2. Literature Review

2.1 Ectoparasites in freshwater aquaculture

Ectoparasites of finfish are diverse, comprising numerous Phyla infecting all species of finfish. Parasite-host relationships in the wild have coevolved and rarely cause substantial injury or mortality (Scholz, 1999). In aquaculture, unfavourable conditions such as crowding, handling, inadequate dissolved oxygen and temperature fluctuations can cause stress in the host (Wedemeyer and Yasutake, 1974; Conte, 2004). This stress, combined with fish being confined, facilitates rapid parasite reproduction that can lead to epizootic infections (Paperna, 1991). Management of parasitic infection is, therefore, often required in aquaculture. Sub-lethal infections can have negative impacts on the host, including reduced growth, altered behaviour, diminished resistance to other stressors and pathogens and reduced marketability (Scholz, 1999). Management of ectoparasites in aquaculture often relies on a 'crisis management' approach, where a chemotherapeutic agent is applied when acute infections triggered by shifts in the parasite-host relationship cause substantial stock loss. Worldwide growth in aquaculture with the emergence of large, mature industries has facilitated the ability for the industry to develop specific, knowledge-based approaches to disease management (Sommerville, 1998a).

2.2 Integrated pest management

Horticulture industries use detailed knowledge of target pest ecology as a basis for plant protection strategies. Integrated pest management utilises all available pest control techniques and integration of prevention measures impeding the development of pest populations and to keep pesticide use to levels that are economically justified and minimise risks to human health and the environment (FAO, 2014a).

Traditional reactive treatment of pests with pesticides in plant production can be effective, although pesticides have environmental risks and social costs (Benbrook et al., 1996). Repeated use of chemical treatments leads to the development of resistance in the target pest, decreasing treatment efficacy (Hoy, 1998). This has motivated the implementation of IPM programs in agricultural industries (Delucchi, 1987). Effective IPM programs require detailed knowledge of the target pest's lifecycle, its ecological and behavioural interactions with the environment and natural controlling factors (Conlong and Rutherford, 2009). Integrated Pest Management does not seek to completely remove a pest from the system but aims to reduce pest numbers to an economically acceptable level (DeWitt et al., 1997). Integrated Pest Management is dynamic and requires constant revision as the relationship between the pest and host changes. A desired outcome of any IPM program is to reduce the volume of pesticide used, which is achieved through careful management, emphasising preventive strategies such as the enhancement of a pest's natural enemies, culture methods and host resistance (Kogan, 1998). If management methods fail and pesticide use is required, biological products, selective pesticides or the lowest effective dose of broad-spectrum pesticides is used (Kogan, 1998) and treatments are applied strategically to interrupt pest lifecycles (Tubbs et al., 2005).

Economic Injury Level (EIL) and Economic Threshold (ET) models are used to determine when control is required (Stern et al., 1959). The EIL is the density of pests that cause damage to the crop that is equal to the cost of managing the pest to that density or lower (DeWitt et al., 1997). The ET, also known as Action Threshold (AT) is the population density at which control measures should be implemented to prevent an increasing pest population from reaching the EIL (Stern et al., 1959). Simple IPM models are based on knowledge of the market value of the crop, population density of

the pest, and the relationship between the density of the pest and the economic damage it causes to the crop (DeWitt et al., 1997). Developing EIL and AT models can be difficult because they rely on detailed knowledge of the population dynamics of the pest (Pedigo and Higley, 1992) and the economic value of pest impacts. Farmers often use pesticides based on experience (DeWitt et al., 1997), but developing a scientifically based AT and applying products strategically provides more detailed information to inform treatment decisions. Effective pest monitoring and identification are required to understand pest densities and to indicate when the AT has been reached and control measures need to be implemented (Kogan, 1998).

2.2.1 Integrated pest management in aquaculture

There are differences between terrestrial agriculture and aquaculture to consider when developing IPM programs specific to aquaculture, which can include: farm design, ecology of the pests, the physical environment and the regulatory use of chemical treatments in different environmental areas (Dumbauld et al., 2006). The principles of IPM have been adapted to aquaculture industries including Pacific oyster *Crassostrea gigas* (see Dumbauld et al., 2006), tiger puffer *Takifugu rubripes* (see Ogawa, 1998) and rainbow trout *Oncorhynchus mykiss* (see Hakalahti-Sirén et al., 2008). The most comprehensive IPM program has been developed to control of sea lice (Caligidae) infecting Atlantic salmon *Salmo salar*, including data on lifecycle, epidemiology, host / parasite and host / environment interactions and the development of multiple treatments targeting specific life stages of the parasite (Sommerville, 1998b).

2.3 Australian freshwater aquaculture industry

In Australia finfish aquaculture is a relatively new industry that is dominated by the production of marine species, with salmonids representing the most valuable species

group followed by tuna and barramundi (ABARE, 2012). Australian freshwater aquaculture has not seen the same growth as mariculture despite having abundant sites with high-quality water available for fish farms (Rowland, 2009). The predominant freshwater species cultured in Australia are rainbow trout, silver perch and barramundi. Barramundi had the highest value of production in 2010 with AUD $32 million produced but production largely occurred in brackish water; rainbow trout had a production value of AUD $5.6 million and silver perch had a production value of AUD $3.5 million (ABARE, 2012), and all other freshwater finfish species had a production value of AUD $7.8 million in 2010 (FAO, 2014b). Freshwater aquaculture systems such as earthen ponds or raceways are often semi-closed, where the incoming water is taken from an adjacent source and the discharge is released back into the same waterway (AQUAVETPLAN, 2004). Hatchery, nursery and grow out stages can also occur in closed systems where the stock and water movement is highly controlled (AQUAVETPLAN, 2004), such as in re-circulation systems (RAS). This project focused on rainbow trout and silver perch culture in semi-closed systems, the two largest freshwater-based aquaculture industries in Australia.

2.3.1 Australian rainbow trout industry

The primary trout species cultivated in Australia is rainbow trout, a cold-temperate species native to the Pacific coast drainages of North America (FAO, 2012). There are two main varieties of rainbow trout: an anadromous marine form ("steelhead") and a fluvial and lacustrine freshwater form, the latter being the primary variety used in aquaculture (Shelton, 1994). Rainbow trout is an ideal species for cultivation (Pillay and Kutty, 2005) and is cultured around the world in areas with a temperate to sub-arctic climate. The main producers are Europe, North America, Chile, Japan and Australia (FAO, 2012). The technology used in commercial cultivation of rainbow

trout is well developed (Sedgwick, 1985; Laird and Needham, 1988; Stevenson, 1980). Cultivation of rainbow trout occurs primarily in concrete or earthen raceways but juveniles can be cultured in flow-through tanks. Brown et al. (1997) identified the seasonal ability to obtain a large volume of good quality water and complying with environmental water quality discharge standards as major constraints on the industry. Recent improvements in feed quality and water filtration and the addition of aerators has facilitated increased fish biomass on farms but with this the occurrence of disease has increased, particularly outbreaks of the ciliate *Ichthyophthirius multifiliis* in water temperatures above 17°C (E. Meggit pers. comm.).

2.3.2 Australian silver perch industry

Silver perch is a Australian native warm temperate fish, endemic to the Murray-Darling River system that is an ideal candidate for culture because they are amenable to high stocking density, grow rapidly, are omnivorous and accept artificial feeds (Rowland and Barlow, 1991). Hatchery technology for silver perch was developed for stocking for recreational fishing (Rowland, 1984) and has been adapted for aquaculture in Australia (Rowland, 2009). Silver perch are also grown in Israel (Barki et al., 2000), China and Taiwan (Rowland and Barlow, 1991). The development of the Australian silver perch industry has been modelled on the highly successful channel catfish *Ictalurus punctatus* industry that dominates freshwater aquaculture production in the southern USA (Walker, 1994). Silver perch are predominantly cultured in earthen ponds; however, alternate technologies such as RAS, concrete raceways and freshwater cages have been trialled (Rowland, 2009). Industry growth has been restricted by poor husbandry and production strategies, difficulties with pond management, stock losses to bird predation and disease and absence of large-scale investment (Rowland and Allan, 2006). Identification of the optimal dietary

requirements of silver perch (Rowland, 2009) combined with an improved feeding strategy (Rowland et al., 2005) has increased farm production. Over-wintering silver perch fingerlings in recirculation systems is also being investigated as a method to reduce winter mortalities caused by disease and predation and to improve growth (Foley et al., 2010). Rowland et al. (2004) suggested production in cages would improve husbandry practices by reducing stress on fingerlings during grading and would provide a physical barrier almost eliminating bird predation. Switching to cage culture could allow silver perch to be stocked in water storage reserves on Australian cotton farms, facilitating increased investment and production (Rowland and Allan, 2006). Culturing fish in freshwater cages reduces spatial movement of the fish, increasing fish biomass, making fish more susceptible to disease (Rowland, 2009).

2.4 Diseases of Australian freshwater aquaculture

There are many diseases described from trout around the world (Laird and Needham, 1988) but Australia is free of a number of important diseases (Morrissy, 2002). Australia's isolation and border controls on biological products has restricted the translocation of diseases (Ward et al., 2003), however, there are parasites present in Australia that can be production limiting. Silver perch are known to be infected by a wide range of diseases (Callinan and Rowland, 1995), including ectoparasites that have had serious impacts on the growth of the industry (Rowland and Allan, 2006). Ectoparasitic infections are persistent in aquaculture systems once established, often occurring as chronic infections with varying degrees of effects on the host. Favourable conditions, can, however trigger an 'acute' phase where rapid proliferation of the parasite causes host mortalities. These parasites often require careful management and chemotherapeutic intervention before potential epizootics

occur. An overview of the common ectoparasites found in freshwater finfish aquaculture in Australia is presented in Table 2.1.

Table 2.1: Common freshwater external parasites of rainbow trout *Oncorhynchus mykiss* and silver perch *Bidyanus bidyanus* present in Australia (adapted from Morrissy, 2002; Rowland et al., 2007).

Parasite	**Pathogen type**	**Rainbow trout**	**Silver perch**
Chilodonella spp.	Protozoan	Susceptible	Susceptible
Ichthyophthirius multifiliis	Protozoan	Susceptible	Susceptible
Trichodina spp.	Protozoan	Susceptible	Susceptible
Saprolegnia parasitica	Oomycete	Susceptible	Susceptible
Lepidotrema bidyana	Gill monogenean	Not susceptible	Susceptible

My project focused on two parasites: *I. multifiliis*, a common parasite infecting rainbow trout and silver perch that requires careful management at water temperatures favourable to rapid proliferation, and *Lepidotrema bidyana*, a chronic infection of silver perch.

2.4.1 *Ichthyophthirius multifiliis*

Ichthyophthirius multifiliis, commonly called 'ich', a ciliate protozoan, is a common cosmopolitan parasite of freshwater teleosts, which occurs from the tropics to temperate regions as far as the Arctic Circle (Matthews, 2005). *Ichthyophthirius multifiliis* infection is characterized by the presence of white spots appearing on the epidermis and/ or gills of infected fish. The mode of entry into the host's epidermis remains unknown, although it has been suggested that the theront enters the host by displacing adjacent epithelial cells (Ewing et al., 1985; Kozel, 1986) and subsequently invading a mucous cell (Buchmann et al., 1999). This is supported by observations that theronts are responsive to host mucous (Lom and Ċerkosová, 1974; Haas et al., 1999; Buchmann and Nielsen, 1999). Infections of *I. multifiliis* in the skin and gills are associated with localized lymphocytic infiltration, focal necrosis and epithelial

proliferation (Maki et al., 2001). Heavy infections (> 1000 parasites per host) induce depletion of energy reserves, cause impaired haematopoiesis and failure of gill epithelia and epidermis to regenerate, resulting in ingress of water, ion imbalance and sensitivity to oxygen tension and uptake (Hines and Spira, 1973a,b; 1974a,b).

Ichthyophthirius multifiliis strains

Subtle variations in morphology of different geographical isolates of *I. multifiliis* led to the suggestion that different strains exist (Nigrelli et al., 1976). Lin and Dickerson (1992) identified and characterised major surface proteins, commonly referred to as immobilization antigens (i-antigens), which are characteristic of different strains. Five i-antigen serotypes, designated A to E, of *I. multifiliis* are described (Dickerson and Clark, 1996). Each of these serotypes expresses different i-antigen polypeptides in the range 40-70 kDa (Dickerson et al., 1993; Lin et al., 1996). Serotypes A, B, C and E co-express two different sized antigens, while serotype D produces a single polypeptide of approximately 55 kDa (Dickerson and Dawe, 1995). Wang et al. (2002) described that the NY1 isolate (a member of serotype A) expressed at least three, and possibly four i-antigen polypeptides. Serotypes A and D are the most predominant (eight out of eleven) of described isolates (Wang et al., 2002). Swennes et al. (2006) reported difference in virulence between two isolates NY1 and G1, serotypes A and D, respectively, NY1 having greater virulence than G1. It is unknown which strain or strains are present in Australia.

Lifecycle of *Ichthyophthirius multifiliis*

The lifecycle of *I. multifiliis* is well described (see reviews by Matthews, 1994; Dickerson and Dawe, 1995). *Ichthyophthirius multifiliis* has a direct lifecycle with three distinct stages: the trophont, which occurs within the host's epidermis; the

tomont, which leaves the host (prototomont) and encysts (tomocyst) in the aquatic environment and undergoes rapid division into daughter cells called tomites, which differentiate into a theront, the free swimming stage that is infective to the host (Matthews, 2005). The development of each stage is influenced by water temperature (Bauer, 1958; Wagner, 1960; Noe and Dickerson, 1995; Aihua and Buchmann, 2001) and salinity (Wagner, 1960; Aihua and Buchmann, 2001). Developmental period and effects of salinity can differ between isolates of *I. multifiliis* (see Aihua and Buchmann, 2001).

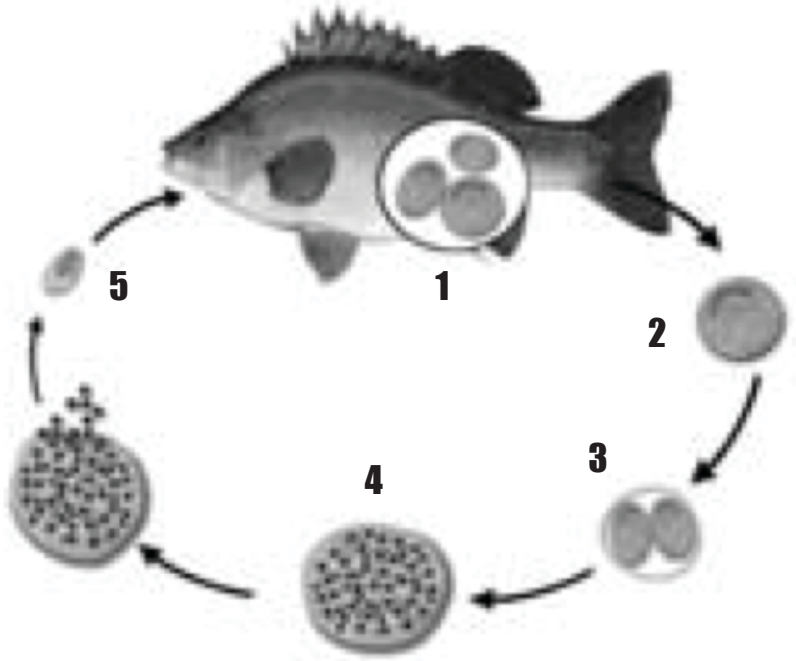

Figure 2.1: Diagrammatic representation of the lifecycle of *Ichthyophthirius multifiliis.* 1 – trophont; 2 – prototomont; 3 – tomocyst; 4 – tomites; and 5 – theronts. Adapted from Read et al. (2007)

The trophont resides and feeds in the epidermis of the host, covered by an epithelial layer that ruptures when the trophont exits (Ewing and Kocan, 1987). Trophonts are spherical and measure 30-1000 µm in diameter, depending on age and maturity (Wagner, 1960). Mature trophonts can develop in 2.5 days at 22°C and 2 days at 27°C (MacLennan, 1942). Parasite emergence can be delayed by reducing

water temperature, with trophonts remaining on the host for 204 days at 9°C compared to only 5-6 days at 25°C (Noe and Dickerson, 1995). Trophonts may exit the host within this period, however only those that attain >100 μm can encyst and continue to develop (MacLennan, 1942; Hines and Spira, 1973a; Ewing et al., 1986).

The tomont is divided into two stages; the prototomont, which is a free-living stage that locates and attaches to a suitable substrate, which then encysts (tomocyst) and produces theronts. The cyst wall can attach to substrates including plants, snail shells and stones (Wagner, 1960). Following encystment, the tomont undergoes rapid sequential binary fission, with no intervening growth, resulting in the production of large numbers of tomites (Matthews, 2005). Tomonts can survive and produce tomites over a wide temperature range, however, at lower temperatures development is delayed and fewer tomites are produced (Aihua and Buchmann, 2001). Each tomite emerges from the cyst as a free-swimming theront.

Theronts locate a new host and are short-lived. Mean life expectancy of a theront is 22.5 h at 20°C, with a significant decline in viability after 12 h (McCallum, 1982). Theront movement is facilitated by somatic cilia; the theronts swim more slowly than their hosts (Matthews, 2005). Theronts are positively phototactic (Wahli et al., 1991), moving towards the surface of the water then using close range chemoattraction (Buchmann and Nielsen, 1999) to stimulate rapid swimming in the vicinity of the host facilitating contact and penetration (Matthews, 1994). Theronts are 30 – 50 μm long (Matthews, 2005), but size varies with the size of the tomont (MacLennan, 1942) and water temperature (Aihua and Buchmann, 2001).

2.4.2 Monogeneans

Monogeneans are parasitic flatworms in the phylum Platyhelminthes. They are mostly external parasites of fish and infect the gills, epidermis, fins, lip folds, skin and other

sites including the crevices of the head, cloaca, branchiostegal membranes and even the stomach (Whittington, 1998). Most monogeneans are hermaphroditic and have a direct lifecycle and a free-swimming larva, the oncomiracidium, which finds and attaches to the host (Cribb et al., 2002). Monogeneans are generally host specific and infect only one or a few closely related host species (Rohde, 1993; Sasal et al., 1999; Desdevises et al., 2001). The strict host specificity demonstrated by most monogeneans reflects coevolution with their hosts that have resulted in a non-pathogenic interaction in wild fish (Paperna, 1984). In aquaculture, fish are kept in confinement with higher stocking densities, facilitating host finding and egg production increasing infection rates which can become epizootic (Thoney and Hargis, 1991).

Monogeneans attach to their host using a haptor, a specialised organ that is specialised and diverse between genera (Kearn, 1994). Sub-lethal high intensity monogenean infections can cause stress, poor feeding response, reduced growth, tissue damage, anaemia and interfere with skin or gill function which can predispose the fish to secondary infections (Paperna, 1991). Severe infections of monogeneans can kill fish, usually because of loss of osmoregulatory capability or severe anaemia (Ogawa, 1996).

Lepidotrema bidyana

Lepidotrema bidyana (Murray) is a monopisthocotylean monogenean in the Diplectanidae (Monticelli). Diplectanids are highly host specific and their phylogeny is well resolved (Desdevises et al., 2001; Domingues and Boeger, 2008). Eight diplectanid species are recorded from Australian freshwater fish, each from only one host species (Fletcher and Whittington, 1998). *Lepidotrema bidyana* is recorded only from the gills of silver perch. *Lepidotrema bidyana* feeds on host mucus and epithelial

cells and is characterised by possessing two pairs of dorsal and ventral hamuli and a dorsal and ventral squamodisc, with rodlet rows and one row of nine spines convergent behind the rodlet rows (Murray, 1931; Young, 1969). Diplectanids are oviparous and have direct lifecycle with no intermediate host, eggs hatch producing oncomiracidia, which locate and attach to the host (Whittington et al. 1999).

Gill dwelling diplectanids can cause variable structural damage to the host. *Diplectanum aequans* (Wagener, 1857) causes major structural damage and marked pathological changes in the gills (Dezfuli et al., 2007; Sánchez-García et al., 2011). *Lamellodiscus* spp. cause only minor structural damage to the gills and appear to have little associated pathology (Katharios et al., 2006; Sánchez-García et al., 2011) but in high intensity infections can decrease host growth (Katharios et al., 2006). Pathological changes associated with *Lepidotrema* spp. infections are not described, but stereomicroscope observations of gill tissue indicated that *L. bidyana* caused epithelial hyperplasia and the formation of white out-growths (Rowland et al., 2006).

2.5 Treatment application methods

In the Australian freshwater aquaculture industry the primary delivery method for chemotherapeutants against ectoparasites is a topical bath or immersion treatment. Medications delivered in-feed are preferred to baths because the whole population is treated synchronously, stress on the fish and labor costs are reduced and delivery is weather independent (Grant, 2002). In-feed medications, however, can have problems associated with delivering the correct dose to all fish within the system caused by hierarchical feeding behaviours, palatability problems (Williams et al., 2007) or inappetence of heavily infected fish (Picón-Camacho et al., 2012b). In freshwater aquaculture bath treatments can be applied as prolonged immersion, short exposure or constant flow treatments.

2.5.1 Prolonged immersion

Prolonged immersion baths are applied directly to the body of water the fish are contained in and the product is left to dissipate through natural decay (Noga, 2000). Prolonged immersions use lower quantities of the chemoteraputant than short exposure or flow-through baths, reducing stress to the host fish and are easier to administer. Disadvantages in prolonged immersions however include: less precise dosing because the fish are exposed to a degradation curve rather than a short exposure to a defined dose, varying degradation rates of chemotherapeutants under different environmental conditions, and fish undergoing treatment are often unable to be removed from the system if showing signs of negative effects. Maintaining effective concentrations in earthen ponds is particularly problematic and can be influenced by precipitation, chemical degradation and/or absorption of chemicals by organic matter, soil, plants and fish (Boyd, 1990; Darwish et al., 2005; Rowland et al., 2007), therefore the dose should be monitored throughout the treatment.

2.5.2 Short exposure bath

Fish being held in large bodies of water such as earthen ponds that are to be treated by short exposure baths need to be removed and placed into a tank or crowded into a liner in the pond and exposed to the chemotherapeutant (Noga, 2000). Stopping the inflow and treating the system can treat fish cultured in small bodies of water such as concrete raceways. Exposures are usually less than 24 h (Noga, 2000). Short exposure baths provide greater control of the dose, and fish can be monitored and removed from the treatment if showing signs of negative effects. Short exposure baths often require husbandry including increased handling to facilitate treatment, which can increase stress and cause physical damage (Grant, 2002). Reducing the volume of

water crowds the fish and it can be hard to maintain satisfactory oxygen levels (Grant, 2002).

2.5.3 Constant flow treatments

Constant flow treatments are used in flow-through systems when it is not possible to stop the water flow (Noga, 2000). An initial dose is added to the system with additional doses applied to add product to untreated incoming water. The dose in constant flow treatments can be influenced by calculation of the system volume, the flow pattern of the system including the geometry and the influence of aerators on flow throughout the system, and the influence of different environmental conditions on product degradation rates (Rach and Ramsey, 2000). If these factors are not adequately controlled, the minimum effective concentration will not be maintained for the treatment period, leading to ineffective treatments (Rach et al., 1997a).

2.6 Chemical treatments for external parasites

The following products are used to treat a wide range of ectoparasites in freshwater aquaculture.

2.6.1 Formalin

Formalin [37% formaldehyde] (FOR), a saturated aqueous solution of formaldehyde gas, is naturally occurring and common at low concentrations in the environment (Hohreiter and Rigg, 2001). Formalin inactivates microorganisms by alkylating the amino and sulfhydral groups of proteins and ring nitrogen atoms of purine bases (Webber et al., 2007). Formalin has been used extensively in aquaculture (Howe et al., 1995) and is recommended for use against protozoan and fungal infections in silver perch (Callinan and Rowland, 1995). Formalin has been used effectively to treat monogeneans (William and Lewis, 1963; Katharios et al., 2006; Rowland et al.,

2006), although there have been reports of ineffectiveness against some monogeneans (Thoney and Hargis, 1991; Sharp et al., 2004). Formalin is also effective against protozoan parasites such as *Ichthyophthirius multifiliis* (see Rowland et al., 2009), *Chilodonella* spp. (see Rowland et al., 2007) and *Trichodina* spp. (see Read et al., 2007).

There are significant limitations to using formalin; it is toxic to phytoplankton, zooplankton and benthic organisms (Birdsong and Avault, 1971; Chiayvareesajja and Boyd, 1993), can induce a stress response in the host (Jørgensen and Buchmann, 2007) and becomes more toxic with increasing water temperatures (Masters, 2004). Formalin has a narrow safety margin and care must be taken to avoid host toxicity (Hohreiter and Rigg, 2001). Formalin is carcinogenic; safety measures are required to protect workers applying the product from formalin exposure (Heit and Riviere, 1995). Formalin must be stored above 4˚C and avoid exposeure to light to prevent polymerisation into paraformaldehyde, which is extremely toxic to fish (Howe et al., 1995). For these reasons formalin is often recommended only as a last resort treatment (Thoney and Hargis, 1991).

Formalin is typically administered at 200 mg/L for 1 h (Noga, 2000). Formalin baths can cause gill necrosis, epithelial separation from lamellar supporting cells and hypertrophy, which impair osmoregulation and gas exchange (Wedemeyer and Yasutake, 1974; Reardon and Harrell, 1990). This can lead to a severe decrease in gas exchange efficiency and eventually cause hypoxia (Ross et al., 1985). There are wide variations in the toxicity of formaldehyde to different species of freshwater fish (reviewed by Hohreiter and Rigg, 2001).

2.6.2 Sodium chloride

Sodium chloride (NaCl) can effectively prevent or treat ectoparasites (Noga, 2000). Exposing freshwater organisms to saline conditions disrupts osmoregulation, resulting in dehydration (Shephard, 1994). Ectoparasites are more severely affected by this process than their hosts due to their higher surface area to volume ratio (Schelkle et al., 2011). Sodium chloride is effective as a high dose short duration bath against monogeneans (Soleng and Bakke, 1997; Read et al., 2007; Schelkle et al., 2011), *Chilodonella* spp. and *Trichodina* spp. (see Rowland et al., 2007). Sodium chloride can also be applied as a low dose long duration bath preventing reinfections of gyrodactylid monogeneans (Soleng and Bakke, 1997; Schelkle et al., 2011), *I. multifiliis* (see Selosse and Rowland, 1990; Mifsud and Rowland, 2008) and *Saprolegnia parasitica* (see Mifsud and Rowland 2008).

Sodium chloride is typically applied at 10 - 30 g/L as a short duration bath and is applied at 2 - 5 g/L for longer duration baths (Noga, 2000). Sodium chloride has a wide safety margin in fish but treatments can be stressful, especially to juveniles (Thoney and Hargis, 1991). Sodium chloride baths can have a varying efficacy between parasite locations; gill monogeneans can withdraw into the inter-lamellar spaces where they may gain extra protection by immersion in mucus (Shephard, 1994) and therefore are more tolerant than skin parasitic species (Thoney and Hargis, 1991). Sodium chloride baths are also restricted to small systems and is not recommended for use in ponds because of the large quantities required and the prolonged adverse environmental impacts of soil salination (Read et al., 2007).

2.6.3 Chloramine-T

Chloramine-T (CAS No. 127-65-1) [*N*-sodium-*N*-chloro-*p*-toluenesulfonamide] (Cl-T), is an *N*-chloro surfactant compound which, when exposed to water, degrades due to nuceophilic substitution to release hypochlorite (OCl^-) and para-

toluenesulfonamide (p-TSA) (Powell and Perry, 1998). The release of hypochlorite is probably the primary mode of therapeutic action (Booth and MacDonald, 1988) and host toxicity (Powell et al., 1995; Powell and Perry, 1996). Chloramine-T is used in aquaculture for a wide range of protozoan infections, including amoebic gill disease (AGD) (Harris et al., 2004), trichodiniasis (Madsen et al., 2000) and *I. multifiliis* (see Cross and Hursey, 1973; Rintamäki-Kinnunen et al., 2005a,b). Mixed infections of bacterial and protozoan pathogens can be treated simultaneously using Cl-T (Ostland et al., 1995).

Chloramine-T is applied as a general parasiticide as a bath at 12 - 20 mg/ l for 60 min on three alternate days or 3 or 4 consecutive days in freshwater fish (Meinertz et al., 2004). Chloramine-T is safe to use within the recommended doses (Sanchez et al., 1997; Gaikowsi et al., 2008; 2009), however, overdose or prolonged exposure causes morphological changes to the gill epithelia of Atlantic salmon, goldfish *Carassius auratus* and rainbow trout (Powell et al., 1995; 1998; Sanchez et al., 1997; Altinok, 2004; Powell and Harris, 2004), which can lead to mortality (Gaikowski et al., 2008). Host toxicity to Cl-T is increases with a higher salinity and oxygen saturation (Powell and Harris, 2004), decreases as stocking density increases (Bills et al., 1988a), and toxicity increases in acidic water and proportional to temperature (Bills et al., 1988b). Fish have varying sensitivity to Cl-T therefore a bioassay is recommended before administration (Gaikowski et al., 2008).

2.6.4 Peracetic acid

Peracetic acid or peroxyacetic acid (CAS No. 79-21-0) (PAA), is a strong oxidant that is widely used as a disinfectant or sterilising agent in the food, beverage, medical and pharmaceutical industries and in the treatment of wastewater (Koivunen and Heinonen-Tanski, 2005). The mode of action of PAA as an antimicrobial agent is

poorly understood but it is speculated that it functions similarly to other peroxides and oxidising agents by disrupting the chemiosmotic function of the lipoprotein cytoplasmic membrane and transport through dislocation or rupture of cell walls (Kitis, 2004). Peracetic acid has been identified as a potential therapeutic for use in aquaculture against ectoparasites and is effective against *I. multifiliis* (see Rintamäki-Kinnunen et al., 2005a,b; Meinelt et al., 2007a,b; Meinelt et al., 2009; Straus and Meinelt, 2009; Sudová et al., 2010; Picón-Camacho et al., 2012a), *Cryptocaryon irritans* (see Picón-Camacho et al., 2011) and *Saprolegnia parasitica* (see Marchand et al., 2012).

Peracetic acid is commercially available as a quaternary equilibrium mixture containing acetic acid, hydrogen peroxide (HP), PAA and water (Kitis, 2004), which degrade into neutral residuals reducing the environmental impact of treatment discharge (Pedersen et al., 2009). Peracetic acid has anti-microbial activity over a wide range of temperatures (Stampi et al., 2001). Disinfection efficiency of PAA increases with decreasing total suspended solids (TSS) and biochemical oxygen demand (BOD) (Kitis, 2004). Peracetic acid degrades more quickly with increasing organic load and fish biomass (Pedersen et al., 2009), and is therefore more effective in low organic content concrete tanks than in high organic content earthen ponds (Rintamäki-Kinnunen et al., 2005a,b).

Peracetic acid is applied as a bath treatment and is recommended at doses between 0.2 – 2.0 mg/L (Pedersen et al., 2009). Overdosing of PAA can cause degeneration and shedding of the gill epithelium (Straus et al., 2012), which can lead to host mortality (Meinelt et al., 2007a; Sortkjær et al., 2008; Straus et al., 2012). Host tolerance to PAA can vary between different species of fish but the no observable

effect concentration (NOEC) generally falls within the recommended dose range (Straus et al., 2012).

2.6.5 Sodium percarbonate

Sodium percarbonate (CAS No. 90569-69-0) (SPC) is a colourless, crystalline and water-soluble solid. When SPC comes into contact with water it dissociates into sodium and carbonate ions and HP (Buchmann et al., 2003), the latter of which is a powerful oxidising agent (Block, 1991) and is the active antiparasitic compound (Heinecke and Buchmann, 2009). Hydrogen peroxide is effective against ectoparasitic infections in aquaculture including monogeneans (see Rach et al., 2000; Buchmann and Kristensson, 2003; Mansell et al., 2005), *Lepeophtheirus salmonis* (see Grant 2002), *Amyloodinium* sp. (see Montgomery-Brock et al., 2001) and *I. multifiliis* (see Buchmann et al., 2003; Heinecke and Buchmann 2009).

Sodium percarbonate is available in commercial solution contain between 25 – 30% active HP. Hydrogen peroxide decomposes into water and carbon dioxide and does not form cumulative or toxic metabolites (Pedersen et al., 2006) and therefore has low environmental impact and treated fish are food safe. Sodium percarbonate decomposes rapidly in aquaculture systems with increasing water temperature and organic load (Møller et al., 2010). Treating fish using liquid HP can be problematic; accurately achieving the target dose is difficult due to the effects of water biogeochemistry (Bishop et al., 1968). There are also workplace health and safety concerns with handling HP and security restrictions on transport and storage of the product (Eul et al., 2001). Sodium percarbonate is a granular solid with lower workplace health and safety risk and no current security sensitivity; its gradual dissociation in water to HP also provides a wider fish safety margin (Buchmann et al., 2003).

Sodium percarbonate is administered as a bath treatment and is recommended at a dose of 50 – 100 mg/L, with no reports of adverse effects on treated fish (Buchmann et al., 2003). The effect of HP released slowly over time from SPC in alkaline water is undescribed in fish. The toxicity of liquid HP is well described in fish and can vary between species (Gaikowski et al., 1999), life stage, and is proportional to water temperature (Rach et al., 1997a). In general, safety ranges reported for freshwater fish exposed to liquid HP fall between 50 and 500 mg/L during 30 to 60 min exposures (Rach et al., 1997a; Lumsden et al., 1998; Gaikowski et al., 1999; Derksen et al., 1999; Thomas-Jinu and Goodwin, 2004; Avendaño-Herrera et al., 2006). Over exposure to liquid HP can cause epithelial lifting and gill necrosis (Johnson et al., 1993; Kiemer and Black, 1997; Tort et al., 2002). Tort et al. (2002) suggested that the physio-chemical water parameters including alkalinity could significantly alter efficacy and host toxicity, potentially resulting in a higher tolerance to HP.

2.7 Treatments for monogeneans

The following treatments are used in aquaculture to treat monogenean infections.

2.7.1 Trichlorfon

Trichlorfon (CAS No. 52-68-6) [dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate] (DEP), a organophosphate insecticide, was developed as a broad-spectrum insecticide (reviewed by Ecobichon, 1996). DEP decomposes in water to the more toxic compound dichlorvos (CAS No. 62-73-7) [2,2-dichlorovinyl dimethyl phosphate] (DDVP), which is the active form, which is taken up by target organisms more quickly because of greater fat solubility which facilitates rapid penetration of biological membranes (Hoffer, 2009). The speed of decomposition of DEP to DDVP

is influenced by temperature, pH and aeration (Samuelsen, 1987). Both DEP and DDVP inactivate the nerve enzyme acetylcholinesterase (AChE) by irreversibly binding to the receptor site, which causes a loss of control of the nervous system (Pope, 1999). Trichlorfon has been recommended for treatment of monogeneans in aquaculture (Sarig et al., 1965; Sarig 1971; Imanda and Muroga, 1979; Schlotfeldt et al., 1995), although it can be ineffective (Obiekezie and Taege, 1991; Janse and Borgsteade, 2003) and resistance is described (Umeda et al., 2006).

Trichlorfon is applied as a dissipative bath treatment at a dose of 0.25 mg/L (Guimarães et al., 2007). Organophosphate exposure has sub-lethal effects on fish kidney tissue and renal function (Veiga et al., 2002) and DEP causes hypertrophy and hyperplasia of epithelial cells in gill lamellae (Capinpin, 1995) and a reduction in AChE activity (Guimarães et al., 2007), which can cause host mortality (Salte et al., 1987; Horsberg et al., 1989). The 96 h LC50 for pacu *Piaractus mesopotamicus* is 0.1906 mg/L DEP (Mataqueiro et al., 2009). Trichlorfon also has potentially adverse ecological impacts (Costello, 1993; Schlotfeldt et al., 1995). Due to these factors care must be taken when applying the product.

2.7.2 Praziquantel

Praziquantel (CAS No. 55268-74-1) [2-(cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a]isoquinoline-4-one] (PZQ), a pyrazinoisoquinoline anthelmintic, was developed for the treatment of a wide range of trematode and cestode infections in humans and animals (Day et al., 1992). The mode of action in PZQ is poorly described, although it causes spastic paralysis of the worm musculature (Cioli and Pica-Mattoccia, 2002). Praziquantel has a chemotherapeutic effect against skin and gill monogenean parasites when administered by bath (Schmahl and Mehlhorn, 1985; Schmahl and Taraschewski, 1987; Chisholm and Whittington, 2002;

Whittington and Ernst, 2002; Janse and Borgsteede, 2003; Forwood et al., 2013) and orally (Hirazawa et al., 2000; Kim et al., 2001; Janse and Borgsteede, 2003; Hirazawa et al., 2004; Williams et al., 2007; Forwood et al., 2013). There have been reports of palatability problems in fish orally administered PZQ (Hirazawa et al., 2004; Williams et al., 2007; Forwood et al., 2013).

Praziquantel has been administered in a wide variety of doses depending on the target helminth species and delivery method. There is little information about the toxic effects of PZQ on fish. Zang et al. (2013) reported a significant change in expression of an inflammatory mediator gene after exposure to 13.5 mg/L PZQ for 48 h in goldfish *Carassius auratus auratus*, which could affect their ability to resist subsequent bacterial pathogens. Nwani et al. (2014) reported micronucleus induction and significant decreases in haematological and biochemical parameters in African catfish *Clarias gariepinus* repeatedly exposed to sub-lethal concentrations of PZQ for 15 d. Sudová et al. (2009) reported light erthropenia and intralienic haemolysis in carp *Cyprinus carpio* 24 hours after a single oral dose of 30 and 50 mg PZQ per kg BW which resolved 96 hours after application, suggesting the effects on the fish were reversible. Praziquantel has a wide safety margin in fish with reported 96 h LC50 values ranging between 50 – 60 mg/L PZQ in freshwater fish (Mitchell and Hobbs, 2007; Nwani et al., 2014).

2.8 Management practices for ectoparasitic infections in Australia

Management practices in Australia are generally reactive to disease and involve the use of chemotherapeutants (Rowland et al., 2007). Management of *I. multifiliis* relies on carefully monitoring stock to achieve early detection and subsequent chemotherapeutic treatment. Monogenean infections are often left unmanaged and the

use of chemotherapeutics is only implemented when the infection appears to cause mortalities (Rowland et al., 2007).

2.8.1 Monitoring *Ichthyophthirius multifiliis* in aquaculture in Australia

Matthews (2005) recommended that monitoring water temperature in spring and early summer in temperate regions could predict and provide some warning for impending epizootics of *I. multifiliis* providing a more targeted approach to control measures. In Australia, Read et al. (2007) recommended daily observation of silver perch for signs of *I. multifiliis* infection such as lethargy and loss of appetite, flashing, ragged fins, raised or broken skin, mild skin haemorrhaging and white nodules on the skin or opaque eyes. Monitoring *I. multifillis* is included in recommended weekly monitoring of Murray cod (*Maccullochella peelii*) fry (Ingram et al., 2005), whereas no specific monitoring programs have been developed for rainbow trout. Detection of *I. multifiliis* can be made by microscopic examination of gill and skin scrapes at 40 – 100x magnification. Trophonts are usually embedded under gill and skin epithelium, and are characterised by a dark granular cytoplasm; a visible horseshoe-shaped (mature trophonts) or spherical (immature trophonts) nucleus; body with uniform cilia (Read et al., 2007).

2.8.2 Prevention strategies for *Ichthyophthirius multifiliis* in Australia

Preventing the introduction of *I. multifillis* into aquaculture facilities relies on adequate biosecurity protocols when adding new fish into the system (Dickerson and Dawe, 1995) and eliminating the pathogen from incoming water (Heinecke and Buchmann, 2009). The Australian trout industry has restricted movement of live fish between farms, but due to a high volume of water and high organic content in the incoming water, micro-filtration is not employed (E. Meggit pers. comm.). Fish mount an immune response and acquire protective immunity to *I. multifiliis* (see

Buchmann et al., 2001) and vaccination has been investigated (reviewed by Matthews 2005). The level of protection obtained depends on the method and route of immunisation, with exposure to live parasites considered the most effective (Dickerson and Clark, 1996). There is currently no commercial vaccine available. On Australian farms, first year fish are briefly naturally exposed to the disease before chemotherapeutic treatment to aid the development of a functional immune response (M. Landos pers. comm.). Mechanical controls such as low-adhesion polymer raceway lining with automated mechanical vacuuming (Shinn et al., 2009) or manipulation of the water velocity (Bodensteiner et al., 2000) have not yet been trialled on Australian trout farms. In-feed probiotics may increase protection and increase survival (Pieters et al., 2008), but validation of the efficacy of probiotics in the field is required.

2.8.3 Current treatments for *Ichthyophthirius multifiliis*

Classically, the most effective compound for the treatment for *I. multifillis* was malachite green oxalate (CAS No. 2437-29-8) [*N*,*N*,*N*′,*N*′-Tetramethyl-4,4′-diaminotriphenylcarbenium oxalate], a diaminotriphenylmethane dye (Wahli et al., 1993). Malachite green has been banned for use in food fish production in most countries (Alderman, 1985; Wahli et al., 1993; Meinelt et al., 2009) because of its possible carcinogenic, mutagenic and teratogenic risks to human health (Srivastava et al., 2004). Since the banning of malachite green the efficacy of many immersion chemotherapeutants have been examined (reviewed by Picón-Camacho et al., 2012b). All alternative chemotherapeutants are effective only against the free-living stages of *I. multifiliis* and not the trophont; therefore an effective treatment program requires at least two applications of treatment. The first treatment targets the free-living stages in the water, while the second treatment targets the free-living stages in the water

derived from trophonts on the host that survived the first treatment. To strategically time the second round of treatments a detailed knowledge of the developmental times of trophont growth and the free-living stages at different water temperatures is required.

In-feed treatments, such as amprolium hydrochloride, vitamin C, quinine, SalarBec, salinomycim sodium and secnidazole reduce trophont burdens but the general inappetence of heavily infected fish complicates delivery of an effective dose in the latter stages of infections (reviewed by Picón-Camacho et al., 2012b). There has also recently been a move to test plant-based products including extracts from garlic (Buchmann et al., 2003), *Mucuna pruriens* (L.) and *Carica papaya* (L.) (see Ekanem et al., 2004), and *Macleaya microcarpa* (Maximowicz) (see Yao et al., 2011), with varying, generally poor, efficacy.

In Australia, *I. multifiliis* is treated using baths of FOR or SPC but there have been reports of ineffectiveness using these chemotherapeutants (E. Meggit pers. comm.). Straus et al. (2009) reported that theronts of the AR1 strain were significantly more resistant to copper sulphate treatment than the AR5 strain, therefore treatment regimes must be designed specifically to target the relevant strain. Achieving and maintaining a MEC and understanding the distribution of the product in the system also influence the effectiveness of a chemotherapeutant (Rach et al., 1997a). Determining if a lack of understanding of dose, poor application or if other factors are the source of the poor efficacy observed, and development of improved methods that address those problems would improve use of FOR and SPC in Australia.

2.8.4 Monitoring programs for *Lepidotrema bidyana* in Australia

There have been a number of methods developed for monitoring monogeneans, which include: sub sampling of a section of gill tissue (Stephens et al., 2003; Katharios et al., 2006; Rowland et al., 2006); bathing the gills (Anshary et al., 2001; Kimura et al., 2006) or the whole host (Williams et al., 2007) in a known effective treatment and filtering the bath solution then counting the parasites from the filtrate, and (2) manually counting all parasites attached to the gills (Sharp et al., 2004; Forwood et al., 2013). Rowland et al. (2007) monitored *L. bidyana* by routine observations of fish for signs of disease and microscopic examination of gills and skin. This comprised daily observations of fish during feeding for unusual behaviour and sampling three to five fish weekly from larval rearing ponds, and monthly sampling of fingerlings and larger fish. Fish showing signs of disease were preferentially sampled. Examination included external observations, full necropsy, including skin mucus scrapes and gill tissue wet mounts viewed microscopically at 40x and 400x magnification.

Monogeneans can be identified by microscopy at 40 – 100x magnification. Adult and juvenile worms show characteristic stretch and recoiling motion (Read et al., 2007). Rowland et al. (2006) estimated prevalence of *L. bidyana* by sampling four to five fish from each pond, removing the left anterior gill arch from each fish and counting the parasites in 5 fields of view at 100x magnification. This technique, however, does not provide an accurate total abundance. Forwood et al. (2012) refined this technique providing an accurate assessment of individual host abundance by counting the total number of individuals on the first left hemibranch (Lp1) and dividing the number by 0.13, which is the average proportional contribution (APC) of Lp1 to the total number of parasites.

2.8.5 Prevention strategies for *Lepidotrema bidyana* in Australia

The primary aim of an aquaculturist is to eliminate the entry of *L. bidyana* into the farming system (Rowland et al., 2007). Read et al. (2007) recommended all silver perch entering a system should be quarantined and prophylactically treated with 2-5 g/L sodium chloride plus 30 mg/L formalin prior to being placed into the culture system. Once *L. bidyana* has established in a system, maximising water quality, ensuring good nutrition and feeding regimes and minimising stress reduces infection and parasite abundance (Rowland et al., 2007). Regular drying of ponds and using calcium hydroxide ($Ca(OH)_2$) or calcium oxide (CaO) on any persistent damp areas will help to eliminate *L. bidyana* from the pond prior to re- filling and restocking with uninfected fish (Read et al., 2007).

2.8.6 Current treatments for *Lepidotrema bidyana*

The current recommended chemotherapeutants for monogeneans infecting silver perch are formalin at temperatures <25°C, and DEP for temperatures >25°C (see Table 2.2). Monogenean eggs are resistant to FOR and DEP and reinfection with *L. bidyana* occurs five to 30 days after treatment based on re-infection rates (Landos et al., 2007). Based on these observations, Read et al. (2007) recommended three treatments 21 days apart to reduce numbers to an insubstantial level. Detailed lifecycle information is required to refine treatment timing.

Table 2.2: Treatment and dose rates used in the management of monogeneans infecting silver perch (*Bidyanus bidyanus*) (Read et al., 2007). DO = Dissolved Oxygen

Treatment	Dose	Length	Culture system	Notes
Formalin	30 mg/L	Prolonged immersion	Tank	Aerate water; no feeding
Formalin	150 mg/L	30 min bath	Tank	
Trichlorfon	0.25 mg/L	Prolonged immersion	Tank	
Salt (NaCl)	15 g/L	1 h bath	Tank	Repeat following day
Trichlorfon	0.5 mg/L	Prolonged immersion	Pond/cage	
Formalin	30 mg/L	Prolonged immersion	Pond/cage	24 h aeration for 4 – 5 days; monitor DO daily

2.9 Summary

In order to be effective, parasite management strategies in aquaculture must move away from a 'crisis management' approach relying on chemotherapeutants to a more rational planned method. The principals of IPM dictate implementation of preventive non-chemical strategies and using chemical intervention strategically and only when required. This approach can effectively reduce pest numbers in aquaculture systems. Development of AT's based on the effect of the parasite on the host will better inform when chemotherapeutic intervention is required. In the event that intervention is required an optimal approach is to deliver a chemotherapeutant at the minimum effective dose to effectively control the parasite, with repeat treatments to interrupt the lifecycle. To effectively deliver the chemotherapeutant, knowledge of the efficacy against the target parasite(s), the parasite lifecycle to inform strategic timing of treatments and how the products interact with the host, within the aquaculture system and the environment are required to optimise efficacy.