Development of a Health Management Strategy for the Silver Perch Aquaculture Industry

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NON-TECHNICAL SUMMARY

2000/267 & 2004/089	Development of a Health Management Strategy for the Silver Perch Aquaculture Industry

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OBJECTIVES:

Objectives of the project during 2001 – 2003:

1) Identify and characterise the causes of winter disease and other important diseases of silver perch.
2) Identify cost-effective control and preventative measures for these diseases.
3) Develop, validate and extend a generic "Health Management Plan" which can be modified to suit the needs of individual farms.

Objectives of the one-year extension in 2004:

1) Evaluate the efficacy of formalin and copper against *Ichthyophthirius multifiliis* infestations and saprolegniosis outbreaks during winter.
2) Produce an updated health management plan for silver perch.
3) Implement and validate a modified health management plan with major silver perch producers.

NON TECHNICAL SUMMARY:

The silver perch (*Bidyanus bidyanus*; family Teraponidae) is an Australian native freshwater fish that is endemic to the Murray-Darling River System. It has long been recognised as having potential for farming. Hatchery techniques were developed in the early 1980's, but it wasn't until the 1990's that research at the Grafton Aquaculture Centre (GAC) demonstrated the species has potential for intensive pond culture. In mid 1990's, a series of workshops transferred technology to industry, and over the last 12 years annual production has increased to around 400 tonnes, with 300 tonnes in NSW and the remainder in Queensland, Victoria and Western Australia. The industry is based on pond culture, but recent research has demonstrated that silver perch also has good potential for cage culture.

In 1998 and 1999, there were reports of disease problems causing significant losses on some commercial silver perch farms. These included regular outbreaks of a fungal disease during winter, particularly in the cooler, inland areas of eastern Australia. Clearly there was a need to identify the major diseases, to determine their causes, and to develop cost-effective control and preventive measures. It was also apparent that an overall strategy for managing health on farms and across the industry was needed. Health management is a concept of dealing with fish health by providing general environmental and culture conditions that reduce the incidence and severity of diseases, and enable rapid and appropriate response to disease outbreaks, optimising fish health and performance.

A project to address silver perch diseases and health management commenced in 2001, and was funded by the former NSW Fisheries, the Aquaculture Initiative, the current NSW Department of Primary Industries (NSW DPI), and the Fisheries Research and Development Corporation. The major objectives of the project were to: (i) identify the cause of the new fungal disease; (ii) identify other major diseases of silver perch, particularly those prevalent in winter; (iii) develop diagnostic, control and preventative measures for the diseases; (iv) develop a generic health management plan for farms; and (v) development of an overall health management strategy for the industry and NSW DPI.

Initially, a total of seven commercial silver perch farms in coastal and western regions of NSW as well as GAC were selected to participate in the project. In addition, disease outbreaks were examined on a further four farms. A generic Health Management Plan (HMP) was developed in the first six months of the project, and consisted of advice on farm design and operation, pond management, fish husbandry, water quality management and disease diagnosis and treatment. The HMP was implemented at GAC and extended to participating farmers. Project staff visited farms monthly in the autumn/winter period, bi-monthly in other seasons, and where possible when disease outbreaks were reported. Data on fish health, water quality and farm operations were collected. After the first year, four of the commercial farms were removed from the project due to limited data collection, withdrawal from the industry or the inappropriate use of chemicals. This reduction in participating farms enabled increased sampling effort on the remaining farms.

The history and current status of infectious diseases in silver perch aquaculture were determined using published scientific records, and data from GAC over the 15 year period 1991 – 2005 and from commercial farms over four years (2001 – 2004). The number of known pathogens and infectious diseases of silver perch has increased from 4 in 1983 to 20 in 2005. Protozoan parasites *Ichthyophthirius multifiliis* (causes the disease ichthyophthiriosis or white spot), *Chilodonella hexasticha* (chilodonellosis), *Trichodina* sp. (trichodinosis) and *Ichthyobodo necator* (ichthyobodosis), and the monogenean gill fluke, *Lepidotrema bidyana*, account for around 80% of all records at GAC and on commercial farms. Ichthyophthiriosis and chilodonellosis are acute diseases that can cause high mortalities if not diagnosed and treated promptly. There were 283 records of pathogens at GAC between 1991 and 2005. In approximately 50% of these cases, action was taken to control disease and this generally involved the use of chemical therapeutants. There was a total of 159 records on commercial farms (2001 – 2004). Two new diseases, winter saprolegniosis (colloquially called winter sap or winter disease) and lepidotremosis (infestations of the gill fluke, *L. bidyana*) were identified. In addition, ichthyophthiriosis was found to be difficult to control at low water temperatures. Besides these, a number of bacterial diseases including columnaris, tail (or fin) rot, streptococcosis, mycobacteriosis and aeromonad dermatitis, and the fungal disease epizootic ulcerative syndrome (EUS or redspot, caused by *Aphanomyces invadans*) were recorded. During the first three years, application of the generic HMP was successful in controlling most diseases; however, winter saprolegniosis and ichthyophthiriosis continued to cause serious problems on some commercial farms during winter.

There was a total of 22 outbreaks of winter saprolegniosis on five of the study farms during the project. A survey of 20 farms found that 15% had outbreaks in the winter of 2002, with the locality of farms ranging from southern Queensland to the Riverina. The disease occurred on at least two farms in coastal regions; however, it was not recorded at GAC over the four years of the project despite the presence of the pathogen. The causative agent of winter saprolegniosis was identified as the pathogenic fungus, *Saprolegnia parasitica*. The timing and severity of winter saprolegniosis varied within and between farms and years. If untreated, winter saprolegniosis can cause 100% mortality. Infections were found on both skin and gill tissue. Most outbreaks commenced at water temperatures below 16°C, and rapid decreases in temperature (e.g., 4° – 5°C in 5 – 7 days) following cold changes during winter were associated with the onset and increased severity of the disease suggesting that immunosuppression is involved in the initiation of winter saprolegniosis in silver perch. There was moderate to severe proliferative branchitis and gill hyperplasia prior to

most outbreaks, and many outbreaks were preceded or accompanied by infestations of the ecto-parasites *I. multifiliis*, *C. hexasticha* and/or *L. bidyana*. It appears that stress and physical damage caused by ecto-parasites is a major predisposing factor for winter saprolegniosis. Physical damage to skin from handling during sampling or partial harvest of ponds also preceded some outbreaks when the water temperature factor was concurrent. Winter saprolegniosis often occurred in ponds with high stocking densities and biomasses (> 20,000 fish/ha and 10 tonnes/ha), high organic loads and poor water circulation. Recommended management actions to assist in the prevention and control of winter saprolegniosis are: reduce fish biomass to < 6 tonnes/ha; ensure fish are free of ecto-parasites; do not over-feed; maintain good water quality; avoid partial harvests; and treat harvested fish in tanks with a continuous bath of 2 – 5 g/L salt.

Experimental work on winter saprologeniosis was limited because of the absence of the disease at GAC, and the lack of opportunities for replication and control treatments on commercial farms. Formalin, copper (as copper sulfate), chloramine-T and extract from barley straw were evaluated *in vitro*, but only formalin (25 mg/L, 35 mg/L) had any inhibitory effects on zoospore production and fungal growth. Formalin was not evaluated for the control of winter saprolegniosis in ponds. The aquatic herbicide diquat was also evaluated *in vitro*, and although it had some inhibitory effects on the growth of *S. parasitica*, it is not recommended for use due to toxicity to silver perch at 10 mg/L, and the presence of residues in muscle tissue for up to at least 27 days post-treatment. A concurrent study at the University of Technology Sydney, demonstrated that a probiotic (*Aeromonas media* strain A 199) was effective in increasing survival of silver perch with saprolegniosis under experimental, laboratory conditions, and is worthy of evaluation in commercial ponds. The following management options were also identified for future evaluation: use of formalin or copper prophylactically or at early stages of infection; use of feed additives such as L-carnitine, β-glucan and ascorbic acid to enhance the immune system; monitoring of *S. parasitica* cysts in ponds for early detection; and the use of cage culture to increase husbandry and production efficiencies.

Experiments were carried out in aquaria, tanks and ponds at GAC to determine the efficacy of copper and formalin in treating ichthyophthiriosis and the depletion rates of these chemicals in earthen ponds. Copper (as copper sulfate) at concentrations of 0.1 – 0.2 mg/L controlled ichthyophthiriosis, but higher concentrations of 0.25 – 1.0 mg/L were toxic to silver perch, and 0.05 mg/L was ineffective. Formalin at a concentration of 30 mg/L controlled ichthyophthiriosis, but 20 and 10 mg/L did not completely control the disease. In earthen ponds containing silver perch, copper (0.2 mg/L) and formalin (30 mg/L) were depleted to or below therapeutic concentrations within 24 and 48 h post-treatment respectively. Using these data, the following modified treatment regimes for copper and formalin were developed: (i) copper, 0.2 mg/L initially, then 0.1 mg/L daily (alkalinity of water must be > 80 mg/L, otherwise copper may be toxic); (ii) formalin, 30 mg/L initially, then 20 – 30 mg/L daily or at least each second day; treatments must be continued until the disease is controlled. These new treatment regimes were validated for the control of ichthyophthiriosis in ponds at GAC. Outbreaks were successfully controlled using either chemical at costs of $486.00/ha/day for formalin and $68.34/ha/day for copper sulfate. A continuous salt (NaCl) bath of 2 g/L is effective in controlling ichthyophthiriosis and preventing saprolegniosis. This treatment is recommended in aquaria, tanks and re-circulating aquaculture systems, but not in ponds because of the large quantities of salt required, the potential for accumulation and the detrimental environmental effects of saline water.

Monogenean gill flukes were not recorded at GAC or on commercial farms in the early 1990's, but have become common over the last 10 years, probably due to the introduction of wild broodfish, the movement of fish between farms, and the difficulty in eradicating the parasites from farms. Mortalities from infestations of gill flukes were generally low (< 5%). Infestations can be controlled in ponds and cages by formalin (30 mg/L) or trichlorfon (0.5 mg/L; Lepidex®), but fish generally became re-infested with *L. bidyana* within 5 – 8 days of treatment at temperatures of

21.6º – 26.8°C, suggesting the eggs are resistant to formalin and trichlorfon. Juvenile stages of *L. bidyana* (length range, 70 – 200 μm) attach to skin and gill tissue, whereas adult flukes (500 – 600 μm) are found primarily on gill tissue. Three consecutive treatments 21 days apart controlled infestations of gill flukes in earthen ponds at GAC. In tanks, 0.25 mg/L trichlorfon (Lepidex® 500) was sufficient to control lepidotremosis. There were no detectable residues of dichlorvos (a metabolic product of trichlorfon) in the muscle tissue of silver perch 6 – 48 days after the first of the three consecutive treatments. Currently, Lepidex® is only registered for use as an insecticide on plants in Australia, but the animal product Neguvon® could be used off-label under veterinary prescription. Care needs to be taken in the use of trichlorfon for the following reasons: (i) it has been reported to have adverse effects on neurological functioning and the immune system of warmwater fish even at low concentrations; (ii) it has adverse effects on pond ecology because of toxicity to decapods and zooplankton; (iii) resistance of *L. bidyana* is a potential problem with repeated use; and (iv) it is harmful to humans if absorbed through the skin or inhaled. Formalin causes a deterioration of water quality, particularly dissolved oxygen at high water temperatures (> 25°C) and so it is only recommended for use at temperatures below 25°C, and even then constant aeration for at least 72 hours is necessary to maintain adequate water quality.

During the project, extension and disease diagnostic support was provided to participating farmers. This support greatly increased their knowledge of silver perch diseases and skills in using microscopes and diagnosing and treating diseases. The need for regular monitoring of fish for disease and the value of good health management and aquaculture practices was demonstrated on participating farms.

To aid on-farm disease diagnosis, a manual "Diagnosis, Treatment and Prevention of the Diseases of the Australian Freshwater Fish Silver Perch (*Bidyanus bidyanus*)" was prepared using the results of this project and previous fish health research. In addition, a generic Health Management Plan is presented as Chapter 10 of this report. These publications will assist in disease diagnosis, treatment and prevention, and general health management on silver perch farms. A summary of key recommendations for the health management of silver perch is provided in Chapter 14 of this report.

During the course of the project, a "Hatchery Quality Assurance Program for Murray Cod (*Maccullochella peelii peelii*), Golden Perch (*Macquaria ambigua*) and Silver Perch (*Bidyanus bidyanus*)" (HQAP) was prepared to address concerns about diseases as well as genetics and trash fish in the native fish hatchery industry. The movement of pathogens and diseases on cultured fish is a world-wide problem, and in Australia there have been reports of diseased fish being stocked onto fish farms and into the wild. The HQAP describes essential criteria for fish hatcheries and provides a basis for best practice, and future accreditation and auditing of hatcheries. Implementation of the HQAP should significantly improve health management on hatcheries and on the silver perch farms receiving fingerlings from commercial hatcheries.

The NSW DPI continues to provide aquaculture extension and disease diagnostic support to silver perch and other aquaculture and fisheries industries. There are also disease diagnostic services provided by commercial companies with highly competent veterinarians who have extensive fish health management experience. Our project, "Development of a Health Management Strategy for the Silver Perch Aquaculture Industry" provides a basis for improved health management across the industry, but success is dependent on the use of all components of the strategy, implementation of major recommendations by farmers, and co-operation between individual silver perch farmers, the industry, the aquaculture associations and NSW DPI.

KEYWORDS: Silver perch, diseases, health management.

1. BACKGROUND AND NEED

Silver perch (*Bidyanus bidyanus*; family Teraponidae) is an Australian native freshwater fish that is endemic to the Murray-Darling River System. It is a high quality, white-fleshed finfish that has long been recognised as having potential for aquaculture (Lake 1967; Barlow 1983; Rowland and Barlow 1991; Rowland 1995a). It is one of the most popular inland fish because of its edible and sporting characteristics. There has been a significant decline in distribution and abundance of silver perch over the last four decades, and it is now a threatened species with the conservation status of "Vulnerable". To protect the stocks, there is a prohibition on the capture of silver perch from rivers and creeks throughout the Murray-Darling River System, and a Recovery Plan has been written for the species (Anon. 2005).

Hatchery techniques involving hormone-induced spawning and extensive larval rearing were developed at the Narrandera Fisheries Centre (formerly the Inland Fisheries Research Station) in the early 1980's (Rowland 1983a, 1983b). Since 1990, research has been done at the Grafton Aquaculture Centre (GAC) and the Port Stephens Fisheries Centre (PSFC) into breeding, domestication of broodfish, production of fingerlings and market-size fish, husbandry, water quality, diseases, off-flavour and purging, nutrition and feeding. The research has demonstrated that silver perch is an excellent species for intensive culture in earthen ponds. Key results at GAC were; high survival rates (> 90%), fast growth rates (2 – 5 g/fish/day) at high stocking densities (20,000/ha) leading to high production rates (10 tonnes/ha/year) (Rowland 1994, 1995b; Rowland et al. 1994, 1995). Results were transferred to industry through a series of workshops, and numerous scientific and technical publications (e.g., Rowland and Bryant 1995; Rowland 1998; Rowland et al. 2002; Rowland and Bryant 2003), and a grow-out industry, based on pond culture commenced in the mid-1990's. A small number of farms have achieved high survival rates and good growth and production rates, demonstrating that the performance of silver perch under research conditions at GAC can be achieved under commercial conditions and in regions of NSW other than the North Coast (Mike Beveridge, Bruce Rhoades, Ian Charles, Calvin Terry, Mark Scifleet, Ray Partridge, personal communication). With relatively low feed ingredient costs compared to other cultured species in Australia (Allan and Rowland 2005), it is clear that silver perch has the potential to form a large industry (> 5,000 tonnes/year) based on high-volume, relatively low-cost production. Recent research has demonstrated that silver perch also has good potential for cage culture, with high survival (> 90%), good growth (1.7 g/fish/day) and high production rates (50 – 90 kg/m^3) (Rowland et al. 2004, 2006).

Currently there are over 150 licensed silver perch growers in all states; however, only about a third of these are producing fish commercially. Although a small number of farms achieve high production rates, many farms are relatively inefficient and are not producing to their potential. Survival, growth and production rates are lower, and FCR's higher than achievable with good husbandry and management. Fish are lost to bird predation, diseases and poor water quality, and these losses have increased production costs, reduced the economic viability of some farms, and limited expansion of the industry.

Diseases, in particular those caused by infectious agents, are recognised as an important threat to the viability of finfish aquaculture. The diseases of silver perch under culture conditions have been described by Ashburner (1983), Rowland (1983b), Rowland and Ingram (1991) and Callinan and Rowland (1995). In 1996/97, a pilot monitoring program of silver perch on farms on the NSW North Coast found that diseases cause significant problems, and that survival and growth rates were reduced by ecto-parasitic infestations and by adverse water quality conditions (Lan Jiang, unpublished data, 1997). In 1998 and 1999, there were reports of disease problems causing large losses on some silver perch farms (Sam Clift, Bruce Rhoades, Mike Beveridge, Andrew Pratt,

personal communication). These included regular outbreaks of fungus during winter, particularly in the cooler, inland areas of eastern Australia. Reports suggested that some, or most of these outbreaks were not just the result of poor husbandry and rough handling. A fungal disease, winter saprolegniosis is a problem in the large channel catfish industry in the USA, and proves difficult to control under commercial conditions (Bly et al. 1992, 1993; Li et al. 1996; Hawke and Khoo 2004; Dr Michael Masser, personal communication). Clearly there was a need to determine the aetiology and pathogenesis of this new disease in the silver perch industry, to identify its cause(s) and to develop cost-effective control and preventive measures.

As the silver perch industry expanded in the late 1990's, the incidence and losses from disease increased, and silver perch farmers became aware of the need for disease research and diagnostic support. Diseases and health management became a high R&D priority. At the time, there was no specific disease research and no validated health management programs or strategies were available to the industry. A project to address these disease issues commenced in 2001, and was funded by the former NSW Fisheries, the Aquaculture Initiative, the current NSW DPI, and the Fisheries Research and Development Corporation. The major objectives of the project were to:

(i) identify the cause of the new fungal disease;
(ii) identify other major diseases of silver perch, particularly those prevalent in winter;
(iii) develop diagnostic, control and preventative measures for the diseases; and
(iv) develop a generic health management plan for farms and an overall health management strategy for the industry.

Health management is a concept of dealing with aquatic animal health by providing general environmental and fish-culture conditions that reduce the incidence and severity of diseases, leading to efficient and economic production of fish. There is a profound and inverse relationship between environmental quality and the disease status of fish (Plumb, 1994), and the concept of health management was first introduced around 50 years ago by Snieszko (1958). The natural resistance of fish to infectious diseases can be enhanced through good facilities and management, or it can be compromised by facilities and practices that create stressful conditions. The old saying "An ounce of prevention is worth a pound of cure" is very applicable to silver perch culture. Good culture conditions optimise survival, growth, food conversion, reproduction and production, and minimise stress and disease problems. Health management encompasses all stages and aspects of the production cycle.

During the first year of the project, a generic Health Management Plan (HMP) based on previous research, established culture practices and various technical publications (Piper et al. 1982; Rowland and Ingram 1991; Callinan and Rowland 1995; Rowland and Bryant 1995; Noga 2000) was implemented at GAC and on some commercial farms, in an attempt to facilitate year-round control of diseases. The approach was successful in controlling the common diseases chilodonellosis, ichthyobodosis, trichodinosis and lepidotremosis; however, winter saprolegniosis and the ecto-parasitic disease ichthyophthiriosis continued to cause major losses on some commercial farms during winter. Both diseases proved difficult to treat effectively at low temperatures because of the relatively complex and slow life cycles of the pathogens, and the presumed depletion of formalin in water. Further work was needed and subsequently carried out to develop effective treatments, and to improve health management in general. Without this work, some farms would have continued to have significant losses to the extent that their economic viability would have been threatened.

This project has provided a basis and the tools for improved health management in the silver perch aquaculture industry. These include:

(i) the identification of two new diseases;
(ii) identification of the pathogen and factors associated with winter saprolegniosis;
(iii) control and preventative measures for most diseases;
(iv) a new, effective treatment regime for ichthyophthiriosis involving the daily application of copper or formalin to overcome the rapid depletion of these chemicals;
(v) a written generic Health Management Plan for farms;
(vi) a Disease Diagnostic Manual; and
(vii) a Hatchery Quality Assurance Program.

To support the industry, the NSW DPI continues to provide extension and disease diagnostic support to silver perch farmers, as well as other industries. Disease diagnostic services are also provided by commercial companies.

Implementation of the major recommendations of this project by farmers, and co-operation between individual silver perch farmers, the industry, the Silver Perch Growers Association and other associations, and NSW DPI will improve health management across the silver perch industry.

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2. OBJECTIVES

2.1. Objectives of the project during 2001 – 2003:

- Identify and characterise the causes of winter disease and other important diseases of silver perch.
- Identify cost-effective control and preventative measures for these diseases.
- Develop, validate and extend a generic "Health Management Plan" which can be modified to suit the needs of individual farms.

2.2. Objectives of the one-year extension in 2004:

- Evaluate the efficacy of formalin and copper against *Ichthyophthirius multifiliis* infestations and saprolegniosis outbreaks during winter.
- Produce an updated health management plan for silver perch.
- Implement and validate a modified health management plan with major silver perch producers.

3. GENERAL MATERIALS AND METHODS

3.1. Silver perch farms

Initially, the Grafton Aquaculture Centre (GAC; see Fig. 3.1) and seven commercial silver perch farms were selected for the monitoring of fish health and water quality in the project. The farms were located throughout NSW, including coastal and inland locations: Grafton, Gloucester (2 farms), Bundarra, Grong Grong in the Riverina (2 farms) and Jerilderie. Participating farmers were contacted verbally and in writing during February and March 2001. After one year, four of the original farms were removed from the project for one or more of the following reasons: (i) unreliable data collection and record keeping; (ii) regular use of unregistered or un-permitted chemicals that compromised the integrity of the research and the project; (iii) withdrawal from the industry. To compensate for the loss of farms, the number of ponds monitored on two farms (Gloucester and Grong Grong) was increased. These changes improved the quality and reliability of data. The final farm visit for the initial component of the project was in September 2003.

Three commercial farms were subsequently selected to participate in the one year extension of the project. One farm was located at Gloucester and two in the Riverina. Low winter water temperatures (< 10°C) were a feature at each locality. One farm had a history of significant losses to diseases in winter (saprolegniosis and ichthyophthiriosis) and the other farms had some problems with ichthyophthiriosis. The farmers were contacted in February/March 2004 to discuss aspects of husbandry, pond management, and disease and water quality monitoring. Aspects of the Health Management Plan (HMP) were recommended for implementation on these farms. There were monthly visits to these farms over the period May – August 2004. In addition, four other commercial farms were visited in NSW to collect data on disease outbreaks.

3.2. Consultation with industry groups

Industry associations [NSW Silver perch Grower's Association (SPGA), Murray Region Aquaculture Association, NSW Aquaculture Association, Aquaculture Association of Queensland] were informed about the project. The project was discussed with farmers at a freshwater aquaculture conference and industry summit at Dubbo in February 2001. In December 2001, a meeting was held with Mark Scifleet (participating farmer and member of the SPGA) to review the project and discuss future directions. In February 2002, the project was reviewed and fully endorsed at a meeting at Narrandera between NSW Fisheries and the native fish industry. A meeting of the project steering group, including three industry representatives was held in Sydney in October 2002. The status of the project, results and plans for future work were discussed. It was agreed that due to significant losses to winter diseases at one farm over the previous two years, and the lack of effective controls for winter saprolegniosis and ichthyophthiriosis, efforts should focus on this and one other farm in the Riverina in the final year.

Updates on the project were published in the SPGA Newsletters. A paper on the initial findings on winter saprolegniosis was given at an international disease conference in Brisbane in 2002. Major findings of the project were presented to industry representatives at the Silver Perch Aquaculture Conference, Grafton, August 2003, the Annual Conference of the Aquaculture Association of Queensland in August 2003, and the SPGA Annual Meeting and Conference at Port Stephens in July 2004.

Consecutive Presidents of the SPGA, Bruce Rhoades (2000 – 2002) and Ian Charles (2003 – present) were co-investigators during the project.

3.3. Routine monitoring

Monitoring on participating farms commenced in April 2001. In general, farms were visited monthly in winter and bi-monthly at other times. Two ponds on each farm, one fingerling and one grow-out, were selected for monitoring. The water quality variables temperature, dissolved oxygen (DO), pH and ammonia were monitored in these ponds three times weekly by the farmer, and these variables plus alkalinity and hardness were monitored by project staff during visits. Five fish were randomly sampled from the ponds. The fish's body and skin and gill tissues were examined for signs of disease, ecto-parasites and fungal lesions. Where appropriate, tissue was excised and stored for later examination and pathology. Moribund and diseased fish in other ponds were also examined. Control methods were discussed with farmers, and approved/legal chemicals were recommended to control disease outbreaks.

3.4. Health Management Plan

A generic Health Management Plan (HMP) was developed in the first six months of the project, and was based on previous research results, established culture practices, technical articles including publications on the diseases of silver perch and other freshwater fish and on the operation and practices at GAC (Piper et al. 1982; Rowland and Ingram 1991; Plumb 1994; Callinan and Rowland 1995; Rowland and Bryant 1995; Noga 2000). The HMP consisted of advice on farm design and operation, pond management, fish husbandry, water quality management and the diagnosis and treatment of diseases. The HMP was extended to participating farmers in an attempt to facilitate year-round control of diseases.

3.5. Project staff and extension

Routine farm visits were carried out by NSW Fisheries staff, mainly the Aquatic Animal Health Veterinary Officer (Matthew Landos) and the Aquaculture Extension Officer (AEO) (Phil Read). This on-farm presence was aimed not only at collecting data and advising farmers of practices under the generic HMP, but increasing the participating farmer's knowledge of silver perch diseases, and their skills in using microscopes, and diagnosing and treating diseases. The need for regular monitoring of fish for disease and for improved health management was demonstrated on the participating farms. The involvement of the AEO in farm visits provided a link between research, management and extension staff of NSW DPI, and ensured that advice to farmers was up-to-date and directly applicable to health management on silver perch farms.

3.6. Grafton Aquaculture Centre

GAC is a model fish farm where research into the culture of silver perch has been carried out since 1990 (Fig. 3.1). GAC provided a comparison to the participating commercial farms in the project. Monitoring of water quality was based on Rowland (1995) and fish were routinely sampled monthly during winter and bi-monthly at other times from the ponds at GAC as well as on commercial farms. Fish health records over the period of 1991 – 2005 were used to determine the incidence of silver perch diseases. The absence of winter saprolegniosis at GAC, and the lack of replication and control treatments on commercial farms restricted experimental work on that disease.

Experimental work on the toxicity, efficacy, depletion and residues of potential therapeutic chemicals was carried out in aquaria, tanks and ponds at GAC, and work on the *in vitro* evaluation of chemicals was done at the Aquatic Animal Health Unit. Details of this research are given in the following chapters of this report.

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Figure 3.1. The NSW Department of Primary Industries' Grafton Aquaculture Centre, a model freshwater fish farm.

4. REVIEW OF THE INFECTIOUS DISEASES OF THE AUSTRALIAN FRESHWATER FISH SILVER PERCH (*BIDYANUS BIDYANUS*) IN AQUACULTURE

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4.1. Abstract

The history and current status of the infectious diseases of silver perch in aquaculture are reviewed. Data were collected at the Grafton Aquaculture Centre (GAC) over 15 years (1991 – 2005) and during a health monitoring program on selected commercial farms in coastal and western regions of NSW over four years (2001 – 2004). The number of known pathogens and infectious diseases has increased from 4 in 1983 to 20 in 2005. Protozoan parasites *Ichthyophthirius multifiliis* (causes the disease ichthyophthiriosis or white spot), *Chilodonella hexasticha* (chilodonellosis), *Trichodina* sp. (trichodinosis) and *Ichthyobodo necator* (ichthyobodosis), and the monogenean gill fluke, *Lepidotrema bidyana*, account for around 80% of all records at GAC and on commercial farms. Ichthyophthiriosis and chilodonellosis are acute diseases that can cause high mortalities if not diagnosed and treated promptly. Gill flukes were not recorded at GAC or on commercial farms in the early 1990's, but have become common over the last 10 years, probably due to the introduction of wild broodfish, the movement of fish between farms, and the difficulty in eradicating this parasite from farms. Winter saprolegniosis (caused by the fungus, *Saprolegnia parasitica*) was first reported in 1998, and has become a serious disease on some farms during winter; this disease was not recorded at GAC. Highest mortalities on commercial farms are caused by ichthyophthiriosis, winter saprolegniosis and chilodonellosis. At GAC, only ichthyophthiriosis, chilodonellosis and one outbreak of mycobacteriosis caused mortality rates over 10%. The fungal disease epizootic ulcerative syndrome (EUS) was initially recorded at GAC in 1991, but has not occurred since 1997 following changes to water management practices. EUS was not recorded on farms in the western region, with one exception following the translocation of fingerlings from a farm in a coastal drainage. The bacterial diseases columnaris, tail rot, streptococcosis, mycobacteriosis and aeromonad dermatitis were recorded, but the incidence was low (< 3%). No viral diseases were recorded, but the epizootic haematopoietic necrosis virus kills silver perch under laboratory conditions and so is a potentially serious pathogen. The use of good aquaculture and health management practices minimise losses from diseases on silver perch farms.

4.2. Introduction

Silver perch (*Bidyanus bidyanus*) is an Australian native warmwater fish endemic to the Murray-Darling River System. Hatchery techniques to produce fingerlings for stock enhancement were developed at the Narrandera Fisheries Centre (NFC) in early 1980's, and a commercial hatchery

industry commenced in 1982 (Rowland 1983; Rowland et al. 1983; Rowland and Tully 2004). Research to develop techniques for production of market-size fish commenced in 1990 at the Grafton Aquaculture Centre (GAC) and results demonstrated that silver perch is an excellent species for intensive culture in earthen ponds and cages (Rowland 1994, 1995a, b; Rowland et al. 1994, 1995, 2004, 2006). Silver Perch Aquaculture Workshops were held in 1994, and an industry based on pond production commenced in the mid-1990's (Rowland and Bryant 1995; Rowland 1998). There has also been interest in silver perch in other countries including the People's Republic of China, Taiwan, Israel, the Philippines and the USA.

Infectious diseases are relatively common in intensive aquaculture industries. Diseases have long been recognised as a major cause of mortality and can significantly limit the performance and production on fish farms (Sarig 1971; Ashburner 1983; Paperna 1991; Plumb 1999). The pathogens and diseases of silver perch under hatchery conditions were originally described by Rowland (1983). Later reports by Rowland and Ingram (1991) and Callinan and Rowland (1995) provided additional information, including descriptions of new diseases and recommendations for treatment and prevention of diseases in silver perch as well as Murray cod (*Maccullochella peelii peelii*) and golden perch (*Macquaria ambigua*). Other publications have provided information on diseases of silver perch (Beumer et al. 1983; Callinan 1986, 1988; Langdon 1990).

There was an increase in the numbers of silver perch farms during the late 1990's and early 2000's as the grow-out industry became established. Production has increased to around 400 tonnes in 2004/05, a majority of which is in NSW (O'Sullivan et al. 2007). Fish health problems have increased as the silver perch industry expanded. The results of a health monitoring project on a silver perch farm in northern NSW found that infestations of parasites limited growth and feeding efficiency (Lan Jiang, unpublished report, 1997). In 1998 and 1999, there were verbal reports of serious disease problems, including a fungal disease causing significant losses on some silver perch farms during winter (Sam Clift, Mike Beveridge, Bruce Rhoades, Andrew Pratt, personal communication). Fungal diseases are known to cause significant mortalities in some freshwater aquaculture industries, and winter saprolegniosis is a problem in the large, pond-based channel catfish industry in the USA (Bly et al. 1993; Li et al. 1996; Noga 2000; Hawke and Khoo 2004; Wise et al. 2004).

In the late 1990's, it was evident that there was a need to review the diseases of silver perch, and identify emerging diseases in the commercial industry. A research project on the health management of silver perch commenced in 2001 with the objectives of: identifying diseases, particularly those causing problems in winter; developing cost-effective control and preventative measures for these diseases; and developing an overall health management strategy for the silver perch industry. This paper reviews the history and occurrence of diseases in silver perch aquaculture using scientific literature, records at GAC and results of the health management project.

4.3. Materials and methods

4.3.1. History of diseases

The following publications were used to determine the history of diseases in silver perch aquaculture; Rowland (1983), Callinan (1986, 1988), Rowland and Ingram (1991) and Callinan and Rowland (1995). Records held by research staff at GAC were used to determine the initial reports and occurrence of diseases at GAC and on commercial farms.

4.3.2. Grafton Aquaculture Centre (GAC)

GAC is located on the North Coast of NSW, and operates primarily as a silver perch aquaculture research facility and hatchery. Research into the pond and cage culture of silver perch has been carried out since 1990, and has included production and husbandry techniques, nutrition and feeding, broodfish management, breeding, larval rearing, fingerling production, water quality, and diseases and health management. A breeding program produces up to 500,000 fingerlings annually. GAC has 19 earthen ponds (0.1 – 0.3 ha surface area), 2 reservoirs (8.5 and 9 ML capacity) and an effluent/settlement dam (43ML). The main water supply is the Clarence River, and all effluent water is stored, settled and either re-used for fish culture or used for irrigation. Guidelines for the husbandry and management of silver perch at GAC are given in Rowland and Bryant (1995) and Rowland and Tully (2004).

4.3.3. Commercial silver perch farms

Most production on silver perch farms is in earthen ponds. Rowland (1995b) recommended a 3-phase production system for silver perch; I – hatchery phase, II – fingerling phase, III – grow-out phase. Some farms combine hatchery, fingerling and/or grow-out phases, while others specialise in hatchery or grow-out. In the health management project, seven commercial farms, located throughout NSW and in both coastal (eastern) and inland (western) regions were initially selected to monitor fish health. After one year, four of the original farms were removed from the project and the number of ponds monitored on two farms was increased. Three farms were subsequently selected to participate in the one year extension of the project. The farms were located near Gloucester, Grong Grong and Howlong. One farm had a history of significant losses to diseases in winter (saprolegniosis and ichthyophthiriosis) and the other farms had some problems with ichthyophthiriosis.

4.3.4. Disease monitoring

4.3.4.1. GAC

Routine water quality and disease monitoring are undertaken year-round at GAC. Water quality monitoring and management are based on Rowland (1995c). To monitor disease, 3 – 5 fish were sampled at varying intervals depending on the culture phase. Larvae and fry were sampled weekly from larval rearing ponds, and fingerlings and larger fish were randomly sampled monthly from each pond using a seine net or cast net. Fish were also opportunistically sampled and examined if there were signs of diseases (e.g., moribund fish, loss of appetite, flashing, abnormal behaviour or colour). Each fish was examined externally, and subjected to a full necropsy, including preparation of skin mucus scrapes and gill tissue wet mounts. The tissue samples were examined microscopically (X40 to X 400) for pathogens and other signs of diseases. Both gill and skin tissues were also excised and fixed in formalin (5%) for subsequent microscopic examination. The presence of pathogens was recorded, and disease control measures implemented when GAC staff consider the level of infestation high enough to cause mortalities.

4.3.4.2. Commercial farms

In general, commercial farms were visited monthly in winter and bi-monthly in other seasons. During years 1 – 3 of the project, five fish were randomly sampled from each of two ponds, one fingerling and one grow-out on each farm. In the fourth year, four ponds on each of two farms and 10 ponds on the remaining farm were monitored. Examination of fish was as for GAC. Where appropriate, excised tissue was stored for later examination and pathology. Moribund and diseased fish in other ponds were also examined. The sampling strategy did not allow seasonal occurrence of diseases on farms to be determined. Control methods were discussed with farmers, and where

necessary approved and legal chemicals were recommended to control disease outbreaks. The water quality variables temperature, dissolved oxygen (DO), pH and ammonia were monitored in these ponds three times weekly by the farmer, and these variables plus alkalinity and hardness were monitored by project staff during visits.

In this review, a record of pathogens did not necessarily mean a clinical disease outbreak. Only the presence of the protozoan parasites *Ichthyophthirius multifiliis* or *Chilodonella hexasticha* on skin or gill tissue invoked immediate control measures to control the acute diseases ichthyophthiriosis and chilodonellosis respectively at GAC. The presence of some common pathogens such as *Trichodina*, *Ichthyobodo* and gill flukes did not always lead to a disease; however, their presence can stress fish, cause physical damage to epidermal tissue, and predispose fish to increased infection and/or other pathogens. Under these circumstances management was improved where possible and the level of monitoring increased. The fungal diseases saprolegniosis and epizootic ulcerative syndrome (EUS), and the bacterial diseases columnaris and tail rot were presumptively diagnosed on-site at GAC and on farms, and confirmed at the Aquatic Animal Health Laboratory, Wollongbar (AAHL). Aeromonad dermatitis, streptococcosis and mycobacteriosis were diagnosed at AAHL. In silver perch, saprolegniosis and winter saprolegniosis have a common pathogen (*Saprolegnia parasitica*) but different causative factors and aetiology.

4.4. Results and discussion

4.4.1. Increase of pathogens and diseases

Ashburner (1983) described diseases in the culture of freshwater fishes, but specific records relating to silver perch were not included. Rowland (1983) reported four common pathogens in the hatchery production of silver perch at NFC between 1978 and 1983; the protozoans *I. multifiliis* and *Trichodina* sp., the copepod *Lernaea* sp. and the fungus *Saprolegnia* sp. During this period, NFC was the only hatchery producing silver perch in Australia, and there were no commercial native fish hatcheries until late 1982 (Rowland and Tully 2004). Beumer et al. (1983) listed the monogenean, *Lepidotrema bidyana*, as well as two species of digenea and four species of nematodes in silver perch in a checklist of parasites of fishes from Australia.

Over the following 12 years, the hatchery industry expanded and the grow-out industry commenced (Rowland and Bryant 1995; Rowland 1998; Rowland and Tully 2004). There was a subsequent increase in the number of pathogens and infectious diseases in silver perch (Table 4.1). Rowland and Ingram (1991) and Callinan and Rowland (1995) added a further nine to the list provided by Rowland (1983), including the common protozoans *C. hexasticha* and *Ichthyobodo necator*, the fungal disease EUS and the bacterial disease goldfish ulcer disease (GUD). GUD has only been recorded once on one inland farm. Records of *Tetrahymena* sp., monogenean gill flukes, and the bacterium, *Aeromonas hydrophila*, were based on limited records and were not considered common at the time. Langdon (1990) reported the protozoans, *Henneguya* sp. and *Eimeria* sp., in silver perch. Between 1995 and 2005, the number of reported diseases and pathogens increased to 20 (Table 4.1). Similar increases of pathogens and diseases associated with the expansion and intensification have been reported in other warmwater aquaculture industries including the channel catfish in the USA and tilapia in various countries (Mitchell 1997; Plumb 1997, 1999; Fitzsimmons 2000; Stickney 2000).

4.4.2. Records and types of pathogens and diseases

There were 283 records of pathogens at GAC between 1991 and 2005. In approximately 50% of these cases, action was taken to control the disease and this generally involved the use of chemical therapeutants. There were 154 records on the participating commercial farms over the period 2001 – 2004. Protozoans and monogeneans accounted for approximately 80% of all records at both GAC

and on commercial farms (Fig. 4.1). The most common protozoans were *Trichodina* sp., *I. multifiliis* and *C. hexasticha* (Fig. 4.2). Trichodinosis is a chronic disease, rarely causing high mortalities (Callinan and Rowland 1995; Read et al. 2007). Ichthyophthiriosis and chilodonellosis are acute diseases and can cause up to 100% mortality on some commercial farms (Table 4.2). Winter saprolegniosis also has the potential to cause very high mortalities in silver perch (Table 4.2).

Bacterial and fungal diseases are not common in silver perch culture. Although the fungal disease winter saprolegniosis accounted for 14.3% of records on commercial farms (Fig. 4.1), including farms in coastal regions (Jeff Guy, Mark Scifleet, personal communication), the disease was not recorded at GAC, despite the presence of the pathogen. The high incidence of protozoan and metazoan diseases in silver perch is in contrast to the channel catfish industry in the USA where bacterial diseases are far more common, comprising up to 32% of recorded cases and accounting for approximately 60% of losses caused by infectious diseases compared to 30% to parasitic infestations and 9% from fungal infections (Duatre et al. 1993; Mitchell 1997; Plumb 1994, 1997; Hawke and Khoo 2004). Parasitic diseases also cause serious mortalities of tilapia in hatchery and rearing facilities (El-Sayed 2006).

4.4.3. Seasonal occurrence and culture phase

Parasitic diseases were recorded in all seasons. The occurrence of the most common pathogens, the protozoan, *Trichodina* sp. and gill fluke, *L. bidyana* is shown in Fig. 4.3. At GAC, *Trichodina* is most common in spring and summer, and flukes in winter and spring. The high incidence of *Trichodina* in these seasons is due to its prevalence on larvae and fry in the eutrophic larval rearing ponds during the hatchery phase which is restricted to spring and summer (Fig. 4.4) (Rowland and Ingram 1991; Thurstan and Rowland 1995). At GAC, the highest incidence of ichthyophthiriosis and chilodonellosis is in winter and spring (Fig. 4.5), and outbreaks of ichthyophthiriosis in fingerlings often occur when water temperatures decline to around 15°C in early winter. Similarly, in Israel *I. multifiliis* usually occurs in late autumn and winter, but outbreaks can occur at any time of the year (Sarig 1971). Rowland and Ingram (1991) reported the period of highest incidence of ichthyophthiriosis at NFC as spring, summer and autumn. NFC operates only as a hatchery, and so this variation in seasonal occurrence between GAC and NFC is probably due to the relatively lower numbers of fish held in ponds at NFC over winter. In general, the incidence of diseases in both the fingerling and grow-out phases are highest in winter and spring, while in summer only trichodinosis is common (Fig. 4.4).

Infectious diseases in warmwater aquaculture in temperate regions are reported to be seasonal, with a peak in spring when water temperatures are between 20º and 28ºC which are optimum for many fish pathogens (Meyer 1978; Plumb 1999). Temperature plays a key role in the pathogenicity of disease organisms, as well as the immune system of fish (Paperna 1991; Plumb 1999; Moore and Hawke 2004; Wedemeyer 1996). The relatively high incidence of diseases in silver perch during winter may be due partly to suppression of the immune system at low temperatures in this warm-water species, and subsequent increased susceptibility to infection by the pathogens *I. multifiliis, C. hexasticha* and *S. parasitica*.

4.4.4. Diseases of silver perch

Detailed descriptions of the diseases of silver perch, plus control and preventative measures are given in Rowland and Ingram (1991), Callinan and Rowland (1995) and Read et al. (2007). Most pathogens and diseases of silver perch are encountered in other warmwater species such as Murray cod, carp (*Cyprinus carpio*), channel catfish (*Ictalurus punctatus*) and tilapia (*Oerochromis*, *Tilapia*) (Sarig 1971; Tucker and Robinson 1990; Hawke and Khoo 2004; Ingram et al. 2005; El-Sayed 2006). The common and important diseases of silver perch are briefly discussed below, grouped by type of pathogen.

4.4.5. Diseases caused by ecto-parasitic protozoans, monogeneans and myxosporidians

Parasitic diseases are a major problem in warmwater fish culture, and translocation of cultured fish has undoubtedly contributed to the world-wide distribution of many ecto-parasites, including those in Australia (Sarig 1971; Paperna 1991; Hoffman 1999; Dr Ilan Paperna, personal communication).

4.4.5.1. Ichthyophthiriosis

Ichthyophthirius multifiliis is a ciliate protozoan that invades the skin and gill tissues of freshwater fish causing the acute disease ichthyophthiriosis, commonly referred to as ich or white spot. It can cause very high mortalities in both warmwater and coldwater species including silver perch, and is particularly common in fingerlings stocked at high densities (Piper et al., 1982; Tucker and Robinson 1990; Rowland and Ingram 1991; Callinan and Rowland 1995; Noga 2000). *Ichthyophthirius multifiliis* is an obligate fish parasite that has a complex, temperature-dependent life cycle (Fig. 4.7). The free-swimming, infestive theront (20 – 40 µm in length) bores under the epithelium of skin and gill tissue, where it feeds and develops into a relatively large trophont (up to 1 mm in diameter) which is visible to the naked eye resulting in the characteristic white spots on skin and gills. Trophonts leave the fish, adhere as tomonts to solid substrates such as pond bottom, cages, tanks and nets, and undergo mitosis before releasing up to 3000 tomites that differentiate into theronts (Paperna 1991). Reproduction may also occur under the epithelium of the fish (Ewing et al., 1986). The length of the life cycle varies from 90 – 96 days at 3° – 5°C, 13 – 14 days at 13° – 15°C, to 3 – 7 days at 20° – 25°C; there is no development below 3°C and over 30°C (Hawke and Khoo 2004; Wise et al. 2004). Only the free-swimming theronts are accessible and susceptible to chemical treatment, and so effective control is dependent on periodic applications or continuous exposure to therapeutants. The disease is difficult to control at low temperatures because of the slow life cycle and rapid depletion of chemicals in water. Salt at concentrations of 2 – 5 g/L is effective in tanks (Selosse and Rowland 1990; Mifsud and Rowland 2007), and a treatment regime involving applications daily or each second day to maintain concentrations of formalin (30 mg/L) and copper (0.1 – 0.2 mg/L) controls ichthyophthiriosis in earthen ponds (Rowland et al. 2007).

4.4.5.2. Chilodonellosis

Chilodonella hexasticha is a ciliate protozoan that causes the acute disease chilodonellosis (Fig. 4.8). The native species silver perch, Murray cod and eastern freshwater cod (*Maccullochella ikei*) are particularly susceptible to this disease under culture conditions (Rowland and Ingram 1991; Rowland et al. 1991; Callinan and Rowland 1995; Ingram et al. 2005) and unless diagnosed and treated promptly can cause high mortalities within several days. *Chilodonella hexasticha* has also been reported to cause fish kills of bony herring (*Nematalosa erebi*) and Murray cod in wild (Langdon et al. 1985; Rowland and Ingram 1991). A single treatment using salt or formalin is usually sufficient to control chilodonellosis, but because a cyst stage of *C. hexasticha* has been reported in the Australian fish *Maccullochella ikei* (Rowland et al. 1991), and infestations of gill parasites can be difficult to control, repeated treatments over several days are recommended to ensure eradication of the parasite (Rowland and Ingram 1991; Read et al. 2007).

4.4.5.3. Trichodinosis

The ciliated protozoan, *Trichodina* sp. is a common gill and skin parasite of freshwater fish which causes the disease trichodinosis (Fig. 4.9). Although Rowland and Ingram (1991) reported high mortality of silver perch fry in a larval rearing pond at NFC due to a heavy infestation of *Trichodina* sp., this parasite rarely causes mortalities of silver perch, and many infestations remain at low levels. *Trichodina* sp. has relatively low pathogenicity and only causes problems when fish are stressed and immuno-suppressed (Noga 2000). Heavy infestations can develop in ponds with a high organic load and very poor water quality, predisposing fish to infection by the fungal and bacterial pathogens (Plumb 1999). Infestations of *Trichodina* are readily treated with a single application of formalin or salt (Callinan and Rowland 1995; Read et al. 2007), and the disease may

be prevented by maintaining good water quality, using appropriate feeding regimes and ensuring tanks are clean.

4.4.5.4. Ichthyobodosis

The flagellate protozoan *Ichthyobodo necator* causes the disease ichthyobodosis (Fig. 4.9). This disease can be difficult to diagnose at low levels of infestation because of the relatively small size of *I. necator* (< 20 μm). Careful preparation of gill tissue, magnification of at least X200 and examination in different fields of view are necessary for accurate diagnosis. Silver perch are relatively susceptible to this disease and if not accurately diagnosed and treated it can cause mortalities. Epizootics usually occur at high stocking densities in tanks and cages, and often follow periods of poor water quality or nutritional stress. *Ichthyobodo necator* can cause high mortalities in channel catfish and tilapia (Plumb 1997; El-Sayed 2006). Ichthyobodosis is controlled by salt or formalin, and the maintenance of good water quality and hygiene are important preventative measures (Read et al. 2007).

4.4.5.5. Henneguya

Henneguya sp. is a myxosporidian that was first recorded at GAC in 2001 (Fig. 4.6) following the introduction of silver perch from a commercial farm in the western region. Most infestations have been light and not caused mortalities or affected growth; however, in one outbreak at GAC, a high infestation rate on large silver perch resulted in chronic mortality over several weeks before the disease was controlled using formalin. Myxosporidians are obligate fish parasites with intermediate hosts such as annelids. The introduction of *Henneguya* to GAC demonstrates the risk of transferring pathogens on live fish, and the importance of effective quarantine to prevent their introduction to farms.

4.4.5.6. Monogenean gill flukes

Monogeneans are common fish parasites with strict host specificity and a non-pathogenic nature which reflects a highly evolved adaptability of their hosts (Paperna 1991). The dactylogyrid infesting silver perch is *Lepidotrema bidyana* (Murray 1931) (Fig. 4.10). Gill flukes were not recorded at GAC between 1990 and 1995 despite routine disease monitoring during production experiments and hatchery operations (Rowland 1994, 1995a, Rowland et al. 1994, 1995). Since the mid 1990's, gill flukes have been common at GAC (Fig. 4.6) and on commercial farms. The introduced of broodfish from the wild, the movement of fish between farms, the intensification of silver perch culture and the difficulty in completely eradicating this parasite are probable causes of the high incidence of gill flukes at GAC and on farms.

Monogeneans cause significant mortalities and economic losses in some warmwater industries (Paperna 1991; Hawke and Khoo 2004; Hanson and Wise 2005). The parasites use a series of hooks to attach to gill and skin tissue and infestations can result in significant tissue damage as well as respiratory stress (Paperna 1991; Hawke and Khoo 2004; El-Sayed 2006). Gill flukes rarely cause mortalities in silver perch, but heavy infestations reduce appetite and growth, cause stress and damage gill filament epithelium. Infestations have been associated with subsequent fungal and bacterial infections, although causation has not been proven. Flukes are readily treated using formalin or trichlorfon, but care needs to be taken using formalin at temperatures around 25ºC and higher because of its adverse affects on water quality (Rowland et al. 2006; Landos et al. 2007a). Eggs of *L. bidyana* are resistant to these chemicals and up to three consecutive treatments may be necessary to control infestations of this parasite (Landos et al. 2007a; Read et al. 2007). Eradication of this parasite from farms is difficult, and control relies on a combination of appropriate use of chemical therapeutants, drying and de-silting ponds and stocking fish that are free of gill flukes.

4.4.5.7. Fungal diseases

Aquatic fungi or molds are ubiquitous, saprophytic organisms that feed on dead organic matter. Some species are pathogenic and responsible for significant losses in freshwater aquaculture industries. Damage to the epidermis and skin caused by physical injury or other pathogens provide a site for infection by fungal spores, and other factors such as immune suppression, high organic loads, poor water quality are also associated with fungal infections (Piper et al. 1982; Noga 2000; Hawke and Khoo 2004). Fungal diseases are very difficult to treat once the infection has commenced, and management needs to be based on prevention (Noga 2000).

4.4.5.8. Winter saprolegniosis

The causative agent of winter saprolegniosis in silver perch is the highly pathogenic fungus, *Saprolegnia parasitica* (Landos et al. 2007b). Winter kill of channel catfish in the USA results from immunosuppression caused by a rapid decrease in water temperature and the presence of high concentrations of zoospores of *Saprolegnia* (Bly et al. 1993). In silver perch, most outbreaks commence at water temperatures below 16°C, and large decreases in temperature (e.g., > 5°C in 7 days) following cold changes during winter are associated with rapid onset and increased severity of the disease, suggesting suppression of the immune system by low and declining water temperature also plays an important role in this disease in silver perch (Landos et al. 2003, 2007b). The timing and severity of winter saprolegniosis varies within and between ponds, farms and years, and mortality rates can approach 100% on some farms. Infections occur on both skin and gill tissue (Fig. 4.11). In addition, many outbreaks are preceded or accompanied by infestations of the ecto-parasites *I. multifiliis*, *C. hexasticha* and/or *L. bidyana*. Stress and physical damage caused by ecto-parasites and partial harvesting of ponds appear to be major predisposing factors for winter saprolegniosis in silver perch, although the disease may occur in the absence of these factors (Landos et al. 2007b). The disease often occurs in ponds with high stocking densities and biomasses (> 20,000 fish/ha and 10 tonnes/ha), high organic loads and poor water circulation. There have been no outbreaks of winter saprolegniosis at GAC despite water temperatures as low as 10ºC in winter and the presence of *S. parasitica*. The aetiology and pathogenesis of winter saprolegniosis in silver perch are still poorly understood, and the disease remains difficult to control (Landos et al. 2007b). Salt (2 – 5 g/L) prevents infection by *S. parasitica*, and is recommended for use in tanks (Mifsud and Rowland 2007). Salt, formalin and copper sulphate were reported to be effective in preventing saprolegniosis in channel catfish (Li et al. 1996), but their efficacy under commercial conditions has not been proven and the disease remains problematic in the catfish industry in the USA (Dr Michael Masser, personal communication). Further research is needed to evaluate the effectiveness of these and other chemicals in preventing and controlling winter saprolegniosis in silver perch in earthen ponds. Recent research under laboratory conditions demonstrated that probiotics have potential for controlling saprolegniosis in silver perch (Lategan et al. 2004), and there is evidence that the feed additive L-carnitine can protect fish exposed to acute cold stress or high levels of ammonia (Harpaz 2005). The potential effects of probiotics and L-carnitine in reducing immunosuppression and the incidence of winter saprolegniosis in silver perch may be worth evaluating.

4.4.5.9. Saprolegniosis

Other than winter saprolegniosis, infections of *Saprolegnia parasitica* are caused principally by physical damage to the epidermis and are characterised by fungal growths that resemble cotton wool. The disease may occur at any temperature and is associated with poor husbandry such as rough handling, netting, over-crowding, lack of anaesthetics during transportation, infestations of parasites, poor sanitation in hatcheries, and damage and stress from bird predation. Saprolegniosis causes fewer problems on farms where good aquaculture and health management practices are used.

4.4.5.10. Epizootic ulcerative syndrome (EUS)

EUS is a serious disease of some estuarine and freshwater fishes caused by the fungus, *Aphanomyces invadans* (Lilley et al. 1998). The disease occurs naturally in coastal drainages of NSW and Queensland, and has not been recorded in the Murray-Darling River System with the exception of infected silver perch transported from a coastal hatchery to a farm on the Murray River (Richard Callinan, personal observation). Silver perch are susceptible to EUS under culture conditions, and outbreaks have occurred on farms on the mid-North Coast and North Coast of NSW and south-eastern Queensland (Callinan and Rowland 1995). Infections of EUS have been associated with high stocking densities, poor water quality, in particular low (< 5) and high (> 9) pH, high organic loads and high turbidity, and a water source containing infected fish. It is likely that any factor that causes acute skin necrosis will increase the susceptibility of silver perch to infection when spores are present. Mortality rates can be high in tanks and but are generally low in ponds (Callinan et al. 1999). At GAC, there have been no mortalities of large silver perch (> 400 g) and generally low levels of mortality in fingerlings. Typical lesions and ulceration of the skin make infected fish unsightly and unmarketable as live or whole product (Fig. 4.12). There is no known treatment for EUS. Many fish recover from this disease, and resolution of lesions and ulcers may take 4 – 6 weeks. The fungus can enter farms in water supplies containing infected fish (Callinan and Rowland 1995; Lilley et al. 1998). The disease was first recorded at GAC in 1991, and most infections followed the use of water from the Clarence River during freshes and floods. Since 1999, water has not been pumped from the river during freshes or floods, and EUS has not been recorded at GAC over the last 7 years (Fig. 4.6).

4.4.5.11. Bacterial and viral diseases

Seven bacterial diseases have been recorded in silver perch (Table 4.1). To date, the incidence has been low (< 3%) and most have not caused high mortalities at GAC or on commercial farms, with the exceptions of an outbreak of mycobacteriosis at GAC and two outbreaks of streptococcosis on commercial farms; one in a tank-based RAS and the other in ponds. There may have been unreported cases of bacterial diseases on commercial farms, particularly aeromonad infections. Bacterial diseases cause significant problems in other warmwater fish culture industries (Plumb 1999; Hawke and Khoo 2004; El-Sayed 2006). A high incidence of mycobacteriosis and streptococcosis restricted the development of silver perch culture in Israel (Dr Sheenan Harpaz, personal communication) and so these diseases may become problematic as the silver perch industry expands and intensifies in Australia. Most bacterial infections are associated with stressful conditions such as poor water quality, rough handling, unsanitary conditions and high stocking densities, and their control is best achieved by using good aquaculture practices (Plumb 1994, 1999).

No viral diseases have been recorded in silver perch. Under experimental conditions, silver perch were shown to be very susceptible to infection by epizootic haematopoietic necrosis virus which causes the disease epizootic haematopoietic necrosis (EHN) (Langdon 1989). Outbreaks of EHN are common in wild populations of redfin (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*) in south-eastern Australia, and so each silver perch farmer should ensure that redfin and trout do not occur in the water supply, and are excluded from the farm. It is possible that silver perch will be susceptible to introduced viruses which are being increasingly reported in Australian native fish (Go and Whittington 2006).

4.4.6. Culture systems

A majority of research and production of silver perch has been in earthen ponds, and so this review applies mainly to diseases in pond culture. Diseases afflicting pond-reared and cage-cultured fish are generally similar, although diseases may occur more frequently in cages because of higher stocking densities, close proximity of fish and cages, and presence of feral fish (Lio-Po and Lim 2002). Recent research at GAC has evaluated the use of the intensive systems of cages and tank-

based re-circulating aquaculture systems (RAS) to produce silver perch. Infestations of the gill fluke, *L. bidyana* and the protozoan, *C. hexasticha* were reported in cage culture, and although successfully controlled using formalin, Rowland et al. (2006) suggested that diseases are a potential problem, particularly in large ponds or open waters with limited or no artificial aeration. Read et al. (unpublished data) found that silver perch perform well in a RAS during the fingerling phase, but in grow-out chronic health problems including infestations of *L. bidyana* and *I. necator* contributed to poor health and performance, suggesting that RAS are unsuitable for the production of large silver perch. Problems with bacterial diseases have been reported in tilapia and striped bass in re-circulating systems (Plumb 1999). Health management and disease control will need to be high priorities in future research to develop production techniques for silver perch in these intensive culture systems.

4.4.7. Health management of silver perch

There is a significant and inverse relationship between environmental quality and the health status of fish (Plumb 1994). Good health management provides environmental and culture conditions that reduce the incidence and severity of diseases, enable rapid and appropriate response to disease outbreaks, and optimise the health, performance and production of cultured fish. Health management begins with site selection and design of farms, and is based on the use of good aquaculture practices and preventative measures, in particular quarantine procedures, maintenance of good water quality, use of high quality feeds and appropriate feeding regimes, regular monitoring of fish for diseases and the strategic use of approved chemicals to control disease outbreaks.

4.5. Conclusions

There has been an increase in the number of pathogens and diseases in silver perch aquaculture over the last 23 years. Protozoans and monogenean gill flukes are the most common pathogens, while the incidence of bacterial diseases is low and no viral diseases have been reported. Only ichthyophthiriosis, chilodonellosis and winter saprolegniosis are common diseases with the potential to cause high mortalities if not diagnosed and treated promptly. Diseases are less of a problem on silver perch farms where good aquaculture practices and health management are used; however, new pathogens and diseases can be expected with increased intensification and expansion of the silver perch industry.

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Table 4.1. Records of pathogens and infectious diseases of silver perch under culture conditions.

Type of organism	Pathogen	Disease	1983[a]	1991 and 1995[b]	2005[c]
Protozoan	*Ichthyophthirius multifiliis*	Ichthyophthiriosis	+	+	+
	Chilodonella hexasticha	Chilodonellosis	-	+	+
	Ichthyobodo necator	Ichthyobodosis	-	+	+
	Trichodina sp.	Trichodinosis	+	+	+
	Tetrahymena sp.	Tetrahymenosis	-	+	+
	Henneguya sp.	Henneguya	-	-	+
	Coccidia sp.	Coccidiosis	-	-	+
Monogenean	*Lepidotrema bidyana* (and *Gyrodactylus* sp.)	Gill flukes	-	+	+
Copepod	*Lernaea* sp.	Anchor worm	+	+	+
	Ergasilus sp.	*Ergasilus*	-	-	+
Fungus	*Saprolegnia parasitica*	Fungus	+	+	+
	Saprolegnia parasitica	Winter saprolegniosis	-	-	+
	Aphanomyces invadans	Epizootic ulcerative syndrome (EUS, red spot)	-	+	+
Bacteria	*Flavobacterium columnae*	Columnaris	-	+	+
	Flavobacterium, Aeromonas and *Pseudomonas*	Tail rot, fin rot	-	+	+
	Aeromonas salmonicida nova	Goldfish ulcer disease	-	+*	+*
	Streptococcis iniae	Streptococcosis	-	-	+
	Mycobacterium spp.	Mycobacteriosis	-	-	+
	Aeromonas hydrophila and spp.	Aeromonad infections	-	+	+
	Chlamydia-like bacteria	Epitheliocystis	-	-	+
Virus	Epizootic Haematopoietic Necrosis Virus (EHNV)	Epizootic Haematopoietic Necrosis (EHN)	-	-	-
Total			**4**	**13**	**20**

[a] Rowland (1983)

[b] Rowland and Ingram (1991) and Callinan and Rowland (1995)

[c] current study

* recorded on one farm in 1988

Table 4.2. Mortality levels associated with silver perch diseases at the Grafton Aquaculture Centre (GAC) and on commercial farms.

Disease	GAC	Commercial farms
Ichthyophthiriosis	++	++++
Chilodonellosis	++	++++
Ichthyobodosis	++	++
Trichodinosis	+	+
Tetrahymenosis	+	+
Henneguya	+	+
Coccidiosis	nr	+
Gill flukes	+	+
Anchor worm	+	+
Ergasilus	+	nr
Fungus	+	++
Winter saprolegniosis	nr	+++
Epizootic ulcerative syndrome (EUS, red spot)	+	+
Columnaris	+	++
Tail rot, fin rot	++	++
Goldfish ulcer disease	nr	+
Streptococcosis	nr	+
Mycobacteriosis	+	nr
Aeromonad infections	+	+
Epitheliocystis	nr	+
Epizootic Haematopoietic Necrosis (EHN)	nr	nr

\+ up to 10% mortality

++ up to 50% mortality

+++ up to 100% mortality (chronic disease)

++++ up to 100% mortality (acute disease)

nr not recorded

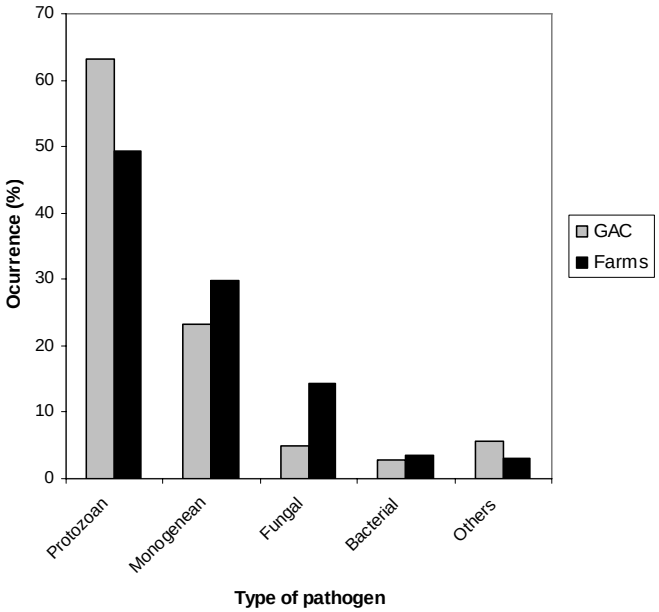


Figure 4.1. Occurrence of different types of pathogens of silver perch at the Grafton Aquaculture Centre (GAC) (283 records; 1991 – 2005) and on commercial farms (154 records; 2001 – 2004).

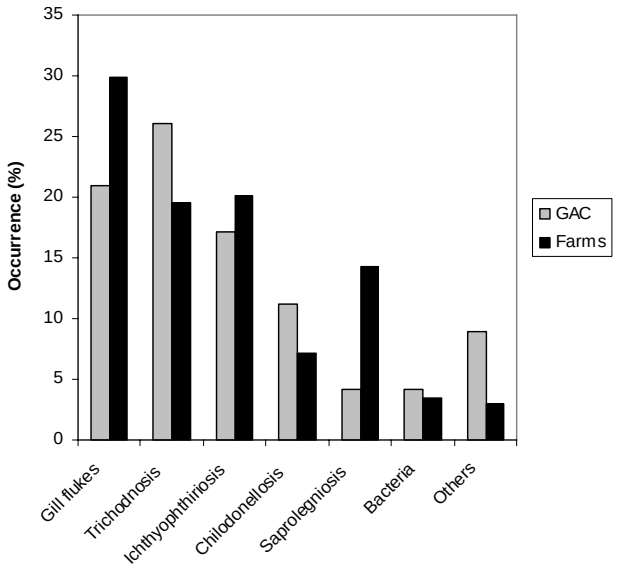


Figure 4.2. Occurrence of the common diseases and pathogens of silver perch at the Grafton Aquaculture Centre (GAC) and on commercial farms.

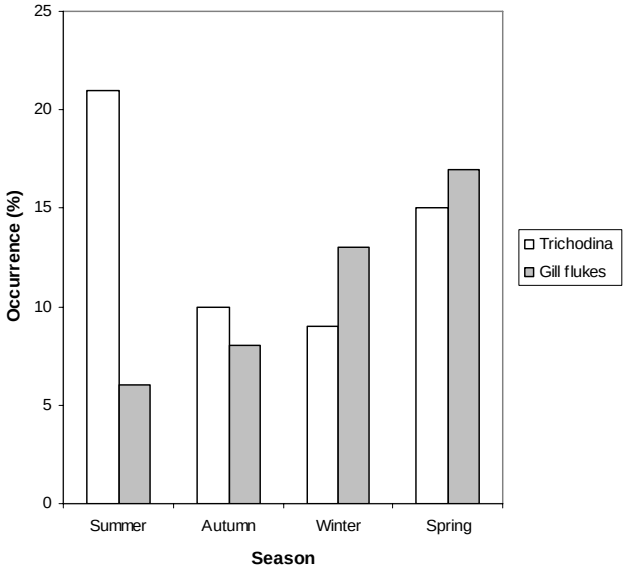


Figure 4.3. Seasonal occurrence of the protozoan parasite *Trichodina* sp. and the monogenean gill fluke *Lepidotrema bidyana* at the Grafton Aquaculture Centre.

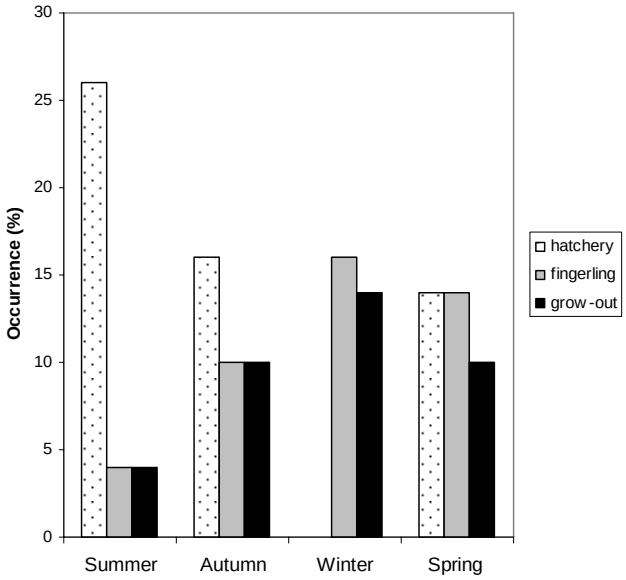


Figure 4.4. Seasonal occurrence of pathogens and diseases in the hatchery, fingerling and grow-out production phases at the Grafton Aquaculture Centre (1991 – 2005).

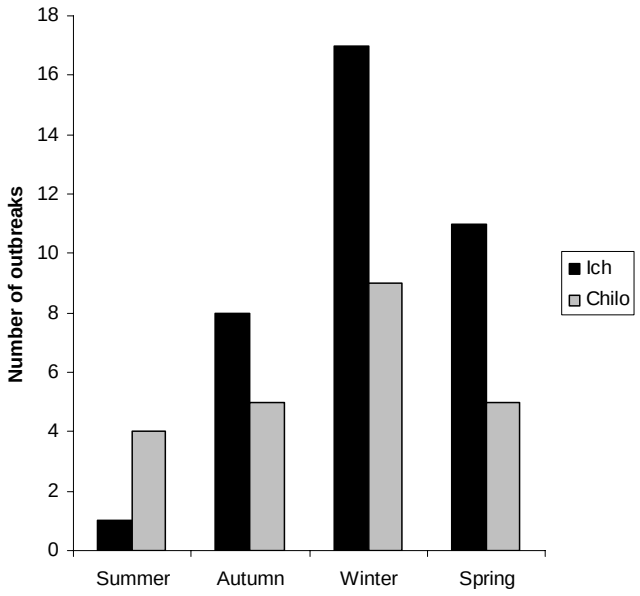


Figure 4.5. Number of outbreaks of the acute infectious diseases ichthyophthiriosis (Ich) and chilodonellosis (Chilo) on silver perch at the Grafton Aquaculture Centre (1991 – 2005).

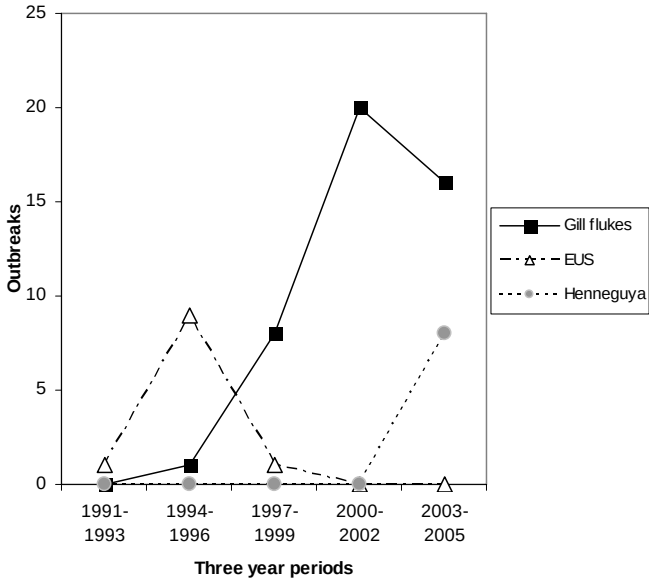


Figure 4.6. Records of gill flukes, the fungal disease epizootic ulcerative syndrome (EUS) and the protozoan *Henneguya* sp. on silver perch at the Grafton Aquaculture Centre (1991 – 2005).

(a)

(b)

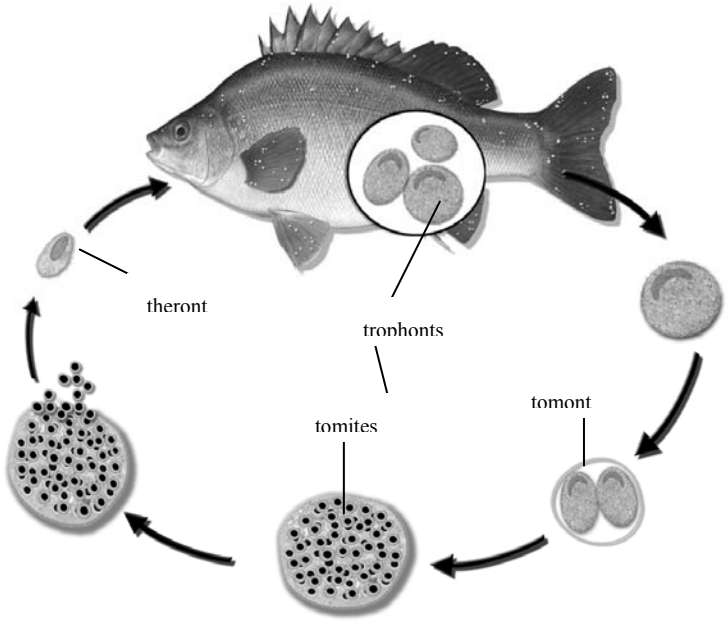
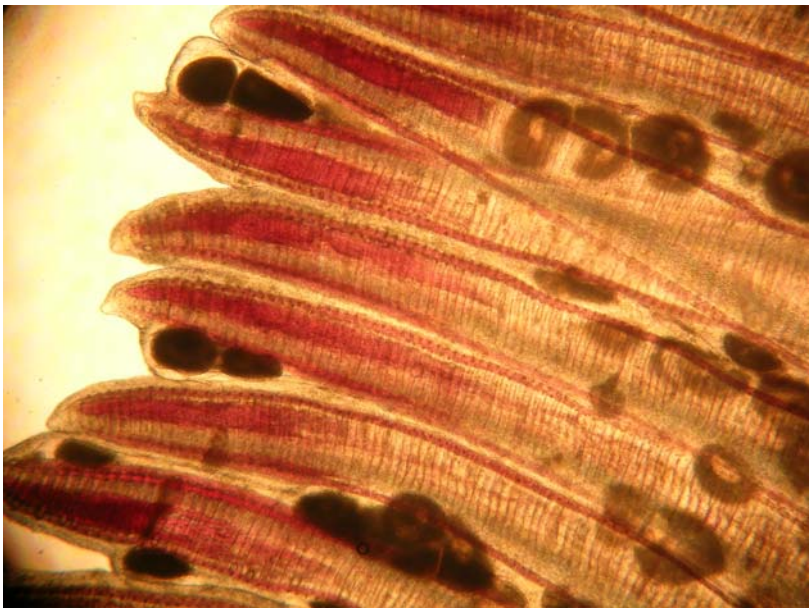


Figure 4.7. The ecto-parasitic protozoan, *Ichthyophthirius multifiliis* which causes the disease ichthyophthiriosis in silver perch. (a) a silver perch with trophonts distinctly visible as white spots on the head and body; (b) life cycle of *I. multifiliis* – stages not drawn to scale; for details see text.

(a)



(b)

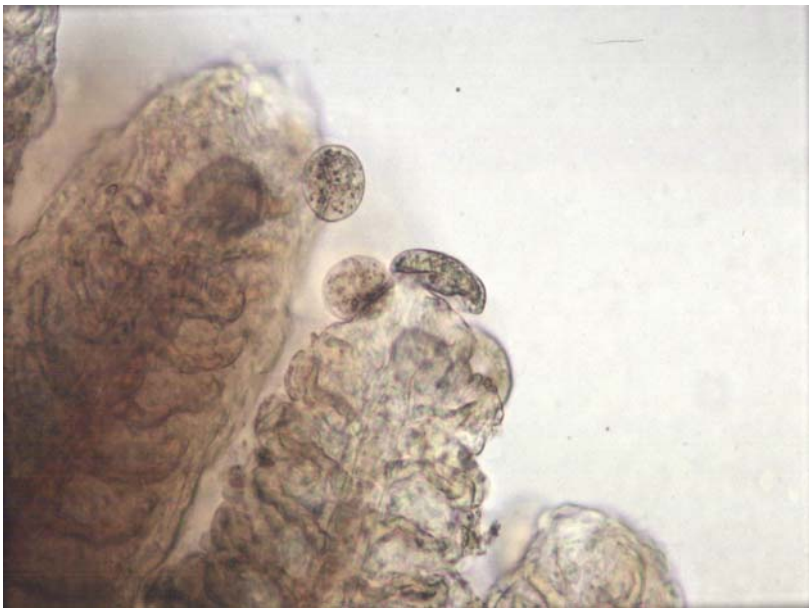
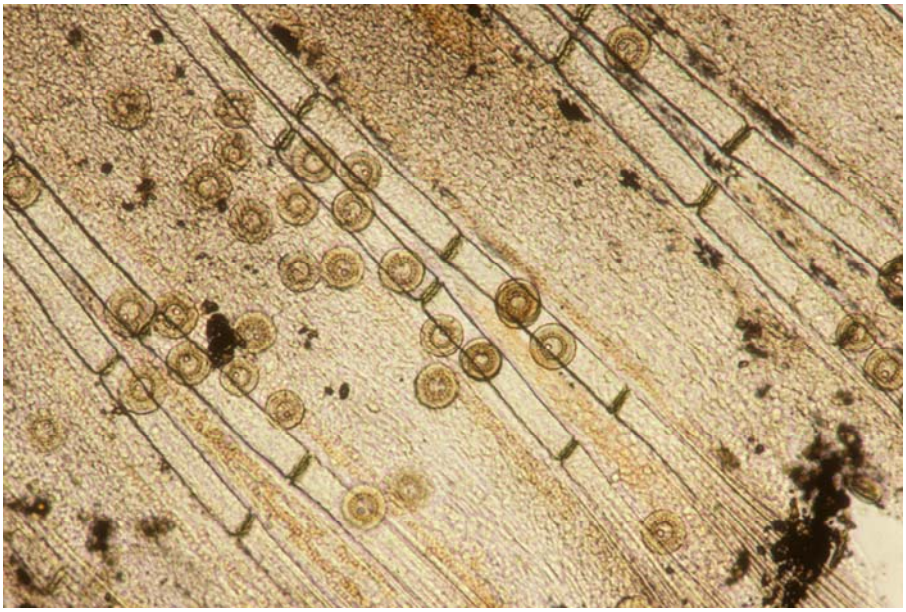


Figure 4.8. The ecto-parasitic ciliated protozoans, *Ichthyophthirius multifiliis* and *Chilodonella hexasticha*. (a) *I. multifiliis* on gill tissue (X200) – note parasites under gill epithelium and the distinct nucleus; (b) *C. hexasticha* on gill tissue (X200) – note the flattened, oval shape and granular appearance of the cytoplasm.

(a)



(b)

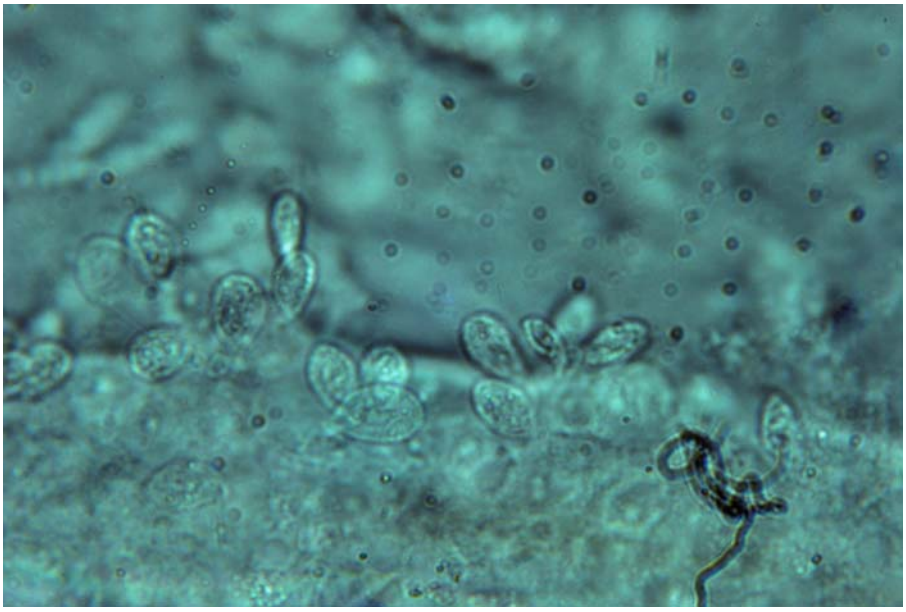


Figure 4.9. Protozoan ecto-parasites of silver perch. (a) the ciliate, *Trichodina* sp. X100; (b) the flagellate, *Ichthyobodo necator* X1000.

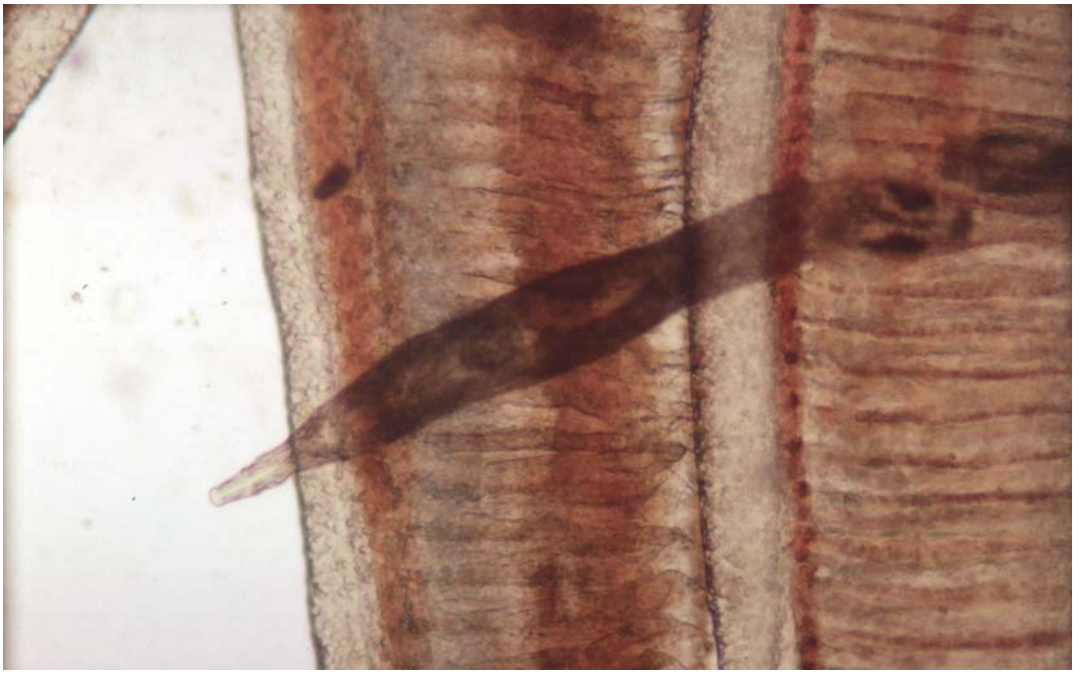


Figure 4.10. The monogenean gill fluke, *Lepidotrema bidyana* (X200) a common parasite of silver perch attached to gill tissue.

(a)

(b)

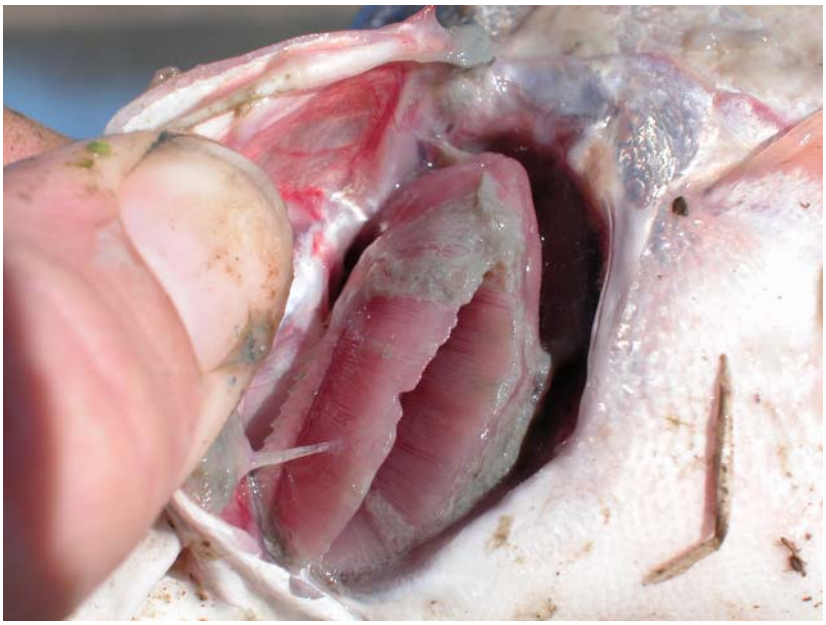


Figure 4.11. Silver perch infected with the fungus, *Saprolegnia parasitica*, the pathogen which causes the disease winter saprolegniosis. (a) pale fungal lesions and abnormally dark skin; (b) fungal lesions on gill tissue.

Figure 4.12. Silver perch fingerling with ulcers that are characteristic of epizootic ulcerative syndrome caused by the fungus, *Aphanomyces invadans*.

5. AETIOLOGY AND PATHOGENESIS OF WINTER SAPROLEGNIOSIS IN SILVER PERCH (*BIDYANUS BIDYANUS)*

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5.1. Abstract

Silver perch aquaculture is a relatively new, pond-based industry in Australia. In the late 1990's, there were reports of fungal infections causing significant losses on some commercial farms during winter. To identify the causative agent and determine the aetiology and pathogenesis of this condition, fish health and water quality were monitored at the Grafton Aquaculture Centre (GAC) and on commercial farms through-out NSW. The pathogen was identified as the oomycete fungus, *Saprolegnia parasitica*. During the study, there was a total of 22 outbreaks of winter saprolegniosis reported on five farms in three regions; mid-North Coast, Northern Tablelands and the Riverina. In winter 2002, winter saprolegniosis occurred on 15% silver perch farms surveyed in NSW and Queensland. Most outbreaks commenced at water temperatures below 16°C, and decreases to below 10ºC were sometimes associated with the onset and increased severity of the disease suggesting that immunosuppression is involved in the initiation of winter saprolegniosis in silver perch. Detection of the disease in the initial stages is difficult in characteristically turbid ponds, where fish are difficult to see and feeding activity is reduced in winter. Sites of initial infection are the distal edge of the opercula, lateral line and abdomen, and early signs include small, raised plaques of clear material on the integument. Some lesions can be associated with mild, diffuse sub-epidermal petechial haemorrhage. Heavily-infected fish often swim slowly near the surface and edges of ponds. Plaques of fungi are clearly visible on de-pigmented areas of the integument and gill, and can appear white to olive green to brown depending on the type and colour of algae and suspended solids in the pond. Mortality rates can be as high as 100%. Large silver perch (> 180 g) were more susceptible than smaller fish. Infestations of the ciliate ecto-parasites *Ichthyophthirius multifiliis* and *Chilodonella hexasticha*, and the monogenean gill fluke, *Lepidotrema bidyana*, were associated with some outbreaks and these pathogens may predispose silver perch to winter saprolegniosis. Other factors associated with outbreaks in ponds were high levels of organic matter, damage to fish during partial harvesting of ponds and pre-existing gill hyperplasia, possibly caused by gill flukes.

Suggested management actions to be taken in late autumn and winter to assist in the prevention and control of winter saprolegniosis are: reduce fish biomass to < 6 tonnes/ha; ensure fish are free of ecto-parasites; do not over-feed; maintain good water quality; avoid partial harvests; harvest ponds with heavily infected fish and treat in tanks with continuous bath of 2 – 10 g/L salt (NaCl) and formalin (30 mg/L). Formalin and copper may control winter saprolegniosis in earthen ponds, but to date their use has not been validated. Other management options worthy of future evaluation are: application of probiotics to ponds to inhibit infection by *S. parasitica* cysts; use of feed additives

such as L-carnitine, β-glucan and ascorbic acid to enhance the immune system of silver perch; monitoring of fungal cysts to aid in early detection; and cage culture to potentially increase the efficiency of feeding, disease control and harvesting of silver perch.

5.2. Introduction

The silver perch (*Bidyanus bidyanus*) is an Australian native freshwater fish that is endemic to the Murray-Darling River System. It has long been recognised as having potential for farming. Hatchery techniques were developed in the early 1980's, but it wasn't until the 1990's that research at the Grafton Aquaculture Centre (GAC) demonstrated the species had potential for intensive pond culture (Rowland 1995a). In the mid 1990's, a series of workshops transferred technology to industry (Rowland and Bryant 1995), and over the last 10 years annual production has increased to around 400 tonnes, with over 300 tonnes in NSW and the remainder in Queensland, Victoria and Western Australia. The industry is based on pond culture, but recent research has demonstrated that silver perch also has good potential for cage culture (Rowland et al. 2004, 2006a) and the over-wintering of fingerlings in re-circulating aquaculture systems (Read et al. unpublished data).

The diseases of silver perch under culture conditions have been described by Rowland (1983), Rowland and Ingram (1991) and Callinan and Rowland (1995). In 1996/97, a pilot monitoring program on a commercial silver perch farm on the NSW North Coast found that infectious diseases caused loss of production, and that survival and growth rates were reduced by ecto-parasitic infestations and by adverse water quality (Lan Jiang, unpublished data, 1997). In 1998 and 1999, there were reports of disease problems causing large losses on some commercial silver perch farms (Sam Clift, Bruce Rhoades, Mike Beveridge, Andrew Pratt, personal communication). These included fungal infections during winter, particularly in the cooler, inland areas of eastern Australia. Reports suggested that some or most of these outbreaks were not just the result of poor husbandry and rough handling, and the disease became known colloquially as "winter disease" or "winter sap". A fungal disease, winter saprolegniosis is a serious problem in the large channel catfish (*Ictalurus punctatus*) industry in the USA, and proves difficult to control under commercial conditions (Bly et al. 1992, 1993; Li et al. 1996; Hawke and Khoo 2004; Dr Michael Masser personal communication). The disease in channel catfish is closely linked to suppression of the immune system caused by a rapid drop in water temperature, maintenance of low temperatures (~ 10ºC) and high levels of fungal zoospores (Bly et al. 1992, 1993, 1994).

The significant losses to fungal infections on some silver perch farms has threatened their economic viability and restricted development of the industry. Clearly there was a need to determine the aetiology and pathogenesis of this disease. The aims of the study reported in this chapter were to: (i) identify the pathogen causing winter saprolegniosis in silver perch; (ii) determine the aetiology and pathogenesis of winter saprolegniosis; (iii) identify environmental factors which may predispose silver perch to fungal infections; and (iv) recommend management actions to prevent and control winter saprolegniosis.

5.3. Materials and methods

5.3.1. Outbreaks of winter saprolegniosis

In June 2000, an outbreak of a fungal disease in a 0.4-ha earthen pond containing 4,000 large (~ 500 g) silver perch on a commercial farm was investigated. The farmer had noted moribund fish in the pond following a rapid decline in water temperature at the beginning of winter. The number of dead fish were counted or estimated daily, water temperature was monitored and farm records were reviewed. The pond was not treated with a chemical therapeutant.

In September 2002, a total of 20 farms (14 in NSW, 6 in Queensland) were survey by telephone to determine the incidence of winter saprolegniosis in the preceding winter. Outbreaks were defined as five or more moribund fish swimming slowly at the surface or edge of the pond with visible white to brownish fungal lesions.

5.3.1.1. Farms and sampling

A study of the infectious diseases of silver perch, with particular emphasis on the winter fungal condition was undertaken over the period 2001 – 2004. Initially, the NSW Department of Primary Industries' Grafton Aquaculture Centre (GAC) and seven commercial farms in locations that represented the wide geographic range of silver perch farms in NSW were involved in the study. The number of farms was reduced after the third year to target three key farms that had disease problems in winter, reliable data collection and good fish husbandry and pond management.

Routine water quality and disease monitoring are undertaken year-round at GAC. Water quality monitoring and management are based on Rowland (1995b). To monitor disease, 3 – 5 fish were sampled at varying intervals depending on the culture phase. Larvae and fry were sampled weekly from larval rearing ponds, and fingerlings and larger fish were randomly sampled monthly from each pond using a seine net or cast net. Fish were also opportunistically sampled and examined if there were signs of diseases (e.g., moribund fish, loss of appetite, flashing, abnormal behaviour or colour). Each fish was examined externally, and then wet mounts of skin and gill tissues were prepared and examined microscopically (X40 to X200) for parasites and fungi, and other signs of disease. Both gill and skin tissues were also excised and fixed in formalin (10% buffered) for subsequent microscopic examination. The presence of pathogens was recorded.

Commercial farms were visited monthly in winter and bi-monthly in other seasons. During years 1 – 3 of the project, five fish were opportunistically sampled using a cast net from each of two ponds, one fingerling and one grow-out on each farm. In the fourth year, four ponds on each of two farms and 10 ponds on the remaining farm were monitored. Examination of fish was as for GAC. Where appropriate, excised tissue was stored for later examination and pathology. Moribund and diseased fish in other ponds were also opportunistically sampled and examined. Temperature, dissolved oxygen, pH and ammonia were monitored in these ponds by the farmer, and these variables plus alkalinity and hardness were monitored by project staff during visits. Where possible, outbreaks of fungal infections reported on other farms not involved directly in the project were investigated and samples collected.

In general, collection of data and information during this study was opportunistic and ad hoc because of the production and economic imperatives on commercial farms. The data from only one farm (Farm A, Tables 5.1 and 5.2), located on the mid-North Coast was used for statistical analysis. On this farm, a total of 97 observations from 12 ponds were taken between February 2003 and September 2004 (Fig. 5.1). Each pond was sampled on five to 10 occasions during the study period with repeat samples on each pond separated by as little as 4 or as many as 283 days. The data consist of the 97 sets of water quality measurements, with each set associated with fish health data.

5.3.1.2. Histopathology and gill condition

Gill and skin tissues from infected fish were processed for microbiology and histology. A standard section of gill tissue was sampled from each of three fish, in each study pond, at each visit. The stains haematoxylin and eosin (H&E) and Gomori methenamine silver (GMS) were used. The percentage of cellular infiltrate/hyperplasia between secondary lamellae was recorded. The gill condition was graded on a scale of 0 to 10. The grading took into account the proportion of gill tissue affected and the severity of hyperplastic change. An estimation was made of the percentage of secondary inter-lamellar space, which had become filled with cellular proliferation (gill % fill). One pathologist did all tissue grading to ensure consistency, with slides read "blind" without

reference to the farm origin. A grade of 0 was given to clinically normal gill tissue, and a grade of 10 to gills with complete fusion of all secondary lamellae.

5.3.1.3. Mycology

Samples for mycology, targeting the earliest skin and gill lesions were collected during outbreaks of saprolegniosis in July 2001 on two commercial farms on the mid-North Coast and the Northern Tablelands. In addition, samples of fungal-like material from wounds caused by bird strikes on three fish were also collected from one pond at GAC.

5.3.1.4. Culture media, sample preparation and culture methods

Culture media were based on Willoughby and Pickering (1977). Sabourauds (S-PS) and Corn Meal Agar (CMA-PS) plates with 250mg/L streptomycin sulfate and 150mg/L Penicillin G to inhibit bacterial growth were used to culture the fungal-like material. Duplicate samples were inoculated onto each type of freshly prepared agar. CMA was chosen with reference to the similar work undertaken by Bly et al. (1992). Plates were incubated aerobically at room temperature of ~ 20^{o}C.

An area of infected skin or gill tissue was rinsed thoroughly with sterile autoclaved water to minimise foreign debris attached to lesion and minimise contamination. Using aseptic technique, a 5 mm square of tissue from the edge of the lesion was dissected and placed in a sterile petri dish and cut into quarters. S-PS and CMA-PS plates were inoculated with two pieces of affected tissue, skin side down on agar, per plate. Inoculated plates were sealed with adhesive tape to minimise contamination risk. A representative section from the same lesion was fixed with 10% buffered formalin for subsequent histological examination with H&E and GMS stains. In the field, plates were stored out of direct sunlight and at 15º – 20^{o}C. Plates were examined daily for 5 days using a dissecting microscope to monitor vegetative growth. Initial observations suggested the possibility of a single type of hypha present in the primary cultures. The majority of primary culture plates grew a pure culture of fungus. Using aseptic technique, in a laminar flow cabinet, a 3 mm agar plug containing hyphal tips was removed from the peripheral area of the hyphal colony and sub-cultured, face down, on S-PS or CMA-PS. This procedure was repeated consecutively until bacteria-free cultures were obtained. Once pure cultures were established, they were moved onto agar that was free of antimicrobials. Plates were successfully stored at 4^{o}C to slow the growth of hyphae without impairing the viability of the organism. Cultures were sub-cultured onto fresh plates every 4 – 6 weeks. For longer term storage, cultures were transferred onto Amyl Media, Sabourauds Dextrose media which contains Gentamicin and Chloramphenicol to inhibit bacterial contamination.

5.3.1.5. Identification of the fungus

To identify the oomycete, hyphae containing agar plugs were placed into sterilised pond water. Zoospores were discharged in the same manner in all samples. These cultures were then refrigerated to try and encourage the formation of sexual stages of the fungi. Sub-cultures of several isolates were sent to Dr Gordon Beakes (Newcastle-upon-Tyne University, United Kingdom) for electron microscopy to identify the species of fungus. Preparation of secondary zoospore cysts involved placing formvar coated 200 mesh copper grids on the bottom of petri dishes containing a 3-day culture of hyphae-invaded hemp/linseed. Grids were gently blotted onto hardened filter paper and then placed onto microscope slides and secured with some double-sided adhesive tape. Slides were coated with a thin layer of palladium at 30ºC in a standard vacuum coating unit. A coat of carbon was also delivered from directly above to stabilise the grids.

5.3.1.6. Data treatment and analysis

Infection levels in ponds were determined from five fish, while the water quality data was from a single sample in each pond. Therefore a degree of pseudo-replication is implicit in the pairing of a single water quality measurement with the five measurements of infection level. It is inappropriate to treat five fish from the same pond as independent indicators of a response to the water quality of that pond. Data on pathogens may differ between fish, and even between skin/gill samples from the same fish. This coupled with the range of possible interactions between gill measurements and skin measurements, lead to the conclusion that counts could lead to non-robust conclusions. Some standardisation was built into the sampling regime, with one person making all the microscopic observations and a standardised section (same size, same anatomical location) being taken from both gill and skin for the wet preparations. An indicator of pond infection status was derived by classifying each pond as "infected" at each sampling date if at least one fish in the sample recorded a positive (non-zero) count on the skin or the gill tissue, and "clean" if there were no pathogens recorded. Associations between pond infection status and the collection of observations on pond quality were then determined. Ponds containing diseased fish were assigned the numeric value of 1 and clean ponds assigned 0. The arithmetic average of those 1/0 observations gives the observed proportion of all ponds with infected fish. Similarly, with ponds classified according to the level of some other variable such as water temperature, the probability of infection at each level can be calculated and the association between disease level and water temperature examined. This process was formalised by fitting a series of logistic regression models to the relationship between the probability of pond infection and each water quality variable. Further information on logistic regression can be found in McCullagh and Nelder (1989). Graphics and modeling were accomplished under the S-Plus software package: S-PLUS 6.2 Release 1 (2003) Insightful Corp., Seattle, WA, USA.

5.4. Results

5.4.1. Outbreaks of winter saprolegniosis

5.4.1.1. Winter 2000

On the farm on the Northern Tablelands (farm B, Table 5.2), moribund fish with numerous fungal lesions appeared after a decline in water temperature of 5.9ºC (13.4ºC to 7.5ºC) over 10 days in early winter; there was total mortality of fish in this pond over the following 14 days (Fig. 5.2).

5.4.1.2. 2001 – 2004

There were 22 outbreaks of winter saprolegniosis recorded on five farms in three regions representing different climatic zones of NSW; mid-North Coast, Riverina and Northern Tablelands (Table 5.1). One farm on the mid-North Coast had 16 (72.7%) of the outbreaks. Winter saprolegniosis was not recorded at GAC. Three of 20 farms (15%) had winter saprolegniosis in at least one pond in the winter of 2002 (Table 5.1).

5.4.2. Clinical signs

In the early stages of an outbreak, fish may not be seen because of the high turbidity of most silver perch production ponds. Initial signs to the farmer may be heavily-infected fish near the surface and edges of the pond. Infected fish are often seen aggregating and swimming very slowly on the surface of the pond or at the edges of the pond, sometimes near an inflow of freshwater (Fig. 5.3a). Some infected fish may be seen rubbing/flashing on objects in the pond, such as aerator ropes and

standpipes. Not all infected and moribund fish are seen near the surface or edges; many remain near or on the bottom after death and are only evident when the pond is drained or netted.

5.4.3. Macroscopic stages of infection

Stage 1 (early/light)*:* Sites of initial infection are the distal edge of the opercula, lateral line, abdomen and fins; early signs include small, raised plaques of clear to pale material; small, localised clear to pale pink lesions on skin, generally on the abdomen and sides (Fig. 5.4); some lesions can be associated with mild, diffuse sub-epidermal petechial haemorrhage; gills pale.

Stage 2 (moderate)*:* pale lesions, some with fungal hyphae visible; skin dark giving fish blotchy appearance; little mucus; soft fins with fungal hyphae visible; some haemorrhaging of skin and fins; some shedding of skin mucus and epithelial tissue; gills pale/brownish with fungal hyphae visible; fish can have lesions on skin or gills, or on both tissues simultaneously.

Stage 3 (severe/heavy)*:* large fungal lesions with hyphae clearly visible on body and head; skin dark, blotchy appearance (Fig. 5.3b); little mucus; skin sloughing-off; may be some erosion of soft fins; gills pale with relatively large areas of brownish fungal hyphae visible (Fig. 5.5).

5.4.4. Gross pathology

Whilst fish are immersed in water, colonies of fungal hyphae appear as small bundles of "cotton wool" attached superficially to skin or gill tissue. Sites of colonisation can vary between outbreaks. The caudal, distal edge of the opercula, the lateral line, abdomen and tail fin were the most common sites of early infection. When infected fish are examined out of the water, the hyphae appear as a raised gelatinous mass which may be translucent white and pale brown (Fig. 5.3b), green or grey depending on the type and amount of algae, suspended solids, detritus and organic matter that become adhered to the hyphal mass. Some fish may exhibit erythema as diffuse sub-epithelial petechial haemorrhage of the ventral abdomen, resulting from physical abrasion. Infected fish stress rapidly when handled, inducing haemorrhaging of the skin, soft ray tissue of the fins and between scales. Rapid blanching of the peripheral edge of the tail fin and skin on the dorsum of the head is also observed, and is likely to be stress-related acute superficial epidermal necrosis.

5.4.5. Histopathology

Skin: severe, diffuse, superficial dermatitis with necrosis and sloughing of the epidermis, associated with presence of large numbers of broad non-septate fungal hyphae; variable scale loss and water logging of tissue beneath erosions; penetration of hyphae does not extend through the stratum compactum. Little to no inflammatory response to presence of invasive mycelium.

Gill: pre-existing severe, diffuse, chronic hyperplastic branchitis with fusion and clubbing of secondary lamellae; in some fish, localised areas of acute, necrosis, sloughing and destruction of gill epithelium associated with invasive non-septate hyphae; in some fish, hyphae may penetrate gill vasculature causing thrombosis and infarction of primary lamellae.

Heart, anterior kidney, posterior kidney, liver, spleen, gastro-intestinal tract, and pancreas: no significant lesions detected.

Histopathology indicated primary mycotic dermatitis and branchitis, and there was no evidence of other infectious agents and no abnormalities of internal organs and tissues.

5.4.6. Mycology

Twelve of 13 primary cultures were macroscopically, microscopically and morphologically identical. The general description is a follows.

Mycelium*:* characterised by stout, tubular, non-septate, variably branching, tapering, multinucleate, 7 – 30 µm diameter hyphae.

Zoosporangia*:* were terminal structures filled with maturing zoospores and generally clavate in shape.

Zoospore discharge*:* rapid release from tip of zoosporangia with most swimming away from the discharge site before encysting. These primary spore cysts either germinated to produce a new mycelium, or generated a motile secondary zoospore (diplanetic). Many of these secondary cysts encysted and subsequently re-emerged (polyplanetic).

Zoospores*:* were ~ 10 µm long, motile biflagellated and pyriform in shape.

5.4.7. Identification

The fungal pathogen associated with winter saprolegniosis was identified as *Saprolegnia parasitica* based on the presence of bundles of very long hooked spines or hairs on the casing of the secondary zoospore cyst (Burr and Beakes 1994; Beakes et al. 1995; Dr Gordon Beakes, personal communication). Cysts of *S. parasitica* viewed using an electron microscope are shown in Fig. 5.6. The long, hooked bundles of the secondary cysts were also observed with phase contrast microscopy of secondary zoospore cysts. Isolates taken from silver perch during outbreaks of winter saprolegniosis in seven ponds, on two farms in the eastern and western drainages in NSW were conspecific. *Saprolegnia parasitica* was also isolated from fungal lesions on wounds caused by bird strikes on silver perch at GAC (Table 5.2). Our findings indicate the widespread distribution of the pathogen in freshwaters in south-eastern Australia.

5.4.8. Factors associated with outbreaks

5.4.8.1. Water temperature

Twenty of the 22 outbreaks commenced at water temperatures lower than 16ºC, and the likelihood of outbreaks increased to above 50% when water temperatures fell under 13ºC (Fig. 5.7). In some outbreaks, significant declines in water temperatures (>4ºC in 24 hours), were associated with rapid onset and increased severity of winter saprolegniosis. Temperature declines of >1.5ºC in the previous 7 days were positively correlated to the occurrence of *S. parasitica* in a pond (Fig. 5.8). Three outbreaks commenced at temperatures above 16ºC (Fig. 5.7).

5.4.8.2. Ectoparasites

Infestations of the ciliated protozoans, *Ichthyophthirius multifiliis*, *Chilodonella hexasticha* and *Trichodina* sp. were associated with several outbreaks of winter saprolegniosis. Saprolegniosis continued even when infestations of *I. multifiliis* were controlled with chemical therapeutants. The monogenean gill fluke, *Lepidotrema bidyana*, was associated with 75% of winter saprolegniosis outbreaks.

5.4.8.3. Fish size and density

There were only two outbreaks of winter saprolegniosis in ponds containing silver perch with mean weights less than 180 g; most outbreaks were in ponds with fish of mean weights of 180 – 500 g (Fig. 5.7). Outbreaks occurred at fish densities of 250 – 900 kg/ML (~ 2.5 – 10.0 tonnes/ha), but outbreaks were more likely to occur at higher densities (Figs. 5.8 and 5.9).

5.4.8.4. Handling of fish

Many of the outbreaks of winter saprolegniosis on Farm A commenced within 48 h of fish being harvested from ponds and stocked into tanks, or released back into the pond after grading or culling (Mark Scifleet, personal communication).

5.4.8.5. Gill hyperplasia

Outbreaks were positively correlated with the degree of pre-existing gill hyperplasia (Fig. 5.10). The cause of this gill hyperplasia is not known; however, the presence of gill flukes is known to be associated with hyperplastic branchitis.

5.5. Discussion

Aquatic fungi or water molds (Class Oomycetes) are ubiquitous saprophytes that feed on dead organic matter. *Saprolegnia* spp. have long been recognised as common and important pathogens of freshwater fishes, and are generally considered to be secondary pathogens, infecting fish after physical damage and/or severe stress provide an entry portal for infection by fungal cysts (Sarig 1971; Noga 2000). There are, however, some circumstances were the host's immune system is suppressed and *Saprolegnia* acts as a primary pathogen (Bly et al. 1994; Pottinger and Day 1999).

Infections of *Saprolegnia* sp. have been previously reported in a number of Australian native freshwater fish under culture conditions, including silver perch (Rowland and Ingram 1991; Callinan and Rowland 1995; Ingram et al. 2005). In the current study, the oomycete fungus, *Saprolegnia parasitica*, was identified as the pathogen causing winter saprolegniosis in silver perch. Lategan et al. (2004) referred to this strain of *S. parasitica* as UTS Spr2. There are different morphotypes and strains of *Saprolegnia parasitica* that are known to vary in their pathogenicity (Pottinger and Day 1999; Fregeneda Grandes et al. 2000, 2001). The high infection and mortality rates (see Fig. 5.2) on some farms suggest that the strain infecting silver perch can be highly pathogenic at low water temperatures. *Saprolegnia parasitica* has also been associated with mycotic dermatitis in bony bream (*Nematalosa erebi*) in the Murray River (Puckeridge et al. 1989). Although silver perch is sympatric with bony bream in western parts of the Murray-Darling River System, infections in silver perch in the wild have not been reported, and at present, the disease is confined to aquaculture facilities.

Winter saprolegniosis is also a serious disease affecting pond-raised channel catfish in the USA (Bly et al. 1992; Hawke and Khoo 2004). Low water temperatures are known to favour the proliferation of *Saprolegnia*, reduce the effectiveness of the immune system and inhibit goblet cell migration to the skin reducing mucus production and the barrier to infection (Bly et al. 1994; Quiniou et al. 1998; Noga 2000; Wise et al. 2004). The disease in catfish is closely linked to suppression of the immune system caused by a rapid drop in water temperature, maintenance of low temperatures (~ 10ºC) and high levels of fungal zoospores (Bly and Clem 1991; Bly et al. 1993, 1994). Most outbreaks of winter saprolegniosis in silver perch were at low water temperatures (< 16ºC) and rapid declines in temperature (e.g., during cold fronts in winter) were sometimes associated with the onset and increased severity of *S. parasitica* infection. Both silver perch and channel catfish are warmwater, temperate species, and it is likely that immunosuppression is also involved in the initiation of winter saprolegniosis in silver perch. The

clinical signs of fungal skin lesions, dry skin, absence of inflammatory response and epithelial destruction seen in silver perch are similar to those reported in channel catfish (MacMillan 1985; Durborow and Crosby 1988). The lack of inflammatory response to the presence of sub-epithelial fungal colonisation suggests the fish are immunosuppressed.

In our study, there were no outbreaks of winter saprolegniosis at locations where winter water temperatures do not fall below 10ºC, such as GAC (Rowland 1995b) and on farms in southern and central Queensland (Michael Hickey, Ross Burton, Stan Moore and Rob Bartley, personal communication). The only outbreak reported from Queensland was on a farm near Stanthorpe (Table 5.1) which is in the southern tablelands region of that state where pond water temperatures can fall below 10ºC for up to 8 weeks in winter (Mike Murphy, Geoff Dongers, personal communication). The presence of immunosuppressed fish and high levels of *Saprolegnia* zoospores result in winter saprolegniosis in channel catfish, whereas if one of these predisposing factors is missing, fish in ponds generally remain healthy (Bly et al. 1993). The absence of winter saprolegniosis on farms in Queensland and at GAC, despite the presence of *S. parasitica* at GAC, may be due partly to the relatively mild winters in these locations and regions.

Monogenean gill flukes have become common ecto-parasites on silver perch over the last 10 years, and although infestations rarely result in mortalities, they are known to cause epithelial hyperplasia in gill tissue and reduced appetite and growth (Rowland et al. 2006b; Read et al. 2007; Rowland et al. 2007). Physical damage of gill tissue caused by monogenean parasites has been linked to infection by bacteria and fungi (Davis 1962; Noble et al. 1963; Pyecroft 1994; Lopez et al. 2002) and so flukes may predispose silver perch to winter saprolegniosis through tissue damage and stress. The hyperplastic gill tissue observed in all silver perch during outbreaks would be consistent with a reduction in both respiratory and metabolic efficiency of gill tissue in infected fish.

Similar culture environments and techniques are used in both the channel catfish and silver perch aquaculture industries; aerated earthen ponds, stocking densities of 10,000 – 30,000 fish/ha, and artificial feeds (28 – 35% protein) (Tucker and Robinson 1990; Rowland 1995a). The resulting high levels of organic matter in these ponds provide ample substrate for ubiquitous saprophytic fungi, and most fungal infections in fish are probably acquired from inanimate sources (Noga 2000). Fungal isolates from silver perch readily colonised fish feed in the laboratory and produced massive numbers of spores within 48 h (Matthew Landos, unpublished data). Handling, crowding, heavy feeding rates, algal blooms and high organic loads are known to increase the risk of saprolegniosis and other fungal diseases (Khoo et al. 1998; Noga 2000). High stocking densities, feeding rates and production rates combine to achieve high biomasses and heavy algal blooms in ponds during the summer, autumn and early winter period on Farm A (Mark Scifleet, personal communication). The combination of low winter temperatures, rapid declines in water temperatures during winter, intensive culture and high organic loads in ponds has probably contributed to the very high incidence of winter saprolegniosis on this farm. In comparison, lower feeding rates were used in autumn and early winter on other silver perch farms with similar stocking densities and water temperature regimes where there have been fewer outbreaks of winter saprolegniosis (Ian Charles, Paul Trevethan, personal communication).

The susceptibility of large silver perch (> 180 g) to winter saprolegniosis may be due to several factors. Large fish have a larger surface area for colonisation of infective *S. parasitica* cysts. Large fish have generally been in ponds for a longer period, and been exposed to high fish biomasses, high feed inputs, high levels of organic matter, and increasingly eutrophic conditions. High feeding rates have preceded outbreaks of winter saprolegniosis on Farm A (Mark Scifleet, personal communication). Although reduced feeding or no feeding may be an option for the prevention of winter saprolegniosis, care needs to be taken because deprivation of feed has been shown to reduce the innate resistance of channel catfish to some infectious diseases (Lim and Klesius 2003; Shoemaker et al. 2003). Because of the role the immune system plays in winter saprolegniosis, it is

recommended that silver perch are fed a restricted ration following Rowland et al. (2005) during autumn and winter.

High levels of un-ionised ammonia have been linked to winter saprolegniosis and other fungal diseases (Khoo et al. 1998; Noga 2000; Hawke and Khoo 2004; Dr Michael Masser, personal communication). In this project, high levels of un-ionised ammonia were recorded in many ponds on commercial silver perch farms. Exposure of silver perch to concentrations of 0.06 mg/L and 0.1 mg/L are known to suppress appetite and reduce growth respectively (Rowland 1995b; Frances et al. 2000). High ammonia can cause gill damage (Noga 2000) and the gill hyperplasia seen in silver perch may have been a result of exposure to high ammonia.

Handling fish, particularly at low water temperatures predisposes silver perch to infection by *S. parasitica*. The use of a prophylactic salt bath (2 g/L NaCl) post-harvest prevents infection by *S. parasitica* (Mifsud and Rowland 2007). A combination of rough handling, rapid confinement and sub-optimal water quality, particularly low dissolved oxygen can lead to spontaneous skin necrosis and blanching of the distal portion of the caudal fin and dorsum of the head in silver perch (Matthew Landos, personal observation). Udomkusonsri and Noga (2005) described these signs and demonstrated that the affected skin is subsequently highly susceptible to colonisation by *Saprolegnia*.

Fungal diseases in warmwater fishes are inherently difficult to treat with chemical therapeutants in ponds, and therefore management must be based on prevention (Noga 2000; Wise et al. 2004). Using the findings of our study, a number of management actions are recommended, and other options considered worthy of evaluation are briefly presented below.

5.5.1. Management in pond culture

The following actions are recommended for late autumn and early winter to assist in the prevention and control of winter saprolegniosis in silver perch.

1. reduce fish biomass to < 6 tonnes/ha;
2. ensure fish are free of ecto-parasites, by increasing the frequency of monitoring susceptible fish to every 14 days;
3. do not over-feed, but continue to feed a high quality ration on a restricted basis;
4. maintain good water quality (avoid low dissolved oxygen, high ammonia, low or high pH);
5. avoid partial harvests and do not return captured fish to ponds;
6. harvest ponds with heavily infected fish and treat in tanks with continuous bath of 2 – 10 g/L salt (NaCl) and formalin (30 mg/L).

5.5.2. Potential therapeutants

Formalin and copper have been shown to inhibit zoospore production and cyst germination in *S. parasitica* (Bly et al. 1996). In channel catfish, formalin is effective as both prophylactic and post-infective treatment, and copper sulfate is effective in preventing infection; however, these treatments have not been validated for large-scale use under commercial conditions in the catfish industry (Li et al. 1996; Dr Michael Masser, personal communication). Formalin and copper have been recently shown to control ichthyophthiriosis in silver perch under both experimental and pond conditions (Rowland et al. 2007), and their strategic use may be of some value in preventing or controlling winter saprolegniosis. However, these chemicals have not yet been validated for use against winter saprolegniosis in silver perch in ponds and further work is warranted.

5.5.3. Probiotics

Probiotics have potential in controlling fungal diseases. Lategan and Gibson (2003) found that an anti-fungal substance produced by *Aeromonas media* strain A199 inhibits the growth of fungus *in vitro*, and that daily applications of A199 contributed to the swift recovery of eels (*Anguilla australis*) infected with *S. parasitica*. Subsequently, Lategan et al. (2004) found that under laboratory conditions, the onset of saprolegniosis in silver perch exposed to high numbers of cysts and zoospores of *S. parasitica* was delayed by A199. Clearly the probiotic developed by Dr Lategan and her co-workers has significant potential for use in the control of winter saprolegniosis in silver perch and further work on its use is warranted. A challenge lies in the maintenance of sufficient levels of the probiotic in the diverse flora of silver perch culture ponds.

5.5.4. L-carnitine, β-glucan and ascorbic acid as a feed additives

L-carnitine is a non-essential organic nutrient that is synthesized from the amino acids lysine and methionine. There is some evidence that the addition of L-carnitine to diets can protect fish from stress associated with low temperatures and high ammonia (Harpaz et al. 1999; Harpaz 2005). Glucans are structural elements of fungal cell walls obtained from baker's yeast that have been shown to elicit host defence mechanisms in a range of animals (Raa et al. 1992). Some studies have demonstrated that the application of β-glucan has significantly increased resistance to infections of micro-organisms in fish, while other studies have found no increase in resistance (Chen and Ainsworth 1992; Raa et al. 1992). Fish lack the ability to biosynthesise ascorbic acid (vitamin C) and so it must be provided in the diet. Some studies have shown improved immune response and disease resistance with high levels of ascorbic acid in the diets of fish, but again some studies have found no benefit in the use of high levels (Li and Robinson 1999). Because immunosuppression and poor water quality play key roles in winter saprolegniosis in silver perch, the addition of L-carnitine, β-glucan or increased levels of ascorbic acid to diets is worthy of evaluation.

5.5.5. Cage culture

Cage culture has features that provide significant benefits over pond culture, and may reduce the incidence of winter saprolegniosis in silver perch. Cage culture enables efficient feeding, hence can potentially reduce wastage and accumulation of uneaten feed and organic matter. Cage culture also provides advantages of ease of sampling and harvesting. The high cost and problems with water quality associated with using formalin and salt to control diseases in earthen ponds may be overcome by isolating cages for disease treatment. Silver perch are known to perform well in cages (Rowland et al. 2004, 2006) and so the potential of cage culture in reducing the incidence of winter saprolegniosis, and/or facilitating its control through good management warrant evaluation on farms where the disease is problematic.

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Table 5.1. Incidence of winter saprolegniosis on commercial silver perch farms.

Years	Farms total no.	Farms with winter sap	Outbreaks of winter sap	Location of farms
2001 – 2004	11[a]	5 (45.5%)	22	Mid-North Coast Northern Tablelands Riverina
2001 – 2004	Farm A		16	Mid-North Coast
2002	20[b]	3 (15%)	3	Mid-North Coast Riverina Southern Queensland (Stanthorpe)

[a] Farms visited by NSW DPI staff as part of routine sampling or in response to reports of disease outbreaks; includes Farm A.

[b] Farmers contacted by telephone in September 2002 (14 in NSW, 6 in Queensland).

Table 5.2. Identification of fungal pathogens from silver perch on farms in NSW. GAC – Grafton Aquaculture Centre.

Farm – location	Number fish	Number ponds	Pathogen	Source of samples
A – mid-North Coast	6	3	*Saprolegnia parasitica*	Lesions during winter saprolegniosis
B – Northern Tablelands	4	4	*Saprolegnia parasitica*	Lesions during winter saprolegniosis
GAC – North Coast	3	1	*Saprolegnia parasitica*	Lesions from wounds caused by bird strikes
Total	13	8		

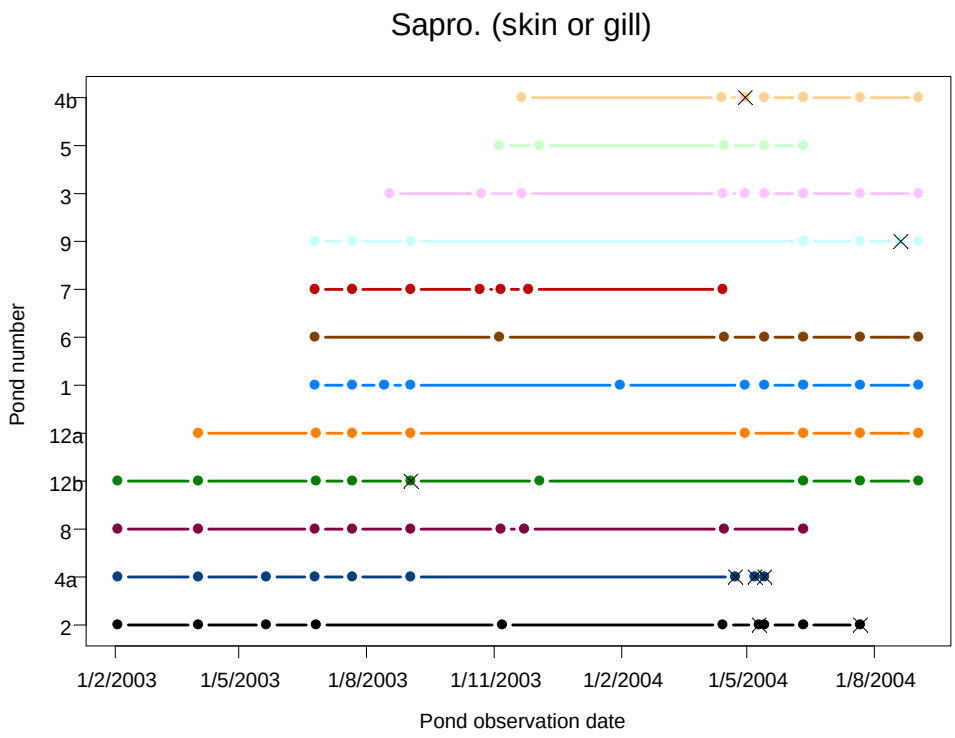
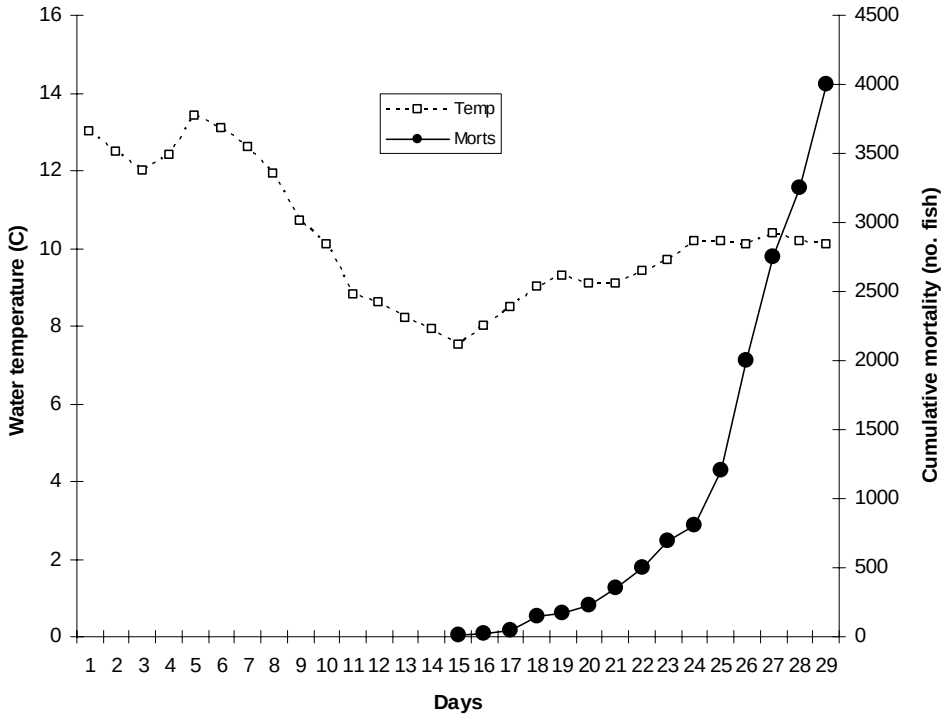


Figure 5.1. Sampling events (dots) on a commercial silver perch farm in NSW. x – signifies outbreak of winter saprolegniosis.

(a)



(b)

Figure 5.2. An outbreak of winter saprolegniosis in an untreated 0.4-ha earthen pond on a commercial silver perch farm. (a) cumulative mortality and water temperature – day1 was 21st May; (b) dead silver perch on the pond bottom.

(a)

(b)

Figure 5.3. Silver perch infected with *Saprolegnia parasitica*. (a) moribund fish near the surface and edge of a pond; (b) infected fish with characteristic fungal lesions on abnormally dark skin.

Figure 5.4. Early signs of winter saprolegniosis – pale pink lesions on skin.

Figure 5.5. Fungal lesions on silver perch gills.

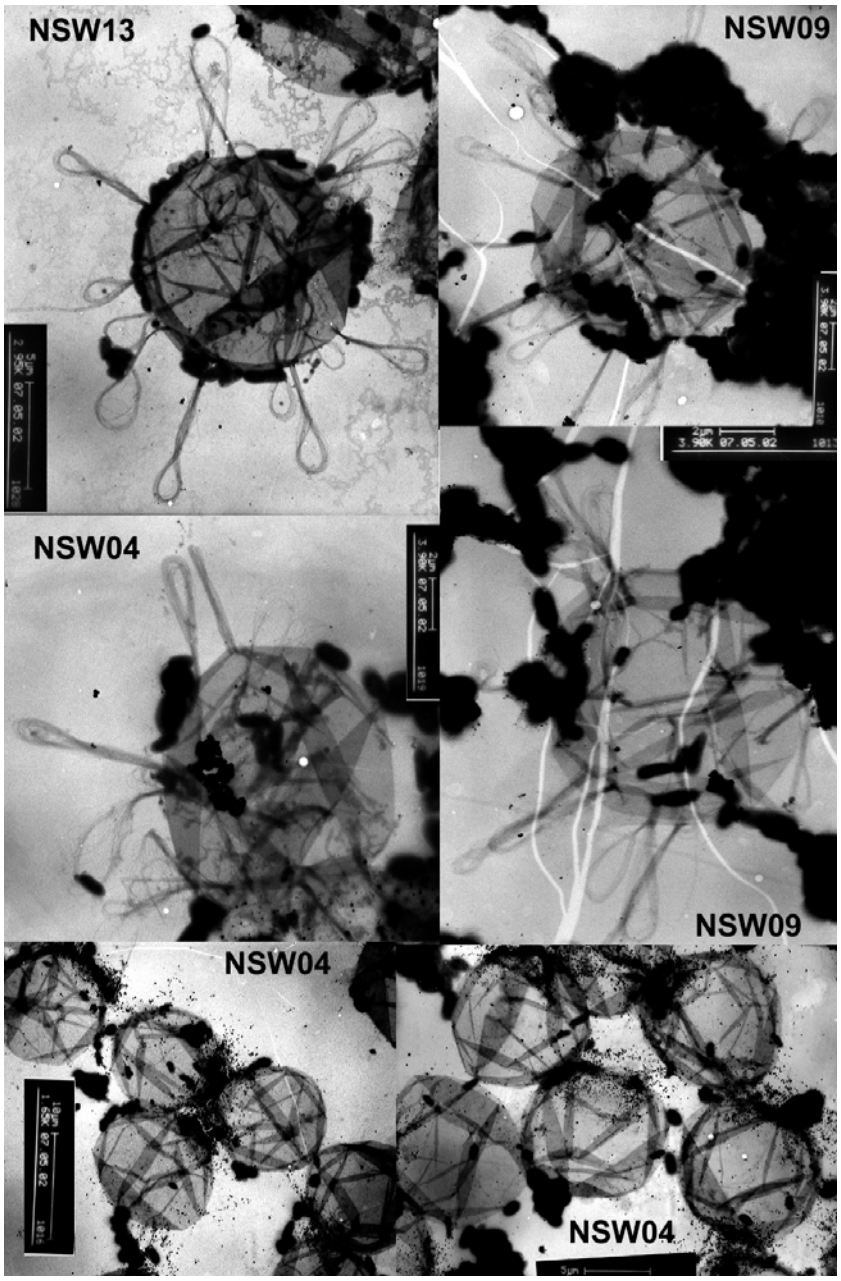


Figure 5.6. Cysts of *Saprolegnia parasitica* viewed using an electron microscope.

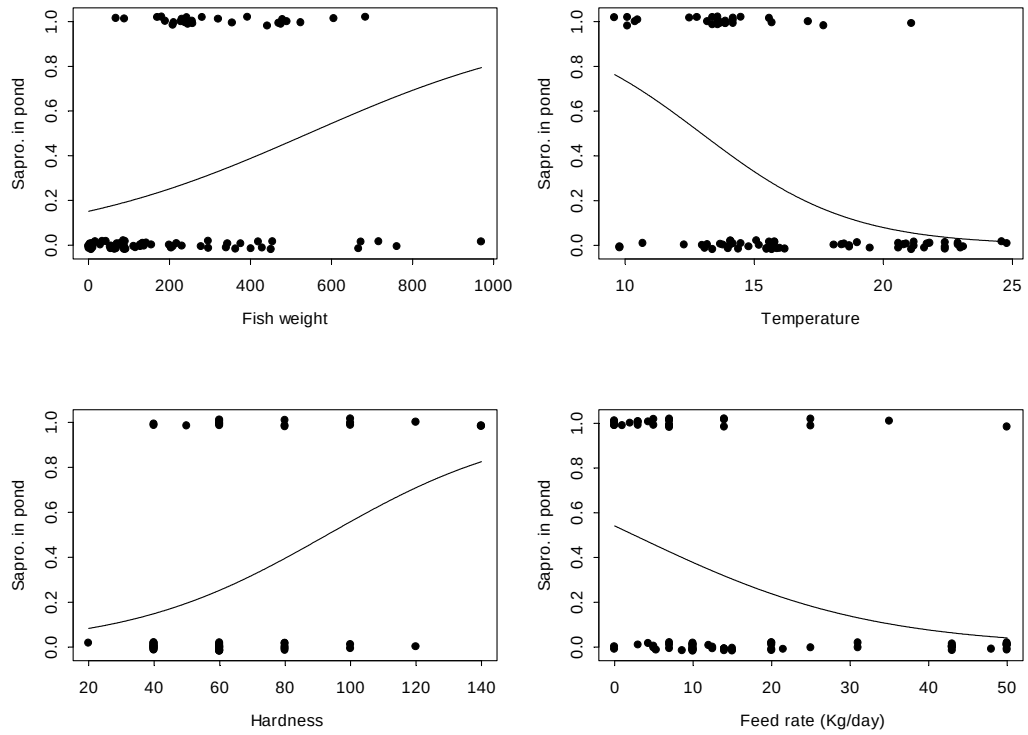


Figure 5.7. Association between the presence of *Saprolegnia parasitica* on silver perch and fish weight, water temperature, water hardness and feeding rate in ponds on commercial silver perch farms in NSW.

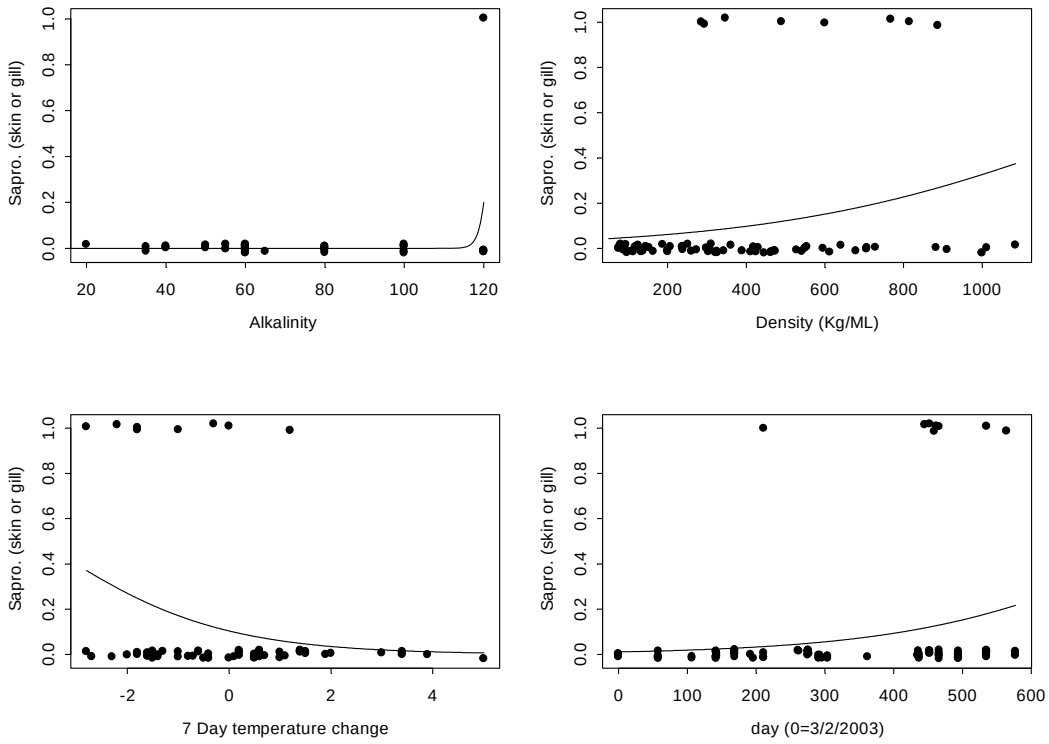


Figure 5.8. Associations between the presence of *Saprolegnia parasitica* on silver perch and alkalinity, fish density, 7-day temperature change and days from commencement of the study in ponds on a commercial silver perch farm in NSW.

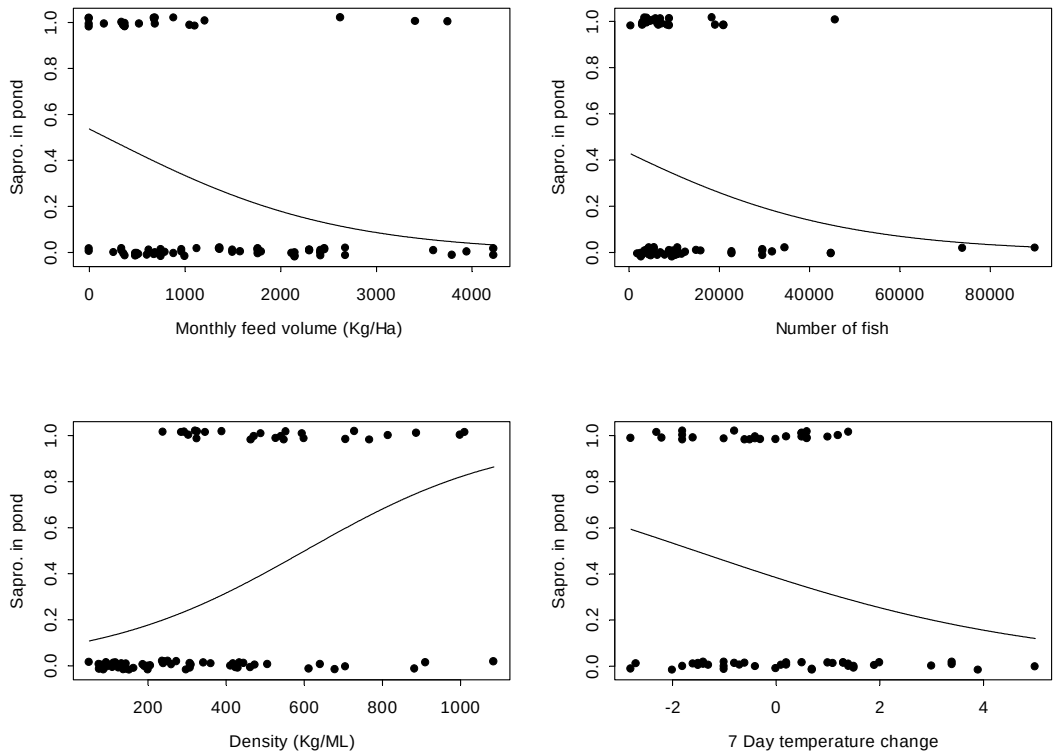


Figure 5.9. Association between the incidence of winter saprolegniosis in ponds and monthly feed volume, number of fish, fish density and 7-day temperature change in ponds on commercial silver perch farms in NSW.

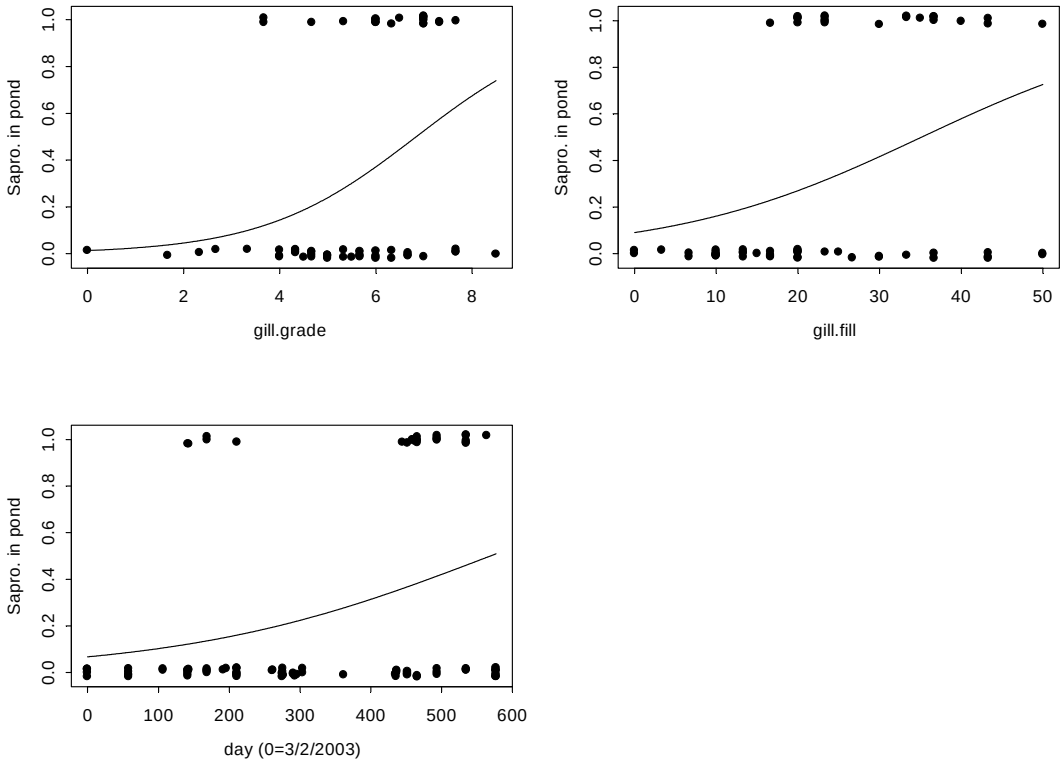


Figure 5.10. Association between the incidence of winter saprolegniosis in ponds and the condition of gill tissue on silver perch and the number of days of culture on commercial silver perch farms in NSW. See text for explanation of gill condition.

6. EVALUATION OF CHEMICAL THERAPEUTANTS

The goal of fish health management is to limit the incidence of infectious diseases and reduce the severity of losses during outbreaks. Health management begins with site selection and design of farms, and is achieved through the use of good aquaculture practices. Chemical therapeutants play a key role in controlling some infectious diseases, particularly acute diseases that can cause very high mortalities if not treated promptly. While good facilities and practices are essential components of health management, the use of chemical therapeutants is necessary in the practical reality of intensive aquaculture where disease outbreaks are inevitable.

Only a relatively small number of chemicals are used in freshwater fish culture throughout the world. Formalin and salt have been used to treat diseases of silver perch under culture conditions for nearly 30 years, but their use is limited to certain diseases, and by practical, environmental and economic factors. In our health management project, two new diseases, winter saprolegniosis and lepidotremosis were identified, and the difficulty of treating the acute disease ichthyophthiriosis at low water temperatures was recognised. To facilitate disease control in silver perch, a number of chemicals were evaluated during the project. The chemicals, which were selected on the basis of current or potential for legal use were; formalin, salt, copper sulfate, diquat, trichlorfon, chloramine-T and extract from barley straw. Research to evaluate these chemicals is reported in the following six sections, 6.1 – 6.6.

6.1. *In vitro* evaluation of the effects of selected chemicals on the oomycete, *Saprolegnia parasitica*, the pathogen which causes winter saprolegniosis in silver perch (*Bidyanus bidyanus*)

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6.1.1. Abstract

Fungal diseases are inherently difficult to treat in fish culture. Winter saprolegniosis, which is caused by the oomycete *Saprolegnia parasitica*, can result in high mortalities and significant economic losses on some silver perch farms. The effects of formalin, copper sulfate, chloramine-T and barley straw extract on various life cycle stages of *S. parasitica* was evaluated in a series of *in vitro* experiments in an effort to identify a chemical therapeutant for winter saprolegniosis. Only formalin demonstrated any inhibitory effects at the levels used. Concentrations of 25 or 35 mg/L formalin inhibited the production of motile zoospores, the growth of hyphae and the establishment of fungal colonies. Copper sulfate (0.1 – 0.4 mg/L), chloramine-T (2.0 and 6.0 mg/L) and barley straw extract (0.25 and 0.5 mg/L) did not affect zoospore motility or the germination of cysts. These results suggest that an evaluation of the use of formalin on farms with a history of winter saprolegniosis is warranted.

6.1.2. Introduction

Winter saprolegniosis is a serious disease of silver perch that is caused by the oomycete, *Saprolegnia parasitica*. Most outbreaks commence at water temperatures below 16°C, and decreases in temperature following cold changes during late autumn and winter are associated with the onset and increased severity of the disease (Landos et al. 2007). Winter saprolegniosis has caused total mortality of fish in earthen ponds, and the disease has lead to significant economic losses on some farms and restricted the development of the silver perch industry (Landos et al. 2007; Rowland et al. 2007a). Fungal diseases in fish are inherently difficult to treat (Noga 2000; Wise et al. 2004). Winter saprolegniosis is a problem in the large channel catfish (*Ictalurus punctatus*) industry in the USA, and proves difficult to control under commercial conditions in earthen ponds (Bly et al. 1992, 1993; Li et al. 1996; Hawke and Khoo 2004; Dr Michael Masser, personal communication).

Under experimental conditions, formalin and copper sulfate have been shown to have some effects on hyphal growth, zoospore production and/or cyst germination in *Saprolegnia* (Bly et al. 1996; Li et al. 1996). Formalin (25 mg/L) was reported to be effective as both a prophylactic and post-infective treatment, and copper sulfate (0.1 mg/L) is effective in preventing saprolegniosis in channel catfish (Bly et al. 1996; Li et al. 1996). Rach et al. (2005) reported increased survival of channel catfish with saprolegniosis following treatment with formalin. However, efficacy under commercial conditions has not been proven and long-term effects of these chemicals in catfish ponds are unknown (Li et al. 1996; Wise et al. 2004). In silver perch ponds, the application of formalin (20 – 30 mg/L) every 3 – 7 days partly controls the disease in some cases, but it is not completely effective in all outbreaks, causes a deterioration of water quality and is expensive (Landos et al. 2007; Rowland et al. 2006; Rowland et al. 2007b).

Several other chemicals have potential for treating fungal diseases in ponds. Barley straw extract has been reported to control algae in freshwater and inhibit the growth of some aquatic saprolegniacious fungi (Ball et al. 2001; Cooper et al. 1997) and chloramine-T is recommended for the treatment of some skin and gill bacterial infections and monogenean parasites (Noga 2000).

This study investigated the effects of formalin, copper sulfate, barley straw extract, chloramine-T, as well as the use of hydrogen chloride to lower the pH on various stages of the life cycle of *S. parasitica*.

6.1.3. Materials and methods

6.1.3.1. Saprolegnia parasitica

Pathogenic isolates were collected from lesions on silver perch during an outbreak of winter saprolegniosis on a commercial farm. The isolates were purified and identified as *Saprolegnia parasitica* as described in Chapter 5.

6.1.3.2. Selected chemicals

The chemicals were selected on the basis of their effectiveness in managing a variety of conditions in aquatic animals as reported in the literature. The chemicals were: formalin, stock solution (BDH, 100% laboratory grade) diluted to the desired concentrations; copper sulfate, with stock solution prepared from laboratory grade reagent cupric sulphate powder ($CuSO_45H_2O$=249.68, 98.5% min. assay); barley straw extract (1g dried barley straw/10L water incubated in aerated water at 18º – 25ºC for 6 weeks, and then filtered through 1.2 µm, then 0.22 µm GF/C Whatman paper); chloramine-T (Nycex-Mavlab) 1000 g/kg; and HCl for lowering the pH of water to achieve a pH of 5.5.

6.1.3.3. Experiment 1 – Effect of formalin on the formation of zoosporangia and subsequent zoosporulation of S. parasitica

Autoclaved silver perch scales were placed onto fresh *S. parasitica* cultures grown on corn meal agar (CMA) and incubated for ~ 48 h at room temperature until they were heavily colonised by vegetative hyphae. Colonisation was confirmed by microscopic examination. Two different concentrations of formalin, 25 mg/L and 35 mg/L were trialled at two different inoculation doses (group A, group B). For group A one hyphae-invaded fish scale was added to each of the treatment flasks, and for group B four hyphae-invaded fish scales were added to each of the treatment flasks. Total flask volume was 10 ml comprised of the treatment and autoclaved pond water. A treatment of autoclaved water only was included as a control. Treatment flasks were incubated for 40 h. The number of zoospores was assessed at 16 h and 40 h after addition of the chemical treatment (see Table 6.1.1). At each time, the total number of motile zoospores observed over a 30 sec period in each of five high powered fields (X40) was assigned a score as follows: 0 zoospores -; 1 – 5 zoospores +; 6 – 10 zoospores ++; > 10 zoospores +++.

6.1.3.4. Experiment 2 – Effects of chemicals on motile zoospores and cysts of S. parasitica

This experiment determined the sensitivity of motile zoospores and cysts to formalin, copper sulfate, barley straw extract and chloramine-T. A stock solution of zoospores was created by placing 20 autoclaved fish scales onto a culture of *S. parasitica* isolate growing on Corn Meal Agar (CMA). Plates were incubated at room temperature for 24 h. Scales were removed from plates and placed in a large tissue flask with 1000 ml autoclaved pond water, incubated for 48 h at room temperature (~ 20^{o}C) to allow for sporulation. The zoospore stock solution was deemed ready for use when more than 10 motile zoospores per high power field (X40) could be observed in a period of 60 s. Zoospore stock solutions were then used immediately for inoculation of the tissue flasks,

ensuring at time zero, there was zoospore motility. Tissue flasks were prepared containing the following individual treatments: 0.1, 0.2 or 0.4 mg/L copper sulfate; 0.25 or 0.5 mg/L barley straw extract; 5, 15, 25 or 35 mg/L formalin; 2 or 6 mg/L chloramine-T; distilled water was used as the control treatment. Each flask was inoculated with 1 ml of the zoospore stock solution. Zoospore motility in each flask was determined after 6 and 24 h. This was assessed utilising the same methodology and classification as in Experiment 1. Only the motility of zoospores was assessed. After 24 h, the liquid from each flask was removed and centrifuged at low speed (2500 rpm). Supernatant was discarded and approximately 0.5 ml of remaining fluid used to resuspend the centrifuged plug. Resuspended material was then spread-plated on Sabouraud's-gentamicin-chloramphenicol agar. Plates were incubated at room temperature (~ 20ºC) and examined daily for 3 days for evidence of germination of cysts and subsequent vegetative growth. After three days the total number of colonies was counted and assigned as score as follows: 0 colonies -; 1 – 5 colonies +; 5 – 10 colonies ++; > 10 colonies +++.

6.1.3.5. Experiment 3 – Effects of chemicals on the vegetative growth of S. parasitica

Cubes of agar (5 mm x 5 mm) were dissected from an advancing edge of a fresh culture (~ 48h) of *S. parasitica* on CMA. Each hyphae-containing agar cube was placed into a tissue flask with 10 ml of one of the following treatments: 15 or 35 mg/L formalin; 2 or 6 mg/L chloramine; control. Autoclaved water was used as a control treatment. The effects of the chemicals on vegetative growth and subsequent zoosporogenesis were assessed by: (i) measuring the extent of vegetative hyphal growth from the edge of the agar after 6, 18 and 24 h (the longest hyphae from all edges of the agar block were measured using a ruler mounted beneath the tissue flask); (ii) assessing the number of zoospores/cysts at 24 and 96 h after incubation as described in Experiment 1.

6.1.4. Results

6.1.4.1. Experiment 1

Zoospore production in the control treatment increased from 1 – 5 spores at 16 h to > 10 after 40 h in both A and B groups (Table 6.1.1). No motile zoospores were observed in the formalin treatments suggesting an inhibitory effect of formalin on vegetative growth and subsequent zoosporogenesis in *S. parasitica*. There were low levels of zoospore production at pH 5.5 in Group A, similar to levels in the control, suggesting no effect of pH 5.5. The high levels in Group B are probably due to the higher inoculation dose of colonised fish scales. The low level of zoospores in the control (Group B) at 16 h suggests that the fish scales used in those flasks had lower levels of vegetative colonisation than those used in the pH 5.5 flasks.

6.1.4.2. Experiment 2

Concentrations of 25 or 35 mg/L formalin appeared to have some temporary inhibitory effect on zoospore mobility, possibly through inducing encystment. The germination of cysts was not affected because viable colonies were grown from cysts that were collected from all groups at the completion of the experiment (Table 6.1.2). The data suggests that these concentrations of formalin are not lethal, only temporarily inhibitory to the development of zoosporangia and the release of motile zoospores. No other treatment was effective in immobilising zoospores, or completely inhibiting the germination of cysts.

6.1.4.3. Experiment 3

A concentration of 35 mg/L formalin appeared to slow the growth of hyphae and the production of zoosporangia in the initial 24 h of the treatment, but zoosporogenesis and zoosporulation continued after 96 h (Table 6.1.3). The vegetative propagation of *S. parasitica* was not inhibited by other treatments.

6.1.5. Discussion

This was a preliminary study to evaluate the potential of selected chemical therapeutants in controlling saprolegniosis in silver perch by exposing various forms of the oomycete, *S. parasitica* to formalin, copper sulfate, chloramine-T and barley straw extract under *in vitro* experimental conditions. The life cycle of Oomycetes is complex and involves vegetative hyphae producing zoosporangia that release motile primary zoospores which are infective, but can also become encysted before forming secondary zoospores. The cysts are also considered infective and are suspected of using their hooked hair-like appendages to attach to fish hosts. Most infections in fish are probably acquired from inanimate sources with fungi sporulating on dead organic matter (Noga 2000). Saprolegniosis may be prevented by inhibiting: growth of hyphae; development of zoosporangia; release of zoospores into water; and/or inhibiting cyst formation and germination.

In our study, only formalin exhibited any inhibitory effects on *S. parasitica*. Concentrations of 25 or 35 mg/L inhibited, within a period of 24 – 40 h, the growth of hyphae and the production of motile zoospores. At a concentration of 35 mg/L, formalin appeared to no longer have an inhibitory effect on vegetative growth and subsequent zoosporogenesis. This may have been due to either the depletion of formalin to sub-lethal concentrations, or the type of substrate used for assessing the activity of formalin on vegetative growth. Rowland et al. (2007b) reported depletion of formalin from concentrations of 25 – 35 mg/L to below 15 mg/L within 48 h in earthen ponds containing silver perch at water temperatures of 13.4° – 16.2°C. The different substrates used in our study (fish scales and CMA) might have affected the outcome because their nutritional value for the growth of *S. parasitica* differ considerably. This may have influenced the sensitivity of either vegetative growth to the effects of formalin (Table 6.1.1) or the organism's ability to recover from the effects of exposure to formalin at these concentrations.

Our overall findings support Li et al. (1996) who found that 25 mg/L formalin was effective as both a prophylactic and post-infective treatment for saprolegniosis in channel catfish under experimental conditions. Bly et al. (1996) found that formalin concentrations over 12.5 mg/L inhibited both zoospore production and cyst germination in *Saprolegnia*. The germination of cysts and recommencement of zoosporulation from zoosporangia after 96 h (Table 6.1.3) may have been due to depletion of formalin to sub-lethal concentrations. In some field trials, the application of formalin to ponds every three of four days appeared to provide partial control of some outbreaks of winter saprolegniosis, but there were still significant losses in most ponds (Bruce Rhoades, personal communication).

Copper sulfate at concentrations of 0.1 – 0.4 mg/L (0.25 – 0.1 mg/L copper) did not prevent the establishment of fungal colonies. Bly et al. (1996) reported inhibitory effects of copper sulfate at concentrations of 12.5 – 200 mg/L, but no effect at 0.1 – 0.25 mg/L, and Li et al. (1996) found that copper sulfate was effective in preventing saprolegniosis in channel catfish at a concentration of 0.1 mg/L, but was ineffective as a post-infective treatment. Levels of 0.25 mg/L copper and higher (> 1.0 mg/L copper sulfate) can't be used because they are toxic to silver perch (Rowland et al. 2007b). Given the *in vitro* results of our study, the benefit of copper reported in the USA may not be a direct result of the effects of copper on the pathogen.

A pH of 5.5 was selected because it is near the lowest tolerable level for freshwater fish (Rowland 1995). A pH of 5.5 did not prevent the production of fungal spores. *Saprolegnia* is known to grow over a wide range of environmental parameters, although its activity is restricted at very high or low pH levels. Barnes et al. (2004) found no difference in attachment or growth of the fungus *Saprolegnia diclina* at pH values ranging from 3 to 10, but significant effects at pH 11 and no fungal activity at pH 2 and 12 up to 200 h post-inoculation. Modification of pond pH would appear to offer no benefit for control of winter saprolegniosis.

Chemical control of saprolegniosis is usually ineffective once an infection is established (Wise et al. 2004). Although formalin and copper sulfate have been shown to prevent winter saprolegniosis in channel catfish under experimental conditions, their efficacy in treating the disease under commercial conditions has not been proven and long-term effects of these chemicals in catfish ponds are unknown (Li et al. 1996; Wise et al. 2004). Formalin and copper sulfate reduce spore numbers, but they do not eradicate *Saprolegnia* from ponds (Wise et al. 2004). However, the strategic use of these chemicals may help alleviate stress and reduce the severity of outbreaks of the disease. Bly et al. (1996) suggested that it may still be worthwhile treating channel catfish ponds which have a history of saprolegniosis immediately after the passage of a severe cold front that results in a rapid decrease in pond water temperature. A similar strategy may be of value for silver perch because of the similarity of the aetiology and pathogenesis of winter saprolegniosis in silver perch and channel catfish, and the inhibitory effects of formalin on the strain of *S. parasitica* that infects silver perch. An alternative strategy is to harvest infected fish and use formalin and/or salt to control winter saprolegniosis in tanks.

6.1.6. Acknowledgements

We thank Dr Michael Masser (Texas A&M University) for information on winter saprolegniosis in the channel catfish industry in the USA, Steve Pepper for technical assistance with the *in vitro* experiments and culture of fungus. We are grateful and sincerely thank Dr Josie Lategan for her comments and advice on a draft of this chapter.

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Table 6.1.1. *In vitro* production of *Saprolegnia parasitica* spores when exposed to formalin, ph 5.5 and control treatments in Experiment 1. Spore numbers: 0, + 1 – 5 spores; ++ 6 – 10 spores; +++ > 10 spores. Group A – 1 fish scale/flask inoculated with fungus; Group B – 4 scales/flask inoculated with fungus. Data are from three replicate flasks.

Treatment	**Number of spores**	
	16 h	**40 h**
Group A		
Control	+	+++
Formalin		
25 mg/L	0	0
35 mg/L	0	0
pH 5.5	+	++
Group B		
Control	+	+++
Formalin		
25 mg/L	0	0
35 mg/L	0	0
pH 5.5	+++	+++

Table 6.1.2. Colonies of *Saprolegnia parasitica* established under different treatments in Experiment 2. For details see text. Number of colonies in each replicate; 0, + 1 – 5; ++ 6 – 10; +++ > 10; where all replicates were the same, only one number given. Data are from three replicate flasks.

Treatment	Hours after inoculation			Growth on Sabourauds
	0	6	24	
Control	+++	+	+	+++
Copper sulphate (mg/L)				
0.1	+++	+++	+++	+++
0.2	+++	+++	+++	+++
0.4	+++	+++	+++	+++
Formalin (mg/L)				
5	0/+++	0/+++	+/++/+++	+/++/+++
15	+++	+++	++/+++	++/++/+++
25	+++	0	0	0/++/++
35	+++	+/+/++	0	0/+/++
Barley straw extract (mg/L)				
0.25	+++	+++	0/+	+/+++
0.50	+++	+++	+/++	+++
Chloramine-T (mg/L)				
2	+++	++/+++	+++	+++
6	+++	++/+++	+++	+++

Table 6.1.3. Growth of hyphae and number of motile zoospores of *Saprolegnia parasitica* exposed to formalin or chloramine-T in Experiment 3. Spore numbers: 0, + 1 – 5 spores; ++ 6 – 10 spores; +++ > 10 spores. Data are means of three replicates.

Treatment	Length of hyphae (mm)		Number of motile zoospores	
	6h	18h	24h	96h
Control	4.0	5.3	+++	+++
Formalin (mg/L)				
15	6.3	8.0	+	+++
35	2.7	2.3	0	+++
Chloramine-T (mg/L)				
2	3.3	5.6	+++	+++
6	4.0	4.7	+++	+++

6.2. Evaluation of diquat for the treatment of winter saprolegniosis in silver perch (*Bidyanus bidyanus*)

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6.2.1. Abstract

Winter saprolegniosis is a serious disease of silver perch caused by the fungus, *Saprolegnia parasitica*. Fungal diseases are inherently difficult to control in ponds. Under laboratory conditions in the USA, the aquatic herbicide diquat has been reported to inhibit the growth of *Saprolegnia* and prevent the onset of saprolegniosis in channel catfish. Because diquat is not registered for use on animals in Australia, it does not have a Maximum Residue Limit in Australia, and so the acceptable residue limit is zero. Trials were carried out to determine the effects of diquat on the growth of *S. parasitica*, and to determine the presence of residues in silver perch tissue after exposure to diquat. In trial 1, *S. parasitica* was cultured in flasks and exposed to concentrations of 0, 0.25, 0.5, 1.0, 2.0 and 10.0 mg/L diquat [diquat dibromide monohydrate (Reglone-Syngenta®)]. The fungus grew at all concentrations, but there was an inhibitory effect on growth with increasing concentrations of diquat. In trial 2, silver perch (range mean weights, 706 – 800 g) were stocked into three 1,000 L fibreglass tanks and treated with 0.5, 1.0 or 10.0 mg/L diquat. There were no mortalities or signs of toxicity at 0.5 or 1.0 mg/L, but there was 25% mortality of fish at 10.0 mg/L diquat. Mean residue levels in muscle tissue of fish treated with 0.5 or 1.0 mg/L were 0.014 and 0.029 mg/kg respectively after 7 days, and 0.003 mg/kg in fish treated with 1.0 mg/L after 27 days. Although diquat has some inhibitory effects on the growth of *S. parasitica*, it is not recommended for use on silver perch due to toxicity at 10 mg/L, and the presence of residues in muscle tissue for up to at least 27 days post-treatment. Diquat is not registered and can't be prescribed for use on food fish in Australia.

6.2.2. Introduction

Winter saprolegniosis is a serious disease of silver perch that is caused by the oomycete fungus, *Saprolegnia parasitica*. Most outbreaks commence at water temperatures below 16°C, and rapid decreases in temperature (e.g., 4° – 5°C in 5 – 7 days) following cold changes during late autumn and winter are associated with the onset and increased severity of the disease (Landos et al. 2007). Winter saprolegniosis can cause total mortality of fish in earthen ponds, and the disease causes significant economic losses on some farms restricting the development of the silver perch industry (Landos et al. 2007; Rowland et al. 2007).

Winter saprolegniosis is also a problem in the large channel catfish (*Ictalurus punctatus*) industry in the USA, but proves difficult to control on commercial farms (Bly et al. 1992, 1993; Li et al. 1996; Hawke and Khoo 2004; Dr Michael Masser, personal communication). Under experimental conditions, formalin (25 mg/L) is reported to be effective as both a prophylactic and post-infective treatment, and copper sulfate (0.1 mg/L) is effective in preventing saprolegniosis in channel catfish (Bly et al. 1996; Li et al. 1996). However, efficacy under commercial conditions has not been proven and long-term effects of the chemicals in catfish ponds are unknown (Li et al. 1996; Wise et al. 2004). In silver perch ponds, the application of formalin (20 – 30 mg/L) every 3 – 7 days partly

controls the disease in some cases, but is not completely effective in all outbreaks, causes a deterioration of water quality and is expensive (Landos et al. 2007; Rowland et al. 2006).

In the USA, the herbicide diquat is known to be effective against some fish diseases including bacterial gill disease and columnaris (Plumb 1994; Noga 2000; Thomas-Jinu and Goodwin 2004). Diquat has an inhibitory effect on *Saprolegnia* growth and is efficacious in preventing the onset of saprolegniosis in channel catfish under experimental conditions (Bly et al. 1996). Despite its high cost, diquat is worth evaluating as a therapeutic agent for winter saprolegniosis in silver perch because of the seriousness of the disease and the use of relatively small ponds in the commercial industry (0.1 – 1.0 ha) compared to the channel catfish industry where ponds are larger than 4 ha in surface area making chemical treatment difficult and costly (Tucker and Robinson 1990; Wise et al. 2004; Thomas-Jinu and Goodwin 2004; Dr Michael Masser, personal communication).

Few chemicals are registered or permitted for use in aquaculture in Australia. Diquat is registered as an aquatic herbicide, but is not registered for use on animals. Chemicals that are not registered have a zero acceptable residue limit. The objectives of this study were to determine: (i) the effects of diquat on the growth of *Saprolegnia parasitica*: and (ii) the presence and levels of residues in the tissue of silver perch after exposure to diquat.

6.2.3. Materials and Methods

6.2.3.1. Trial 1

Cultures of the fungus, *Saprolegnia parasitica*, were established in flasks using Sabourauds (S-PS) and Corn Meal Agar (CMA-PS) with 250mg/L streptomycin sulfate and 150mg/L Penicillin G to inhibit bacterial growth. Duplicate samples were inoculated onto each type of freshly prepared agar. CMA was chosen with reference to the similar work undertaken by Bly et al. (1992). Flasks were aerobically incubated at room temperature ~ 20^{o}C. Cultures of *S. parasitica* were exposed to diquat concentrations of 0, 0.25, 0.5, 1.0, 2.0 or 10.0 mg/L. Growth of the fungus, as determined by follicle length was evaluated after 20, 40, 120 and 140 h.

6.2.3.2. Trial 2

Silver perch (range mean weights, 706 – 800 g) were harvested from a 0.1 ha earthen ponds; the fish had never been exposed to diquat. After harvest, 16 fish were placed into each of three 1,000 L fibreglass tanks and given a continuous 5 g/L salt (NaCl) bath for 24 h to reduce stress, kill ecto-parasites and prevent fungal infection (Rowland and Ingram 1991). Water was exchanged (100%) in each tank following the salt bath and the tanks refilled. The fish were then held in the aerated, static tanks and diquat (diquat dibromide monohydrate (Reglone-Syngenta®) added to achieve concentrations of 0.5, 1.0 or 10.0 mg/L; there was one tank per treatment. The range of the water quality variables were: temperature 16.0º – 17.6º; dissolved oxygen 8.9 – 9.7 mg/L; pH 7.4 – 9.0. After three days, a continuous flow of 5 L/min of freshwater was provided to each tank. Fish were not fed. Three fish were randomly sampled from each tank 7 and 27 days post-treatment. The fish were filleted (skin-on) and the fillets frozen. Fillets and skin were homogenised and analysed for diquat residues using a Liquid Chromatography Mass Spectrophotometer (Advanced Analytical Australia Pty Ltd, 11 Julius Avenue, North Ryde, NSW, 2113).

6.2.4. Results and Discussion

This was a preliminary study to evaluate the potential of the aquatic herbicide diquat to control saprolegniosis in silver perch. Saprolegniosis in silver perch is caused by the Oomycetes fungus *Saprolegnia parasitica*. Herbicides have potential to control fungal diseases because Oomycetes

are known to have phylogenetic origins with the chromophyte algae rather than true fungi (Beakes 1989). Although *S. parasitica* grew at diquat concentrations of 0.25 – 10.0 mg/L, maximum follicle length of the fungus decreased with increasing concentrations of diquat over 140 h (Fig. 6.2.1), suggesting an inhibitory effect of the herbicide on fungal growth. The trend suggests that concentrations of diquat higher than 10.0 mg/L may have prevented fungal growth. Bly et al. (1996) reported that diquat concentrations of 0.25 and 0.5 µl/L significantly suppressed zoospore production, but those authors did not evaluate effects of diquat on fungal growth. Thomas-Jinu and Goodwin (2004) found that diquat was a highly effective bath treatment at 5.4 mg/L for the bacterial disease columnaris in channel catfish, and stated that because of its high cost of around US$9,000 in a 1 ha pond at this concentration, it is likely to be economically feasible only in tanks or raceways.

There were no mortalities or signs of toxicity (abnormal behaviour, swimming or colouration) in the silver perch exposed to 0.5 or 1.0 mg/L diquat, but there was 25% mortality at the higher concentration of 10.0 mg/L (Table 6.2.1). Toxicity ($95LC_{50}$) in other species varies markedly amongst species from 10 mg/L in rainbow trout to 245 mg/L in bluegill (Morgan and Brunson 1989, cited in Bly et al. 1996) and levels reported for fish kills range from 90 to 723 mg/L (Langdon 1988). The toxicity of 10 mg/L to silver perch and the high costs of diquat treatments, suggest that this chemical is not appropriate for further consideration for the treatment of winter saprolegniosis in silver perch.

Diquat residues were detectable in silver perch muscle/skin tissue for up to 27 days post-treatment (Table 6.2.1). Because diquat is not registered for use on animals in Australia, it does not have a Maximum Residue Limit, and so the acceptable residue limit is zero. The detection of residues in silver perch muscle tissue means that diquat cannot be prescribed or used in silver perch culture.

Diquat is a Poison Schedule 6 chemical that is registered for use in the aquatic environment on duck weeds, red azolla, water hyacinth, salvinia, water lilies, water lettuce, cattail and pond weeds. It is a dangerous chemical that is poisonous if absorbed by skin, inhaled or swallowed (®Syngenta; product label). Cross and Needham (1988) suggest that diquat is so dangerous it should not be used for control of diseases in fish.

6.2.5. Conclusion

Although diquat has a limited inhibitory effect on the growth of *Saprolegnia*, it is not recommended for use in silver perch culture at the present time because of its toxic effects at 10 mg/L, its persistence in silver perch tissue for at least 27 days post-treatment, and the risks that the chemical poses to the fish farmer. The product is not registered and cannot currently be prescribed for use on silver perch in Australia.

6.2.6. Acknowledgements

We thank Dr Michael Masser (Texas A&M University) for information on winter saprolegniosis in the channel catfish industry in the USA, staff at the Advanced Analytical Australia Pty Ltd for analysis of diquat residues, and Lee Cook (NSW Department of Primary Industries) for advice on the use of chemicals in aquaculture and comments on a draft of this chapter. Thanks also to Dr Josie Lategan for comments on the chapter.

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Table 6.2.1. Survival of silver perch and residues in muscle and skin tissue after exposure to different concentrations of the aquatic herbicide diquat.

Concentration of diquat (mg/L)	Survival (%)	Days post-initial exposure	Residue (mg/kg)
0.5	95	7	0.014
1.0	100	7; 27	0.029; 0.003
10.0	75	27	Not determined

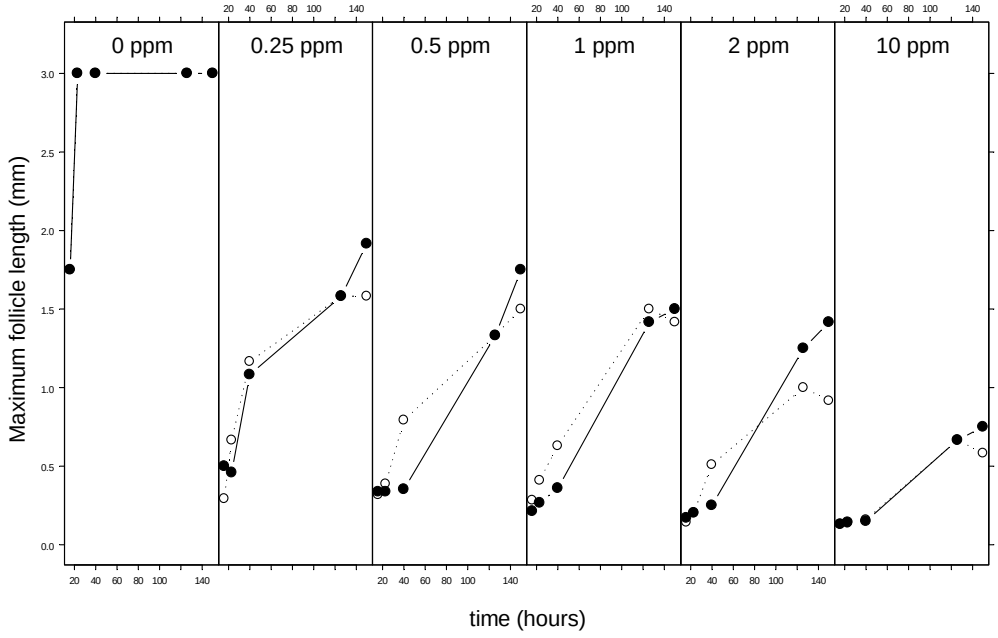

Figure 6.2.1. Growth of the fungus, *Saprolegnia parasitica*, exposed to concentrations of 0, 0.25, 0.5, 1.0, 2.0 and 10.0 ppm of the aquatic herbicide diquat in flasks.

6.3. Use of salt to control ichthyophthiriosis and prevent saprolegniosis in silver perch (*Bidyanus bidyanus*)

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6.3.1. Abstract

The diseases ichthyophthiriosis and saprolegniosis are caused by the ecto-parasitic protozoan, *Ichthyophthirius multifiliis*, and the fungus, *Saprolegnia parasitica* respectively. Both diseases can cause high mortalities of the Australian freshwater fish silver perch (*Bidyanus bidyanus*) and are difficult to control, particularly at low water temperatures. The efficacy of salt (NaCl) in controlling and preventing these diseases was evaluated in aquaria and tanks. Concentrations of 2 or 3 g/L salt controlled infestations of *I. multifiliis*, and fish were free of both theronts and trophonts by day 8 at temperatures of 17.3° – 21.3°C and day 6 at 19.2° – 23.5°C. Fish treated with 1 g/L salt remained infested and all fish in a control treatment (0 g/L salt) died. Although the mean survival rates of infested fish at pH levels of 5 or 6 were only 13.9% and 7.6% respectively, there were no theronts or trophonts on surviving fish after 12 days. Silver perch harvested from a pond and treated with 2 or 3 g/L salt did not become infected with *S. parasitica* and survival was 100%, whereas 16.6% of untreated (0 g/L salt) fish became infected and survival was only 66.7%. A concentration of 2 g/L NaCl is recommended for use in tanks, aquaria and re-circulating aquaculture systems to control ichthyophthiriosis and to prevent saprolegniosis in silver perch.

6.3.2. Introduction

The ecto-parasitic ciliate protozoan, *Ichthyophthirius multifiliis*, and the water mold or fungus, *Saprolegnia parasitica*, are responsible for the diseases ichthyophthiriosis (also called white spot or ich) and saprolegniosis respectively. Both diseases are common in most freshwater fishes, and can cause high mortalities in cultured silver perch (*Bidyanus bidyanus*) (Rowland and Ingram 1991; Callinan and Rowland 1995), particularly in late autumn, winter and early spring when water temperatures are below 16°C (Landos et al. 2007; Rowland et al. 2007).

The control of many fungal and parasitic diseases in freshwater aquaculture has traditionally relied on malachite green and formalin. However, malachite green is no longer approved for use on food fishes in most countries because of its teratogenic and mutagenic properties, and formalin is costly to use and can cause a deterioration of water quality (Leteux and Meyer 1972; Alderman 1985; Schlotfeldt et al. 1995; Noga 2000; Rowland and Tully 2004; Rowland et al. 2006). Salt (NaCl) is a safe, relatively inexpensive parasiticide and osmoregulatory aid that is widely used in freshwater fish culture (Selosse and Rowland 1990; Wurts 1995; Seim et al. 1997). Fungal infections are inhibited by prolonged immersion of fish in salt concentrations of 1 to 5 g/L (Li et al. 1996; Noga 2000), although some studies have found it less effective than formalin and hydrogen peroxide at inhibiting growth of *Saprolegnia* on fish eggs (Waterstrat and Marking 1995; Schreier et al. 1996).

Selosse and Rowland (1990) found that 5 g/L salt was effective in controlling infestations of *I. multifiliis* on four species of Australian native fish, including silver perch. Subsequently, Rowland and Ingram (1991), Callinan and Rowland (1995) and Ingram et al. (2005) recommended the use of 5 g/L NaCl to treat ichthyophthiriosis and fungal infections, and salt baths have been routinely used to control these diseases and as a post-harvest treatment in Australian native fish.

The effectiveness of salt to control ichthyophthiriosis may vary with fish species, salt concentration and/or strain of *I. multifiliis*. There are various strains of *I. multifiliis* which differ in their ecology,

including susceptibility to salinity (Allen and Avault 1970; Aihua and Buchmann 2001). Allen and Avault (1970) indicated that levels of salinity of 1 g/L and higher are effective against *I. multifiliis*; however, Aihua and Buchmann (2001) reported that theronts were produced and released at salinities up to at least 5 g/L, and Wagner (1960) found that theronts were released at 10 g/L in a German strain of *I. multifiliis*. Wagner (1960) also reported that *I. multifiliis* cysts die at a pH of 5.5. A concentration of 3 g/L salt was ineffective in controlling ichthyophthiriosis in channel catfish (Tieman and Goodwin 2001) and Miron et al. (2003) reported low survival of silver catfish (*Rhamdia quelen*) infested with *I. multifiliis* when treated with 0, 1 or 2 g/L salt, but 100% survival at 4 g/L salt.

In Australia, there are two main concerns with the use of salt in freshwater aquaculture: (i) the environmental impact of saline effluent water on receiving waters; and (ii) the welfare of farm workers under Occupational Health & Safety regulations when handling 25 kg bags of salt. The effective use of salt concentrations lower than 5 g/L would reduce environmental impacts, the quantities of salt handled and used, and the cost of disease treatments. The aims of our study were to determine: (i) the effectiveness of salt concentrations lower than 5 g/L in controlling ichthyophthiriosis and preventing saprolegniosis; and (ii) the effects of pH levels around 5 and 6 on *I. multifiliis.*

6.3.3. Material and methods

The research was done at the NSW Department of Primary Industries' Grafton Aquaculture Centre. Water from the Clarence River was stored in a large earthen reservoir, and filtered to 80 μm using a sand filter and cartridge filters prior to use in the aquaria. An Horiba U-10 meter (Horiba Ltd, Kyoto, Japan) was used to monitor temperature, dissolved oxygen (DO), pH and salinity each day. Fish were naturally infested with *I. multifiliis*.

6.3.3.1. Experiment 1

Twenty-two silver perch (mean total length 127 mm, range 98 – 164 mm; mean weight 25 g, range 10 – 52 g), lightly infested with *I. multifiliis* were stocked into each of 12, 50 L glass aquaria and treated with 0 (control), 1, 2, or 3 g/L salt (NaCl) for 16 days. There were three randomly-assigned replicate aquaria for each treatment. Salinities were achieved by the addition of coarse pool salt (initially on day 1). Subsequently on days 3 and 8, water was added to each aquarium to replace that lost due to evaporation, and the salt concentration adjusted to nominal levels. On day 13, 5 g agricultural limestone ($CaCO_3$) was added to each aquarium to compensate for a decline in pH. Dead fish were removed daily from each aquarium. Multiple nets and sterilisation of equipment with chlorine were used to ensure individual aquaria were not contaminated with *I. multifiliis*.

Biopsies of gill and skin tissues were undertaken from two fish in each aquarium prior to the addition of salt on day 1 to determine the initial level of infestation of *I. multifiliis*. Subsequently, two fish were sampled from each aquarium every 3 or 4 days. The left anterior gill arch and a scraping of mucus from the left side of each fish, including the caudal fin were placed on a slide, covered with a coverslip and examined microscopically at X100. All theronts and trophonts in each sample were counted. Survival rates were determined using the expected number of fish remaining at termination, taking into account sampled fish.

6.3.3.2. Experiment 2

Thirty silver perch (mean length 73 mm, range 54 – 126 mm; mean weight 3.7 g, range 1.2 – 19.4 g), heavily infested with *I. multifiliis* were stocked into each of 20, 50 L aquaria and treated with 0 (control), 1 or 2 g/L salt, or pH 5 or pH 6 for 12 days. There were four replicate aquaria for each treatment. In the pH treatments, pH was monitored in each aquarium at least twice daily and adjusted as required by the addition of hydrochloric acid. Management of aquaria and monitoring

of water quality were as described for Experiment 1, and one fish was sampled from each aquarium daily for examination of gill and skin tissue.

6.3.3.3. Experiment 3

Silver perch were harvested from an earthen pond using a seine net, and transported live without anaesthetic to the hatchery building over a period of approximately 15 min; the use of anaesthetic during transportation is normal practice. A total of 120 fish (mean length 332 mm, range 300 – 350 mm; mean weight 514 g, range 379 – 650 g) was randomly selected and then 12 fish were stocked into each of 8, 1,000 L circular, fibreglass tanks for 18 days. Tanks were treated with 0 (control), 1, 2 or 3 g/L salt within 30 minutes of stocking. There were two replicate tanks for each treatment. Tanks were aerated using diffused air, and there was no water exchanged. Fish were observed daily and two fish were removed from each tank every three days, anaesthetised and inspected for lesions and signs of fungal infection such as pale skin, haemorrhagic areas, raised scales, fungal growths. Dead fish were removed daily.

6.3.3.4. Data analysis

One way analysis of variance was used to determine the effects of salt concentration and pH levels on survival in each experiment, and on the incidence of fungal infection in Experiment 3. Percentage data was arc-sin transformed before analysis.

6.3.4. Results

6.3.4.1. Experiment 1

Salt concentration had a significant effect ($P<0.05$) on survival. Survival rates of silver perch treated with 0, 1, 2 and 3 g/L salt were 66.7, 96.7, 96.7 and 100% respectively (Table 6.3.1). Fish treated with 2 or 3 g/L salt were free of both theronts and trophonts by day 8, but fish in 1 g/L salt remained infested (Table 6.3.1; Fig. 6.3.1). All silver perch in one control aquarium had died by day 15, but some fish survived in the remaining two control aquaria. The level of infestation decreased in these two aquaria between days 11 and 16 (Fig. 6.3.1) during a period when the pH had declined and remained below 6.5 for 6 days; the pH in the third replicate aquaria, where all fish died remained above 7 (Fig. 6.3.2). These data suggest that low pH may have prevented re-infestation by theronts. Ranges of water quality variables were: temperature 17.3° – 21.3°C; DO 6.8 – 10.9 mg/L; pH see Fig. 6.3.2.

6.3.4.2. Experiment 2

Levels of salt and pH had a significant effect ($P<0.01$) on survival. Survival at 2 g/L salt was 96.7% and all fish were free of theronts and trophonts on day 12, whereas survival rates at 0 or 1 g/L salt or pH 5 or pH 6 were only 0, 8.7, 13.9 and 7.6% respectively (Table 6.3.2). All fish in the control treatment had died by day 3, and most fish in 1 g/L salt by day 6; all surviving fish remained infested at day 12 (Table 6.3.2; Fig. 6.3.3). Although survival rates at pH 5 and pH 6 were only 13.9% and 7.6% respectively, surviving fish were free of theronts and trophonts on day 12 (Table 6.3.2; Fig. 6.3.3). Ranges of water quality variables were: temperature 19.2° – 23.5°C, DO 7.29 – 9.08 mg/L; pH in the non-adjusted aquaria 7.1 – 7.8; pH 5 treatment 4.5 – 6.3, and pH 6 treatment 5.2 – 6.7.

6.3.4.3. Experiment 3

Salt concentration had a significant effect ($P<0.05$) on survival and the incidence of fungal infection. There were no signs of infection and no mortalities of fish treated with 2 or 3 g/L salt, whereas in the control treatment (0 g/L salt), post-harvest survival was 66.7% and there was a 16.6% incidence of fungal infection in the surviving fish (Table 6.3.3). The incidence of fungal

infection in fish treated with 1 g/L salt was 4.2% and survival 91.7%. Ranges of water quality variables were: temperature 16.7° – 20.4°C; DO 7.6 – 11.4 mg/L.

6.3.5. Discussion

This study demonstrated that a concentration of 2 g/L salt is sufficient to control ichthyophthiriosis in silver perch. This is lower than the concentration of 5 g/L salt previously reported by Selosse and Rowland (1990), but in that study the effectiveness of lower levels was not evaluated. A concentration of 1 g/L was insufficient to completely control the infestation, with re-infestation by theronts still evident. Higher salt concentrations are required to control ichthyophthiriosis in channel catfish (5 g/L) and silver catfish (4 g/L) (Tieman and Goodman 2001; Miron et al. 2003), and in both those studies 2 g/L salt was not effective. Various strains of *I. multifiliis* are susceptible to different salinities. Wagner (1960) found survival of *I. multifiliis* and release of theronts at 10 ppt with a German strain, and Aihua and Buchmann (2001) found that there was still development and theront production at 5 g/L with a Danish strain. Our results suggest that the strain of *I. multifiliis* infesting silver perch in Australia is relatively susceptible to salt.

Results of Experiments 1 and 2 suggest that low pH has some effect on *I. multifiliis*. Although survival was low at pH 5 and pH 6, all surviving fish were free of theronts and trophonts on day 12. The high level of fish mortality may have been due to a very high initial level of infestation in Experiment 2 (see Fig. 6.3.3), and/or fluctuations in pH to levels above the nominal levels during the first 5 days that may have provided the opportunity for re-infestation of the host fish. The success of treating ichthyophthiriosis is partly dependent on the severity of the infestation, with heavy infestations having a poor prognosis (Wise et al. 2004). Wagner (1960) reported that *I. multifiliis* cysts die at pH 5.5. Observations in this study indicate that theront production was inhibited either by the pH affecting the trophont as it left the host, the encysted tomont and/or the infestive theronts. The significantly lower survival at pH 5 and pH 6 compared to the 2 g/L salt, may have been partly due to the ease of maintaining the nominal salt concentrations compared to maintaining pH levels.

Fungal infections in freshwater fish are very difficult to treat under culture conditions and effective management is usually dependent on prevention (Noga 2000; Wise et al. 2004). Callinan and Rowland (1995) recommended that a bath of 5 g/L for several days would prevent fungal infections in silver perch, and Noga (2000) stated that infections are inhibited by concentrations > 3 g/L. In the current study, we demonstrated that a relatively low salt concentration of 2 g/L was sufficient to prevent infection by *S. parasitica* and reduce post-harvest mortalities of silver perch in tanks.

Salt is well known for its positive influences on freshwater fish osmoregulation and physiology that lead to reduced stress, reversal of ionic losses, increased mucus production and healing of damaged skin tissue (Piper et al. 1982; Selosse and Rowland 1990; Wurts 1995; Tumbol et al. 2001; Wise et al. 2004). We have further demonstrated its role in disease control. The use of 2 g/L salt to control ichthyophthiriosis and prevent saprolegniosis in silver perch in tanks will significantly decrease the quantity of salt used, improve occupational health and safety of farm workers, reduce costs of disease control, and reduce salt concentrations in effluent waters from silver perch farms.

6.3.6. Acknowledgements

We thank Peter Boyd and Ralf Jahrling for technical assistance with harvesting the fish, setting up experiments and recording some of the data, and Annette Gorham and Astrid Green for translating the paper by Wagner (1960) from German into English. We thank Drs Geoff Allan and Wayne O'Connor for comments on a draft of this chapter.

6.3.7. References

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Table 6.3.1. Survival of silver perch fingerlings (mean weight, 25.0 g) and level of infestation of *I. multifiliis* at different salt concentrations after 16 days at temperatures of 17.3° to 21.3°C. Data are means±SD of three replicate aquaria.

Parameter	**Salt concentration (g/L)**			
	0	**1**	**2**	**3**
Survival (%)	66.7±47.1	96.7±4.7	96.7±4.7	100
Number of *I. multifiliis*				
theronts	1.3±0.8	25.1±10.2	0	0
trophonts	0.5±0.5	10.2±7.3	0	0

Table 6.3.2. Survival of silver perch fingerlings (mean weight, 3.7 g) and level of infestation of *I. multifiliis* at different salt concentrations and pH levels after 12 days at temperatures of 19.2° to 23.5°C. Data are means±SD of four replicate aquaria.

Parameter	**Salt concentration (g/L)**			**pH**	
	0	**1**	**2**	**5**	**6**
Survival (%)	0	8.7±15.1	96.7±4.7	13.9±13.0	7.6±13.2
Number of *I. multifiliis*					
theronts	-	0	0	0	0
trophonts	-	43	0	0	0

Table 6.3.3. Survival of silver perch (mean weight, 514 g) and incidence of *Saprolegnia* infection at different salt concentrations after harvest from a pond. Data are means±SD of two replicate tanks.

Parameter	**Salt concentration (g/L)**			
	0	**1**	**2**	**3**
Survival (%)	66.7±8.4	91.7	100	100
Incidence of fungal infection (%)	16.6±2.4	4.2	0	0

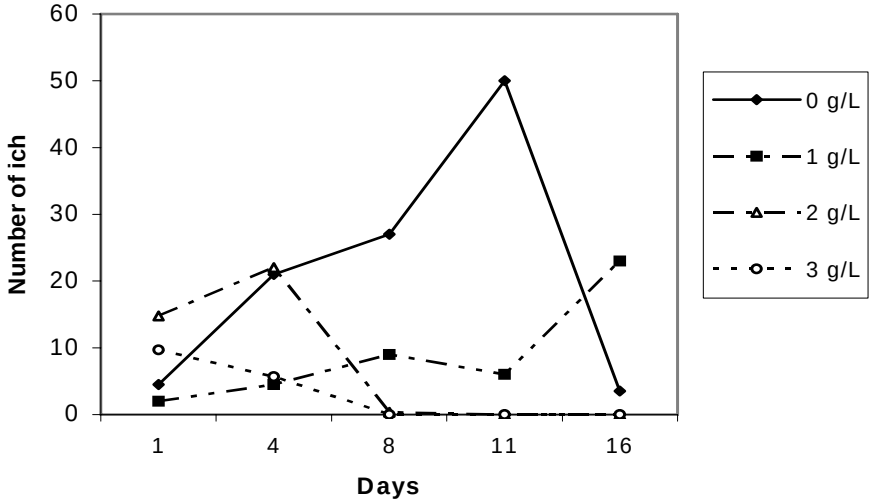

Figure 6.3.1. Numbers of *Ichthyophthirius multifiliis* on silver perch fingerlings (mean weight, 25.0 g) treated with different concentrations of salt for 16 days in aquaria at 17.3º to 21.3ºC in Experiment 1. Data are means of three replicate aquaria.

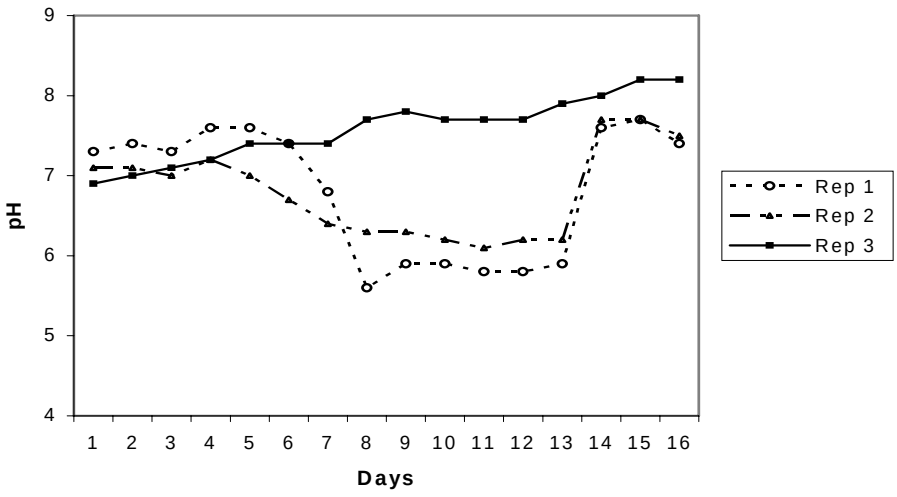

Figure 6.3.2. Levels of pH over 16 days in the three replicate aquaria of the control treatment in Experiment 1.

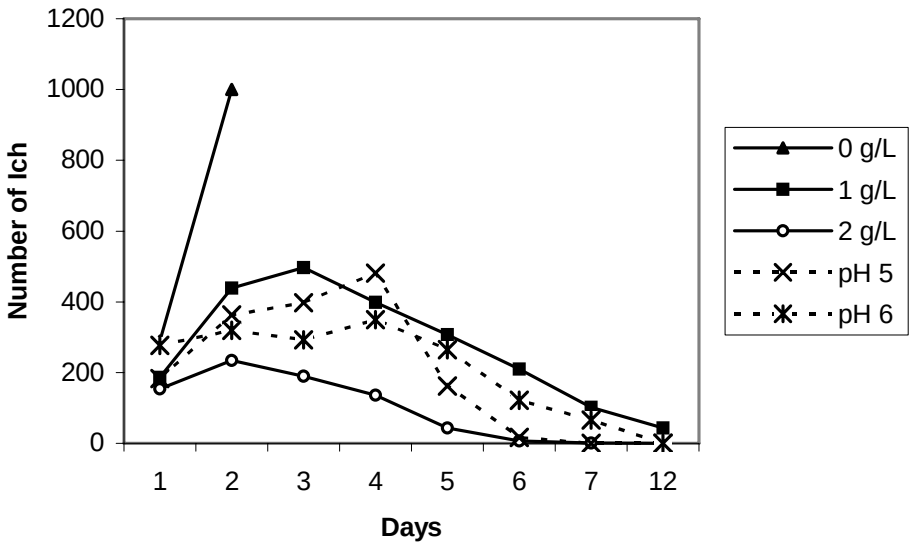

Figure 6.3.3. Numbers of *Ichthyophthirius multifiliis* on silver perch fingerlings (mean weight, 3.7 g) treated with different concentrations of salt and different levels of pH for 12 days in aquaria at 19.2º to 23.5ºC in Experiment 2. Data are means of four replicate aquaria.

6.4. Use of formalin and copper to treat ichthyophthiriosis in the Australian freshwater fish silver perch (*Bidyanus bidyanus*)

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6.4.1. Abstract

Infestations of the protozoan parasite, *Ichthyophthirius multifiliis*, cause the serious disease ichthyophthiriosis (ich or white spot) in freshwater fishes throughout the world. Formalin is a recommended treatment for ichthyophthiriosis in the Australian fish silver perch (*Bidyanus bidyanus*), but the disease is difficult to control in ponds particularly at low water temperatures because of the complex life cycle of *I. multifiliis* and the depletion of chemical therapeutants after application. Experiments were carried out to develop an improved treatment regime for formalin and to evaluate copper as a therapeutant. Silver perch fingerlings infested with *I. multifiliis* were stocked into 55 L aquaria at temperatures of 14.8° – 17.6°C and alkalinities of 70 – 110 mg/L. Formalin (34 – 38% formaldehyde) or copper (24.5% copper sulfate) were added to the aquaria and then monitored and re-adjusted to nominal concentrations daily. A concentration of 30 mg/L formalin controlled ichthyophthiriosis, but fish treated with 20 mg/L remained infested with theronts and trophonts on day 17; survival at both concentrations was 100%. A concentration of 10 mg/L formalin did not control ichthyophthiriosis and all fish were dead from the infestation by day 17. Fish treated with 0.1 or 0.2 mg/L copper were free of theronts and trophonts by days 17 and 14 respectively, and survival was 100%. Survival at 0.05 mg/L copper was 100%, but fish remained infested. At 0.25 mg/L copper, survival was 82.5% and there were no theronts or trophonts on gill and skin tissues of fingerlings by day 14. There was total mortality of fish treated with 0.5 or 1.0 mg/L copper suggesting these concentrations are toxic to silver perch. All fish in infected-control treatments died. In earthen ponds containing silver perch, copper applied to achieve a concentration of 0.2 mg/L was depleted to below 0.1 mg/L within 24 h, and 30 mg/L formalin was depleted to concentrations below 15 mg/L within 48 h. Treatment regimes involving daily applications of formalin (30 mg/L initially, then 25 – 30 mg/L daily) or copper (0.2 mg/L initially, then 0.1 – 0.15 mg/L daily) controlled ichthyophthiriosis in silver perch in earthen ponds at costs of $AUD486.00 and $AUD68.34/hectare/day respectively. This study has developed a new formalin treatment regime for the control of ichthyophthiriosis, and demonstrated that copper sulfate has potential as chemical therapeutant for this serious disease of silver perch.

6.4.2. Introduction

Ichthyophthirius multifiliis is a holotrichous protozoan that invades the skin and gill tissues of freshwater fish causing the important disease ichthyophthiriosis, commonly referred to as ich or white spot. It is one of the most common and serious parasitic diseases of freshwater fishes in aquaculture, and can cause high mortalities in both warmwater and coldwater species, particularly in fingerlings stocked at high densities (Sarig 1971; Piper et al., 1982; Hoffman 1991; Paperna 1999; Noga 2000). Ichthyophthiriosis is a common disease of the Australian warmwater fish silver perch (*Bidyanus bidyanus*) under culture conditions (Rowland 1983; Rowland and Ingram 1991; Callinan and Rowland 1995; Rowland et al. 2007).

Ichthyophthirius multifiliis is an obligate parasite that has a complex, temperature-dependent life cycle. The free-swimming, infestive theront (20 – 40 µm in length) bores under the epithelium of skin and gill tissue, where it feeds and develops into a relatively large trophont (up to 1 mm in diameter) which is visible to the naked eye resulting in the characteristic white spots on skin and gills. Trophonts leave the fish, adhere as tomonts to solid substrates such as pond bottom, cages, tanks and nets, and undergo mitosis before releasing up to 3000 tomites that differentiate into theronts (Paperna 1991; Hoffman 1999). Reproduction may also occur under the epithelium of the fish (Ewing et al. 1986, 1988). The length of the life cycle varies from 90 – 96 days at 3° – 5°C, 20 days at 10ºC, 13 – 14 days at 13° – 15°C, to 3 – 7 days at 20° – 25°C; there is no development below 3°C and over 30°C (Sarig 1971; Hawke and Khoo 2004; Wise et al. 2004). Free-swimming theronts are susceptible to chemical treatment (Piper et al. 1982; Hoffman 1999; Paperna 1991) and so effective control is dependent on periodic applications or continuous exposure of the parasite to chemical therapeutants.

Malachite green was a common, highly effective, low-cost treatment used to control many parasitic and fungal diseases, including ichthyophthiriosis in freshwater fishes until the early 1990's; however, it is a potential carcinogen and tetratogen, and it is no longer permitted for use on food fishes in most countries (Sarig 1971; Hoffman and Meyer, 1974; Meyer and Jorgenson, 1983; Alderman 1985; Meyer and Schnick 1989; Callinan and Rowland 1995; Schlotfeldt et al. 1995). Subsequently there have been numerous studies to evaluate alternatives to malachite green for the control of ichthyophthiriosis (e.g., Alderman 1992; Griffin 1989; Selosse and Rowland 1990; Wahli et al. 1993; Schlenk et al. 1998; Bodensteiner et al. 2000; Tieman and Goodwin 2001; Lin et al. 2003; Miron et al. 2003). Few chemicals are registered for use with food fishes in Australia. Formalin can be used by members of the National Aquaculture Council, including members of the NSW Silver Perch Growers Association under an Australian Pesticides and Veterinary Medicines Authority permit – PER8853. Formalin is also approved for use as an aquatic therapeutant in both the US and Canada (Schnick et al. 1997). However, formalin is costly to use, adversely affects some aquatic organisms, and causes a deterioration of water quality, particularly at high temperatures (Meyer and Schnick 1989; Boyd 1990; Noga 2000; Rowland et al. 2006). Current recommended treatments for ichthyophthiriosis for various species involve the periodic application of formalin, e.g., three treatments on alternate days, each third day, every 5 – 7 days (Sarig 1971; Piper et al. 1982; Hoffman 1999; Callinan and Rowland 1995; Noga 2000; Wise et al. 2004). Such treatment regimes are not always successful in controlling ichthyophthiriosis in silver perch and there have been large losses on some commercial farms, particularly during winter (Rowland et al. 2007; Ian Charles, Mark Scifleet, personal communication). An improved formalin treatment regime is required for the effective control of ichthyophthiriosis in silver perch.

Other chemicals recommended as therapeutants for ichthyophthiriosis include salt, potassium permanganate and copper sulfate (Selosse and Rowland 1990; Straus 1993; Schlenk et al. 1998; Noga 2000; Miron et al. 2003). Salt controls ichthyophthiriosis in silver perch in tanks and aquaria (Selosse and Rowland 1990; Mifsud and Rowland 2007), but the large-scale use of salt in ponds would be impractical and environmentally unsound. Potassium permanganate is strongly influenced by detoxification and so effective concentrations are difficult to maintain in fish ponds (Straus and Griffin 2001, 2002). Copper sulfate is registered as an algacide, but is also used to control ecto-parasites, including *I. multifiliis* in channel catfish in the USA (Tucker and Robinson 1990; Straus 1993). Copper can be difficult to use because its toxicity is closely linked to water quality, particularly alkalinity, and it has a low therapeutic index (Straus and Tucker 1993; Noga 2000; Straus 2006). However, the successful use and relatively low cost of copper sulfate to control ichthyophthiriosis in some freshwater fish (Piper et al. 1982; Tucker and Robinson 1990; Noga 1990) warrants its investigation for use in silver perch.

Aims of this study were to: (i) determine the efficacy of various concentrations of formalin and copper in treating ichthyophthiriosis under experimental conditions; (ii) determine the patterns of

depletion of formalin and copper concentrations in earthen ponds; and (iii) develop and validate new formalin and copper treatment regimes for controlling ichthyophthiriosis in silver perch.

6.4.3. Materials and methods

6.4.3.1. General

This study was done at the Grafton Aquaculture Centre (GAC). Fish husbandry, pond management and water quality monitoring followed Rowland (1995 a, b). Experiments 1 and 2 were done in a temperature-controlled room, subjected to natural photo-period. Aquaria were aerated continuously with diffused air. Water from the Clarence River was stored in a large earthen reservoir, and filtered to 80 μm using a sand filter and cartridge filters prior to use in the aquaria. An Horiba U-10 meter (Horiba Ltd, Kyoto, Japan) was used to monitor temperature, dissolved oxygen (DO) and pH. Total ammonia-nitrogen (TAN) was determined using Nessler Reagent. Total alkalinity was determined using a Hach colorimeter.

6.4.3.2. Diseased fish – maintenance and monitoring

Silver perch fingerlings were infested naturally with *I. multifiliis*, and subsequently a stock of infested fish was held in two 500 L fibreglass tanks for use in Experiments 1 and 2. In addition, two tanks of un-infested fish were held in two 9,000 L tanks to provide fish for un-infested control treatments and for periodic addition to the infested stock. Multiple nets and sterilisation of equipment using chlorine were used to ensure aquaria were not contaminated with *I. multifiliis*. Parasites were monitored by microscopic examination of gill and skin tissue. Fish were euthanased by severing the spinal column with a scalpel (fingerlings) or sharp knife (larger fish). The left, anterior gill arch was removed from each fish. In fingerlings, mucus was scraped from the total left hand side and caudal fin, and in larger fish the scraping was along the lateral line and caudal fin. The tissue samples were placed on microscope slides, covered with a coverslip and examined at 100X magnification.

6.4.3.3. Application and measurement of formalin and copper

Three batches of formalin were used; (i) Unilab Formaldehyde Solution® (34 – 38% formaldehyde; Ajax Chemicals) in aquaria; (ii) Formalin® (400 g/L formaldehyde; Deltrex Chemicals) in pond trial 1; (iii) Formaldehyde Solution® (30 – 60% formaldehyde; Orica Australia Pty Ltd) in pond trials 2 and 3. The form of copper was reagent grade copper sulfate pentahydrate (24.5% copper, Cu^{2+}, by weight). Concentrations of both chemicals were achieved by addition of the appropriate quantity of chemical based on the volume of water in each aquarium or pond. After the initial application to aquaria, concentrations were measured within 2 h and, if needed adjusted to the nominal concentration by addition of water or chemical. In ponds, initial concentrations of formalin and copper were based on estimates of pond volume. After the initial application to ponds, the concentrations were measured, but there was no attempt to adjust to nominal concentrations. Copper sulfate was dissolved in water prior to application. Formalin and copper sulfate were applied directly to the water flow in front of the aerator in each pond. To increase the total alkalinity in ponds to 50 – 80 mg/L, agricultural limestone ($CaCO_3$) was placed in a hessian bag attached to the walkway of each pond. Ponds were aerated for 24 h/day during the trials. Formalin was measured using a Formaldehyde Test Kit (Merck®RQ Flex2) and copper was measured using a spectrophotometer (Hach®DR/EL 2000).

6.4.3.4. Efficacy of formalin and copper

Experiment 1

Silver perch fingerlings (mean length 97.9 mm; mean weight 10.8 g) infested with ***I. multifiliis*** were stocked into 55 L glass aquaria at 16 fish/aquarium, and treated with concentrations of 0, 0.25, 0.5 and 1.0 mg/L copper. Treatments and replicates were randomly allocated to aquaria, and there was an additional control treatment (0 mg/L copper) containing un-infested fingerlings. There were four replicates of each treatment. Total alkalinity in all aquaria was maintained at 80 – 110 mg/L using sodium bicarbonate, and temperature was maintained at 14.8° – 16.8°C. The concentration of copper was monitored and adjusted to the nominal concentration twice daily. Fish were observed daily, the presence of trophonts noted, and dead fish removed. Three fish were sampled from each aquarium every 7 days, and skin and gill tissue examined for theronts and trophonts. Survival was expressed as a percentage of expected survivors taking into account sampled fish. Treatments were considered effective if ***I. multifiliis*** was absent from gill and skin tissues of fish surviving the particular treatment.

Experiment 2

Silver perch fingerlings (93.0 mm; 7.9 g) infested with ***I. multifiliis*** were stocked into 55 L glass aquaria at 16 fish/aquarium, and treated with concentrations of 0.05, 0.1 or 0.2 mg/L copper or 10, 20 or 30 mg/L formalin. There were three randomly-allocated, replicate aquaria for each concentration and an untreated control containing infested fish. Total alkalinity was maintained at 70 – 90 mg/L and temperature at 16.4° – 17.6°C. Management of copper and alkalinity was as in Experiment 1, and formalin was monitored and adjusted to the nominal concentration once daily.

6.4.3.5. Depletion of formalin and copper in ponds

Formalin – trials 1, 2 and 3

In trial 1, formalin (400 g/L formaldehyde; Deltrex Chemicals) was added to achieve a concentration of 30 mg/L in 4, 0.1-ha earthen ponds each containing 1,500 fish (range mean weights, 136.1 – 164.2 g; estimated biomasses 2.0 – 2.5 tonnes/ha). Mean water temperature was 14.7ºC. Formalin concentration was measured at 4, 8, 12 and 20 h post-treatment. In trial 2, formalin (30 – 60% formaldehyde; Orica Australia Pty Ltd) was added to 3, 0.1-ha earthen ponds containing 400 fish (range mean weights, 720 – 1,100 g; estimated biomasses, 2.9 – 4.4 tonnes/ha) and to 3, 0.1-ha ponds without fish. Mean water temperature was 24.1ºC. In trial 3, formalin (30 – 60% formaldehyde; Orica Australia Pty Ltd) was added to 3, 0.1-ha earthen ponds containing 400 fish (793 – 1,211 g; range biomasses 3.2 – 4.8 tonnes/ha) and in 3, 0.1-ha ponds without fish. Mean water temperature was 14.6ºC. In trials 2 and 3, formalin concentration was determined 15 min after application, then 24 and 48 h post-treatment.

Copper – trials 1 and 2

In trial 1, copper sulfate was added to achieve a copper concentration of 0.2 mg/L in 3, 0.1-ha earthen ponds containing 400 fish (range mean weights, 698 – 801 g; estimated biomasses, 2.8 – 3.2 tonnes) and in 3, 0.1-ha ponds without fish. Mean temperature was 20.9ºC (19.5º – 22.4ºC). In trial 2, copper sulfate was added to achieve a copper concentration of 0.2 mg/L in 3, 0.1-ha earthen ponds containing 400 fish (range mean weights, 774 – 876 g; estimated biomasses, 3.1 – 3.5 tonnes/ha) and to 3, 0.1-ha ponds without fish. Mean water temperature was 14.8ºC (13.3º – 16.6ºC). In each trial, copper concentration was determined 15 min after application, then 24 and 48 h post-treatment.

6.4.3.6. Validation of treatment regimes

Outbreaks of ichthyophthiriosis on silver perch in 2, 0.1 ha earthen ponds were treated with formalin or copper. Concentrations of formalin and copper were monitored daily and returned to the nominal concentration by the addition of the appropriate amount of formalin or copper sulfate. Both ponds were aerated continuously during treatment, which were continued until the outbreaks were controlled. Three fish were sampled from each pond every 3 – 6 days, and the total number of trophonts and theronts on gill and skin tissue was determined.

Trial 1

The mean weight and biomass of silver perch in pond #1 were 487.3 g and 645.8 kg (6.5 tonnes/ha). Pond #1 was initially treated with 30 mg/L formalin.

Trial 2

The mean weight and biomass in pond #2 were 406.6 g and 906.3 kg (9.1 tonnes/ha). The pond was initially treated with 0.2 mg/L copper. Alkalinity in the pond was maintained between 90 and 105 mg/L using agricultural limestone ($CaCO_3$).

6.4.3.7. Statistical analysis

One-way analysis of variance was used to determine the effects of copper concentration on survival in Experiment 1, and to compare the concentrations of formalin and copper in ponds with and without fish at 24 h and 48 ha post-treatment. Two-way analysis of variance was used to determine the effects of formalin and copper concentrations in Experiment 2. Percentage data were arc-sin transformed before analysis.

6.4.4. Results

6.4.4.1. Efficacy of formalin and copper

Water quality variables in both experiments (Table 6.4.1) were within acceptable levels for silver perch (Rowland 1995b).

Experiment 1

Copper concentration had a significant effect ($P<0.01$) on survival of silver perch fingerlings (Fig. 6.4.1). All fish treated with 1.0 or 0.5 mg/L copper died within 5 and 15 days respectively, and fish in the infested control were dead after 12 days (Fig. 6.4.2). On day 15, there were no *I. multifiliis* on gill or skin tissue of fish treated with 0.5 mg/L copper. Survival at 0.25 mg/L copper on day 15 was 82.5% (Fig. 6.4.1) and there were no theronts or trophonts on gill and skin tissue of surviving fingerlings by day 14. There were no mortalities in the un-infested control. These data indicate that concentrations of 0.5 and 1.0 mg/L are toxic to silver perch. Signs of copper toxicity were a spiralling swimming action, loss of equilibrium, lethargy, listlessness, dark colouration and gasping.

Experiment 2

The concentration of formalin or copper had a significant effect ($P<0.01$) on survival of silver perch fingerlings. All fish in the control and 10 mg/L formalin treatments were dead by day 17 (Fig. 6.4.3). Fish treated with 0.2 or 0.1 mg/L copper were free of theronts and trophonts on days 14 and 17 respectively, as were those treated with 30 mg/L formalin on day 17 (Fig. 6.4.4). Survival at these concentrations was 100%. Survival rates at 0.05 mg/L copper and 20 mg/L

formalin were 100%, but all fingerlings in these treatments remained infested with *I. multifiliis* theronts and trophonts on day 17 (Fig. 6.4.4).

6.4.4.2. Depletion of formalin and copper

Formalin

In trial 1, a concentration of 30 mg/L formalin declined to a mean of 18.9 mg/L by 20 h post-treatment (Fig. 6.4.5). In trials 2 and 3, there were no significant differences between the concentrations after 24 h, but after 48 h concentrations in ponds containing fish were significantly (P<0.01) lower than in ponds with no fish (Fig. 6.4.6). There were unexpectedly large variations between the nominal (30 mg/L) and achieved concentrations after the application of formalin; in trial 2, formalin concentrations were 38 mg/L in both treatments and in trial 3, despite the addition of similar amounts of formalin as in trial 2, concentrations were only 25 mg/L (Fig. 6.4.5). This variation may have been due to different concentrations of formaldehyde in the two separate batches of formalin used. In trials 2 and 3, concentrations of formalin were below 20 mg/L within 48 h in ponds containing fish (Fig. 6.4.6).

Copper

Copper declined rapidly in both trials. Concentrations were significantly lower (P<0.01) in the ponds containing fish at both 24 and 48 h post-treatment (Fig. 6.4.7). After 24 h, mean copper concentrations in ponds with fish were only 0.06 and 0.03 mg/L. There were no variations in the nominal (0.2 mg/L) and achieved concentrations, except in the no-fish treatment in trial 1 where the mean concentration was 0.23 mg/L. The pattern of copper depletion was similar at 14.8ºC and 20.9ºC (Fig. 6.4.7).

6.4.4.3. Validation of treatment regimes in ponds

When diagnosed, the level of infestation was far higher in pond #1 (>1000 parasites on 3 fish) than in pond #2 (40 parasites on 3 fish) (Fig. 6.4.8).

Trial 1

Formalin had controlled the outbreak in pond #1 by day 22 at water temperatures of 19.2° – 23.9°C, although the level of infestation was low by day 15 (Table 6.4.2; Fig. 6.4.8a). Survival was 55.7%, and the cost of treatment was $AUD486.00/ha/day.

Trial 2

Copper had controlled the outbreak in pond #2 by day 21 at 13.7° – 17.0°C and the level of infestation was low by day 16 (Table 6.4.2; Fig. 6.4.8b). Survival was 93.5%, and the cost of treatment was $AUD68.34/ha/day (Table 6.4.2).

6.4.5. Discussion

Both formalin and copper controlled ichthyophthiriosis in silver perch, but success was dependent on the maintenance of efficacious concentrations of each chemical in both aquaria and ponds. A concentration of 30 mg/L formalin prevented re-infestation of silver perch by theronts, but fish treated with 20 mg/L remained infested with both theronts and trophonts, and those treated with 10 mg/L died as a result of the infestation. Concentrations of formalin in ponds declined to below 20 mg/L within 48 h (Figs. 6.4.5 and 6.4.6). Temperature is known to influence the depletion rate of formalin (Helms 1967; Pedersen and Pedersen 2006), and the significantly faster depletion in ponds containing silver perch compared to ponds without fish (Fig. 6.4.6) suggests that fish biomass and

organic matter may also play a role, possibly through bacterial mediation (Masters 2004). The application of formalin daily or at least on alternate days is necessary to achieve efficacious levels in ponds. Other studies have also reported success with daily applications of formalin, and ineffectiveness of concentrations below 25 mg/L and other treatment regimes. A single application or an application every other day of 25 mg/L formalin did not control ichthyophthiriosis in channel catfish (*Ictalurus punctatus*), whereas daily application of this dosage was effective (Leteux and Meyer 1972; Tieman and Goodwin 2001). A treatment of 25 mg/L formalin for 4 h, 4 d/week did not control ichthyophthiriosis in channel catfish in raceways (Bodensteiner et al. 2000). The relatively rapid depletion of formalin to non-efficacious levels partly explains the difficulties in treating ichthyophthiriosis in ponds, particularly at low water temperatures when the life cycle of *I. multifiliis* may take two or more weeks. Periods of sub-lethal concentrations of formalin (e.g., when treatments are 3 or more days apart) would provide opportunities for re-infestation by theronts and continuation of the parasite's life cycle.

A treatment regime involving the application of 30 mg/L initially, followed by 25 – 30 mg/L daily for 15 days controlled ichthyophthiriosis in silver perch in an earthen pond (9.1 tonnes/ha) at a cost $AUD486.00/ha/day (Table 6.4.2). Besides the high cost, there are several other disadvantages of using formalin in earthen ponds. Formaldehyde is a strong reducing agent, combining with oxygen to yield formic acid, which in turns combines with oxygen to yield carbon dioxide and water; the process may be mediated by bacterial metabolism (Helms 1967; Noga 2000; Masters 2004). Rowland et al. (2006) reported deterioration in water quality, including a decline in dissolved oxygen and pH at temperatures around 24°C following the application of 30 mg/L formalin to ponds stocked with silver perch. In addition, toxic effects of formalin adversely affect aquatic organisms including algae, insects and other invertebrates (Bills et al. 1977; Boyd 1990), and formalin can cause damage to gills, eyes and liver, as well as anaemia and hypoglycaemia in some fishes (Wedemeyer 1971; Cruz and Pitogo 1989; Omoregie et al. 1994). Despite these disadvantages, formalin is recommended for the control of ichthyophthiriosis in silver perch at water temperatures up to 25°C because it can be legally used, it is an effective therapeutant, and continuous aeration maintains adequate dissolved oxygen concentrations in ponds (Rowland et al. 2006).

Copper has a relatively narrow therapeutic range of 0.1 – 0.2 mg/L for the control of ichthyophthiriosis in silver perch. Fish treated with a lower concentration of 0.05 mg/L remained infested, while there was 15% mortality of fish treated with 0.25 mg/L and total mortality of fish treated with 0.5 and 1.0 mg/L, presumably due to toxic effects of copper. Our results are similar to findings with channel catfish where Straus (1993) reported that a concentration of 0.15 mg/L prevented infestation by theronts, and Schlenk et al. (1998) found that all theronts were killed at copper concentrations greater than 0.05 mg/L, and that 0.4 mg/L and higher concentrations of copper sulfate controlled ichthyophthiriosis. Noga (2000) recommended concentrations of copper be maintained between 0.15 and 0.2 mg/L. Toxic levels of copper vary between freshwater species (Moore 2005; Straus 2006) and Moore (2005) reported 96-hour LC_{50} values for copper toxicity of 0.71 mg/L for channel catfish and 0.69 mg/L for blue catfish (*Ictalurus furcatus*). The toxic levels in our study were lower than those reported for silver perch cultured in Taiwan where there was 10% – 95% mortality at copper sulfate concentrations ranging from 1.35 – 2.97 mg/L [~ 0.34 – 0.74 mg/L copper] at a temperature of 20°C (Lin et al. 2003).

Although there are many factors including hardness, pH, temperature, form of copper and organic content of ponds which influence the toxicity of copper to fish, it is generally considered that the most important is total alkalinity which inversely affects copper toxicity through its influence on the proportion of free copper available (Boyd 1979, 1990; Straus and Tucker 1993; Wurts and Perschbacher 1994; Darwish et al. 2005; Perschbacher 2005). Most fish culture and disease texts recommend treatment with copper should only proceed if total alkalinity is above 20 mg/L and preferably above 50 mg/L (Noga 2000). We maintained alkalinity levels of 70 – 110 mg/L in

experiments in aquaria and ponds. Our data support Straus and Tucker (1993) and Schlenk et al. (1998) who suggested that the effective dose of copper sulfate to control ichthyophthiriosis is lower than the 1.0 mg/L per 100 mg/L total alkalinity that is recommended in some texts (e.g., Brown and Gratzek 1980; MacMillan 1985; Tucker and Robinson 1990).

Copper is a difficult chemical to use for the control of fish diseases because of its low therapeutic index and rapid depletion in pond water. Copper sulfate quickly dissolves to yield cupric ions (Cu^{2+}) and sulfate, and it is the cupric ions which are toxic to fish, algae, protozoans and invertebrates (Boyd 1990). Copper is absorbed by plants, and the cupric ions precipitate as copper oxide and are then absorbed on colloidal clay and organic matter in the ponds (Boyd 1990; Darwish et al. 2005; Silapajarn and Boyd 2006). The toxicity of copper, as well as its effectiveness as a parasiticide decrease for a given dose as the total alkalinity and pH of waters increases (Boyd 1990; Straus and Tucker 1993). Previous studies have reported a rapid decline of copper in ponds, including to background levels within 48 h (McNevin and Boyd 2004; Darwish et al. 2005). In our study, copper declined from 0.2 mg/L to around 0.05 mg/L within 24 h in ponds containing fish (Fig. 6.4.7). Silver perch remain infested at a concentration of 0.05 g/L, and so unless the level is increased daily, sub-lethal concentrations of copper would enable re-infestation by *I. multifiliis* theronts. A treatment regime of 0.2 mg/L followed by 0.15 mg/L for 5 days and then 0.1 mg/L for the next 16 days controlled ichthyophthiriosis in a pond with alkalinities of 90 – 105 mg/L at a cost of #AUD68.34/ha/day (Table 6.4.2). While this treatment regime was successful in our study, care must be taken because of the many variables that influence copper toxicity and the large variation between ponds even on the same farm, particularly in water quality, fish biomass and organic matter. The use of a spectrophotometer is recommended for silver perch farmers to enable copper concentrations to be accurately monitored and maintained between 0.1 and 0.2 mg/L.

Some previous studies have suggested that the long-term use (up to 3 years) of therapeutic levels of copper sulfate does not have detrimental effects on fish or ponds. Weekly application of copper sulfate (0.12 mg/L copper) reduced the incidence of off-flavours in channel catfish under both experimental and commercial conditions, and increased stability of production and economic returns compared to un-treated ponds (Tucker et al. 2001; Schrader et al. 2005). However, Rábago-Castro et al. (2006) reported that the prophylactic use of copper sulfate suppressed growth of channel catfish, and damage to gills and internal organs can occur when fish are exposed to low concentrations of copper for extended periods of time (Cardeilhac et al. 1979; Moore 2005). Caution needs to be taken when using copper sulfate because of these potential adverse affects, its low therapeutic index and algacidal properties. Low dissolved oxygen and even oxygen depletion can occur after application, and massive, copper-related fish kills in catfish ponds have resulted from mistakes in dose calculation (Boyd 1990, 2005). Accurate estimates of pond volumes and calculation of copper doses are essential to avoid such problems.

Carbonell and Tarazona (1992) found no accumulation of copper in rainbow trout and concluded there is no hazard to consumers from the use of copper sulfate as a fisheries therapeutant. The rapid decline of copper in the aquatic environment suggests that the use of copper sulfate to control ichthyophthiriosis and other diseases in silver perch will not result in significant environmental problems on farms, at least in the short-term.

Copper sulfate is currently not registered for use on animals in Australia, and so cannot be prescribed and used legally at this time. Its legitimate use will require the issue of a minor use permit by the Australian Pesticides & Veterinary Medicines Authority.

6.4.6. Acknowledgements

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Table 6.4.1. Water quality in 55 L aquaria stocked with silver perch fingerlings infested with *I. multifiliis* and treated with different concentrations of formalin and copper in Experiments 1 and 2. Data are ranges.

Variable	Experiment 1	Experiment 2
Temperature (°C)	14.8 – 16.8	16.4 – 17.6
Dissolved oxygen (mg/L)	7.2 – 10.1	3.0 – 9.6
pH	7.8 – 8.0	6.7 – 7.9
Total alkalinity (mg/L)	80 – 110	70 – 90
Total hardness (mg/L)	120 – 160	120 – 160

Table 6.4.2. Control of ichthyophthiriosis in two 0.1-ha earthen ponds at GAC using formalin or copper. Water quality data are means with ranges in parentheses.

Parameter	Formalin (pond #1)	Copper (pond #2)
Treatment regime		
Initial	30 mg/L	0.2 mg/L
daily	25 – 30 mg/L	0.15 mg/L for 5 d, 0.10 mg/L for 16 d
Days to eradication		
theronts	5	15
trophonts	22	21
Cost (AUD$/ha/day)	486.00	68.34*
Water quality		
temperature (ºC)	21.3 (19.2 – 23.9)	15.3 (13.7 – 17.0)
dissolved oxygen (mg/L)	5.7 (4.6 – 8.3)	10.0 (9.3 – 10.7)
pH	6.8 (6,5 – 7.3)	8.0 (7.4 – 8.3)
alkalinity (mg/L)	–	97.3 (90 – 105)

* includes cost of copper sulfate and lime

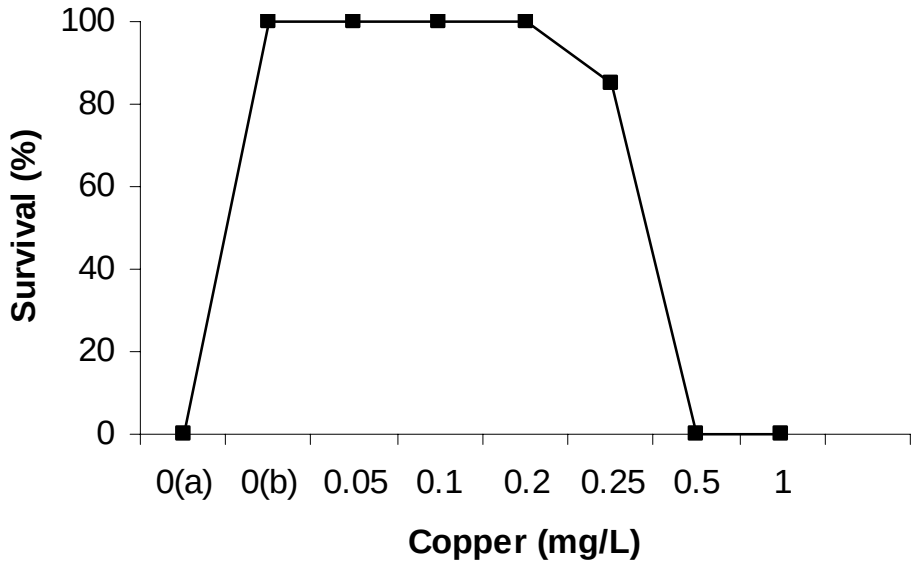


Figure 6.4.1. Survival of silver perch fingerlings (mean weights, 10.8 and 7.9 g) infested with *Ichthyophthirius multifiliis* and treated with different concentrations of copper in Experiments 1 and 2. In the control treatments, fingerlings in 0(a) were infested, and fingerlings in 0(b) were un-infested.

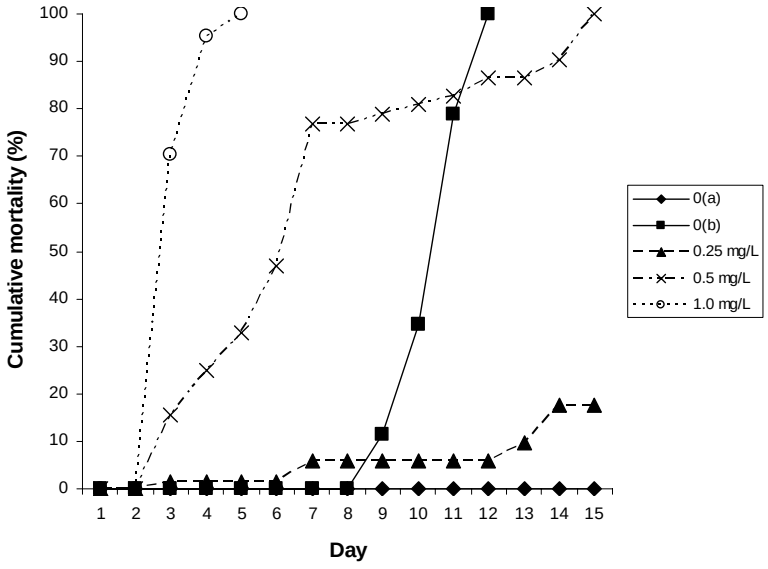


Figure 6.4.2. Cumulative mortality of silver perch fingerlings infested with *Ichthyophthirius multifiliis* and treated with different concentrations of copper in Experiment 1. 0(a) – un-infested fish, 0(b) – infested fish.

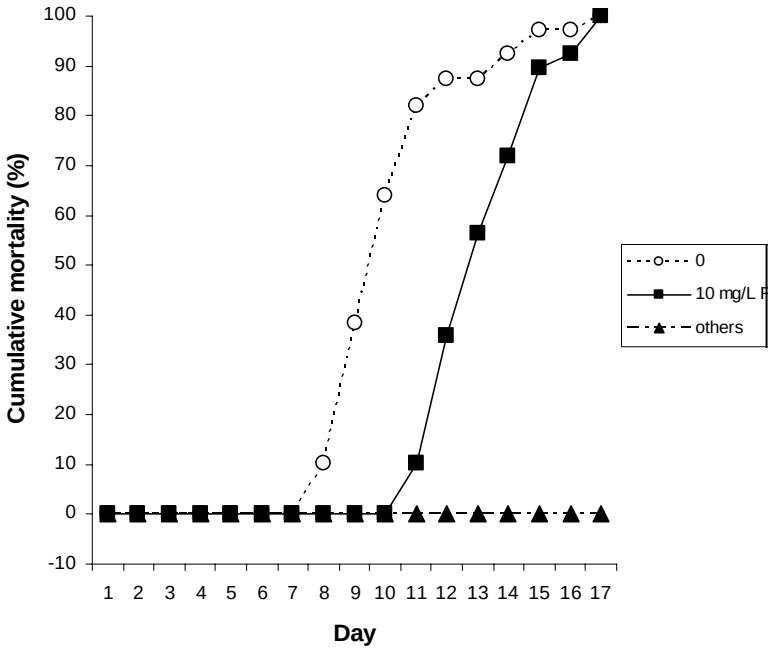


Figure 6.4.3. Cumulative mortality of silver perch fingerlings infested with *Ichthyophthirius multifiliis* and treated with 0 or 10 mg/L formalin and other concentrations of formalin (20, 30 mg/L) or copper (0.05 or 1.0 mg/L) in Experiment 2.

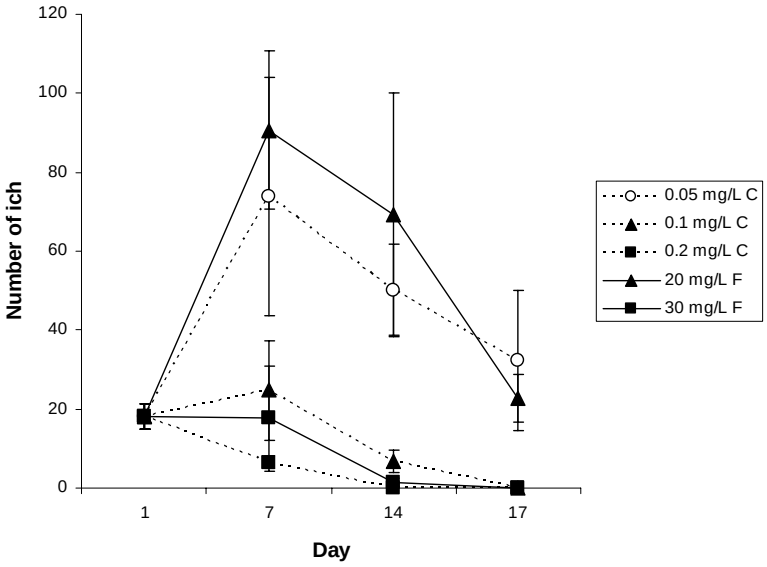


Figure 6.4.4. Number of *Ichthyophthirius multifiliis* (ich; theronts and trophonts) on silver perch fingerlings treated with 0.05, 0.1 or 0.2 mg/L copper or 20 or 30 mg/L formalin in Experiment 2. Data are means (±SE) of 3 replicate aquaria.

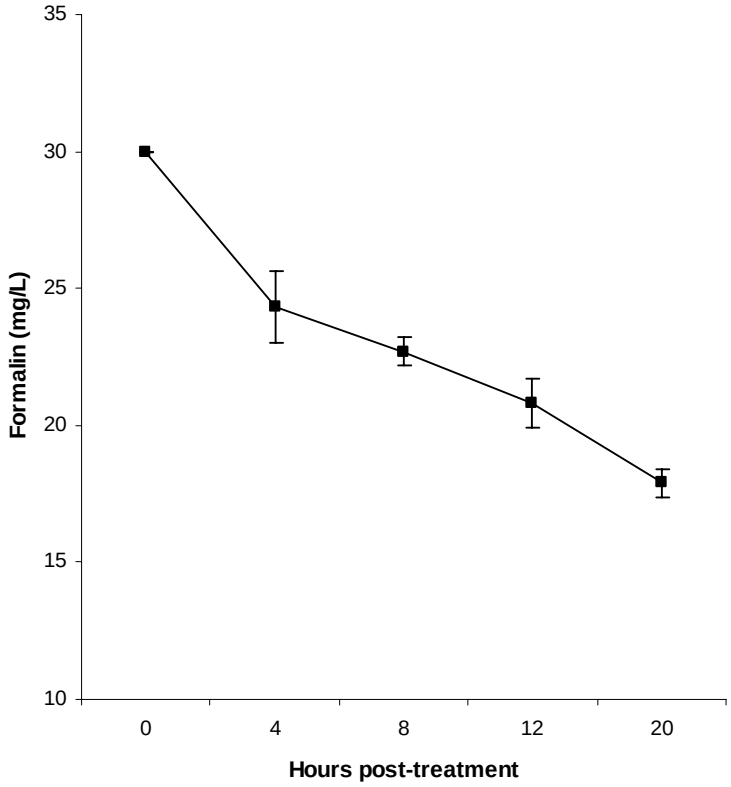


Figure 6.4.5. Depletion of formalin in aerated, 0.1-ha earthen ponds containing silver perch (2.0 – 2.5 tonnes/ha) at a temperature of 14.7ºC. Data are means±SD of four replicate ponds.

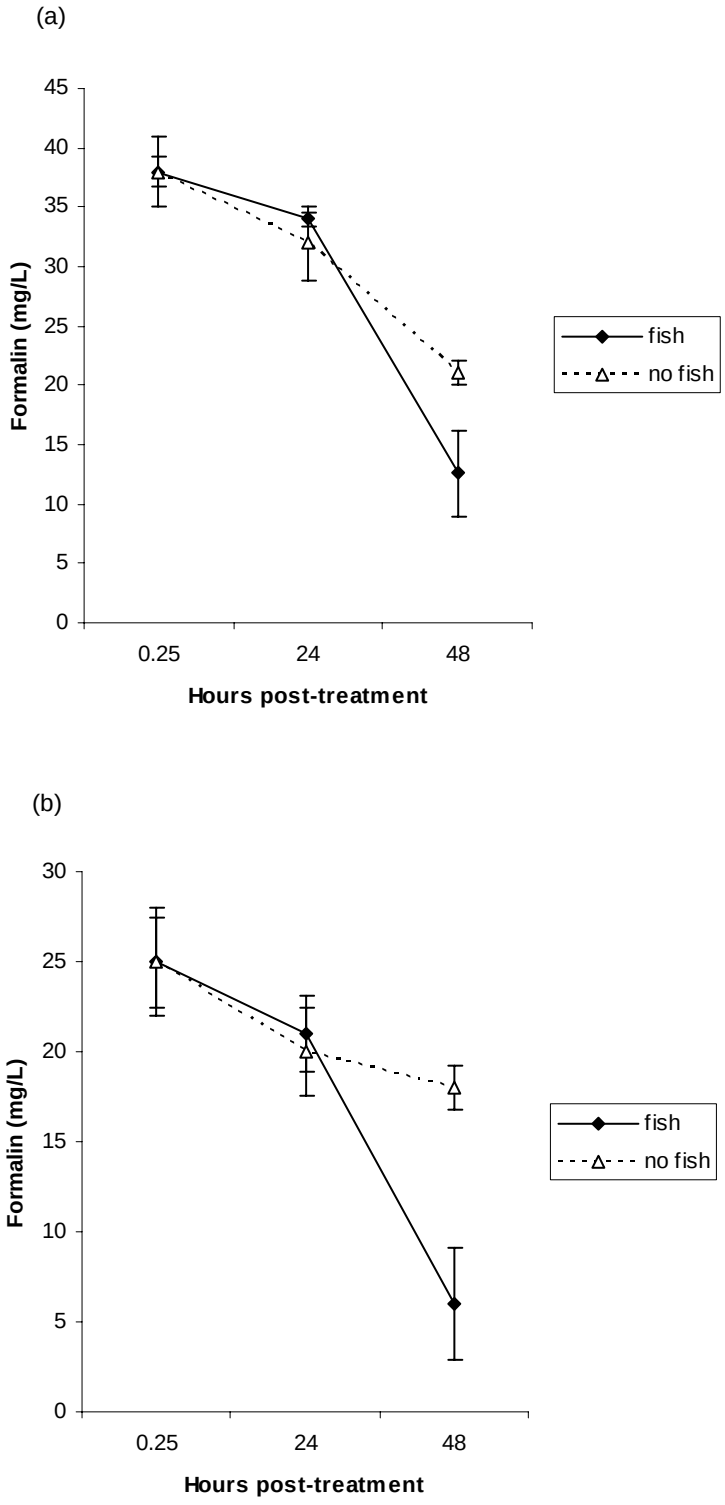


Figure 6.4.6. Depletion of formalin in aerated, 0.1-ha earthen ponds with fish and without fish at (a) trial 2 – mean water temperature 24.1º (23.3º – 24.8ºC) and (b) trial 3 – 14.6ºC (13.4º – 16.2ºC). Data are means±SD of three replicate ponds.

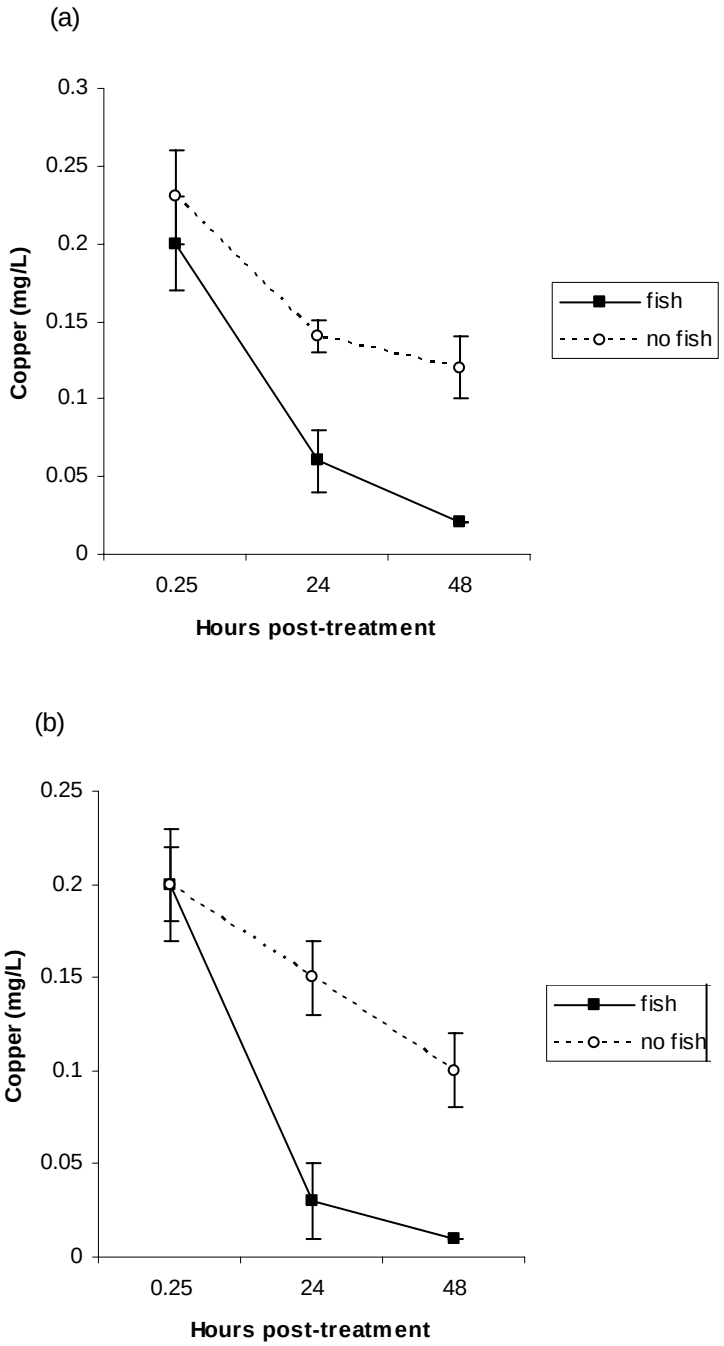
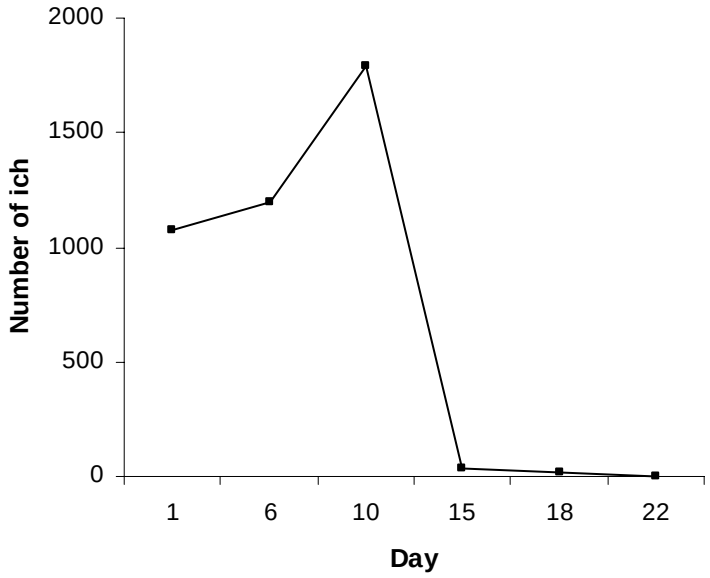


Figure 6.4.7. Depletion of copper in aerated, 0.1-ha earthen ponds with and without fish at (a) trial 1 – mean water temperature 14.8ºC (13.3º – 16.6ºC) and (b) trial 2 – 20.9ºC (19.5º – 22.4ºC). Data are means±SD of three replicate ponds.

(a)



(b)

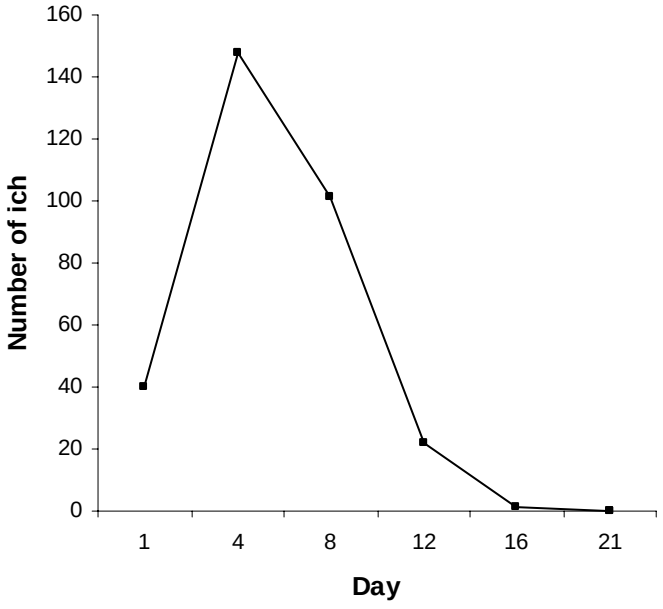


Figure 6.4.8. Number of *Ichthyophthirius multifiliis* (ich) on silver perch in ponds treated daily with (a) formalin – pond #1 and (b) copper – pond #2. Data are total numbers of theronts and trophonts on gill and skin tissue of three fish from each pond.

6.5. Effects of formalin on water quality and parasitic monogenean gill flukes on silver perch (*Bidyanus bidyanus*) in earthen ponds

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6.5.1. Abstract

Infestations of parasitic monogenean trematodes or gill flukes (*Lepidotrema bidyana* and *Gyrodactylus* sp.) on freshwater silver perch (*Bidyanus bidyanus*) in earthen ponds were treated with formalin (37% formaldehyde). Concentrations of 30 and 40 mg/L formalin were effective, but fish in ponds treated with 20 or 25 mg/L remained infested. At temperatures of 24.1° – 26.9°C, concentrations of 30 or 40 mg/L formalin caused dissolved oxygen (DO) to decline from 10.1 –11.9 mg/L to 3.0 – 3.3 mg/L and 1.2 – 1.7 mg/L respectively, within 36 – 42 h of treatment. In addition, pH declined from 7.2 – 8.4 to 6.3 – 6.7, within 36 h and turbidity decreased over 48 h. In the ponds where DO was 1.2 – 1.7 mg/L, silver perch showed signs of severe stress, but continuous aeration (10 hp/ha) for three days and inflow of well-oxygenated water for 6 – 8 h prevented mortalities. At temperatures of 13.2° – 15.7°C, concentrations of 30 or 40 mg/L formalin caused DO to decline from 9.0 – 10.0 mg/L to 6.0 – 8.1 mg/L and pH from 7.0 – 7.3 to 5.9 – 6.6 within 72 h. Total ammonia-nitrogen increased over 72 h in ponds treated with 30 or 40 mg/L formalin. Fish became re-infested with *L. bidyana* in all ponds within 30 days of treatment. A concentration of 30 mg/L formalin is recommended as a treatment for monogeneans on silver perch in ponds, but aeration is necessary to maintain adequate water quality at higher temperatures.

6.5.2. Introduction

The freshwater fish silver perch (*Bidyanus bidyanus*) is an excellent species for intensive culture in earthen ponds and cages (Rowland 1995a; Rowland et al. 1995; Rowland et al. 2004, 2006) and an industry based on pond production is developing in Australia (Rowland 1998). There is also interest in silver perch in other countries including the People's Republic of China, Taiwan, Israel, the Philippines and the USA.

Infestations of monogenean trematodes have been reported in some freshwater fishes in Australia (Rowland and Ingram 1991), and Murray (1931) named the trematode *Lepidotrema bidyana* from the gills of silver perch. Monogenean trematodes are commonly called gill flukes. Callinan and Rowland (1995) did not report gill flukes in silver perch, and previous studies at the Grafton Aquaculture Centre (GAC) failed to find these parasites on fingerling or larger fish despite routine disease monitoring (Rowland et al. 1994, 1995; Rowland 1995c). However, since the mid 1990's, there has been an increase in the incidence of gill flukes at GAC and on commercial silver perch farms in Australia (Rowland et al. 2007). Monogeneans are known from many freshwater and marine fishes through-out the world, and can cause serious disease problems in aquaculture (Paperna 1963; Sarig 1971; Buchmann et al. 1993).

Trichlorfon [dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate] and its active ingredient dichlorvos have been recommended and widely used for the treatment of monogenean trematodes

(Sarig et al. 1965; Sarig 1971; Imanda and Muroga 1979; Schlotfeldt et al. 1995), but there are reports of ineffectiveness, resistance and toxicity (Goven et al. 1980; Obiekezie and Taege 1991; Janse and Borgsteade 2003), and there are concerns of possible adverse ecological effects (Costello 1993; Schlotfeldt et al. 1995; Noga 2000). Other chemicals have been evaluated as treatments for monogeneans, and while some such as mebendazole and niridazole are reported to be successful and non-toxic (Mellergaard 1990; Tojo et al. 1993a) others such as toltrazuril have been successful in some trials (Schmahl et al. 1988) but not in others (Buchmann et al. 1990; Tojo et al. 1993b).

Formalin (37% formaldehyde) is an effective therapeutant for some fish diseases, and it is recommended for the treatment of protozoan and fungal diseases in silver perch (Callinan and Rowland 1995; Rowland et al. 2007). Noga (2000) recommended formalin, along with other chemicals for infestations of monogeneans, but Schlotfeldt et al. (1995) stated that formaldehyde has only limited effect on *Dactylogyrus*. Few chemicals are registered for use with food fishes in Australia. Formalin can be used by members of the National Aquaculture Council, including members of the NSW Silver Perch Growers Association under an Australian Pesticides and Veterinary Medicines Authority permit – PER8853. Formalin is also approved for use as an aquatic chemo-therapeutant in both the US and Canada (Schnick et al. 1997).

Formalin is reported to adversely affect water quality and some aquatic organisms, and is usually not recommended for use in large ponds because of these factors and its high cost (Meyer and Schnick 1989; Boyd 1990; Noga 2000). However, few studies have reported the effects of different concentrations on water quality in freshwater ponds, and the effectiveness of formalin to treat infestations of monogenean gill flukes in silver perch is unknown. The aims of our study were to determine: (i) the effects of formalin on water quality in earthen ponds; and (ii) effects of formalin on infestations of monogenean gill flukes on silver perch.

6.5.3. Material and methods

6.5.3.1. General

This study was done at the NSW Department of Primary Industries' Grafton Aquaculture Centre (GAC). Silver perch fingerlings (mean weight, 58.5g) were stocked into 9, 0.1-ha earthen ponds at a density of 15,000 fish/ha and cultured for 12 months. Fish (150 g) were also stocked into a 0.32-ha pond at a density of 27,000 fish/ha. Trials 1 and 2 were done in the 0.1-ha ponds and Trial 3 in the 0.32-ha pond. Fish husbandry and pond management procedures followed Rowland (1995a). The ponds were aerated with 1 hp paddlewheel aerators at rates of up to 10 hp/ha for at least 8 h daily between 00.00 and 08.00 h. Fish were fed a diet formulated for silver perch (34% digestible protein and 14 MJ/kg energy). Each pond was surveyed when empty two months prior to the study to provide accurate estimates of pond volumes and formalin concentrations at different water levels.

6.5.3.2. Water quality and disease

Routine monitoring of water quality followed Rowland (1995b). An Horiba U-10 meter was used to monitor temperature, dissolved oxygen (DO), pH and turbidity at a depth of 1 m from the walkway of each pond. Total ammonia-nitrogen (TAN) was determined using Nessler Reagent. Fish health was monitored by examining gill and skin tissue from four or five fish from each pond at various intervals (see Table 6.5.1). The left, anterior gill arch was removed from each fish and all parasites in 5 fields of view at 100X magnification were counted; the prevalence of monogeneans (% of fish with parasites) and the mean number of parasites per field of view were determined.

6.5.3.3. Formalin treatments

Infestations of gill flukes were treated by applying formalin directly to the water flow in front of an aerator in each pond. Concentrations of formalin were based on estimates of pond volume. Aerators were then run for 24 h/d for three days. The effects of formalin concentration on DO, pH, turbidity and TAN at each monitoring period in Trials 1 and 2 were determined using a one-way analysis of variance.

Trial 1

Water temperatures of 24.1° – 26.9°C. Ponds were treated with 30 or 40 mg/L formalin and there were four replicate ponds for each concentration. DO and pH were monitored immediately prior to treatment (0 h) then 12, 24, 30, 36, 42 and 48 h post-treatment. Turbidity was monitored prior to treatment and then at 24, 48 and 72 h. TAN was monitored prior to the application of formalin and then 72 h post-treatment. In ponds where DO was < 2 mg/L, well-oxygenated water from an aerated, earthen reservoir was added for periods of 6 – 8 h at a rate of about 5 L/s.

Trial 2

Water temperatures of 13.2° – 15.7°C. Ponds were treated with 20, 30 or 40 mg/L formalin and there were three replicate ponds for each concentration. DO and pH were monitored each 24 h for 5 and 6 days respectively, and turbidity was monitored for 4 days.

Trial 3

Water temperatures of 20° – 23°C. The pond was treated with 25 mg/L formalin.

6.5.4. Results

6.5.4.1. Fish

The mean weights and estimated production rates of silver perch in the 0.1-ha ponds during the treatments were 215.1 – 247.6 g and 2.8 – 3.4 tonnes/ha in March (Trial 1) and 416.1 – 437.0 g and 5.9 – 6.6 tonnes/ha in July (Trial 2). Survival rates in these ponds at the completion of the culture period ranged from 91.3% to 94.7%. The mean weight and estimated production rate in the 0.32-ha pond during Trial 3 in October were 179.0 g and 1.6 tonnes/ha.

6.5.4.2. Monogenean gill flukes

The monogenean gill flukes, *L. bidyana* (Diplectanidae) and *Gyrodactylus* sp. (Gyrodactylidae) were found on silver perch. Most parasites were located on gill tissue, and infestations consisted predominately of *L. bidyana*. Fish stocked in December were free of parasites, but within 8 weeks all fish sampled had monogeneans on gill tissue (Fig. 6.5.1). In Trials 1 and 2, concentrations of 30 or 40 mg/L formalin eliminated parasites from all fish within 1 day (Table 6.5.1). In Trial 1, some fish were re-infested in April, 30 days after treatment and by June the prevalence of parasites had returned to 100% (Fig. 6.5.1). In Trial 2, fish treated with 20 mg/L formalin remained infested, and although the mean number of monogeneans was lower after 1 day, it had returned to pre-treatment levels by day 7 (Table 6.5.1). Prevalence of monogeneans in July was 33% (Fig. 6.5.1) because the fish treated with 20 mg/L formalin remained infested. Prevalence across all ponds was 41% – 78% from August to December (Fig. 6.5.1), but during this period the mean number of parasites on fish were generally lower than in June (Table 6.5.1). In Trial 3, a concentration of 25 mg/L formalin had not eliminated all parasites after 1 day (Table 6.5.1).

6.5.4.3. Water quality

At temperatures of 24.1° – 26.9°C, both formalin concentration and the length of the post-treatment period affected DO. In ponds treated with 30 mg/L, DO declined by 7.8 – 8.9 mg/L to minima of 3.0 – 3.3 mg/L and in ponds treated with 40 mg/L DO declined by 8.5 – 12.0 mg/L to minima of 1.2 – 1.7 mg/L within 42 h of treatment (Fig. 6.5.2). DO was significantly lower ($P<0.01$) after 36 and 42 h in ponds treated with 40 mg/L compared to ponds treated with 30 mg/L (Fig. 6.5.2). Values of pH declined to 6.3 – 6.7 within 36 h (Fig. 6.5.2) but were not affected ($P>0.05$) by the concentration of formalin. Turbidity decreased over 48 h, before increasing at 72 h, and was not affected ($P>0.05$) by formalin concentration (Fig. 6.5.2). TAN increased significantly ($P<0.05$) from means of 0.4 and 0.6 mg/L in ponds treated with 30 and 40 mg/L respectively, to 1.4 mg/L under both treatments.

At temperatures of 13.2° – 15.7°C, DO in ponds treated with 30 and 40 mg/L formalin declined reaching minima of 7.2 and 6.7 mg/L respectively, 72 h after treatment (Fig. 6.5.3). DO was significantly lower ($P<0.05$) in ponds treated with 40 mg/L compared to 20 mg/L. Values of pH declined to 6.4 – 6.6 after 72 h, and were not affected ($P>0.05$) by formalin concentration. Turbidity increased after treatment (Fig. 6.5.3); there were large variations between ponds, but no significant effect ($P>0.05$) of formalin concentration at each monitoring period. Mean values of TAN rose from 1.2, 0.7 and 1.0 mg/L to 1.6, 2.4 and 2.9 mg/L in ponds treated with 20, 30 or 40 mg/L formalin respectively, with significant differences ($P<0.01$) at both 30 and 40 mg/L.

6.5.4.4. Silver perch and low dissolved oxygen

In ponds where DO was 1.2 – 1.7 mg/L, silver perch showed signs of severe stress by piping at the surface and swimming listlessly near the edges and in the flow created by the aerators. In an effort to save the fish, well-oxygenated water was added to these ponds at a rate of approximately 5 L/s for a period of 6 – 8 h commencing when the signs of stress were first seen. There were no mortalities.

6.5.5. Discussion

Relatively few *L. bidyana* were found on the skin of silver perch and so it appears to be primarily a gill parasite as reported for dactylogyrids in other species of freshwater fish (Yamaguki 1968; Sarig 1971; Shaharom-Harrison 1986; Hoffman 1999). Despite the prevalence of gill flukes, survival rates of silver perch were high and there were no mortalities associated with the infestations in our study. Microscopic examination of gill tissue indicated that the parasites cause epithelial hyperplasia with the formation of white out-growths on the distal half of gill filaments similar to those described by Putz and Hoffman (1964) in fallfish (*Semotilus corporalis*) infested with *Dactylogyrus corporalis*. Physical damage caused by monogenean parasites has been linked to infection by bacteria and fungi, and infestations of protozoans (Davis 1961; Noble et al. 1963; Pyecroft 1994; Lopez et al. 2002) and so further research is needed to determine the role that *L. bidyana* and other monogeneans play in silver perch diseases, particularly the problematic mycotic diseases winter saprolegniosis and epizootic ulcerative syndrome (Callinan and Rowland 1995; Landos et al. 2003; Landos et al. 2007a).

A concentration of 30 mg/L formalin is an effective treatment for monogeneans on silver perch, but concentrations of 25 and 20 mg/L are not completely effective. Brown and Gratzek (1980) also suggested that 25 mg/L formalin is not effective in removing monogenetic trematodes. Although effective, 40 mg/L formalin caused a significant deterioration of water quality at temperatures over 24°C with DO declining by 8.5 – 10.7 mg/L to stressful levels of 1.2 – 1.7 mg/L within 36 h of treatment. However, following treatment with 30 mg/L formalin at these high temperatures, adequate DO (> 3.0 mg/L) was maintained by continuous aeration for three days in ponds containing up to 6.6 tonnes/ha of fish. Helms (1967) using data from aquaria, also found that

oxygen demand from formalin begins to be exerted about 24 h after application, and that concentrations greater than 30 – 40 mg/L for 2 days at 21°C will result in oxygen concentrations sufficient to kill fish and other aquatic life. Formaldehyde is a strong reducing agent, combining with oxygen to yield formic acid, which in turn combines with oxygen to yield carbon dioxide and water; the process may be mediated by bacterial metabolism (Helms 1967; Noga 2000; Masters 2004).

Formalin is toxic to phytoplankton. Chiayvareesajja and Boyd (1993) found decreased chlorophyll *a* after the application of formalin to freshwater ponds, and severe depletion of oxygen followed formalin treatment of ponds containing heavy phytoplankton blooms and fish being fed artificial feed (Boyd 1990). The ponds in our study contained relatively large blooms of phytoplankton and high turbidity prior to Trial 1, and the decrease in turbidity and decline in pH (Fig. 6.5.2) were probably due to a rapid loss of phytoplankton following the application of formalin and the subsequent reduction of photosynthetic activity. Formalin also kills zooplankton and benthic organisms (Birdsong and Avault 1971) and so the increasing TAN after both treatments was probably caused by the decay of phytoplankton and other aquatic organisms. The low pH values ensured concentrations of un-ionised ammonia (NH_3-N) would remain well below toxic levels (Rowland 1995b).

The prevalence of monogeneans increased from 0 to 100% in 8 weeks at temperatures of 23.7° – 30.7°C (mean, 27.1°C) and in 12 weeks at 17.3° – 28.4°C (21.5°C) (Fig. 6.5.1). Mellergaard (1990) also reported re-infestation of eels (*Anguilla anguilla*) by the oviparous monogenean *Pseudodactylogyrus* 8 – 12 weeks after double treatments with mebendazole, and suggested that eggs may have a longer hatching time than stated in the literature. In *Dactylogyrus* and *Pseudodactylogyrus* spp., the period from oviposition to hatching is 3 – 6 days; the oncomiracidium attaches to gill tissue within 24 h of hatching and develops into a mature parasite in 6 – 9 days (Putz and Hoffman 1964; Mellergaard 1990). At 22°C, the life cycle from egg to egg-producing adult in *D. corporalis* is 13 – 14 days (Putz and Hoffman 1964). Our findings suggest that two or more treatments of formalin may be required to prevent re-infestation of silver perch by the oviparous *L. bidyana*, and Landos et al. (2007b) confirmed that three consecutive treatments of trichlorfon, 21 days apart controlled an infestation of *L. bidyana* in silver perch in a pond at water temperatures of 21.6º – 26.8ºC.

Formaldehyde degrades in the aquatic environment, although there is much variation attributed to differences in water chemistry and organic content (Masters 2004). A concentration of 50 mg/L is not detectable after 2 – 6 days at 20°C (Ueno et al. 1984; Jung et al. 2001), and the rate of degradation is accelerated by aeration and higher temperatures (Xu and Rogers 1995; Jung et al. 2001). Formaldehyde occurs naturally in animal tissues as a product of normal metabolism, and formalin treatment does not result in levels of formaldehyde above the normal range of endogenous concentrations in fish (Sills and Allen 1979; Ueno et al. 1984; Owen et al. 1990; Subasinghe and Yusoff 1993; Jung et al. 2001). Consequently formalin is an effective, short-term parasiticide, with no major concerns about chemical residues in fish flesh or the pond environment.

6.5.6. Acknowledgements

We thank Dr Craig Hayward for identification of *L. bidyana* and advice on the taxonomy of monogeneans. Joel Crispin, Daniel Kratz, Hussarn Jordan and Todd Rowland provided assistance with the management of ponds and sampling fish. We thank Dr Dick Callinan for information on diseases in silver perch, and Drs Wayne O'Connor and Geoff Allan for comments on a draft of the paper.

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Table 6.5.1. Monogenean gill flukes on silver perch in ponds before and after treatment with different concentrations of formalin. Data are mean number of parasites on gill tissue per field of view (magnification of X100). See text for details of the trials. ns – not sampled.

Trial	Formalin	Days post-treatment							
no.	(mg/L)	0	1	2	7	30	60	90	120
1[a]	30	5.8	0	ns	0	2.1	4.1	7.7	-
	40	7.0	0	ns	0	2.7	3.3	6.8	-
2[b]	20	10.2	5.4	6.3	10.4	11.3	6.3	5.3	4.0
	30	10.3	0	ns	0	3.2	10.3	5.7	5.8
	40	8.3	0	ns	0	2.1	4.6	4.9	3.1
3[c]	25	8.9	2.2	ns	1.9	3.4	3.9	ns	ns

[a] 4 replicate ponds for each formalin concentration

[b] 3 replicate ponds for each formalin concentration

[c] 1 pond treated with 25 mg/L

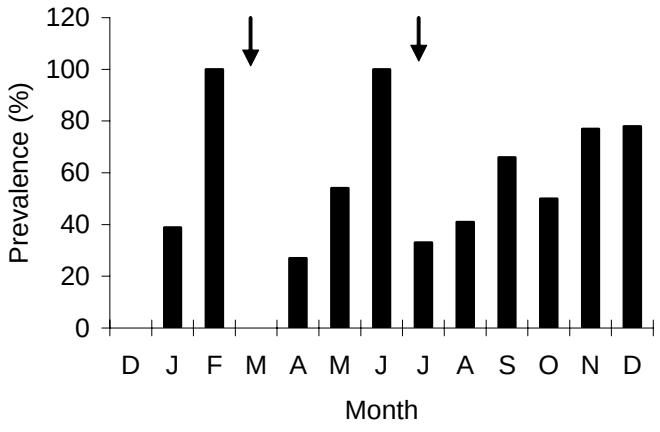

Figure 6.5.1. Prevalence of monogenean gill flukes on gill tissue of silver perch stocked at a density of 15,000 fish/ha in 0.1-ha earthen ponds. Ponds were treated (see arrows) with formalin at the beginning of March (Trial 1; 30 or 40 mg/L) and July (Trial 2; 20, 30 or 40 mg/L).

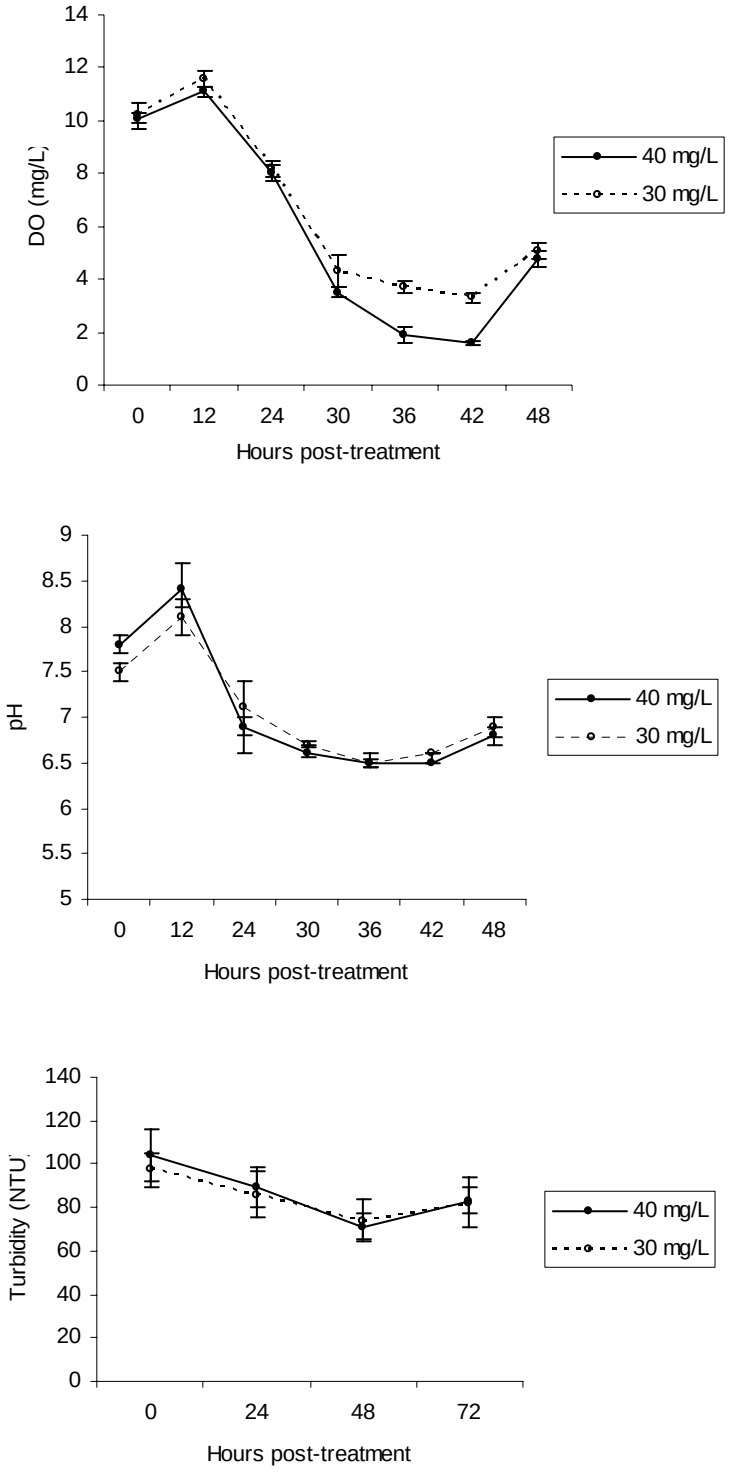

Figure 6.5.2. Dissolved oxygen (DO), pH and turbidity in 0.1-ha earthen ponds treated with formalin at concentrations of 30 or 40 mg/ L and temperatures of 24.1° – 26.9°C. Data are means ± SE.

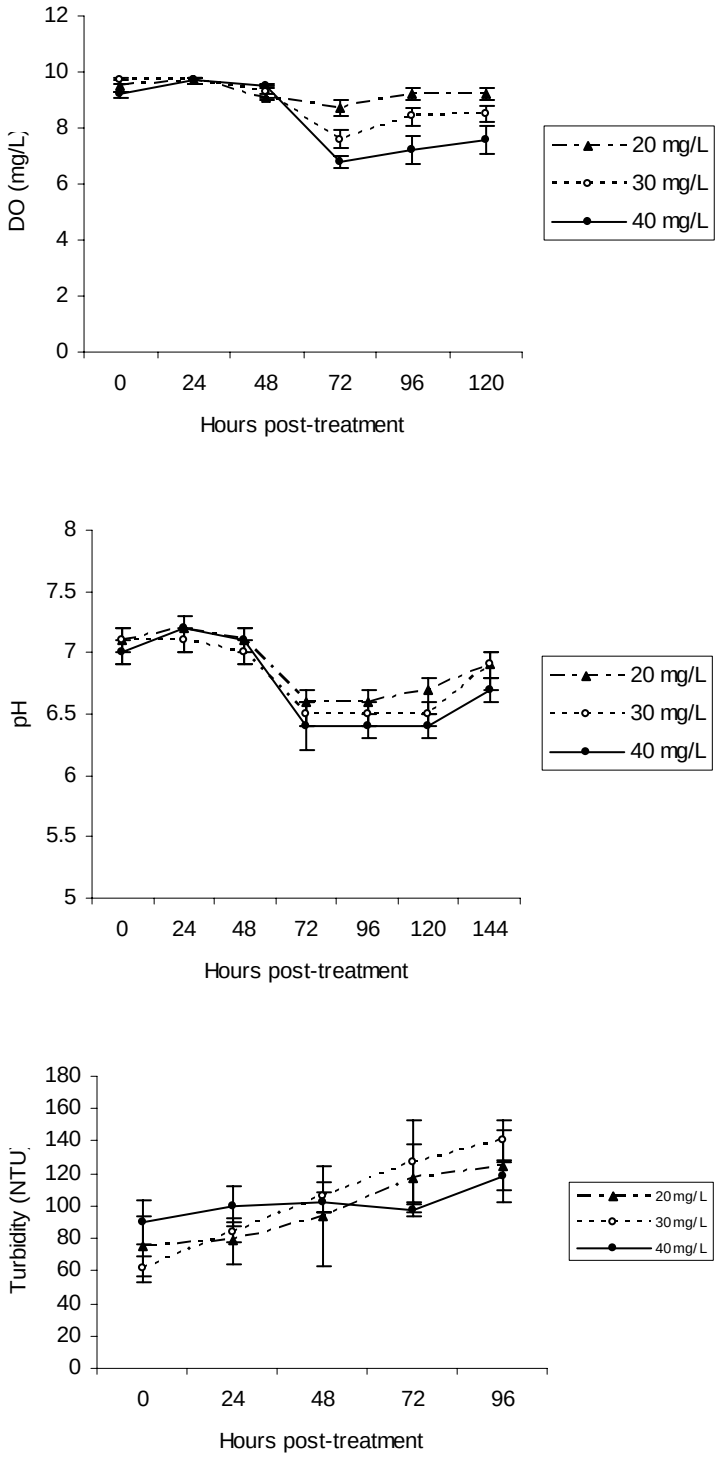

Figure 6.5.3. Dissolved oxygen (DO), pH and turbidity in 0.1-ha earthen ponds treated with formalin at concentrations of 20, 30 or 40 mg/L and temperatures of 13.2° – 15.7°C. Data are means ± SE.

6.6. Evaluation of trichlorfon to treat infestations of the monogenean gill fluke, *Lepidotrema bidyana*, in silver perch (*Bidyanus bidyanus*)

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6.6.1. Abstract

Infestations of the monogenean gill fluke, *Lepidotrema bidyana*, cause the disease lepidotremosis in silver perch. Formalin is a recommended treatment for lepidotremosis, but it is costly and adversely affects water quality in ponds, particularly at high temperatures. The aims of this study were to: (i) evaluate the organophosphate trichlorfon as an alternative therapeutant for lepidotremosis; (ii) to determine patterns of infestation of *L. bidyana*; and (iii) to analyse silver perch muscle tissue for dichlorvos, the breakdown product of trichlorfon. In tanks, 0.25 mg/L trichlorfon (Lepidex® 500) controlled lepidotremosis, but in ponds a concentration of 0.5 mg/L was required to eradicate all adult and juvenile gill flukes. Fish in ponds and cages became re-infested with juvenile flukes within 5 days and with gravid adults by 8 days at temperatures of 21.6º – 26.8°C, suggesting the eggs are resistant to trichlorfon. Three consecutive treatments of 0.5 mg/L trichlorfon, 21 days apart, controlled an infestation of *L. bidyana* in silver perch in an earthen pond at temperatures of 17.1º – 24.7°C. Juvenile stages of *L. bidyana* (length range, 70 – 200 μm) attach to skin and gill tissue, whereas adult flukes (500 – 600 μm) are found primarily on gill tissue. There were no detectable residues of dichlorvos in the muscle tissue of silver perch 6 – 48 days after the first of the three consecutive treatments. Trichlorfon is an effective therapeutant for the control of monogenean gill flukes in silver perch. Lepidex® is only registered for use as an insecticide on plants in Australia, but the animal product Neguvon® could be used off-label under veterinary prescription. Any other form of trichlorfon would require the issue of a permit by the Australian Pesticides and Veterinary Medicines Authority.

6.6.2. Introduction

Monogenean trematodes or gill flukes are common fish parasites with strict host specificity and a non-pathogenic nature which reflects a highly evolved adaptability to their hosts (Paperna 1991). Murray (1931) named the dactylogyrid trematode from the gills of silver perch, *Lepidotrema bidyana*, and infestations of this parasite cause the disease lepidotremosis in silver perch. Infestations of gill flukes were reported in some freshwater fishes at the Narrandera Fisheries Centre by Rowland and Ingram (1991), but flukes were not recorded at the Grafton Aquaculture Centre (GAC) between 1990 and 1995 despite routine disease monitoring during silver perch production experiments and hatchery operations (Rowland et al. 1994, 1995; Callinan and Rowland 1995). Since the mid 1990's, gill flukes have been common at GAC and on commercial silver perch farms, and the increased incidence is probably due to the introduction of broodfish from the wild, the movement of fish between hatcheries and farms, intensification of silver perch culture, and the difficulty in completely eradicating this parasite from farms (Rowland et al. 2007a).

Monogeneans cause significant mortalities and economic losses in some warmwater aquaculture industries (Paperna 1991; Hawke and Khoo 2004; Hanson and Wise 2005). The parasites use a series of hooks to attach to gill and skin tissue and infestations can result in significant tissue damage as well as respiratory stress (Paperna 1991; Hawke and Khoo 2004). Gill flukes rarely

cause mortalities in silver perch, but heavy infestations reduce growth, cause stress and damage gill filament epithelium, predisposing silver perch to fungal and bacterial infection (Rowland et al. 2007a).

Few chemicals are registered for use with food fishes in Australia. Formalin can be used by members of the National Aquaculture Council, including members of the NSW Silver Perch Growers Association under an Australian Pesticides and Veterinary Medicines Authority permit – PER8853. Rowland et al. (2006) found that formalin is an effective treatment for infestations of *L. bidyana*, but that it causes a significant deterioration of water quality in ponds, in particular dissolved oxygen, pH and ammonia at temperatures around 25ºC. Formalin also adversely affects some aquatic organisms and is usually not recommended for use in large ponds in the USA because of these factors and its high cost (Meyer and Schnick 1989; Boyd 1990; Noga 2000). Clearly there is a need for an alternative treatment for lepidotremosis in silver perch, particularly for use at high water temperatures.

The organophosphate pesticide trichlorfon [dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate] and its active metabolite dichlorvos have been recommended and widely used for the treatment of monogenean trematodes in fish culture (Sarig et al. 1965; Sarig 1971; Imanda and Muroga 1979; Schlotfeldt et al. 1995; Noga 2000) and so trichlorfon is a potential alternative treatment to formalin for lepidotremosis in silver perch. The aims of this study were to: (i) evaluate trichlorfon (Lepidex® 500) as a treatment for lepidotremosis; (ii) determine patterns of infestation by *L. bidyana*; and (iii) analyse silver perch muscle tissue for dichlorvos, the active breakdown product of trichlorfon.

6.6.3. Materials and methods

This study was carried out at the NSW Department of Primary Industries' Grafton Aquaculture Centre (GAC). Monitoring of water quality followed Rowland (1995) and details and diagnosis of lepidotremosis in silver perch are given in Read et al. (2007). Microscopic examination of gill and skin tissues was used to confirm the disease; approximately 10 mm of the left anterior gill arch was excised from each fish, and mucus and scales were taken from the length of the lateral line on the left side of each fish using a scalpel. The samples were mounted on a microscope slide and examined at a magnification of X100. All gill flukes on each slide were counted and the level of infestation determined as follows; + light < 10 flukes, ++ moderate 11 – 50, +++ heavy 51 – 100, ++++ very heavy > 100.

6.6.3.1. Trial 1

Silver perch infested with gill flukes were collected from an earthen pond at GAC, and 165 fish were placed into each of two 9,000 L fibreglass tanks. Fish were given a continuous 5 g/L salt (NaCl) bath for 2 days to reduce stress and prevent fungal infection (Rowland and Ingram 1991). The water in each tank was then exchanged (100%) and on day 1, tank A was treated with 30 mg/L formalin and tank B was left untreated as a control (Table 6.6.1). The tanks were static and aerated. Tank A was retreated with 30 mg/L formalin on day 4. On day 9, water was exchanged (100%) in both tanks, and tank A was treated with 0.25 mg/L trichlorfon (Lepidex® 500, Newfarm Chemicals). Six fish were sampled from each tank on days 8 and 10, and gill and skin tissues were examined microscopically for gill flukes.

6.6.3.2. Trial 2

Silver perch (range mean weights, 282.4 – 315.0 g) were stocked at a density of 50 fish/m^3 into 6 adjacent 1m^3 floating cages in a 0.32-ha earthen pond. The pond was aerated with a 2 hp paddlewheel aerator for 8 h per day from 00.00 to 08.00 h. On day 1, three cages were stocked with fish that had been treated with 0.25 mg/L trichlorfon and were free of gill flukes ("clean" fish); the other three cages were stocked with fish that were heavily infested (+++) with gill flukes

("infested" fish) as a potential source of pathogens. Three silver perch were sampled from each "clean" cage 5, 8 and 10 days after stocking, and gill and skin tissues were examined microscopically. The number of gill flukes on tissue sampled from each fish was recorded, and the length of five gill flukes on both skin and gill tissues of each fish were measured using an eye-piece micrometer.

6.6.3.3. Trial 3

Silver perch (mean weight, 301 g) heavily infested (++++) with gill flukes were taken from a tank and stocked at densities of 25 – 50 fish/m^3 into 8 adjacent 1m^3 floating cages in a 0.32-ha earthen pond. On day 1, the pond was treated with 0.25 mg/L trichlorfon and fish were sampled and gill and skin tissues examined on days 2 and 6. The pond was then retreated with 0.5 mg/L trichlorfon on days 6, 27 and 48 (i.e., 3 treatments, 21 days apart). A total of six fish were sampled from at least 2 cages on days 7, 17, 21, 28, 34, 49 and 69 and tissues were examined microscopically for gill flukes.

6.6.3.4. Trial 4

Fifty silver perch (mean weight, 301 g) that were heavily infested (++++) with gill flukes were placed into a 1,800 L aerated, fibreglass tank. The tank was treated with 0.5 mg/L trichlorfon on days 1, 14 and 25. Water was exchanged (100%) in the tank immediately prior to each treatment. Fish were sampled from the tank on days 7, 13, 21, 35 and 49, filleted and the muscle tissue frozen until analysed for dichlorvos at the Environmental Chemistry Laboratory (NSW Agriculture Diagnostic and Analytical Services, Wollongbar, NSW, 2477).

6.6.4. Results

6.6.4.1. Trial 1

On day 8, silver perch in tank A remained infested with low numbers of gill flukes despite two treatments with 30 mg/L formalin on days 1 and 4 (Table 6.6.1). There were no gill flukes on silver perch on day 10, following treatment with 0.25 mg/L trichlorfon.

6.6.4.2. Trial 2

At stocking on day 1, "clean" fish were free of gill flukes, while those in adjacent cages were very heavily infested. On day 5, juvenile flukes (lengths 150 – 200 µm) were present on both skin and gill tissues of the previously "clean" fish (Table 6.6.2). On day 8, juvenile and adult flukes were present, with one gravid fluke, and on day 10, approximately 50% of the flukes were gravid (Table 6.6.2). All adult flukes were on gill tissue. Ranges of water quality variables were; temperature 21.6º – 26.8ºC, pH 6.6 – 8.3, dissolved oxygen 6.7 – 8.4 mg/L, and total ammonia-nitrogen 0.2 – 0.6 mg/L.

6.6.4.3. Trial 3

Silver perch were heavily infested with gill flukes on day 1, prior to the first treatment. There was a decrease in the level of infestation following application of 0.25 mg/L trichlorfon, but some fish remained lightly infested on days 2 and 6. A concentration of 0.5 mg/L trichlorfon eradicated all flukes on gill and skin tissues within 24 h, and was an effective, short-term control of lepidotremosis (Table 6.6.3). Fish became re-infested within 11 and 7 days of the first and second trichlorfon treatments, respectively but no gill flukes were found on silver perch 21 days after the third consecutive treatment of 0.5 mg/L trichlorfon (Table 6.6.3). Ranges of water quality variables were; temperature 17.1º – 24.7ºC, pH 7.3 – 9.6, dissolved oxygen 6.6 – 10.2 mg/L and total ammonia-nitrogen 0.2 – 1.0 mg/L.

6.6.4.4. Trial 4

Silver perch were held at 20º – 21ºC. The Limit of Reporting (LOR) for dichlorvos residues in 0.1 mg/kg. There were no detectable residues of dichlorvos (i.e., < 0.1 mg/kg) in silver perch muscle tissue 6 and 13 days after the first treatment, 7 days after the second treatment, and 10 and 24 days after the third treatment, i.e., 6 – 48 days after first exposure of live silver perch to 0.5 mg/L trichlorfon (Table 6.6.4).

6.6.5. Discussion

Trichlorfon is an effective therapeutant for the control of infestations of the gill fluke, *L. bidyana* in silver perch. A concentration of 0.25 mg/L killed all adult and juvenile flukes on silver perch in tanks, but this concentration was not completely successful in a pond where 0.5 mg/L was required to eradicate all flukes on fish. Efficacious concentrations and treatment regimes in earthen ponds can vary because of precipitation, chemical degradation and/or absorption of chemicals by organic matter, soil, plants and fish (Boyd 1990; Darwish et al. 2005; Rowland et al. 2007b). Temperature, pH and aeration may also affect the rate of degradation of organophosphates, and the half-life of trichlorfon is less than one day in a pond with a pH of 9 during summer (Langdon 1988; Noga 2000). Trichlorfon should be applied in the early morning to maximise the period with an effective concentration because temperatures and pH usually increase during the day in silver perch ponds (Rowland 1995).

Formalin at a concentration of 30 mg/L did not completely control lepidotremosis in the tank (Table 6.6.1). Rowland et al. (2006) had reported that this concentration was effective, but that 25 mg/L did not completely control lepidotremosis in silver perch in earthen ponds. The depletion of formaldehyde in water is influenced by factors such as temperature, aeration, microbial activity and the presence of fish (Masters 2004; Pederson and Pederson 2006; Rowland et al. 2007b), and vigorous aeration in the tanks may have reduced formalin to ineffective levels before all gill flukes were killed.

Species and even populations of monogeneans can differ in their sensitivity to chemical treatments (Noga 2000). Although a wide range of concentrations of trichlorfon have been reported or recommended for the control of monogenean gill flukes in freshwater fishes, the relatively low concentrations (0.25 – 0.5 mg/L) required to control infestations of *L. bidyana* in silver perch using long-term baths were similar to those reported for monogeneans in other warmwater fishes (Sarig et al. 1965; Schlotfeldt et al. 1995; Noga 2000; Lio-Po and Lim 2002).

Silver perch became re-infested within 10 days of treatment suggesting that trichlorfon does not kill the eggs of the oviparous *L. bidyana*. Rowland et al. (2006) found that the prevalence of monogeneans on silver perch increased from 0 to 100% in 8 weeks at temperatures of 23.7° – 30.7°C (mean, 27.1°C) and in 12 weeks at 17.3° – 28.4°C (21.5°C). Mellergaard (1990) also reported re-infestation of eels (*Anguilla anguilla*) by the oviparous monogenean *Pseudodactylogyrus* 8 – 12 weeks after double treatments with mebendazole. In *Dactylogyrus* and *Pseudodactylogyrus* spp., the period from oviposition to hatching is 3 – 6 days; the oncomiracidium attaches to gill tissue within 24 h of hatching and develops into a mature parasite in 6 – 9 days (Putz and Hoffman 1964; Mellergaard 1990). At 22°C, the life cycle from egg to egg-producing adult in *D. corporalis* is 13 – 14 days (Putz and Hoffman 1964). We found that the period from egg to gravid adult in *L. bidyana* is 8 – 10 days at 21.6º – 26.8ºC. Rowland et al. (2006) suggested that two or more treatments of formalin may be required to prevent re-infestation of silver perch by the oviparous *L. bidyana*, and our study has demonstrated that three consecutive treatments of trichlorfon, 21 days apart prevented re-infestation in an earthen pond.

There are important issues to be considered when using trichlorfon to control lepidotremosis in silver perch. Trichlorfon is toxic to some fish larvae (Flores-Nava and Vizcarra-Quirox 1988) and

its effects on larval and juvenile silver perch are not known. Concentrations of 0.25 and 0.5 mg/L trichlorfon were reported to cause leucopenia and lymphocytopenia, and to reduce brain acetylcholinesterase activity in common carp, and so have the potential to impair neurological functions and reduce disease resistance (Chandrasekara and Pathiratne 2005). Repeated use of trichlorfon in silver perch ponds should be avoided. Organophosphates can cause asphyxiation in fish (Noga 2000) and so ponds and tanks should be well aerated during treatment.

Application of trichlorfon to ponds may alter the pond ecology. There are reports about possible adverse ecological effects, and trichlorfon is reported to be acutely toxic to freshwater decapods and some species of zooplankton (Schlotfeldt et al. 1995; Noga 2000; Qin and Dong 2004). There have also been reports of ineffectiveness, reduced efficacy, resistance and toxicity of trichlorfon (Goven et al. 1980; Langdon 1988; Obiekezie and Taege 1991; Costello 1993; Roth et al. 1993; Janse and Borgsteade 2003). Goven et al. (1980) found that a dosage 100 times the commonly recommended dosage of 0.25 mg/L was required to remove trematodes from infested goldfish. Drug resistance of monogenean trematodes is a potential problem in freshwater fish culture (Goven et al. 1980; Roth et al. 1993).

A strategy of alternating treatments of formalin and trichlorfon may prevent the development of resistance in *L. bidyana*, reduce possible adverse effects on the neurological functions and the immune system, and limit adverse environmental effects in silver perch ponds. Formalin could be used at low temperatures (e.g., < 20º) and trichlorfon at higher temperatures where formalin is known to cause a deterioration of water quality in silver perch ponds (Rowland et al. 2006). Winter saprolegniosis is linked to immunosuppression at low water temperatures in channel catfish and silver perch (Bly et al. 1993, 1994; Landos et al. 2007) and so formalin should be the chemical used to reduce numbers of gill flukes in autumn to avoid any immunosuppressive effects of trichlorfon prior to winter.

Trichlorfon is not registered for use on food fish in Australia and only one product, Neguvon® which is registered for use as an insecticide on animals, can be used for control of gill flukes on fish under veterinary prescription. Trichlorfon does not have a Maximum Residue Limit in Australia, and so the acceptable residue limit is zero. No dichlorvos residues were detectible in silver perch tissues between 6 and 24 days after exposure to trichlorfon (Table 6.6.4). Organophosphates are potentially dangerous chemicals because they can induce neurotoxic poisoning in humans, and trichlorfon is a possible tetratogen (Noga 2000). Consequently, trichlorfon must be handled with great care by fish farmers.

6.6.6. Conclusions

Trichlorfon is a potential alternative treatment to formalin for the control of lepidotremosis in silver perch; however, the chemical is not registered and only Neguvon® can be used under veterinary prescription. Care needs to be taken in the use of trichlorfon for the following reasons: (i) it has been reported to have adverse effects on neurological functioning and the immune system of warmwater fish even at low concentrations; (ii) it has adverse effects on pond ecology because of toxicity to decapods and zooplankton; (iii) resistance of *L. bidyana* is a potential problem with repeated use; and (iv) it is harmful to humans if absorbed through the skin or inhaled. Trichlorfon also has potential for adversely affecting other aquatic organisms if released into the environment.

6.6.7. Acknowledgements

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6.6.8. References

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Table 6.6.1. Treatment of an infestation of the gill fluke, *Lepidotrema bidyana*, on silver perch in 1,800 L fibreglass tanks using formalin or trichlorfon. Numbers of gill flukes on gill and skin tissues; + < 10, ++ 11 – 50.

Day	Number of gill flukes		Comments
	Tank A	Tank B	
1	++	++	Tank A – 30 mg/L formalin Tank B – not treated
2			Water exchanged (100%)
4			Tank A – 30 mg/L formalin Tank B – not treated
5			Water exchanged (100%)
8	+	++	
9			Tank A – 0.25 mg/L trichlorfon Tank B – not treated
10	0	++	Tank A – fish free of gill flukes Tank B – fish still infested

Table 6.6.2. Infestation of silver perch in cages by the gill fluke, *Lepidotrema bidyana*, at temperatures of 21.6º – 26.8ºC. Data are means of fish in 3 replicate cages. Day 1 – day of stocking.

Day	Number of flukes on "clean" fish		Description of gill flukes
	gills	skin	
1	0	0	No flukes
5	1.6	0.2	Juvenile flukes only; 150 – 200 µm in length
8	10.9	2.9	Adult and juvenile flukes; 150 – 500 µm in length; all adults on gill tissue only; juveniles on gills and skin; one gravid fluke
10	17.0	1.1	Adult and juvenile flukes; 70 – 600 µm; 50% gravid; all adults on gill tissue

Table 6.6.3. Treatment of an infestation of the gill fluke, *Lepidotrema bidyana*, on silver perch in a 0.32-ha earthen pond at temperatures of 17.1º – 24.7ºC. Numbers of gill flukes on gill and skin tissues; + < 10, ++ 11 – 50, +++ > 50.

Day	Treatment – trichlorfon(mg/L)	Level of infestation
1	0.25	+++
2		+
6	0.5	+
7		0
17		+; adults and juveniles; gravid adults
21		++; mainly adults; gravid adults
27	0.5	
28		0
34		+
48	0.5	
49		0
69		0

Table 6.6.4. Dichlorvos residues in silver perch muscle tissue following treatment with trichlorfon to control an infestation of the gill fluke, *Lepidotrema bidyana*, in a 1,800 L fibreglass tank at 20º – 21ºC.

Day	Trichlorfon(mg/L)	Dichlorvos(mg/kg)
1	0.5	
7		< 0.1
13		< 0.1
14	0.5	
21		< 0.1
25	0.5	
35		< 0.1
49		< 0.1

7. AQUACULTURE EXTENSION AND DISEASE DIAGNOSTIC SUPPORT

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7.1. Summary

The role of extension and its importance in supporting aquaculture development was demonstrated in our project to develop a health management strategy for the silver perch industry. Winter saprolegniosis, a new fungal disease of silver perch, is a good example of how aquaculture industries can be challenged with diseases not previously encountered. The NSW Department of Primary Industries (NSW DPI) aquaculture extension officers assisted with the collection and collation of data, and facilitated communication between farmers, aquaculture managers and researchers at the Grafton Aquaculture Centre and the Aquatic Animal Health Unit. This co-operative approach enabled on-farm investigations of winter saprolegniosis and other diseases, and provided opportunities for technology transfer, leading to an overall improvement of aquaculture practices and health management on participating farms. Technical publications resulting from the project, including this Fisheries Research and Development Corporation Final Report, a Disease Diagnostic Manual, a generic Health Management Plan and a Hatchery Quality Assurance Program have been prepared. Extension continues to be provided by aquaculture staff, and disease diagnostic support is available from NSW DPI veterinary laboratories. A combination of research, management, extension and disease diagnostic support is fundamental to the development of sustainable, economically-viable aquaculture industries.

7.2. Introduction

Extension has played a critical role in the development and sustainability of aquaculture and agriculture industries in Australia and other countries. The channel catfish (*Ictalurus punctatus*) industry in the USA is an example of how the potential of an industry has been realised through sound investment, relative research, marketing and a well-resourced extension service (Masser 2004).

Extension encompasses more than technology transfer from the researcher to the farmer. It facilitates partnerships between government departments, industry, fish health specialists, investors, aquaculture service providers and the community. An extension service acts as a conduit for information transfer between personnel involved in R&D, planning, development and operation of aquaculture projects.

This chapter briefly summarises the NSW aquaculture industry, extension services provided by NSW DPI, and the role of extension in the development of the silver perch (*Bidyanus bidyanus*)

aquaculture industry and in the project to address winter disease problems. The current disease diagnostic and health management support services provided by NSW DPI are briefly outlined.

7.3. NSW Aquaculture industry

The oyster industry is the oldest, largest and most valuable aquaculture industry in NSW. Over 30 estuaries are used to culture Sydney rock oysters (*Saccostrea glomerata*), Pacific oysters (*Crassostrea gigas*) and flat oysters (*Ostrea angasi*). There is also an emerging industry for Akoya pearls (*Pinctada imbricata*). The newer sectors of the NSW aquaculture industry are; marine prawns, freshwater fish [silver perch, Murray cod (*Maccullochella peelii peelii*), barramundi (*Lates calcarifer*)] and marine finfish and worms. Prawn farming is based in the estuaries of the Richmond River and Clarence River on the North Coast, with six farms producing 300 – 400 tonnes of black tiger prawn (*Penaeus monodon*) annually. Silver perch farms are located throughout the state including parts of the Murray-Darling Basin, the Hunter Valley, and the Central and North Coast. Annual production of silver perch is approximately 300 tonnes, with a further 100 tonnes produced in Queensland and other states. Over half the product is sold live into Sydney, Canberra and Melbourne. The production of barramundi and Murray cod occurs mainly in recirculating aquaculture systems (RAS). A number of farms produce ornamental fish [koi carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*)] and commercial production of rainbow trout (*Oncorhynchus mykiss*) is restricted to the cooler waters of the Northern and Southern Tablelands. A small marine fish industry produces snapper (*Pagrus auratus*), yellow-fin bream (*Acanthopagrus australis*) and mulloway (*Argyrosomus japonicus*) using earthen ponds and sea cages. A marine, bait worm farm (family Onuphidae), utilises the cooling water of the Eraring power station, Lake Macquarie, to produce live and preserved worms. Mussel farms (*Mytilus* sp.) operate in Twofold Bay and Jervis Bay. Over the past 25 years the coastal regions of NSW have undergone rapid demographic change, and there has been increasing pressure from development and urbanisation. Numerous reserve, wilderness and National Park areas have been established. As a result of these changes, it is likely there will be significant challenges to the establishment of marine aquaculture facilities on the NSW coast in the future (Glendenning and Read 2003). In addition, any deterioration of water quality in coastal estuaries could negatively impact on existing aquaculture projects.

7.4. NSW Aquaculture Extension Service

Aquaculture extension has been a function of NSW Fisheries and the NSW Department of Primary Industries for many years. Originally researchers and managers extended information to industry through farm visits, conferences and key publications (e.g., Rowland 1983a, b; Rowland and Ingram 1991; Rowland and Bryant 1995; Callinan and Rowland 1995; Rowland 1998a, b; Rowland et al. 2002). In the mid-1990's, one dedicated aquaculture extension officer was appointed to support the emerging silver perch industry. Under the NSW Government's Aquaculture Initiative, two extension officers (focussing on the non-oyster sector) and one aquatic animal health specialist were appointed in 2001. After the Aquaculture Initiative, one dedicated position was retained by the NSW DPI. Species groups supported were marine finfish, freshwater finfish and crayfish, eels, native fish hatcheries, marine worms, prawns and ornamental fish. Culture systems used for these species vary and include earthen ponds, tanks, RAS, raceways, cages, and fabricated ponds.

Aquaculture is a technically difficult industry for new operators, much more so than most established agricultural industries. The knowledge base is limited because of the infancy of the industry in Australia. The failure to plan carefully and implement good aquaculture practices has left some aquaculture businesses struggling to produce fish and be profitable. The "new" fish farmer must rapidly gain some understanding of fish biology and physiology, water quality, ecology, diseases and basic laboratory techniques. Poor site selection has resulted in the failure of some projects (e.g., limited water supply, poor quality water, unsuitable soils for pond construction,

unsuitable climate). In an attempt to avoid such failures, NSW DPI staff offer to "case manage" aquaculture investors through the preliminary site assessment and early developmental stages. Not all farmers seek this support. Extension by technically-competent officers can assist newcomers with site selection, design and operation of facilities and the implementation of good aquaculture practices and production strategies that will minimise problems and avoid project failures.

7.5. Silver perch aquaculture

The aquaculture potential of silver perch has long been recognised (Rowland 1995, 1998). It represents one of the few species in NSW with the potential to become a very large aquaculture industry (e.g., > 5,000 tonnes annually). This potential is primarily due to the following.

1) The physiological attributes of the species: schooling fish; hardy; rapid growth; high fecundity; omnivorous; white flesh, with few bones and high levels of omega oils.
2) Well-established hatchery techniques and good culture performance: guaranteed fingerling supply; high survival; fast growth; good feed conversion; adapts well to intensive culture in ponds (> 10 tonnes/ha) and cages (50 – 100 kg/m^3); fingerlings do well in RAS.
3) Abundant sites based available with high quality water from rivers, creeks and bores.
4) Established live fish market and potential for developing markets for whole and processed fish in the major capital and regional cities.
5) A sustainable culture method: the industry is ecologically-sustainable through nil discharge of effluent; it utilises a static pond system which limits water use; and returns are high (~ $3,000/ML) and superior to most forms of irrigated agriculture.

The silver perch industry grew steadily between 1993 and 2003, but growth has been stagnant in NSW at around 300 tonnes over the last three years (Fig. 7.1). Production is based on aerated, static (no/low water exchange) earthen ponds. Although there have been improvements in efficiencies and production on some farms, many farms that were established in the mid and late 1990's have not realised their potential. In addition, some very good farmers have left the industry for personal reasons. The following have been constraints to industry development.

1) Poor site selection and design of some farms.
2) Inexperience of farmers and the use of inappropriate practices have lead to poor fish performance, low production, high production costs and lack of profitability of some farms.
3) Undercapitalization and underestimation of the time and capital required to develop a fish farm and make it a commercial success.
4) Reliance on fingerlings from commercial hatcheries, leading to little or no control over genetics, health, condition of fingerlings and the time of supply.
5) High production costs, particularly labour, electricity and feed (a general characteristic of aquaculture in Australia).
6) Failure to appreciate that a fish farm is labour-intensive, and very difficult to operate as a secondary business.
7) Changing markets which now require larger fish and therefore increased costs and risks to the farmer.
8) The wide geographic distribution of farms in eastern Australia (from the Murray River in the south to Bundaberg in the north) has lead to fragmentation of the industry, little or no communication between farmers (with exceptions in localised areas), variation in priorities and approaches of farmers in different regions, and a lack of co-ordinated promotion and marketing programs.

7.6. Extension and the silver perch industry

In the early stages of industry development, extension concentrated on the transfer of production technology to farmers. Hatchery techniques were developed and transferred to industry in the early 1980's (Rowland 1983a, b). Research into grow-out to market-size for human consumption

commenced in 1990, and a series of workshops at Grafton and Narrandera in 1994 was an important starting point for the broader industry. There was an urgent need for farmers to have a good understanding of the basics of site selection, farm design, fish husbandry and production techniques, water quality, diseases and health management, diets and feeding, business plans, and various potential problems such as bird predation and off-flavour. These subjects were presented at the 1994 workshops and in the subsequent Proceedings (Rowland and Bryant 1995). Attempts were made to link farmers with the existing native fish hatchery sector to ensure an adequate supply of fingerlings for grow-out, and technology on post-harvest management (e.g., fish killing, icing and packaging, live fish transport) was extended to farmers. In the following years, technology transfer was provided through regional workshops, demonstrations at research facilities, telephone discussions, written publications, the Internet and on-farm consultations. A number of marketing strategies and reports were also developed with industry during this period. Consultative meetings between Government staff and industry were held each year to enable open discussion and to facilitate industry development. In 2003, a Silver Perch Aquaculture Conference, organised by NSW Fisheries and the NSW Silver Perch Growers Association, was held at Grafton to update farmers on research results, to introduce new technology such as cage culture, RAS and raceway systems, to discuss Safe Food Regulations, quality assurance program and marketing, to review the health management project and the hatchery quality assurance program, and to facilitate communication (Cheetham 2003; Rowland and Bryant 2003; Mosig 2004).

Despite the strong technical base and Government support, the industry had not developed to the extent predicted (Rowland and Bryant 1995; see Fig. 7.1), and new challenges have arisen. There have been disease problems during winter in some areas (see following), feed costs have risen, and markets are now demanding larger fish (> 600 g). Farmers are faced with longer growing periods, increased pond biomasses, higher costs of production, and increased risks associated with disease and/or water quality. The extension service has become more focussed on issues impacting directly on production efficiencies and profitability. Extension staff played an important role in a project funded by the Fisheries Research and Development Corporation to address winter disease problems and develop a health management strategy for the silver perch aquaculture industry.

7.7. Extension and the "winter disease" project

Expansion and intensification of the silver perch industry in the late 1990's resulted in increased problems associated with larger biomasses in ponds and longer growing periods. Some farmers were reporting significant losses to diseases in the winter. There appeared to be a higher incidence of infestations of ecto-parasites, particularly the protozoans, *Chilodonella hexasticha* and *Ichthyophthiriasis multifiliis*. Winter saprolegniosis or "winter disease" as it was colloquially known, was first reported in the late 1990's, and came to prominence in the winter of June 2000 when there were high mortalities at a silver perch farm in northern NSW. During this outbreak, the following observations were made and information collected by NSW DPI research and extension staff:

- Fish behaviour and appearance: large fish (> 500 g) were seen schooling near the surface and edges of ponds, often near in-coming water; fish had a distinct blotchy appearance with whitish fungal growths on the head, body, tail and abnormally dark skin; there were also fungal infections on the gills of many fish.
- Water quality: the outbreak was preceded by a severe cold change which caused a decline in water temperature from 14.0º to 8.8ºC over 7 days; high levels of ammonia (total ammonia ~ 6 mg/L) in some ponds; heavy blooms including blue-green algae, with secchi disc readings of < 10 cm in most ponds.
- Parasites: there were infestations of the protozoan parasites *Trichodina* sp., *Ichthyobodo necator*, and the gill fluke *Lepidotrema bidyana* on fish sampled from ponds.
- Pond sediment: there were deep (< 60 cm) anaerobic layers on the bottom of some ponds thought to be a result of excess feed.

- Fish in a majority of the ponds were infected with fungus, including fingerlings (50 – 100 g).
- There was total mortality of fish in several ponds.

The losses on this farm were significant. Over the following three years, winter saprolegniosis was reported on farms in the Riverina, Northern Tablelands and Gloucester districts of NSW. These areas typically experience "harsh" conditions with severe cold changes and rapid decreases in water temperature during winter.

Over the course of the winter disease and health management project, extension officers worked closely with researchers and other staff at the Grafton Aquaculture Centre (GAC) and the Aquatic Animal Health Unit. NSW DPI staff organised consultative meetings with the NSW Silver Perch Growers Association to prioritise actions to address the impacts of diseases in winter. Research to determine the pathogen, aetiology and pathogenesis of winter saprolegniosis, as well as identify preventative and control measures was a high priority. Farmers willing to participate in the project were contacted to explain the aims of the project and to outline the necessary commitment. During the course of the project, the aquaculture extension officer provided field support with fish sampling, water quality monitoring, skin and gill preparations and specimen collection. This field work also offered the opportunity to visit other farmers not participating in the project, to extend and discuss other aspects of fish husbandry and production. At times, the extension officer was the first point of contact for non-participating growers reporting disease problems. The extension officer kept farmers updated on the project's progress.

The pathogen that causes winter saprolegniosis was identified as the ubiquitous fungus, *Saprolegnia parasitica*, which for decades has been implicated in fish mortalities in aquaculture in other countries. Species susceptible to infection by *Saprolegnia* include salmon, carp, channel catfish, trout, eels and ornamental fish. The extension officer assisted in the collation of information from overseas fish health specialists, technical publications and scientific journals, and was involved in some of the experimental work at GAC. The project determined the aetiology and pathogenesis of winter saprolegniosis and identified various management options.

A team effort involving scientists, technicians, extension officers, managers and silver perch farmers lead to the development of a health management strategy for the silver perch industry. Resulting technical publications include this Fisheries Research and Development Corporation Final Report, a Disease Diagnostic Manual (Read et al. 2007), a generic Health Management Plan (Chapter 10 this report) and a Hatchery Quality Assurance Program (Rowland and Tully 2004). Extension continues to be provided by NSW DPI aquaculture staff. The combination of research, management, extension and disease diagnostic support is fundamental to the development of sustainable, economically-viable aquaculture industries.

7.8. Disease diagnostic services

NSW DPI offers a disease diagnostic service and advice on health management and biosecurity. The service is available through two of its Regional Veterinary Laboratories. Details on the preparation and submission of samples and specimens are given in Read et al. (2007), to be found in the NSW DPI Veterinary Laboratory Manual (available on-line at http://www.dpi.nsw.gov.au/agriculture/vetmanual) and further information is available from the laboratories – see below for contact details. Fees are involved for some services.

Disease investigation should form a routine component of aquaculture production and farm management, and costs associated with such investigation need to be factored into the costs of production. All unexplained mortalities need to be investigated thoroughly and such investigation forms part of the permit requirements for aquaculture in NSW. Timing is generally critical when dealing with disease problems in aquaculture, and the sooner unusual mortalities are investigated,

the sooner appropriate management decisions can be made. Appropriate measures can then be implemented to treat or manage the problem and thereby minimise mortalities and losses. Such health investigation is also vital to avoid recurrence of similar problems in the future.

Disease investigation is one aspect of production that is frequently neglected, as the returns to investment are not always immediately apparent. However, it is also one aspect that, if neglected will often result in catastrophic consequences. All successful long-term aquaculture business ventures understand the importance of regular health investigation, and the returns from investing in monitoring and health investigation.

Regular health investigation is also invaluable in guiding appropriate management decisions. Investment in this area is therefore likely to lead to long term increases in efficiency of production and overall profitability.

The address and contact details for the two Regional Veterinary Laboratories in NSW which offer disease diagnostic services for aquatic animals are as follows:

Menangle Regional Veterinary Laboratory
Woodbridge Rd
Menangle, NSW, 2570
Telephone – (02) 4640 6327
Fax – (02) 4640 6400
Officer in Charge, Keith Walker

Wollongbar Regional Veterinary Laboratory
Bruxner Hwy
Wollongbar NSW 2477
Telephone – (02) 6626 1261
Fax – (02) 6626 1276
Officer-in-Charge, Graeme Fraser

Disease diagnostic services and advice on health management are available from a number of experienced veterinarians with commercial companies. For further information see the Austasia Aquaculture Trade Directory.

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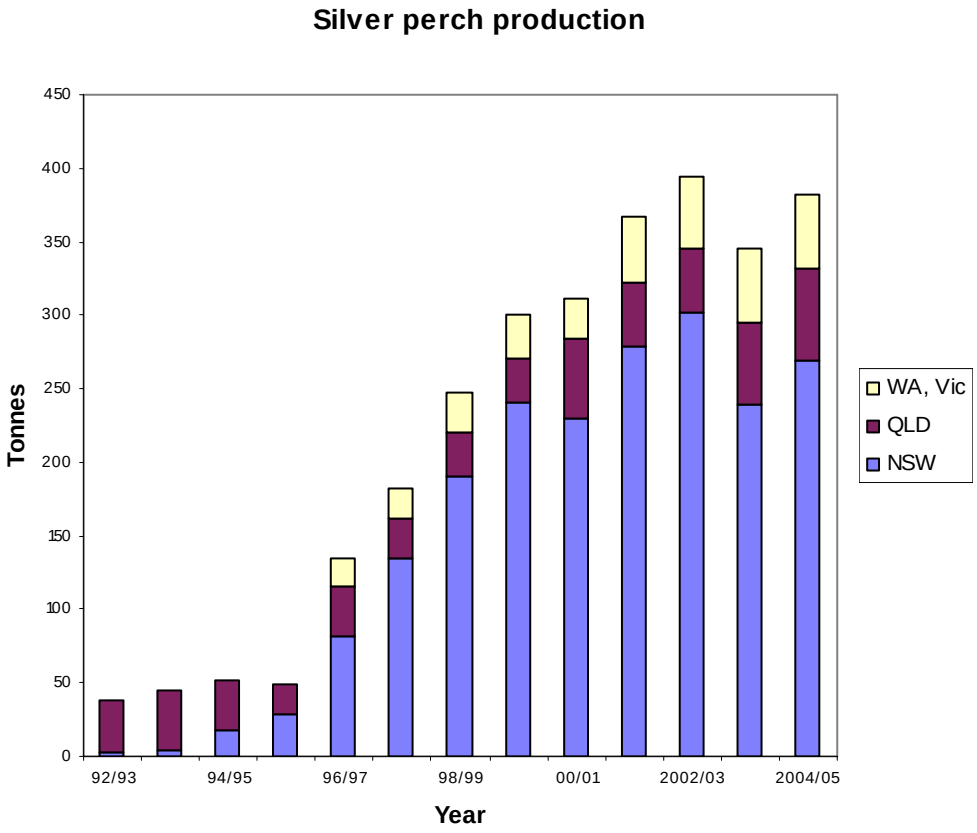

Figure 7.1. Silver perch production.

8. DIAGNOSIS, TREATMENT AND PREVENTION OF THE DISEASES OF THE AUSTRALIAN FRESHWATER FISH SILVER PERCH (*BIDYANUS BIDYANUS*) – A MANUAL *(TABLE OF CONTENTS ONLY)*

Phil Read[1,a], Matthew Landos[2,b], Stuart J. Rowland[1] and Charlie Mifsud[1]

[1] NSW Department of Primary Industries, Grafton Aquaculture Centre, PMB 2, Grafton, NSW, 2460
[2] NSW Department of Primary Industries, Aquatic Animal Health Unit, Wollongbar, NSW, 2477
Current address:
[a] NSW Department of Primary Industries, PO Box 530, Coffs Harbour, NSW, 2450
[b] Future Fisheries Veterinary Services, PO Box 364, Lennox Head, NSW, 2478

This is a Disease Diagnostic Manual that has been published separately to the FRDC Final Report. The manual is an aid to the diagnosis and treatment of silver perch diseases. It contains photographs of pathogens and diseased fish, and is an on-the-spot reference for typical signs of silver perch diseases. The manual also has directions for the treatment and prevention of all diseases, syndromes and conditions that have been reported in silver perch culture. The table of contents of the manual is given below.

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Copepods
Anchor worm – *Lernaea* sp.
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Bacterial diseases
Columnaris – *Flexibacter columnaris*
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Viral diseases
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Chemical use and legislation (January 2007)

Information required to prevent, diagnose, treat and control disease

Calculations, treatments and dose rates

Glossary

References and further reading

9. HATCHERY QUALITY ASSURANCE PROGRAM FOR MURRAY COD (*MACCULLOCHELLA PEELII PEELII*), GOLDEN PERCH (*MACQUARIA AMBIGUA*) AND SILVER PERCH (*BIDYANUS BIDYANUS)*

Stuart J. Rowland[1] and Patrick Tully[2]

[1] *NSW Department of Primary Industries, Grafton Aquaculture Centre, PMB 2, Grafton, NSW, 2460*
[2] *NSW Department of Primary Industries, Port Stephens Fisheries Centre, PMB 1, Nelson Bay, NSW, 2315*

The NSW Department of Primary Industries' Hatchery Quality Assurance Program was published in 2004, and distributed to industry, state and Commonwealth Government aquaculture managers and scientific staff including FRDC, and other interested persons. Below is a summary of the publication.

9.1. Summary

Techniques for the large-scale hatchery production of the Australian native fishes Murray cod (*Maccullochella peelii peelii*), golden perch (*Macquaria ambigua*) and silver perch (*Bidyanus bidyanus*) were developed at the Narrandera Fisheries Centre in the early 1980's, and commercial hatcheries began to produce and sell fingerlings in 1982/83. Around 30 hatcheries in NSW, Queensland and Victoria produce between 5 and 8 million fish annually. The fish are sold to stocking groups, State and Territory Governments for stock enhancement, farm dam owners, commercial fish farms, and a small number to the aquarium trade. In addition, around 2.5 million fish are produced by Government hatcheries for conservation and stock enhancement. Over the last 25 years, the regular stocking of native fish into impoundments and rivers has established large, popular recreational fisheries and contributed significantly to the conservation of these species.

In recent years, there have been concerns about some aspects of native fish hatcheries, in particular genetics, diseases and trash fish. Research has found closely related species and subspecies of Murray cod, golden perch and silver perch in other drainages and discrete populations within the Murray-Darling River System. Populations (or strains) are genetically distinct and usually differ in other biological attributes. These differences reflect natural selection and adaptation to local environments, and so mixing of populations through inappropriate stockings may have serious long-term effects and reduce the "fitness" of the endemic, wild populations. The transfer of pathogens and diseases through aquaculture has been a major problem world-wide. Diseases that are transferred on hatchery fish, may reduce post-stocking survival and introduce new diseases to regions and farms. There have been examples of this in eastern Australia. Native fish hatcheries have been implicated in the translocation of non-endemic fish such as banded grunter which is now found as far south as the Clarence River in NSW. Continuation of poor practices may have serious long-term biological consequences for populations and species, and hinder the development of sustainable and economically-viable aquaculture industries.

To address these concerns, NSW Department of Primary Industries has developed a Hatchery Quality Assurance Program (HQAP) for use by Government and commercial hatcheries. The HQAP describes key features of native fish hatcheries and identifies *Essential Criteria* and *Recommended Criteria* for site selection, design and operation, and the management of broodstock, breeding programs, water quality and fish health. Breeding programs need to be closely linked to

stocking programs to meet genetic goals. *Essential Criteria* are the basis for accreditation and auditing, and hatcheries in NSW that produce and sell Murray cod, golden perch and silver perch fingerlings for stock enhancement, conservation and commercial grow-out will be required to be accredited in accordance with this HQAP.

10. HEALTH MANAGEMENT PLAN

10.1. Introduction

Silver perch (*Bidyanus bidyanus*) is an Australian native freshwater fish that is endemic to the Murray-Darling River System. It is a high quality, white-fleshed finfish that has long been recognised as having potential for aquaculture. Hatchery techniques involving hormone-induced spawning and extensive larval rearing were developed at the Narrandera Fisheries Centre (formerly the Inland Fisheries Research Station) in the early 1980's to produce fingerlings for stock enhancement and conservation. Since 1990, research has been done at the NSW Department of Primary Industries' Grafton Aquaculture Centre (GAC) and the Port Stephens Fisheries Centre into breeding, production of fingerlings and market-size fish, husbandry, water quality, diseases, off-flavour and purging, nutrition and feeding. A grow-out industry, based on pond culture commenced in the mid-1990's and currently produces around 400 tonnes annually.

Diseases, in particular those caused by infectious agents, are recognised as an important threat to the viability of finfish aquaculture. The diseases of silver perch under culture conditions have been studied for nearly 30 years. As the silver perch industry expanded in the late 1990's, the incidence and losses from diseases increased, and health management became a high R&D priority. At the time, there was no specific disease research, and no validated health management plans or strategies were available to the industry. To address these issues, a project "Development of a Health Management Strategy for the Silver Perch Aquaculture Industry" was funded by the former NSW Fisheries, the NSW Department of Primary Industries (NSW DPI) and the Fisheries Research and Development Corporation. The seasonal incidence of diseases at GAC over a 15 year period (1990 – 2005) was determined, and major diseases of silver perch on commercial farms were identified, including two new diseases, winter saprolegniosis caused by the fungus, *Saprolegnia parasitica*, and lepidotremosis caused by infestations of the monogenean gill fluke, *Lepidotrema bidyana*. The project also developed diagnostic, control and preventative measures for silver perch diseases.

This Health Management Plan is based on results of the project, as well as past research into the diseases and culture of silver perch. It provides guidelines for the monitoring, treatment and prevention of diseases, as well as for site selection, design and operation of farms, water quality monitoring and management, husbandry and production techniques, and nutrition and feeding. All these aquacultural practices form the basis of health management.

10.2. Health management

10.2.1. Introduction

Health management is a concept of dealing with aquatic animal health by providing general environmental and fish-culture conditions that reduce the incidence and severity of diseases, leading to efficient and economic production of fish. The concept of health management in fish culture was first introduced around 50 years ago by Snieszko (1958) when he proposed that the natural resistance of fish to disease could be enhanced through good management, or compromised by facilities and practices that create stressful conditions. The old saying *"An ounce of prevention is worth a pound of cure"* is very applicable to silver perch farming. Health management commences with the selection of a site with an abundant supply of good quality water. If a farm is unable to provide optimum water quality and culture conditions because of poor site selection and

design, then no level of disease monitoring, treatment and management can compensate for this basic short-coming. Health management encompasses all aspects of the production cycle by combining a well designed farm with Good Aquaculture Practices to minimise stress and health problems, and to optimise survival, growth, food conversion, reproduction and production.

10.2.2. Maintaining health

In the aquatic environment, there is a profound and inverse relationship between environmental quality and the physiological condition and disease status of fish. As culture conditions deteriorate (poor water quality, high stocking densities, rough handling etc.) the incidence and severity of silver perch diseases increase. The fungal disease winter saprolegniosis is linked to low and declining water temperatures in winter, infestations of ecto-parasites, high organic loads in ponds and/or rough handling. Another fungal disease, epizootic ulcerative syndrome (EUS) can follow exposure of silver perch to poor water quality, particularly very high or low pH. Outbreaks of the ectoparasitic diseases white spot (ichthyophthiriosis), chilodonellosis and ichthyobodosis often follow declining or fluctuating water temperatures, periods of low dissolved oxygen (DO) and poor nutrition. Bacterial diseases such as tail rot, columnaris, and aeromonad dermatitis are associated with low oxygen, high temperatures, high organic loads and rough handling. The immune response of fish to pathogens is adversely affected by malnutrition and nutritional deficiencies. Well-conditioned silver perch are less likely to be affected by pathogens than poor or emaciated fish; it is these fish that are usually the first to become infested with parasites and die in a disease outbreak. Maintaining good health not only reduces losses to diseases, but improves growth, food conversion and production rates.

10.2.3. The environment

In fish culture, there are constant interactions between the fish, the pathogens and the environment. The susceptibility of fish is influenced by their genetic strain, age, size, condition, nutritional status, immune status and general physiological status. For example, silver perch fingerlings are more susceptible to white spot than larger fish, which in turn are more susceptible to winter saprolegniosis than fingerlings; however, under certain conditions all silver perch can get either disease. Factors influencing pathogenicity include the number, genetic strain and virulence of the pathogens present. Although some pathogens are highly virulent making silver perch susceptible (white spot, chilodonellosis), these and other diseases are enhanced by environmental stress. Most outbreaks of infectious diseases in silver perch are caused by unsuitable or deteriorating culture conditions, in particular:

- exposure to low DO, high or low pH, high nitrite, high organic loads;
- rapid decline of water temperature during winter;
- physical damage and stress during stocking, sampling and harvesting;
- physical damage and stress from bird predation.

10.2.4. Stress

Stress is a general physiological reaction to trauma, or to a physical or physiological insult to the body that impairs normal functioning and reduces performance and chances of survival. Fish react to stress in different ways, depending on the species of fish, and the severity and length of exposure to the stressor. Acute stress caused by factors such as netting, sudden and rough handling, low dissolved oxygen and the presence of hydrogen sulphide, may result in rapid death, whereas chronic stress caused by unsuitable culture facilities (e.g., large silver perch perform poorly in tank-based re-circulating aquaculture systems), very high stocking densities, aggression, poor nutrition or marginal water quality will result in reduced feed intake and/or conversion, reduce growth and lower resistance to infectious diseases. Because the aquatic environment is dynamic, freshwater fish are continually adapting physiologically; however, under intensive conditions, environmental changes are sometimes severe and rapid.

Common stressors in intensive pond, cage and tank culture of silver perch are:

- poor water quality, in particular rapid changes in variables, very high or low temperatures, low DO, low or high pH, high un-ionised ammonia, high nitrite;
- rough handling causing loss of mucus and damage to skin and fins;
- poor nutrition;
- social hierarchies with aggressive fish dominant over subordinate individuals in cages and tanks;
- high light intensities in tanks;
- sudden changes in the culture environment;
- some chemicals such as heavy metals, pesticides;
- birds such as cormorants, darters and pelicans.

To minimise stress, silver perch should be provided with the following basic conditions:

- good water quality;
- appropriate stocking densities in each production phase;
- appropriate quantities of a nutritionally-complete diet;
- a stable culture environment;
- limited physical disturbance and careful handling during stocking, sampling, grading and harvesting (this can be achieved by combining care and concern for the fish, patience, use of knotless nets and anaesthetics);
- protection from bird predation, especially fingerlings.

10.2.5. Exposure to pathogens

Infectious diseases are caused by exposure of fish to pathogenic organisms such as protozoans, bacteria and fungi. Fish pathogens are natural inhabitants of freshwater aquatic environments, and can remain viable under a range of conditions. Water is an excellent medium for transferring organisms from fish to fish, from reservoir to ponds and tanks, from ponds and tanks to equipment such as nets, bins and so on. Consequently infectious agents can spread rapidly on a farm and between fish in a pond or tank. Obligate pathogens, i.e., those dependent on a fish host, can be highly virulent, and at times can initiate a disease outbreak in apparently normal, "unstressed" fish. White spot is such an example in silver perch, with healthy populations of fingerlings sometimes succumbing to infections of the pathogen, *Ichthyophthirius multifiliis*. Opportunistic or facultative pathogens do not require a host to survive and replicate, and can live without fish; they usually only cause disease in stressed, weakened or damaged fish. Fish and facultative pathogens often co-exist without any interactions. Low numbers of some protozoans and monogeneans (e.g., *Trichodina*, gill flukes) are often present, and it is only when the fish's immune system or other defensive mechanisms such as mucus and skin are compromised or damaged that the pathogen can infect the fish and cause a disease. Most diseases occur only when the pathogens reach a specific level. Facultative pathogens are very difficult to eliminate from the fish culture environment. The ubiquitous nature of saprophytic fungi such as *Saprolegnia parasitica* is a reason that fungal diseases are common and difficult to prevent and control in freshwater fishes.

Primary aims of the silver perch farmer should be to prevent the entry of pathogens to the farm, and to prevent exposure of fish to pathogens. Methods of prevention include:

- screening incoming surface waters to prevent the entry of trash fish that can introduce and harbour pathogens;
- ensure the effluent-settlement dam is free of trash fish, and do not stock with any fish if it is used as an alternative water supply;
- use quarantine procedures for <u>all</u> fish, including fish coming onto the farm from the wild or other farms, fish harvested from any pond/cage/tank that are to be stocked into another pond/cage/tank on the farm, and fish to be transported from the farm;

- use prophylactic chemical therapeutants during quarantine (salt, formalin);
- use prophylactic chemical therapeutants on broodfish, and at times when disease is known to occur, e.g., on fingerlings when water temperature declines through 15°C to prevent white spot;
- grade and segregate size/age classes;
- dry, de-silt and spell all ponds between crops, and the reservoir(s) each 1 – 3 years;
- disinfect ponds with lime following bacterial diseases, or where ponds can't be dried;
- disinfect equipment used to handle fish during disease outbreaks;
- remove moribund and dead fish, and dispose of appropriately;
- exclude birds from ponds, particularly fingerling ponds.

In fish culture industries overseas, other methods are also used to reduce or prevent exposure of fish to pathogens including disease-free certification of fish and eggs, disease inspections, vaccination, segregation of species, and eradication of diseased populations (certain bacterial and viral diseases only).

10.2.6. Prevention and control of diseases

Prevention of disease is achieved by reducing or eliminating the introduction of pathogens to the farm or certain parts of the farm, and/or by eliminating environmental and husbandry conditions that predispose silver perch to disease. Methods for preventing the entry of pathogens and exposure of fish to pathogens are given in the previous section. Prevention is also based on good husbandry and practices including:

- maintaining good water quality;
- ensuring good nutrition and feeding;
- routine disease monitoring, involving sampling of fish and examination of gill and skin tissues for pathogens;
- careful handling of fish at all times to minimise stress.

Control of disease is usually considered to be the reduction of disease to a level that is economic and biologically manageable. In general, it is extremely difficult to completely eradicate disease from a farm, and so health management plans are based on prevention and control.

Control of diseases involves the following actions:

- identifying signs and pathogens;
- prompt diagnosis of the disease;
- application of appropriate chemical therapeutants;
- quarantine of diseased fish in-situ in ponds/tanks;
- improvement of water quality;
- post-treatment monitoring of health and water quality.

10.2.7. Nutrition and feeding

Good nutrition is essential for normal survival, growth and reproduction of all animals. This is especially the case in intensive fish culture where nutrition is largely dependent on artificial diets. Generally diets fed to fish in cages and tanks needs to be higher quality than those fed to fish free-ranging in ponds where stocking densities are lower and there is usually access to some natural food. Manufactured feeds are formulated to contain the essential ingredients of proteins, fats, carbohydrates, fibre, vitamins and minerals. A deficiency of any of these basic nutrients may result in nutritional disease or lowered resistance to infectious diseases.

Besides using high quality formulated feeds for silver perch, effective delivery of the feed is essential to ensure fish receive the appropriate amounts of nutrients. Both overfeeding and under-feeding can be detrimental. Under-feeding reduces growth and can lead to lowered resistance to diseases. Over-feeding can cause a deterioration of water quality, in particular low DO, high

ammonia and high organic loads in ponds. Such conditions are known to predispose silver perch to a number of diseases. The need for close attention to nutrition and feeding cannot be over-emphasised.

10.2.8. Genetics and breeding

Certain species and strains of fish are more susceptible to some diseases than others, e.g., amongst Australian native fish, silver perch and Murray cod (*Maccullochella peelii peelii*) are far more susceptible to chilodonellosis than golden perch (*Macquaria ambigua*). Heritability for disease resistance has been demonstrated in some fish species (often in association with increase growth and performance), and so under controlled culture conditions some increased resistance to disease can be expected from breeding programs. Currently research into genetic improvement in silver perch is monitoring the disease status of hybrids, and of selected and unselected fish of the Murray River and Cataract Dam strains. These populations or strains are genetically distinct and have some different biological attributes.

In silver perch breeding programs, all broodfish are required to be tagged so that individuals can be identified. Certain abnormalities known to be carried by a small number of individual fish within certain strains of silver perch (e.g., paint-brush tails in the Cataract strain) can be eliminated by culling fish carrying the traits. Disease resistance varies between individual fish, and so the same principle of selection in broodfish may apply in silver perch. Although disease susceptibility/resistance between the progeny of breeders is somewhat difficult to determine, particularly where fingerlings from numerous parents are pooled, the high fecundity of silver perch makes the use of this strategy feasible in future breeding programs.

10.2.9. Role of the farmer

Many people have gone into silver perch farming without really knowing what is required to establish and maintain a successful fish farm. Aquaculture is a new industry in Australia and very few people have relevant experience. The farming of warmwater species like silver perch is very demanding and requires significant capital investment and technical knowledge, plus full-time commitment. During critical periods of the production cycle, the farmer must maintain close attention to numerous aspects of the farm; it is a 24/7 operation. At high water temperatures fish are growing fast, biomasses increasing rapidly, feed inputs are high, water holds less oxygen, fish require more oxygen, water quality can deteriorate rapidly, plankton can bloom and then crash, and diseases can progress rapidly. At low temperatures fish are stressed, immune systems are suppressed, certain pathogens are virulent and diseases can be difficult to treat. The farmer needs to appreciate these complexities to successfully manage the farm.

Silver perch farmers should develop and maintain microscopy skills to facilitate rapid on-farm diagnosis of diseases. They should be aware of chemicals that can be used legally and the appropriate storage and use of these chemicals. There are relatively few drugs and chemicals that can be used in aquaculture. Farmers should keep up-to-date with disease and chemical issues through their grower's associations and Government scientists and extension officers.

Good records are essential for a successful aquaculture business. Production information should include stocking densities, survival rates, growth rates, feed rates and quantities, food conversion ratios, harvest quantities, production rates, water quality records for each culture facility, and health records. The latter should include mortality information (numbers, sizes, dates), diseases, treatments and results of treatments. The records can be used to facilitate assistance from fish health experts, to identify critical periods and diseases on the farm, and to identify which culture conditions are conducive to disease and which can be altered or eliminated in future.

While there are many general principles that apply to all silver perch farms, each farm will have its own features, peculiarities and problems that need to be identified and managed.

10.3 Disease monitoring and diagnosis

10.2.10. Introduction

Disease monitoring and diagnosis are integral parts of health management that should be implemented on a regular basis, not just when there is a disease outbreak. Health management on silver perch farms must involve routine sampling of fish and microscopic examination of gill and skin tissues for pathogens and other signs of disease. Farmers who only take action when moribund or dead fish appear will have difficulty in controlling diseases, particularly diseases such as white spot, chilodonellosis and winter saprolegniosis. If these diseases are detected early, losses can be minimised or even prevented. At GAC, if only one or several specimens of either of the protozoans, *Ichthyophthirius multifiliis* (white spot) or *Chilodonella hexasticha* (chilodonellosis) is found during routine examination of gill and skin tissues, control and management actions are implemented immediately in that particular pond or tank, and fish in other facilities are subsequently monitored closely over the following days and weeks. By the time moribund and dead fish are found, disease outbreaks are usually well underway, the level of infestation is usually high, the fish are stressed, the outbreaks are difficult to control, and there is a likelihood of high mortalities. If silver perch are heavily infested with *I. multifiliis* or infected with *Saprolegnia parasitica* the prognosis for control is poor.

THE TIME AND EFFORT SPENT REGULARLY SAMPLING AND MONITORING FISH FOR DISEASE IS REWARDED WITH HIGHER SURVIVAL, IMPROVED FISH HEALTH, BETTER PERFORMANCE AND INCREASED PROFITABILITY IN THE LONG TERM.

10.2.11. Monitoring

10.2.11.1. Signs of disease

Close, daily observations of fish and facilities are essential management practices. The farmer needs to be able to differentiate between normal fish behaviour and culture conditions, and those that are abnormal. Experience provides the best basis for recognition. Often early signs of poor water quality and disease involve subtle changes in feeding behaviour, swimming patterns of fish or the appearance of water. Common and important signs in silver perch are:

- loss of appetite;
- other changes in feeding behaviour;
- abnormal or unusual colour, appearance – pale, dark or blotchy skin, frayed fins;
- abnormal behaviour – flashing, gasping at surface, flighty and/or rapid movements, spiralling swimming action, decreased swimming activity, swimming in a head-up or head-down position;
- lack of response to stimuli;
- fish congregating near surface, edges and/or aerators;
- moribund or dead fish;
- increasing numbers of dead fish.

It must be appreciated that silver perch can change behaviour without being diseased or stressed (e.g., after feeding strongly at the surface, fish may start to feed mid-water even at similar water temperatures). The farmer needs to trust in results of water quality and disease monitoring – if there are no pathogens or other signs of disease, and if the water quality is good, it can be assumed all is

OK. If the farmer is still concerned, the level of water quality and disease monitoring can be increased.

10.2.11.2. Fish

Routine or targeted monitoring of fish for disease enables early diagnosis and treatment of diseases. This monitoring involves both observation of fish and microscopic examination of skin and gill tissues for pathogens and other signs of disease. Fish can be sampled using hand nets, cast nets, seine nets and traps; cast nets are quick and efficient. The following guidelines are recommended:

- careful observation of fish daily during feeding; looking for unusual behaviour, feeding patterns, colour of fish etc;
- sample 5 larvae/fry/fingerlings each 7 days from rearing ponds;
- sample at least 3 fish monthly from each fingerling and grow-out facility;
- sample at lest 3 fingerlings from each culture facility when water temperatures decline through 15°C in autumn and early winter (examine tissue for white spot);
- sample large fish from ponds (on farms with a history of winter saprolegniosis) when water temperatures fall below 16°C in late autumn or early winter, or following rapid declines in water temperature (e.g., 4° – 5°C over a period of a 5 – 7 days) that are characteristic of a cold changes during winter;
- sample at least 3 fish from ponds during and after use of chemicals to control a disease to confirm efficacy of the treatment.

10.2.11.3. Water quality

Maintaining water quality is important in preventing disease; however, some changes are natural and cannot always be controlled. Unavoidable changes, e.g., decrease in DO after a series of hot, cloudy, windless days, rapid decline in temperature during cold changes, "crash" of algae (pond water changing from green to clear or tea colour as the algae dies) leading to low DO and high ammonia, can lead to stressful conditions that predispose fish to disease. It is important that the farmer monitors these changes in water quality (DO, temperature, pH and TAN) because infestations of protozoans and fungal infections can occur after such events. By monitoring water quality, the farmer can be prepared for potential disease events and take appropriate management actions. Absolute levels of the variables and changes-over-time help identify deteriorating water quality, or on the other hand eliminate poor water quality as a cause of abnormal behaviour. Farmers must understand the daily and seasonal dynamics of the key water quality variables so they can evaluate data collected during routine monitoring.

10.2.12. Diagnosis

Guidelines for the diagnosis of silver perch diseases are given in the disease diagnostic manual by Read et al. (2007). The manual contains photographs of pathogens and diseased fish, and serves as an on-the-spot reference to typical signs of silver perch diseases. The manual also has recommendations for the treatment and prevention of all diseases, syndromes and conditions that have been reported in silver perch.

10.2.12.1. Reactions – what to do!

The following figure "Disease Diagnostic Process" outlines the basic actions to be taken. The first and immediate reaction to changes in fish behaviour, moribund fish and/or changes to the appearance of water is to check the water quality and use records to determine trends over the last 3 – 7 days. Water quality is quickly and easily checked. If water quality is eliminated as a cause of abnormal fish behaviour, fish should be sampled and gill and skin tissues examined for signs of disease. Look for external signs of disease or damage, e.g., appearance and colour of skin, bird strikes, and then carefully examine gill and skin tissues for pathogens. If present, moribund fish should be sampled because they are the best indicators of problems and disease status.

Most disease outbreaks in silver perch culture involve the common diseases and pathogens – during the early stages of an investigation look for the obvious!

Changes in the appearance of both skin and gill tissue are common during a disease event; however, determining the aetiological agent(s), or causes, is occasionally difficult due secondary invasion of pathogens or other organisms, or the manifestation of overt signs caused by systemic disease. Changes in skin can include hyperaemia, haemorrhaging, ulceration, erosion, changes in pigment or thickening of the epithelium. Diseased gill tissue often shows hyperplasia and hypertrophy, causing cell growth and fusion between the secondary lamellae. Skin and gill tissue can have parasites and bacteria present without causing clinical disease; the interpretation of their significance will depend upon other clinical findings; this comes with experience.

Silver perch farmers must learn to accurately diagnose the common infectious diseases on-the-spot so that prompt control actions can be taken; this is critical in the case of acute diseases. Few veterinarians are trained in fish diseases or are readily available, and so physical assistance is usually not available to farmers – they must do it themselves!

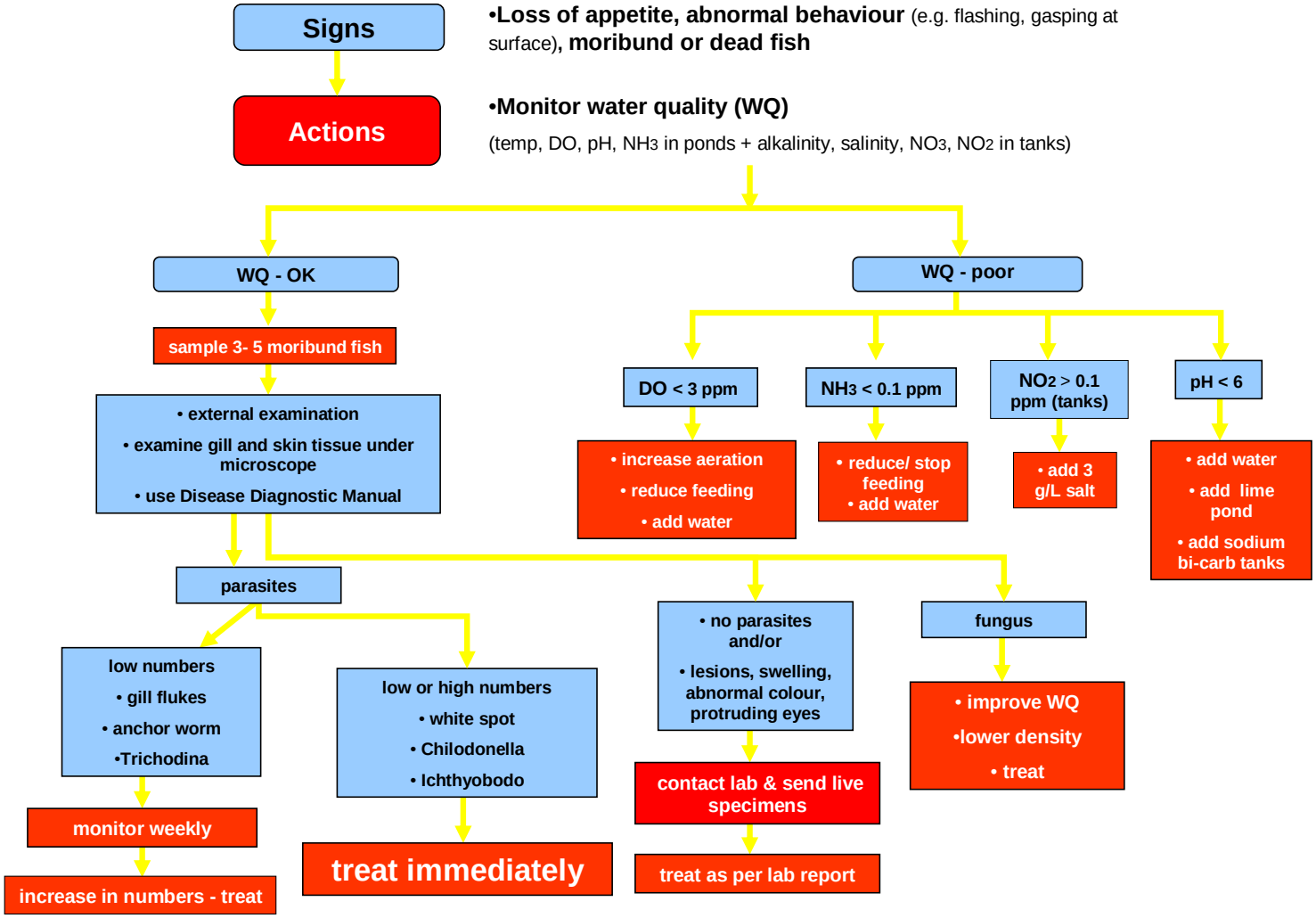
10.2.13. Essential and recommended criteria

The essential and recommended criteria in this and following sections of the Health Management Plan are from the Hatchery Quality Assurance Program (Rowland and Tully 2004) and provide the basis for accreditation and auditing of native fish hatcheries in NSW. They are included in this Health Management Plan because they are fundamental to efficient farms and good practices.

10.2.13.1. Essential criteria for the monitoring and diagnosis of disease

- *Specific laboratory area*: closed room, bench, sink, water supply, lighting.
- *Binocular microscope*: high power, internal light source, mobile stage, three objectives able to view at a magnification of X40, X100, X200, X400.
- *Appropriate level of knowledge to correctly identify the causative agents of disease*:
- *References to assist in the diagnosis of disease*: Rowland and Ingram (1991), Callinan and Rowland (1995), Noga (2000), Read et al. (2007).
- *Data sheets*: detailing rearing facility, date, species, number and size of fish sampled, type and number of pathogens; data sheets showing regular monitoring of fish health.
- *Sampling equipment*: seine net, cast net, lift net, plankton net, traps, sled with net; sterilising chemicals (formalin, chlorine) for use on equipment between batches.
- *Dissecting and laboratory equipment*: scalpels, scissors, probes, forceps, microscope slides, distilled water, petri dishes, vernier callipers, cover slips, knives, cutting board, scales, ruler, gloves.
- *Formalin and plastic containers*: to preserve tissues and/or specimens for despatch to laboratories for pathology.
- *Plastic bags (heavy duty), oxygen and boxes or bins*: for despatch of live and moribund specimens to laboratories for pathology and histology to aid in diagnosis of bacterial and other diseases. *Esky for the transport of chilled specimens*: to laboratories for pathology and histology.
- *Esky for the transport of chilled specimens*: to laboratories for pathology and histology.

Disease Diagnostic Process



10.4 Infectious diseases and treatments

10.2.14. Introduction

Fish under culture conditions are susceptible to infectious diseases caused by many different types of organisms, as well as non-infectious diseases that are caused by environmental conditions such as low oxygen and poor nutrition. Research into the culture of silver perch commenced in the late 1970's and so there have been records of diseases of silver perch for around 30 years. The expansion of the silver perch grow-out industry since the mid 1990's has seen a corresponding increase in the incidence of diseases, plus several new diseases and pathogens including winter saprolegniosis, gill flukes, and various syndromes and conditions. There are now around 20 recorded infectious diseases of silver perch (Table 10.1).

The common diseases of silver perch are caused by protozoans, monogeneans, fungi and bacteria. Protozoans are usually single-celled organisms that reproduce by binary fission and have specialised organelles such as cilia or flagella for locomotion. When present in large numbers, they greatly impair the epithelium, particularly of gill tissue. Some protozoans feed on the cells and mucus, while others cause physical injury, and some may produce toxins. Protozoans cause more diseases in silver perch culture than any other group of organisms. Monogeneans are flatworms (trematodes) commonly called flukes, and each species usually has a single host fish species. Flukes attach to gill and skin tissue using a large posterior, adhesive organ consisting of suckers and hooks. Fungi or water molds are ubiquitous in freshwaters, and form a large group of saprophytic organisms that feed opportunistically on dead organic matter. Fungi are important pathogens of fish, and are generally consider to be secondary pathogens, with infections often following physical damage; however, they can act as primary pathogens in association with severe stress and immune suppression. Fungal infections are inherently difficult to treat because of the complex biology of aquatic fungi. Bacteria form a large group of small, ubiquitous organisms that play a key role in nature. Bacterial cells are simple, do not contain complex organelles and can divide rapidly.

Infectious diseases have had a significant impact on commercial production and the viability of some silver perch farms through induced stress on fish, loss of growth and production, death of stock, and high costs of treatments. The common diseases of silver perch are briefly discussed below, and management options for some important diseases are given.

10.2.15. Diseases caused by ecto-parasitic protozoans, monogeneans and myxosporidians

Parasitic diseases are a major problem in warmwater fish culture, and translocation of cultured fish has undoubtedly contributed to the world-wide distribution of many ecto-parasites, including those in Australia. Silver perch diseases caused by protozoans and monogenean gill flukes account for around 80% of all records at GAC and on commercial farms.

10.2.15.1. White spot (ichthyophthiriosis)

Ichthyophthirius multifiliis is a ciliate protozoan that invades the skin and gill tissues of freshwater fish causing the acute disease ichthyophthiriosis, commonly referred to as ich or white spot. It can cause very high mortalities in silver perch and is particularly common in fingerlings stocked at high densities. *Ichthyophthirius multifiliis* is an obligate fish parasite that has a complex, temperature-dependent life cycle (Fig. 10.1). The free-swimming, infestive theront (20 – 40 µm in length) bores under the epithelium of skin and gill tissue, where it feeds and develops into a relatively large trophont (up to 1 mm in diameter) which is visible to the naked eye resulting in the characteristic white spots on skin and gills. Trophonts leave the fish, adhere as tomonts to solid substrates such as pond bottom, cages, tanks and nets, and undergo mitosis before releasing up to 3000 tomites that

differentiate into theronts. Reproduction may also occur under the epithelium of the fish. The length of the life cycle varies and may take 90 – 96 days at 3° – 5°C, 20 days at 10°C, 13 – 14 days at 13° – 15°C, 3 – 7 days at 20° – 25°C, and 3 – 4 days at 25º – 30ºC; there is no development below 3°C and over 30°C. Only the free-swimming theronts are accessible and susceptible to chemical treatment, and so effective control is dependent on periodic applications or continuous exposure to therapeutants. The disease is difficult to control at low temperatures because of the slow life cycle and rapid depletion of some chemicals in water. Salt at concentrations of 2 – 5 g/L is effective in tanks, and a treatment regime involving applications daily or each second day to maintain concentrations of formalin (30 mg/L) or copper (0.1 – 0.2 mg/L) controls ichthyophthiriosis in earthen ponds (Table 10.2).

10.2.15.2. Chilodonellosis

Chilodonella hexasticha is a ciliate protozoan that causes the acute disease chilodonellosis (Fig. 10.2). The native species silver perch, Murray cod and eastern freshwater cod (*Maccullochella ikei*) are particularly susceptible to this disease under culture conditions and unless diagnosed and treated promptly can cause high mortalities within several days. *Chilodonella hexasticha* has also been reported to cause fish kills of bony herring (*Nematalosa erebi*) and Murray cod in wild. A single treatment using salt or formalin is usually sufficient to control chilodonellosis, but because a cyst stage of *C. hexasticha* has been reported in eastern freshwater cod (see Fig. 10.2), and infestations of gill parasites can be difficult to control, repeated treatments over several days are recommended to ensure eradication of the parasite (Table 10.2).

10.2.15.3. Trichodinosis

The ciliated protozoan, *Trichodina* sp., is a common gill and skin parasite of silver perch which causes the disease trichodinosis (Fig. 10.3). This parasite rarely causes mortalities of silver perch, and many infestations remain at low levels. *Trichodina* sp. only causes problems when fish are stressed and immuno-suppressed. Heavy infestations can develop in ponds with a high organic load and very poor water quality. Infestations of *Trichodina* are readily treated with a single application of formalin or salt (Table 10.2), and the disease may be prevented by maintaining good water quality, using appropriate feeding regimes and ensuring tanks and cages are clean.

10.2.15.4. Ichthyobodosis

The flagellate protozoan, *Ichthyobodo necator*, causes the disease ichthyobodosis (Fig. 10.4). This disease can be difficult to diagnose at low levels of infestation because of the relatively small size of *I. necator* (< 20 µm). Careful preparation of gill tissue, magnification of at least X200 and examination in different fields of view are necessary for accurate diagnosis. Silver perch are relatively susceptible to this disease and if not accurately diagnosed and treated it can cause mortalities. Epizootics usually occur at high stocking densities in tanks and cages, and often follow periods of poor water quality or nutritional stress. Ichthyobodosis is controlled by salt or formalin (Table 10.2), and the maintenance of good water quality and hygiene are important preventative measures.

10.2.15.5. Henneguya

Henneguya sp. is a myxosporidian (Fig. 10.5) that was first recorded at GAC in 2001 following the introduction of silver perch from a commercial farm. Most infestations have been light and not caused mortalities or affected growth; however, in one outbreak at GAC, a high infestation rate on large silver perch resulted in chronic mortality over several weeks before the disease was controlled using formalin. Myxosporidians are obligate fish parasites with intermediate hosts such as annelids. The introduction of *Henneguya* to GAC demonstrates the risk of transferring pathogens on live fish, and the importance of effective quarantine to prevent their introduction to farms.

10.2.15.6. Gill flukes

Monogenean gill flukes are common fish parasites with strict host specificity and a non-pathogenic nature which reflects a highly evolved adaptability of their hosts. The dactylogyrid infesting silver perch is the oviparous *Lepidotrema bidyana* (Fig. 10.6). Gill flukes were not recorded at GAC between 1990 and 1995 despite routine disease monitoring during production experiments and hatchery operations. Since the mid 1990's, gill flukes have been common at GAC as well as on commercial farms. The introduction of broodfish from the wild, the movement of fish between farms, the intensification of silver perch culture and the difficulty in completely eradicating this parasite are probable causes of the high incidence of gill flukes.

Flukes use a series of hooks to attach to gill and skin tissue (see Fig. 10.6) and infestations can result in significant tissue damage as well as respiratory stress. Gill flukes rarely cause mortalities in silver perch, but heavy infestations reduce appetite and growth, cause stress and damage gill filament epithelium. Infestations have been associated with subsequent fungal and bacterial infections, although causation has not been proven. Flukes are readily treated using formalin or trichlorfon (Table 10.2), but care needs to be taken using formalin at temperatures around 25ºC and higher because of its adverse affects on water quality. Eggs of *L. bidyana* are resistant to these chemicals and up to three consecutive treatments 1 – 3 weeks apart, may be necessary to control infestations of this parasite. Eradication of this parasite from farms is difficult, and control relies on a combination of appropriate use of chemical therapeutants, drying and de-silting ponds and stocking fish that are free of gill flukes.

10.2.16. Fungal diseases

Aquatic fungi or molds are ubiquitous, saprophytic organisms that feed on dead organic matter. Some species are pathogenic and responsible for significant losses in freshwater aquaculture industries. Damage to the epidermis and skin caused by physical injury or other pathogens provide a site for infection by fungal spores, and other factors such as immune suppression, high organic loads and poor water quality are also associated with fungal infections. Fungal diseases are very difficult to treat once the infection has commenced, and management needs to be based on prevention.

10.2.16.1. Winter saprolegniosis

The causative agent of winter saprolegniosis in silver perch is the fungus, *Saprolegnia parasitica*. Most outbreaks commence at water temperatures below 16°C, and declining temperatures (e.g., > 5°C in 5 – 7 days) following cold changes during winter are associated with increased severity of the disease, suggesting suppression of the immune system also plays an important role in this disease in silver perch. The timing and severity of winter saprolegniosis varies within and between ponds, farms and years, and mortality rates can approach 100% on some farms (see Fig. 10.7). Infections occur on both skin and gill tissue (Fig. 10.7). In addition, many outbreaks are preceded or accompanied by infestations of the ecto-parasites *I. multifiliis*, *C. hexasticha* and/or *L. bidyana*. Stress and physical damage caused by ecto-parasites and partial harvesting of ponds appear to be major predisposing factors for winter saprolegniosis in silver perch, although the disease may occur in the absence of these factors. The disease often occurs in ponds with high stocking densities and biomasses (> 20,000 fish/ha and 10 tonnes/ha), high organic loads and poor water circulation. There have been no outbreaks of winter saprolegniosis at GAC despite water temperatures as low as 10ºC in winter and the presence of *S. parasitica*. The aetiology and pathogenesis of winter saprolegniosis in silver perch are still poorly understood, and the disease remains difficult to control. Salt (2 – 5 g/L) prevents infection by *S. parasitica*, and is recommended for use in tanks. Formalin and copper sulfate were reported to be effective in preventing saprolegniosis in channel catfish in the USA, but their efficacy under commercial conditions has not been proven and the disease remains problematic in the catfish industry. Further research is needed to evaluate the

effectiveness of these and other chemicals in preventing and controlling winter saprolegniosis in silver perch in earthen ponds. Recent research under laboratory conditions demonstrated that probiotics have potential for controlling saprolegniosis in silver perch, and there is evidence that the feed additives L-carnitine, ß-glucan and vitamin C can protect fish exposed to acute cold stress or high levels of ammonia and/or enhance the immune system in fish.

10.2.16.2. Saprolegniosis

Other than winter saprolegniosis, infections of *Saprolegnia parasitica* usually follow physical damage to the epidermis and are characterised by fungal growths that resemble cotton wool. The disease may occur at any temperature and is associated with poor husbandry such as rough handling, netting, over-crowding, lack of anaesthetics during transportation on farms, infestations of parasites, poor sanitation in hatcheries, and damage and stress from birds. Saprolegniosis causes fewer problems on farms where good aquaculture and health management practices are used.

10.2.16.3. Epizootic ulcerative syndrome (EUS or red spot)

EUS is a disease of some estuarine and freshwater fishes including silver perch caused by the fungus, *Aphanomyces invadans* (Fig. 10.8). The disease occurs naturally in coastal drainages of NSW and Queensland, and has not been recorded in the Murray-Darling River System with the exception of infected silver perch transported from a coastal hatchery to a farm on the Murray River. Silver perch are susceptible to EUS under culture conditions, and outbreaks have occurred on farms on the mid-North Coast and North Coast of NSW and south-eastern Queensland. EUS has been associated with high stocking densities, poor water quality, in particular low (< 5) and high (> 9) pH, high organic loads and high turbidity, and a water source containing infected fish. It is likely that any factor that causes acute skin necrosis will increase the susceptibility of silver perch to infection when spores are present. Mortality rates can be high in tanks and but are generally low in ponds. At GAC, there have been no mortalities of large silver perch (> 400 g) and generally low levels of mortality in fingerlings. Typical lesions and ulceration of the skin make infected fish unsightly and unmarketable as live or whole product (Fig. 10.8). There is no known treatment for EUS. Many fish recover from this disease, and resolution of lesions and ulcers may take 4 – 6 weeks. The fungus can enter farms in water supplies containing infected fish. The disease was first recorded at GAC in 1991, and most infections followed the use of water from the Clarence River during freshes and floods. Since 1999, water has not been pumped from the river during freshes or floods, and EUS has not been recorded at GAC over the last 7 years.

10.2.17. Bacterial and viral diseases

Seven bacterial diseases have been recorded in silver perch (Table 10.1). To date, the incidence has been low (< 3%) and most have not caused high mortalities at GAC or on commercial farms, with the exceptions of an outbreak of mycobacteriosis at GAC and two outbreaks of streptococcosis on commercial farms; one in a tank-based RAS and the other in ponds. There may have been unreported cases of bacterial diseases on commercial farms, particularly aeromonad infections. Bacterial diseases cause significant problems in warmwater fish culture industries in other countries. A high incidence of mycobacteriosis and streptococcis restricted the development of silver perch culture in Israel and so these diseases may become problematic as the silver perch industry expands and intensifies in Australia. Most bacterial infections are associated with stressful conditions such as poor water quality, rough handling, unsanitary conditions and high stocking densities, and their control is best achieved by using good aquaculture practices.

No viral diseases have been recorded in silver perch. However, under experimental conditions, silver perch were shown to be very susceptible to infection by the epizootic haematopoietic necrosis virus (EHNV) which causes the disease epizootic haematopoietic necrosis (EHN). Outbreaks of EHN are common in wild populations of redfin (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*) in south-eastern Australia, and so each silver perch farmer should ensure

that redfin and trout do not occur in the water supply, and are excluded from the farm. It is possible that silver perch will be susceptible to introduced viruses which are being increasingly reported in Australian native fish.

10.2.18. Specific recommendations for white spot, chilodonellosis, lepidotremosis and winter saprolegniosis

10.2.18.1. White spot (ichthyophthiriosis or ich; caused by the ecto-parasitic protozoan, Ichthyophthirius multifiliis)

- Treat immediately white spot is diagnosed; disease can progress rapidly causing high or total mortality; check fish in all other ponds and tanks on farm for the disease.
- Outbreaks can occur at any time of year; often associated with decline of water temperatures through 15°C, particularly on fingerlings; can also be prevalent in larval rearing and fingerling ponds at 25° – 30°C.
- Maintain good water quality; poor water quality (e.g., low DO) is a major factor in outbreaks.
- Ensure adequate nutrition.
- Monitor fingerlings when temperatures reach 15°C in autumn and winter; monitor fry and fingerlings weekly in summer.
- Ensure reservoir is free of trash fish.
- Parasite has a complex, temperature-dependent life cycle; only free-swimming stages susceptible to treatment; need to treat at least for duration of cycle (e.g., 3 – 4 days at 25° – 30°C, up to 3 weeks at 10°C).
- Treatments:
    - salt (NaCl) – 2 g/L, continuous in tanks;
    - formalin (37% formaldehyde) – 30 mg/L initially, then maintain between 20 and 30 mg/L daily; aerate 24 h/day and monitor water quality daily;
    - copper (~ 25% in copper sulfate) – 0.1 – 0.2 mg/L active ingredient, achieved by adding 0.2 mg/L copper initially, then around 0.1 mg/L daily; monitor copper daily; alkalinity must be > 50 mg/L, preferably > 80 mg/L; beware of DO depletion as copper is an algacide;
    - continue treatment until the disease is controlled.

10.2.18.2. Chilodonellosis (caused by the protozoan, Chilodonella hexasticha)

- Treat immediately *Chilodonella* is found; check fish in all other ponds and tanks on farm.
- Outbreaks can occur at any time of year and progress very rapidly causing high mortalities; most prevalent in winter and spring.
- Maintain good water quality.
- Ensure adequate nutrition.
- Ensure reservoir free of trash fish.
- An encysted stage has been reported and a follow-up treatment(s) may be necessary.
- Treatments:
    - salt (NaCl) – 10 g/L for 1 h in tanks, flush and repeat following day; 5 g/L continuous in re-circulating aquaculture systems;
    - formalin (37% formaldehyde) – 30 mg/L in ponds (temperature < 25^{o}C) and tanks;
    - copper (~ 25% in copper sulfate) – 0.2 mg/L copper active ingredient; alkalinity must be > 50 mg/L, preferably > 80 mg/L; beware of DO depletion as copper is an algacide.

10.2.18.3. Lepidotremosis (infestations of the gill fluke, Lepidotrema bidyana)

- Common parasite in silver perch.
- More prevalent in winter and spring; numbers generally build up slowly.

- Mortalities unusual, but infestations predispose fish to winter saprolegniosis and bacterial infections.
- Treatments do not kill egg stage; up to 3 consecutive treatments, 7 – 21 days apart may be necessary to eradicate.
- Treatments:
    - formalin (37% formaldehyde) – 30 mg/L (< 25°C);
    - trichlorfon – 0.5 mg/L active ingredient in ponds; 0.25 mg/L in tanks.

10.2.18.4. Winter saprolegniosis (winter sap or winter disease; caused by the fungus, Saprolegnia parasitica)

- Outbreaks commence at water temperatures < 16°C, often after rapid drop in temperature, e.g., up to 5°C over 3 – 7 days in response to cold changes in winter.
- Can cause total mortality if not treated, or if diagnosis and treatment are delayed.
- If possible, maintain 'lighter' pond biomasses (< 6 tonnes/ha) during winter.
- Do not over-feed in mid to late autumn (April – May).
- Ensure fish are free of ecto-parasites going into winter, particularly *I. multifiliis*, *C. hexasticha* and *L. bidyana*.
- Monitor fish weekly for fungal infections when temperatures < 18°C, and following rapid decreases in water temperature.
- Maintain ponds at maximum depth to buffer changes in water temperature and to assist fish acclimate to changes.
- Remove any fish having fungal growth from ponds.
- Where possible, harvest market-sized fish before winter; have sufficient tanks for quarantine or purging.
- Avoid partial harvesting of ponds in winter; if unavoidable, remove all fish captured and do not return any fish caught in the seine net or other harvesting equipment to the pond.
- Emergency harvest if a significant outbreak is imminent and hold fish in a continuous bath of 2 – 5 g/L salt.
- Treatments – the use of formalin or copper has not been validated for the control of winter saprolegniosis. The following treatments are recommended for ichthyophthiriosis and may be of value for winter saprolegniosis:
    - salt (NaCl) – 2 – 5 g/L, continuous in tanks to prevent infection – salt has limited effect onestablished infections;
    - formalin (37% formaldehyde) – 30 mg/L initially, then maintain between 20 and 30 mg/L by adding 15 – 20 mg/L daily; aerate 24 h/day and monitor water quality daily;
    - copper (~ 25% in copper sulfate) – maintain concentrations of 0.1 – 0.2 mg/L active ingredient, by adding 0.2 mg/L initially, then around 0.1 mg/L daily or each second day; monitor copper daily; alkalinity must be > 50 mg/L, preferably > 80 mg/L; beware of DO depletion as copper is an algacide.
    - continue treatment until the disease is controlled.

10.2.19. Essential criteria for treatment of diseases

- *Appropriate chemicals on farm*: have commonly chemicals on-hand for use when required, e.g., salt, formalin.
- *Known volumes of ponds and tanks*: at different depths of water so that correct dosage of each chemical can be applied without delay.
- *Known quantities of each chemical to be applied to each pond and tank at certain volumes and dosage*: so that correct dosage of each chemical can be applied without delay.
- *Microscopic examination of fish tissues post-treatment to ensure chemical has controlled disease*.
- *Records of chemicals applied to ponds*.
- *Knowledge of OH&S issues in relation to chemicals*.

Table 10.1. Infectious diseases and pathogens of silver perch.

Type of organism	Pathogen	Disease	Comment
Protozoan	*Ichthyophthirius multifiliis*	Ichthyophthiriosis (white spot, ich)	Common; acute
	Chilodonella hexasticha	Chilodonellosis	Common; acute
	Trichodina sp.	Trichodinosis	Common; chronic
	Ichthyobodo necator	Ichthyobodosis	Not common; potentially acute
	Tetrahymena sp.	Tetrahymenosis	Rare
	Coccidia sp.	Coccidiosis	Rare
Myxosporidian	*Henneguya* sp.		Uncommon
Ecto-commensal ciliate	*Ambiphyra* sp.		Rare
Monogenean	*Lepidotrema bidyana*	Gill flukes	Common; chronic
	Gyrodactylus sp.	Gill flukes	Rare
Copepod	*Ergasilus* sp.	*Ergasilus*	Rare
	Lernaea sp.	Anchor worm	Uncommon eastern drainage; may be common in parts of western drainage where carp in water supply
Fungus (mold)	*Saprolegnia parasitica*	Fungus Winter saprolegniosis	Ubiquitous pathogen Acute on some farms
	Aphanomyces invadans	Epizootic ulcerative syndrome (EUS, red spot)	Eastern drainage only; not common; notifiable disease
Bacteria	*Flexibacter columnaris*	Columnaris	Bacterial diseases are uncommon in silver perch culture
	Flexibacter, Aeromonas, Pseudomonas	Tail rot, fin rot	
	Aeromonas salmonicida nova	Goldfish ulcer disease	
	Streptococcis iniae	Streptococcosis	
	Mycobacterium spp.	Mycobacteriosis	
	Aeromonas hydrophila and other spp.	Aeromonad dermatitis	
	Chlamydia-like bacteria	Epitheliocystis	
Virus	Epizootic Haematopoietic Necrosis Virus (EHNV)	Epizootic haematopoietic necrosis (EHN)	Not recorded naturally in silver perch

Table 10.2. Recommended chemical treatments for the common and important infectious diseases of silver perch. For information on these and other diseases see Read et al. (2007).

Disease	Chemical	Treatment and comments
Chilodonellosis	Formalin	Ponds/cages 25 – 30 mg/L: 1 treatment usually sufficient; may need to retreat ponds after 3 – 4 weeks. Use as prophylactic for broodfish in winter (June) and early spring (August). Apply morning; distribute dose around pond and in aerator current; maintain 24 h aeration for 4 – 5 days; use 25 mg/L if water temperature > 25^0C; decreases dissolved oxygen (DO) so monitor daily and provide additional aeration if necessary. May reduce algae and zooplankton in ponds. Tanks 25 mg/L: continuous bath for 24 h; flush (exchange 100% water) and repeat following day; aerate well; do not feed; observe fish regularly during treatment; monitor DO, ammonia and nitrite in re-circulating aquaculture systems (RAS). Or 150 mg/L: 60 mins, flush well on completion; 1 treatment usually sufficient; not to be used on larvae or fry. May inhibit nitrification in RAS.
	Salt	Tanks 10 g/L; 1 h, flush and repeat following day; aerate well; do not feed. May inhibit RAS.
Ichthyophthiriosis (white spot, ich)	Formalin	Ponds/cages/tanks 25 – 30 mg/L; maintain levels between 25 and 30 mg/L until disease controlled; monitor formalin concentrations daily and retreat daily or each 2nd day; see previous comments about pond application of formalin.
	Copper	Ponds/cages/tanks 0.1 – 0.2 mg/L; maintain levels between 0.1 and 0.2 mg/L until disease controlled; monitor concentrations daily and retreat daily or each 2nd day; dissolve copper sulfate before application; alkalinity must be > 50 mg/L, otherwise copper toxic – concentrations of 0.25 mg/L and higher copper toxic to silver perch; continuous aeration; monitor DO, pH and ammonia; copper sulfate is an algacide and decay of algae can cause low DO.
	Salt	Tanks 2 – 5 g/L; continuous until disease controlled; may be up to 28 days at 15ºC or lower; flush tanks and retreat if high organic load.

Table 10.2. Cont'd

Disease	Chemical	Treatment and comments
Ichthyobodosis	Formalin	Ponds/cages 25 – 30 mg/L: 1 treatment usually sufficient; may need to repeat after 1 or 2 days; see previous comments about pond application of formalin. Tanks 25 mg/L: continuous bath for 24 h; flush (exchange 100% water) and repeat following day; aerate well; do not feed; observe fish.
	Salt	Tanks 10 – 13 g/L; 1 h, flush; repeat following day.
Trichodinosis	Formalin	Ponds/cages/tanks 15 – 25 mg/L: 1 treatment sufficient; may need to retreat larval rearing ponds after 2 – 4 weeks; see previous comments about pond application of formalin.
	Salt	Tanks 10 g/L; 1 h, flush; 1 treatment usually sufficient.
Gill flukes	Formalin	Ponds/cages/tanks 30 mg/L; 3 consecutive treatments 1 – 3 weeks apart to prevent re-infestation; see previous comments about pond application of formalin.
	Trichlorfon	Ponds/cages 0.5 mg/L; 3 consecutive treatments 1 – 3 weeks apart to prevent re-infestation; apply in morning; continuous aeration. Tanks 0.25 mg/L; continuous bath for 24 h; weekly treatments for 3 – 4 weeks may be necessary for control in RAS.
Saprolegniosis#		#Fungal disease difficult to treat once infection commenced; chemicals reduce new infections by killing infectious zoospores; management should be based on prevention.
	Formalin	Ponds/cages 30 mg/L: maintain 20 – 30 mg/L until disease controlled; need to monitor formalin and retreat daily or each 2nd day; see previous comments about pond application of formalin. Tanks 25 mg/L; continuous bath; flush tanks and retreat daily; aerate well; do not feed. 50 mg/L; 1 h bath; flush after treatment; retreat daily.
	Salt	Tanks 2 g/L; continuous, indefinite bath; flush and retreat if high organic load; preventative treatment only – not a control; may need 5 g/L in heavy infections associated with winter saprolegniosis.

Table 10.2. Cont'd

Disease	Chemical	Treatment and comments
Epizootic ulcerative syndrome (EUS)[#]		#Fungal disease difficult to treat once infection commenced; chemicals reduce new infections by killing infectious zoospores; management should be based on prevention. Ponds/cages Maintain good water quality.
	Salt	Tanks 10 g/L; 1 h, then 5 g/L continuous; no known treatment for infected fish; salt reduces new infections; maintain good water quality; aerate well; do not feed.
Bacterial diseases Columnaris Tail rot Aeromonad infections Gold fish ulcer Streptococcosis	Oxytetracycline	Tanks 20 mg/L; continuous bath 7 days at > 20ºC or 10 days at < 20ºC; maintain low light; good aeration; water exchange (100%) each 2 – 3 days to dilute bacterial load and improve water quality; retreat immediately.
	Salt	Tanks 2 – 5 g/L; continuous bath with antibiotic; flush tanks and retreat if water quality deteriorates; aerate well; do not feed; treatment does not kill bacteria – prevents fungal infection, reduces stress.

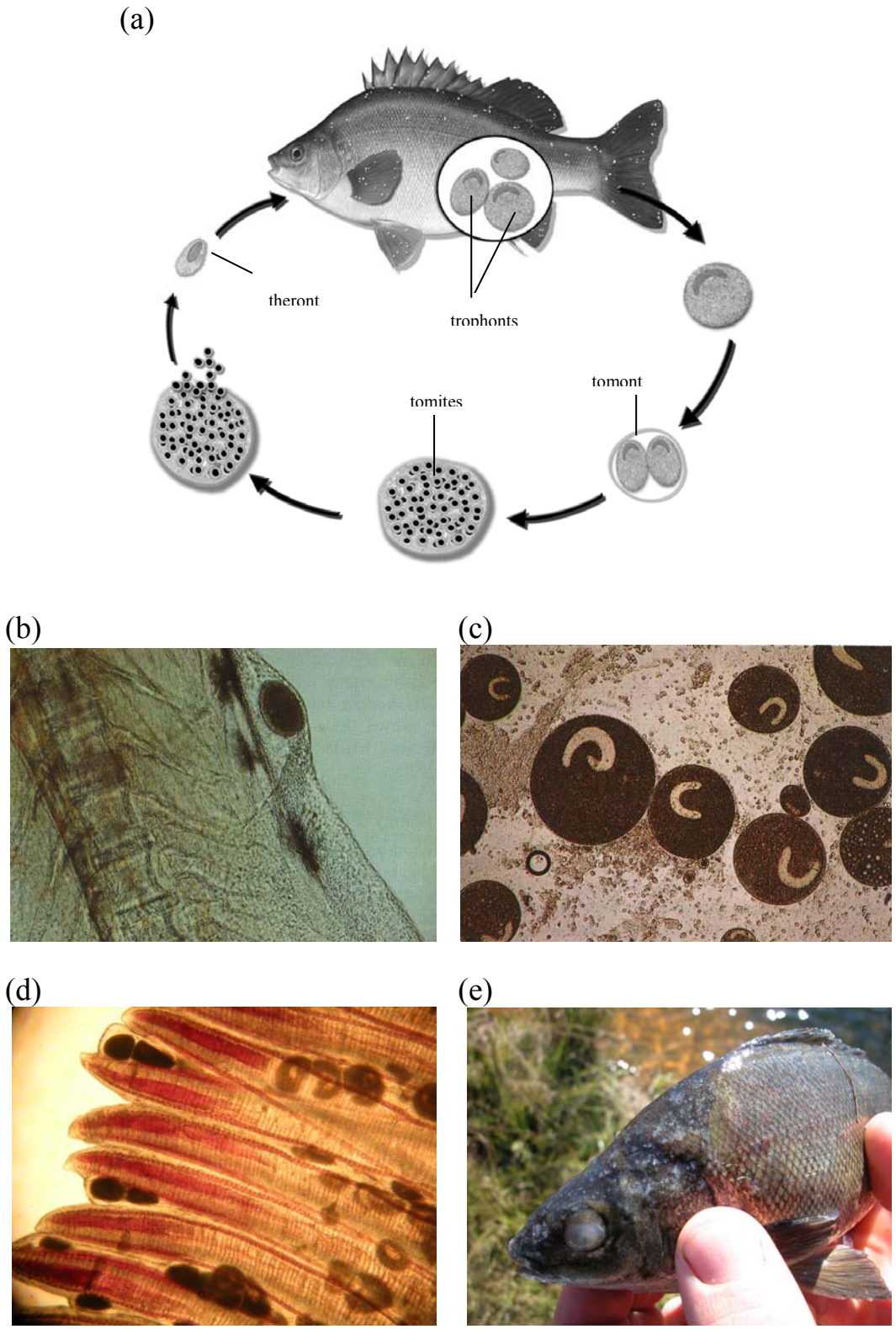


Figure 10.1. Ichthyophthiriosis or white spot – a serious ecto-parasitic disease of silver perch caused by the ciliated protozoan, *Ichthyophthirius multifiliis*. (a) life cycle; (b) trophont under skin epithelium (200X); (c) trophonts with horse-shoe shaped nuclei (nuclei not always evident) (X200); (d) trophonts on gill tissue (X100); (e) a heavily-infested silver perch with characteristic white spots and cloudy eyes.

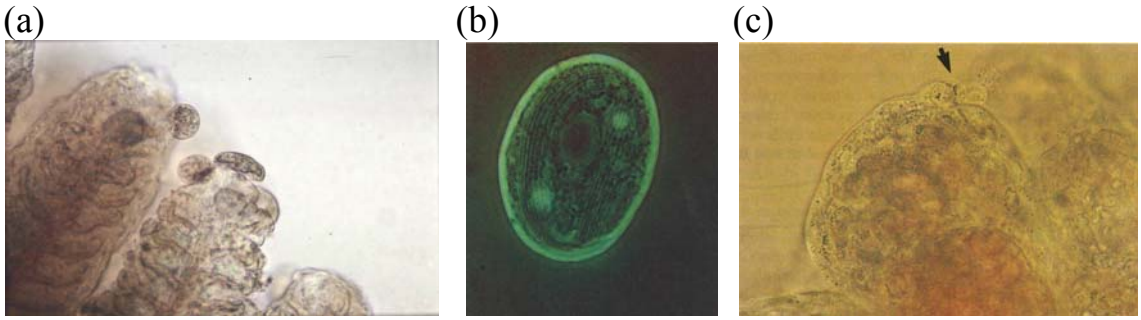


Figure 10.2. *Chilodonella hexasticha* – a ciliated protozoan parasite which causes the disease chilodonellosis. (a) parasites on gill tissue (X200); (b) *C. hexasticha* – note bands of cilia, granular cytoplasm, central dark nucleus (X 1000); (c) cyst with ciliates emerging (arrow) (X400).

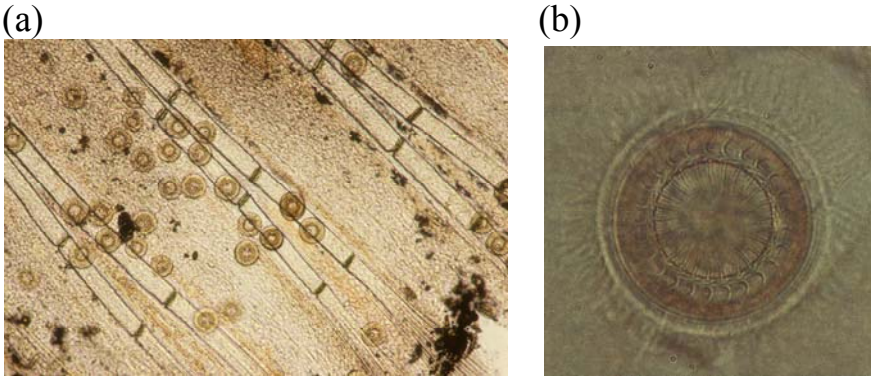


Figure 10.3. *Trichodina* sp. a common ecto-parasite of silver perch. (a) infestation on a fin (x100); (b) characteristic appearance showing cilia around edge and denticular ring (X400).

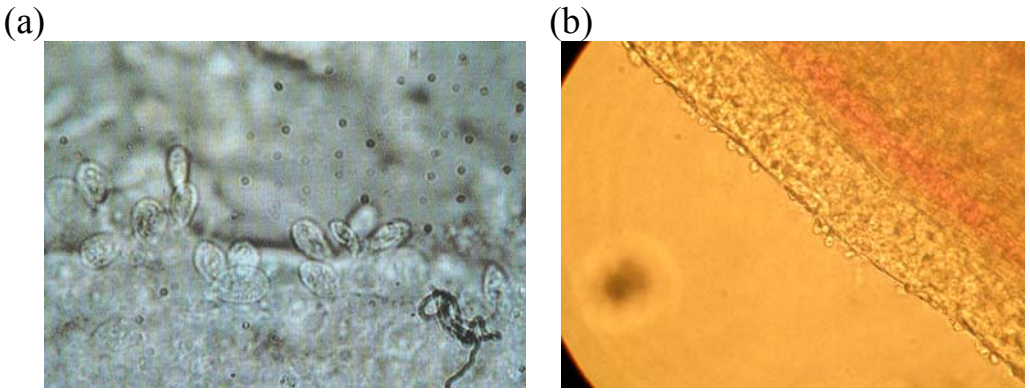


Figure 10.4. *Ichthyobodo necator* – a small flagellate protozoan which causes the disease ichthyobodosis. (a) attached to skin tissue showing characteristic oval shaped (X1000); (b) heavy infestation with parasites clearly visible on the edge of gill tissue (X400).

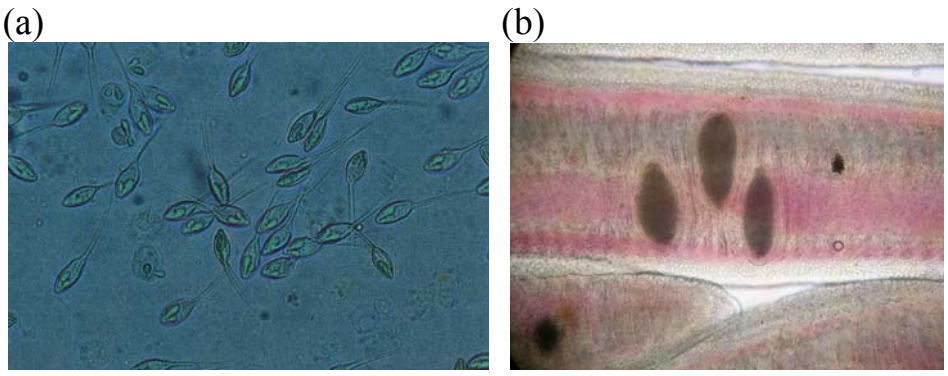


Figure 10.5. The myxosporidian *Henneguya* sp. (a) free-swimming spores (X400); (b) cysts in gill tissue (X100).

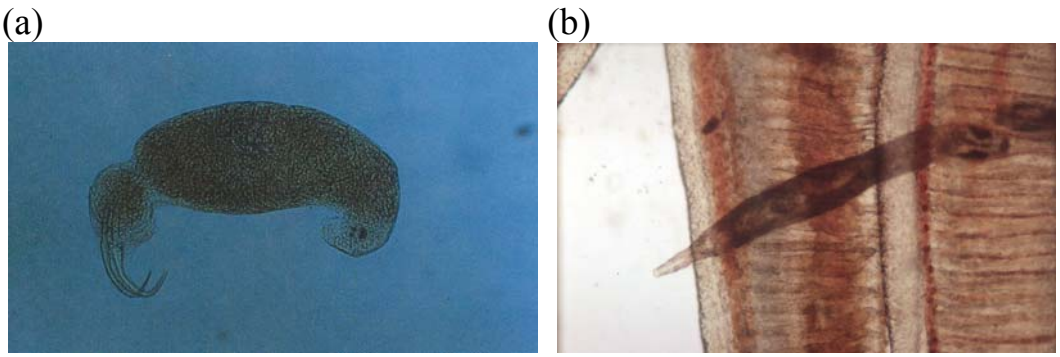


Figure 10.6. Monogenean gill flukes. (a) *Lepidotrema bidyana* the common gill fluke of silver perch showing hooks for attachment and eye spots (X400); (b) fluke attached to gill tissue (X100).

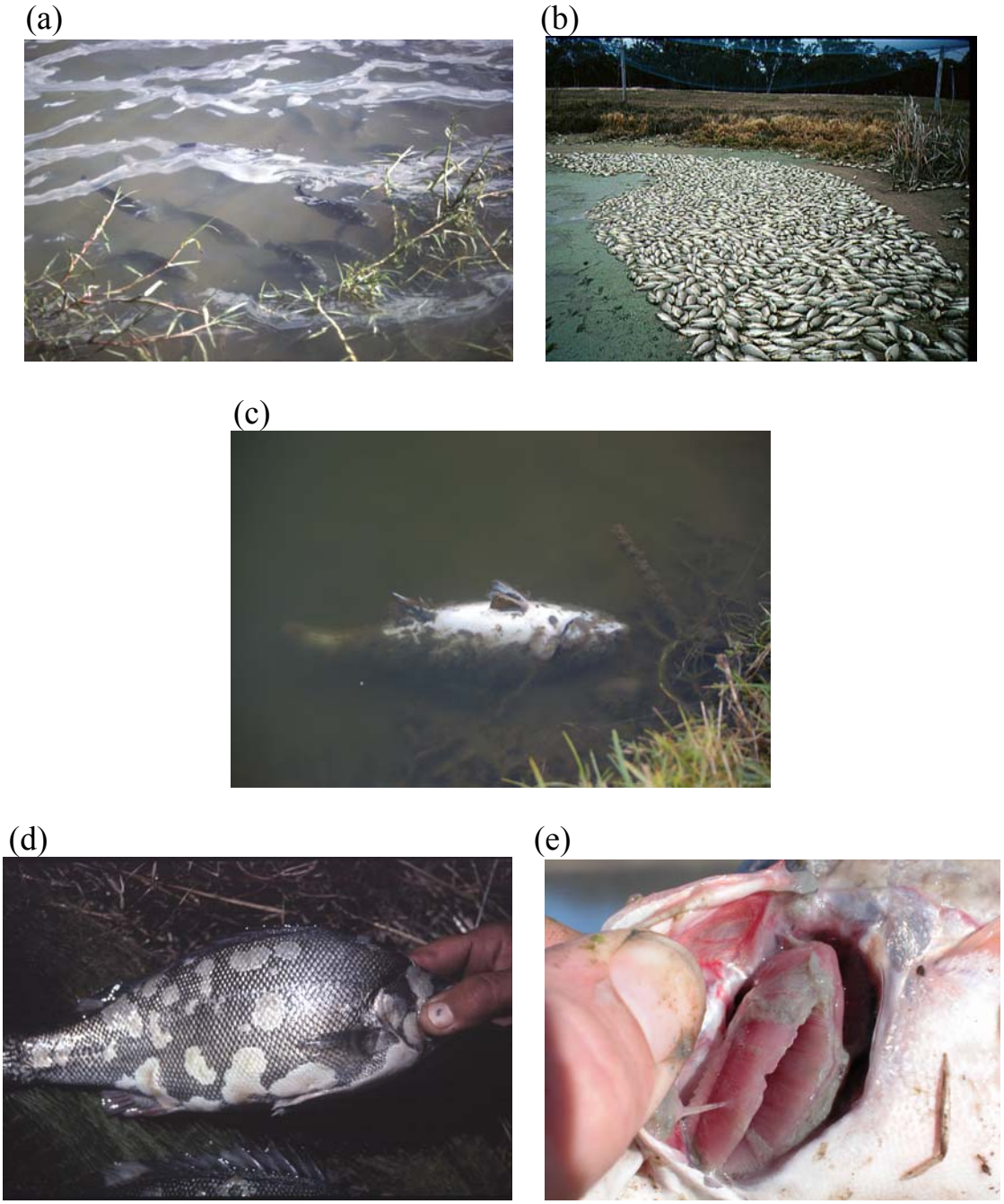
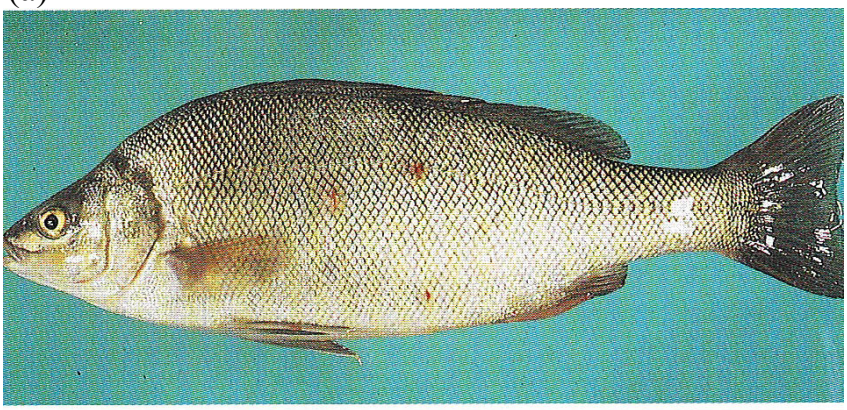
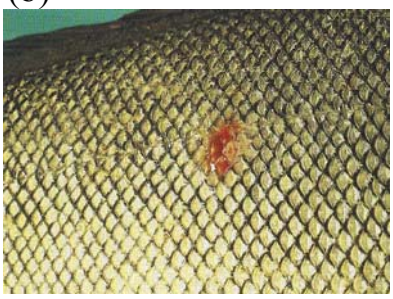


Figure 10.7. Winter saprolegniosis – a disease of silver perch caused by the fungus *Saprolegnia parasitica*. (a) infected fish near the edge of a pond; (b) the disease can cause total mortality of silver perch; (c) dead silver perch heavily infected with fungus; (d) moribund silver perch showing characteristic fungal lesions and abnormally dark skin; (e) gill tissue infected with *S. parasitica*.

(a)



(b)



(c)

Figure 10.8. Epizootic ulcerative syndrome (EUS or red spot) – a disease of silver perch caused by the fungus, *Aphanomyces invadans*. (a) silver perch with EUS; (b) a small lesion characteristic of EUS; (c) fingerlings with large ulcers caused by EUS.

10.3. Chemicals

10.3.1. Introduction

Chemicals play an important role in aquaculture. They are used to facilitate handling fish, control reproduction, improve water quality, promote plankton blooms, sterilise pond bottoms and equipment, and to prevent and control both diseases and aquatic plants. Chemical therapeutants are used to control some infectious diseases, particularly acute diseases that can cause very high mortalities of not treated promptly. While good facilities and practices are essential components of health management, the use of chemical therapeutants is necessary in the practical reality of intensive aquaculture where disease outbreaks are inevitable.

10.3.2. Regulation of chemical use

In Australia, the Australian Pesticides and Veterinary Medicines Authority (APVMA) regulates chemicals used in agriculture, including aquaculture. Other relevant legislation in NSW includes the *Stock Medicine Act 1989* and the *Food Act 2003*. Fish farmers need to familiarise themselves with the rules and regulations of these Acts and any amendments in order to use chemicals legally and responsibly. Several departments [e.g., Office of Chemical Safety, NSW DPI, NSW Food Authority, and Food Standards Australia New Zealand (FSANZ)] work closely with the APVMA on issues dealing with chemical evaluation and chemical residues in food. Further information can be obtained at www.apvma.gov.au, www.foodstandards.gov.au and www.dpi.nsw.gov.au.

Few chemicals are registered for aquaculture in Australia. To be approved for use in food animals, a drug must generally undergo rigorous testing of its efficacy in treating specific diseases in each species at specific dosages and routes of administration. Data must be obtained on residue dynamics, safety for the operator and consumer, and effects on the environment. This can require years of experimental trials and high costs. At the end of the process registered drugs must be used only in accordance with the label to treat the species on the label, at the directed dose rates.

The APVMA may allow the use of unregistered chemicals, or registered chemicals off-label, under a minor use permit (MUP). The issuing of MUPs is a "temporary" approval system chemicals when no alternative treatment is registered. The APVMA still carries out a risk assessment before issuing an MUP.

It is important to verify with the APVMA the applicability and validity of any MUP (this can be done using their web site) and/or to verify with NSW DPI under what conditions a chemical can be used. Some chemicals registered for use on other food-producing animals (e.g., chickens, sheep or cattle) may be used off-label with written veterinary directions (prescription). Some chemicals used only for water treatment and with no residue concerns can be exempt from registration (see some specific examples in Table 10.3.)

10.3.3. Industry practices and image

Australian aquaculture promotes itself as a "clean, green" industry. The use or detection of illegal residues of chemicals will jeopardise the future of an individual farm, a species, an industry and Australian aquaculture in general. Although the issue of chemical residues in fish tissues is more applicable to grow-out industries directly producing fish for human consumption, fish hatcheries are also subject to the same standards of chemical usage in Australia. Adherence to the use of chemicals as directed by the APVMA is a legal requirement of fish farmers.

10.3.4. Chemicals recommended for disease control in silver perch (Table 10.2 – Section 10.4)

10.3.4.1. Formalin

Formalin is an aqueous solution of formaldehyde gas (usually around 37%) which is recommended for use in silver perch culture to control some diseases. It can be used under the APVMA permit number PER8853. Formalin has some disadvantages. It is relatively costly to use in ponds (~ $480/ha/day) and adversely affects water quality. Formaldehyde is a strong reducing agent, and the application of formalin to water reduces dissolved oxygen, particularly at high temperatures (> 25ºC). It also reduces pH and increases ammonia through its toxic effects on phytoplankton, zooplankton, aquatic insects and other invertebrates. Formalin can cause damage to fish gills, eyes and liver, as well as induce anaemia and hypoglycaemia in some fish species. Despite these disadvantages, formalin is recommended for use at water temperatures up to 25°C because: (i) it can be legally used; (ii) it is an effective therapeutant for some diseases, and (iii) continuous aeration usually maintains adequate DO in ponds and tanks. Formalin is rapidly depleted in water, and so must be applied daily or each second day to maintain effective concentrations. Formalin is a volatile and irritating chemical, and may cause hypersensitivity and lung damage in humans. It needs to be carefully handled in well-ventilated areas, and protective clothing, gloves and mask should be worn. Formalin should be stored in a tightly sealed container, in the dark in a well-ventilated area, and above 4ºC to prevent the formation of paraformaldehyde, a white precipitate which may be toxic to fish.

10.3.4.2. Copper [as copper sulfate ($CuSO_4$)]

Copper sulfate is an aquatic algacide, which also controls some fish diseases caused by protozoans. It is also reported as having some bactericidal value. Copper sulfate contains approximately 24.5% copper and calculations should be based on this figure when determining the doses for application. Recommended concentrations of copper for control of some diseases in silver perch are 0.1 – 0.2 mg/L, and concentrations of copper of 0.25 mg/L and higher are toxic to silver perch i.e., > 1.0 mg/L copper sulfate. Copper is rapidly depleted in ponds, and so the treatment regime usually requires daily application to maintain effective concentrations until the disease is controlled. Copper concentration should be measured daily using a reliable instrument. Alkalinity has a major effect on copper toxicity; the lower the alkalinity, the more toxic the copper. Alkalinity should be accurately measured prior to copper sulfate application and must be > 50 mg/L and preferably > 80 mg/L. Alkalinity can be increased by the addition of calcium carbonate ($CaCO_3$) or sodium bicarbonate ($NaHCO_3$). Toxic levels of copper can cause gill damage and disrupt the fish's ionic regulation. Clinical signs of copper toxicity in silver perch include loss of appetite, spiralling swimming action, loss of equilibrium, listlessness, fish swimming at pond edges, gasping and dark colouration of skin. The copper sulfate should be thoroughly dissolved before being applied to ponds (or tanks) and care should be taken to disperse the copper over the entire pond area to avoid the creation of "hot spots". Copper may kill aquatic plants, and their subsequent decay will decrease DO. Consequently, DO should be monitored regularly and aeration should be maintained during and after the treatment until concentrations return to expected levels. There is currently an application to the APVMA for a permit to use copper sulfate for the control of some silver perch diseases.

10.3.4.3. Salt

Salt (NaCl) is a safe, relatively inexpensive chemical that prevents and controls some diseases of silver perch. It is also well known for its positive influences on osmoregulation and physiology in freshwater fish that lead to reduced stress, reversal of ionic losses, increased mucus production and

healing of damaged skin tissue. Coarse or pool salt can be used, and un-iodised table salt is suitable for small-scale facilities such as aquaria. The dose can be deposited directly into tanks and allowed to dissolve. Salt is not used in ponds because of the large quantities required, its accumulation due to evaporation, and detrimental environmental effects on receiving waters.

10.3.4.4. Trichlorfon

Trichlorfon [dimethyl (2,2,2-trichloro-hydroxyethyl) phosphonate] is an organophosphate that can be used to control infestations of some ecto-parasites in silver perch. Care needs to be taken in the use of trichlorfon for the following reasons: (i) it has been reported to have adverse effects on neurological functioning and the immune system of some warmwater fish even at low concentrations; (ii) it has adverse effects on pond ecology because of toxicity to decapods and zooplankton; (iii) development of resistance in gill flukes with repeated use. Degradation of trichlorfon increases with increasing temperature and pH, and with aeration and light, and so it should be applied early in the morning to maintain effective concentrations for as long as possible. Trichlorfon must be handled with great care because organophosphates can induce neurotoxic poisoning in humans. There is currently an application to the APVMA for a permit to use trichlorfon for the control of some silver perch diseases.

10.3.4.5. Oxytetracycline

Oxytetracycline is a broad spectrum antibiotic that is effective against a number of bacterial diseases of silver perch. It is relatively stable in water and is used in long-term baths (7 – 10 days) in tanks and aquaria, and can also be used to medicate feeds. Disease resistance to antibiotics is a significant problem in aquaculture, and they must only be used under veterinary prescription to control a diagnosed bacterial disease. Antibiotics must not be used prophylactically because continued use promotes resistance. There is currently an application to the APVMA for a permit to use oxytetracycline for the control of some silver perch bacterial diseases.

10.3.5. Other chemicals

10.3.5.1. Benzocaine and Aqui-S

Benzocaine and Aqui-S are permitted and registered fish anaesthetics respectively. Benzocaine does not dissolve easily in water and the preparation of stock solutions in alcohol is recommended. Anaesthetics play an important role in health management by reducing stress and physical damage during examination, transportation, grading, stocking, breeding and general handling.

10.3.5.2. Human chorionic gonadotrophin (HCG)

HCG is a synthetic gonadotrophin used to induce final oocyte maturation, ovulation and spawning in silver perch.

10.3.6. Chemicals that must not be used

10.3.6.1. Malachite green

Malachite green is an arylmethane dye ($C_{23}H_5N_2$) used to stain leather and acrylics in the textile industries. It was a common, effective and low-cost treatment for many ecto-parasitic and fungal diseases of freshwater fish. However, it has been recognised as a respiratory poison, tetratogen and suspected carcinogen, and is no longer permitted for use on food fishes in most countries including Australia. Malachite green stains all objects and persists in tissues for long periods of time. It should not be used in the silver perch industry; other chemicals such as salt, formalin and copper

sulfate can be used to treat silver perch diseases that were previously controlled using malachite green.

10.3.6.2. Chloramphenicol

Chloramphenicol is an antibiotic that is used in the last line of defence against some human diseases. Consequently it should not be used in aquaculture, and its use in animal industries is highly illegal in most countries. Chloramphenicol is hazardous to a small percentage of humans because it can cause aplastic anaemia which is often fatal.

10.3.6.3. Nitrofurans

Nitrofurans are synthetic antimicrobials that are effective against some common pathogens of fish. However, they are carcinogenic, genotoxic and mutagenic and are strictly illegal for use in most countries.

Table 10.3. Current status of some chemicals in silver perch aquaculture as determined by the Australian Pesticides and Veterinary Medicines Authority (APVMA).

Category	Chemical#	Use/comment
Registered	Aqui-S	anaesthetic
	Human chorionic gonadotrophin (HCG)	spawning induction
Permit	Formalin	external parasites, fungal infections
	Benzocaine	anaesthetic
	Oxytetracycline*	antibiotic
	Copper sulfate*	external parasites, fungal infections
	Salt*	external parasites, prevention of fungal infections, reduction of stress
	Trichlorfon*	gill flukes
Exempt	Calcium carbonate, calcium hydroxide, calcium oxide, calcium sulphate, magnesium carbonate	increase pH, increase alkalinity, improve buffering, calcium source, sterilise pond bottom
	Zeolite	absorption of ammonia
	Aluminium sulphate, ferric chloride	flocculation of suspended clay colloids
	Fertilisers – organic and inorganic	promotion of plankton blooms

Check with APVMA for expiry dates of permits.

* Applications for MUPs have been submitted to APVMA (January and February 2007).

10.4. Site selection

10.4.1. Introduction

The success of a fish farm is dependent on many factors, beginning with the selection of a suitable site, and the design and construction of facilities that enable efficient and economical operation. Poor site selection and design will inevitably lead to failure. The selection of a site with an abundant supply of good quality water is the first and most important step in establishing a silver perch farm. Other important factors to be considered when selecting a site are: the suitability of soils for pond construction; topography; susceptibility of the site to flooding; availability of 3-phase electricity, labour, equipment suppliers, services, and transport for feed and fish; proximity to customers and stocking sites; ability to secure the site against poaching and theft.

10.4.1.1. Location

The farm should be located in a region with an appropriate temperature regime. Although silver perch is a temperate, warmwater species and can tolerate a wide range of water temperatures (2º – 38ºC), the temperature range for optimal growth is thought to be 23º – 28ºC. Ideally water temperatures should be in the range 18° – 30°C for seven or more months of the year to maximise the growing period and minimise the time taken to produce market-size fish. Low water temperatures (< 10ºC) are thought to compromise the immune system of silver perch, increasing susceptibility to winter saprolegniosis and other diseases. In addition, some diseases such as white spot and winter saprolegniosis are difficult to treat at low temperatures.

For ecological and biosecurity reasons, silver perch should be cultured within the Murray-Darling River System, where it is endemic. Silver perch is not found naturally in rivers in the eastern drainage, and escapees from fish farms have the potential to become established in rivers and creeks where they will interact with endemic fish fauna. Farms in the eastern drainages must ensure they meet the permit requirement that prohibits the release of effluent waters to natural waterways.

10.4.1.2. Water supply

The abundance and quality of water are major factors determining the success of a fish farm; a regular, abundant supply of good quality water is essential. The supply must be guaranteed during drought periods, which can last for many years in inland regions of Australia. Seasonal changes in quantity and quality also need to be considered during site selection. It is emphasised that no amount of understanding, monitoring and management will compensate for an inadequate water supply. Large, permanent rivers and creeks are the most commonly used sources. Surface waters are ideal for ponds because they are usually good quality and contain natural organisms such as zooplankton that play a key role in the rearing of larvae in earthen ponds. Under-ground water has a number of features that make it particularly suitable for use in fish culture: usually regular and dependable; free of pathogens, organic, agricultural or industrial pollution, and suspended solids; relatively constant temperature; free of trash fish and other undesirable organisms. Under-ground water may have excessively high or low alkalinity and/or hardness, be deficient in oxygen, saturated with nitrogen, or contain relatively high concentrations of carbon dioxide, and other harmful gases such as methane and hydrogen sulphide, and minerals such as iron, lead, zinc and copper. Only some of these limitations can be over-come by storing and aerating the water before use. Consequently, it is very important to have the water thoroughly analysed and evaluated by an expert before a commitment is made to use an under-ground supply. Water quality criteria for silver perch and other native freshwater fishes are given in Table 10.4.

Rainfall is characteristically unreliable in much of inland Australia and so any venture that considers the use of rain run-off as the major supply (i.e., catchment and storage in a reservoir)

should seriously estimate the requirements and water budget. Annual water budgets for 1 ha of grow-out ponds at different locations in NSW range from about 35 ML at Grafton on the North Coast to 46.5 ML at Bourke in the drier, western part of the state. The quality of water in a large catchment dam will deteriorate as the level falls and aquatic organisms and suspended solids become concentrated. Water from domestic supplies should be avoided as it is expensive and usually contains chemicals such as chlorine which is toxic to fish. However, domestic supplies (after de-chlorination) are suitable where large quantities are not required, e.g., for quarantine or purging. The water entering a hatchery must be of high quality and free of sewage, heavy metals, oils, pesticides, chlorine, methane, hydrogen sulphide and other poisonous substances. The use of eutrophic water (i.e., high in nutrients) will lead to an increased incidence of some parasitic diseases, as well as excessive algal blooms and subsequent problems with water quality. The cost of supplying water to the site may be a major factor determining the economic viability of a farm. Pumping costs are high and must be minimised. Obviously the farm should be as close as possible to its water supply. Gravity flow should be utilised where possible because it is very efficient and cheap.

10.4.1.3. Soils

Ponds should be constructed from impervious soils to eliminate or at least minimise the loss of water by seepage; clay or clay loams are ideal. A proposed site should be surveyed for gravel or sand layers, rock strata or other soil characteristics that may interfere with water holding capacities. Dispersive or flocculative soils can lead to embankment failure or "tunnelling". Advice on the suitability of soils for pond construction should be sought from appropriate experts. If the land was previously used for cropping, the soil should be tested for pesticide residues. Areas with acidic soils should be avoided because of resulting low pH and alkalinity in impounded water. Areas with high ground water cause problems because it is difficult or impossible to build ponds. If they can be built, they cannot be completely drained and dried; essential operations for efficient pond management.

10.4.1.4. Size and topography

The area of land selected for a farm should be large enough to accommodate the maximum number of ponds, a reservoir, an effluent/settlement dam, and buildings (hatchery, office, laboratory, tanks, storage, workshop etc.). Future expansion should be considered when selecting the site. The land should be relatively flat and ideally slope gently away from the source of water or reservoir, to facilitate the gravity supply of water.

10.4.2. Essential and recommended criteria

10.4.2.1. Essential criteria

- *Abundant supply of good quality water*
  - see Table 10.4 for water quality criteria for native fish;
  - for details of key water quality variables see Rowland (1995c) and Rowland (1998);
  - surface waters (rivers, creeks, lakes, canals) can be used for all purposes (ponds, tanks,
  - spawning, incubation), but are especially important for larval rearing because these waters usually contain plankton that "seed" the ponds;
  - underground waters (bores, wells) can be used for all purposes if suitable quality;
  - especially suitable for tanks, quarantine, purging off-flavours, spawning, incubation, and in some cases where quality allows, for larval rearing ponds.
- *Out of reach of the Probable Maximum Flood level:* to prevent the escape of fish during floods.
- *Soils that are suitable for pond construction*: to minimise construction costs; minimise seepage and enable efficient use of water.

10.4.2.2. Recommended criteria

- 3-phase power.
- Annual water budget of at least 40 ML/ha/year.
- Annual water temperature range within 10º to 30ºC.
- Combination of surface and underground water supplies: for pond and hatchery supplies respectively.
- Close proximity to water supply (e.g., within 2 km for surface supply).
- Available land: large enough to accommodate the maximum number of ponds, a reservoir, an effluent/settlement dam, and buildings (hatchery, office, laboratory, tanks, storage, workshop etc.) and to allow for future expansion.
- Topography: the land should be relatively flat and ideally slope gently away from the source of water or reservoir, to facilitate the gravity supply of water.
- Soils analysed for pesticide residues.

10.4.2.3. Not Recommended

- *Run-off water as the major water supply*: i.e., run-off, surface water captured in a large dam(s).

10.4.2.4. Other factors to consider

- Availability of suitable manpower to operate the farm.
- Proximity to equipment suppliers and service industries.
- Availability of transport for fish and feed.
- Proximity to customers and stocking sites.
- Ability to secure the site against poaching and sabotage.

Table 10.4. Water quality criteria for silver perch farms. Values are ranges, maximum levels for heavy metals and minimum and maximum levels for other variables. After Rowland and Tully (2004).

Variable	Values	Comments
Alkalinity (mg/L)	20 – 400	
Hardness (mg/L)	50 – 400	Alkalinity, hardness, conductivity and metals are relatively stable variables that "characterise" water. Waters that are very alkaline (> 500 mg/L) and/or hard (> 500 mg/L) are unsuitable for native fish and may cause slow growth, tissue damage, morbidity and mortality. Heavy metals can have sub-lethal or toxic effects on native fish at concentrations above those listed.
Conductivity (µS/cm)	0 – 3,500	
Heavy metals (mg/L)		
cadmium	0.003	
calcium	10 – 160	
copper	0.006	
iron	0 – 0.5	
lead	0.03	**Variables in bold <u>must</u> be analysed during site selection.**
manganese	0 – 0.01	
mercury	0.002	
zinc	0.05	
pH	6.5 – 8.5	pH, dissolved oxygen, ammonia and nitrite are relatively unstable variables and can change rapidly from acceptable to stressful or lethal levels under certain conditions. Some underground waters can be very low in pH and oxygen, and high in nitrogen, hydrogen sulphide, carbon dioxide, ammonia and nitrite because of anoxic conditions and high pressure. Vigorous aeration of water when it reaches the surface increases oxygen and pH, and releases hydrogen sulphide, carbon dioxide, and nitrogen as gases. Consequently analyses of these variables may not accurately determine the suitability of water for fish culture.
Dissolved oxygen (mg/L)	> 5.0	
Phosphorus	0.01 – 3.0	
Nitrogen	< 105% saturation	
Hydrogen sulphide (mg/L)	< 0.002	
Total ammonia (mg/L)	< 3.0	
Un-ionised ammonia (mg/L)	< 1.0	
Nitrite (mg/L)	< 4.0	

10.5. Design and operation

10.5.1. Introduction

A silver perch farm must be well designed to enable efficient and environmentally-sound operation, the maintenance of fish health and the production of high quality fingerlings and market-sized fish. The basic components of a silver perch farm are: water supply; reservoir; earthen ponds (0.1 – 0.5 ha surface area) for broodfish, larval rearing, and fingerling production; earthen ponds for grow-out, either free-ranging in ponds or in cages in the ponds; an effluent-settlement dam; a building with facilities such as tanks for spawning, egg incubation and quarantine; laboratory; office; support buildings; and associated electricity, plumbing, pumps, air blowers, filters, vehicles and other equipment. Re-circulating aquaculture systems (RAS) can be used for quarantine, broodfish, over-wintering fingerlings and purging.

Reservoirs, ponds and tanks must be screened to prevent the entry of "trash" fish to the farm and the escape of fish from individual facilities. It is very important that trash fish such as trout, redfin (*Perca fluviatilis*), mosquito fish (*Gambusia holbrooki*), goldfish (*Carassius auratus*), noxious species such as carp (*Cyprinus carpio*) and banded grunter (*Amniataba percoides*), as well as non-target fish such as eels and gudgeons that may be present in the water supply, are excluded from the reservoir and ponds. If present these fish can: (i) prey on larvae, fry and fingerlings; (ii) stress broodfish, fry and fingerlings; (iii) carry pathogens and cause disease; (iv) compete for food; (v) contaminate batches of fingerlings dispatched from the hatchery. Fish in the reservoir and the effluent-settlement dam act as a source of pathogens and subsequently increase the incidence of disease on a farm.

10.5.2. Reservoir

The reservoir receives and stores water from the major source(s). It enables control of both supply and quality, exclusion of trash fish and other unwanted aquatic organisms, and efficient delivery of water to ponds and the hatchery. The reservoir must be kept free of fish, particularly silver perch (as the culture species) and other fish such as carp that are known to carry pathogens that cause disease in silver perch and other native fish.

The reservoir also provides a reserve of water at critical times, e.g., during power failure, pump break-down and flood. Reservoirs are normally earthen, with separate and screened inlets and outlets. Water from under-ground supplies can be stored in earthen, fibreglass, plastic or concrete reservoirs. Reservoirs should be aerated to prevent stratification and to maintain good water quality.

10.5.2.1. Essential Criteria

- *Screened inlet for surface waters*: 500 μm screen to prevent the entry of trash fish (all life cycle stages, including larvae) and other aquatic organisms into the reservoir; robust screen, e.g., 2m x 2m stainless steel.
- *Screened outlet for all waters*: 5 mm to prevent the escape of trash fish from the reservoir.
- *Capacity to drain and dry reservoir*: for the following reasons: (i) to remove all fish, especially trash fish; (ii) to desiccate pathogens; (iii) to enable disinfection, e.g., application of lime to the substrate; (iv) to enable silt to be removed and/or the substrate to be tilled or scraped; (v) to enable repairs and general maintenance.

10.5.2.2. Recommended Criteria

- *Located and constructed to enable gravity flow*: efficient, reliable and economical delivery of water to all facilities.
- *Capacity should exceed twice volume of largest ponds*: e.g., if largest pond holds 5 ML, reservoir should be at least 10 ML.
- *Aeration*: e.g., paddlewheel or diffused aerator, to maintain good water quality and prevent or reduce stratification.
- *Back-up water supply for hatchery building*: an elevated reservoir to ensure water is available under gravity during power failure.

10.5.3. Earthen ponds

Earthen ponds are the basic production unit on silver perch farms, and are principally used to hold broodfish, rear larvae, produce fingerlings and market-size fish. Ponds must have appropriate plumbing to enable efficient management of water, and screened outlets to prevent the escape of fish. Cage culture can be carried out in ponds.

10.5.3.1. Essential Criteria

- *Drainable by gravity*: drainage line from deepest section of pond.
- *Separate inlet and outlet*.
- *Screened (500 – 1,000 µm) inlet if water directly from surface supply*: to prevent the entry of trash fish.
- *Screened outlet structure*: 1 mm^2 when larvae stocked, 6 mm for fry and fingerlings, 6 – 25 mm for broodfish and large fish; to enable the draining of ponds and to prevent the escape of fish during harvest, water exchange and over-flow.
- *Harvest sump, supplied with fresh water*: for all ponds, to reduce stress and physical damage to fish, and to provide good quality water during harvest.
- *Aeration for intensive culture*: 3-phase power; to maintain optimal water quality.
- *Records*: for each pond to include the following – date, species, number of fish, size of fish, activity (stocking, feeding, harvest, sampling), information on disease, chemical treatments, general comments.
- *Larval rearing ponds should be dry before use*: (i) to enhance the production of plankton, particularly the zooplankton on which larvae feed; (ii) to desiccate pathogens; (iii) to treat with lime if necessary; (iv) to provide conditions for good water quality through the oxidation of sediments; (v) to reduce or eliminate predators such as large aquatic insects and dragon fly larvae; (vi) to ensure there are no trash fish in the ponds when larvae are stocked; (vii) to reduce or prevent growth of macrophytes.

10.5.3.2. Recommended Criteria

- *Shape*: square (0.1 ha surface area) or rectangular (0.2 – 0.5 ha).
- *Size*: broodfish 0.05 – 0.1 ha; larval rearing 0.1 – 0.5 ha; fingerling and grow-out 0.1 – 0.5 ha.
- *Outlet tower – concrete*: also called a monk or penstock; contains boards to control water level and screens to prevent escape of fish; vertical PVC pipes are acceptable as outlets, but are not recommended.
- *Inlet pipe*: 150 mm (6") diameter, with valve.
- *Outlet pipe*: at least 150 – 200 mm (6" – 8") diameter; a diameter of 500 mm (20") enables rapid water exchange and facilitates external drain harvest of fish.
- *Internal sump*: e.g., 50 cm deep, 50 cm wide, 20 m long for 0.1 – 0.2 ha ponds; for collection of fish after the pond is drained.
- *External sump*: for collection of fish outside the pond.

- *Overhead netting of larval rearing and fingerling ponds*: to prevent bird predation.
- *Ponds to be drained and dried annually or at least each 2 – 3 years*: for the following reasons; (i) to remove all fish; (ii) to desiccate pathogens; (iii) to treat with lime if necessary; (iv) to enable silt to be removed and/or the substrate to be tilled or scraped; (iv) to enable repairs and general maintenance; (v) to provide conditions for good water quality; (vi) to reduce or prevent growth of macrophytes.
- *Larval rearing ponds dry over winter and early spring*: to facilitate plankton blooms and good water quality.
- *Plant a crop (wheat, oats, rye grass) every 2 years in larval rearing ponds during late autumn and winter, burn-off stubble*: to use phosphorus bound-up in the soil and assist in promotion of rotifer blooms.
- *Lime bottom of larval rearing ponds*: especially if alkalinity is low (< 20 mg/L); may promote rotifer blooms and reduce problems with fairy shrimp and clam shrimp; 500 – 1,000 kg/ha agricultural limestone ($CaCO_3$).
- *Vehicular access to all banks.*

10.5.4. Effluent-settlement dam

All silver perch farms are required to have an effluent-settlement dam as part of the permit requirements. The effluent-settlement dam received all effluent water from the ponds, hatchery and other facilities, except water containing salt – there should be a separate dam for any water with salt. The settled water can be re-used for fish culture or used for irrigation.

10.5.4.1. Essential Criteria

- All silver perch farms must have an effluent-settlement dam.
- Twice volume of largest pond: if the largest pond contains 5 ML, the effluent-settlement dam should be 10 ML or larger.
- Ability to screen overflow/outlet to prevent escape of fish.

10.5.4.2. Recommended Criteria

- Earthen dam.
- Does not receive run-off.
- Pump that can return water to reservoir and/or ponds.
- Ability to drain and dry: (i) to remove all fish, especially trash fish; (ii) to desiccate pathogens; (iii) to enable silt to be removed and/or the substrate to be tilled, scraped or limed; (iv) to prevent excessive growths of macrophytes such as milfoil (*Myriophyllum* spp.); (v) to enable repairs and general maintenance.
- Aeration: to maintain optimum water quality.
- Effluent to enter over wide area: to facilitate the settlement of solids.
- Salt-evaporative pond(s): separate to effluent-settlement dam; to receive water containing salt from hatchery and quarantine facilities.

10.5.5. Building(s), infrastructure and equipment

Building(s) on silver perch farms contain fish breeding facilities, quarantine facilities and associated power and plumbing systems, a laboratory, office(s), stores and records.

10.5.5.1. Essential Criteria

- *Filtration/screening of all surface waters and water from earthen reservoirs that enter the hatchery building*: sand filtration and/or cartridge filters of 100 µm; under-ground water directly from bore may not need to be filtered, but may need to be stored and aerated.
- *Spawning tanks*: 1,000 – 5,000 L; recommended with temperature control.

- *Quarantine tanks*: 1,000 – 10,000 L; separate from spawning tanks.
- *Each tank with own water supply.*
- *Drainage system to remove all over-flow or drained water from tanks and other facilities*: water to effluent-settlement dam or salt-evaporative pond.
- *Aeration of all tanks*: high-volume, low-pressure blower(s); diffused air through airstones.
- *Screened outlets in all tanks*: screen size depends on fish, e.g., maximum of 25 mm for broodfish, 6 mm for fry/fingerlings.
- *Incubation facilities*: may be tanks or aquaria for silver perch eggs; screens 500 μm for silver perch.
- *Laboratory facilities*: specific area containing bench, sink, water, microscope(s), balances, reference material, records; ideally a separate, closed room.
- *Refrigeration*: for chemicals such as hormones.
- *Office.*
- *Support buildings and workshop:* for maintenance and to house vehicles, feed, and general equipment.
- Storage areas for chemicals and inflammable liquids: to satisfy OH&S requirements.

10.5.5.2. Recommended Criteria

- *Circular tanks*: fibreglass or plastic; with central drain for self-cleaning; internal or external standpipe.
- *RAS*: for quarantine, holding broodstock or over-wintering fingerlings.
- *3-phase electrical system*: power for heaters, aerators, welding equipment.
- *Emergency oxygen supply*: bottled oxygen.
- *Computer system*: for general office use and access to e-mail and Internet.
- *Telephone and fax.*

10.5.6. Generator for emergency power supply

Electricity is essential for the operation of a fish farm. It is strongly recommended that each farm has a generator to provide electricity during failure of mains power. The generator should be large enough to power all essential equipment (e.g., blowers, pumps, aerators, heaters), and with the capacity to cover any future expansion of the farm.

10.5.7. Staff

Aquaculture is a intensive animal industry, and the success of native fish farms is dependant on high quality staff that have a good understanding of fish biology, and the technical and practical aspects of aquaculture. On smaller farms, staff need to be "all-rounders" with a combination of technical and practical skills, while on large farms there may be scope for specialisation, with trained personnel concentrating on the more technical aspects such as hormone-induced breeding, water quality and health management. There are a number of courses available at Technical and Further Education (TAFE) facilities and universities – see Austasia Aquaculture Trade Directory (2007) for information.

10.5.8. Duty of animal care

Farmers have a duty to provide appropriate facilities and care for silver perch. Directions for animal care on research institutions in Australia such as the NSW Department of Primary Industries' fisheries R&D facilities are provided by the National Health and Medical Research Council (NHMRC) and appropriate Animal Care and Ethics Committees. The NHMRC directs that *"the overall condition and management of facilities must permit effective maintenance and*

servicing and be compatible with maintaining the animals in good health". The NSW DPI – Fisheries Animal Care and Ethics Committee (ACEC) has provided written guidelines for fish care (see Barker et al. 2002). The ACEC and NSW DPI institutions are responsible for ensuring that facilities are appropriately staffed, designed, constructed, equipped, operated and maintained to achieve a high standard of animal care.

10.5.9. Disease problems in tank culture and purging systems

Diseases are common in tanks and purging systems because of high stocking densities. Purging (called 'conditioning' in the Silver Perch Quality Assurance Program), or the removal of off-flavours from fish, ensures a uniform, high quality product for the market. Purging requires harvested fish to be held in tanks for up to 7 days in clean, well-aerated water. Three management options are generally used: (i) a static system using periodic, 'batch,' water exchange; (ii) a recirculating system using tanks, filtration and UV units; (iii) a combination of the two. Generally, disease problems are more prevalent in recirculating systems under the following conditions: (i) where there is minimal or zero water exchange; (ii) when poor husbandry practices have been used during the harvest; (iii) in poorly designed systems; (iv) where the system has not been 'conditioned' to assimilate sudden increases in ammonia and nitrite when large numbers of harvested fish are placed in the tanks. Bacterial and fungal diseases are the most common health problems.

10.5.9.1. Signs

Clinical signs include chronic mortality (5 – 10 fish/day, starting 2 – 3 days post-harvest) frayed fins, blotchy skin, haemorrhaging, white patches on the head and caudal peduncle, dark skin, swimming into currents and lethargy.

10.5.9.2. Prevention of disease in purging systems

- Do not harvest diseased fish.
- Do not harvest fish from ponds with poor water quality.
- Do not feed fish for 2 –3 days prior to harvest (prevents fouling of water and high ammonia post-harvest).
- Implement good harvest procedures (use oxygen, low stocking densities and anaesthetics in transport).
- Stock fish at rates < 50 kg/m^3 in clean, well-aerated water post-harvest.
- Exchange water in tanks constantly during harvest, then ~ 80% of water (in static and recirculating systems) the day after harvest, and then periodically (> 30%; every 2^{nd} to 3^{rd} day) to remove organic matter, dilute bacterial loads and flush ammonia.
- Hold fish in continuous bath of 2 g/L salt to prevent fungal infections, reduce stress and prevent nitrite poisoning; re-salt following water exchange.
- Measure water quality regularly; DO, pH, salinity and ammonia.
- Do not feed fish in the purging system.
- Regularly clean/scour purging system including pipes, tanks and bio-filters.
- Leave water circulating permanently through bio-filtration units and maintain some load in the system, i.e., between major harvests (desiccation of the system will kill nitrification bacteria).

Holding some fish permanently in recirculating systems will help 'condition' or keep a load on the bio-filtration units; however, the filter's nitrification potential (the process of changing ammonia to nitrite and nitrate) is severely compromised following the introduction of large biomasses of fish (and subsequently ammonia) and any other sudden alterations to water quality (e.g., increased salinity). Nitrification is further compromised when bio-filters are too small and are unable to assimilate the ammonia load, and/or the bio-filter has been contaminated with organic matter

(mucus, scales, grass, and suspended solids) due to inadequate mechanical filtration (pre-biofilter) or water exchange.

10.5.10. Disease problems in cage culture

Cage culture of finfish is growing rapidly throughout the world. Recent research has demonstrated that silver perch perform very well in cages, with high survival, fast growth and high production rates. This farming technique has significant potential for silver perch and enables the use of existing water bodies such as gully dams and irrigation storages. Cage culture also provides potential advantages over pond culture including ease of observation, feeding, sampling and harvesting fish, and reduction or prevention of bird predation. However, diseases can cause significant losses of fish because the high stocking densities and close proximity of cages facilitate rapid spread of pathogens. In cage culture research at GAC, there have been no losses of fish to diseases due to rapid diagnosis and treatment. Only two diseases have been encountered, chilodonellosis and lepidotremosis, and both were successfully controlled by applying formalin or trichlorfon to the pond. Diseases are a potential problem in the cage culture of silver perch particularly in large ponds and open waters with limited or no artificial aeration.

10.5.11. Aquatic plants and fish health

The presence of large, aquatic plants (macrophytes) in aquaculture ponds is undesirable. Plant species including filamentous algae (*Cladophora* sp.), milfoil (*Myriophyllum* sp.), cumbungi (*Typha* sp.), ribbonweed (*Vallisneria* sp.) and pondweed (*Hydrilla* sp.) have been problematic in ponds on some silver perch farms. While some plant species are restricted to shallow areas of ponds, submerged species such as ribbonweed and pondweed are capable of colonising the entire pond including deeper sections. Some species of duckweed (Lemnaceae) are capable of rapid propagation, budding from adult plants and covering entire surfaces of ponds in a few days. Macrophytes can have a significant effect on the maintenance of fish health and the treatment and control of disease in the following ways.

- Interfere with pond management and feeding.
- Impact on water quality (clear water, compete with phytoplankton for nutrients, cause abnormally high pH levels).
- Restrict circulation of oxygenated water to parts of the pond including the bottom.
- Crowd the fish and enhance disease transfer, particularly ecto-parasites.
- Restrict water flow and therapeutic chemical distribution and concentrations.
- Reduce chemical concentrations due to high organic load.
- Provide havens for fish and parasites which may remain untreated.
- Contribute to oxygen depletion and high TAN levels when they decompose.
- Contribute to water loss through evapo-transpiration.
- Hinder or prevent sampling and harvesting.

10.5.11.1. Management and prevention

- Limit shallow areas.
- Dry and de-silt ponds every 1 – 3 years.
- Harvest problem weeds as soon as they appear especially prior to their seeding.
- Increase phytoplankton turbidity through correct feeding regimes and/or application of fertiliser.
- Use a registered herbicide.
- Avoid the contamination of waters from other locations (e.g., fish transportation water).
- Exclude aquatic birds and farm stock such as cattle from ponds.

10.5.12. Birds and fish health

Birds cause a number of serious problems on fish farms. Some species are extremely efficient predators of fish and if given the chance, can take most fish from a pond. The most damaging birds on silver perch farms are the little black cormorant (*Phalacrocorax sulcirostris*) which preys on small fingerlings (3 – 75 g) and the large black cormorant (*P. carbo*) which takes fish from 55 g to around 400 g. Both species of cormorants hunt in flocks, with up to 100 or more birds. In addition, the darter (*Anhinga melanogaster*) and pelicans can take silver perch from ponds. Besides reducing survival, bird predation severely stresses surviving fish, and increases their susceptibility to disease. Birds also introduce pathogens to the farm from the wild, and can spread pathogens from pond to pond. Efforts should be made to minimise the number of predatory birds on farms. Fingerling ponds should be completely netted to exclude birds.

10.6. Water quality

10.6.1. Introduction

High quality water is a basic pre-requisite for good health of silver perch. Poor water quality will either kill eggs, larvae or fish directly, or cause stress leading to reduced feeding, slow growth, inhibition of gonadal development and reproduction, suppression of the immune system and increased susceptibility to disease.

Although water chemistry is complex, the suitability of water for fish culture is governed by relatively few variables. Water quality in freshwater aquaculture is discussed in detail by Boyd (1982, 1990) and in relation to silver perch by Rowland (1995c, 1998).

10.6.2. Water quality variables

Key variables and relevant values are listed in Table 10.4 – Section 10.6. The relatively stable variables of alkalinity, hardness, conductivity and metals need to be analysed during site selection; however, they are not greatly influenced by fish culture activities and so do not need to be regularly monitored (other than alkalinity in RAS). Temperature influences all chemical and biological processes, and has direct effects on dissolved oxygen concentration (DO), pH and ammonia and must be monitored regularly. These variables are unstable and significantly influenced by fish culture activities; DO, pH and ammonia can change from acceptable levels to stressful or lethal levels within several days, particularly in summer. DO is the most important and limiting factor in intensive aquaculture; it fluctuates diurnally, weekly and seasonally. In ponds, DO is lowest near dawn, whereas maximum levels of pH and ammonia usually occur in mid-afternoon. The water quality monitoring program used at GAC is given in Table 10.5 and the recommended levels and levels for concern of key variables are given in Table 10.6.

Silver perch, like other native freshwater fish endemic to the Murray-Darling River System, can be considered relatively hardy in terms of water quality, and few fish are lost because of poor water quality on farms with a good water supply, appropriate facilities and good management.

10.6.3. Water quality management

There is a close link between water quality and fish health – the susceptibility of fish to disease is greatly increased by poor water quality. Water quality can deteriorate rapidly in larval rearing ponds where there are often large blooms of phytoplankton and zooplankton. Plankton blooms can "crash" causing a decline in DO due to decay of dead plankton and reduced photosynthetic production of oxygen by algae. In grow-out ponds where the biomass of fish can exceed 10 tonnes/ha towards the end of a production period, DO is often low because of the high demand by fish and the decay of faeces, uneaten food and other organic matter. The weather also influences

water quality. DO will decline during a series of still, cloudy days because of reduced photosynthesis at low light levels and reduced diffusion of oxygen at the air/water interface.

10.6.3.1. Good Aquaculture Practices to assist in managing water quality

- Aerate all facilities – ponds nightly at least 00.00 – 08.00 h, paddlewheel or propeller-aspirator aerators, aeration rate 5 – 10 hp/ha.
- Daily inspection of ponds.
- Regular observation of fish.
- Monitor water quality regularly (see Table 10.5).
- Keep good records.
- Use recommended feeding regimes.
- Spell and dry ponds every 1 – 3 years.
- Dry larval rearing ponds during winter and early spring.
- Appropriate use of chemicals.

10.6.3.2. Actions that can be taken by the farmer to combat poor water quality are:

- Decrease or stop feeding.
- Increase aeration to 24 h/day.
- Increase aeration by adding an extra aerator to the ponds.
- Put a flow of fresh water into the pond to top-up or over-flow.
- Exchange water, remove and then replace water.
- Increase monitoring to daily.
- Decrease stocking density.
- Ensure ponds are dried between crops.

10.6.4. Criteria for water quality management

10.6.4.1. Essential Criteria

- *Water quality meter(s) and equipment*: with the capacity to monitor temperature, DO, pH, total ammonia-nitrogen (TAN), conductivity/salinity; un-ionised ammonia (NH_3) is calculated using TAN, temperature and pH.
- *Regular monitoring of water quality*: data sheets and records showing regular collection of water quality data from all ponds, tanks and other facilities.
- *Aeration*: of all ponds, tanks, incubation, quarantine and purging facilities.
- *Capacity to exchange water rapidly (in all facilities)*: flows to tanks (1,000 – 10,000 L) should be around 15 L/min, with a capacity up to 250 L/min to enable tanks to be filled quickly.

10.6.4.2. \Recommended Criteria

- Formaldehyde test kit to monitor formalin concentrations, and spectrophotometer to monitor copper: during treatment for some diseases.
- Trained technical staff with a good understanding of water quality in aquaculture.
- Regular calibration and maintenance of water quality meter(s) and equipment: according to manufacturers guidelines.
- Use of appropriate fertilisation regimes for larval rearing ponds; for recommendations see Rowland (1983, a, b), Boyd (1990), Thurstan and Rowland (1995).
- Equipment to monitor alkalinity, hardness.

Table 10.5. Monitoring program for water quality in ponds at GAC.

Season, months, temperatures	**Days**	**Time of day (h)**	**Variables**
Spring – summer – early autumn	Monday Wednesday Friday	06.00 – 08.00	DO, temperature, pH
September – April		15.00 – 16.00	DO, temperature, pH, ammonia*
> 20º			
Late autumn – winter	Monday Thursday	06.00 – 08.00	DO, temperature
May – August		14.00 – 15.00	DO, temperature, pH, ammonia*
< 20º			

*ammonia once weekly if at acceptable levels.

Table 10.6. Recommended and levels for concern of temperature, dissolved oxygen (DO), pH, total ammonia (TAN) and un-ionised ammonia (NH_3) for silver perch.

Variable	**Recommended**	**Levels for concern**
Temperature (°C)	10 – 30	< 5; > 32
DO (mg/L)	> 5.0 (afternoon) > 3.0 (near dawn)	< 3.0
pH	7.0 – 9.5	< 5.0; > 10.0
TAN (mg/L)	< 2.0	> 3.0
NH_3 (mg/L)	< 0.1 (long-term)	> 1.0

10.7. Production and husbandry

10.7.1. Production phases

A 3-phase production strategy is recommended for silver perch: I – hatchery phase; II – fingerling phase; III – grow-out phase (see Fig. 10.9). This strategy should be used for culture in ponds, cages and tanks. The hatchery phase involves hormone-induced breeding and larval rearing and usually lasts for 6 – 10 weeks by which time the fry are around 30 mm and 0.5 g. Larval rearing ponds are stocked at high densities (~ 100 larvae/m^2) and large numbers of fry can be produced with good survival e.g., > 30% survival can lead to the production of > 100,000 fry/pond (0.3 ha). Relatively high stocking densities (20,000 – 150,000 fish/ha in ponds; 500 – 1,000 fish/m^3 in cages and tanks) are recommended for the fingerling phase to maximise production per unit area, and to ensure efficient management and utilisation of pond/cage/tank space. A large size range develops during the fingerling phase; this is innate in most fish species both under aquaculture conditions and in the wild. At the completion of this phase, fingerlings should be harvested, quarantined and graded before being stocked for grow-out. Size variation during the grow-out of silver perch is much lower. There is scope for flexibility within and between the phases on farms; however, if combined, e.g., larval rearing and fingerling phases or fingerling and grow-out phases, there are management difficulties and inefficiencies, and fish health may be compromised.

10.7.2. Single-batch system

It is recommended that a single-batch system is used where each pond has only fish of the same age or batch. The fish in each pond are harvested completely, and the pond drained and dried before the next batch of fish is stocked.

10.7.3. Stocking densities

Stocking density is one of the most important variables in silver perch culture. In ponds, it has a significant effect on the growth of fingerlings; those stocked at 20,000 fish/ha will grow faster than those stocked at 80,000 – 150,000 fish/ha. In cages, density affects survival and growth during grow-out. There is aggression between fish stocked in cages at densities of 25 – 50 fish/m^3 resulting in lower survival than fish stocked at low (10 fish/m^3) or high densities (100 – 200 fish/m^3). As stocking densities increase, production and production rates increase, but risks associated with disease and poor water quality also increase. Recommended stocking densities for silver perch are given in Table 10.7.

10.7.4. Growth and temperature

Although silver perch can tolerate a wide range of water temperatures (2º – 38ºC), the range for optimal growth is 23º – 28ºC. Silver perch feed and grow well when temperatures are over 18ºC, but appetite decreases and growth slows at temperatures under 18ºC and over 30ºC. There is little growth at temperatures under 15ºC. Consequently, the length of the growing season and time to reach market size for silver perch is determined by the temperature regime in a region. Longer growing seasons usually mean shorter periods to market-size, and lower production costs and fewer risks. On the NSW North Coast silver perch grow well for 7 – 8 months of the year, whereas in southern NSW this period may be only 5 – 6 months. Silver perch on southern farms may take much longer, up to 2 – 3 years to reach market-size compared to northern farms. In the Bundaberg area of Queensland, temperatures are 18º and higher throughout the year, and silver perch can reach market-size in 12 months. However, farms in that region can have difficulties with high temperatures in summer that reduce appetite and growth, and can cause water quality problems, particularly low dissolved oxygen.

10.7.5. Size of fingerlings at stocking

The size of fingerlings at stocking has a significant influence on growth and production during the grow-out phase. Large fingerlings (e.g., 50 – 100 g) will reach market-size much faster than small fingerlings (1 – 5 g). However, larger fingerlings take more time, are more costly to produce and more difficult to transport because of their higher biomass. Under current industry practices, large fingerlings are not produced by hatcheries, and small fingerlings are usually not available for stocking until mid to late summer. As a consequence many farmers, particularly those in southern NSW and Victoria are forced to hold much of their crop into or through a second winter before fish reach market size (> 500 g). The practice of carrying silver perch over two winters significantly increases production costs and risks associated with diseases and bird predation.

The over-wintering of fingerlings at elevated water temperatures, e.g., in RAS, provides advantages such as increased growth, reduced risk of bird predation, and fewer disease problems. Fingerlings can be grown from 5 g to 50 g at temperatures around 24ºC in RAS over a three month period during winter. Advanced fingerlings (50 – 100 g) can reach 500 g in a 5-month period when water temperatures exceed 20ºC and good aquaculture practices are used. A production strategy of over-wintering fingerlings and stocking advanced fingerlings in spring has the potential to significantly reduce the period and cost of production, and risks associated with winter diseases in regions with relatively long, cold winters.

10.7.6. Low water temperatures

Besides reducing growth in temperate species such as silver perch, low water temperatures can also suppress the immune system, making fish more susceptible to some diseases. Immune suppression is involved in the initiation of the fungal disease winter saprolegniosis in silver perch. In addition, the parasitic disease ichthyophthiriosis is difficult and costly to treat at low temperatures because of the complexity and length of the life cycle of *Ichthyophthirius multifiliis* and the rapid depletion of chemical therapeutants formalin and copper in the aquatic environment.

10.7.7. Quarantine

Quarantine is the term used to describe restrictions that are placed on the movement of fish into, or out of a facility. It is a tool for: (i) preventing pathogens and diseases entering the farm; (ii) preventing the spread of diseases on the farm; and (iii) preventing diseased fish leaving the farm.

Each silver perch farm should routinely use quarantine procedures for all batches of fish:

- new broodfish from the wild or other farms;
- fingerlings from hatcheries;
- fry at the end of the hatchery phase;
- fingerlings at the end of the fingerling phase;
- fish harvested from any pond/cage on the farm that are to be restocked into ponds, cages or tanks on the farm;
- fingerlings or larger fish that are to be despatched for sale to another farm or stocked into the wild or farm dams;
- fish that are suspected or known to be diseased.

Quarantine involves the holding of fish in isolation in tanks under low-stress conditions where they are readily observed, treated and handled. Features of silver perch quarantine facilities are:

- circular tanks (1,000 – 10,000 L) with a supply of high quality water and diffused air from a blower – tanks on a farm may be multi-purpose, e.g., used for spawning in the breeding season and then quarantine at other times;
- self-cleaning tanks with a circular flow and a central, bottom drain that is screened;

- relatively dark tanks (e.g., black, dark blue) that are partially covered to lower light intensity – aids in reducing stress;
- ability to rapidly drain and fill tanks;
- isolated, quiet area of the facility;
- equipment such as nets, buckets and bins restricted to the quarantine area and disinfected regularly.

Fish should be checked for disease (close observation of fish and microscopic examination of gill and skin tissues) as soon as possible after being placed in quarantine tanks. Water should not be exchanged or the tanks drained until the disease status of the fish is known. Appropriate chemical therapeutants should be used if fish are diseased. It is recommended to hold fish in quarantine in 2 – 5 g/L salt for at least 5 days to reduce stress, prevent fungal infection after handling; these concentrations will also kill some ecto-parasites. Do not feed fish in quarantine. Fish should spend at least 5 days in quarantine. Fish should be checked immediately prior to stocking or despatch to ensure they are disease-free.

10.7.8. Use of anaesthetics for handling silver perch

Anaesthetics play a key role in the health of silver perch by reducing stress and physical damage during farming practices such as sampling, handling, injecting hormones for induced spawning, grading and harvesting. Once fish are stressed or damaged, their susceptibility to infection by parasitic, bacterial or fungal pathogens is greatly increased. The anaesthetics that are registered or permitted for use in aquaculture, Aqui-s and benzocaine, can be used on all sizes of silver perch from fry to large broodfish. Low levels of anaesthesia in silver perch are achieved using around 20 mg/L benzocaine and high levels by using 40 – 50 mg/L benzocaine. Key times when anaesthetics should be used are:

- transporting fish after harvest from ponds/cages to the quarantine tanks or hatchery (low anaesthesia);
- transporting fish from quarantine tanks to ponds/cages/tanks for stocking (low);
- grading, counting or sorting fish (low);
- broodfish – examination, sampling eggs and milt, injection of hormones (high).

10.7.9. Grading

Variation in the size of fish in any group is natural. There is always a large size range established during larval rearing and the fingerling phases, but generally less during grow-out. Besides the innate variation in fingerlings, other factors that can influence size range are genetics, stocking density, social hierarchies and associated aggression and subordination, and inappropriate feeding practices.

Grading reduces size variation, facilitates good growth and efficient feeding, and reduces competition and aggression between different-sized fish. If silver perch are not graded at the end of the fingerling phase, the large size range is exacerbated during grow-out creating management problems, inefficiencies in production and feeding, and potentially compromising health. Grading into three relative size classes of small, medium and large at the completion of the fingerling phase is sufficient for silver perch. After grading, the small fingerlings usually grow as fast or faster (in terms of specific growth rate) as the medium and larger fingerlings. Fish can be graded by eye and hand, and while this is acceptable for small batches it can be time-consuming and impractical for large batches or on large farms. Various types of graders, including mechanised graders based on parallel bars or slits appropriately spaced are available.

Silver perch should be graded:

- at the completion of the hatchery phase;
- at the completion of the fingerling phase;
- post-harvest for market.

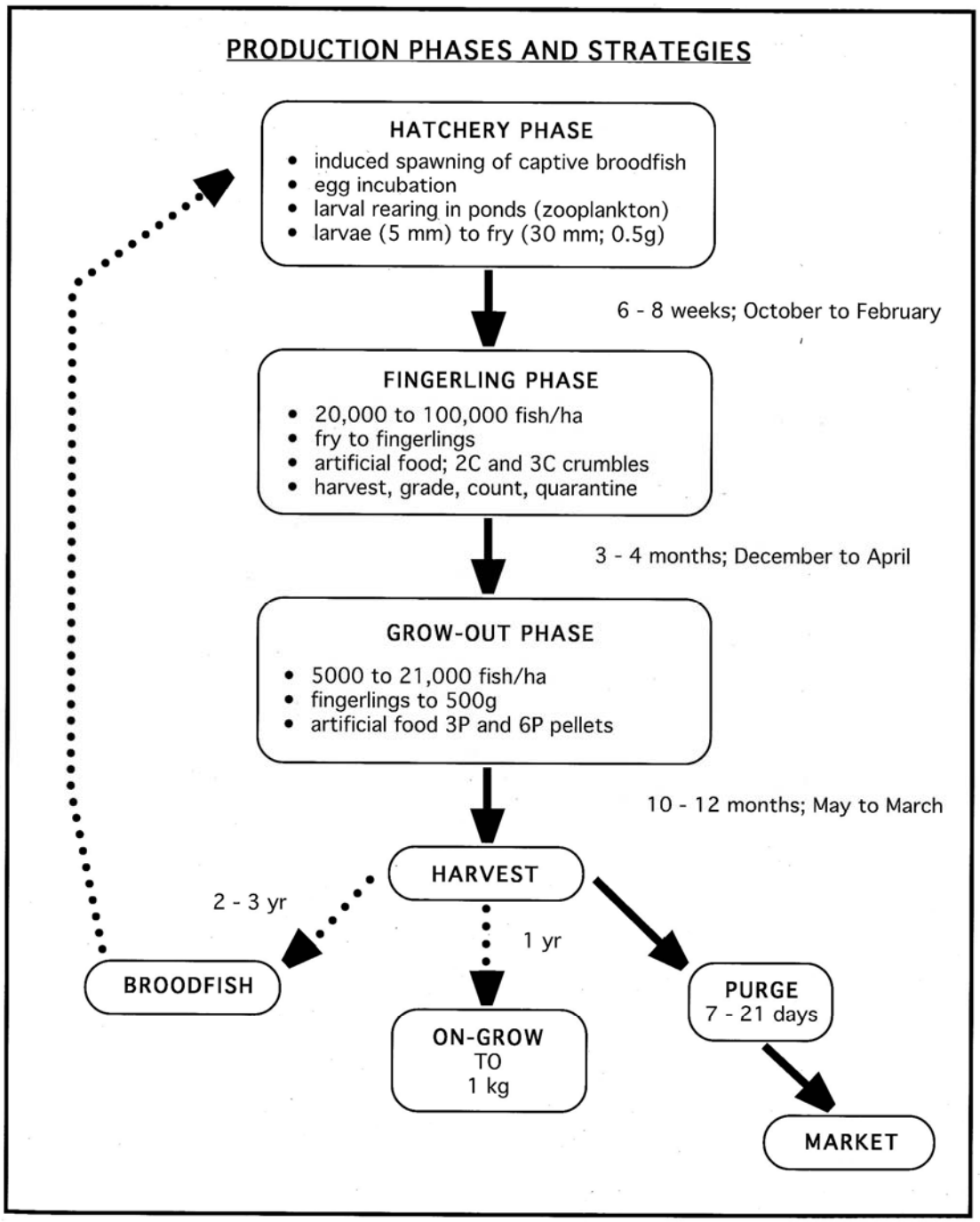


Figure 10.9. Production phases for silver perch (from Rowland 1995b).

Table 10.7. Recommended stocking densities for silver perch.

Production phase	Culture facility	Stocking densities
Fingerling	Ponds	20,000 – 150,000 fish/ha
	Cages	500 – 1,000 fish/m^3
	Tanks in RAS	500 – 1,000 fish/m^3
Grow-out	Ponds	10,000 – 20,000 fish/ha
	Cages	500 fish/m^3 to 250 g 200 fish/m^3 to 500 g 100 fish/m^3 to 800 g
	Tanks	Not recommended for grow-out

10.8. Nutrition and feeding

10.8.1. Introduction

Feeding is one of the most important activities in fish farming. In silver perch, feeding accounts for around 20% of total production costs. The efficient delivery of high quality feeds is essential for high survival, fast growth, high production rates, low food conversion ratios, maintenance of good water quality, fish health and economic viability. The use of poor or inappropriate diets and poor feeding practices adversely affects fish performance, and increases the cost of production. Adequate nutrition is required for normal functioning of the fish's immune system. Poor nutrition leads to immuno-suppression, increasing susceptibility to infection and disease.

10.8.2. Silver perch diets

Silver perch is an omnivorous species and unlike most other fish currently cultured or considered for culture in Australia, it is efficient at digesting carbohydrate, especially starch. This means starch-rich plant ingredients can successfully be used to replace protein as an energy source. The best protein content for silver perch on low energy diets (13 MJ/kg digestible energy), medium energy diets (15 MJ/kg digestible energy) and high energy diets (17 MJ/kg digestible energy) are 24.7%, 26.1% and 30.1% (digestible protein) respectively. If fish are fed restrictively, that is less than they will eat by choice, the best protein contents for diets are higher than when fish are fed to satiation. Diets with excess protein and energy are unnecessarily costly and may cause problems such as high levels of body fat, liver dysfunction and poor health in silver perch.

In addition to cost advantages of feeding an omnivorous species, extensive research to determine nutritional requirements and evaluate agricultural ingredients has led to the development of practical, least-cost diets for silver perch. In these diets, most or all of the imported fish meal can be replaced with Australian agricultural products such as meat and poultry meals, lupins, canola, peas and wheat; silver perch perform well on diets with only 0 – 5% fish meal. In the early days of the silver perch industry, steam-pressed pellets were used, but now high quality extruded, pellets that are slow-sinking or floating are available.

10.8.3. Feeding

10.8.3.1. Regimes

Over-feeding wastes feed and adversely affects water quality, while under-feeding results in reduced growth, longer periods to market-size, increased costs and increased susceptibility to disease. Different feeding strategies are used in aquaculture and include feeding on demand, feeding to satiation, and feeding a restricted ration based on a proportion of body weight (e.g., 3% body weight per day). Satiation can be difficult to determine in the characteristically turbid silver perch ponds where not all fish feed at the surface. It can also be time-consuming, and can increase labour costs and the overall cost of production. A feeding strategy for silver perch has been developed based on restricted rations for fingerling, market-size silver perch and broodfish. Recommended feeding rates and frequencies are given in Table 10.8. These feeding rates are close to satiation. Silver perch, including small fingerlings, do not need to be fed more that twice daily for optimal growth and food conversion. Silver perch should be fed at least 6 days/week for optimal growth. Vehicle-mounted blowers are used to deliver the feed to fish in ponds, and fish in cages and tanks are hand-fed.

10.8.3.2. Feeding behaviour

Although some silver perch feed aggressively at and near the surface, particularly in the warmer months, many fish feed mid-water and so slow-sinking or a mixture of floating and sinking pellets are recommended for this species to ensure all fish receive their daily ration. Feeding behaviour and activity of silver perch vary with temperature, stocking density and genetic strain, and are also influenced by fish health, water quality, turbidity, availability of natural food, time of day, cloud cover, wind, and bird predation.

10.8.3.3. Feeding times

As with other animals, feeding activities in fishes exhibit distinct daily patterns; however, many studies have shown that regulated feeding in aquaculture overrides the various natural rhythms associated with appetite, feeding and digestion. Consequently, feeding silver perch only during daylight hours will not adversely affect performance. Recommended feeding times when the frequency is twice daily are 08.00h and then 15.00 – 17.00h in the afternoon. Prior to the morning feed, the farmer must ensure there are adequate oxygen concentrations in ponds bearing in mind that minimum levels occur near dawn. The daily ration can be divided evenly between morning and afternoon feeds. Feeding time during winter, when the frequency is once daily, is 15.00 – 17.00h; the period when the highest temperature and oxygen levels occur.

10.8.3.4. Regular adjustment of ration

Where restricted rations are used, the amount of feed that should be fed changes daily because of fish growth, and so the effective use of feeding tables is dependent on the regular estimate of biomass and adjustment of daily rations. Biomass can be estimated by a number of techniques: (i) using known growth rates; (ii) assuming a certain food conversion ratio (FCR) e.g., 2.0; (iii) by sampling fish. At GAC, fish are sampled, weighed and the daily ration adjusted each 2 weeks for small fingerlings and 4 weeks for fish larger than 50g. In ponds, 100 – 200 fish (usually 5 – 10% of the crop) are sampled using a seine net. In experimental cages ($1m^3$) all fish are removed and bulk-weighed, but in large cages under commercial conditions a randomly selected sample of around 10% would be sufficient to estimate biomass.

10.8.3.5. Maximum daily input of feed to ponds

As fish grow and the pond biomass and daily ration increase, the increasing quantity of feed applied to ponds causes a deterioration of water quality, in particular, dissolved oxygen (DO) concentrations decrease and concentrations of total ammonia-nitrogen (TAN), and usually un-ionised ammonia (NH_3) increase. To ensure the maintenance of good water quality, it is recommended that total feed inputs into silver perch ponds do not exceed 150 kg/ha/day in small ponds (< 0.3 ha) and 100 kg/ha/day in larger ponds. Farmers must ensure they have adequate aeration in ponds, i.e., around 10 hp/ha, the ability to exchange water and reliable water quality monitoring equipment. Farmers must be cautious when feeding ponds with a large biomass of fish and dense blooms of phytoplankton during summer. Under these circumstances water quality can deteriorate rapidly, necessitating a reduction or cessation of feeding. It may also be necessary to reduce feeding if the weather changes quickly, e.g., if a cold snap occurs, lowering the water temperature by several °C over a few days.

10.8.3.6. Feeding before and during winter

Activity and appetite in silver perch decline as temperatures fall below 20°C, but fish will continue to feed at temperatures down to at least 9°C. Feeding in winter can prevent weight loss, achieve small weight gains, and keep fish healthier than those that are not fed.

10.8.3.7. Purchase and storage of feed

Farmers should order feed well before their on-farm supplies are exhausted. On arrival, the feed should be checked for appropriate type and size, and lack of contamination such as mold. All feed should be stored under conditions that are rodent proof, and cool (< 15°C) with low humidity. Feed should be used within 3 months of purchase.

10.8.3.8. Management

The feeding strategy in Table 10.8 is recommended as a guideline only for silver perch farmers. Feeding is an important management practice, and there is significant variation in fish behaviour and feeding activity between ponds on any one farm. Farmers need to combine careful observation of feeding activity with other practices, particularly the monitoring and management of water quality and disease.

Table 10.8. Recommended feeding rates and frequencies for silver perch.

Fish (g)	**Water temperature °C**						
	< 9	**9 – 12**	**12 – 15**	**15 – 18**	**18 – 21**	**21 – 25**	**25 – 30**
1 – 15	0.5 3d/w	0.5/1x	1.0/1x	2.0/1x	3.0/1x	7.5/2x	7.5/2x
15 – 50	0.5 3d/w	0.5/1x	1.0/1x	2.0/1x	2.5/1x	5.0/2x	7.5/2x
50 – 250	0.5 3d/w	0.5/1x	1.0/1x	1.5/1x	2.5/1x	4.0/2x	5.0/2x
250 – 500	0.5 1d/w	0.5/alt.d	0.5/1x	1.0/1x	2.0/1x	3.0/2x	2.0/2x
> 500	0.5 1d/w	0.5/alt.d	0.5/1x	1.0/1x	1.0/1x	1.5/1x	1.0/1x
Broodfish	0.5 1d/w	0.5/alt.d	0.5/1x	1.0/1x	2.0/2x spring and early summer; 2.0/1x other seasons	2.0/2x spring and early summer; 2.0/1x other seasons	1.0/1x

Feeding rate = % body weight per day; feeding frequency = no. of feeds per day, i.e., once (1x) or twice (2x) e.g., fish to be fed 5.0% body weight, twice daily (i.e., 5% distributed over 2 feeds) = 5.0/2x

Table 10.9. Recommended feed particle sizes for silver perch.

Fish size		**Size (mm) and type of feed**	
total length (mm)	**weight (g)**	**diameter**	**crumble (c) or pellet (p)**
15 – 25	0.5	0.6	c
25 – 35	0.5 – 1.0	1.0	c
35 – 50	1 – 2	1.5	c
50 – 75	2 – 5	2.0	c
75 – 100	5 – 10	1.5	p
100 – 125	10 – 25	2.0	p
125 – 175	25 – 100	3.0	p
175 – 275	100 – 350	4.0	p
> 275	> 350	6.0	p

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11. BENEFITS

11.1. Publications, recommendations and diagnostic support

Publications arising from this project are:

1. "Development of a Health Management Strategy for the Silver Perch Aquaculture Industry" – FRDC Final Report, including a Health Management Plan and Key Recommendations.
2. "Diagnosis, Treatment and Prevention of the Diseases of the Australian Freshwater Fish Silver Perch (*Bidyanus bidyanus*)" – a disease diagnostic manual.
3. "Hatchery Quality Assurance Program for Murray Cod (*Maccullochella peelii peelii*), Golden Perch (*Macquaria ambigua*) and Silver Perch (*Bidyanus bidyanus*)" – this HQAP has been published and distributed; recommendations in the HQAP are currently being implemented on native fish hatcheries in NSW.

These three publications will be distributed to members of the silver perch and other freshwater aquaculture industries, aquaculture managers, scientists and extension officers in NSW and other states, FRDC and other interested people and groups. The publications will directly benefit silver perch farmers and the industry as a whole by providing the basis for good health management. The diagnostic manual and the Health Management Plan will also be up-to-date references for veterinarians providing disease diagnostic support from the NSW DPI veterinary laboratories at Menangle and Wollongbar, other veterinarians involved in fish disease diagnosis and health management, and tertiary courses in aquaculture at TAFE and universities.

11.2. Improvements on farms

The silver perch aquaculture industry will benefit from the results of this project at several different levels. At the farm level, farmers who participated directly in the project are now aware of the need and value of routine monitoring of fish for signs of disease. These farmers have increased knowledge and greater skills through their direct involvement in the project and contact with extension and research staff. New skills include; sampling fish, excision and preparation of skin and gill tissues, use of microscopes to examine tissue samples, recognition and identification of pathogens, and diagnosis of diseases. Improved diagnostic ability will enable rapid and accurate diagnosis of infectious diseases on farms in the future. Early detection of diseases, particularly acute infectious diseases such as ichthyophthiriosis and chilodonellosis is essential for control and to minimise losses. Higher survival rates resulting from improved diagnostic abilities will lead to increased levels of production and higher production rates, with few or no extra inputs. Preventative measures recommended in the publications should also lead to a reduction in the incidence of diseases and contribute to improved fish health on farms in the future.

The cost of using formalin can be very high, and so the recognition of copper and trichlorfon as cost-effective, alternative therapeutants for some silver perch diseases will help reduce these costs. The subsequent increases in efficiencies should lead to higher returns and increased economic viability of individual farms. In the past, significant losses on some farms have limited production and threatened their viability, as well as restricted industry development.

Good health management, as outlined in the Health Management Plan and the Disease Diagnostic Manual, reduces the incidence and severity of diseases, as well as optimising the health, performance and production of cultured fish. Healthy, unstressed fish perform much better than unhealthy fish; they feed aggressively, convert food efficiently and grow to their potential.

Improved health management and fish performance lead to shorter production periods and reduced costs. All these improvements should lead to an increase in the economic viability of silver perch farms over the next 5 – 10 years.

11.3. Improvements across the industry

Improvements at the farm level have been reflected across the industry. The silver perch industry has grown from 250 tonnes in 1998/99, prior to the commencement of the project, to around 400 tonnes. Over this period, production rates on farms in NSW increased significantly from 3.4 to 6.2 tonnes/ha/year in 2005/06 (Fig. 11.1). Increasing production rates reflect increasing efficiencies. The higher production rates reflect lower mortalities and increased survival of silver perch in response to improved disease control and health management. Besides better health management, it is also likely that improvements in farming and husbandry practices (e.g., use of appropriate feeding regimes, regular water quality monitoring, grading of fingerlings) following advice from extension officers and research staff during the project have also contributed to these increases. The total annual production of silver perch has not increased since 2003, due to the loss of three key farms from the industry (for family and personal reasons), and significant losses due to winter saprolegniosis on some farms in several years. However, the relatively high mean production rate of 6.2 tonnes/ha/year in 2005/06 suggests that efficiencies have increased across the industry, and it is expected that overall production will increase in future years with the adoption of recommendations from this study.

11.4. Chemical permits

There are few chemicals registered or permitted for use in aquaculture in Australia. Data collected during the project on efficacy, depletion rates and residues of formalin, copper, salt and trichlorfon are being used in applications to the Australian Pesticides and Veterinary Medicines Authority (APVMA) for minor use permits. In addition, supporting information on the general use of these and other chemicals under culture conditions has been supplied to the APVMA and the Department of Environment. These chemicals will play an important role in disease control and health management in the silver perch aquaculture industry.

11.5. Hatchery sector

Hatchery production is the basis on any aquaculture industry, and the supply of high quality seedstock is essential for success at both the farm and industry levels. Although most silver perch hatcheries produce good quality fingerlings, in the past, some hatcheries have produced poor quality fish that have caused significant problems, restricted production on individual farms, and subsequently hindered industry development. Features of poor quality fingerlings have included: infestations of parasites; bacterial infections; unweaned and ungraded fingerlings in poor condition; abnormalities. Some hatcheries have supplied batches contaminated with trash fish (e.g., banded grunter in consignments from hatcheries in Queensland). In addition, the production of fingerlings with “poor” genetics (e.g., low genetic variation, inbreeding) can result in inferior fish which grow slowly and have decreased resistance to disease. Hatcheries must have high quality broodfish, and use appropriate breeding programs. Fingerlings from hatcheries have the potential to introduce pathogens onto farms and into the wild, and so the hatchery industry has significant responsibilities towards both the aquaculture industry and the conservation of wild populations. The Hatchery Quality Assurance Program (HQAP) provides a basis for significant improvements in the hatchery sector. Requirements for broodfish management and breeding programs, as well as water quality monitoring and health management are given. Under the HQAP, each batch of fingerlings leaving a hatchery must be checked for disease, abnormalities and trash fish before dispatch. Implementation of the HQAP should result in healthy, high quality fingerlings and improved fish health across the industry. The risks of introduction of pathogens and trash fish to the farms and to the wild will be

greatly reduced under the HQAP, and provide significant benefits to both the aquaculture industry and wild stocks.

11.6. Other species and industries

The pathogens and diseases of silver perch are also found in other native freshwater fish of the Murray-Darling River System such as Murray cod and golden perch, the Barcoo grunter (called jade perch in the aquaculture industry) from the Lake Eyre Drainage, and in rainbow trout and other salmonids. All these freshwater species are cultured in south-eastern Australia. The results and recommendations of our project are directly applicable to these species and industries, and will benefit the native fish sector and possibly the salmonid sector of the Australian aquaculture industry.

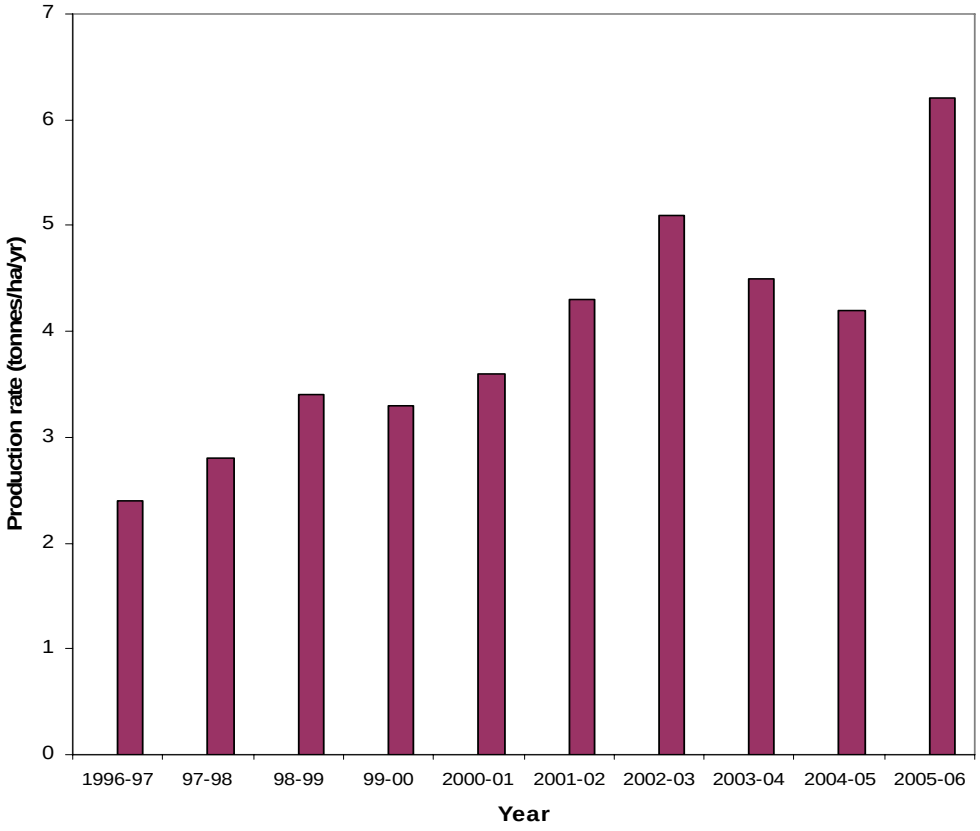

Figure 11.1. Production rates of silver perch in earthen ponds on commercial farms in NSW. Data are means of farms that produced 2 tonnes or more.

12. FURTHER DEVELOPMENT

12.1. Publications and extension

Results of the project and key recommendations for health management of silver perch will be extended to industry through the publications, on-farm visits, workshops, attendance at annual conferences of industry associations (NSW Silver Perch Growers Association, Aquaculture Association of Queensland, NSW Aquaculture Association), and communication with farmers and extension officers in Queensland and Victoria.

12.2. Future research – winter saprolegniosis and ichthyophthiriosis

Although the common diseases of silver perch were identified and appropriate control measures developed during the project, the fungal disease winter saprolegniosis remains a threat to some farms in colder regions. Management actions to reduce the incidence of winter saprolegniosis have been recommended (see Chapter 14 – Key Recommendations). In addition, the following options were considered worthy of future evaluation as preventative and/or control methods for the disease: (i) the strategic use of formalin and copper sulfate; (ii) the use of probiotics, particularly the probiotic, *Aeromonas media* strain A199 developed by Dr Josie Lategan and her colleagues at the University of Technology, Sydney; (iii) the use of feed additives such as L-carnitine, β-glucan or ascorbic acid to improve the immune response and increase resistance to pathogens; and (iv) the use of cage culture to reduce the incidence of diseases and facilitate control through efficient management, good water quality and reduced organic matter in ponds. The monitoring of fungal zoospores and cysts in ponds may also be of value in predicting impending outbreaks.

Experimental work on winter saprolegniosis in ponds was limited during the project due to the absence of the disease at the Grafton Aquaculture Centre (GAC) and the lack of opportunities for replication and control treatments on commercial farms because of production and economic constraints. Future research work should focus on farms with a high incidence of the disease (e.g., Farm A, mid-North Coast – Chapter 5) and at facilities with equipment to culture and study aquatic fungi such as *Saprolegnia parasitica* and staff that specialise in mycotic and/or microbial diseases of fish.

Ichthyophthiriosis is a difficult disease to treat during winter, and new, cost-effective treatment regimes involving formalin and copper sulfate have been identified. The regimes were validated in ponds at GAC, and in ponds on a commercial farm, but further validation would be valuable. The new regimes are now used at GAC, and their future use on commercial farms will be monitored by research and extension staff.

12.3. Current silver perch research

Research projects at GAC are:

1. *Genetic Improvement Program for Farmed Silver Perch* – funded by NSW DPI.
2. *Evaluation of the Potential for Aquaculture on Cotton Farms; cage culture of silver perch* – funded by the Cotton Catchment Communities Co-operative Research Centre (Cotton CRC) and the University of New England with possible future links to the Seafood CRC.

Disease and health management is a component in the Cotton CRC cage culture project, and provides opportunities for further validation of new treatment regimes. The proposal to link this cage culture project with the new Seafood CRC, will enable a continuation of silver perch disease and health management work by NSW DPI.

13. PLANNED OUTCOMES

Planned outcomes of FRDC Projects Nos 2000/267 and 2004/089 have been achieved.

The aetiology and pathogenesis of the fungal disease winter saprolegniosis are now known. Fungal diseases in fish culture are inherently difficult to treat because of the ubiquitous nature of aquatic fungi, the complexity of their life cycles and the depletion of chemicals used as therapeutants in the aquatic environment. Management of fungal diseases is best achieved through prevention. A good example of this is the significant reduction in the incidence of epizootic ulcerative syndrome at the Grafton Aquaculture Centre (GAC) achieved through altered water management practices. Although cost-effective chemical treatments were not developed for winter saprolegniosis, factors predisposing silver perch to infection were identified and a number of management actions to assist in the prevention and control of the disease have been recommended. In addition, directions for further work have been suggested.

The major infectious diseases of silver perch were identified. There are now 20 diseases, compared to only four reported in the early 1980's. Several new diseases to silver perch culture, winter saprolegniosis and lepidotremosis (infestations of gill flukes), were addressed during the project. The seasonal occurrence of infectious diseases under research conditions at GAC over a period of 15 years was quantified, and this information will assist in future health management of silver perch both at GAC and on commercial farms. Methods for the prevention and control of silver perch diseases were developed. Depletion patterns of both formalin and copper in earthen ponds were quantified and used to develop new treatment regimes to control the problematic disease ichthyophthiriosis. The use of these regimes was validated in ponds at GAC and on a commercial farm. Copper was shown to be significantly cheaper than formalin in controlling ichthyophthiriosis in earthen ponds, and so is a cost-effective therapeutant for this disease. Formalin and the organophosphate trichlorfon were found to be effective chemical therapeutants for lepidotremosis, and salt was shown to control ichthyophthiriosis and prevent saprolegniosis in tanks. Salt is an excellent therapeutant for some silver perch diseases (and those of other freshwater fishes), as well as being a stress-reducing agent for freshwater fish, and so is recommended for use in quarantine and in re-circulating aquaculture systems. Data on the efficacy, depletion and residues of these chemicals have been used in applications to the Australian Pesticides and Veterinary Medicines Authority (APVMA) for minor use permits.

There is now an increased awareness of diseases and health management amongst silver perch farmers. Those farmers who participated directly in the project are fully aware of the need and value of routine monitoring of fish for signs of disease. The saying "*An ounce of prevention is worth a pound of cure*" is very applicable to the health management of silver perch. The farmers have significantly increased knowledge and skills, and improved diagnostic ability. Extension of results to other farmers in NSW, Queensland and Victoria will be facilitated by the publications and extension by research and extension officers in each state. Although the impact of the project may not be clearly evident for a number of years, the relatively high production rate of 6.2 tonnes/ha/year on commercial farms in NSW in 2005/06 may be a reflection of improvements in silver perch health management.

An overall health management strategy for the silver perch aquaculture industry is based on the FRDC Final Report (including the generic Health Management Plan), the Disease Diagnostic Manual, the HQAP, extension of technology and information by NSW DPI research and extension staff, a disease diagnostic service offered by staff at two NSW DPI veterinary laboratories, and support from NSW DPI aquaculture management staff.

The project has provided a basis for improved health management across the industry, but success is dependant on the use of all components of the strategy, implementation of major recommendations by farmers, and co-operation by between individual silver perch farmers, the industry, industry associations and the NSW DPI.

14. CONCLUSIONS – KEY RECOMMENDATIONS FOR HEALTH MANAGEMENT

Good health management provides environmental and culture conditions that reduce the incidence and severity of diseases, enable rapid and appropriate response to disease outbreaks, and optimise the health, performance and production of cultured fish. Health management begins with site selection and design of farms, and is based on the use of good aquaculture practices and preventative measures, in particular quarantine procedures, maintenance of good water quality, use of high quality feeds and appropriate feeding regimes, regular monitoring of fish for diseases and prompt, appropriate action to control disease outbreaks. The following recommendations for health management have been developed from our health management project and past research into the culture and diseases of silver perch.

14.1. Site selection, farm design and operation

Silver perch should be cultured in a region with a suitable climate for the species, ideally within the water temperature range of 10° – 30°C, and with temperatures higher than 18ºC for 7 or more months of the year. There is an increased likelihood of disease problems, as well as shorter growing seasons in regions with relatively long, cold winter, and where-ever water temperatures fall below 10ºC. The site must have an abundant supply of high quality surface and/or underground water; advantages of underground water include no pathogens, no pollutants, no off-flavour compounds and relatively constant temperature. Farmers should use the following recommendations for the design and operation of fish farms. Earthen ponds: construct from impervious soils; rectangular (0.2 – 0.3 ha recommended for fingerlings and grow-out); separate inlet and outlet (> 150 mm diameter); screened outlet; ability to drain water from surface or bottom; harvest sump; known pond volumes at different water depths; dry and de-silt each pond between crops. All ponds and tanks should be aerated (ponds, 5 – 10 hp/ha). Cages can be used for the production of fingerlings and market-sized fish. Reservoir: located so that water can be moved using gravity; aerate; keep free of fish which are a potential source of pathogens; screen inlet and outlet; dry and de-silt every 2 – 4 years. All effluent water from ponds and the hatchery should be held in an effluent-settlement dam, which should be kept free of trash fish if water is recycled. Farms should have a generator to provide emergency power during failure of mains power. Birds (e.g., cormorants, darters, pelicans) can significantly reduce survival through predation, and can severely stress and damage fish, cause poor growth and transfer pathogens to the farm and from pond to pond. Birds should be excluded by netting ponds, particularly fingerling ponds.

14.2. Production phases, stocking and feeding

Use a 3-phase production system: I – hatchery phase (6 – 10 weeks); II – fingerling phase (3 – 6 months); III – grow-out phase (6 – 12 months). Use a single-batch system in which only fish of the same age/batch are stocked in any pond or cage. Fish should be stocked at recommended densities. Harvest and quarantine between production phases. Grade fish at least once after the fingerling phase; stock fish of similar sizes after grading. Consider over-wintering fingerlings at elevated water temperatures (e.g., in a tank-based, re-circulating aquaculture system, or ponds in a hot-house system) to increase growth, eliminate bird predation, reduce the length of the production period and reduce the incidence of diseases in winter. Check all fish for parasites and signs of disease before stocking on the farm, or transportation off the farm. Use anaesthetics when handling, grading, transporting and stocking fish to reduce stress and physical damage. Use high quality, silver perch or native fish feeds. Use recommended feeding regimes and feed 6 days/week. Do not overfeed, particularly in autumn. Store feeds under cool, dry conditions.

14.3. Water quality

Water quality is a basis of good fish health. Poor water quality, in particular low dissolved oxygen (DO), high un-ionised ammonia, and high, low or broadly fluctuating pH predispose fish to disease. The important water quality variables (temperature, DO, pH, total ammonia (TAN) and un-ionised ammonia (NH_3) should be monitored at least 3 times weekly at temperatures > 20°C and twice weekly < 20°C in all ponds; monitor more often if water quality is poor or there are signs of disease. Know the dynamics and critical levels of DO, pH, TAN and NH_3 for silver perch.

14.4. Quarantine

Quarantine procedures play an important role in health management by reducing the incidence of diseases on fish farms. Quarantine all new fish (e.g., fingerlings from hatcheries, broodfish from the wild) coming onto the farm; place in static, aerated tanks and treat prophylactically with a continuous bath of 2 g/L salt to reduce stress, control some parasites and prevent fungal infection; minimum of 7 days (at around 20ºC). Locate tanks in an area with relatively low light intensity, and partially cover tanks. Observe fish closely each day. Check gill and skin tissue from a minimum of 3 fish for parasites and signs of disease to determine the need for treatment with higher concentrations of salt (e.g., to control chilodonellosis) or formalin (to kill gill flukes) or other chemicals. Ensure there are no pathogens on fish before over-flowing or draining the quarantine tanks. Quarantine all fish after harvest from ponds and cages, and before re-stocking. Ensure fish are free of parasites and signs of disease before stocking, transport or marketing. Do not feed in quarantine, unless fish are being held for 3 or more weeks prior to restocking. Maintain good water quality. Ensure re-circulating systems are kept clean and filters are regularly backwashed.

14.5. Disease monitoring, diagnosis and control

Effective disease control requires close, daily observation of fish, and regular sampling and microscopic examination of gill and skin tissues for pathogens and other signs of disease. Changes in appearance and behaviour are early signs of many diseases. Always be looking for key signs – loss of appetite, flashing, abnormal swimming behaviour, abnormal skin colour, moribund and dead fish. Monitor fry and fingerlings each 2 weeks, and larger fish at least monthly; sample at least 3 fish, in particular moribund fish from each batch/pond/tank/cage. Early, on-farm diagnosis of winter saprolegniosis, ichthyophthiriosis and chilodonellosis is essential to minimise losses. Monitor fish post-treatment to gauge effectiveness of treatment and to ensure control.

14.6. Chemicals

Chemicals must be in one of the following categories for legal use in silver perch culture: (i) registered (e.g., Aqui-s, Human Chorionic Gonadotrophin); (ii) off-label or minor use permit (e.g., formalin); (iii) prescribed by a veterinarian (oxytetracycline, copper sulfate, trichlorfon); (iv) emergency permit. Some chemicals are exempt (e.g., fertilizers, lime) and others that were previously used in aquaculture (e.g., malachite green, nitrofurans) are not permitted for use on food fish because of concerns about their effects on human health. Use registered or permitted chemicals only at recommended concentrations and under prescribed conditions. Have the regularly-used chemicals on-farm; some pathogens can increase rapidly (2 – 3 days) to levels that cause high mortalities, and so must be treated promptly to prevent or reduce losses. Establish a working relationship with a veterinarian or NSW DPI staff who are able to assist with disease diagnosis, prescription of therapeutants and provide advice on health management of fish. Know the volumes of all ponds and tanks, and the amounts of chemicals required to achieve recommended concentrations. Appropriate protective clothing should be worn when handling and applying chemicals. Formalin decreases DO and pH, and increases ammonia, particularly at high temperatures (> 25°C); aerate ponds for 24 h/day commencing before the first application and

continuing for up to 7 days after the last application at all water temperatures (summer and winter). The alkalinity of water influences the toxicity of copper to fish and so copper sulfate should only be used in waters with alkalinities > 50 mg/L and preferably > 80 mg/L; ponds can be limed (using agricultural lime, $CaCO_3$) to increase alkalinity prior to and during copper treatment. Copper sulfate is an algacide and may cause oxygen depletion following the death and decay of algae; aerate ponds as required. Formalin and copper are rapidly depleted in water (rate is influenced by fish biomass, organic matter and temperature) and so these chemicals need to be applied daily or each second day to maintain effective concentrations. It is recommended that laboratory equipment (e.g., spectrophotometer, test kits) is used to monitor concentrations of formalin and copper during treatment.

14.7. Specific recommendations for important diseases

14.7.1. Winter saprolegniosis (winter sap or winter disease; caused by the fungus, Saprolegnia parasitica)

Outbreaks commence at water temperatures < 16°C, often after rapid drop in temperature, e.g., up to 5°C over 3 – 7 days in response to cold changes in winter.

- Can cause total mortality if not treated, or if diagnosis and treatment are delayed.
- If possible, maintain 'lighter' pond biomasses (< 6 tonnes/ha) during winter.
- Do not over-feed in mid to late autumn (April – May).
- Ensure fish are free of ecto-parasites going into winter, particularly *Ichthyophthirius*, *Chilodonella*, gill flukes.
- Monitor fish weekly for fungal infections when temperatures < 16°C, and following rapid decreases in water temperature, particularly on farms with history of the disease.
- Maintain ponds at maximum depth to buffer changes in water temperature and to assist fish acclimate to changes.
- Remove any fish with fungal growth from ponds.
- Where possible, harvest market-sized fish before winter; have sufficient tanks for quarantine or purging.
- Avoid partial harvesting of ponds in winter; if unavoidable, remove all fish captured and do not return any fish caught in the seine net or other harvesting equipment to the pond.
- Emergency harvest if a significant outbreak is imminent and hold fish in a continuous bath of 2 – 5 g/L salt.
- Treatments – the use of formalin or copper has not been validated for the control of winter saprolegniosis:
    - salt (NaCl) – 2 – 5 g/L, continuous in tanks to prevent infection – salt has limited effect on established infections;
    - formalin (37% formaldehyde) – 30 mg/L initially, then maintain between 20 and 30 mg/L by adding 15 – 20 mg/L daily; aerate 24 hours/day and monitor formalin and water quality daily;
    - copper (25% of copper sulfate) – maintain concentrations of 0.1 – 0.2 mg/L active ingredient, by adding 0.2 mg/L initially, then around 0.1 mg/L daily or each second day; monitor copper daily; alkalinity must be > 50 mg/L, preferably > 80 mg/L; beware of DO depletion as copper is an algacide.
    - continue treatment until the disease is controlled.

14.7.2. Ichthyophthiriosis (white spot or ich; caused by the ecto-parasitic protozoan, Ichthyophthirius multifiliis)

- Treat immediately white spot is found; disease can progress rapidly causing high or total mortality; check fish in all other ponds and tanks on farm for the disease.
- Outbreaks can occur at any time of year; often associated with decline of water temperatures through 15°C, particularly on fingerlings; can also be prevalent in larval rearing and fingerling ponds at 25° – 30°C.
- Maintain good water quality; poor water quality, particularly low DO is a major factor in outbreaks.
- Ensure adequate nutrition.
- Monitor fingerlings when temperatures reach 15°C in autumn and winter; monitor fry and fingerlings weekly in summer.
- Ensure reservoir is free of trash fish.
- Parasite has a complex, temperature-dependent life cycle; only free-swimming stages susceptible to treatment; need to treat at least for duration of cycle (e.g., 4 days at 25° – 30°C, up to 3 weeks at 10°C).
- Treatments:
    - salt (NaCl) – 2 g/L, continuous in tanks;
    - formalin (37% formaldehyde) – 30 mg/L initially, then maintain between 20 and 30 mg/L daily; aerate 24 hours/day and monitor formalin and water quality daily;
    - copper (25% in copper sulfate) – 0.1 – 0.2 mg/L active ingredient, achieved by adding 0.2 mg/L copper initially, then around 0.1 mg/L daily; alkalinity must be > 50 mg/L, preferably > 80 mg/L; monitor copper daily; beware of DO depletion as copper is an algacide;
    - continue treatment until the disease is controlled.

14.7.3. Chilodonellosis (caused by the ecto-parasitic protozoan, Chilodonella hexasticha)

- Treat immediately *Chilodonella* is found; check fish in all other ponds and tanks on farm.
- Outbreaks can occur at any time of year and progress very rapidly causing high mortalities; most prevalent in winter and spring.
- Maintain good water quality.
- Ensure adequate nutrition.
- Ensure reservoir free of trash fish.
- An encysted stage has been reported and a follow-up treatment(s) may be necessary.
- Treatments:
    - salt (NaCl) – 10 g/L for 1 hr in tanks, flush and repeat following day; 5 g/L continuous in re-circulating aquaculture systems;
    - formalin (37% formaldehyde) – 30 mg/L in ponds (temperature < 25^{o}C) and tanks;
    - copper (~25% in copper sulfate) – 0.2 mg/L copper active ingredient; alkalinity must be > 50 mg/L, preferably > 80 mg/L; beware of DO depletion as copper is an algacide.

14.7.4. Lepidotremosis (infestations of the monogenean gill fluke, Lepidotrema bidyana)

- Common parasite in silver perch.
- More prevalent in winter and spring; numbers generally build up slowly.
- Mortalities unusual, but infestations predispose fish to winter saprolegniosis and bacterial infections.
- Treatments do not kill egg stage; up to 3 consecutive treatments, 7 – 21 days apart may be necessary to eradicate.
- Treatments:
    - formalin (37% formaldehyde) – 30 mg/L (< 25^{o}C);
    - trichlorfon – 0.5 mg/L active ingredient in ponds; 0.25 mg/L in tanks.

14.8. Support services provided by the NSW Department of Primary Industries and commercial companies

14.8.1. Aquaculture Extension

Information on all aspects of silver perch culture is available from staff at the Grafton Aquaculture Centre (GAC). GAC is a model freshwater fish farm. Information and advice on permits and aquaculture policies is available from management staff at the Port Stephens Fisheries Centre (PSFC). Advice is available on site selection, design and operation of farms, pond management, recirculating systems, all aspects of pond and cage culture, production strategies, water quality, diseases and health management, artificial diets and feeding, growth and performance, harvesting, post-harvest management and purging, and equipment and service providers. Useful information can be found on the web at *www.fisheries.nsw.gov.au*, links – "aquaculture" and "extension service".

14.8.1.1. Contact Details

GAC

Tel: (02) 6640 1690; Fax: (02) 6644 7879.

PSFC

Tel: (02) 4982 1232; Fax: (02) 4982 1107.

Disease Diagnostic Services – NSW DPI and Commercial Veterinary Laboratories

NSW DPI provides a disease diagnostic service to the aquaculture industry through two of its Regional Veterinary Laboratories. Fees are involved for some services. The address and contact details for these services are as follows.

Menangle Regional Veterinary Laboratory
Woodbridge Rd
Menangle, NSW, 2570
Telephone – (02) 4640 6327
Fax – (02) 4640 6400
Officer-in-Charge, Keith Walker

Wollongbar Regional Veterinary Laboratory
Bruxner Hwy
Wollongbar NSW 2477
Telephone – (02) 6626 1261
Fax – (02) 6626 1276
Officer-in-Charge, Graeme Fraser

Disease diagnostic services and advice on fish health management are available from a number of competent veterinarians with commercial companies. For further information see the Austasia Aquaculture Trade Directory or visit their website *www.AustasiaAquaculture.com.au*

14.8.2. Silver Perch Disease and Health Management Publications

"Diagnosis, Treatment and Prevention of the Diseases of the Australian Freshwater Fish Silver Perch (Bidyanus bidyanus)" (Read, Landos, Rowland and Mifsud 2007).

This manual is an aid to the diagnosis and treatment of silver perch diseases. It contains photographs of pathogens and diseased fish, and serves as an on-the-spot reference for typical signs of silver perch diseases. The manual also has directions for the treatment and prevention of all diseases, syndromes and conditions that have been reported in silver perch culture.

"Health Management Plan" (see Chapter 10 of this report)

This Plan provides an outline of key aspects of site selection, design and operation, and good aquaculture practices, as well as aspects of health management and the diseases of silver perch. It should be used in conjunction with the disease diagnostic manual for the management of health on silver perch farms.

"Hatchery Quality Assurance Program for Murray Cod (Maccullochella peelii peelii), Golden Perch (Macquaria ambigua) and Silver Perch (Bidyanus bidyanus)" (Rowland and Tully 2004).

This publication describes key features of the design and operation of native fish hatcheries, and the management of broodfish, genetics, breeding programs, water quality and fish health. It provides the basis for accreditation and auditing of native fish hatcheries in NSW.

14.8.3. References and Further Reading

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15. APPENDICES

15.1. Intellectual Property

All information brought into this project or developed during the project is public domain.

15.2. Staff

15.2.1. NSW Department of Primary Industries

Dr Stuart Rowland – Principal Investigator
(Senior Research Scientist, NSW DPI, Grafton Aquaculture Centre)

Dr Dick Callinan – Co-Investigator (2000 – 2004)
(Senior Research Scientist, NSW DPI, Aquatic Animal Health Unit)

Dr Geoff Allan – Co-Investigator
(Research Leader, Aquaculture, NSW DPI, Port Stephens Fisheries Centre)

Matthew Landos – Veterinary Officer – Aquatic Animal Health (2001 – 2005)
(NSW DPI, Aquatic Animal Health Unit)

Charlie Mifsud – Senior Fisheries Technician
(Grafton Aquaculture Centre)

Mark Nixon – Fisheries Technician
(Grafton Aquaculture Centre)

Peter Boyd – Fisheries Technician
(Grafton Aquaculture Centre)

Philip Read – Aquaculture Extension Officer
(Grafton Aquaculture Centre)

Pat Tully – Aquaculture Manager
(Port Stephens Fisheries Centre)

Steve Pepper – Technician
(Aquatic Animal Health Unit)

15.2.2. Industry

Bruce Rhoades – Co-Investigator (2000 – 2002)
(President, NSW Silver Perch Grower's Association)

Ian Charles – Co-Investigator (2003 – present)
(President, NSW Silver Perch Grower's Association)

15.3. Publications

Landos M., Callinan, R., Rowland S., Read P., Boyd, P., Nixon M., Mifsud C., Glendenning D. and Beakes G. (2003) (Abstract). Winter disease in farmed silver perch (*Bidyanus bidyanus*) in New South Wales, Australia. In, Proceedings of the 5th Symposium on Disease in Asian Aquaculture, November 2002, Gold Coast, Australia.

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No. 1 Andrew, N.L., Graham, K.J., Hodgson, K.E. and Gordon, G.N.G., 1998. Changes after 20 years in relative abundance and size composition of commercial fishes caught during fishery independent surveys on SEF trawl grounds. Final Report to Fisheries Research and Development Corporation. Project No. 96/139.

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