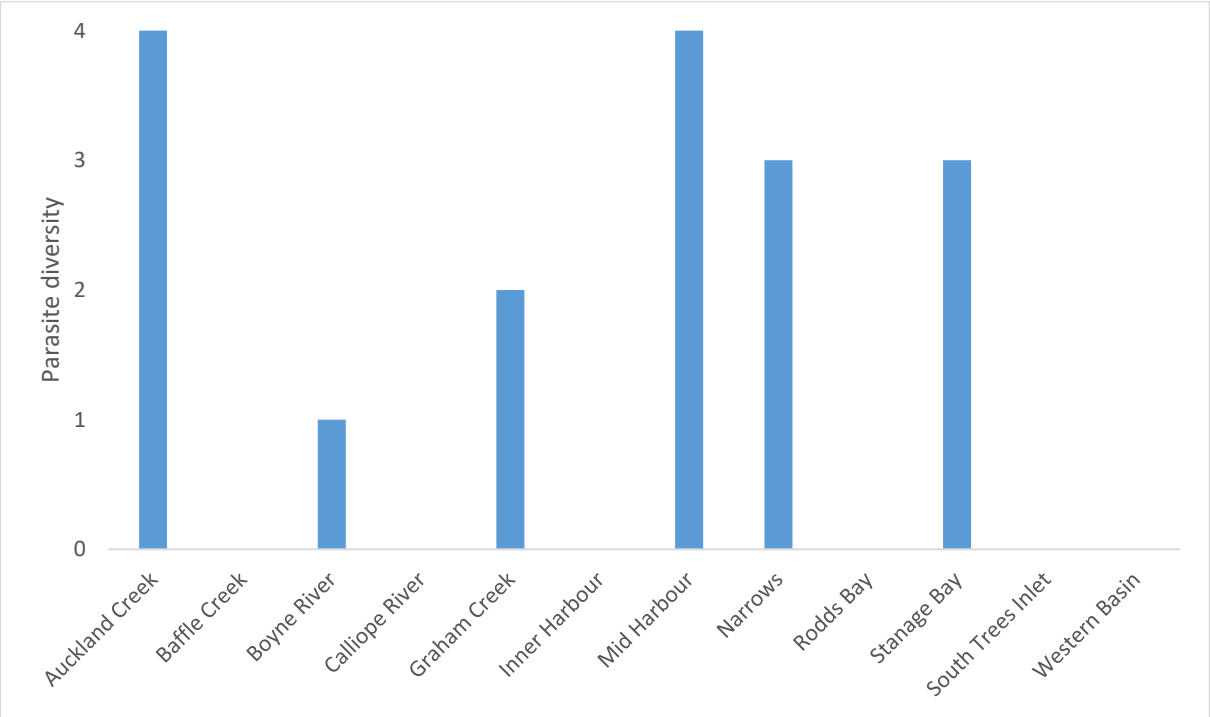


Figure 11. Parasite diversity in *L. vaigiensis*. Bar size represents the total parasite taxa types found per site.

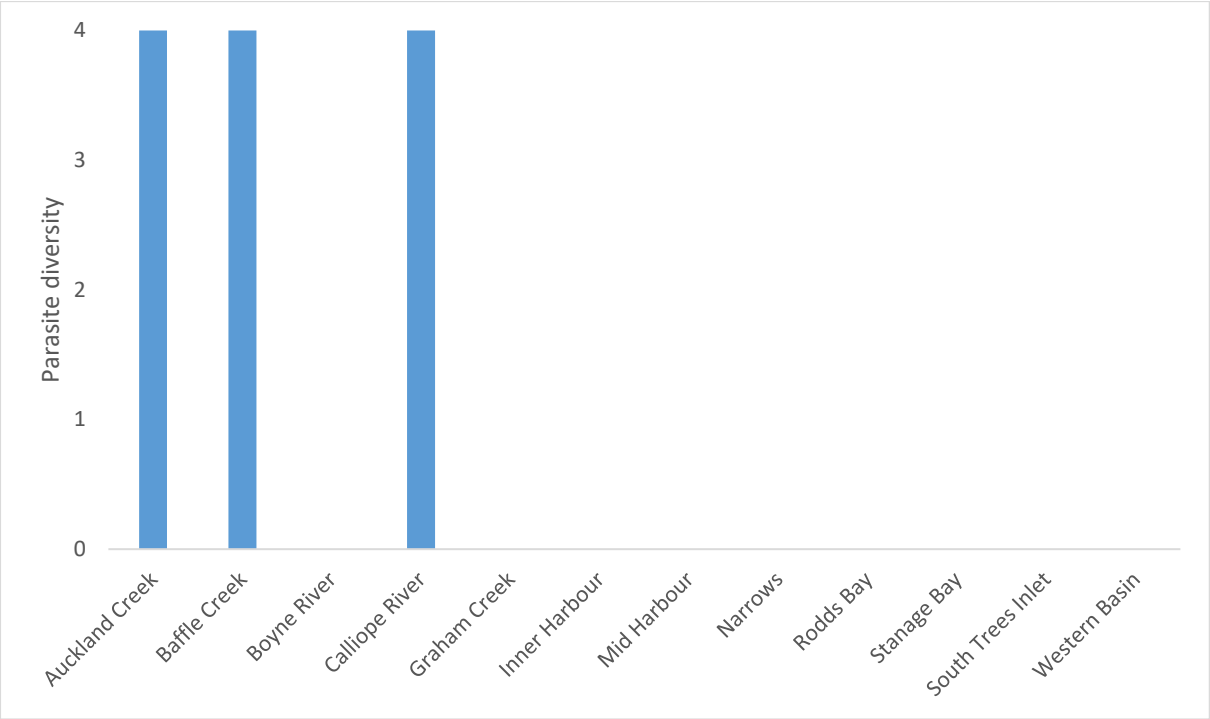


Figure 12. Parasite diversity in *M. cephalus*. Bar size represents the total parasite taxa types found per site.

Appendix 7: Fish Histopathology Summary Report

Central Queensland University (CQ University) – Gladstone Healthy Harbour Partnership (GHHP)

Fish Histopathology Summary Report

Abstract

A initial pilot study on the histopathology of several fish species sampled in April 2018 from Gladstone Harbour revealed a range of cellular changes in the gill, liver, pancreas, skin and muscle tissues sampled. Histopathology lesions included inflammation, degeneration, pigment accumulation, hyperplasia, granulomas, necrosis, metaplasia and neoplasia. Some of these changes are associated with parasites while most changes did not have an apparent infectious causation. A semi-quantitative assessment of the lesions provided a rate of lesions useful in comparing the severity of histopathology between individual fish, fish species, and sample locations, including fish sampled from the reference sites. A Relative Fish Health Index (RFHI) based on the histopathology lesion rates of the fish provides a tool to infer trends or patterns in fish health for the locations studied.

Aspects of the study can be improved in areas of sample collection and fixation of tissues to exclude post mortem degradation artifacts, inclusion of a broader range of organ and tissue types as well as selection of reference sites with fish that have even lower rates of histopathology. The usefulness of the RFHI can also be improved by increasing the numbers of fish examined per species and per location, especially in the context of a long-term multi-year study.

Introduction

Gladstone Harbour has a program for fish sampling and fish health indicators required for the Gladstone Harbour Report Card, as part of the Gladstone Health Harbour Partnership (GHHP) initiative. One of the projects in fish is conducted by the School of Health, Medical and Applied Sciences, CQ University (CQU), in which a pilot histopathology assessment of specific tissues is performed. Fish are sampled from various locations by CQU and histopathology processing and examination of tissues is conducted by Biosecurity Sciences Laboratory (BSL) of Biosecurity Queensland, Department of Agriculture & Fisheries. This report is a summary of the histopathology results obtained from the examination of fish tissues submitted by CQU.

Methods and materials

Fish were euthanased and tissue samples were collected either immediately post euthanasia on the sampling boat or several hours later with fish placed in ice and transported to a laboratory by CQU. 10% formalin preserved gill, liver (with embedded pancreas), skin and muscle tissues from barramundi (*Lates calcarifer*), pikey bream (*Acanthopagarus pacificus*), yellowfin bream (*A. australis*), sea mullet (*Mugil cephalus*) and diamond scale mullet (*Liza valgiensis*) were submitted to BSL. Information pertaining to the species of fish, tissue type, fish identification number, site and date of sampling were provided on the specimen advice sheet of BSL. A total of 46 fish were sampled in April 2018 and 135 tissue samples were submitted in May 2018 to BSL.

At BSL, tissues were processed by National Association of Testing Authorities (NATA) accredited histological methods. This involved (1) trimming of tissues into histology cassette tissue holders with sample specific identification numbering , (2) decalcification of tissues where required e.g. skin with scales, (3) processing in a series of alcohols to dehydrate the tissues, (4) embedding into paraffin wax, (5) microtome sectioning to 4 µm thin tissue sections, (6) placement on glass slides with sample specific identification numbering (7) processed for haematoxylin & eosin staining and (8) cover-slipped on the

same glass slides. Tissue slides were examined under light microscopy and tissue histological changes interpreted by a veterinary pathologist experienced in fish histopathology.

Results

Results of individual fish histopathology are detailed in the Appendix of 20 pathology reports. A summary of the key histopathology findings and qualitative interpretations are collated in Table 1. Photo-micrographs of the normal tissues and typical histopathological lesions are presented in Figures 1-30. A semi-quantitative analysis of the histopathological lesions is presented in the Excel file : CQU – Gladstone fish Histopathology Score Card.

Table 1. Key histopathology findings in GHHP fish survey – April 2018

BQ Job no./ CQU reference	Fish species, fish no. & location	Key histopathological changes and qualitative interpretation
P18-02259/ RB1BA	*Lates calcarifer* x1 Rodd's Bay, Gladstone	1. Gill fixation could be improved – sample and place gill samples in fixative as soon as fish is dead/euthanased. The aneurysms may reflect a toxic change or insult to the delicate gill lamellae or a sequel to rough handling of the fish during capture – given the limited distribution, the latter reason is more probable. A single monogenean fluke is incidental. 2. Muscle is generally normal, there is one incidental, probable traumatic (and chronically healed) lesion. 3. The liver has low level inflammation of hepatic veins. If more widespread, it suggests injury to the liver (perhaps irritating substances carried by and leaking from the blood vessels into the liver parenchyma). Some hepatocytes have accumulation of cytoplasmic pigment – suggesting that liver cells are processing metabolic or absorbed substances. The melanomacrophage centres in the liver are considered to be repositories of effete substances being metabolically recycled or stored. High numbers and darkly stained MMCs generally suggest physiologically stressed fish that live in contaminated environments. The MMCs level in this sample is low-moderate and light brown – indicating a lower level of metabolic stress. There is adequate lipid storage indicating an adequate plane of nutrition or feeding. 4. The pancreatic islets in the liver are normal. This is consistent with good nutritional condition of the fish. 5. There is no evidence of bacterial, viral, parasitic or fungal disease in the tissues examined.
P18-02260/ GC15BA	*L. calcarifer* x1 Graham Creek, Gladstone	1. Gill fixation could be improved – sample and place gill samples in fixative as soon as fish is dead/euthanased. There is a low level of parasitism (2 parasite types) in the gills which does not appear to be correlated to significant inflammatory changes in the filaments and a generalised mild to moderate hyperplastic response. This suggests that the gills are irritated by some other environment cause(s)/factor(s). 2. The liver significant level of inflammation affecting hepatic veins, some exocrine pancreatic islets and within the parenchyma amongst hepatocytes. This suggests injury to the liver (perhaps irritating substances carried by and leaking from the blood vessels into the liver parenchyma). Some hepatocytes have accumulation of cytoplasmic pigment – suggesting that liver cells are processing metabolic or absorbed substances. The melanomacrophage centres in the liver are considered to be repositories of effete substances being metabolically recycled or stored. High numbers

		and darkly stained MMCs generally suggest physiologically stressed fish that live in contaminated environments. The MMCs level in this sample is low and but the presence of melanin suggests a moderate level of metabolic stress. There is adequate lipid storage indicating an adequate plane of nutrition or feeding. 3. Muscle is normal. 4. There is no evidence of bacterial, viral or fungal disease in the tissues examined.
P18-02261/ IH1BA,IH3BA IH4BA,IH13BA	*L. calcarifer* x4– Inner Harbour, Gladstone	1. Gill fixation could be improved – sample and place gill samples in fixative as soon as fish is dead/euthanased. There is a low level of parasitism – mainly a trematode metazoan in the gills. There is marked inflammatory response (branchitis) in the gills, which maybe the fish recruiting these leucocytes (lymphocytes, other mononuclear leucocytes and some eosinophilic granulocytes (eosinophils) to the gills tissues in response or readiness for gill injury. Normally, a small number of inflammatory cells in the gills is expected, but the numerous numbers seen in all 4 fish suggests that the gills are irritated by some other environment cause(s)/factor(s) in this location of sampling. However, there is no gill necrosis, and this may mean that the irritants are absorbed through the gill interphase, into the internal tissue compartment via the blood vessels. 2. The liver had significant levels of inflammation affecting mainly the exocrine pancreatic islets, and less so the hepatic veins and within the parenchyma amongst hepatocytes. There is also cell apoptosis (programmed cell death to remove injured cells) within the hepatocyte parenchyma (possibly injured hepatocytes). The pancreatic inflammation is very severe/generalised in 1 or 4 fish, but more focally present in the other 3 fish livers. Because in each pancreatic islet, there is a blood vessel, this suggests injury to the liver perhaps by irritating substances carried by and leaking from the blood vessels into the liver parenchyma via the pancreatic islets. There is no apparent necrosis of the pancreatic islets, which if this occurred, will release digestive enzymes to the surrounding tissue, and would serve as an alternate explanation for the inflammation. Some hepatocytes have accumulation of cytoplasmic pigment – suggesting that liver cells are processing metabolic or absorbed substances. The melanomacrophage centres in the liver are considered to be repositories of effete substances being metabolically recycled or stored. High numbers and darkly stained MMCs generally suggest physiologically stressed fish that live in contaminated environments. The MMCs level in this sample is low and but the presence of melanin suggests a moderate level of metabolic stress. There is low to adequate lipid storage indicating a sub-optimal plane of nutrition or feeding in 3 of the 4 fish. Taken together, the inflammatory changes in both gill and liver tissues are unusual for this species of fish (we don't see these changes in cultured barramundi, unless there is an infectious agent involved e.g. bacteria or virus), and therefore suggest that the fish are being exposed to irritants or toxic compounds – absorbed through the gills and transported to the liver for metabolic processing. 3. Muscle is normal. There is no evidence of bacterial, viral or fungal disease in the tissues examined.
P18-02262/ NW4BA	*L. calcarifer* x1, The Narrows, Gladstone	1. The gills are essentially normal, with incidental presence of a trematode-like metazoan. 2. The liver has low level of inflammation affecting some exocrine pancreatic islets and a hepatic vessel. This suggests injury to the liver (perhaps irritating substances carried by and leaking from the blood vessels into the liver parenchyma). Some hepatocytes have

		accumulation of cytoplasmic pigment – suggesting that liver cells are processing metabolic or absorbed substances. The melanomacrophage centres in the liver are considered to be repositories of effete substances being metabolically recycled or stored. High numbers and darkly stained MMCs generally suggest physiologically stressed fish that live in contaminated environments. The MMCs level in this sample is low and but the presence of melanin suggests a moderate level of metabolic stress. There is reduced lipid storage indicating a reduced plane of nutrition or feeding. 3. Muscle has some degeneration and inflammation. 4. There is no evidence of bacterial, viral or fungal disease in the tissues examined.
P18-02263/ MH9BA	*L. calcarifer* x1, Mid-harbour, Gladstone	1. Gill fixation reasonable. There is an incidental level of parasitism – mainly a trematode metazoan in the gills. There is mild inflammatory response (branchitis) in the gills, and mild interlamellar epithelial hyperplasia, which is almost normal gill structure. The aneurysms are probably handling related. 2. The liver had apoptotic changes generally amongst the hepatocytes, suggesting an increased turnover of cells. This is the main change of concern, suggesting reduced hepatocyte health. The inflammatory changes of the pancreas and hepatocyte lobules are considered mild and focal. The melanomacrophage centres in the liver are considered to be repositories of effete substances being metabolically recycled or stored. High numbers and darkly stained MMCs generally suggest physiologically stressed fish that live in contaminated environments. The MMCs level in this sample is very low and suggests a low level of metabolic stress. There is low lipid storage indicating a reduced plane of nutrition or feeding in fish. 3. Muscle is normal. There is no evidence of bacterial, viral, parasitic or fungal disease in the tissues examined.
P18-02264/ AC5BA, AC6BA, AC7BA, AC14BA	*L. calcarifer* x4, Auckland Creek, Gladstone	1. The liver pathology of these barramundi are quite different from other barramundi examined. There is metaplastic, preneoplastic hepatoma and/or pancreatic fibromatous neoplastic change in the liver of 1 of 4 fish and metaplastic liver change in 3 of 4 fish. Focal hepatitis and/or hepatocellular apoptosis (programmed cell death) is noted in 4 of 4 fish. There are also parasitic granulomas, other granulomas (no observed pathogen) in the liver and pancreas in 4 of 4 fish. Pancreatitis is less severe than fish from other sites and occurred in 4 of 4 fish. To sum-up, there are several abnormal processes happening in the liver and the associated pancreatic islets that are more non-infectious (than infectious) in nature – i.e. the metaplasia, preneoplasia and neoplastic changes, the apoptosis of cells and non-pathogen associated inflammation. 2. There is generally a milder change to the gill inflammation and similar but low grade gill hyperplasia pathology compared to fish from other sites. 1 of 4 fish had significantly increased levels of trematode infection, but even that probably does not explain the generalised branchitis. 3. The nutritional condition of the fish as taken from the level of liver lipid vacuolation is considered as reduced or suboptimal. 4. Taken together, environmental (non-infectious) factors potentially altering the structure and function of the liver/pancreas and gills of the barramundi in this location remain to be defined. These fish are not healthy.
P18-02265/ STI14BA	*L. calcarifer* x1, South Trees Inlet, Gladstone	1. The main disease is that of a parasitic, granulomatous hepatitis – on the basis of some granulomas still with an encysted trematode-like metazoan. Inflammation and MMCs are associated with the

		parasitic disease. The fish liver did not have any pancreatic tissue – which could have been replaced by these multiple, extensive granulomas, however the nutritional plane is still sufficient – there is fat vacuolation storage in the hepatocytes. 2. The gills have a low level of branchitis and hyperplasia. 3. Muscle is normal, and skin dermis is normal.
P18-02266/ BR2BA	*L. calcarifer* x1, Boyne River, Gladstone	1. The gills have some changes of moderate inflammation and mild hyperplasia. Incidental (different type of) myxosporean encysted. 2. The liver has melanomacrophage centres, considered to be repositories of effete substances being metabolically recycled or stored. High numbers and darkly stained MMCs generally suggest physiologically stressed fish that live in contaminated environments. The MMCs level in this sample is moderate (relatively more and larger MMCs) and with melanin, which suggests a moderate level of metabolic stress. There is adequate lipid storage and adipose tissue around the liver, indicating an adequate plane of nutrition or feeding. 3. There is no evidence of bacterial, viral or fungal disease in the tissues examined.
P18-02268/ WB1PB	*Acanthopagrus pacificus* x1, Western Basin, Gladstone	1. The gills are autolyzed. Fixation as soon as the fish is euthanased is need to preserve gill integrity for proper histopathology. 2. There is significant pancreatitis (a pathology seen in barramundi from various sites in the harbour), but in pikey bream is mostly mediated by a different leucocyte type (the eosinophil) compared to the barramundi (the lymphocyte). Eosinophils usually attend to parasitic diseases, but no parasites are observed. The cause of this inflammation is unknown. 3. The liver has melanomacrophage centres, considered to be repositories of effete substances being metabolically recycled or stored. High numbers and darkly stained Melanomacrophage centres (MMCs) generally suggest physiologically stressed fish that live in contaminated environments. The MMCs level in this sample is low, which suggests are not under metabolic stress. There is adequate lipid storage around the pancreatic islets of the liver, indicating an adequate plane of nutrition or feeding. 4. There is no evidence of parasitic, bacterial, viral or fungal disease in the tissues examined.
P18-02269/ CR1YB	*A. australis* x1, Western Basin, Gladstone	1. The gills and liver tissues need to be preserved as soon as the fish is euthanased to prevent autolysis. 2. There is very low grade pancreatitis. 3. There is no evidence of bacterial, parasitic, viral or fungal disease in the tissues examined.
P18-02273/ SB3BA, SB4BA, SB5BA, SB7BA and SB8BA	*L. calcarifer* x5, Stanage Bay, near Rockhampton	1. There is no evidence of bacterial, viral or fungal disease in the tissues examined. 2. There could be a number of artifactual changes (not true pathology) in these samples. The main one is of enlarged, glassy looking hepatocytes in 4 of 5 fish and gill oedematous change in the gills of 1 of 5 fish. Attention to prompt fixation of these tissues as soon as the fish have died is important to exclude tissue artifacts that develop after death. 3. The gills are essentially normal with exception of some inflammation not related to the low level parasites there. 4. There is significant pancreas pathology – inflammatory and granulomatous reactions of 2 fish, inflammation without

		granulomas in 2 fish and 1 fish with absence of the pancreas in the liver. 5. There is significant liver pathology – focal hepatitis with some necrotic hepatocytes in 5/5 fish, a portal hepatitis with many granulomas in 1/5 fish. No existing pathogen is observed in the granulomas and they are chronic changes. There is also hepatocellular apoptosis (individual cells dying) and some of this apoptotic change may be related to fixation artifact. It is subtle, but present. 6. The levels of melanomacrophage centres (MMCs) are relatively low in 3 of 5 fish, and a little more prominent in 2 of 5 fish, suggesting some level of increased stressors for the fish. Couple that with a fairly ordinary level of lipid vacuolation of the liver, makes the nutritional status not that great. 7. This is a reference site, but the fish have a level of different pathologies – in some respects quite similar (or even more severe) to the study site barramundi, which makes the site difficult to use as comparison to the study site.
P18-02274/ CR3SM, CR11SM, CR12SM, CR13SM, CR14SM, CR15SM	*Mugil cephalus* x6, Calliope River, Gladstone	1. Some of the fish did not have proper fixation of the gills and/or liver. This should be avoided by immediate collection of the tissues as soon as the fish has died or been euthanased. 2. The gills have branchitis. There is no obvious pathogen-related inciting cause, and the interpretation is that these inflammatory cells have been primed to be at an increased level in the gill tissues for non-pathogen inciting cause(s). 3. Gill chloride cells are increased in number. These cells manage a high salt load in higher salinity water by enhancing the excretion of excess salts from the fish tissues through the gills. If the mullet have come into the river recently from the higher salinity environment, this is normal. But if the mullet are in a low salinity environment for quite some time, then this is could be abnormal. 4. Liver changes are relatively mild – a low grade inflammation of the portal areas, but the apoptosis of cells in the parenchyma suggests an increased turnover of hepatic cells in 4 fish with 2 fish not assessed due to inadequate liver fixation. 5. Overall, the nutritional status is adequate (lipid vacuolation in hepatocytes) and the levels of melanomacrophage centres (MMCs) are relatively mild, suggesting a low level of stressors to the fish. 6. There is no evidence of bacterial, parasitic, viral or fungal disease in the tissues examined.
P18-02275/ AC4SM	*M. cephalus* x1, Auckland Creek, Gladstone	1. The fish did not have proper fixation of the gills and/or liver. This should be avoided by immediate collection of the tissues as soon as the fish has died or been euthanased. 2. The gills have branchitis. There is no obvious pathogen-related inciting cause, and the interpretation is that these inflammatory cells have been primed to be at an increased level in the gill tissues for non-pathogen inciting cause(s). 3. Gill chloride cells are increased in number. These cells manage a high salt load in higher salinity water by enhancing the excretion of excess salts from the fish tissues through the gills. If the mullet have come into the river recently from the higher salinity environment, this is normal. But if the mullet are in a low salinity environment for quite some time, then this is could be abnormal. 4. There is an unusual fibrosis and hyperplasia of the bile duct connective tissues. Bile duct hyperplasia has been reported in fish

		that reside in polluted sites, if a pathogen cause has been excluded. Due to the suboptimal liver fixation, this cannot be confirmed. Future samples in this location and for this species is required.
P18-02276/ BC6SM,BC27SM,BC38SM, BC39SM,BC40SM	*M. cephalus* x5, Baffle Creek, near Bundaberg	1. The main pathology of concern although much milder than that of Gladstone fish in terms of numbers of lesions is the liver inflammatory foci around the blood vessels and in the liver parenchyma. The level of melanomacrophage centre (MMC) formation is deemed low, suggestive of a relatively benign level of stress on the fish and the liver nutritional level is adequate. 2. The liver cellular apoptosis may be a fixation artifact, given the information that fish could be dead for several hours before tissue sampling and fixation. This issue will need to be taken into account, when I synthesize all of the histological observations, so as to be able to report on true pathology, in the summary report for all the samples. It is important to emphasize that processing of fish should be : caught live fish kept alive, euthanased, then tissues sampled into formalin, all within a ½ hour per fish, so as to avoid any significant artifacts for histopathology assessment. 3. The gills show a lot of inflammatory cells suggestive of a severe 'resident' branchitis, that is immune cells that have migrated there is readiness for a rapid immune response – but there is no apparent observable injury threat to the gill tissues. Lamellar structures are generally normal which suggest good water quality.
P18-02277/ NW3DM, NW1DM	*Liza valgiensis* x2, The Narrows, Gladstone	1. The gills show a lot of inflammatory cells suggestive of a 'resident' branchitis, that is immune cells that have migrated there is readiness for a rapid immune response – but there is no apparent observable injury threat to the gill tissues. Lamellar structures are generally normal which suggest good water quality. 2. Gill chloride cells are abundant in these fish – which is normal if they are in a high salinity area. 3. There is very low grade liver vasculitis and hepatitis, although the lipid storage is reduced and in 1 of 2 fish the level of MMCs is increased, suggesting some stress on the fish. 4. The liver cellular apoptosis may be a fixation artifact, given the information that fish could be dead for several hours before tissue sampling and fixation. This issue will need to be taken into account, when I synthesize all of the histological observations, so as to be able to report on true pathology, in the summary report for all the samples. It is important to emphasize that processing of fish should be : caught live fish kept alive, euthanased, then tissues sampled into formalin, all within a ½ hour per fish, so as to avoid any significant artifacts for histopathology assessment. 5. Muscle of 1 fish has a localised, incidental myxosporean encysted plasmodium.
P18-02278/ MH6DM, MH7DM, MH12DM	*L. valgiensis* x3, Mid Harbour, Gladstone	1. The liver pathology of significance in these mullet are the melanomacrophage centres (MMCs). They are more prominent, and along with hepatocytes accumulating similar brown effete material as the MMCs, suggest a level of stressors affecting the fish which may influence a low nutritional level with minimal liver fat storage. 2. Myxosporean infections at very low level occur in the gills and muscle – usually this group of parasites are tissue-specific, so it probably 2 different myxosporeans here in one fish. One exception is a fish with significant lamellar fusion, branchitis and pseudocysts formation – which are patchy and likely to be myxosporean parasitism related. There is also an incidental epitheliocystis infection in the gill of one fish.

		3. Of note, there is largely an absence of inflammatory changes in the pancreas, liver or blood vessels of the liver, seen in other fish in this region.
P18-02279/ AC9DM, AC11DM, AC12DM	*L. valgiensis* x3, Auckland Creek, Gladstone	1. Gill and liver fixation could be improved in 1 fish. 2. The main lesion of note in 1 fish is a neoplastic lesion affecting 1 gill filament. 3. The liver changes of an inflammatory nature are very mild and the level of melanomacrophage centres (MMCs) development is low grade – low stress on the fish, which is supported by the high lipid storage in the hepatocytes.
P18-02280/ BR6DM, BR16DM	*L. valgiensis* x2, Boyne River, Gladstone	1. Gill pathology of significance includes a generalised branchitis not associated with any pathogen and unrelated to the myxosporean in 1 fish or the epitheliocystis in another fish. Aneurysms of the gills may be related to water quality e.g. toxicants. Gill fixation can and should be improved – gills collected and preserved in formalin within ½ hour or less after fish has died. 2. Liver pathology of significance in only 1 fish with liver sample is inflammation of various liver structures, not related to any pathogen(s). 3. Of note, there is also inflammation of blood vessels in the muscle tissue of the fish with the liver inflammation.
P18-02281/ SB2DM	*L. valgiensis* x1, Stanage Bay, near Rockhampton	1. This one fish is largely normal with minor changes to the liver. Liver fixation could be improved by having the liver sample collected and preserved in formalin within ½ hour or less after fish has died.
P18-02282/ GC7DM	*L. valgiensis* x1, Graham Creek, Gladstone	1. This one fish has significant pathology in gills, liver, muscle and skin tissues. 2. With the exception of the liver myxosporean parasite, the inflammatory reactions in these tissues are quite severe and may be environmentally related (cause unknown) and probably fairly recent. 3. The inflammatory lesions in the liver and gills are a common theme across the fish sampled from the Gladstone harbour sites, with relatively milder expressions of these in the reference sites (although numbers of fish from the reference sites need to be higher for a more valid comparison).

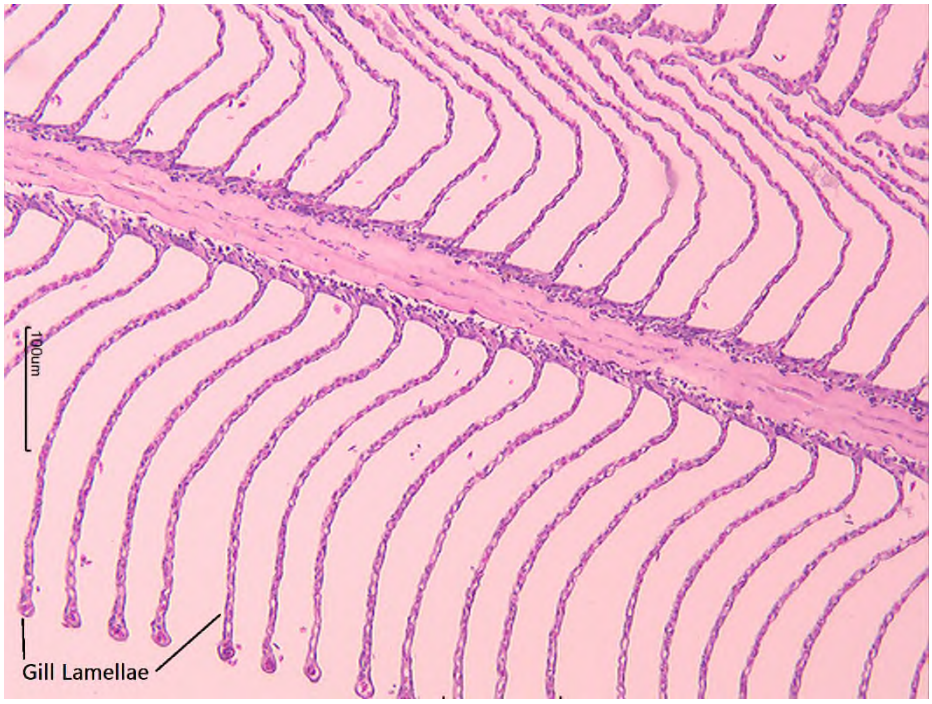


Fig. 1. P18-02281 Stanage Bay *L. valgiensis,* SB2DM. Gills - Haematoxylin & Eosin (H&E). Relatively normal portion of a gill filament showing the delicate gill lamellae.

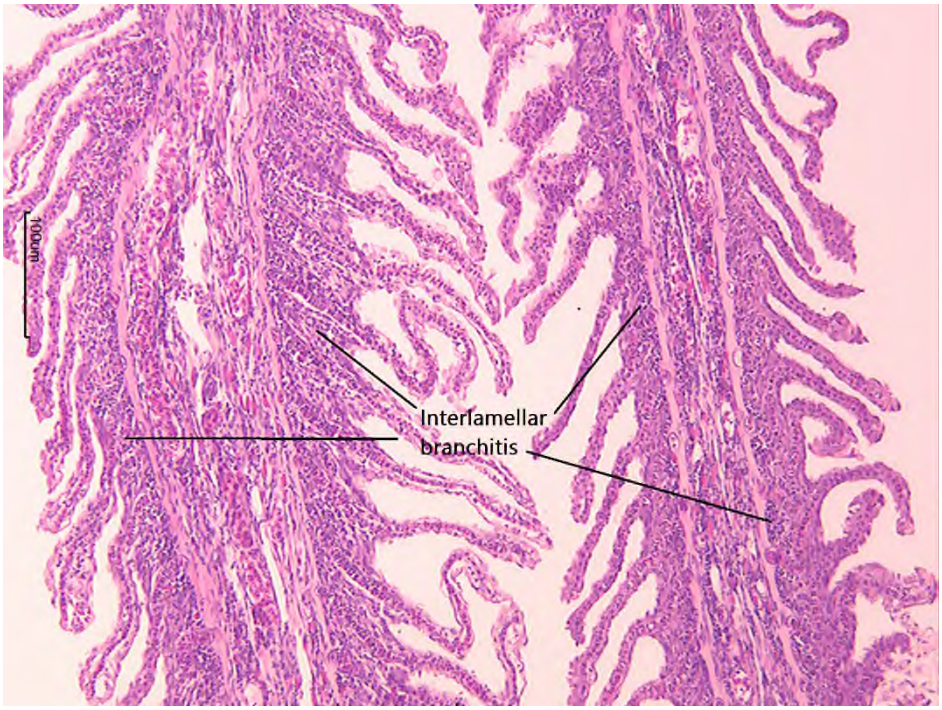


Fig. 2. P18-02261 Inner Harbour *L. calcarifer*, IH13BA. Gills – H&E. Two gill filaments showing severe interlamellar branchitis. Note the abundance of dark-staining and infiltrating cells which thickens the base of the lamellae and reduces the interlamellar space.

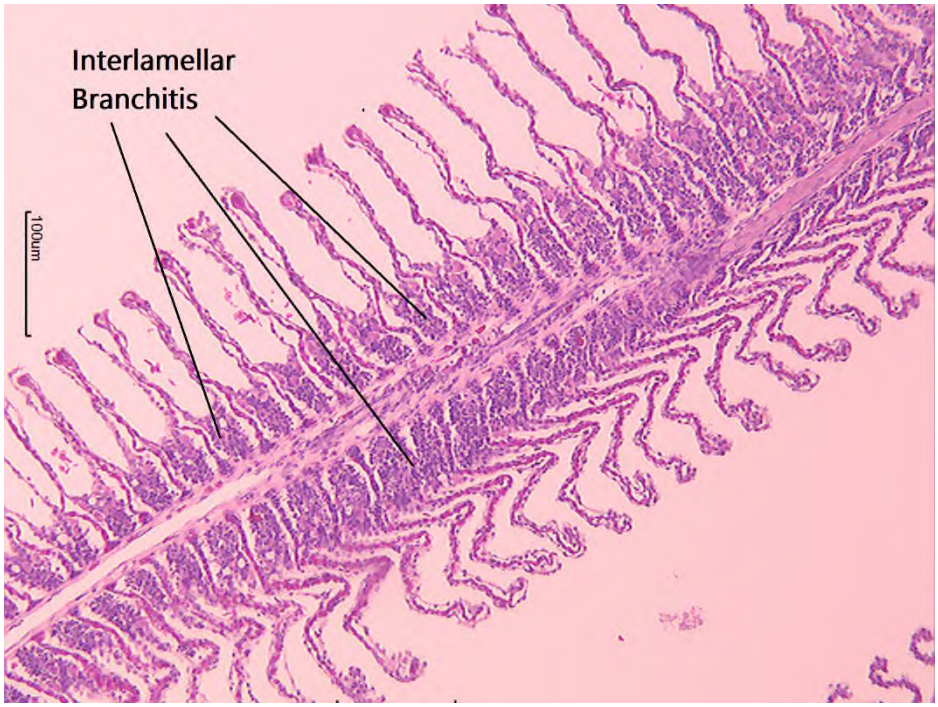


Fig. 3. P18-02274 Calliope River *M. cephalus* CR11SM. Gills – H&E. Moderately severe interlamellar branchitis with infiltration of inflammatory cells that have dark staining nuclei.

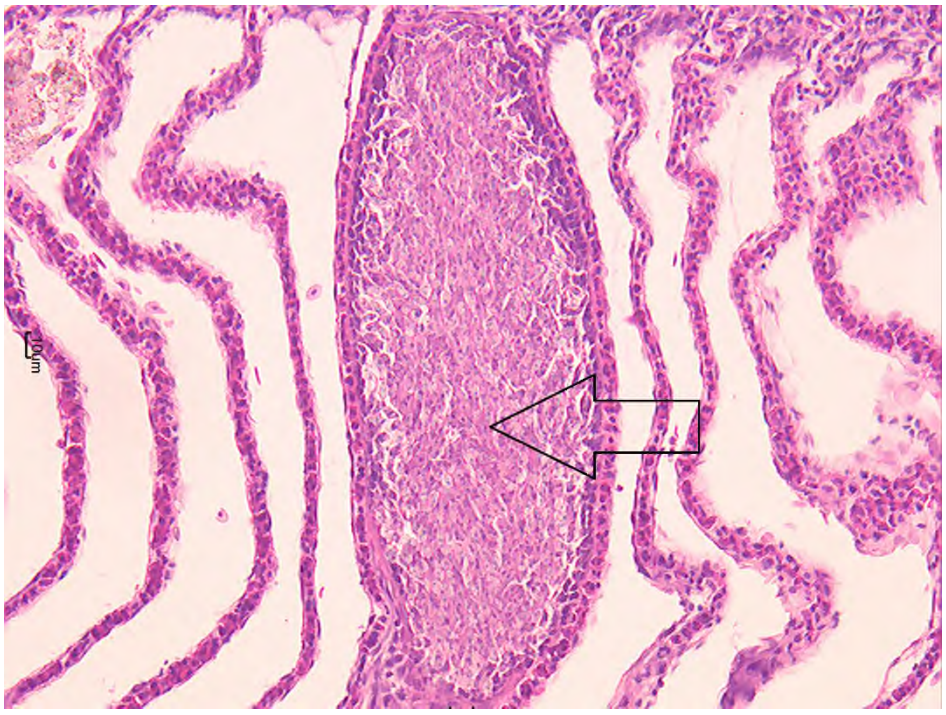


Fig. 4. P18-02264 Auckland Creek *L. calcarifer* AC5BA. Gills – H&E. A myxosporean plasmodium containing numerous *Henneguya-like* spores (arrow).

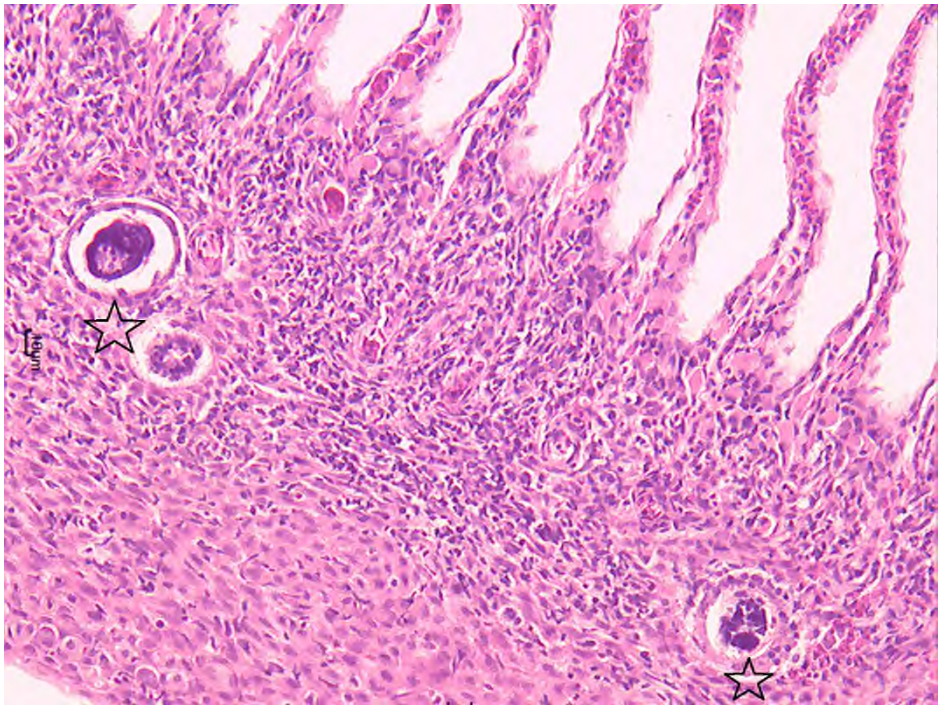


Fig. 5. P18-02264 Auckland Creek *L. calcarifer* AC5BA. Gills – H&E. Encysted trematode parasites (stars) in the gill epithelium.

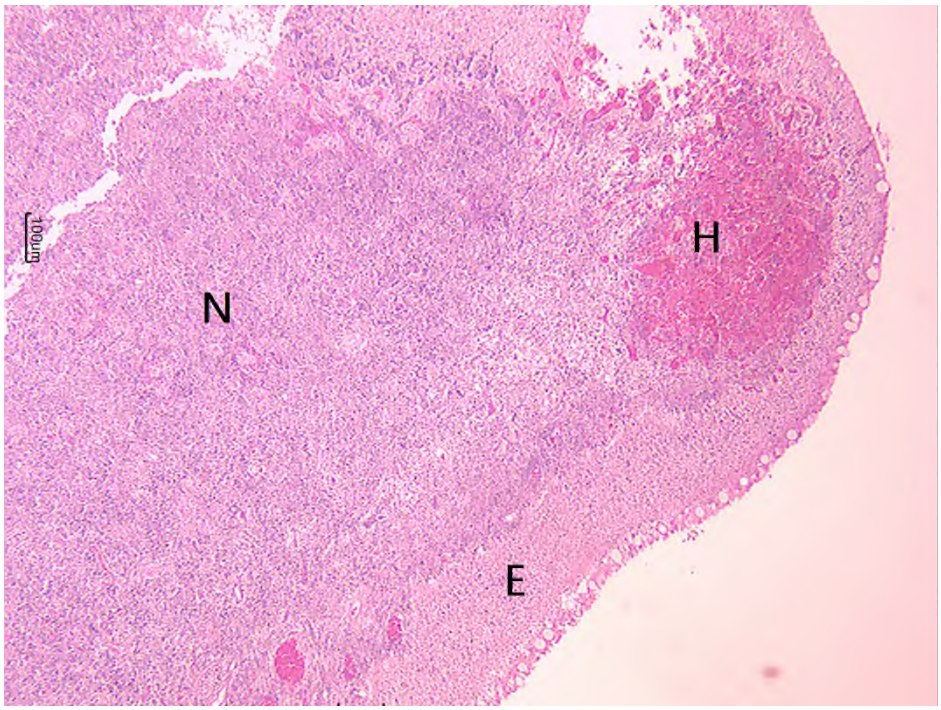


Fig. 6. P18-02279 Auckland Creek. *L. valgiensis* AC11DM. Gill – H&E. This severely enlarged distal portion of a gill filament has neoplastic cells (N), haemorrhaging (H) and an encapsulating thickened layer of epithelium (E).

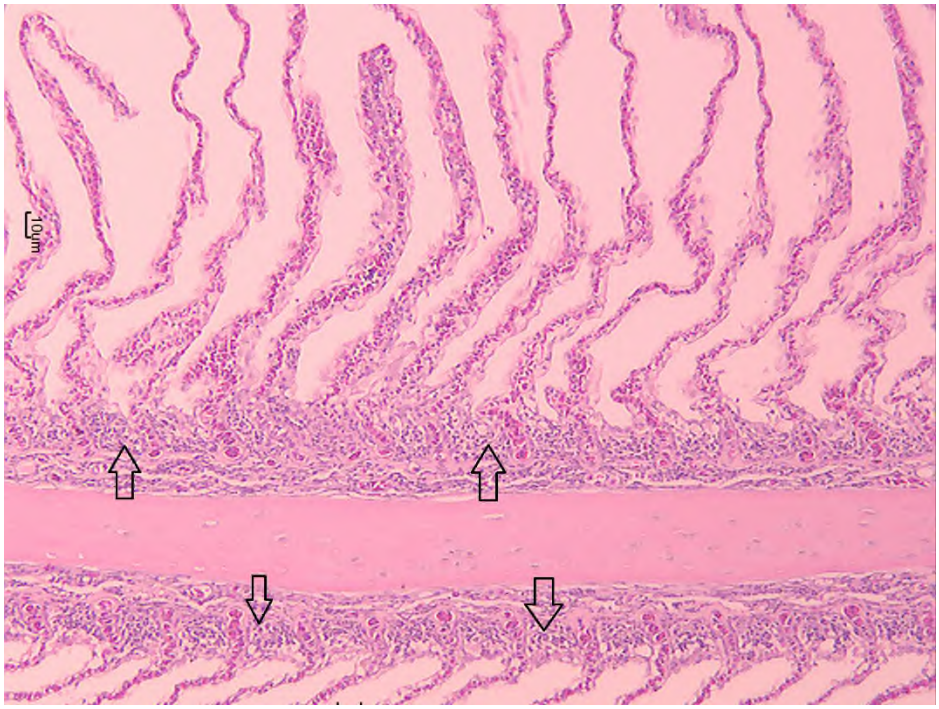


Fig. 7. P18-02266 Boyne River *L. calcarifer* BR2BA. Gills – H&E. Mild interlamellar epithelial hyperplasia (arrows) in a gill filament.

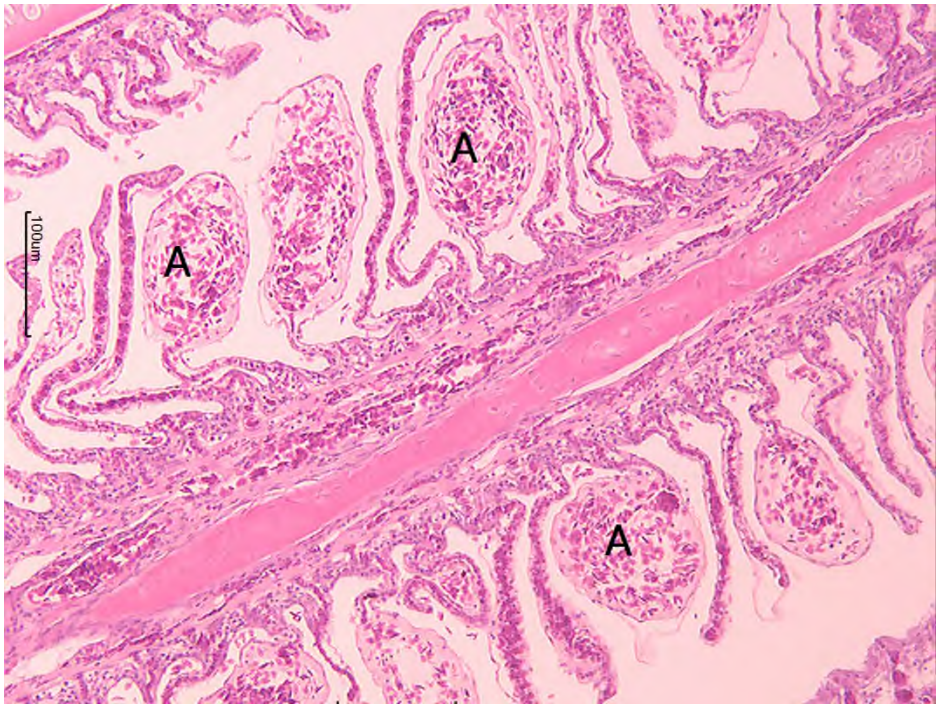


Fig. 8. P18-02273 Stanage Bay *L. calcarifer* SB8BA. Gills – H&E. Aneurysms (telangiectasis) affecting the lamellae (A).

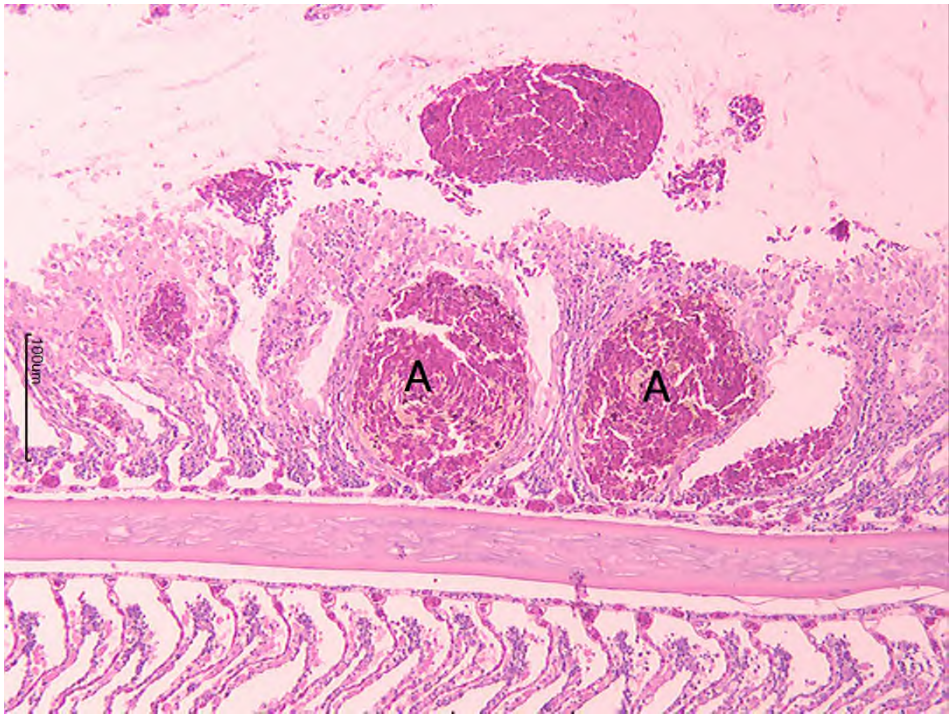


Fig. 9. P18-02280 Boyne River *L. valgiensis* BR6DM. Gills – H&E. Gill lamellae with aneurysm lesion (A).

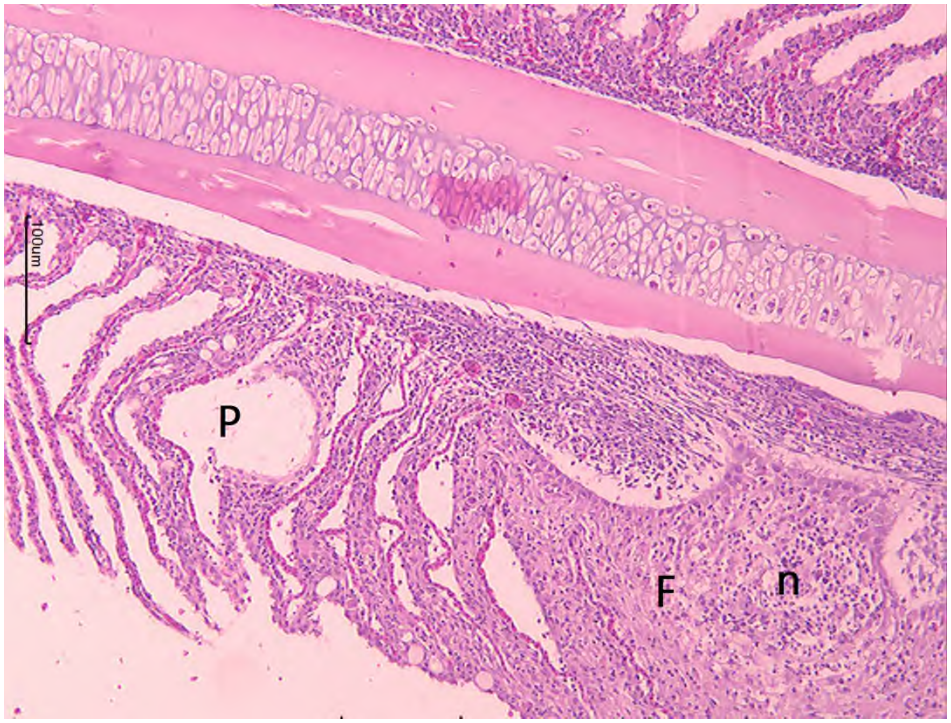


Fig. 10. P18-02278 Mid harbour *L. valgiensis* MH12DM. Gills – H&E. There is lamellar fusion (F), formation of pseudocysts (P) and necrosis (n) in this gill filament section.

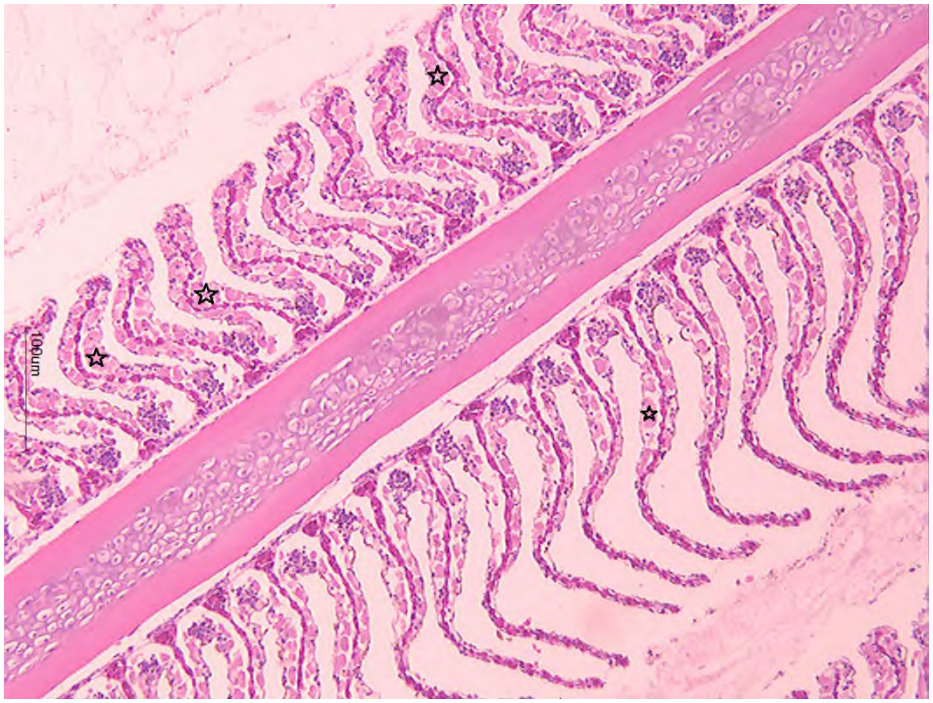


Fig. 11. P18-02277 The Narrows *M. cephalus* NW3DM. Gills – H&E. The gill lamellae have an over-abundance of eosinophilic cells which are the chloride cells (stars). Chloride cells are involved in osmotic regulation.

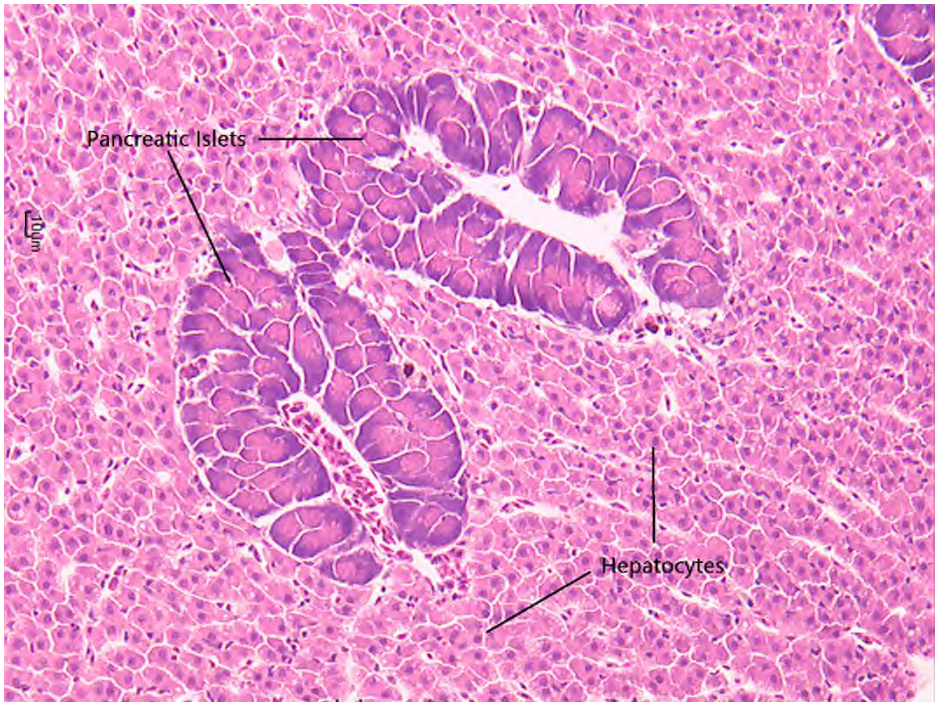


Fig. 12. P18-02268 Western Basin *A. pacificus* WB1PB . Liver – H&E. Normal hepatocytes and normal intrahepatic pancreatic islets.

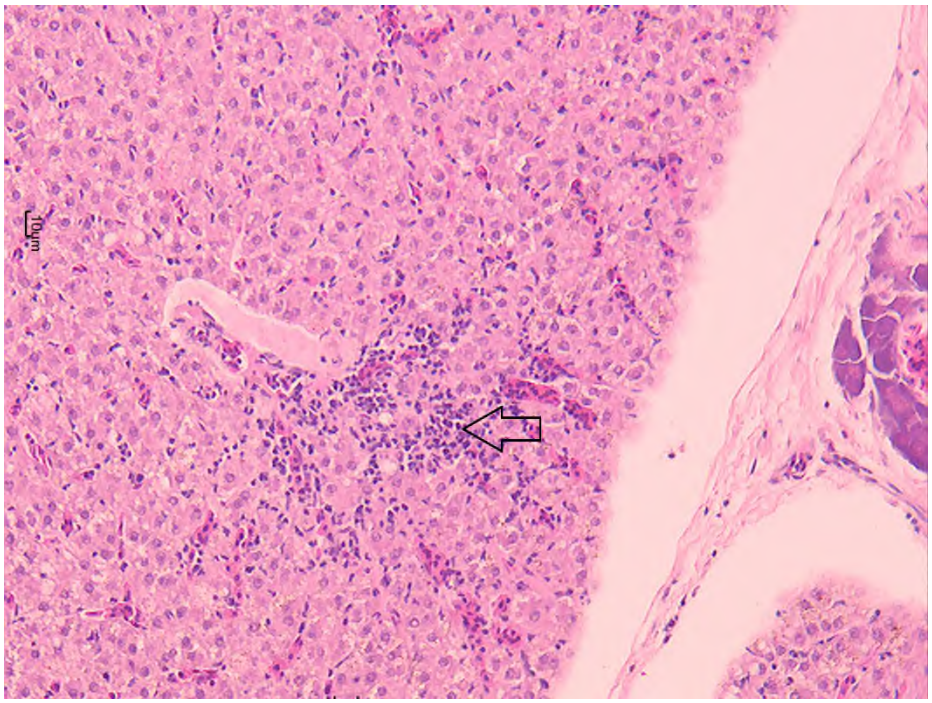


Fig. 13. P18-02260 Graham Creek *L. calcarifer* GC15BA. Liver – H&E. A focus of hepatitis due to infiltrating inflammatory cells (arrow).

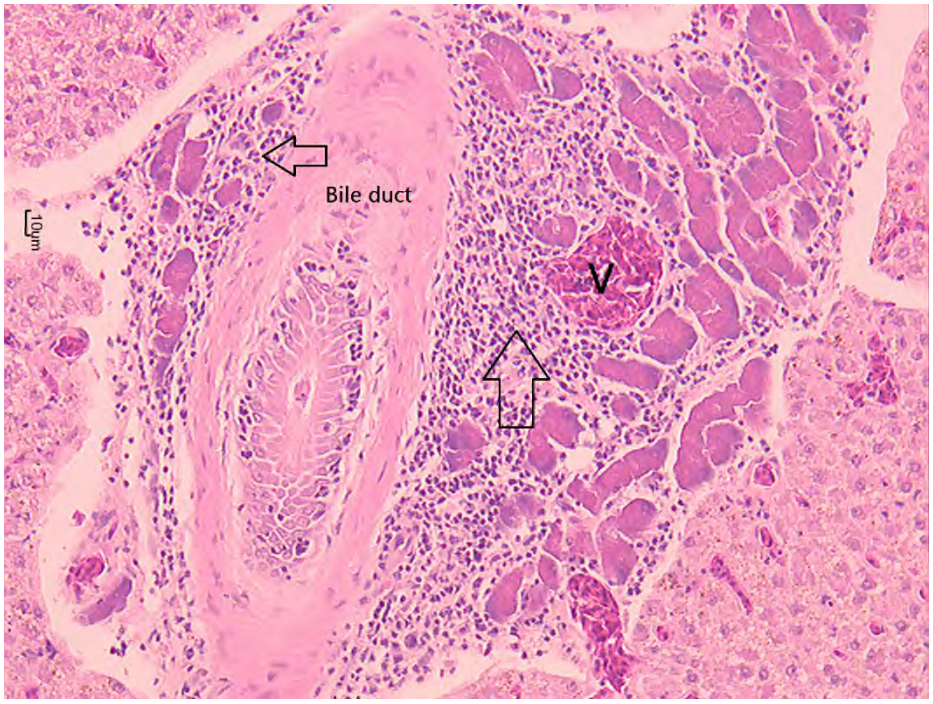


Fig. 14. P18-02262 The Narrows *L. calcarifer* NW4BA. Liver – H&E. Inflammation (arrows) of the pancreatic islet focused on the blood vessel (V). Infiltrating inflammatory cells also surround the bile duct. This lesion is a combination of a vasculitis and pancreatitis.

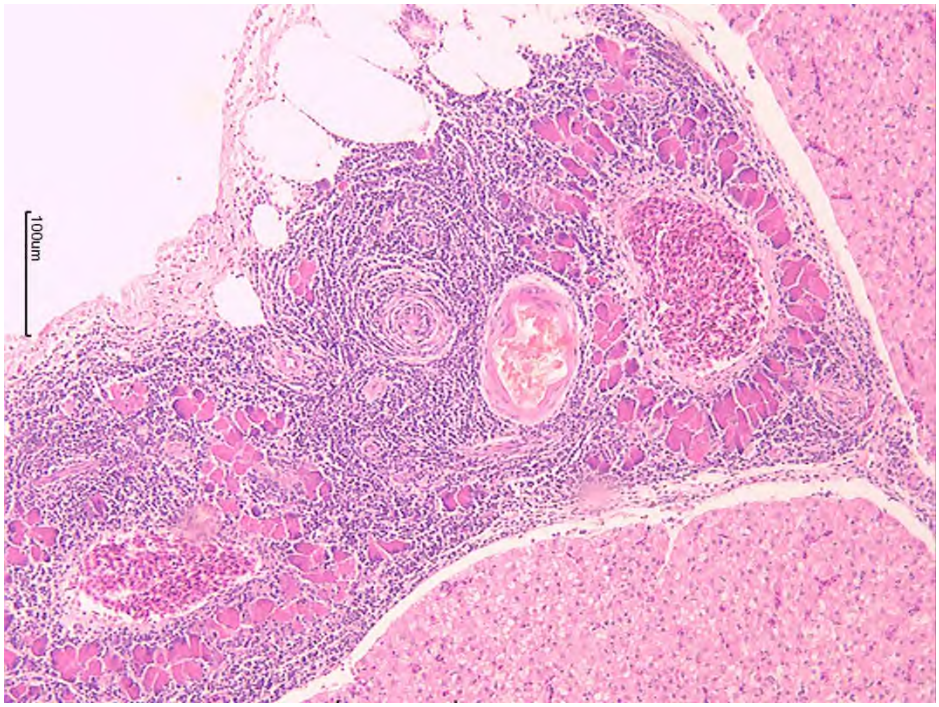


Fig. 15. P18-02261 Inner harbour *L. calcarifer* IH13BA. Liver – H&E. Severe multifocal pancreatitis.

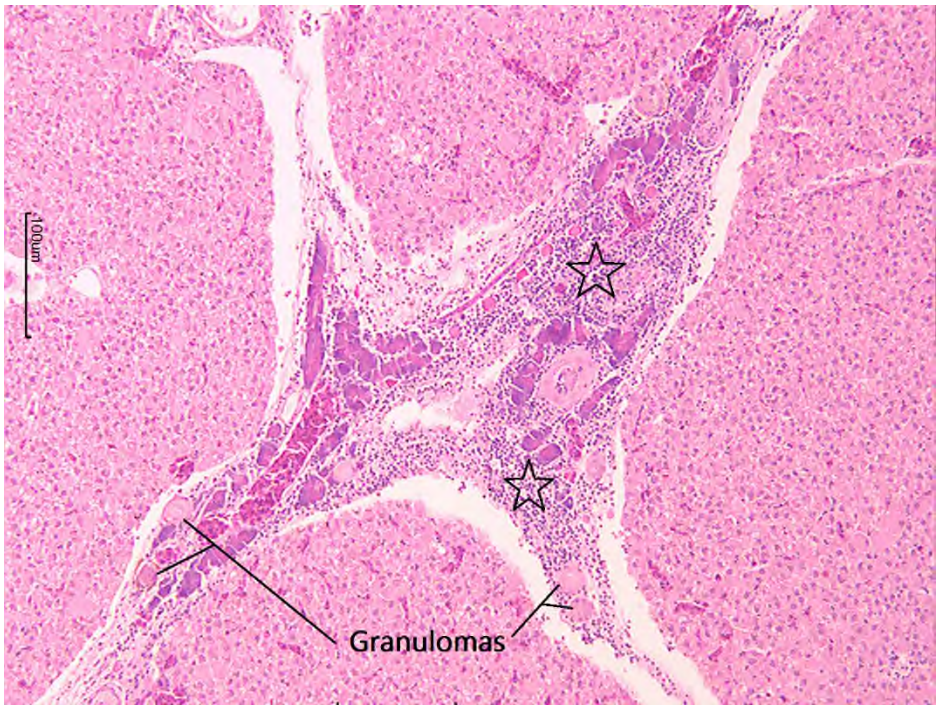


Fig. 16. P18-02273 Stanage Bay *L. calcarifer* SB3BA. Liver – H&E. Pancreatitis with loss of islet cells (stars) and also several granulomas in the inflammed pancreatic islet.

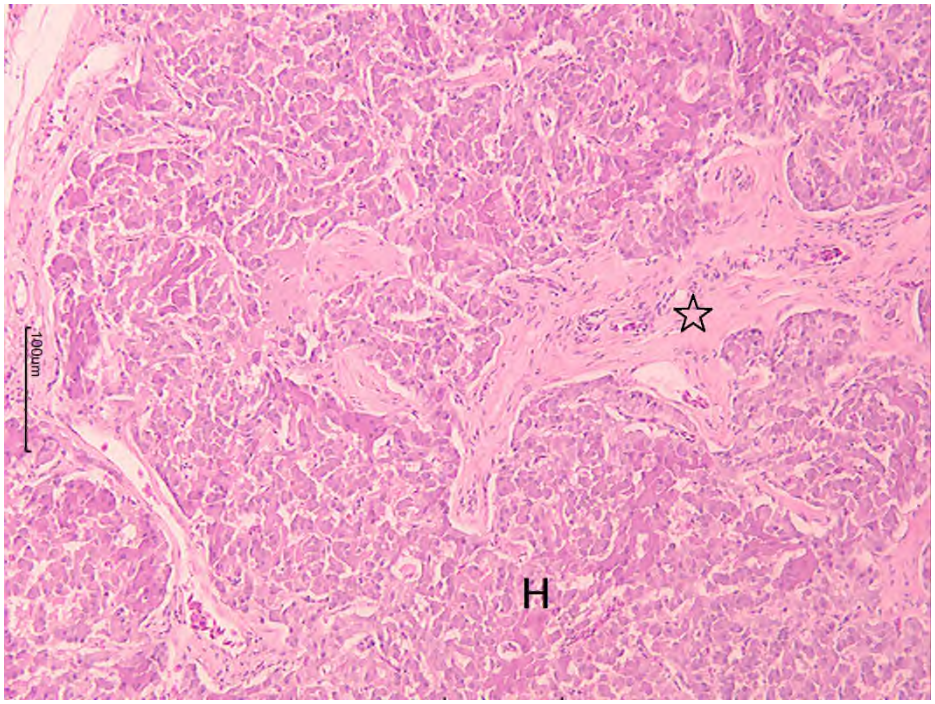


Fig. 17. P18-02264 Auckland Creek *L. calcarifer* AC5BA. Liver – H&E. Section of pleomorphic hepatocytes (H), loss of hepatic cord structure and fibrous trabeculae structures (star), a pre-neoplastic hepatoma-like change.

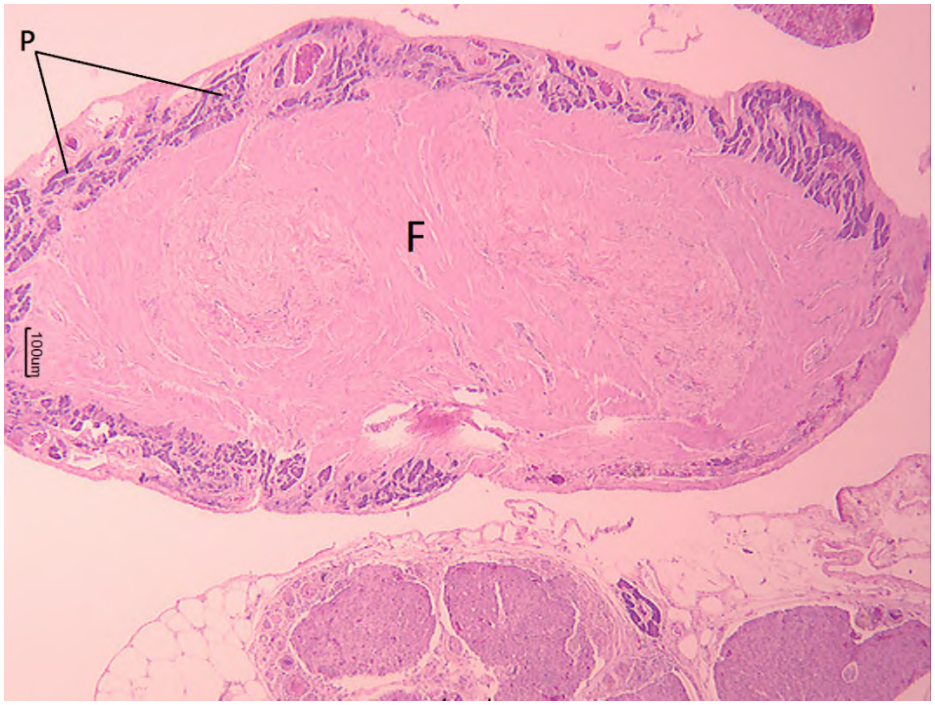


Fig. 18. P18-02264 Auckland Creek *L. calcarifer* AC5BA. Liver – H&E. A pancreatic fibroma (F) with a rim of pancreatic islet tissue (P).

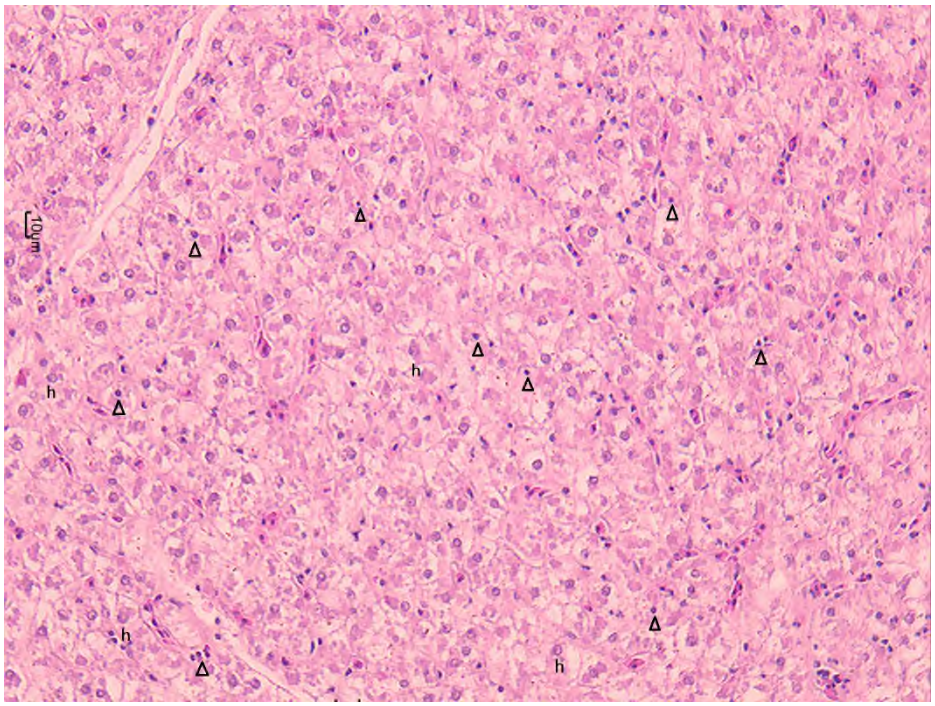


Fig. 19. P18-02263 Mid harbour *L. calcarifer* MH9BA. Liver – H&E. Apoptosis of hepatocytes with nuclei of cells becoming small, dark and condensed (arrow heads), compared to normal hepatocytes (h).

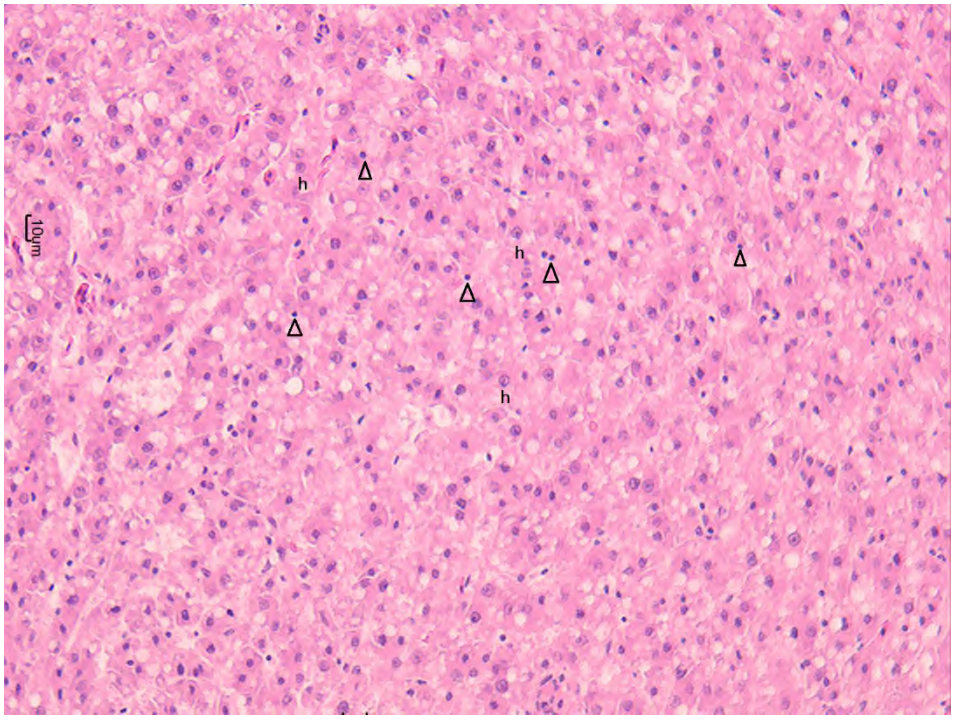


Fig. 20. P18-02274 Calliope River *M. cephalus* CR3SM. Liver – H&E. Apoptosis of hepatocytes with nuclei of cells becoming small, dark and condensed (arrow heads), compared to normal hepatocytes (h).

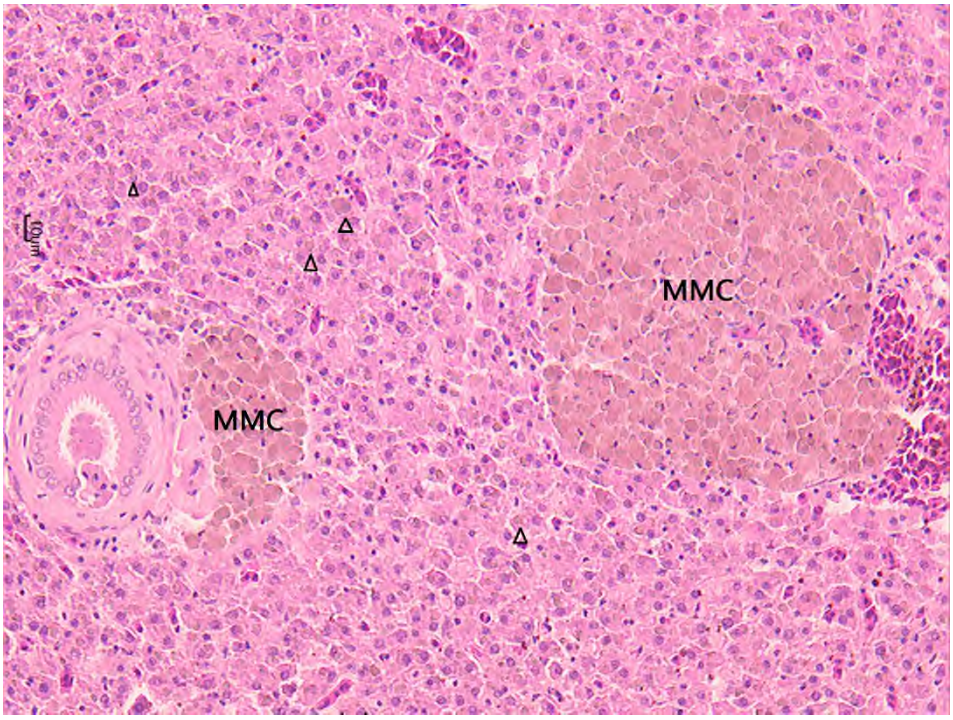


Fig. 21. P18-02278 Mid harbour *L. valgiensis* MH6DM. Liver – H&E. Note the melanomacrophage centres (MMCs) with brown pigment in melanomacrophage cells and also similar pigment in hepatocytes (arrow heads)

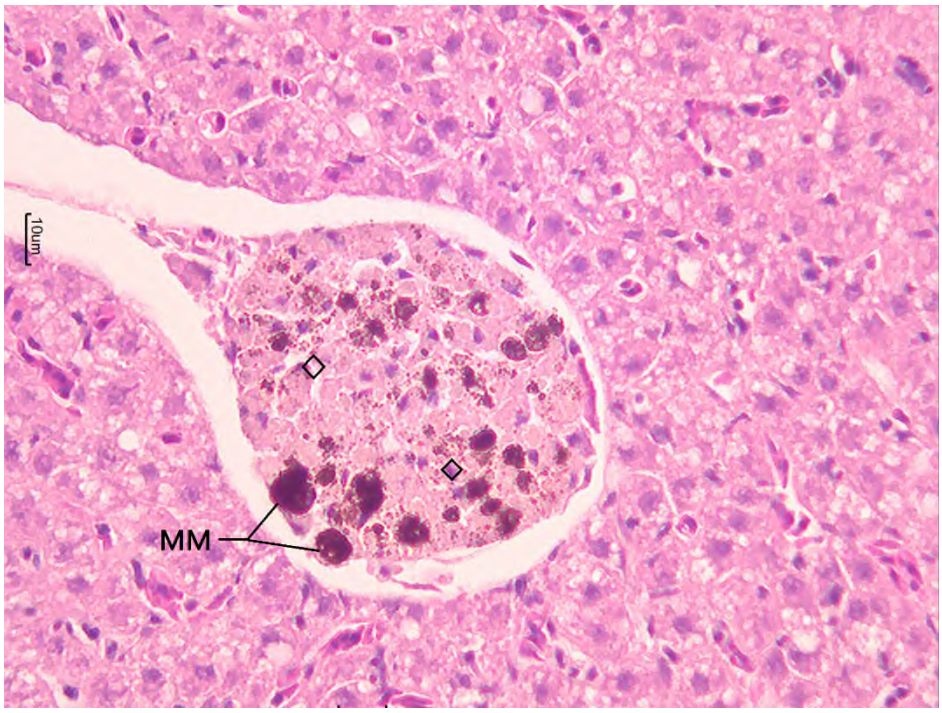


Fig. 22. P18-02260 Graham Creek *L. calcarifer* GC15BA. Liver – H&E. A melanomacrophage centre (MMC) with melanomacrophage cells (MM) possessing melanin granules, some melanin is dispersed (diamond) in the MMC.

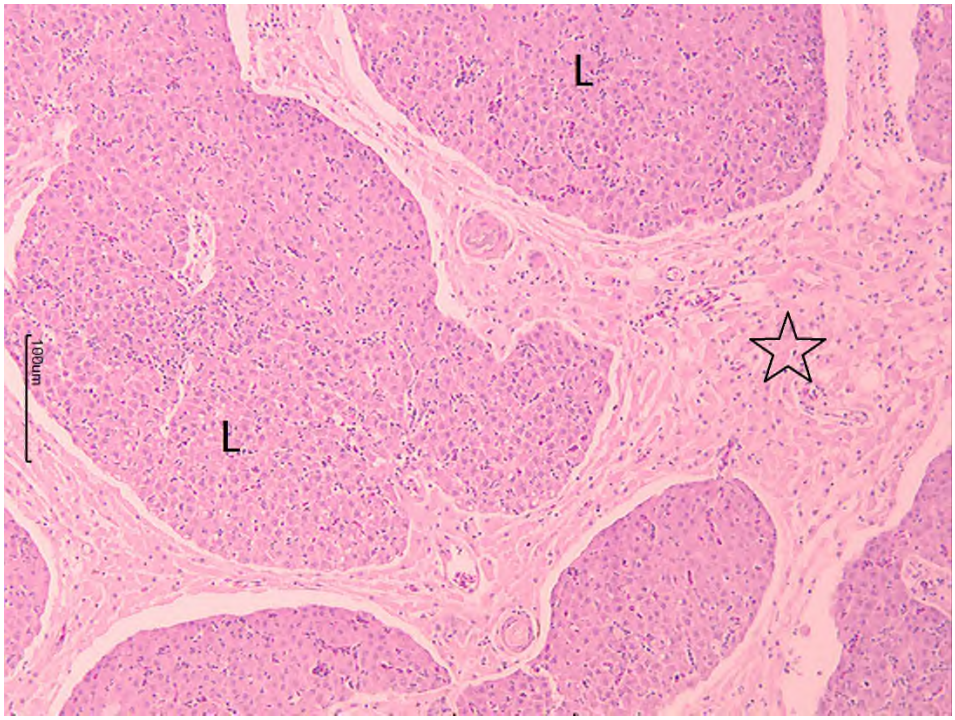


Fig. 23. P18-02264 Auckland Creek *L. calcarifer* AC14BA. Liver – H&E. Metaplasia of the interlobular tissue which is highly thickened (star), dividing the liver into smaller lobules (L).

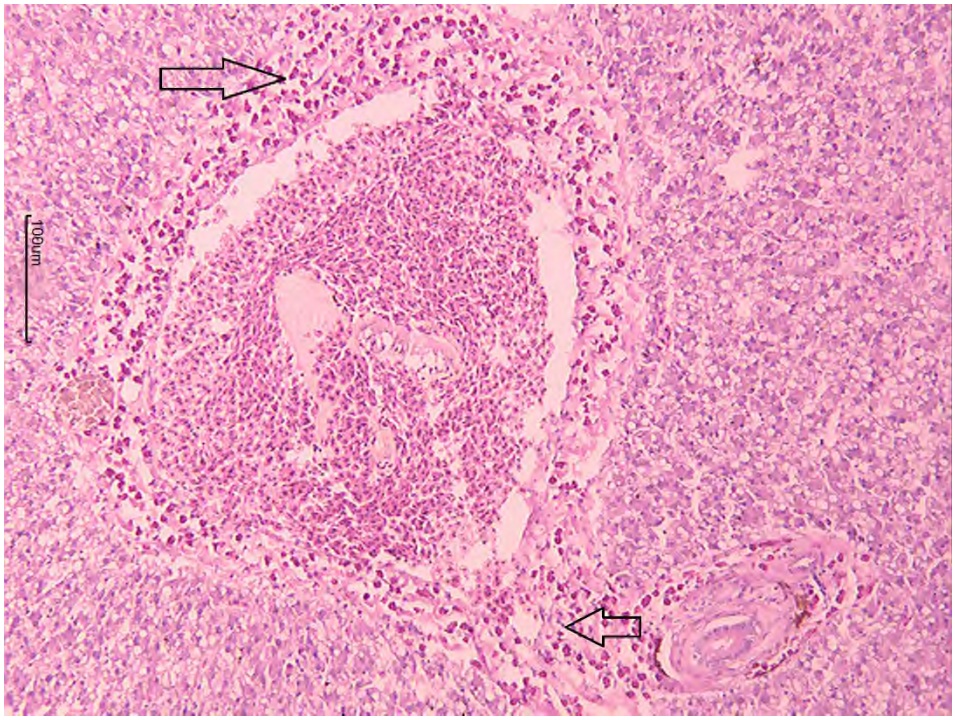


Fig. 24. P18-02274 Calliope River *M. cephalus* CR12SM. Liver – H&E. There is vasculitis involving many eosinophilic (arrows) inflammatory cells surrounding the hepatic blood cell.

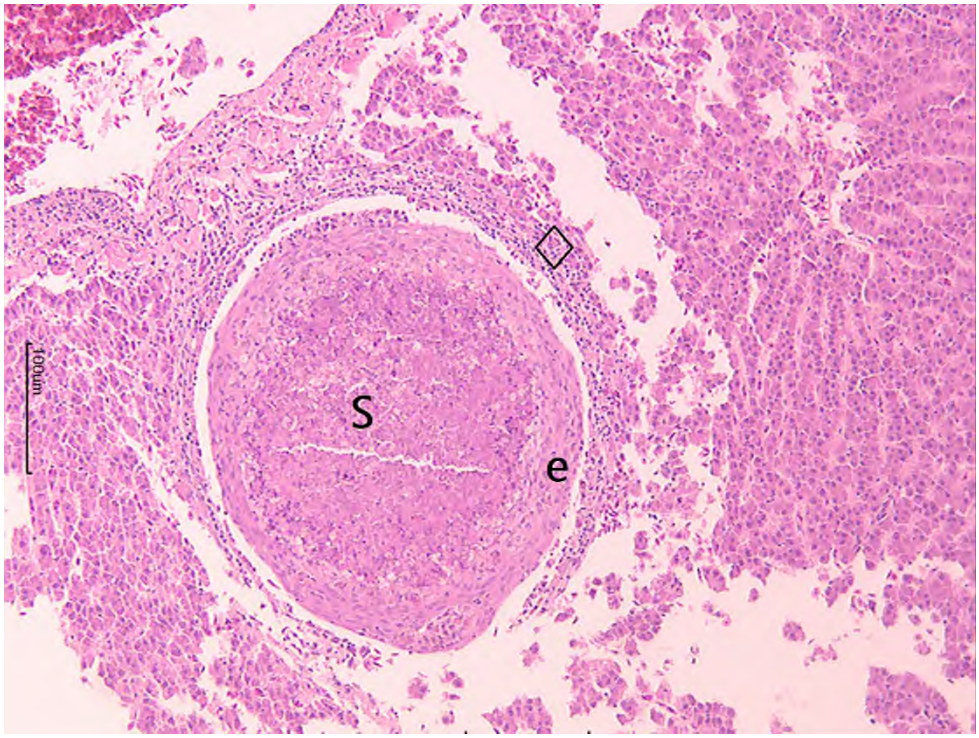


Fig. 25. P18-02282 Graham Creek *L. valgiensis* GC7DM. Liver – H&E. A large granuloma lesion encapsulating (e) a central core of myxosporean spores (S), and surrounding by inflammatory cells (diamond).

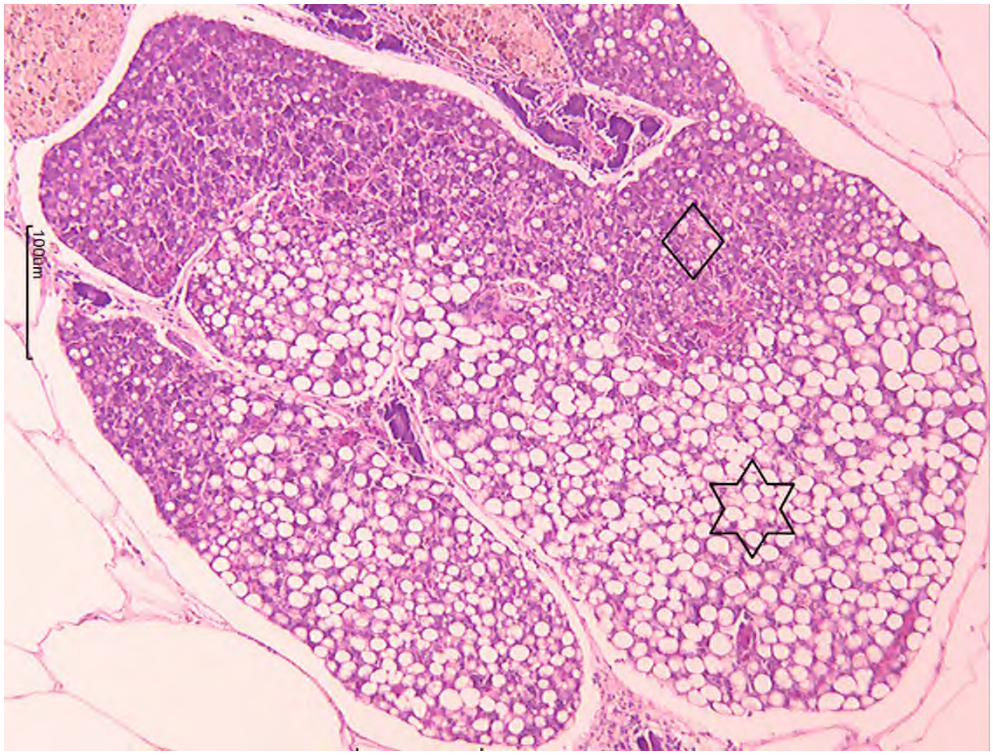


Fig. 26. P18-02266 Boyne River *L. calcarifer* BR2BA. Liver – H&E. A portion of liver with extremely fatty hepatocytes (star) compared to a section with fewer fat vacuolated hepatocytes (diamond).

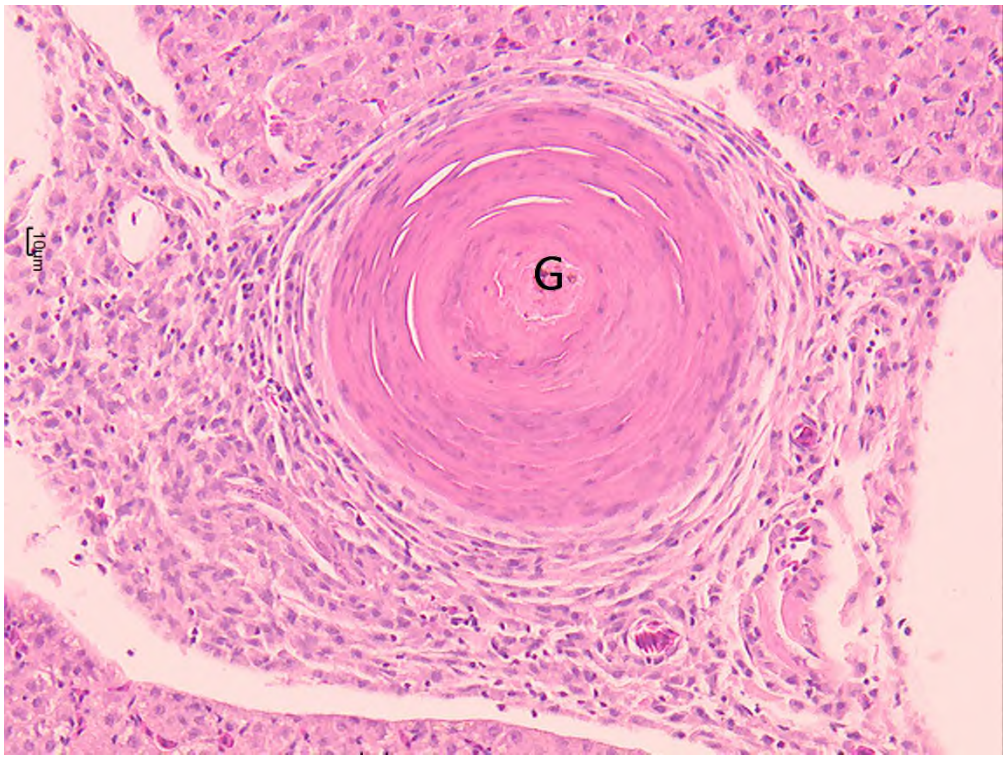


Fig. 27. P18-02260 Graham Creek *L. calcarifer* GC15BA. Liver – H&E. A large granuloma (G) which has an effete centre of eosinophilic cell debris and encapsulating fibrous tissue.

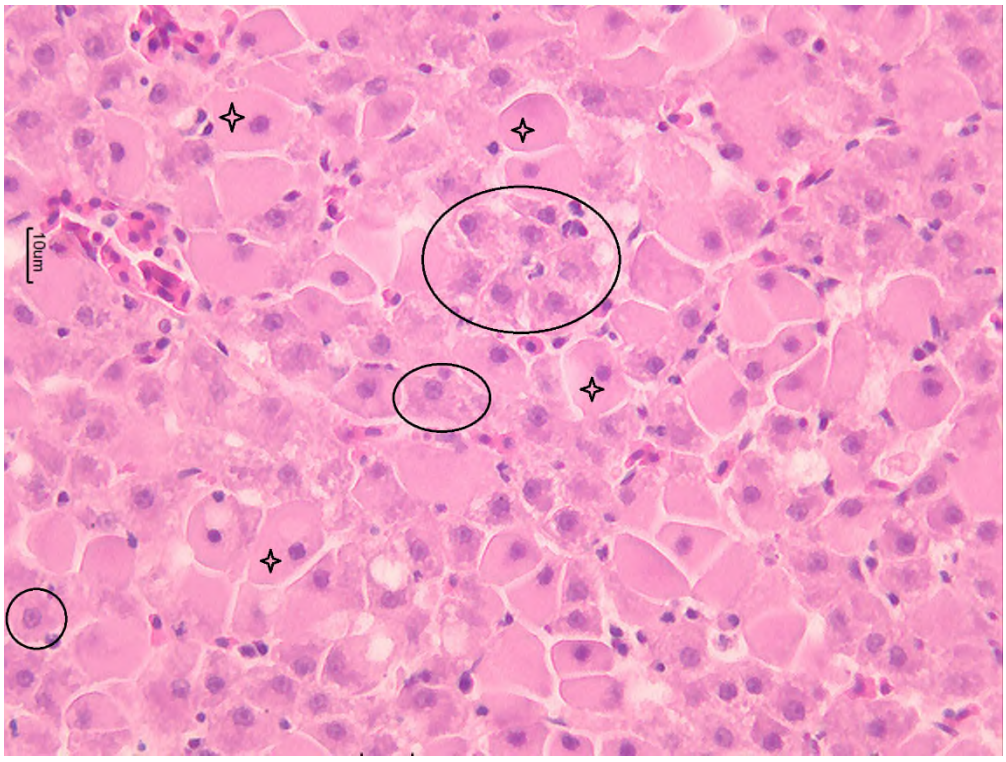


Fig. 28. P18-02273 Stanage Bay *L. calcarifer* SB4BA. Liver – H&E. There are glassy-appearing hepatocytes (stars) that have a smaller condensed nucleus, enlarged cytoplasm. This in comparison to normal hepatocytes (circled) in which the nucleus has a clear centrally located nucleolus and a more granular appearing cytoplasm with some detail. This may be an issue with delayed fixation of the liver after death of the fish – but would need proper fixation to be sure in future samplings of fish from this site.

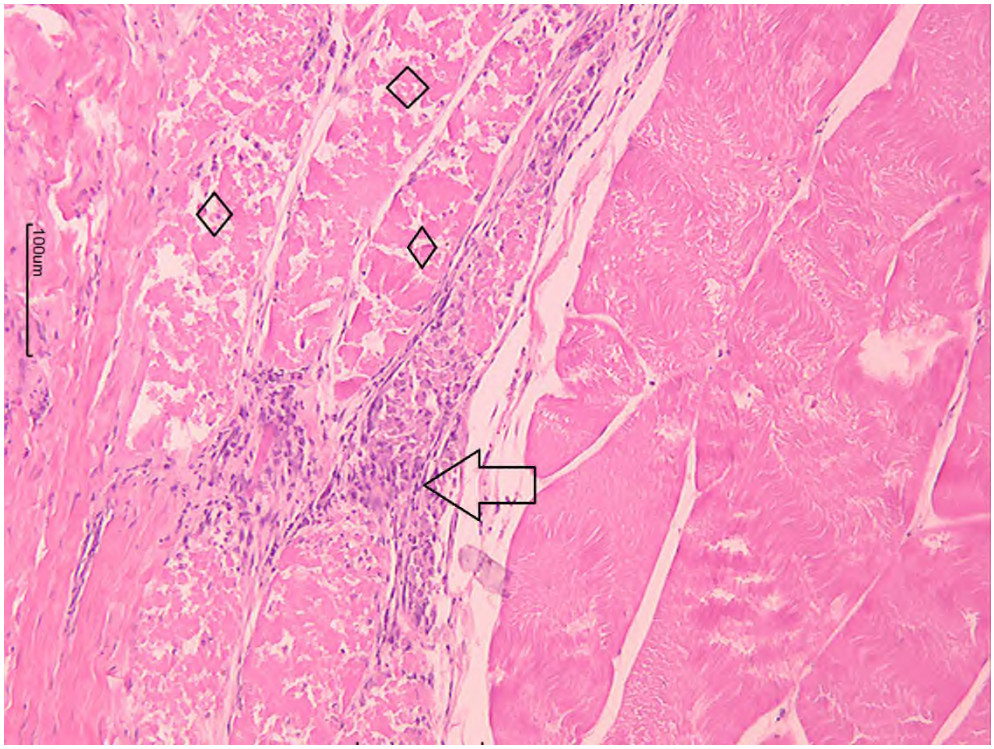


Fig. 29. P18-02262 The Narrows *L. calcarifer* NW4BA. Muscle – H&E. Myositis (arrow) with infiltrating inflammatory cells, and several muscle fibres undergoing degeneration (diamonds).

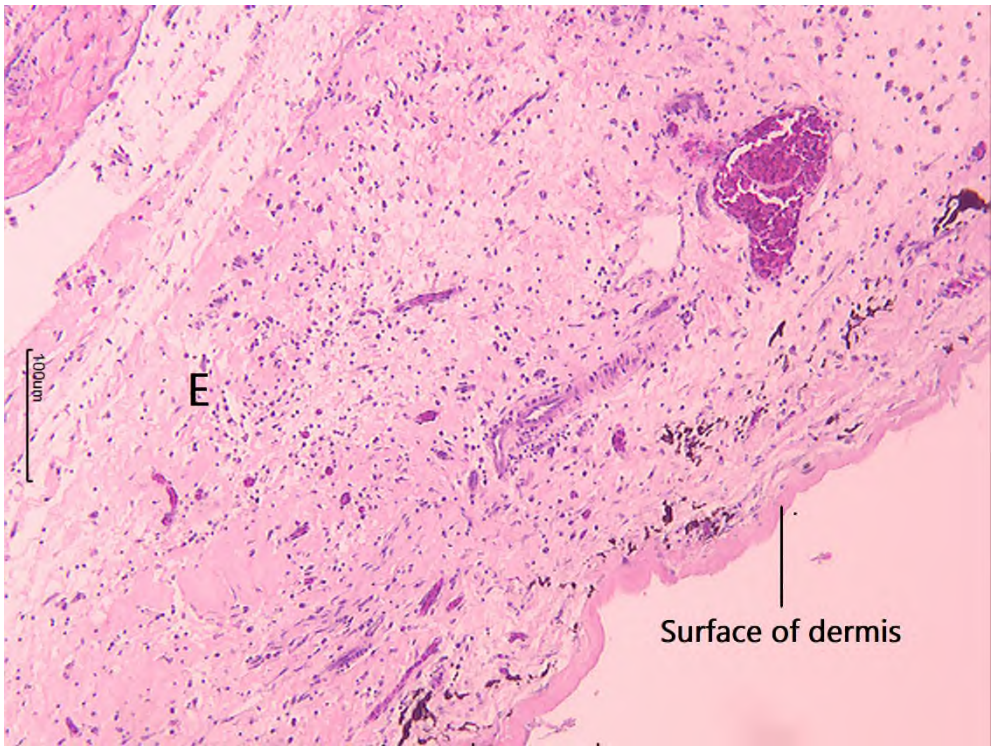


Fig. 30. P18-02282 Graham Creek *L. valgiensis* GC7DM. Skin – H&E. Epidermis has been removed during sampling. The layer of dermal skin is oedematous (E) appearing a loose tissue rather than a more compact dermis.

Lesion Score and Relative Fish Health Index

A lesion score on the histopathological lesions observed is based on interpretation of the lesions as being incidental (score of 0.50), low severity (1.00), low to moderate severity (1.50), moderate severity (2.00), moderate to high severity (2.50) and high severity (3.00). This provides a semi-quantitative assessment of the histopathology – a necessary step to compare and measure changes in fish health.

The limitations of this approach are :

- Scoring is dependent on the subjective assessment of the fish pathologist. The more experienced the pathologist, the more reliable the assessment.
- Scoring can be biased towards the knowledge of fish species which are familiar to the fish pathologist, and extrapolation to less familiar species is required and may not be as accurate.
- Lesion significance depends on the environmental, clinical history, sample handling or husbandry contexts of the fish examined. This information can often be in-complete, resulting in under or over-interpretation of the lesion severity.

A number of approaches are required to minimise the potential errors arising from in-accurate lesion scoring, and these include :

- Sampling a high number of fish over an extending period of time. This allows patterns of significant lesions to be more clearly defined, and helps to exclude incidental findings.

- Quality of fish tissue sampling in the entire process from fish capture, necropsy, fixation and histological processing must be optimised.

- The range of tissue types included in the histopathology assessment.

- Information about the environment (water chemistry and quality), clinical history (including evidence of fish mortalities, fish body condition, fish abundance) and any significant fish health event should be available and included in the final analysis of fish lesions.

In this 1st sampling pilot study, a number of issues have been identified which influences the exercise of lesion scoring, including –

- Delayed collection and fixation of fish tissues after the fish had died (any period more than a few minutes to ½ hour), particularly for gills but also some liver sections. This can create post-mortem autolytic changes which can mask true pathology or present as 'untrue' or artifactual pathology.
- Removal of the epidermis of the skin. This removes any evidence of surface active pathogens or lesions.
- Limited range of tissues. This limits the clinical picture of the entire fish.

Taking these limitations into account, the lesion scoring exercise was conducted to provide a limited and initial comparative view of fish lesions; serving as a basis for future refinement of the process.

The results of individual lesion scoring is provided in the excel file : CQU – Gladstone Fish Histopathology Score Card – 24.7.2018. Fish with few and less severe lesions would achieve a low lesion score, while fish with many and severe lesions obtain a high numerical lesion score. The total lesion score for a fish is the sum of lesion scores and the maximum lesion score is the sum of lesions x 3.00. For liver fat lipid storage vacuolation, no fat vacuoles indicate a low nutritional status and would score as a lesion at 3.00,

while inadequate vacuolation a 2.00 and adequate vacuolation a zero (normal). Table 2 summarises the lesion score for fish from the various sampling locations.

Table 2. Histopathological lesion scores of fish

Location	Fish species	Fish ID. No.	Total Lesion Score*
Rodd's Bay	*Lates calcarifer*	RB1BA	6.00
Graham creek	*Lates calcarifer*	GC15BA	14.50
Inner harbour	*Lates calcarifer*	IH1BA	14.00
	Lates calcarifer	IH3BA	10.50
	Lates calcarifer	IH4BA	10.50
	Lates calcarifer	IH13BA	15.50
The Narrows	*Lates calcarifer*	NW4BA	13.00
Mid-harbour	*Lates calcarifer*	MH9BA	14.50
Auckland Creek	*Lates calcarifer*	AC5BA	35.50
	Lates calcarifer	AC6BA	15.00
	Lates calcarifer	AC7BA	18.00
	Lates calcarifer	AC14BA	19.50
South Trees Inlet	*Lates calcarifer*	STI14BA	14.50
Boyne River	*Lates calcarifer*	BR2BA	9.50
Western Basin	*Acanthopagrus pacificus*	WB1PB	6.00
Calliope River	*A. australis*	CR1YB	2.00
Stanage Bay	*Lates calcarifer*	SB3BA	20.50
	Lates calcarifer	SB4BA	16.00
	Lates calcarifer	SB5BA	12.00
	Lates calcarifer	SB7BA	14.50
	Lates calcarifer	SB8BA	12.50
Calliope River	*Mugil cephalus*	CR3SM	5.00
	Mugil cephalus	CR11SM	11.50
	Mugil cephalus	CR12SM	1.00
	Mugil cephalus	CR13SM	7.50
	Mugil cephalus	CR14SM	7.00
	Mugil cephalus	CR15SM	4.00
Auckland Creek	*Mugil cephalus*	AC4SM	7.00
Baffle Creek	*Mugil cephalus*	BC6SM	10.50
	Mugil cephalus	BC27SM	4.00
	Mugil cephalus	BC38SM	12.00
	Mugil cephalus	BC39SM	5.00
	Mugil cephalus	BC40SM	8.50
The Narrows	*Liza valgiensis*	NW3DM	14.50
	Liza valgiensis	NW1DM	12.50
Mid-Harbour	*Liza valgiensis*	MH6DM	9.50
	Liza valgiensis	MH7DM	12.50
	Liza valgiensis	MH12DM	12.00
Auckland Creek	*Liza valgiensis*	AC9DM	5.00
	Liza valgiensis	AC11DM	12.00
	Liza valgiensis	AC12DM	0.50
Boyne River	*Liza valgiensis*	BR6DM	4.00
	Liza valgiensis	BR16DM	15.50
Stanage Bay	*Liza valgiensis*	SB2DM	5.50
Graham creek	*Liza valgiensis*	GC7DM	23.00

* fish with more lesions that are more severe obtain a higher lesion score. **These lesion scores should not be considered as definitive of the whole fish organ systems.**

The Relative Fish Health Index (RFHI) is formulated as -

RFHI = 1-(total lesion score divided by maximum lesion scores possible).

Table 3. summarises the RFHI for fish examined.

Table 3. Relative Fish Health Index (RFHI)

Location	Fish species	Fish ID. No.	Relative Fish Health Index@
Rodd's Bay	*Lates calcarifer*	RB1BA	0.94
Graham creek	*Lates calcarifer*	GC15BA	0.87
Inner harbour	*Lates calcarifer*	IH1BA	0.87
	Lates calcarifer	IH3BA	0.90
	Lates calcarifer	IH4BA	0.90
	Lates calcarifer	IH13BA	0.86
The Narrows	*Lates calcarifer*	NW4BA	0.88
Mid-harbour	*Lates calcarifer*	MH9BA	0.87
Auckland Creek	*Lates calcarifer*	AC5BA	0.67
	Lates calcarifer	AC6BA	0.86
	Lates calcarifer	AC7BA	0.83
	Lates calcarifer	AC14BA	0.82
South Trees Inlet	*Lates calcarifer*	STI14BA	0.87
Boyne River	*Lates calcarifer*	BR2BA	0.91
Western Basin	*Acanthopagrus pacificus*	WB1PB	0.94
Calliope River	*A. australis*	CR1YB	0.98
Stanage Bay	*Lates calcarifer*	SB3BA	0.81
	Lates calcarifer	SB4BA	0.85
	Lates calcarifer	SB5BA	0.89
	Lates calcarifer	SB7BA	0.87
	Lates calcarifer	SB8BA	0.88
Calliope River	*Mugil cephalus*	CR3SM	0.95
	Mugil cephalus	CR11SM	0.89
	Mugil cephalus	CR12SM	0.99
	Mugil cephalus	CR13SM	0.93
	Mugil cephalus	CR14SM	0.94
	Mugil cephalus	CR15SM	0.96
Auckland Creek	*Mugil cephalus*	AC4SM	0.94
Baffle Creek	*Mugil cephalus*	BC6SM	0.90
	Mugil cephalus	BC27SM	0.96
	Mugil cephalus	BC38SM	0.89
	Mugil cephalus	BC39SM	0.95
	Mugil cephalus	BC40SM	0.92
The Narrows	*Liza valgiensis*	NW3DM	0.87
	Liza valgiensis	NW1DM	0.88
Mid-Harbour	*Liza valgiensis*	MH6DM	0.91
	Liza valgiensis	MH7DM	0.88
	Liza valgiensis	MH12DM	0.89

Auckland Creek	*Liza valgiensis*	AC9DM	0.95
	Liza valgiensis	AC11DM	0.89
	Liza valgiensis	AC12DM	1.00
Boyne River	*Liza valgiensis*	BR6DM	0.96
	Liza valgiensis	BR16DM	0.86
Stanage Bay	*Liza valgiensis*	SB2DM	0.95
Graham creek	*Liza valgiensis*	GC7DM	0.79

@ A higher RFHI is achieved for fish with fewer and less severe histopathological lesions. **These RFHI scores should not be considered as inferring a definitive state of health of the fish sampled.**

Discussion

The RFHI provides a way to compare the health parameters of fish based in this case on the histopathology lesion scores. Individual fish, fish species, fish locations, fish capture dates can be compared using the RFHI. A higher RFHI means a lower number and severity of histopathology lesions. Factors that can influence the RFHI include :

(1) Quality of the sample - autolysis could mask lesions, thus artificially increase a fish's RFHI or create 'false lesions' and decrease the RFHI.

(2) Numbers of fish sampled and the range of different lesions present. Here, more different lesion types can diminish the contribution of a particular lesion to the overall RFHI.

(3) Severe disease agents of both infectious and non-infectious nature generally will reduce the RFHI.

(4) A lack of infectious and non-infectious stressors will generally increase the RFHI.

(5) For particular scenarios which look at estimating the impact to fish health of specific pathogens or pollutants, the RFHI will need to have categorisation of lesions into acute non-reversible lesions and chronic reversible/reparable lesions. These are then assessed separately as RFHI-acute vs RFHI-chronic. This will avoid a dilution effect of the acute lesions by the chronic lesions that render a false negative result i.e. a relatively high RFHI when a fish kill has occurred. In a fish kill, the RFHI-acute should be very low for affected fish , while in surviving fish, the RFHI-chronic should be higher.

When using the RFHI for comparative or trend analysis of histopathology, it important to select control or reference fish species and especially locations where the RFHI is close to 1.00 and is stable over time. The RFHI is not an absolute measure of the health status of fish. A more reliable RFHI is derived when high numbers of fish and all major organs/tissues are examined. The fewer the variety of organs/tissues assessed, the less reliable is the RFHI. The value of the RFHI is in the following :

(1) Provide a reproducible method of semi-quantitatively measuring the relative health status of fish populations in ecolocations over a temporal and spatial scale.

(2) Correlate factors that influence the health of fishes, those factors that increase, versus those that decrease the RFHI.

(3) It is one way to estimate the impact of remediation strategies that alter factors to improve general fish health status in an ecolocation of interest over a specified temporal scale.

Concluding remarks

This pilot histopathology assessment of fish health represents one component of a range of methods that can be employed to establish baseline knowledge of the health of fish living in the Gladstone Harbour environment, compared to reference sites, over a predetermined period of time. Should an adverse environmental event or events occur, determining the cause(s) of such would be easier and more definitive with this type of baseline health information.

These early results suggest that fish in the wild or natural environment of Gladstone and the reference sites of Stanage Bay or Baffle Creek, are not entirely free of histopathological lesions or tissue changes. What these changes mean, can be better understood with continued surveillance, looking for quantifiable trends and patterns. Correlation of histopathology with results from water quality and other biomonitoring indicators would be the next logical step in this environment health-risk assessment exercise, with the overall aim of achieving a health harbour.

Prepared on 27 July 2018 by :

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