FINAL REPORT

aquatic animal health subprogram

Improved fish health management for integrated inland aquaculture through Better Management Practices (BMPs)

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February 2014

FRDC Project No. 2010/036

FRDC
FISHERIES RESEARCH & DEVELOPMENT CORPORATION

THE UNIVERSITY OF MELBOURNE

Department of Environment and Primary Industries

State Government Victoria




ISBN 978-1-74326-574-1

Improved fish health management for integrated inland aquaculture through Better Management Practices 2010-036

2014

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Bradley, T., McCowan, C., Cohen, S., Ingram, B., Green, C. and Mansell, P. 2014, *Improved fish health management for integrated inland aquaculture through Better Management Practices*, Department of Environment and Primary Industries, Melbourne, December.

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Objectives

1 Determine risk factors and prevalence of diseases resulting in reduced production on inland integrated aquaculture farms.

2 Develop fish health and biosecurity better management practices (BMPS) for inland integrated aquaculture industries

3 Examine the effect of different standard farmer treatments and frequencies on fish mortality, weight and health under controlled RAS conditions.

Non-technical summary

OUTCOMES ACHIEVED TO DATE

The project outputs have contributed to or will lead to the following outcomes:

1. Determine risk factors and prevalence of diseases resulting in reduced production on inland integrated aquaculture farms. The project has determined the major causes of mortality events from information provided by farmers and the prevalence and cause of the most important production - limiting factors.

2. Develop fish health and biosecurity better management practices (BMPs) for inland integrated aquaculture industries. The project developed BMPs for inland integrated aquaculture and other associated resources.

3 Examine the effect of different standard farmer treatments and frequencies on fish mortality, weight and health under controlled RAS conditions. The project compared three commonly used farm chemicals and determined the effects on fish in a controlled trial.

The health and production of Murray cod grown on integrated aquaculture farms in the eastern states of Australia was examined during this 2 year project. Eighty – five farm submissions were received with over 400 fish being dissected and examined for diseases. Most of these fish were presumed to be healthy, however there were some submissions from farms where fish were affected by disease or mortality events. The most common health problem seen under the microscope was problems with the gills (in 81% of submissions) and this was most commonly associated with the parasite *Chilodonella.* Farm data was collected, inputted into a computer and analysed from 6 of the project farms. The completeness of this data varied amongst the farms but included mortality rates, water quality parameters and

treatments used. It was clear that many of the farms do not collect data of a sufficient quality to generate stock mortality rates and basic financial information for the farms. Most of the major mortality events reported during the project were believed to be caused by management rather than disease events. For example overdosing fish with chemicals during treatments and equipment failure when power was lost. Some disease events were investigated and the cause determined through the project.

A Better Management Practices (BMPs) manual was developed as part of the project. This large and detailed document covers a range of topics pertaining to Murray Cod health and production. There are a series of standard operating procedures and appendices that address topics such as how to submit fish to a laboratory and use of chemicals. Through the project other materials were developed including a video presentation on fish dissection and a poster on the appearance of common parasites.

A treatment trial was conducted on healthy fish with the most commonly used chemicals (formalin, hydrogen peroxide and salt). This trial aimed to determine if any of the chemicals at varying frequency caused problems with the growth, skin and gills of the fish. The trial found that formalin used every 3 days caused the highest mortality rate. Generally treating fish every 10 days did not affect mortality when compared with control fish.

It is apparent from the results of this project that in integrated Murray Cod systems infestations with *Chilodonella* is the greatest cause of mortality and reduced production. Further work into effective treatment regimes for this parasite should assist in addressing this problem. The data quality collected by farmers varied widely. Simple systems should be developed on farms where they don't exist already to ensure a better understanding of fish health and production. Although this industry is small, openness and cooperation amongst farms could assist the growth of the industry as a whole.

Acknowledgments

The authors wish to acknowledge the assistance of Fisheries Victoria and FRDC in funding this project. We also wish to acknowledge the assistance of the Murray Cod farmers involved in the study (who shall remain anonymous) and the staff of Agribio Victoria and Irymple DEPI for assisting in providing fish, technical and logistic support. We also wish to acknowledge the kind support of Terry Miller and his staff at James Cook University for assistance with molecular typing of *Chilodonella* spp. Dr. Ian Beveridge of the University of Melbourne very kindly assisted in parasite identification.

Abbreviations

DEPI – Department of Environment and Primary Industries

DO – Dissolved oxygen

Ich - *Ichthyophthirius multifiliis*

ppm - parts per million

ppt – parts per thousand

RAS – Recirculating aquaculture system

Executive Summary

Murray Cod (*Maccullochella peelii*) integrated aquaculture is a sustainable and developing industry in inland Australia using farm dams on horticulture and grazing enterprises to rear Murray Cod. In the first study of its kind in the Murray Cod industry, the Department of Environment and Primary Industries (DEPI) and the University of Melbourne have collaborated to examine the major causes of death and disease on integrated inland farms. Over a period spanning more than 2 years Murray Cod integrated aquaculture farms were visited and samples of fish and farm data were collected. A census of farms providing detailed information about each individual enterprise was undertaken at the commencement of the project and baseline farmer knowledge was assessed using a simple quiz. The project was conducted across Victoria, NSW and Queensland and was finalised in September 2013. The initial impetus for this project was anecdotal farmer reports that they were losing up to 60% of their stock in the first 2 weeks of life on the farm. Because these farmers are located remote to veterinary services and laboratories they are not generally equipped with the usual supports and tools that other aquaculture enterprises may enjoy. This problem is compounded by the fact that Murray Cod aquaculture is usually a secondary enterprise for the farmers and most of them do not have skills or knowledge in the area.

Background

Murray Cod are classified as a threatened, iconic, Australian native species and the species is also considered a good candidate for freshwater aquaculture. Farmed Murray Cod is a premium product in domestic markets with production annually in 2010/11 of 55.4 tonne in Australia worth $1 209 800 (this figure includes Mary river Cod and sleepy Cod). A relatively strong recirculating aquaculture systems (RAS) sector currently exists in Victoria and NSW for this species. These enterprises generally concentrate on rearing finfish specifically and do not report major health problems over the life of the production cycle. This is in stark contrast to the open water integrated aquaculture enterprises currently operating in Australia. In recent years it has become apparent that although some innovative grow - out systems were being developed in Victoria there was insufficient support for farmers who were investing in expensive infrastructure and were unfamiliar with rearing young fish. This problem was exacerbated by the geographical isolation of the farmers and the expense involved in engaging veterinary consultants. Many of these farmers were uncertain how to detect and prevent diseases on farm, undertake basic microscopy and submit specimens to a laboratory. These farms also generally did not have in place basic systems for recording mortalities, illness or production data. Where such data was recorded it was usually not utilised in any meaningful way.

Although major diseases have been documented for silver perch and to a lesser extent Murray Cod there are unique challenges in integrated systems where there is little control over water quality and supply as it is provided primarily for horticultural practices. Ideally farmers should be able to accurately identify major disease syndromes and risk factors affecting the productivity of their farms and be aware of when they are likely to occur relative to the major production events in the fish life cycle, climatic conditions and relative to water movements. The ability to mitigate these predicted disease/reduced production events would enable higher production levels.

Aims/objectives

1 Determine risk factors and prevalence of diseases resulting in reduced production on inland integrated aquaculture farms.

2 Develop fish health and biosecurity better management practices (BMPS) for inland integrated aquaculture industries

3 Examine the effect of different standard farmer treatments and frequencies on fish mortality, weight and health under controlled RAS conditions.

Methods

This project was composed of three separate parts: the histopathology report from routine monitoring and disease events; the farm surveillance report and the toxicity trial.

Histopathology study:

Fish were collected routinely from all project farms either on a quarterly or monthly basis during farm visits depending on location. Fish were either examined on farm for parasites using wet preparations of skin and gill tissue or transported live to the laboratory where such examinations were undertaken. Bacterial cultures were taken and all fish were dissected and standard tissues preserved for histopathology. Results from the histopathology were conveyed to farms verbally and in writing. In some cases virology, special staining and speciation of parasites was undertaken. Farmers were also able to submit samples where there was a disease process suspected and results were reported back in a timely fashion.

Farm surveillance

At the commencement of the project a census was completed of participating project farms and a wider pool of RAS enterprises. At this time a quiz was undertaken attempting to assess farmer knowledge of basic diseases and suitable water quality conditions. Where available farm data was collected with the quality varying according to existing practices of the farmers. Generally farms that provided data recorded basic mortality figures, water quality, treatments and feed rates. Farmers were provided with opportunities to use templates for recording data with varying uptake of this opportunity.

Toxicity trial:

A small trial was undertaken to assess the effect of commonly used chemicals at rates and frequencies utilised by farmers on the health of Murray Cod held under controlled conditions. The chemicals used were salt, hydrogen peroxide and formalin at 3 and 10 day intervals. The outcomes measured were mortality, weight, length and skin and gill changes. This trial ran for a period of 31 days following an acclimatisation period.

Results/key findings

Histopathology study:

Over 400 Murray Cod were submitted to the laboratory for examination during this project. Generally the majority of submissions did not have growth of bacteria from kidney cultures. In cases where there was growth it was not associated with disease. Where there were disease investigations done there were no potentially causative bacteria recovered where cultures were submitted however these cases did not present as likely to have a positive bacterial culture.

The most common site of damage in the submitted fish was the gills with the parasite *Chilodonella* being the most common cause of this damage. There were often marked changes to the epithelial covering of the gills that would undoubtedly limit the fish's ability to take up oxygen. However it was remarkable that these fish were submitted as clinically unaffected during routine monitoring. It is likely that they can handle very low levels of oxygen exchange due to their sedentary lifestyle under farm conditions. Also, farms used aeration devices to maintain dissolved oxygen level. The second most common finding was non-specific dermatitis in the skin.

Farm surveillance:

Results from the farm census and farmer quiz conducted at the beginning of the project are provided and give some insight into a large portion of the integrated aquaculture Murray Cod sector. Results from the farmer quiz illustrate that at the commencement of the project there was a low knowledge base in the area of wet microscopy in particular. A small collection of repeated quizzes at the end of the project illustrated that qualitatively there was a strong improvement in test results and arguably farmer knowledge in those tested.

Mortality, water quality, parasite load, production and treatment data were collected from 6 farms for varying periods of time during the project. Mortality was plotted against water quality variables such as temperature, pH and dissolved oxygen. Mortality rates in general were not as high as those predicted from the farmer census. *Chilodonella* sp. was clearly the most prevalent parasite occurring throughout the year and associated with very low to very high mortalities on farms. The intensity and incidence of infection with *Chilodonella* was associated with a range of variables including temperature, pH, nitrate and alkalinity.

Toxicity trial:

The toxicity trial found that mortality rates were higher in fish treated every 3 days when compared with those treated every 10 days. Treatment with formalin every 3 days yielded the highest percentage mortality. In general, across all groups there was a qualitative increase in severity of histopathological lesions associated with treatment type and interval.

Implications for relevant stakeholders

Industry: This project has illustrated the poor quality of data collected by Murray Cod farms enrolled in the project. It would appear that there is a strong need for transparent benchmarking of the costs and returns experienced by members of the industry that would enable the whole industry to move towards more profitable production using a more collaborative approach. Further resources should be directed towards elucidating rational control and treatment regimes for *Chilodonella* infections and further skill development in fish health for farmers.

Government/policy makers: For the continued development of the Murray Cod industry further technical support should be provided to these isolated farmers and further resources directed towards elucidating rational control and treatment regimes for *Chilodonella* infections.

Recommendations

This project has demonstrated that there is still much work to be done in the small, integrated Murray Cod aquaculture industry in the areas of *Chilodonella* management (control and prevention), data recording practices and marketing.

Keywords

Murray Cod, *Maccullochella peelii*, aquaculture, farm surveillance, histopathology, *Chilodonella* spp.

Introduction

Murray Cod (*Maccullochella peelii*) are an iconic native freshwater fish cultivated throughout Australia using a diversity of aquaculture techniques. This threatened fish has very good aquaculture prospects and small quantities are sold into domestic markets (Gooley and Rowland 1993, Ingram *et al.* 2005a). In recent years integrated aquaculture enterprises have developed where large water bodies such as irrigation dams used for horticulture crops are stocked with fish. Within these farms, the aquaculture enterprise is often secondary to another primary horticultural or other farm enterprise such as sheep or table grapes. These systems are particularly well suited to horticultural enterprises with large water holdings as a means of adding value to the scarce water resource. As the agricultural component of these farms takes precedence, there are inherent constraints to this type of aquaculture which can include limited and/or variable water quality supplies, irrigation demands, as well as limited or intermittent staff resources and skills. The production of fingerlings for stock enhancement purposes has been established commercially since the 1980s. More recently a range of different culture techniques have been established for grow-out including cages in dams and free ranging fish in pond systems.

It is useful to document the diseases and potential growth limiting health issues such as parasites in Murray Cod before developing management strategies for farm enterprises. Although Murray Cod in dedicated recirculating aquaculture systems (RAS) have proven to be hardy and disease resistant, the very different conditions faced by fish in open, integrated aquaculture dam systems have not been fully investigated. Farmers engaging in integrated grow-out aquaculture tend to have limited access to local aquatic veterinary and laboratory services. Furthermore these farmers tend to be developing skills in the area of fish health themselves so they may be unable to readily undertake routine health checks (such as gill and fin clips). Estimates gained from a census of integrated aquaculture farmers indicates there are mortality events that can kill up to 60% of newly introduced stock (see Farm Surveillance Section of this project). In any livestock enterprise such losses place a heavy financial burden on farmers. The cause of these mortality events may not be known. The intention of the histopathology study was to document the health problems of Murray Cod on project farms via routine monitoring of apparently healthy fish at regular intervals and during disease events wherever possible. The aim was not only to elucidate the causes of disease and mortality events but also to gather baseline data about the pathological and microbiological features of Murray Cod on project farms. To this end we undertook a two year survey of fish health using histological and bacteriological examination of tissues acquired either routinely throughout the year or during a disease outbreak.

Over the last half century the gradual intensification of various agricultural industries has required that there are clear measures of farm performance in terms of stock health and productivity. This has resulted in the collection and analysis of large volumes of data in industries such as poultry meat and pig production. Well recognised industry production standards are known and can be used by individual companies and farmers for benchmarking purposes. This is not the case with the small and disparate Murray Cod industry. In some cases, particularly in open or cage dam culture, the mortality rate of stock is not known or recorded. This is complicated by the predatory nature of Murray Cod where mortalities can be consumed by cage/pond mates and the need to move fish for grading. Water quality parameters are collected regularly and are useful tools in RAS systems for maintaining optimal water quality for fish health however this is not the case in open and cage grow- out systems where some enterprises did not record any data at all.

The major impediment to recording or collating data is a lack of time according to the farmers. As Murray Cod aquaculture is often a secondary and much smaller part of the farm business than the primary horticulture interest this behaviour may be difficult to change. On only some of the farms were sufficient details recorded to provide a mortality rate. This requires knowledge of the number of fish within a cage or pond which in most aquatic and terrestrial industries would be considered essential data. The practice of often moving fish means that it requires some effort to estimate total fish numbers. It is hoped that as farmers are provided with the clear benefits for recording more information they will have an appropriate incentive to do so.

Surveillance of farmed Murray Cod has confirmed the ubiquity and importance of parasites of the gills, particularly *Chilodonella* spp. but half of the submissions with gill lesions were not associated with any clear aetiology. Farmers regularly treat fish to remove or reduce parasite burdens, using varying protocols based on reagents with different but potentially damaging effects on fish tissues, particularly paraformaldehyde (formalin), hydrogen peroxide (peroxide) and sodium chloride (salt). Regular treatment, particularly at high rates of repetition, are a possible cause or contributor to gill lesions that may be affecting fish health and growth. The finding that one farm using chemical baths at high rates suffered an outbreak of epizootic ulcerative syndrome, a disease associated with secondary infection of damaged skin by an oomycete *Aphanomyces invadans,* raised the question of the role of these chemicals in predisposing fish to this infection. An experiment was designed to investigate the effects of chemical treatments on fish growth and survival and on the health of gills and skin when used at strengths and frequencies commonly applied on farms included in the project. Research of this nature has not been previously conducted in Murray Cod before.

Objectives

1 Determine risk factors and prevalence of diseases resulting in reduced production on inland integrated aquaculture farms.

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3 Examine the effect of different standard farmer treatments and frequencies on fish mortality, weight and health under controlled RAS conditions.

Methods

Histopathology study

Participating farms were visited on a regular schedule and a selection of fish taken for gross and histological examination. Extra samples were taken off-schedule during episodes of increased stock mortality. Date and site of collection were noted for analysis of disease patterns.

Bacteriology: Swabs were taken of kidney and of gross lesions where present, and cultured under aerobic conditions for known freshwater pathogens.

Histopathology: A full range of tissues was fixed in formalin, processed in routine fashion and examined by light microscopy. Lesions were described and documented and further stains performed as necessary.

Comparison of gill clip and histological examination for the diagnosis of chilodonellosis was performed on 176 fish for which both sets of information were available. The gill clips were performed by a trained staff member on farm or at a local laboratory. In some cases fish were anaesthetised prior to removing gill tissue, in others they were not. Comparisons were made only on a positive/negative basis per fish.

The diagnostic sensitivity and predictive values of gill clips, using histological examination as the gold standard, were calculated in standard fashion with 95% confidence intervals.

Chilodonella speciation: Gill samples were collected from a selection of fish into RNA later and stored at -20^{0}C. Accumulated samples were sent to Dr Terry Miller, James Cook University, for speciation of attached *Chilodonella* using silver stains and molecular genomics.

Samples (tissues and swabs) were taken into viral transport medium and stored at -80^{0}C for future use if required.

Farm Surveillance

Farm census and farmer quiz

A census of farms was conducted at the start of this project during April 2011. This census was both qualitative and quantitative and comprised approximately 180 questions that were dichotomous, categorical or open ended in nature. The questions assessed a range of areas including farmer knowledge and experience, physical aspects of the enterprise, biosecurity activities, other agricultural pursuits, estimated prevalence of a range of common diseases, treatments regimes etc. Nearly all surveys were conducted face to face with one distant farm (Queensland) conducted by phone. Each survey took approximately 2 hours to complete and was accompanied by an assessment of the farmer's knowledge with the "Farmer Quiz" (the quiz), which was completed concurrently.

The aim of the quiz was to get a baseline indication of the ability of farmers to recognise some common health issues they may come across with a practical, paper- based assessment. The quiz comprised a series of images taken from the view looking down a dissecting microscope with questions referring to structures, parasites and artefacts in the field of vision. There were also questions on the pathology of a pictured skin lesion, how to best submit fish and how to prevent the disease on farm. There were a series of questions on normal water quality parameters and how and why they should be changed. In total there were 16 questions. The quiz was conducted face to face without prior knowledge that it was to be conducted.

Farm surveillance data

Data was collected from all project farms, where available, for the duration of the project. The parameters mortality (numbers/cage or tank), water temperature, pH, dissolved oxygen levels, salinity and other parameters were collected

from farms where recorded. Where available results of gill/skin fresh examination under a microscope, treatment regimes and feed levels were also collected. Data was recorded on paper sheets by the farmer and copied during farm visits or in the case of Farm 6 provided electronically. In some instances (Farms 2 and 3) some water quality data was recorded electronically for some periods on water loggers located in the dam.

The relationships between incidence and intensity of infection with *Chilodonella* and environmental variables was examined for cages in open water pond culture systems. There was usually only one water quality monitor per pond on farms and the data collected from this was assigned to each cage on the farm. Using data from farmer gill and skin smear preparations, incidence in a cage was recorded as either "1" if any one slide was positive for *Chilodonella*, or "0" if all slides were clear of *Chilodonella*. For gill and skin smear preparations where the number of *Chilodonella* were recorded (intensity of infection), the median value was used and then grouped as follows: Nil - no *Chilodonella* present, low - up to 10 *Chilodonella* present, medium – between 10 and 30 *Chilodonella*, high- greater than 30 *Chilodonella* or "severe infections" noted. Incidence and intensity data were compared with environment data recorded on the day of observation as well as the rate of change in each variable over the preceding seven data records. The latter was undertaken for logged data only (temperature, dissolved oxygen, pH and conductivity). For display purposes only, environmental data was also summarised as ranges. In order to explore correlations between intensity of infection and the environmental data, intensity ranges were classed as follows: Nil=0, Low=1,Medium=2,High=3.

Toxicity trial

Stock

Murray Cod fingerlings (6-8 months old) were sourced from a local commercial hatchery and held at the Snob's Creek Hatchery, Department of Environment and Primary Industries (DEPI) in Snobs Creek, Victoria, Australia, for a total of 31 trial days.

Experimental design

Trials were conducted using a re-circulating aquaculture system (RAS).160L fibreglass 't' tanks with black plastic custom-made covers were supplied with 2 mineral based air stones and custom made bio-filters with 2-4 mm diameter polystyrene beads (approximate bead volume of 2100 L).

Water was sourced directly from the Snobs Creek at approximately 9L/min (540L/hr) with a water turnover of 3.4 times per hour, physically filtered using a 4mm coarse filter screen and a drum filter at 600 µm and heated to an average of 25 C^0. A gas ducted system maintained the air temperature at an average of 25 C^0. Dissolved oxygen (DO ppm), salinity (ppt), and temperature (C^0) were tested daily. The pH was also tested daily. Water hardness was tested at the start of the experiment and ammonia was tested weekly.

A total of 16 fish were allocated to each of 23 holding tanks. A single group was allocated to be negative control; the fish were not moved or exposed to chemicals. Remaining groups were moved to treatment tanks at either 3 or 10 day intervals and were either "sham treated" (not exposed to chemicals) or received a "treatment" regime consisting of immersion for 1 hour in a bath of formalin (200ppm), hydrogen peroxide (peroxide, 200ppm Deltrex 50%), or salt (10 ppt Olsson's Pacific Fine Salt). Each group was exposed to only one treatment chemical and fish were returned to their original tanks between treatments. There were two sham treated groups, one for each treatment frequency. Chemical exposures were performed in triplicate.

At the start of the trial (day 1), fish were individually measured from tip of the nose to the tail fin in centimetres, and weighed in grams. Six fish from each tank were euthanased and sampled for gill and skin histology. Mortalities from each tank were recorded daily. The experiment finished on day 31 with euthanasia of remaining fish and histological sampling.

Sampling

Individual fish were sedated with AQUI-S (175 ppm), euthanasia performed. (DPI FISH AEC SOP 23) and allocated to a sample number according to a randomised key with the collector blinded to the number allocated to each fish. The 2nd and 3rd gill leaflets and a left flank portion of skin dorsal to the vent including the lateral line were collected and fixed in neutral buffered 10% formalin. Fixed tissues were processed routinely for light microscopy and sections stained with haemotoxylon and eosin (H&E) in routine fashion.

Histological examination and tissue scoring

Qualitative histological assessment of gills and skin was made, comparing fish at the beginning and end of the trial and between treatment groups.

Sections from two fish chosen at random from each tank were scored according to the system of Bernet *et al.* (1999). Briefly, pathological processes are separated in to five major groups, for example, circulatory disturbances, inflammation etc. and defined histological indicators of each group are assessed with weights allocated to each indicator as a marker of importance, with 1 being reversible changes of relatively little significance individually and 3 indicating a change that is likely to be irreversible. So, within "inflammation", "exudate", if present, is scored as 1, and "infiltration" as 2.

Control slides were scored unblinded for reference. All other tissues were scored with the operator blinded to the treatment group from which they came. A computer generated grid (Image J National Institute of Health, USA) was used on each slide in conjunction with a simple random square sampling pattern of 10 randomly allocated areas.

A selection of 10 gill and 10 skin slides was rescored, blinded to both treatment group and original sample number, on a second occasion to permit calculation of scoring reliability.

Statistical methods

Statistical analysis was performed using Microsoft Excel, IBM SPSS 21.0, IBM SYSTAT 13, WinPepi and Statacorp Stata 12.1. For all tests, significance was set at p=0.05

Normality: Data for mortality rate, fish length and fish weight were tested for normality of distribution (Shapiro-Wilks test) and homogeneity of variance (Levene's test). All failed to demonstrate normality ($p<0.05$) or homogeneity ($p<0.05$) and non-parametric testing was used for further analysis.

Weight and length: Fish weights and lengths at the first and second sampling periods (days 1 and 31) were compared using a Mann Whitney U test. Weights for different treatment groups at the end of the trial were compared.

Mortality: A univariate exploratory analysis of independent variables was performed with significant ($p\leq0.05$) independent variables retained for further generalised linear model building.

A maximum likelihood sum of squares type III error model was built, controlling any direction of effects errors. These models also determined the significance of any cluster effects between tanks.

Generalised linear mixed models (GLMM) were created for fish mortality rate at 3 and 10 day frequencies using a backward stepwise method with a random intercept. The random variable was tank and the outcome variable was mortality rate. Independent variables used were frequency (3 or 10 days), chemical (Formalin, H_20_2, and Salt), and/or sampling period (day 1 or day 31). Sham treatment (3 days) was used as the control.

Models were checked by plotting the predicted values over the residuals.

A one-way analysis of variance (ANOVA) model for mortality was built using a type III error adjusted sum of squares model. Individual treatment variables consisted of frequency and chemical. Sham treatment at 3 days functioned as the control group.

Type 1 error control was performed using a Tukey's Honest Significant Differences test for multiple pairwise comparisons between treatments.

Histopathology: Repeatability of the scoring was measured using Lin's Concordance Correlation Coefficient.

Results

Histopathology study

Bacteriology:

A total of 65 submissions included swabs for bacterial culture. No growth was seen from 34 (52%) and mixed growth, probably indicating field contamination, from 12 (18.5%). Nineteen samples (29%) returned culture of organisms identifiable as possible pathogens of fish or mammals, but there was no obvious association with disease.

Routine culture identified a range of organisms; only *Aeromonas hydrophila* and *Aeromonas sobria* were identified in more than one accession. *Acinetobacter* sp. was identified three times, but not speciated so it is not certain that this was in every case the same organism. *Pseudomonas* sp. was cultured twice – once was speciated to *P.fluorescens*, the other was not speciated. Single identifications of *Achromobacter xylosoxidans*, *Plesiomonas shigelloides* and *Streptococcus iniae* were made. The significance of these organisms is uncertain; they were not associated with disease in these fish.

Histopathology and lesions:

A total of 85 submissions ranging from two to nine fish each (totalling 404 fish) were examined histologically.

GILLS - Gill lesions were the most commonly seen abnormality. Epithelial hypertrophy and hyperplasia, with partial or complete lamellar fusion and often associated with lymphocytic or lymphohistiocytic inflammatory infiltrates (branchitis) affected 68 of 84 submissions (81%), with all farms and all times of year represented. Aetiology was not always evident

ie lesions were idiopathic, but sometimes saucer shaped protozoa consistent with *Chilodonella* spp. were clearly associated with the change (Figure 1a, b).

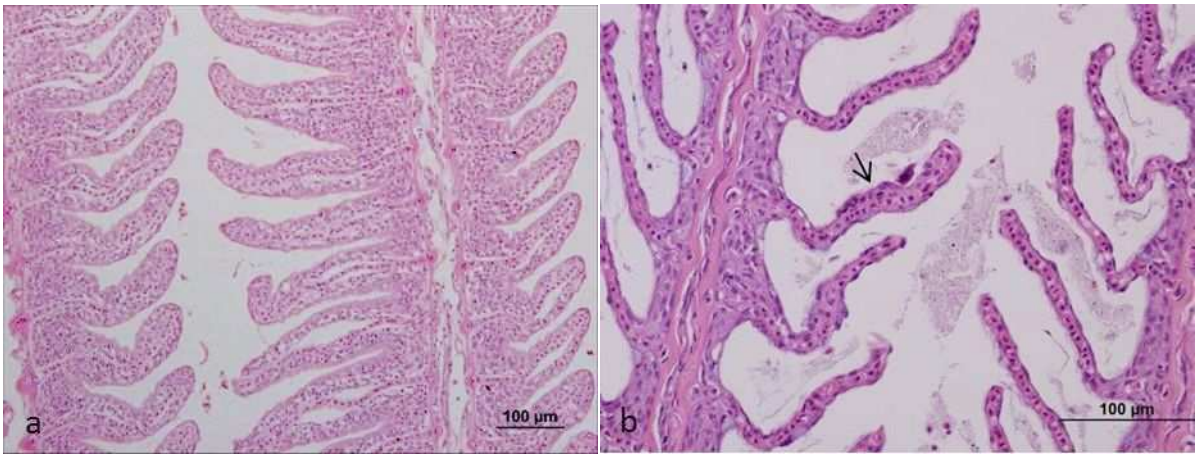

Figure 1. a) Epithelial hyperplasia (here diffuse), often associated with lymphocytic infiltrates in the lamina propria (branchitis), was a common finding. b) In some fish there was a clear association with *Chilodonella* spp. and epithelial changes. The arrow demonstrates epithelial hyperplasia at a site of parasite attachment.

Chronic changes affected some fish, with extensive epithelial hyperplasia and metaplasia leading to extensive lamellar fusion and epidermalisation (Figure 2). Inflammation was often mild in these gills and aetiology rarely evident although *Chilodonella* was occasionally present.

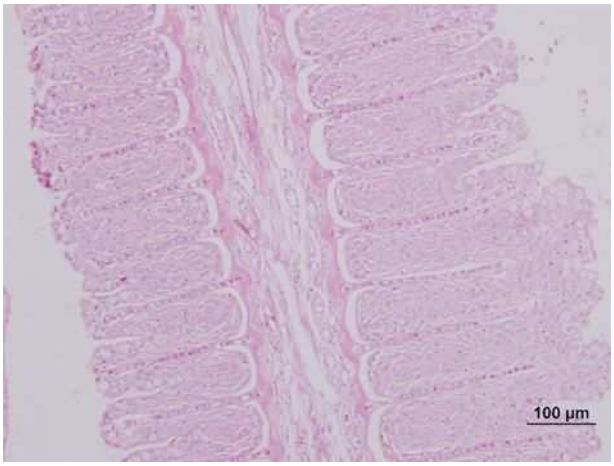

Figure 2. Chronic changes in gills could be extensive and severe, affecting all lamellae with epidermalisation and fusion. There is little inflammation, in this case virtually none, associated with these changes.

Of the specific aetiologies, *Chilodonella* was the most common, with 34 of 84 farms (40.5%) having one or more positive fish on histological examination. In most submissions there were both affected and unaffected fish; only in 8 cases (10% submissions, 23.5% of *Chilodonella* submissions) were all fish that were examined positive for protozoa. *Chilodonella* infestations were seen at all times of year, although none were seen in summer 2011/12 (December to February), with the first 2012 cases occurring in late March.

Epitheliocystis was present in 26 submissions (31%), ergasilid copepods in 9 (10%) and *Trichodina* sp. in 8 (9.5%). As for *Chilodonella*, infection rate within the submission was usually not 100%. Copepod infestation clustered in the second half of 2012, affecting one farm particularly (Figure 3 a-e).

Fungal or oomycete infection was seen in association with gill infarction, whether as cause or effect was uncertain (Figure 4). Infarction is reputedly common in fish gills (Dr Hugh Ferguson, *pers comm*), although only two instances were recognised in this survey, and secondary infection is likely.

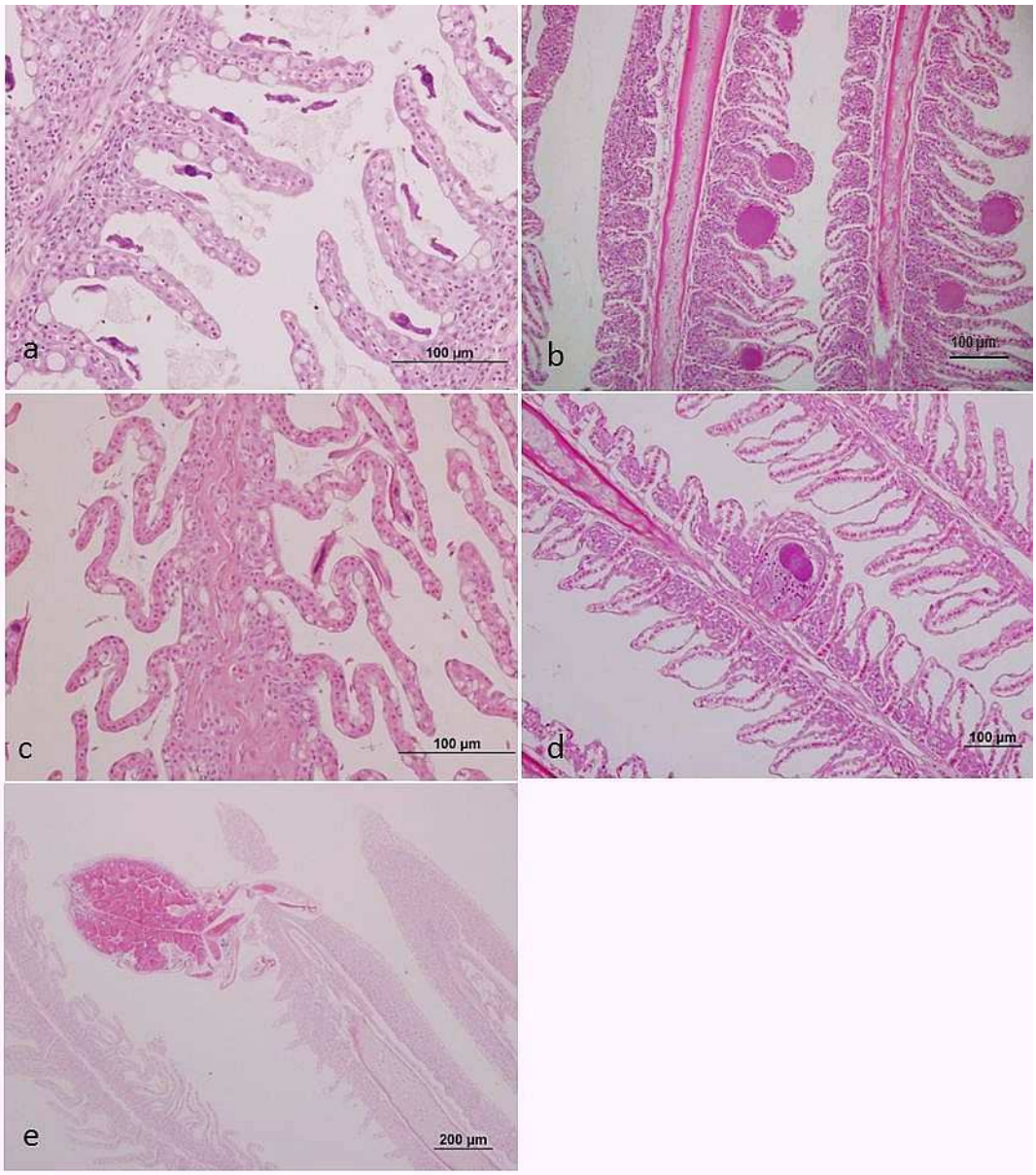

Figure 3. Parasites seen in gills of Murray Cod in this survey included a) *Chilodonella* spp., b) *Epitheliocystis,* c) *Trichodina sp,* d) *Ichthyophthirius multifiliis* and e) ergasilid copepods. Except for *Chilodonella*, most caused little if any inflammatory reaction beyond the local site of damage.

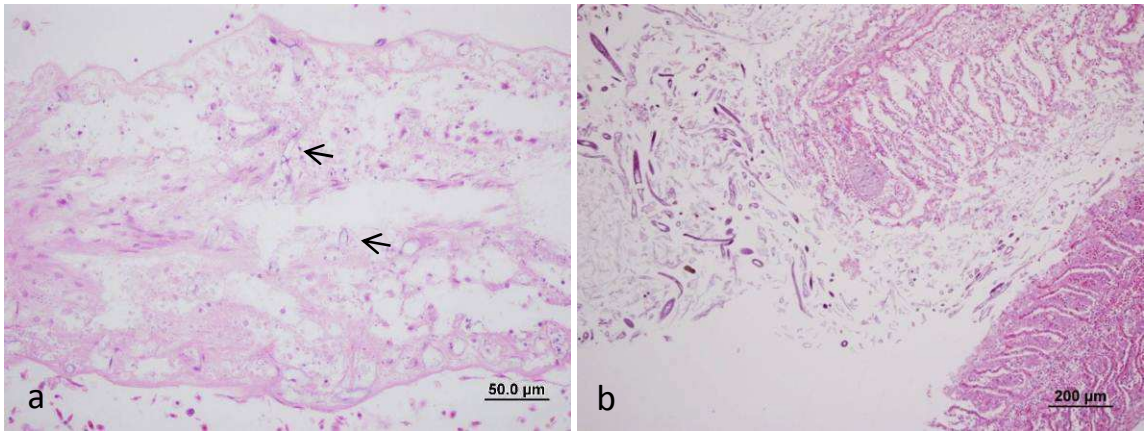

Figure 4. Hyphae (arrows) were seen in necrotic and devitalised lamellae. b) Hyphae sometimes formed mats across the necrotic surface. Remaining viable gill was not normal, but showed no evidence of mycotic invasion.

Telangiectasis (capillary dilation or aneurysm in lamellae) was seen in 14 (16.7%) of fish (Figure 5).

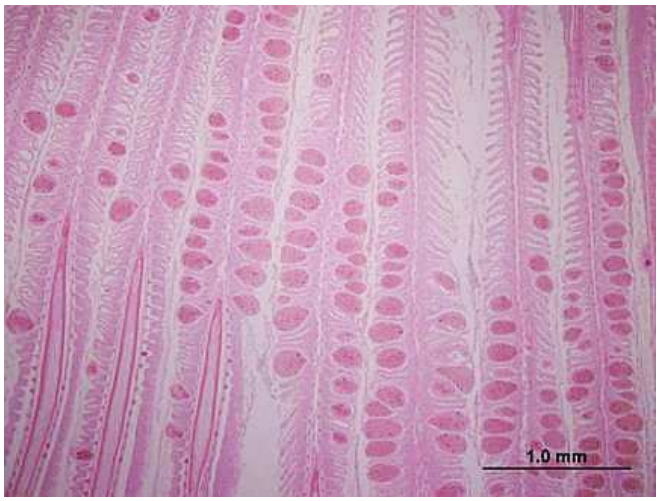

Figure 5. Telangiectasis ranged from mild, showing a few small expanded vessels, to florid and extensive, with dilated capillaries in most or all lamellae.

In two submissions, both from the same farm, gills had extensions of lymphoma. These were RAS fish; pond-raised fish on the same farm were unaffected (Figure 6).

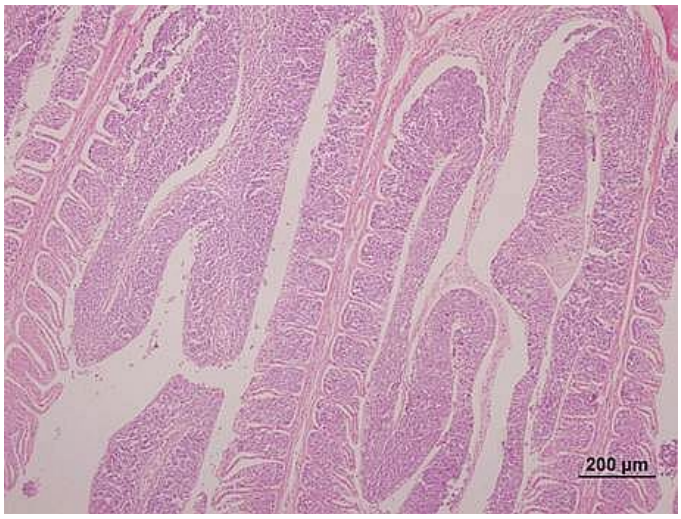

Figure 6. Lymphoma in the gills is a rare extension from a primary site. Dense sheets of small lymphocytes infiltrate the gill arch and lamellar lamina propria, obliterating the epithelium and stroma.

SKIN AND BODY WALL (Figure 7) - Lesions of skin and subcutis were next most prevalent, with 49 submissions (58%) having changes. Lymphocytic dermatitis was seen in 34 submissions (41% of submissions, 69% of dermal lesions) and ulcerative dermatitis in 7 (14% and 8%). As with gills, aetiological agents were rarely identified but parasites, including *Chilodonella*, ich (*Ichthyophthirius multifiliis*) and copepods were seen in 6 submissions (12% and 7%). Secondary infections with oomycetes consistent with *Saprolegnia* sp. were seen in 7 cases (14% and 8%) and one farm had an outbreak of epizootic ulcerative syndrome (*Aphanomyces invadans*).

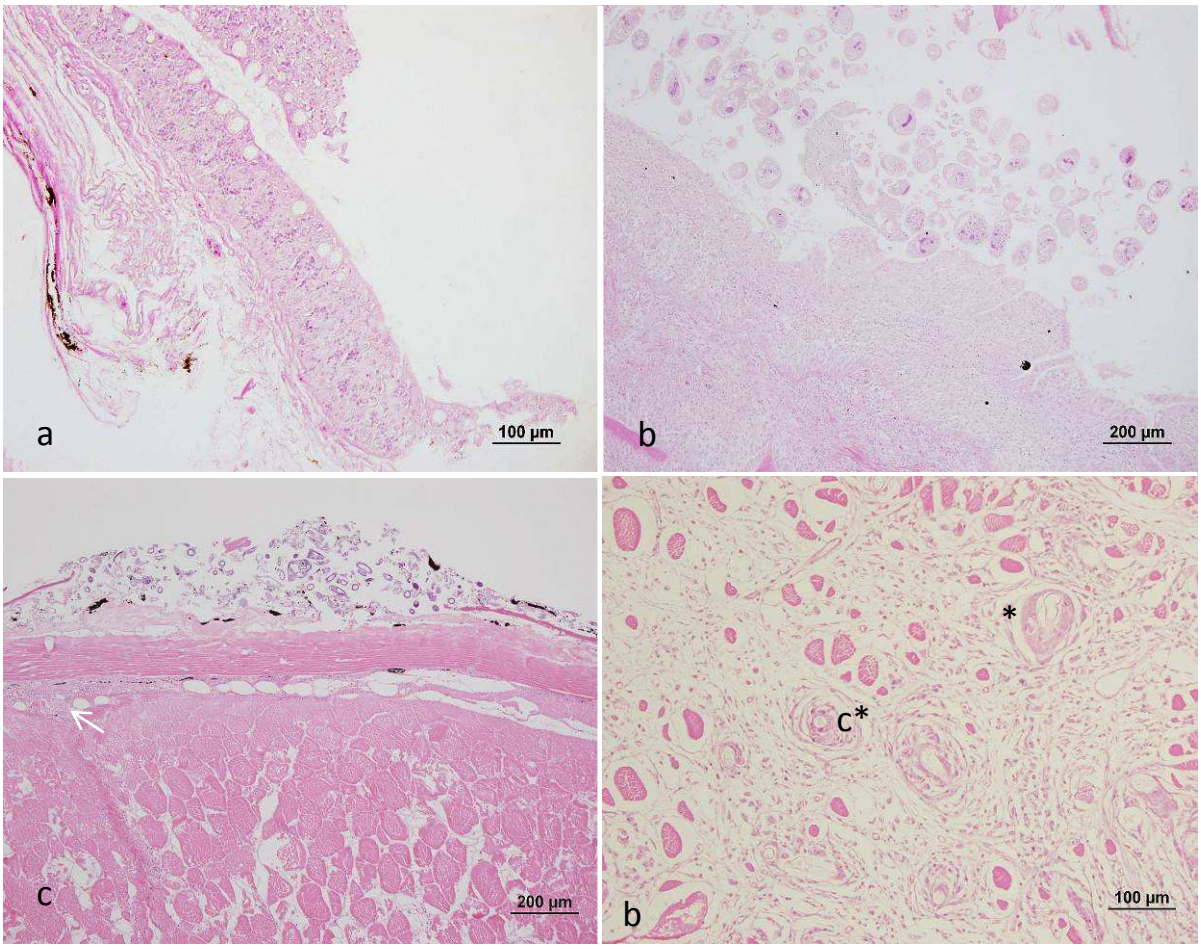

Figure 7. Lesions of skin and muscle a) The most common lesion of skin was non-specific lymphocytic dermatitis, generally mild and often more prominent in the basal layers b) *Ichthyophthirius multifiliis* was sometimes seen, particularly on the fins c) *Saprolegnia* sp. was usually associated with loss of epithelium, as seen here. Muscle necrosis and mild myositis (arrow) were uncommon. d) *Aphanomyces invadans.* Oomycete hyphae with characteristic encircling granulomatous response (*) are accompanied by muscle degeneration and a moderate lymphocytic inflammatory infiltrate.

INTESTINES (Figure 8) – Intestines were the visceral site showing most lesions, with 38 of 84 submissions (45%) exhibiting intestinal lesions in one or more fish. Parasitism was the most frequent specific finding, with 15 accessions (18% of submissions, 39% of intestinal lesions) demonstrating nematodes in the intestinal lumen, 6 (7% and 15.8%) having coccidia and 2 (2% and 5%) having cestodes. Seventeen accessions (20% and 45%) showed lymphocytic infiltrates in one or more regions of the intestinal tract, generally of mild to moderate degree. Ulceration was seen in 4 fish, with two having intestinal ulcers and two having rectal ulcers. In a separate submission, two fish of three had intestinal rupture, with one of these having oomycetes and ich - like protozoa within the lesion.

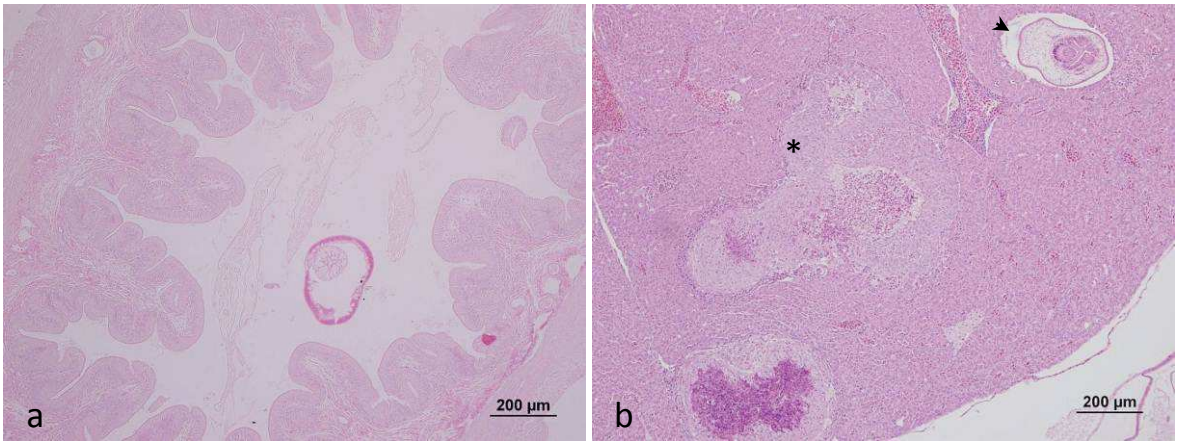

Figure 8. a) Nematode profiles in the intestinal lumen were not associated with mucosal damage b) Cestodes (arrow head) in fingerling liver created necrotic foci with histiocytic reaction (*) along their migratory path.

KIDNEYS (Figure 9) - Although 22 submissions (26%) showed renal lesions, no one cause was dominant, with 6 cases of focal granulomata, 5 of myxozoan infection and 3 each of acute multifocal tubular necrosis, haematopoietic necrosis and membranous glomerulopathy.

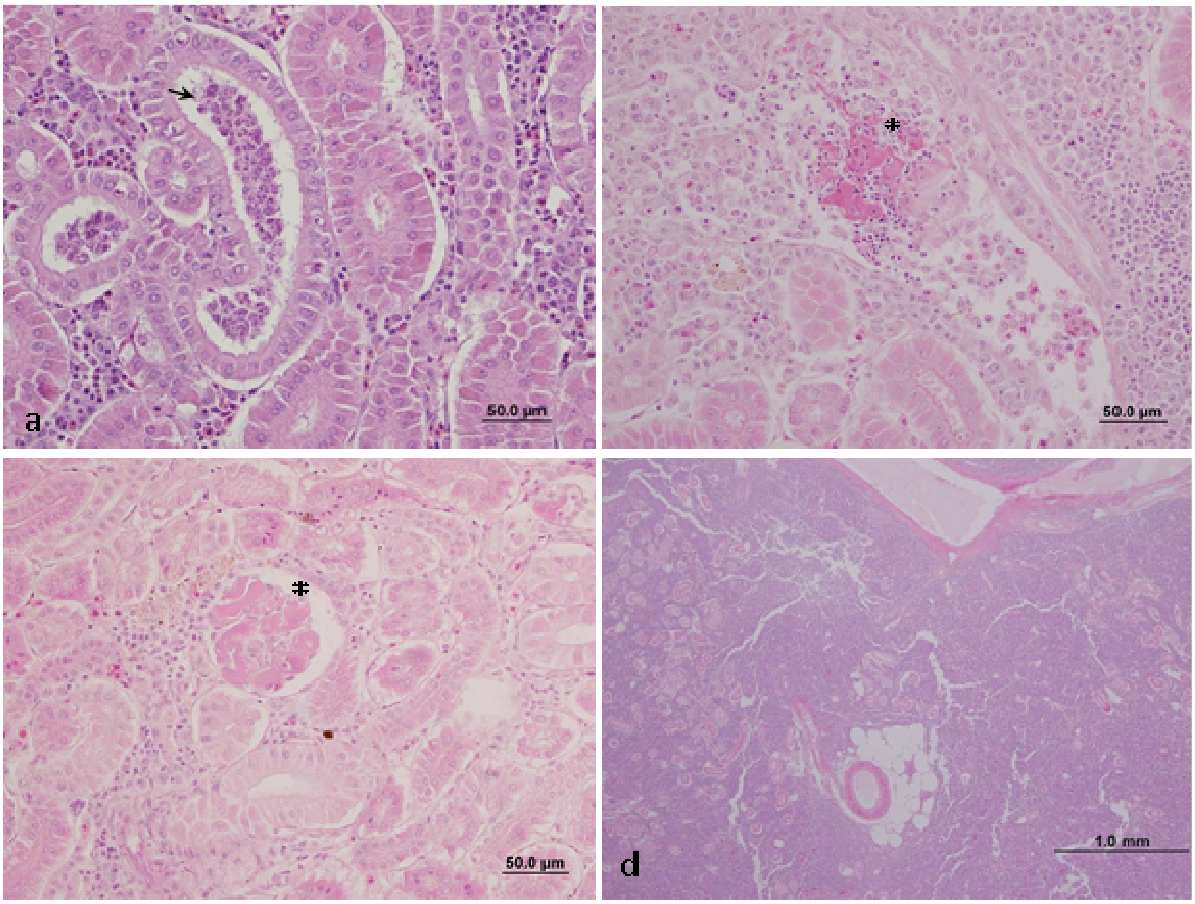

Figure 9. Renal lesions a) Myxozoa were seen in tubular lumina, apparently without effect on the epithelium. b) Epithelial necrosis (*), when present, was generally mild but could be associated with sloughed and degenerate epithelium and a moderate inflammatory reaction in a multifocal and segmental pattern. c) membranous glomerulopathy (*) was seen as thickened glomerular basement membrane, without associated proteinuria or nephropathy in most cases. d) Lymphoma was seen in the kidney at all levels in one fish with affected gills.

Acid fast staining did not highlight mycobacteria in the granulomatous lesion; in one fish weakly acid fast organisms were seen and PCR and sequencing found a close match to an undescribed environmental organism, Bacterium strain 77003 (GenBank Accession # AF227847).

One of the fish with gill lymphoma had neoplasia also surrounding renal tubules.

OTHER SITES -Focal and locally diffuse lymphohistiocytic infiltrates were also seen in other viscera, but rarely demonstrated mycobacteria on special staining. They were the main lesions of coelomic tissue, particularly mesentery, with 6 instances (7%) of locally extensive steatitis, 6 focal or multifocal granulomatous lesions and 2 examples (2%) of fat necrosis.

Other visceral lesions were sporadic and uncommon. Lymphocytic infiltrates in the interstitium of the exocrine pancreas (Figure 10), sometimes associated with acinar necrosis, were seen in 6 cases (7%) and was associated with similar infiltrates in the epicardium and around the bulbus arteriosis in three cases. Histiocytic and granulomatous infiltrates were also seen in hearts, rarely.

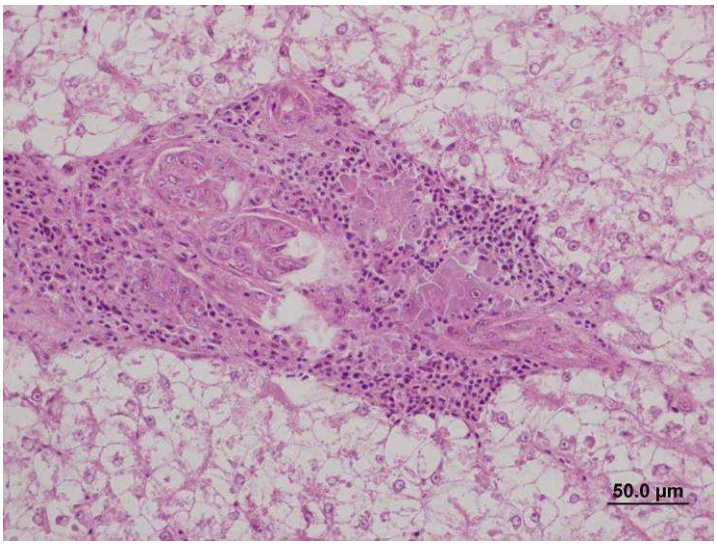

Figure 10. Lymphocytic infiltrates in pancreas were rare findings.

Eyes were not submitted from every fish so the frequency of lesions cannot be determined. However, of those examined, most showed keratitis and/or corneal erosion or ulceration. Only one fish had intraocular inflammation (Figure 11).

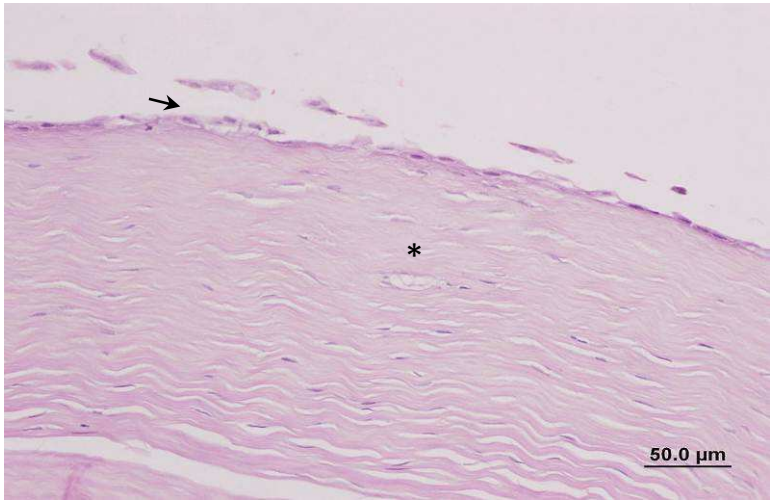

Figure 11. Corneal changes were common. Epithelium was frequently roughened or lost (arrow), with stromal vascularisation (*) and increased cellularity.

Comparison of histology and gill clip:

In total 176 fish were examined by both gill clip and histopathology. The results are given below in Table 1 using histopathology as the gold standard.

Table 1. Results for 176 fish that were tested for chilodonellosis by both gill clip and histological examination (histo) of gills

	Positive histo	**Negative histo**	**TOTAL**
Positive gill clip	20	9	29
Negative gill clip	22	125	147
	42	134	176

Diagnostic parameters for gill clip are (95% CI):

Diagnostic sensitivity = 0.48 (0.32 – 0.63);

Diagnostic specificity = 0.93 (0.87 – 0.97);

Positive predictive value = 0.69 (0.49 – 0.84);

Negative predictive value = 0.85 (0.78 – 0.90).

Using the more sensitivity method of diagnosis (histopathology) it was ascertained that 24% of individual fish examined had evidence of *Chilodonella.*

Chilodonella speciation:

Using silver nitrate impregnation and light microscopy (Figure 12a), Dr Miller has identified parasites on one gill as *Chilodonella piscicola* and confirmed this with amplification and sequencing of key genomic sequences to create a dendrogram of relationships between this and other *Chilodonella* species isolated in Australia and elsewhere (Figure 12b). Further submissions totalling 10 gill samples revealed parasites of identical DNA sequence from a range of farms and dates.

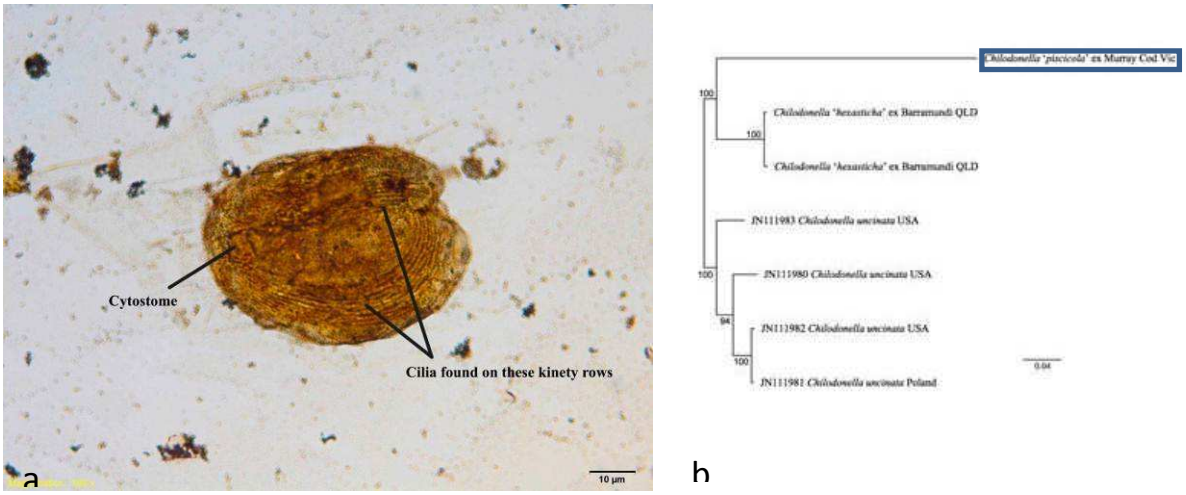

Figure 12. a) Silver impregnation shows cytological features of *Chilodonella* sp. consistent with *C. piscisola.* b) Relationship of the sequence amplified from Murray Cod *Chilodonella* with *C.hexasticha* isolated from Australian barramundi and *C.uncinata* from international specimens. *Images courtesy of Dr Terry Miller, School of Marine and Tropical Biology, James Cook University, Townsville, Qld.*

Farm Surveillance

Farm census

In total 20 surveys were completed. The results provided in this report refer only to 7 grow – out open aquaculture farms. These farms comprise some (but not all) of the 6 "project" farms that were part of the histopathology project. A summary of the 6 project farm characteristics is provided in Table 2. There were various other enterprises undertaken on the integrated aquaculture farms such as horticultural enterprises: vineyards, olives, forestry and cereal crops, or livestock production such as beef and sheep enterprises.

The range of ages that farmers had been farming Murray Cod varied from 1.5 – 40 years with a median of 6 years. The education skills of managers varied from Year 10 at secondary school to tertiary qualifications with just over half the farmers stating they had some form of aquatic health training although this wasn't further specified.

Seed stock supply for open pond farms came from 3 suppliers (or was self - supplied) and weighed (at stocking) between 100g and 600g. Initial stocking densities varied from 4 – 30kg/m^3 with final densities ranging from 7 to 50 kg/m^3. Three of the 7 farms in this category graded prior to stocking and checked the health of new stock visually. Four farms treated new stock with a range of treatments including formalin, peroxide and salt. Only one of these farms quarantined new stock; most farms expressed confidence in the seed supplier and lack of facilities as reasons why they did not quarantine.

Feed was supplied to grow-out fish by hand or auto-feeder with 2 farms using feeding frames. The stated percentage of body weight fed per day (by those farms able to provide this figure) was between 0.5% and 5% with a calculated feed conversion efficiency (FCR) of 1 – 1.3. Across all farms the majority sourced feed from 2 suppliers.

Inflowing water was treated by one farm (screening) and 3 of the 7 farms had storage or settlement ponds for inflowing water. Four farms treated ponds before stocking with weed killer and lime and none of the farms treated cages or nets before or during culture. Three farms tested the quality of the water supply for a range of parameters. All farms tested the quality of water in the ponds when fish were resident for dissolved oxygen, temperature and pH, in most farms daily. Other parameters: nitrite, nitrate, alkalinity, ammonia etc. were tested less frequently but this varied between farms. Five of the farms improved water quality using methods such as paddle wheels and blowers. Out - flowing water was treated on 4 of the farms by screening or settlement ponds and then used in horticultural enterprises in 5 of the farms; one farm recycled and reused the water and one farm disposed of waste water to a stream.

Reported mortality rates varied considerably. Across the whole production cycle fish deaths varied from “low” to greater than 70%. However the general consensus amongst those farms experiencing losses (the majority) was that most losses occurred in the first 2 weeks of life in the dam (20% to 60% losses) from causes such as shock and parasites whereas in the mid to late production cycle losses were much lower (<10%). Mortality and other observations such as feeding behaviour were generally made on paper and farmers wished to use this data but in some cases were not sure how to do so.

Four farms checked fish health 2 – 3 times per week by lifting the cages out of the water. Health was also checked by performing skin and gill scrapes and observing behaviour at feeding by some farmers. Generally farmers stated that they could detect disease in their fish from their own experience. Technical information about fish health came from a range of sources: government, the internet, other farmers, consultants and books. Nearly all the farms had a microscope (6 of 7) with all having a compound and one farm a dissector as well. The frequency of use of microscopes varied widely amongst farmers and training in microscopy had been provided by DEPI (formerly DPI) or TAFE institutes. Some of the farms employed a vet (3 of 7) but generally only requested visits once per year.

Table 2. Summary of 6 project farm characteristics

Farm	**1**	**2**	**3**	**4**	**5**	**6**
Farming system	Cages in dam	Cages in dam	Cages in dam	RAS	RAS and Pond	Cages in dam
Annual production	TBD	3.4 tonnes	10 tonnes	8 tonnes	3.5 tonnes	TBD
Fish life stages	Stockers	Broodstock/ larvae to stockers	Stockers	Broodstock larvae to stockers	Larvae, fingerlings, stockers	Stockers
Water source	River water via channel	River water via channel	River water via channel	Bore	Bore and settlement pond	River water via channel
Water pre-treated	No	No	No	Yes	Yes	No
On-farm health checks	Yes	Yes	Yes	Yes	No	Yes
Mortalities event >20%	No	No	Yes	No	Yes	No
Operating post-study	No	Yes	No	Yes	Yes	Yes

Disease prevention on the survey farms (6 of 7) was instituted with measures such as preventative treatment and restricting entry of other species. It was acknowledged that the latter is very hard to do.

The results for the reported occurrence of the specified diseases (a range of common parasites, “sudden death” and “red spot”) varied considerably across farms. These results should be considered in light of the “farmer quiz” which found that many farmers could not accurately identify some common parasites or artefacts in a microscope field at the start of the project. It may be that there is misdiagnosis of parasites and other diseases occurring where the results are not verified. Most (6 of 7) of the grow-out farms reported experiencing *Chilodonella* at different times of the year with an occurrence classified from rare (less than once per year) to very common (occurrence > 3 episodes per year) . The reported treatment was salt, formalin and peroxide in a range of dosage regimes. Ich (*Ichthyophthirius Multifiliis)* (4 of 7 farms) occurred rarely with low severity and generally in the warmer months. This was treated as for *Chilodonella, Lernaea* (4 of 7) occurred in the warmer months rarely to frequently (1 – 3 episodes per year) and was believed to be low severity (<10% mortality). Hydrogen peroxide and chlorine treatments were used for the treatment of this parasite. Sudden death (a diagnosis that was not further refined) occurred on 2 farms and was believed to be of low severity. Aggression was believed to occur frequently but with low severity on 5 of the farms usually occurring post grading or later in the cycle when fish size varies. The solution for aggression was believed to be grading however this was constrained in open pond culture systems.

There was a limited ability for farms to quarantine sick fish with only 2 of the farms having the capacity to do this. All farmers buried their dead fish on the farm and only one of the farms stated it had a biosecurity plan although this was not seen.

Annual production figures varied from 3.4 to 20 tonne per year (including RAS but not figures for the hatcheries). The length of production had a range of 7 months to 17 months for the grow-out facilities. Production costs were not known or stated by most farmers but were estimated to be $8 per fish over 6 months for one farm. The minimum selling price to wholesalers or processors over the 2011/12 production cycle was $16.5 – $17/kg with a maximum of $22 – $27/kg.

Farmer quiz

In total 15 farmer assessments from a range of farms were completed. One farmer refused to complete an assessment.

The quiz was presented in 2 parts:

i) Microscopy and diseases for which farmers received scores between 15% and 65% (median 58%)

ii) Water quality where farmers scored between 33% and 100% (median 67%)

It is apparent that farmers had a greater knowledge of water quality parameters than issues relating to fish disease and identification. Although most of the farmers have access to microscopes, in general they had difficulties recognising basic structures in the provided field of view including common parasites and an air bubble artefact. Quite a few of the farmers did not know the correct way to submit fish to a laboratory to enhance the chances of a diagnosis. Those managers who were not involved in the daily running of the farm were understandably less able to accurately complete questions on water quality.

The same quiz was re- administered to the ongoing project farmers in September this year (some two and a half years after the first test). As there were only 7 grow – out farms participating in the project this reduced the pool of potential farmers to be retested. There had also been numerous staff movements over this period so there was only retest data available from 3 farmers. Although the numbers are low, it was interesting to note, even as a qualitative observation, that there was a strong improvement in results for these 3 farmers with an increase from 55% to 92% correctly answered questions.

Farm surveillance data

The nature and quality of the data available varied substantially across the project farms. Four of the farms that provided fish for the project did not record any data at all.

During the period of the whole project a total of 6 farms provided data. Farm 3 closed down following a severe disease outbreak and provided a limited data set. Farm 4 only recorded data for the purposes of the project for a period of 6 months. Farm 6 was a new entrant towards the end of the project and so also had a smaller data set. As previously noted, the quality of the data provided limits the conclusions that can be drawn.

Mortality data

Mortality as either raw numbers or rates is illustrated plotted against a range of water quality variables, causes of mortality and treatment types in Figures 13 – 19.

Farm 1

Farm 1 is a cage - culture farm with 8 cages. Mortality rates and events with different water quality parameters are shown in Figure 13. There were major fish losses that occurred on this farm related to a power failure affecting DO levels and treatment of channel water with copper sulphate to reduce algae infestation. Other smaller causes of reduced numbers included management failures such as loss of fish from cages when there were holes in the nets and overdosing with treatment chemicals. Overall cumulative mortality rates for this farm from March 2012 – May 2013 totalled 22% based on farmer mortality figures.

The amount of Murray Cod sold by Farm 1 in kilograms totalled 4679kg during the period of investigation.

Farm 2

Farm 2 is a cage- culture farm with 16 cages. This farm has been in production for over 10 years. Some water quality data was retrieved from *in-situ* water loggers. Figure 14 displays mortalities versus temperature and DO. Total mortality rates could not be calculated for this farm.

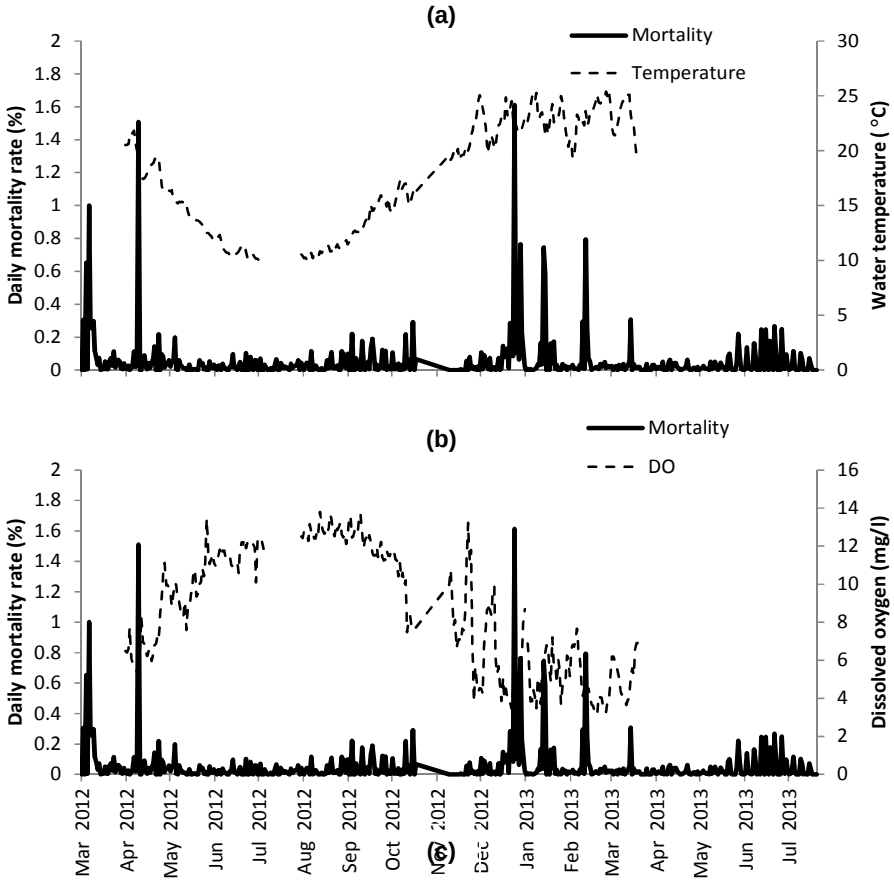

Figure 13. Daily mortality rate combined for all cages vs (a) water temperature, (b) DO, (c) mortality events Farm 1

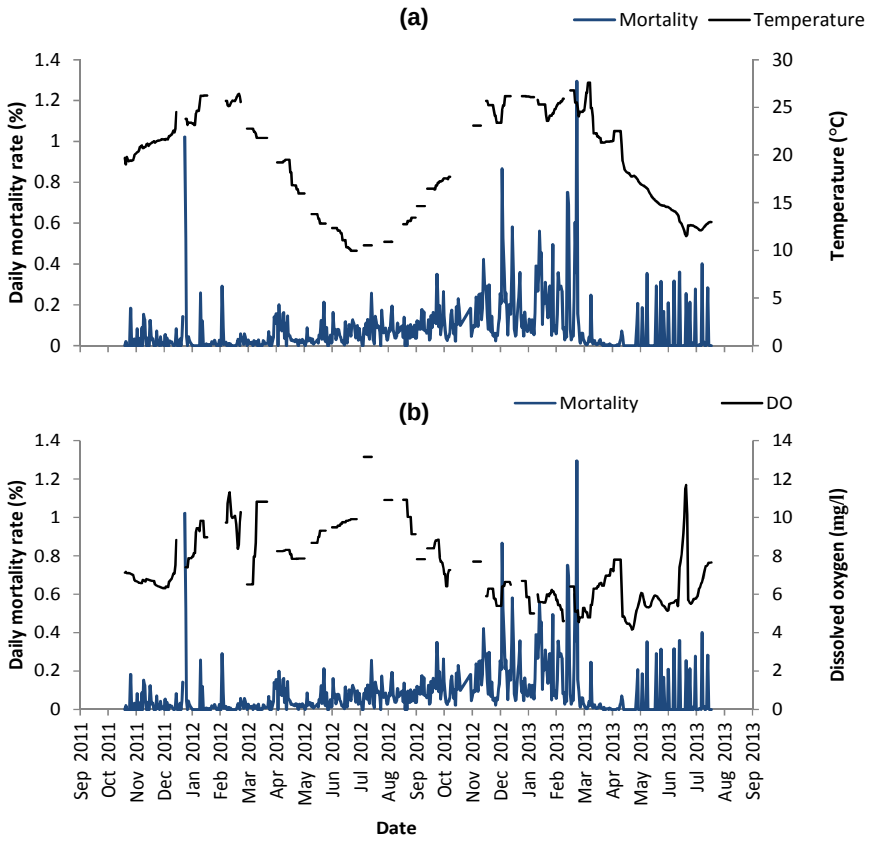

Figure 14. Daily mortality rate vs (a) water temperature and (b) dissolved oxygen for Farm 2. The maximum daily mortality occurred on 03/01/12 (4.5%) when the farm was unattended and the cause was unknown. This data has been removed from the charts to increase the readability of the scale.

Farm 3

Farm 3 had 4 lines of cages (annotated as 1 – 4 with 20 cages in total). Data for this farm was recorded from September 2011 until February 2012. Approximately 13,500 mortalities occurred during December 2012 (Figure 15). All remaining fish died by the end of January 2012 (records ceased from mid - January), most probably due to *Aphanomyces invadans* (red spot) which was diagnosed by histopathological analysis. Prior to this event *Chilodonella* was frequently encountered, but mortality was less severe.

Selected water quality parameters during the study period are presented in Figure 16. Mortality during December and January was associated with lowered pH and DO. Treatment for *Chilodonella* was occurring very frequently during this time and presumed to be a factor related to disturbance of the epithelium and infection with *Aphanomyces.*

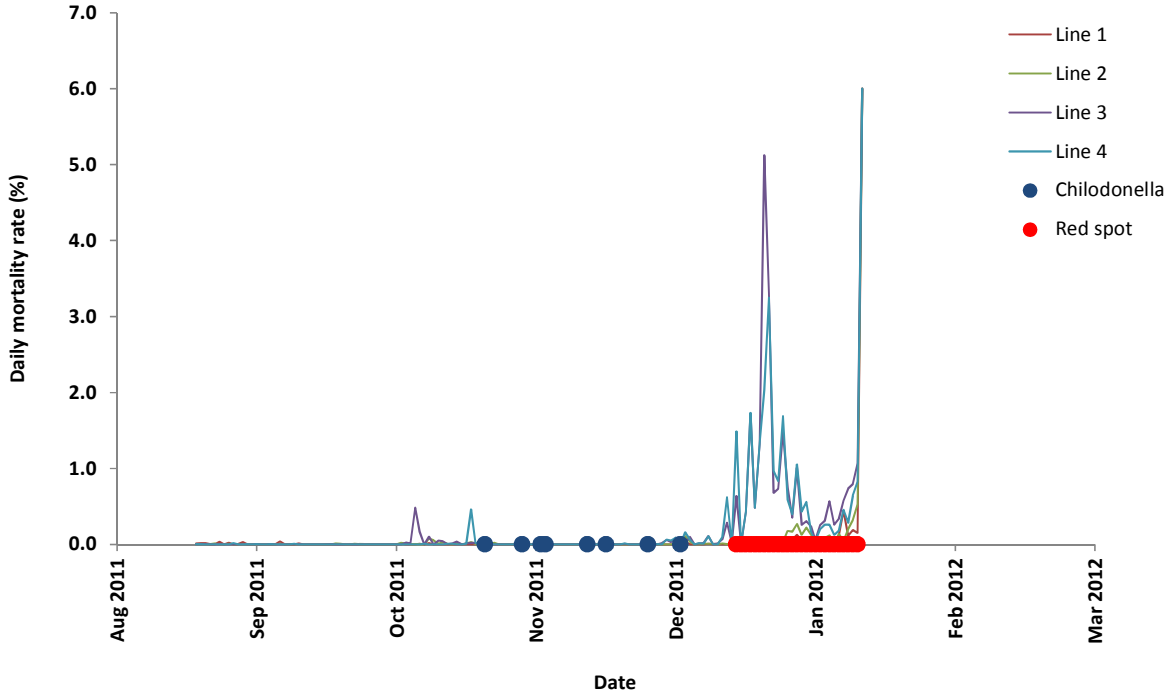

Figure 15. Daily mortality rate of Murray Cod occurring in lines of cages 1 – 4 Farm 3. Red circles indicate fish affected with red spot, blue circles indicate when *Chilodonella* was detected in fish.

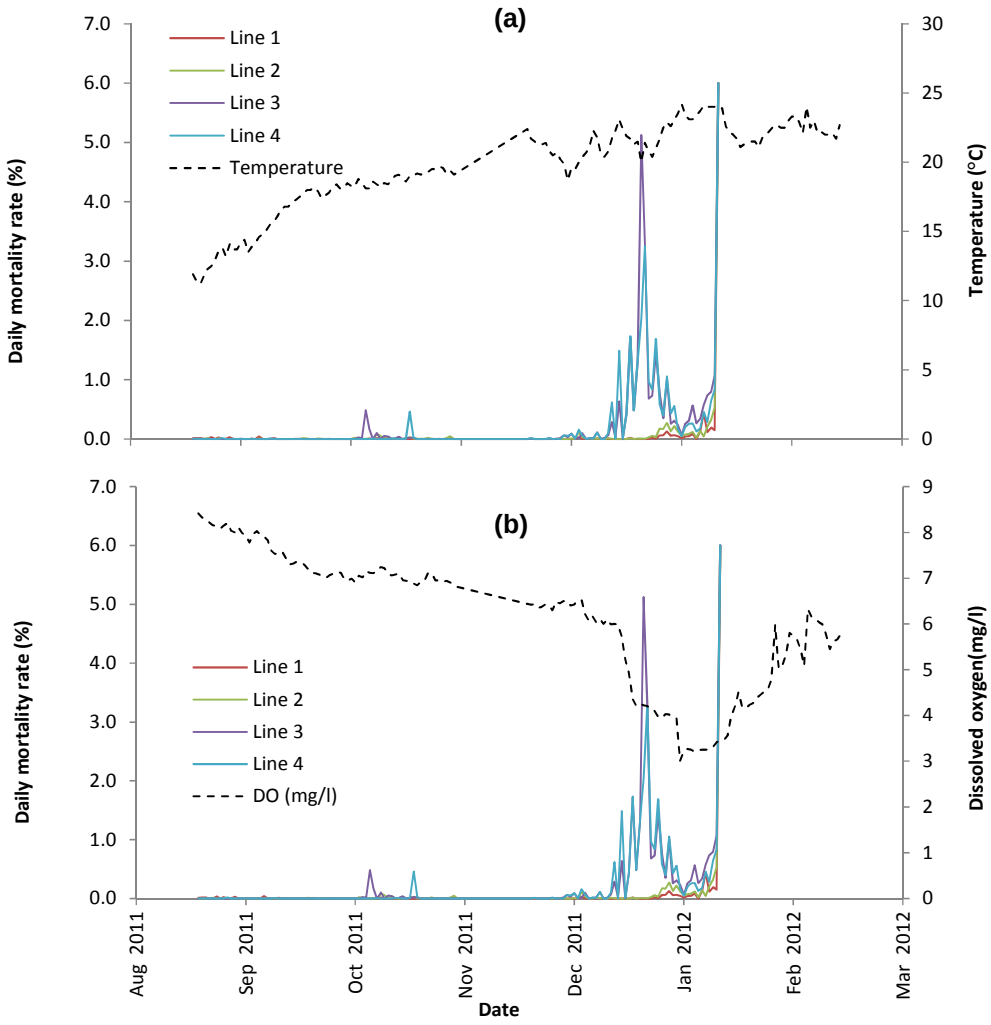

Figure 16. Farm 3 cage lines 1 – 4 daily mortality rates vs (a) temperature, (b) dissolved oxygen

Farm 4

Farm 4 is a RAS farm with data collected from 29/06/12 to 06/12/12. As can be seen, this farm experienced very low mortality rates in common with other Murray Cod RAS farms (Figure 17). Total farmer recorded mortalities was less than 1% from June to December in 2012. The average size of fish in tanks was 518g with an average of 216 animals per tank (median 270). Farm 4 only treated with formalin (50ppm) and Chloramine T during July and August 2012.

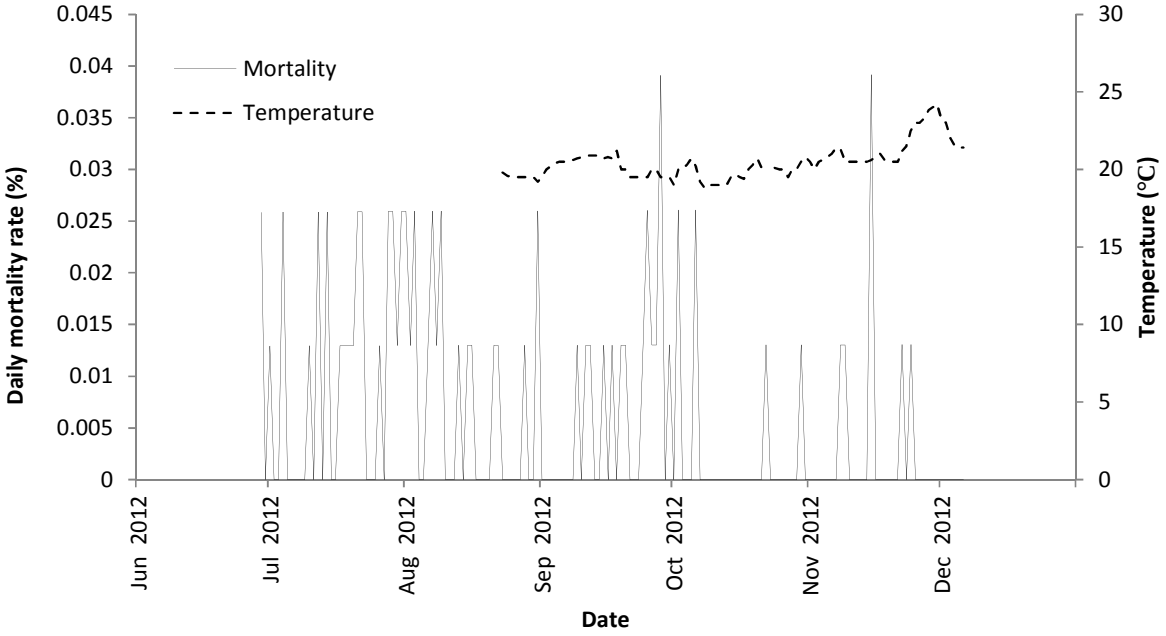

Figure 17. Daily mortality rate for all tanks combined vs water temperature for Farm 4.

Farm 5

Farm 5 is a combined open dam system and RAS enterprise. Data was collected from August 2011 until May 2013. The data presented below refer to the RAS part of the farm only as data for the pond system was not available. Mortality rates could not be calculated for this farm, instead number of mortalities per day are presented. The large spike in mortalities in December 2011 was due to an overdose with formalin and low DO levels (Figure 18).

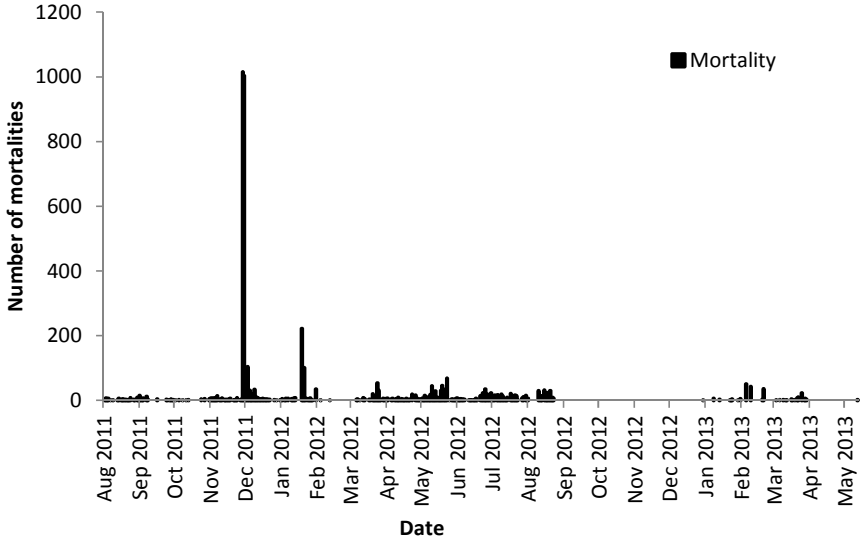

Figure 18. Raw mortality count for Farm 5

Farm 6

Farm 6 is the most recent addition to the project with data collected from December 2012 to late August 2013. This farm has a large dam with 8 cages. Algal blooms were reported to occur on 28/01/13 and 17/03/13. Elevated mortality occurred in early December and early January however the mortality rates experienced on this farm are very low compared to other open dam systems. In total 839 mortalities were recorded (Figure 19). The causes of these mortalities was believed to be parasitic in origin . Over the recording period, the average mortality occurring in each tank was 104 fish with a range of 74-161. The total mortalities recorded across the period were 4%. This farm has not experienced any problems with *Chilodonella.*

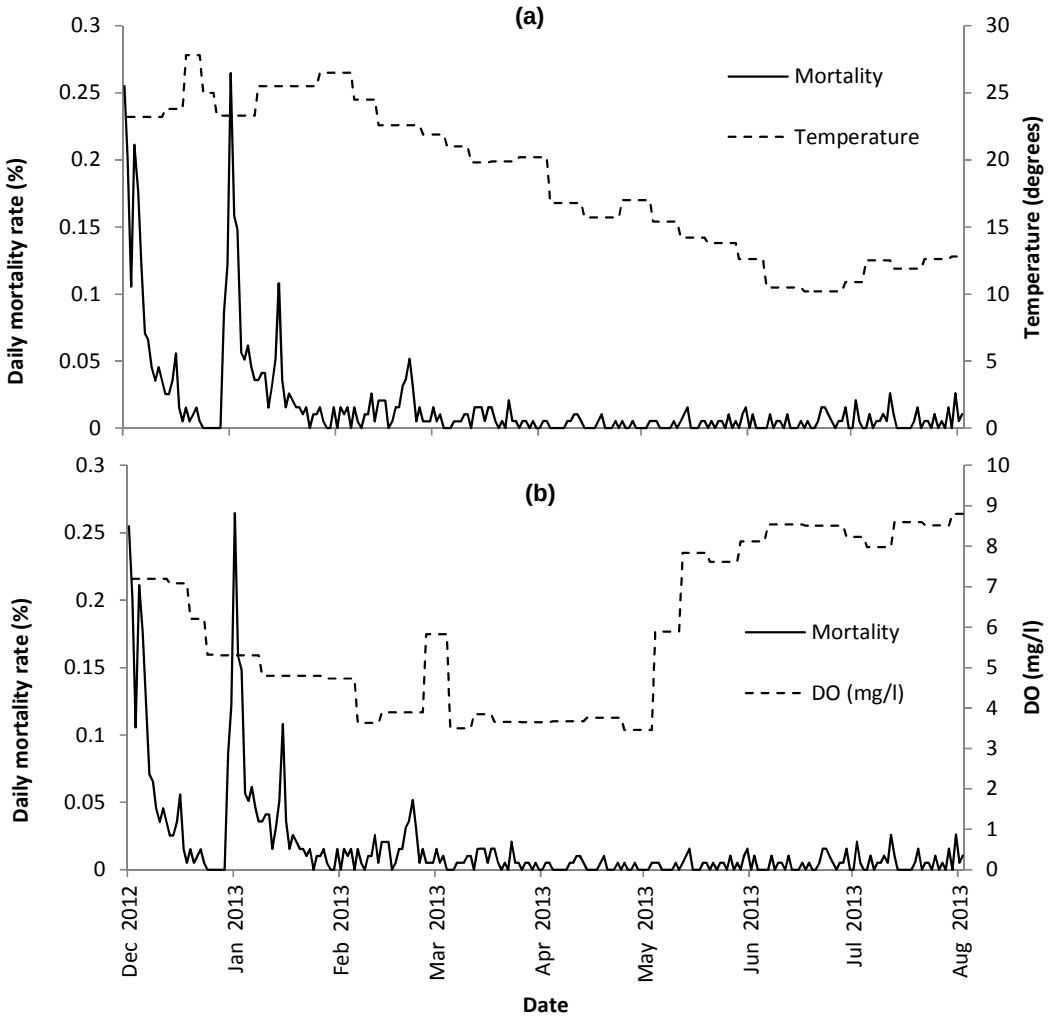

Figure 19. Daily mortality rate vs (a)temperature and (b) dissolved oxygen (DO) for combined cages (1 – 8) Farm 6

Water quality parameters

Water quality variables recorded from open water cage farms (4 farms) and RAS farms (2 farms) are presented in Table 3. The table highlights the differences seen in water quality parameters between open water and RAS. Notably, water temperature was less variable than in RAS, DO was more variable in open water and pH was lower in RAS. The parameters seen here were within the bounds of what has been reported previously for Murray Cod farming (Ingram *et al.* 2012).

Table 3. Water quality parameters measured at farms growing Murray Cod in open waters and in RAS. Values represent mean ± standard error with range in brackets

Parameter	Open water	RAS
Water temperature (^{o}C)	18.4 ± 0.05 (9.5-27.8)	20.9 ± 0.02 (17.2-27.2)
DO (ppm)	7.1 ± 0.03 (3-18.8)	5.7 ± 0.04 (3-8.7)
DO (percent saturation)	no data	70 ± 0.4 (41-106)
pH	7.4 ± 0.01 (5.2-9.74)	6.2 ± 0.03 (4-8)
Conductivity (µs/cm)	640 ± 5 (160-5532)	no data
Salinity (ppt)	0.3 ± 0.01 (0-2.98)	1.5 ± 0.05 (0.2-8.8)
TAN (ppm)	1.66 ± 0.11 (0.01-9.84)	0.38 ± 0.13 (0.25-0.5)
Nitrate (ppm)	0.81 ± 0.01 (0.01-2.36)	2.63 ± 2.38 (0.25-5)
Alkalinity (ppm)	43 ± 1 (23-95)	44 ± 2 (17-107)
In vivo Chlorophyll a (*µ*g/L)	33.2 ± 0.5 (10.1-54)	no data
Secchi Disk depth (cm)	77 ± 2 (10-99)	no data

Chemical usage

The main chemicals used by open system farmers in this project were hydrogen peroxide (H_2O_2), formalin, copper sulphate ($CuSO_4$) and salt (Table 4). These were generally used alone, but in some instances were combined. Formalin, salt and H_2O_2 were used to treat fish in cages whereas $CuSO_4$ was used to treat the dam in which the cages were located. The percentage of treatments for each chemical and concentrations of chemical used are presented in Table 4.

Table 4. Chemical usage (%) for four commercial Murray Cod farms

Culture system	Chemical	Percent of treatments (%)	Dosages (% of treatments)
Open water	H_2O_2	30	100-199 ppm (1) 200-299 ppm (98) 300-399 ppm (1)
	Formalin	27	100-199 ppm(9) 200-500 ppm (91)
	$CuSO_4$	23	0.13-0.15 ppm (7) 0.25-0.35 ppm (93)
	Salt	17	5-9 ppt (0.6) 10 ppt (97.5) 11-12 ppt (1.9)
	Other combinations	1	Formalin & H_2O_2, formalin & salt
	H_2O_2 & Salt	2	H2O2 (200 ppm) & salt (10 ppt)
RAS	Chloramine-T	2	No data provided
	Formalin	87	50 ppm
	Salt	4	10 ppt
	Unknown	7	

Most chemicals (H_2O_2, formalin and salt) were used to treat *Chilodonella* and other ciliated protozoans (Trichodina and Ich) (Table 5). $CuSO_4$ was extensively used on one farm to treat redspot and on other farms to treat ich (white spot). Metazoan parasites (*Ergasilus*, *Lernaea* and monogeneans) were rarely encountered and tended not to be treated.

Table 5. Chemicals used at the time when different parasites and diseases were observed in health checks (results summarised for 3 open water farms)

Chemical	*Chilodonella*	Redspot	*Trichodina*	Ich	*Ergasilus*	Monogenea	*Saprolegnia*	*Lernaea*
$CuSO_4$	0	98	0	2	0	0	0	0
Formalin	100	0	0	0	0	0	0	0
Formalin & Salt	100	0	0	0	0	0	0	0
H_2O_2	67	0	0	11	0	0	0	22
Salt	100	0	0	0	0	0	0	0

In RAS, formalin was used in 87% of treatments followed by salt and Chloramine-T in 4% and 2% of treatments, respectively.

The time between re-treatment of cages on open water systems varied considerably over the year, with treatments being more frequent in warmer months. On average, cages were retreated less than every 6-12 days from November to June, and 14-23 days from July to October (Figure 20).

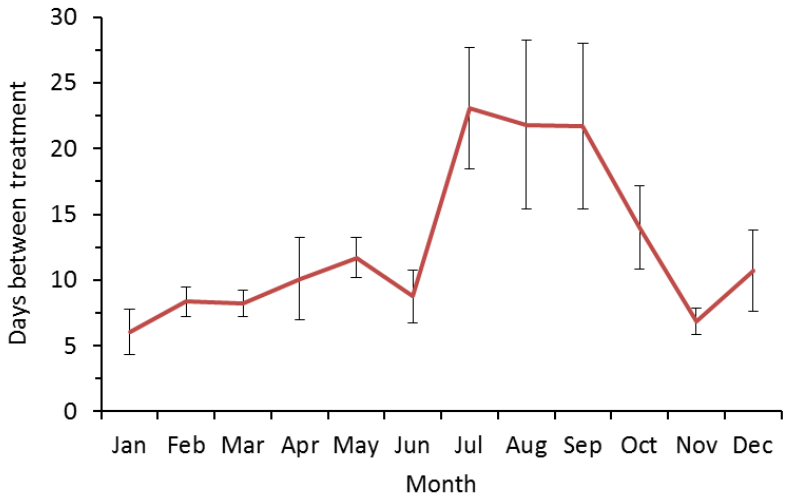

Figure 20. Time between chemical retreatment of cages in open water Murray Cod farming systems (mean of cages from 3 farms ± s.e.).

Disease checks conducted on farm

Chilodonella was by far the most frequently observed parasite during health checks of Murray Cod from three open water farms followed by *Trichodina* and Ich (Figure 21). Metazoan parasites, *Ergasilus*, monogeneans and *Lernaea*, were rarely recorded as having occurred on the farms.

Chilodonella was observed in Murray Cod health checks in all months of the year, especially through spring and summer (October to March) (Figure 22). *Trichodina* was also observed in most months of the year, but was most prevalent during summer and early autumn (December – March). Ich was observed mainly in January and April. *Ergasilus* was most commonly observed in March.

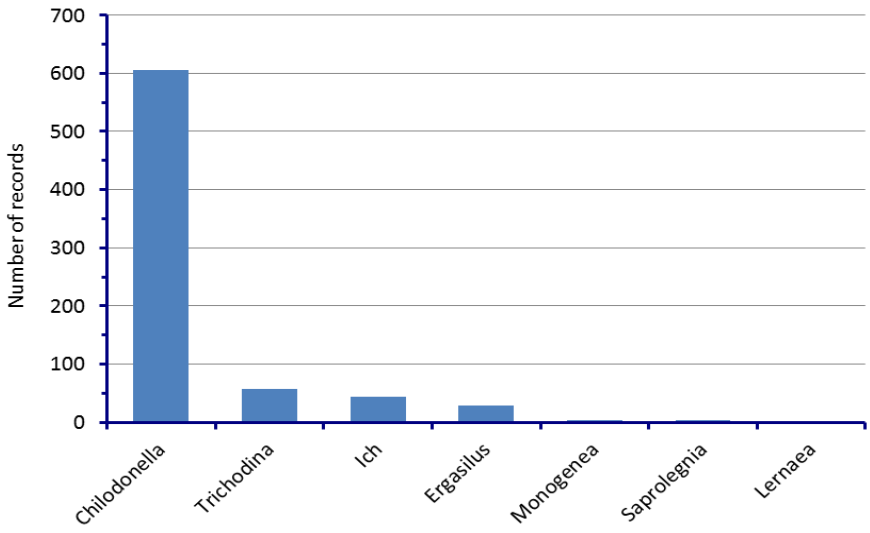

Figure 21. Number of records of parasites and diseases in health checks on Murray Cod farmed in open waters.

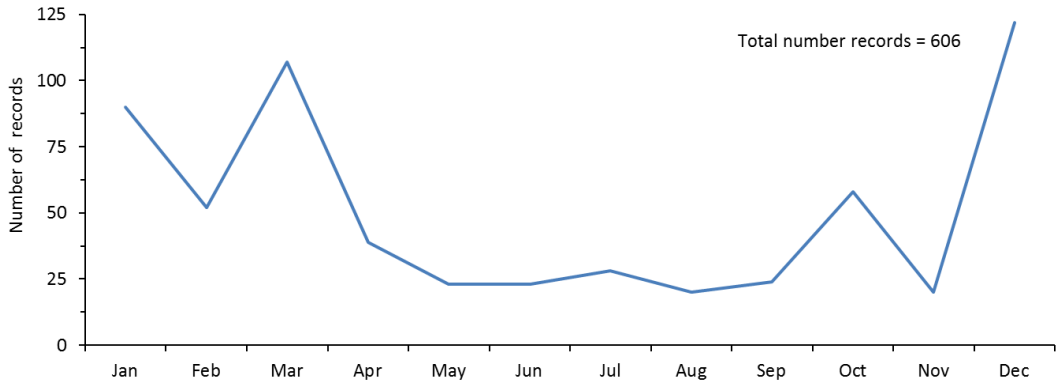

Figure 22. Number of times *Chilodonella* observed in Murray Cod health checks in each month of the year

In order to compare mortality levels in cages across farms, the level of mortality in each cage was grouped into the following categories,

Nil	no mortalities
Low	up to 10 mortalities per cage per day
Medium (Med)	11-25 mortalities per cage per day
High	26-100 mortalities per cage per day
Very High (V. High)	>100 mortalities per cage per day

Chilodonella was associated with low to very high levels of daily mortalities in cages, but was also frequently observed on fish on days when no mortalities occurred in cages (Figure 23). *Trichodina*, Ich and *Ergasilus* were observed on days when mortalities were mainly low or nil. The presence of Monogenea, *Lernaea* and saprolegnia were generally not associated with mortality. Redspot, which was observed on one farm only, was present when very high mortalities occurred (data excluded).

Both the incidence and intensity of infection with *Chilodonella* in open water cages was greater in warmer water, and when the pH was higher (Table 6). Intensity of infection also appeared to increase with increasing water temperature and increasing pH. Intensity of infection was greater at higher alkalinity values and lower nitrate values. No other clear correlations were apparent with other environmental variables.

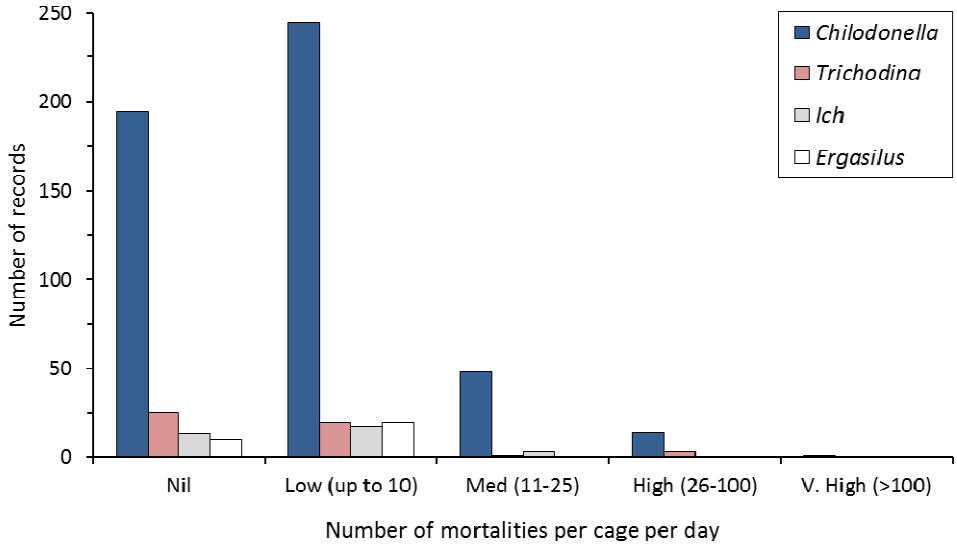

Figure 23. Number of records when different parasites were observed in Murray Cod farmer health checks and the level of mortality (per cage per day) at the time of the observations

Table 6. Incidence and intensity of infection of *Chilodonella* for different environmental variable in open water cages. Significant (P<0.05) Pearson correlation results presented for intensity of infection data.

Variable	**Range**	**Number of records**					
		Incidence	Intensity of infection				
			Nil	Low	Med.	High	Sign. Pearson Corr.
Temperature (^{o}C)	<12	22	89	13	3	2	Rho = 0.15383
	12-15.9	35	135	10	2	13	P = 0.0002
	16-19.9	54	100	16	5	7	No. obs = 579
	20-23.9	154	84	17	13	11	
	>24	50	38	12	7	2	
Change in Temperature	-1	126	250	27	17	12	Rho = 0.09286
	0	76	88	21	11	4	P = 0.0202
	1	138	143	27	6	19	No. obs = 625
DO (ppm)	<5	68	28	2	0	6	
	5-7.9	140	134	31	19	10	
	8-10.9	62	162	20	7	4	
	>11	45	122	15	4	15	
Change in DO	-1	148	174	38	11	14	
	0	94	150	20	9	11	
	1	101	166	19	15	10	
pH	<6	4	4	1	0	0	Rho = 0.20711
	6-6.9	9	9	1	1	0	P = <0.0001
	7-7.9	88	270	20	13	5	No. obs = 518
	>8	169	132	31	5	26	
Change in pH	-1	28	62	3	0	0	Rho = 0.10896
	0	188	290	28	2	26	P = 0.0189
	1	26	46	2	1	4	No. obs = 464
Conductivity (µs/cm)	<250	53	62	25	17	1	
	250-499	11	4	1	0	3	
	500-750	194	334	25	2	28	
	>750	30	15	2	0	2	
Change in conductivity	-1	139	162	18	1	22	
	0	1	5	0	0	0	
	1	120	231	15	2	11	
TAN (ppm)	<0.25	11	4	4	4	1	
	0.25-0.74	0	0	0	0	0	
	>0.75	0	0	0	0	0	
Nitrate (ppm)	<0.75	9	1	4	4	1	Rho = -0.69017
	>0.75	2	3	0	0	0	P = 0.0090
							No. obs = 13
Alkalinity (ppm)	<50	2	3	0	0	0	Rho= 0.49027
	>50	20	16	10	9	1	P = 0.0015
							No. obs = 39

Toxicity trial

Water Quality

Throughout the study water quality parameters remained within acceptable limits for Murray Cod aquaculture.

Fish weight and length

Mann-Whitney testing showed that fish lost weight between the start (1st sampling) and the end (2nd sampling) of the trial ($p<0.01$) (Table 7), but there was no apparent difference in degree of weight loss between groups (Figure 24). No significant growth (change of length) had occurred.

Table 7. Mann Whitney Test results of fish weight and length

Parameter	Mann-Whitney U	Z	p-value
Weight (grams)	4588.50	-5.49	<0.01
Length (cm)	7019.50	-1.19	0.24

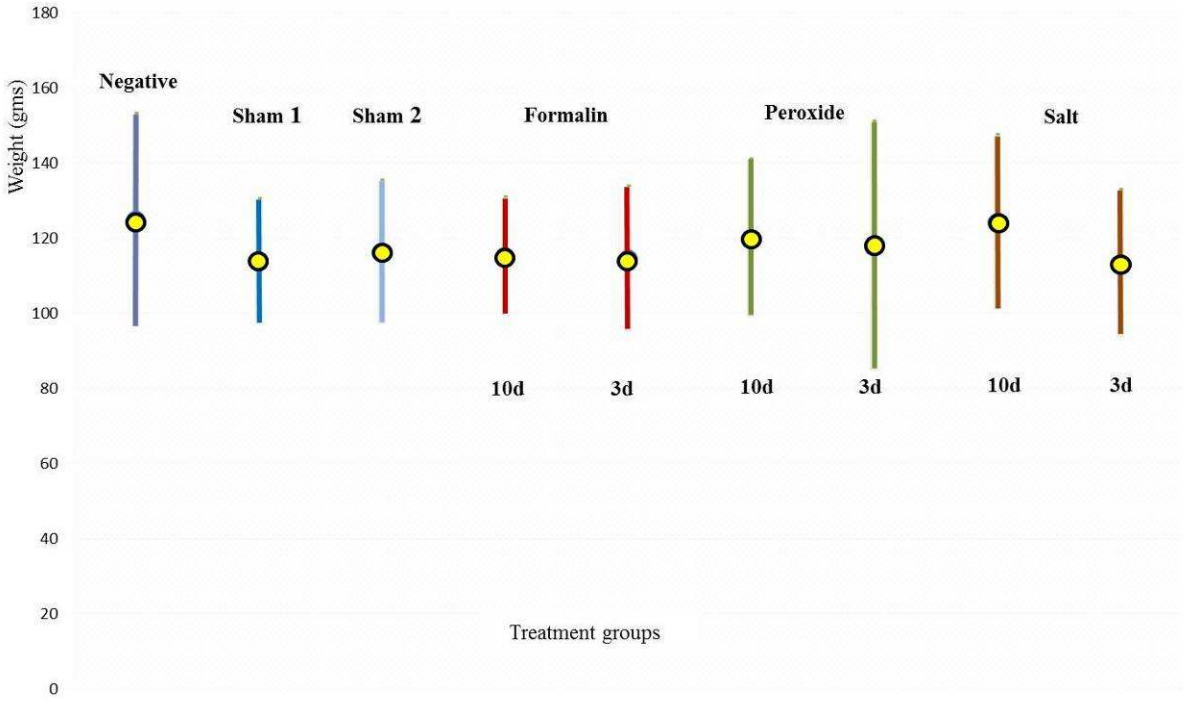

Figure 24. Mean (yellow circles) and standard deviation for fish weights of each treatment group at the end of the trial period shows similarity between groups.

Mortality Results

Table 8 summarises the final mortality rates for each treatment group. Mortality rates were higher for fish treated every 3 days than for those on 10 day treatment cycles. The highest mortality rate occurred in fish treated with formalin every 3 days and lowest in fish treated with formalin every 10 days.

Numbers of mortalities for control groups, both negative and sham treated, were intermediate. A single mortality event in which 4 of 9 remaining fish in the 10 day sham control fish died on one day increased the death rate of that group to be the highest of any 10 day treatment cycle. Five fish also died in one event in the 3 day frequency group, but overall mortality remained below that of both formalin and peroxide for this treatment frequency.

Table 8. Summary of Total Fish Mortality Percentage in for Experimental Therapeutic Regimens Including RAS Water and Control (Number of fish in each group indicated in parentheses)

Treatment	No movement	3 Days	10 Days
No chemical	40% (10)	60% (10)	40% (10)
Formalin	-	80% (30)	10% (30)
Peroxide	-	67% (30)	27% (30)
Salt	-	30% (30)	27% (30)

GLMMs for fish mortality at 3 and 10 day frequencies indicated that tank cluster effects were not significant (tank variance=5.63x10-12, S.E. 1.13x10-12).

A one-way ANOVA confirmed that the treatment regime significantly affected ($p<0.001$) the rate of fish mortalities.

The dataset was split into two models one for fish treated every 3 days and another for fish treated every 10 days. Both models were used to determine significant predictors of fish mortality and the presence of tank clustering effects. Neither model had significant clustering effects (3 day model between-tank variance=5.63x10-12 and 10 day model between-tank variance=3.26x10-15).

The 3-day GLMM model demonstrated that frequent treatment of fish every 3 days is a significant predictor of fish mortality when compared to controls. Fish treated with formalin are 5 times more likely to experience mortalities than fish treated with either RAS water, H_20_2 or salt. The 10-day GLMM did not reveal any significant predictors of fish mortality when compared to control fish.

Post hoc multiple pairwise comparisons between treatment regimes were made using a Tukey's Honest Significant Differences test confirming that fish treated with formalin or peroxide every 3 days had the highest rates of mortality and the greatest differences in mean mortality from other groups.

Individual mortalities were not further investigated, but autopsies were performed on the 4 fish from the sham treated 10 day group that died in one event shortly before the end of the trial. Gross appearance of the dead fish was consistent with bullying; Murray Cod are aggressive and territorial fish. Autopsy showed acute bacterial septicaemia, with granulomatous inflammation and fibrin leakage in lymphoid tissues and moderate abundance of intracellular gram negative bacteria.

Histopathology

Concordance of scoring was not significant for either skin (Lin's concordance correlation Coefficient was 0.548, [95% CI=0.24,0.76]) or gills (Lin's concordance correlation coefficient was <0.00, [95% CI=-0.68,0.68]). No further statistical testing was performed.

Gills: Gill changes were evident in fish on day 1 (Figure 25). There was one or several of mild to moderate, multifocal and segmental to diffuse epithelial hypertrophy, multifocal partial to full thickness lamellar fusion, generally with mild, mainly lymphocytic or lymphohistiocytic inflammatory infiltrates in the associated lamina propria, mild to moderate infections with epitheliocystis and, rarely, multifocal moderate telangiectasis. Changes were generally mild to moderate in degree and were patchy across sections, with some gills being essentially normal with rare, mild change and others showing coalescing to diffuse lesions, particularly epithelial hypertrophy.

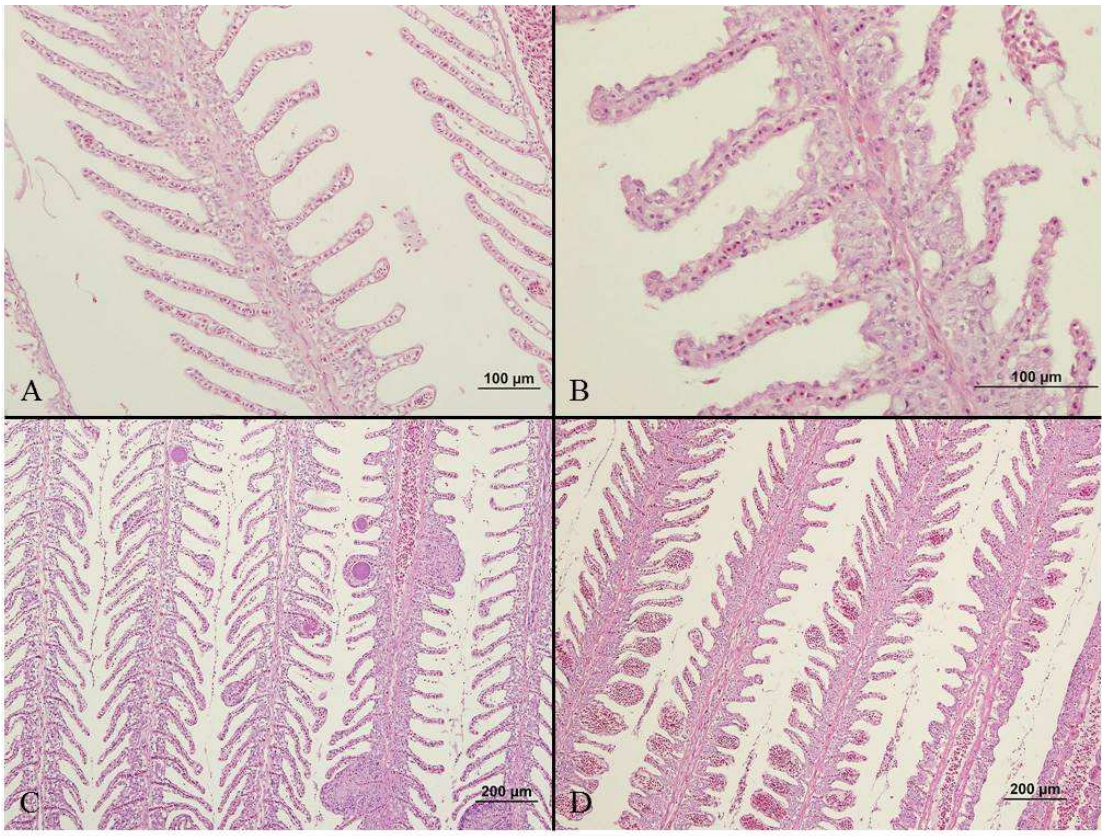

Figure 25. Gills at day 1 of sampling ranged from a) mostly normal to b) showing diffuse epithelial hypertrophy c) multifocal fusion and lymphocytic inflammation- Epitheliocystis was a common finding in association with other changes or alone - and/or d) telangiectasis.

In all groups of fish sampled at the end of the trial period there was an increase in lesion severity overall, although in each group some fish continued to have minor changes only (Figure 26). Epithelial hyperplasia was more likely to be diffuse in fish treated with formalin at 3 day intervals than other treatments, and telangiectasis was prominent in most fish treated with salt at 3 day intervals. These findings were not confined to these treatments, however, and even control fish showed increased evidence of gill lesions. One control fish had acquired a mild infestation with *Chilodonella* sp.

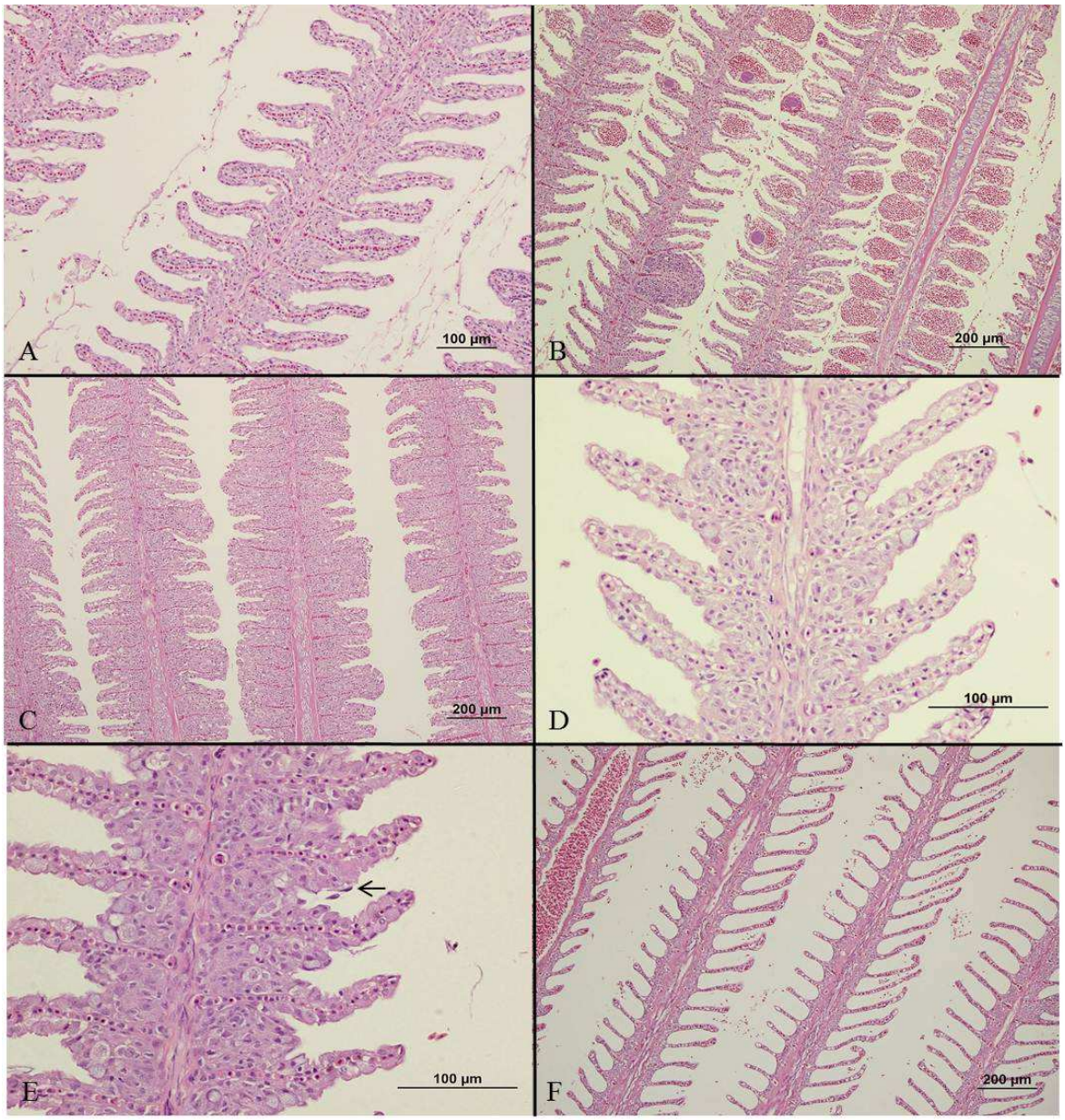

Figure 26. Lesions seen in gills from fish at the end of the trial included a) diffuse epithelial hypertrophy with lymphocytic infiltrates (Formalin 10 days) b) marked telangiectasis (Salt 3 days) c) and d) hyperplasia, lamellar fusion and lymphohistiocytic infiltration (c- Salt 3days, d- Peroxide 10) e) *Chilodonella*.(arrow) (control) f) Some gills remained relatively unaffected. (Formalin 10 days)

Skin: At the beginning of the experiment, there was mild to moderate focal, multifocal or diffuse lymphocytic infiltration (Figure 27) of the epidermis in about 50% of fish.

Few differences were seen at the end of the trial, with all changes in all groups limited to mild lymphocytic infiltrates. At least 50% of specimens showed no infiltrates or other changes.

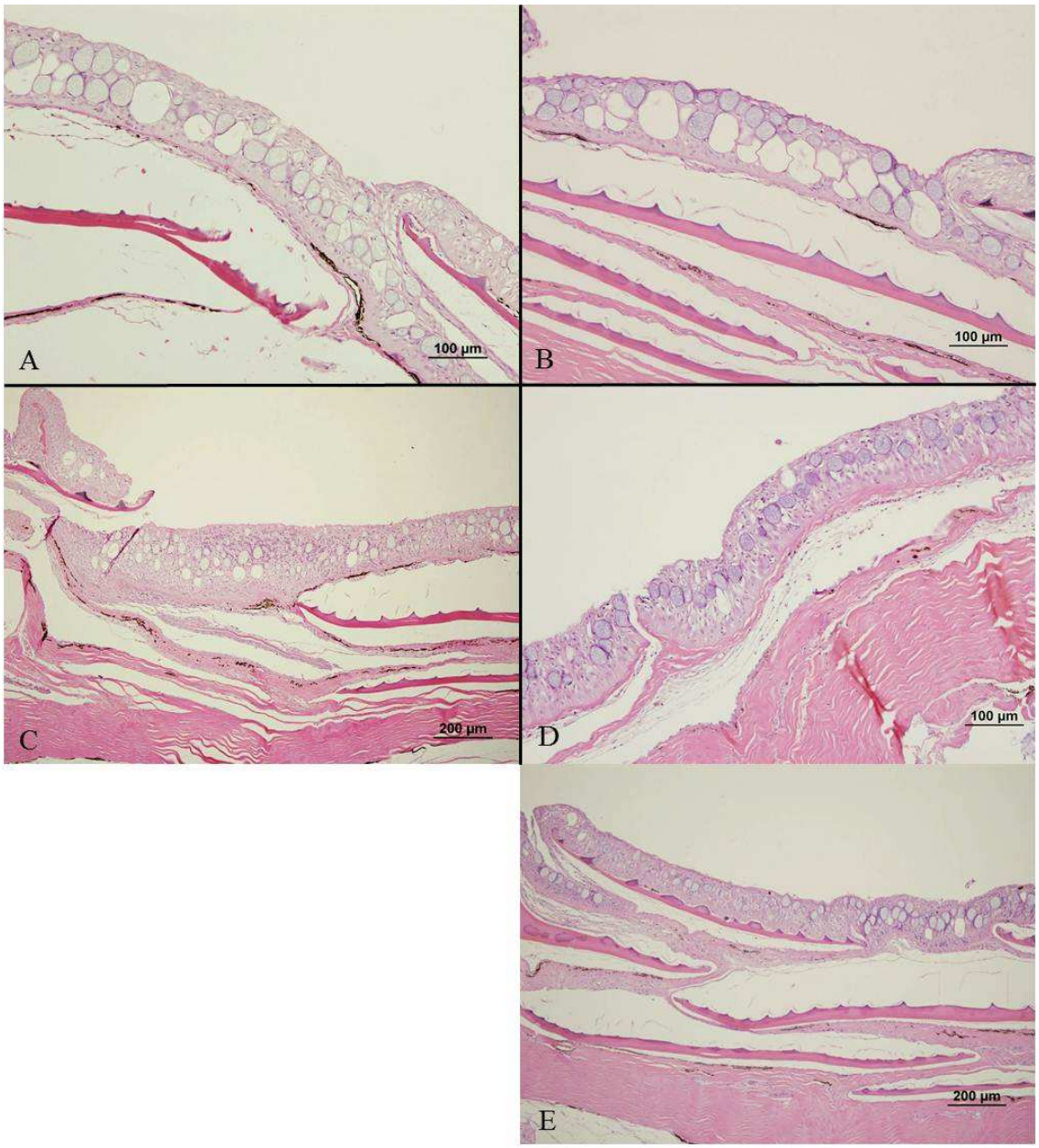

Figure 27. Skin at day 1 a) with mild diffuse lymphocytic infiltrates and c) focal lymphocytic infiltrate, and day 30 showing b) no abnormalities (Formalin 3 days) d) diffuse lymphocytic infiltrates(Formalin 3 days and e) mild diffuse lymphocytic infiltrates (Peroxide 3 days)

Discussion

Histopathology study

Bacteriology

Septicaemia was not encountered in this survey and routine bacteriology was generally unremarkable. Several of the organisms isolated from kidney swabs are recognised as agents of human diarrhoea, including *Aeromonas hydrophila* and *Plesiomonas shigelloides* (Parker and Shaw 2011, Kaiser and Surawicz 2012), and *Acinetobacter baumannii* is an emerging hospital pathogen with an increasing degree of multidrug resistance (Adegoke *et al.* 2012, McConnell *et al.* 2013). *Achromobacter xylosoxidans* is also a cause of disease, rare and of low pathogenicity, particularly in patients with indwelling catheters or cystic fibrosis (Duggan *et al.* 1996, Tokuyasu *et al.* 2012). These bacteria, however, are generally considered to be ubiquitous environmental opportunists; they are not notably associated with disease in fish, nor with aquaculture practices. Although one farm in the survey uses reclaimed sewerage water in the dams for fish,

there was no evidence that these fish were more likely to yield cultures of human faecal pathogens than fish from other farms.

In contrast, *Aeromonas sobria*, the other bacterium isolated on more than one occasion, has been shown to have the potential to act as a primary pathogen in perch (Wahli *et al.* 2005). However, the fish from which it was isolated in this survey did not have evidence of associated disease.

Histopathology and lesions

Infestation of gills with *Chilodonella* spp. proved to be the major problem encountered in this survey, supporting the farmers' beliefs that this is their most important issue. *Chilodonella* has previously been considered to be a seasonal problem, and in the first year of the survey we did not have any cases presented from December until late March, but in the second year we documented infestation in all months. Sixty - two species of this protozoan are documented (http://www.catalogueoflife.org/col/search/all/key/*Chilodonella*/match/1 accessed October 9th, 2013) and, although most are free living (Migala and Kazubski 1972), at least two parasitic species are known and it is possible that the diffuse spread of detection in this survey indicates a multispecies infestation. This has implications for management and therapeutics. Morphological and genetic analysis indicate that one known pathogenic species, *C.piscisola,* is present. Previous studies have indicated a second species, *C. hexasticha,* has caused significant mass mortalities in Murray Cod (Rowland and Ingram 1991).

Much about the epidemiology of infestation is unknown, at least with regards to dam raised, farmed fish. The source of parasites for naïve fish is unknown. *Chilodonella* is generally considered to be a significant parasite only of farmed fish, with wild fish rarely carrying heavy burdens (Langdon *et al.* 1985), but farmers consider feral fish in the dams to be possible reservoirs of disease. The nets of the pond cages are also suspected of harbouring parasites; experiments to test these hypotheses have been planned. As *Chilodonella* spp have been found in river sediment (Madoni 2006), the pond floor should also be tested. However, fish from recirculating systems also acquire these parasites suggesting direct water borne infestation. Once the source of *Chilodonella* has been identified, preliminary management strategies can be implemented and experiments designed to refine them.

Greek workers note differing sensitivities to various parasites between fish species, with mullet being very sensitive to *Chilodonella* and catfish to ich (Athanassopoulou *et al.* 2004). The frequency and, sometimes, severity of parasite burdens suggests that Murray Cod are also very sensitive to *Chilodonella* infestation, but our survey was not designed to test this. Rowland and Ingram (1991) found *Maccullochella* species, including eastern freshwater Cod (*M. ikei*) and trout Cod (*M. macquariensis*) to all be highly susceptible to infestation by *Chilodonella*. There can be very wide differences in numbers of parasites and epithelial damage between fish of the same farm cohort. Parasite burdens are variable, both from month to month and farm to farm, and between fish from the same farm, with some fish having heavy burdens and marked gill damage while cohort fish have no detectable parasites and normal gill epithelium.

Finnish researchers consider age and species of fish, season and tank type to be critical to the build- up of gill parasites in farmed salmonids (Rintamaki-Kinnunen and Valtonen 1997), but we cannot confirm this in Murray Cod. It is likely that stress, including handling and stocking density, will be found to be important, but the aggressive temperament of Murray Cod makes testing different stocking densities difficult.

A study looking at the effect of temperature on parasitic infestations found that *Chilodonella* was not affected either way (Karvonen *et al.* 2010) contradicting the previously perceived seasonal incidence of this parasite and suggesting a possible interaction of season through effects on the fish rather than the protozoan, possibly by way of immunocompromise or general metabolic response to decreased temperature. We have attempted to match farmer reports of *Chilodonella* infestation to water temperatures (see Farm Surveillance) and other parameters but not within the regular surveillance project. This would be interesting work for future surveillance programs. If a transmission model can be developed, then studies with fish held at different temperatures are also possible.

In acutely affected gills *Chilodonella* was clearly associated with epithelial changes histologically particularly with epithelial hypertrophy. Similar changes were seen in uninfested fish however. Since the relationship of sampling to effective treatment was sometimes not known, this absence of parasites may have been a result of recent parasite clearance, but the damage is not specific to or pathognomonic for *Chilodonella* infestation. The lesion has been likened to that caused by osmotic imbalance (Langdon *et al.* 1985), which may reflect the pathogenesis of disease related to *Chilodonella* infestation.

The reversibility of these changes has not been directly studied, but the presence of fish with excellent gill structure in cohorts where *Chilodonella* is clearly present and has been present for extended periods may indicate that restoration of normal structure is possible if the infestation is cleared, at least for some individuals and hypertrophy is generally reversible upon removal of the stimulus. Some fish, however, had some or all of extensive lamellar fusion, extensive epithelial hyperplasia and metaplasia leading to epidermalisation, which are likely to be permanent changes. That these findings were often apparently incidental in fish that were considered to be in good condition raises interesting speculation on the ability of Murray Cod, generally a sedentary fish in the wild, to deal with restricted oxygen intake but also confuses the issue of cause in cases of respiratory disease.

Using only the surveillance data, the direct effect of *Chilodonella* on the fish is difficult to assess. While *Chilodonella* is undoubtedly a significant pathogen in fish, including Murray Cod, most submissions with *Chilodonella* reported the fish to be growing well, although in all cases regular treatment was occurring which modified the parasitic burden. One farmer did note gasping of fish at the surface during a period when he felt that infestation was severe, but gills of these fish showed severe and chronic damage histologically, which may have contributed to respiratory distress. Another farmer noted decreased response to treatment during a period when copper sulfate was being added to the water and oxygen levels were low, with improved control of *Chilodonella*, as determined by on - farm monitoring, as oxygen levels increased. *Chilodonella* numbers during the perceived difficult period were low on histology, with mild to moderate gill changes. As the parasite control improved, gill changes were more severe, suggesting an effect of the increased

application of control chemicals or perhaps the copper sulfate. A toxicity trial using common treatment chemicals was undertaken to examine the effects of therapy on gills and skin.

Increased mortality is often blamed on *Chilodonella* without further laboratory investigation. On one occasion a farmer reported at the monthly surveillance visit that he had lost most of his fish to an “alarm failure” and most of the remaining fish to *Chilodonella*. The samples received in that submission showed fish to have healthy gills and no detectable parasites which suggests good recovery of structure and function following formalin treatment, but no laboratory samples were received during the mortality event to confirm that *Chilodonella* was indeed present in large numbers. Examination of gills from dead fish may be misleading as to *Chilodonella* numbers; *C. uncinata* has been reported to accumulate on dead mosquito larvae, presumably feeding on the dead tissues or the bacteria on the tissues (Spring and Zufall 2013) as, perhaps, may other non - pathogenic *Chilodonella* species.

Other parasites were associated with local damage and inflammation in the gills, particularly copepods. They were relatively uncommon, possibly as a secondary effect of regular *Chilodonella* treatment, with epitheliocystis being the most frequently seen. This bacterium has been linked to morbidity and mortality when seen in high numbers (Nowak and LaPatra 2006), but is generally considered to be incidental. *Ichthyophthirius multifiliis* was seen in gills in association with skin infestation, but not alone. While some fish had multiple parasites infesting or infecting the gills, there was no subjective evidence that infestation with one increased the prevalence of others In particular, there was no obvious increase in secondary parasitic infestations in fish with *Chilodonella*. No statistical analysis was performed on these data because of the uncontrolled nature of the sampling.

Finding lymphoma in the gills of multiple fish on one farm was particularly interesting. Gill involvement of lymphoma has been reported in a yellow rasbora (*Rasbora lateristriata*) (Smith *et al.* 1936) and a Mexican tetra (*Astyanax mexicanus*) (Schlumberger and Lucke 1948), but is rare. The fish in our survey were being held in RAS tanks with constant exposure to dilute formalin to control *Chilodonella*. Fish on the same farm but retained in dams and having intermittent *Chilodonella* treatment using various chemicals did not have lymphoma raising the possibility of chemical carcinogenesis. The role of formalin as a carcinogen in mammals is controversial (Golden *et al.* 2006, McGregor *et al.* 2006, Bosetti *et al.* 2008, Duhayon *et al.* 2008, Zhang *et al.* 2009, Nielsen and Wolkoff 2010, Zhang *et al.* 2010), and no studies on the carcinogenicity of aldehydes to fish have been performed. The affected cohort grew to market weight – this farm specialises in larger fish and the subjects were 2 to 3 years old – and no further tumours have been reported by the farmer since that year group went to market.

Alternatively an infectious carcinogenic agent could be involved. Outbreaks of a transmissible lymphoma are reported in esocid fish (Mulcahy 1963, Mulcahy and O'Leary 1970), but only fixed tissue was submitted so no testing for possible viral involvement could be performed. Lymphocyte markers for fish tissues are not available, so further tumour typing could not be done. Tissue submission from affected RAS fish was limited, but the pattern of tumour invasion suggested that the primary site was thymus or possibly head kidney.

Body wall, including skin and muscle, was the next most frequently affected body system after gills. Most affected fish had mild, non - specific lymphocytic dermatitis, possibly related to water issues or other external stimulus. Although a small number had detectable parasitism, particularly ich or *Chilodonella*, most did not and there was no clear suggestion of a relationship between parasitism of gills and dermatitis, except that, as mentioned, no fish had gill ich without skin infestation. For the remaining fish, ulcerative lesions with secondary oomycete infection were the predominant finding, with no primary cause for the ulceration detectable.

One farm had an outbreak of *Aphanomyces invadans* (EUS) following increasingly frequent treatments for *Chilodonella*. EUS is also thought to be a secondary invader, requiring skin damage for entry into the muscle (Kiryu *et al.* 2003, Oidtmann 2012), and we hypothesised that the frequent bathing in irritant solutions may have damaged the skin sufficiently to permit this. However, other farms treated at the same frequency without disease, and the toxicity trial undertaken did not demonstrate extensive skin alterations, so the reason for this event is still unclear.

Of viscera, intestines and hind kidney were the organs of most interest. As with gills, non- specific lesions predominated in intestines, and of specific aetiologies, parasitic infestation were the most frequent, with nematodes being relatively common. One of these nematodes was collected whole at autopsy and identified by Dr Ian Beveridge of the University of Melbourne as *Spirocamallanus murrayensis*. Cestodes were seen uncommonly, in liver of two fingerlings in one submission as well as intestines of older fish on two occasions. Whether these were the same species is uncertain. A specimen collected at autopsy from the intestine was identified by Dr Beveridge as *Bothriocephalus acheilognathi*, the Asian fish tapeworm, a widespread parasite native to the Asian grass carp but now found worldwide, and known to be present in the Murray Darling-basin in association with the movements of introduced carp (Dove and Fletcher 2000).

There was no evidence that enteric parasitism was directly affecting the health of the fish, although the necrotic and granulomatous lesions in the livers of the fingerlings were severe and may have led to complications in time. In other aquaculture systems the Asian tapeworm is associated with poor growth and control through anthelmintics and control of copepod intermediates in the water is practised (Hansen *et al.* 2007, Zargar *et al.* 2012).

Coccidia, previously identified as *Goussia lomi* (Philbey and Ingram 1991) were seen in a small number of fish. Prior report of this parasite in Murray Cod comments noted that it is found in aquarium reared fish but not pond fish, with some mortality in fry. Affected fish in this survey were clinically healthy pond fish approaching market weight.

The demonstration of ulceration of intestine or rectum in multiple fish in apparently adequate health and rupture of the intestine in one was unexpected, even though very uncommon. No cause was evident. The rupture was found to have *Ichthyophthirius multifiliis* and oomycetes in the perirupture tissues, possibly suggesting recent ingestion of a cage mate, but whether this was related in any way to the rupture is uncertain.

Renal lesions also included parasites, presumed to be myxozoans and not clearly causing any effect on the fish. Membranous glomerulopathy was seen in two fish and three had acute tubular epithelial necrosis, with no cause evident.

Granulomata were found in several organ systems, generally in different fish with few having lesions in more than one organ. Acid fast staining did not indicate mycobacterial infection, although *Mycobacterium marinum*, the usual agent in fish, is known to react poorly to standard ZN stains. However, in one instance a weakly positive, beaded filamentous organism was seen in the middle of a large renal granuloma. No tissue was available for culture, but PCR amplified a 16S sequence that matched one deposited in Genebank for an otherwise undescribed environmental bacterium. The location of this organism suggests some role in granuloma formation, underlining the fact that the granulomatous reaction is not pathognomonic for mycobacteria, although they remain the most usual cause.

The finding of *C. piscicola,* which is generally considered to withstand colder water temperatures, is interesting. Previous studies have indicated that *C. hexasticha,* has caused significant mass mortalities in Murray Cod (Rowland and Ingram 1991).

The results for specificity and negative predictive value of the gill clip as a diagnostic test versus histopathology were high. It would appear that both the likelihood of a negative result in fish known to be free of *Chilodonella* (as determined by histology) and the probability that the fish did not have *Chilodonella* when the gill clip was negative were high. However the results for detecting the presence of *Chilodonella* from gill clips (when compared with histopathology) were not so favourable. In other words there was a high rate of false negatives where the investigator did not call a wet preparation positive although there were *Chilodonella* detected using histopathology. There could be several reasons for this finding. Wet preparations of gills were generally performed in the lab following transportation and usually anaesthetising of fish. During this time it is likely that *Chilodonella* dropped off or left the gill detecting that the local environment has changed or the *Chilodonella* themselves are affected by the anaesthetic and are lost from gill tissue. It is also likely that if more superficial sections of gill are removed for gill clip then *Chilodonella* located more deeply in the tissue will not be apparent. Farmers generally become very efficient at removing a section of gill from a live fish and examining it immediately so the sensitivity and subsequent positive predictive value would most likely be higher on farm than what was found in these results. Histopathology also provides a much greater area of tissue for examination that gill clip. In some cases only one protozoan was seen on one lamella of one gill.

Although we did not attempt to compare parasite loads on different gill arches it was evident from the sections seen that mild to moderate parasitism could be quite local in extent, and damage to the gills could be patchy. Sampling could, coincidentally, fail to capture parasites, particularly if only partial arch samples are taken or with the limited tissue available from a gill clip. This work has highlighted that sampling multiple fish and from different areas of the gill will increase the probability of detecting parasites where they are present.

The failure of histology to find parasites where they were seen on gill clip may be due to the length of time that the fish was anaesthetised or dead before the gill was fixed and the uneven nature of *Chilodonella* infestations both on individual fish and within a group. There is no information on the effect of gill clip or other disturbance on the remaining *Chilodonella* populations and there is also a possibility that handling transport and clipping may sometimes hasten detachment of parasites.

Gill clips in this survey were generally examined by trained investigators. Farmers new to aquaculture will not be equally competent to identify parasites accurately on microscopy which will result in a lower sensitivity and specificity of this examination method as a tool in routine farm management. However, gill clips remain the only *in vivo* test for gill parasitism so it is important to assist new entrants into this field. Materials were prepared during this project to assist farmers with identifying common parasites by gill clip.

Better understanding of *Chilodonella* and its interactions with fish would be of considerable benefit in formulating diagnostic protocols as well as management strategies.

Farm Surveillance

Farmer census

This project has provided a highly comprehensive review of the Murray Cod grow-out industry across Australia as it currently stands. The industry is small and volatile and as such the conclusions to be drawn from the farmer census of the industry are limited. Over the life of this project some farms closed and new entrants into the industry appeared.

From farmer information provided in the census it appears that there is limited ability or inclination to undertake some basic biosecurity practices such as quarantining stock when they are sick or before they are placed in the facility with existing fish. Inflowing water is generally not treated in any way or tested for water quality parameters. It is not normal practice to grade fish prior to stocking or check their health which is a major limitation in circumstances where the quality of purchased stock is poor.

Farming of Murray Cod in irrigation dams is considered by most farmers as a secondary production system while irrigation of crops is the primary use of the water. Because of this, water use in the dams is managed according to irrigation needs, In summer exchanges rates are high, which may increase the risk of introduction of parasites to the dams. In contrast, water exchange in winter is minimal or even nil. In this case there is a risk that water quality may deteriorate if nitrogenous wastes build-up.

Farmer quiz

It was apparent from the initial farmer quiz that basic knowledge in wet preparation microscopy was missing amongst many of the farmers as illustrated by the poor results (15% – 65% of quiz questions answered correctly). There was a

better understanding of water quality parameters and how to adjust them for optimal environmental conditions, particularly amongst the RAS – enterprise farmers. These poor results were seen as a good opportunity for developing skills in basic microscopy that the farmers could use on farm. The benefits in improving microscopy skills are numerous, particularly as very few of the farms used veterinary services, farms were located long distances from laboratory support and most fish health problems experienced by farms relate to parasites which are best diagnosed on farm with fresh preparations.

Although there were very few farmers retested with the quiz, it is very encouraging to note that in those that were retested there were great improvements in the results. It is unlikely that the farmers recalled the details of the repeated quiz and the correct answers were not provided nor was the quiz left with the farmers after the initial attempt. Given the poor performance of the farmers in the microscopy part of the quiz, it is hoped that the various resources and contact/mentoring with project staff has improved farmer skills in this area.

Farm surveillance data

The quality of farm surveillance data collected from the project farms varied considerably. It was an intention of this project to provide data templates and recommend fish data collection software options for the farmers. However uptake of these tools by project farmers was minimal. Those that collected data preferred to retain their pre-existing systems. The various data management software packages available are generally quite expensive and have limited utility for the particular conditions encountered in Murray Cod integrated aquaculture.

One of the primary aims of this project was to ascertain whether anecdotally high mortality rates on farms could be verified. In the census, farmer - reported mortality rates varied significantly (from low to greater than 70% in early life). In these cage – culture and open systems there may be some issues with data quality: it is often difficult for farmers to see dead fish, observations are sometimes made irregularly and predation of dead fish is very common. In the census the farmers reported that most deaths occurred soon after stocking. The quality of data and constant movement of fish makes ascertaining mortality rates accurately very difficult. Mortality rates varied between the farms with the higher rates seen in the cage grow - out systems as would be expected. There was a range of total mortality rates (across different time periods) from 4% to 100%. Although the data quality is not necessarily high, it appears that generally (excluding Farm 3) the mortality levels were lower than those quoted by the farmers in the census. Farmers have commented that it is not until the stock are removed for marketing that accurate figures on weights or biomass (but not necessarily numbers) of stock are known. Furthermore this project did not follow fish from initial stocking to harvest for all the farms, the period when most mortalities are reported to occur. Due to the practice of grading stock and moving between cages without clear accounting for these movements at all times it is difficult for some farmers to assess mortality rates and production statistics.

It was not possible to conduct more sophisticated analysis of the mortality and water quality data due to concerns with data quality. For this reason simple graphical representation of the information is provided. Generally it appears that there are higher mortality rates in the warmer months when DO levels are lower.

Further interrogation of mortality event data has illustrated that on the occasions where a cause of a major mortality event was known and recorded, human error was often involved. This was particularly evident in Farm 1 which was a newer enterprise. In biological systems many elements are interrelated, thus loss of power causing aerators to fail and DO levels to drop may then precipitate *Chilodonella* infestations which ultimately cause fish losses. On Farm 3 where there were major problems with red spot and *Chilodonella* resulting in the closure of the farm, over-treating with formalin for this parasite most probably precipitated the loss in epithelial integrity and subsequent red spot infection. A stronger collaboration between farmers and higher level of support within the industry may assist new comers in not repeating the mistakes of other farmers.

Water quality parameters gained from farmer measurements and in some cases in situ water loggers have generated data that can be added to current information. Previous work has documented industry standards and provided water quality data for integrated aquaculture farms (Gooley and Gavine 2003, Ingram *et al.* 2005b, Gooley *et al.* 2007). However, this project is the first to investigate interactions between water quality, disease and mortality of Murray Cod farmed in open water culture systems.

This project has been able to elucidate from the enrolled farms what treatment chemicals are used, including dose rates and for what purpose. This information does not document efficacy but rather what works for the farmer by trial and error following a health assessment of the fish. As would be expected the treatment frequency is much higher in the warmer months (down to treating every 4 days) when there tends to be heavier burdens of parasites. Farmers need to be constantly mindful of the effects of chemicals on the integrity of fish epithelium and therefore ramifications to overall fish health. This information should be useful to entrants in this industry as a guide for common treatment practices.

Data generated by this project confirmed that *Chilodonella* was far and away the greatest health issue facing integrated aquaculture farms. From farmer records of parasites and diseases encountered on wet preparations of gills and observations of fish health (where recorded), it was seen that all other health issues were dwarfed in comparison to *Chilodonella.* It also appears that *Chilodonella* tends to occur all throughout the year with the highest incidence recorded in the warmer months. It is likely that farmers under-reported problems with saprolegnia as it tends to be a minor but endemic health problem in the cooler months. The constant presence of *Chilodonella* on most farms also affected its association with daily recorded mortalities; this parasite was associated with nil to high fish mortality rates due to its ubiquitous nature, although it is clearly a major problem faced by farmers. In contrast red spot (EUS) was associated with very high mortalities due to its overwhelming presence on one farm which resulted in the death of all stock and closure of the farm. Other parasites did not seem to be associated with high mortality rates of stock.

Incidence and intensity of infection with *Chilodonella* in open water cages was correlated with increasing temperature and pH. Season trends in *Chilodonella* have been observed in other species. for example, the occurrence of *Chilodonella hexasticha* on rainbow trout in farms in Serbia was highest in May (Spring) (Nikolić *et al.* 2006). However, the trend observed in the present study may be confounded by species resolution. *C. hexasticha* and *C. piscicola* have different temperature preferences, *C. hexasticha* prefers warmer temperatures (up to 31^{o}C) whereas *C. piscicola* prefers cooler temperatures (Kazubski and Migala 1974, Rintamaki *et al.* 1994). Both species have been described from Murray Cod, *C. piscicola* in the present study and *C. hexasticha* in previous studies (Rowland and Ingram 1991).

It is unclear why intensity of infection correlated with pH as unlike temperature, pH does not vary seasonally. Barker and Cone (2000) found that the abundance, and prevalence of the gill parasites *Pseudodactylogyrus anguillae* and *Ergasilus celestis* on eels (*Anguilla rostrata*) were positively correlated with pH, suggesting these parasites are sensitive to acidic waters. In the present study, intensity of infection with *Chilodonella* was greater at higher alkalinity values. Similar findings have been found with other parasite species. For example, infection of silver catfish (*Rhamdia quelen*) by ich was less severe at a pH of 5, but high water hardness increased intensity of ich trophonts (Garcia *et al.* 2011). Although pH and alkalinity are related, they are measuring different aspects of water quality, alkalinity being a measure of the water's ability to buffer against rapid pH changes. In aquaculture ponds, high alkalinity values are associated with more productive waters (Boyd 1990), which may also benefit growth of *Chilodonella*. It is also surprising that DO levels did not appear to play a role in intensity and infection with *Chilodonella* as it is usually assumed that low DO levels result in increased physiological stress on fish and a greater susceptibility to parasite infection. These associations warrant further investigation given the importance of this parasite. The lack of *Chilodonella* and low mortality rates on Farm 6, which has been operational for 9 months, may support the proposition that *Chilodonella* builds up over time in a system and then is extremely difficult to eliminate. Given that this parasite is ubiquitous and occurs on a wide range of fish hosts, and that incoming water is not treated to remove *Chilodonella*, successful control of this most problematic of parasites remains challenging.

It was very difficult to obtain good quality production data for this project. The ability to remain financially viable in any food production venture requires basic knowledge of the returns and costs of production. In this small Murray Cod aquaculture sector farmers were either unwilling or unable to provide information about the volumes of stock produced, prices obtained per kg (although some enterprises did provide this data) and other relevant production parameters. In integrated aquaculture systems horticulture or agricultural stock production is usually the primary enterprise. This reduces the relative importance of Murray Cod aquaculture and the farmer's commitment to profitable fish production. The industry is hindered by its small size, lack of clear markets and rivalry amongst the different players.

Toxicity trial

Farmed fish are commonly exposed to chemicals, particularly formalin, peroxide or salt, for control of parasites. These chemicals have different modes of activity; formalin is a cross linking agent, causing cross links between aldehyde groups that are initially reversible, although becoming irreversible with chronicity, hydrogen peroxide is an oxygen radical that causes oxidative damage before breaking down to water and oxygen, and salt is an osmotic agent, causing ionic fluxes across permeable membranes.

All chemical treatments are likely to have damaging effects on the fish tissues most exposed to them, that is, gills and skin, and movement to and from treatment tanks is a stress on the fish that may also lead to growth interruption through stress or tissue damage through handling. Comparison of fish with other aquatic organisms suggests that fish are sensitive indicators of pollutants such as formalin and phenol (Tišler and Zagorc-Končan 1997).

Exposure to the chemicals themselves may trigger stress reactions in fish. Bowers *et al.* (2002) found that peroxide exposure did increase markers of stress in salmon, but the effect was transient, lasting less than 24 hours. However, investigation of aquatic pollutants has shown that effects vary with species, at least in severity (Nero *et al.* 2006, Kelly and Janz 2009, Saenphet *et al.* 2009, Troncoso *et al.* 2012, Pereira *et al.* 2013), and stressors and pathogens should be tested directly on the species of interest.

Fish in this trial did not increase in length and showed significant weight loss at the end of the treatment period, with no difference seen between treatments, frequencies or control groups. This is in contrast to reports from salmonid trials; Speare *et a.l* (1999) found that rainbow trout continue to grow throughout a tank acclimatisation period and following immersion in a peroxide bath, although bathing slowed growth compared to controls (Speare *et al.* 1999). It is likely that factors other than movement and chemical exposure are affecting Murray Cod in the first month after transfer to new facilities potentially both limiting growth and predisposing them to develop disease. It was not the focus of this project to look at stressors in tank cultured fish and further investigation of this effect was not undertaken, but it is possible that Murray Cod, being naturally solitary fish, are more susceptible to crowding stress than other commercially raised fish.

The mortality rate in the group of fish that were neither moved nor treated was comparatively high, comparable to groups undergoing frequent movement and exposure to irritant chemicals. Investigation of sporadic mortalities was limited but suggested that aggression and subsequent septicaemia could play a part in fish deaths in sham treated tanks. Murray Cod are territorial fish and aggressive, requiring care to maintain a stocking rate which suppresses the bullying response. How and whether this behavioural characteristic interacts with the stress of movement or with chemical exposure stress remains unknown. Most deaths in unexposed fish occurred late in the trial period, which could indicate a period of settling and overcoming stress factors before asserting territoriality. Moving fish could exacerbate the behaviour, or minimise it as fish continually re-establish boundaries.

Although no chemical or treatment frequency on its own had any detectable effect on fish health compared with controls, there was an interaction with them, with movement to formalin on a 10 day cycle being mildly protective, although the reason for this is unclear.

Gill morphometric changes have been used to assess wild fish exposure to a range of irritants and pollutants including oil sands (Nero *et al.* 2006), heavy metals (Kelly and Janz 2009) and acidity (Saenphet *et al.* 2009) and non-specific contamination (Costa *et al.* 2009). Histological examination is an inherently qualitative discipline, as morphological responses to challenge follow a continuum of degree. Some changes are of more importance to the subject's subsequent health. Scoring systems have been designed to attempt to quantify these responses in a way that highlights consequences and permits comparisons between individuals or groups. Bernet's system was chosen because it grades presence of changes with the importance being given to the expected reversibility of the change, as well as the degree of change. The final score achieved is a relative indicator of overall health risk posed by the changes seen.

However, in our hands this system was not reproducible, with concordance between trials showing no significance. Factors of note include the patchy nature of the lesions, particularly in gills although both tissues showed some degree of regionality. Examination of selected fields may skew the results if the only region of higher grade is not included in one of the trials. Technical considerations such as gill orientation on the slide are also of importance, as some changes can be difficult to assess if, for example, lamellae are folded or the filament cut obliquely; concordance was better for skin, which is more easily oriented. Undoubtedly experience of the operator will also have some effect in this discordance too; extreme grades (mild and severe) are generally not challenging, but the intermediate stages are subjective and must be continually compared to chosen standards to maintain some repeatability.

Previous trials to examine treatment effects on fish have generally investigated the effect of chemicals on freshwater ornamental or salmonid teleosts (Das and Srivastava 1978, Zaccone 1981, Speare *et al.* 1997, Speare *et al.* 1999, Santos *et al.* 2012), although euryhyaline (Yoshikawa *et al.* 1993) and marine (Larsen *et al.* 2012) fish, including seawater adapted salmon (Nowak *et al.* 2013) have been investigated. As with general pollutants, species sensitivity to parasiticides varies (Santos *et al.* 2012). Murray Cod are naturally adapted to freshwater environments that undergo large fluctuations in water quality seasonally, and might be expected to have some degree of resilience, at least where osmotic factors are concerned. It is difficult to ascribe significance to the telangiectasis seen in the salt treated fish, although it was more apparent than in other groups, but the change in osmolarity applied is both large and rapid and possibly overwhelms the normal control mechanisms.

All treatments caused a subjective increase in epithelial hypertrophy, hyperplasia and lamellar fusion, in contrast to the reported responses of salmonids (Speare *et al.* 1997), at least with respect to formalin bathing, which show slight and insignificant changes in these parameters. This may suggest some degree of sensitivity in Murray Cod and may be important to further examine.

Our surveillance work suggested that Murray Cod may be a species highly susceptible to *Chilodonella* sp. infestation, which supports the views of Rowland and Ingram (1991). In this experiment we have not been able to link treatment for this parasite to mortality or specific tissue injury, but the increased changes in gill epithelium may indicate a need for care in planning therapeutic protocols and in understanding parasite epidemiology with a view to supplementing these with environmental management. The absence of effect on skin does not support a role for parasite treatment in the development of EUS on the farm that suffered this outbreak.

Conclusion

Gill lesions are the greatest threat to fish health and growth in farmed Murray Cod. This survey has confirmed the importance of *Chilodonella* spp. and highlighted some areas of potential management importance that need structured investigation, such as the source of parasites for naïve fish, factors affecting disease development after infestation and factors affecting response to treatment. Gills autolyse quickly after death of the fish and submission or examination of dead fish is not rewarding. Shipping of live fish to the laboratory is expensive and labour intensive for the farmer so farm collection of tissues will improve diagnostic capabilities. Encouraging farmers to acquire microscopes and become competent in their use will also improve management decision making.

The farm census and farmer quiz demonstrated that there is a limited amount of knowledge of basic farm biosecurity and ability of farmers to recognise common parasites and structures seen in wet preparations. For a small subset of farmers that were retested there was a dramatic improvement in diagnostic skills utilising microscopy. There was limited good quality data available for this project and little enthusiasm from farmers to improve data collection and utilisation. There were concerns with data quality generated from the farm surveillance part of this project. Some farms do not record (or were not willing to share) basic mortality and production data which raises concerns about how the farms can operate profitably or plan across the production cycle. Generally the ability of farmers in integrated aquaculture to account for the movement of their fish was poor. *Chilodonella* is clearly the health issue that most concerns farmers when considering what they see during health checks, what they treat for and what tends to be associated with fish mortalities across the year.

It is likely that Murray Cod are quite susceptible to chemical treatments based on the physiological response seen in gill tissue. This coupled with the apparent susceptibility to *Chilodonella* infestation exacerbates the effect of this parasite in this species of fish.

Implications

Industry:

1. This project has shown that diseases, mainly *Chilodonella*, play an important role in limiting production and productivity, especially on open water farms.

2. The project has also found that some farm management and husbandry practices, or lack thereof, have at times contributed to mortalities on farms. This is also illustrated by the poor quality of data collected by Murray Cod farms enrolled in the project, which limited the ability to identify key factors and trends affecting production. The development and application of Better Management Practices to farms may assist in redressing some of these issues.

3. It would appear that there is a strong need for transparent benchmarking of the costs and returns experienced by members of the industry that would enable the whole industry to move towards more profitable production using a more collaborative approach.

4. Farmers should be encouraged to perform brief autopsies on farm, particularly during mortality events, following the techniques demonstrated on the video produced during this project to allow submission to the laboratory of un-autolysed material which will facilitate diagnosis.

Government/policy makers:

1. For the continued development of the Murray Cod industry further technical support should be provided to these isolated farmers and further resources directed towards elucidating rational control and treatment regimes for *Chilodonella* infections.

Recommendations

Suggestions for further research requirements stemming from the findings in this project are outlined below.

Further investigation into the epidemiology and pathogenesis of *Chilodonella* in Murray Cod gills should be undertaken as a matter of importance. Experiments have been planned to investigate the role of feral fish, dam sludge and cage netting in transmission. The interaction of environmental factors such as oxygen and water temperature should also be investigated once a reliable transmission mode is established. These experiments may also provide information to farmers of other fish species.

Development of “freeware” software that is simple and effective for recording fish health and production data.

Further development

This project has demonstrated that there is a major issue with *Chilodonella* infestation in Murray Cod grown in integrated aquaculture systems. This finding has been verified from both farmer and laboratory assessments. Further, it is clear that the current therapeutic regimes practiced on farm cause discernible damage to fish gill tissue. From farmer reports and histopathological observation current chemical treatments are not effective in controlling *Chilodonella* in these systems. Further research is required into the most effective means of controlling this parasite which may involve elucidating the epidemiology of the organism.

The development of a collaborative approach across the Murray Cod integrated aquaculture farms would be highly beneficial to the advancement of the industry. This would include undertaking benchmarking activities, sharing of knowledge across industry members and a unified marketing approach.

Extension and Adoption

The student and project team have conducted over 75 individual farm visits and the project team has had close telephone/email communications with all enrolled farmers.

Two versions of the Better Management Practices (BMPs) for Murray Cod health has been published during the study. Version 1.0 was disseminated to farmers in 2012. Version 2.0 will be disseminated to farmers, along with the final report.

> Ingram, B., Gooley, G., Bradley, T., Ho, H. and Cohen, S. (2012). *Fish health better management practices for Murray Cod farming. Version 1.0*. Fisheries Victoria Technical Report No. 76. 60 pp.
>
> Ingram, B., Gooley, G., Bradley, T., Ho, H. and Cohen, S. (2014). *Fish health better management practices for Murray Cod farming. Version 2.0*. Fisheries Victoria Science Report in press

Farmers also received other project material including, two farmer newsletters, a laminated Murray Cod fish health poster, a fact sheet on *Chilodonella* and a review of aquaculture farm management software.

Veterinary pathology and ancillary staff at Agribio Bundoora (DEPI laboratory) have greatly improved their proficiency in processing and reading pathology slides and culturing swabs for bacteriology.

Two fish health workshops were conducted, *19th March 2012, Mildura and 28 March 2012-Rutherglen.* Cohen, S. (2012).

> Presentation to Murray Cod farmers. In *Fish health Workshop - update on FRDC project (19th March 2012, Mildura and 28 March 2012-Rutherglen)*.

Other presentations:

> Cohen, S. (2012). Fishing for information. An epidemiological study into the health and management of integrated aquaculture of Murray Cod. In *University of Melbourne PhD confirmation seminar (21 Feb 2012, Werribee)*.
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> Cohen, S. Fishing for information in irrigation dams. *Australian Aquaculture Association of Queensland (10th August 2013, Childers)*

Project materials developed

Key project materials developed were:

Ingram, B., Gooley, G., Bradley, T., Ho, H. and Cohen, S. (2012). *Fish health better management practices for Murray Cod farming. Version 1.0*. Fisheries Victoria Technical Report No. 76. 60 pp.

Ingram, B., Gooley, G., Bradley, T., Ho, H. and Cohen, S. (2014). *Fish health better management practices for Murray Cod farming. Version 2.0*. Fisheries Victoria Science Report in press

"What's wrong with my Cod" – A1 laminated poster illustrating basic Murray Cod health conditions pictorially (Appendices)

Chilodonella Fact Sheet (Appendices)

Farmer newsletters x 2.

Appendices

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Intellectual property

This project has not developed any intellectual property (IP) that needs protecting.

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Poster: What's wrong with my Cod?

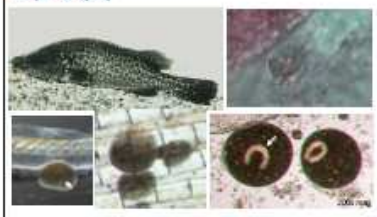
What's wrong with my cod?

A microscopic view of common Murray cod ailments

Project outline

The Murray cod health project is a collaboration between DEPI and the University of Melbourne that has been funded by the Victorian Government and the Fisheries Research and Development Corporation (FRDC). This project is investigating health problems in Murray cod and the information that farmers record about their Murray cod culture systems (both grow-out and RAS). The aim of the project is to better inform farmers about how to prevent and treat losses due to disease and other health issues in their fish.

Ich (white spot)



Description: large ciliate that burrows under the skin and appears as distinctive white spots measuring 0.5–1 mm in diameter.
At high magnification (x200) distinctive "horse-shoe" shaped macronucleus (arrow) may be seen. The surface of parasite is covered with cilia.
Where found: body surface and gills of all aged fish (including larvae)
Best viewed: fresh wet preparation, with either a dissecting microscope (x40) or a compound microscope (x100–400).

Trichodina

Description: an active swimmer with a spinning motion. Body is disc-shaped (flattened or domed)with a distinctive fringe of cilia around edge.
Where found: body surface and gills of all aged fish (including larvae).
Best viewed: fresh wet preparation, with a compound microscope (x100–400).

Chilodonella

Description: distinctive gliding movement. The body is round to asymmetrically oval (heart-shape) and slightly flattened. The bottom surface has two characteristic belts of cilia rows.
Where found: body surface and gills of all aged fish (including larvae)
Best viewed: fresh wet preparation, with a compound microscope (x100–400).

Anchor worms and gill maggots

Anchor worm
Description: Large copepod parasite (up to 22 mm) . The anchor-shaped head of the parasite is embedded into the body of the fish while the abdomen and greenish-coloured eggs are exposed.
Where found: body surface of juveniles, sub-adults and adults.

Gill maggot
Description: Large copepod parasite (2-3 mm) of gills . This parasitic copepod attaches by wrapping its second antennae (arrow) around the gill filament. Heavy infestations may result in secondary infections by fungi.
Where found: Gills of juveniles, sub-adults and adults.

EUS (epizootic ulcerative syndrome -red spot disease)

Description: Initially appears as a red spot, which progresses to red lesions and ulcers which may extend deeply into the muscle. Caused by the fungus Aphanomyces. Since other diseases can also cause ulceration, special laboratory tests are required for diagnosis.
Where found: body surface of juveniles, sub-adults and adults.

Sap (Saprolegnia)

Description: Patches of fungi that look like patches of "cotton wool" to the naked eye. Caused by Saprolegnia spp. Diagnosed by examination under high magnification which reveals fungal hyphae between 10 – 30 micron in size.
Where found: body surface and gills of all fish (including larvae).
Best viewed: Naked eye, and fresh wet preparation, with a compound microscope (x100–400).

Healthy gills

Description: Healthy gills appear red in colour. The gill filaments are usually well-defined without any swelling or clubbing of the tips.

Non-healthy gills

Description: Unhealthy gills may be discoloured or pale pink in colour. The gill filaments are often not-well defined. Filaments may appear swollen, or have clubbed tips. There may be an excess amount of mucus and "debris" present. There may be abnormal growths also present. Gills may be eroded in places.

Artefacts (bubbles)

Air bubbles
Description: Air bubbles (arrow) appear as a thick black circle with a clear centre, often seen in tissues samples on microscopes slides over which a coverslip has been placed.

Debris
Description: Debris may accumulate amongst gil filaments of fish when infections occur. Debris is made of up of mucous, particles from the water and other unidentifiable material.

Sample submission

Want to know what's wrong with your fish?
Make sure you send good specimens

- Accurate diagnosis of a disease is dependant on the quality of the specimens submitted for examination, (the fresher the samples the better), and the accompanying documentation describing the event.
- Live sick, fish are best to submit.
- Submit live samples in well-sealed insulated containers with sufficient water and with oxygen added.
- Otherwise submit moribund or very recently dead fish (less 24 hours) on ice.
- Tissue samples fixed in 10% formalin can also be sent. Use sturdy well-sealed containers.
- Select transport method that will ensure samples arrive in a timely manner.
- Contact the DEPI aquatic veterinarian or laboratory (see contact details right) before submitting samples to obtain details on sample preparation and submission procedures.

Fish autopsies

Dissecting a freshly euthanased fish. Note equipment being used. Secateurs for cutting the gill operculum and other hard parts

Instruments useful for fish autopsies include, disposable food-handling gloves , white board or tray, pointed forceps, scissors, scalpel, kitchen/garden shears, Petri dishes for holding samples for examination under low magnification (< 40x), and microscope slides and coverslips for examination of tissue under high magnification (100-1,000x).

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Further information:

Ingram, B., Gooley, G., Bradley, T., Ho, H. and Cohen, S. (2012). *Fish health better management practices for Murray cod farming. Version 1.0. Fisheries Victoria Technical Report No. 76. 60 pp.*

FRDC FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Department of Environment and Primary Industries — State Government Victoria

Fact sheet: *Chilodonella*, a major parasite of Murray Cod

Chilodonella

A major parasite of Murray cod

What is *Chilodonella*?

Chilodonella (Figure 1) is a genus of ciliated protozoa; that is a single-celled animal with rows of cilia on parts of the cell surface. Chilodonella causes the disease chilodonelliasis, which accounts for many major mortality events in farmed and aquarium fish.

Chilodonella is one of the most common and major parasites causing disease and mass mortality of Murray cod

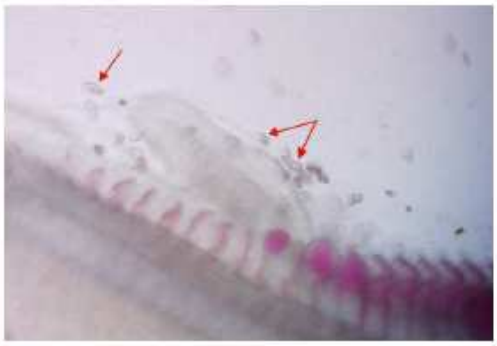

Figure 1. *Chilodonella* (arrows) on gills of Murray cod (400x mag.)

Biology of *Chilodonella*

Chilodonella comprises mostly free-living species. Two species, however, are parasitic on freshwater fish, *C. piscicola* and *C. hexasticha*. Distinguishing *C. piscicola* and *C. hexasticha* requires detailed examination of live and stained specimens at high magnification (1,000x mag.) Both species have been reported from Murray cod. Fortunately species identification is not required for diagnosis and treatment of chilodonelliasis.

The body of *Chilodonella* is round to asymmetrically oval or heart-shaped (Figure 2). The top surface is slightly convex while the bottom surface (oral side) is flat or slightly concave. The bottom surface has two characteristic belts of ciliary bands and a cytostome (mouth) used for feeding (Figure 2).

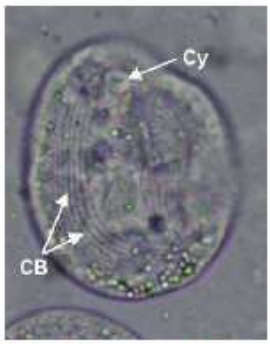

Figure 2. *Chilodonella* showing ciliary bands (CB) and cytosome (Cy) (1,000x mag.)

Chilodonella reproduces by dividing in half to create two new cells. In this way, under favourable conditions, *Chilodonella* populations can build up rapidly in a very short period of time.

Chilodonella may encyst when conditions are unfavourable, and in this stage may be able to survive for long periods in in water or on the bottom of ponds.

What fish are affected?

In Australia, *Chilodonella* has been reported from numerous species including Murray cod, trout cod, eastern freshwater cod, Macquarie perch, golden perch, silver perch, bony bream, brown trout and rainbow trout.

Chilodonella infests all sizes (ages of fish) of Murray cod from larvae to broodstock.

In most cases mass outbreaks occur in aquaculture operations, though outbreaks have been implicated in the mass mortalities of bony bream and Murray cod in the wild.

FRDC Fisheries Research & Development Corporation

Department of Environment and Primary Industries — State Government Victoria

Chilodonella. A major parasite of Murray cod

When does *Chilodonella* occur?

Mortalities associated with *Chilodonella* have occurred over a wide temperature range, from slightly above zero for *C. piscicola* to 31°C for *C. hexasticha*.

In a health survey of farmed Murray cod, conducted as part of a collaborative project between, DEPI, the Fisheries Research and Development Corporation (FRDC) and the University of Melbourne, *Chilodonella* were observed in all months of the year and was the most common parasite seen and causing problems on farms. Traditionally farms experience more problems in the warmer months with this parasite. However more recently there have been reports of severe outbreaks in mid winter. Whether different species are causing problems at different times of the year is not known.

How does *Chilodonella* affect the fish?

Chilodonella are thought to feed directly on epithelial cells of the host.

The most significant damage occurs on the gills. The thin respiratory epithelium becomes covered with a proliferation of epithelium. Gill filaments may become fused together and in extreme infestations soft tissue is lost, leaving just the cartilaginous filament ray. Infestations reduce the respiratory surface of the gills, which can lead to suffocation. Osmotic balance may also be affected.

Large infestations may also cause secondary bacterial infections.

What are the signs of infestation by Chilodonella?

Gross signs associated with presence of *Chilodonella* are typical of other external parasite infestations:

- Fish cease feeding.
- There appears to be increased mucous production as the fish attempts to shed parasites.
- The skin may become dark grey patchy or mottled in appearance and develop an opaque slimy layer. In severe infestations the skin may appear "tattered", and scales may become detached. The eyes may become opaque.
- The gills of heavily infested fish are clogged with mucus and the filaments may appear swollen and fused together.

Fish may be seen and "flashing", scraping themselves against the bottom and other surfaces.

Fish may become listless or uncoordinated, and show signs of breathing difficulty, such as flaring their opercula, an increased rate of opercula ventilation, and gasping at the surface. Moribund fish appear emaciated.

Severe damage can occur before gross signs become apparent. In heavy infestations the parasite may cover the body surface in a continuous layer.

How to diagnosis

Microscopic examination of smears of skin and gill tissues is required for diagnosis. Place freshly collected samples of skin scrapes and gill tissue on a microscope slide and view at >200x magnification. Movement on the slide is strong evidence that there may be parasites.

Key features that distinguish *Chilodonella* from other parasites are

- Size: 30-80 µm long (2-5 x larger than blood cells)
- Appearance: flattened oval or "heart-shape", with cilary rows on the flattened side.
- Motion: distinctive gliding motion.

Management

Take steps to prevent *Chilodonella* entering culture systems. Consider quarantine and prophylactic treatment of all new stock, sterilise inflow water and reduce/avoid stressful episodes.

When the parasite is detected treat promptly with registered or approved chemicals. Ensure the water is well-aerated or oxygenated.

In cage systems fish can become reinfected with *Chilodonella* very quickly and treatments may need to be applied often (every 5–7 days) to manage the parasite.

Further information

Call **136 186** from **anywhere in Australia 8am – 6pm.**

Published by the Victorian Government Department of Environment and Primary Industries Melbourne, August 2013.

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