10. General Discussion

There are several areas that require investigation when developing IPM programs against target parasites in aquaculture systems. Effective IPM programs require accurate monitoring programs, a detailed knowledge of the parasite's ecology and host interactions to develop prevention strategies, and when these measures are ineffective the strategic use of chemical treatments. There is limited knowledge in these areas specific to *L. bidyana* and the majority of information on *I. multifiliis* is based on overseas isolates, which can affect the accuracy of treatment timing. The development of IPM programs in Australia is vital, including understanding how programs for different parasites can interact.

A primary goal of an aquaculture producer is to limit the impact of a parasite in the culture system (Meyer, 1991). Freshwater aquaculture systems are often semi-closed, which provides capacity to limit the entry of the parasite to the farm using biosecurity strategies, however, once a parasite is established in a system chemical intervention is often required. My project contributed information to the development of IPM programs for *L. bidyana* and *I. multifiliis* through developing improved techniques for surveillance; elucidating key areas of parasite ecology and host interactions; clarifying lifecycle dynamics to facilitate strategic timing of repeat treatments; and improving delivery of chemical treatments.

10.1 IPM plans for *Ichthyophthirius multifiliis*

10.1.1 Development of monitoring programs

Effective monitoring is an essential part of an IPM program but is unique to each parasite; ectoparasitic infections require routine monitoring for the detection of the

parasite to prevent epizootics. Chapter 3 described the microhabitat of *I. multifiliis* trophonts on rainbow trout and identified the dorsal region as the preferred settlement site. A skin scrape from this region therefore provides the highest likelihood of detecting *I. multifiliis*. Monitoring programs for *I. multifiliis* are linked to changes in water temperature, which are critical for parasite development and infection windows (Chapter 3) and management decisions are often based on predicted rising water temperature.

10.1.2 Strategic timing of treatment

Strategically timing treatments for *I. multifiliis* relies on having an accurate understanding of temperature-development profiles for each life stage of the parasite. On Australian trout farms treatment for *I. multifiliis* was often applied over a week, with treatments on Monday, Wednesday and Friday without taking water temperature into account (E. Meggit, pers. comm.). Using SPC, the treatment interval (time it takes for a tomont to settle and produce theronts) at 17°C is 23 – 29 h (Chapter 3), which means with the above treatment timing tomonts can complete their lifecycle, producing theronts and reinfecting fish before the second treatment has been applied. Using SPC at 12°C, the treatment interval is 42 – 54 h (Chapter 3), which falls within the treatment regime used prior to my work, but SPC has low efficacy against tomonts at 12°C (Chapter 4) and therefore treatments should be applied 12, 42 and 54 hours after the initial treatment to maximise efficacy.

10.2 IPM plans for *Lepidotrema bidyana*

10.2.1 Development of monitoring programs

Parasites that have low pathogenicity require routine monitoring to check if 'action thresholds' have been breached. There is an accurate subsampling method for routine

monitoring of *L. bidyana* in aquaculture facilities (Forwood et al., 2012); this method is also accurate when abundance is low, such as in post treatment evaluation (Chapter 7). Further work is required to determine the number of fish required to be sampled to make accurate predictions of the average intensity within an aquaculture system and, indeed if such prediction can be made, to investigate specific lifecycle data such as parasite growth rate, time to maturity, egg production and time to hatching at different temperatures to model population growth.

10.2.2 Setting action thresholds

The development of 'action thresholds' requires a detailed knowledge of population dynamics of the parasite, to determine the relationship between parasite intensity and the impact on the host. Chapter 6 described the attachment and pathology of *L. bidyana*: attachment penetrates the gill epithelium causing up to 36 epithelial punctures per parasite, tearing and distorting the epithelium and causing mild gill epithelial hyperplasia. High intensity infections by *L. bidyana* could have negative effects on host growth, feeding behaviour, haematocrit levels, oxygen uptake capacity and stress and facilitate the entry of secondary pathogens but further work is required to define the relationships between parasitism and its effects.

10.2.3 Strategic timing of treatments

The timing of treatments for monogeneans is dependent on the culture system. Fish in semi-closed systems such as sea cages are continually exposed to parasites from external sources and therefore must be continually monitored and managed applied based on the parasite's lifecycle under different environmental conditions (Tubbs et al., 2005). In semi-closed systems, such as freshwater systems where water is sourced from the environment and returned, exposure to parasites from external sources, such as infected wild fish, is minimal. Therefore, in the case of *L. bidyana,* application of a

treatment with 100% efficacy prior to re-stocking in parasite free ponds and improved biosecurity should eliminate the need for repeated treatments.

10.3 Treatment delivery

A key goal of IPM programs is to limit the amount of chemical treatment applied to the system, and when intervention is required the lowest effective dose of treatment is used (Kogan, 1998). Management of *I. multifiliis* on trout farms and *L. bidyana* on silver perch farms has been poorly planned and has resulted in treatments being ineffective, which often leads to the use of repeated treatment in an attempt to improve efficacy. The incorrect target dose, inaccurate delivery leading to under dosing, or inappropriate application methods, can also reduce efficacy.

10.3.1 Minimum effective concentrations

One of the aims of this study was to find a chemotherapeutant that could be used to effectively treat *I. multifiliis* and *L. bidyana*. Chapter 3 determined MECs for SPC and FOR over a 1 h period against the free-living stages of *I. multifiliis*. Chapter 8 investigated the efficacy of SPC and FOR and six alternative chemotherapeutants for the treatment of *L. bidyana*. Treatment of mixed infections of monogeneans and protozoans of rainbow trout using HP is successful (Rach et al., 2000). Ostland et al. (1995) used FOR and CL-T to treat a mixed infection of *Flavobacterium branchiophilum* and *Ichthyobodo necatarix*, which significantly reduced the infections but was not 100% effective. In this study MECs for FOR and SPC against *I. multifiliis* were ineffective at reducing a *L. bidyana* infection. The anthelmintic PZQ was the only treatment that was found to be effective against adult *L. bidyana* as a short-term bath. Silver perch need to be held for prolonged treatment or a second treatment to target juvenile worms that survived the first dose.

10.3.2 Host safety

In vitro results suggested that the MEC for FOR against *I. multifiliis* is below the current recommended dose of 200 mg/L for 1 h (Noga, 2000). The effects on the gills of rainbow trout at that dose are well defined (Speare et al., 1997) and such treatments are likely to be safe. *In vitro* results for SPC indicated that at low water temperatures (12°C) the MEC against *I. multifiliis* exceeded that of the maximum allowable dose in Australian freshwater aquaculture of 100 mg/L for 1 h (APVMA: PER12944). Pathological changes in fish exposed to liquid HP treatment are well described (Johnson et al., 1993; Kiemer and Black, 1997; Tort et al., 2002), however, there was little information on the effect of HP gradually released from SPC over time. Chapter 5 described the effect on the gills of rainbow trout of a SPC treatment regime using histology. Repeated doses of 250 mg/L SPC for 1 h induced a significant increase in lamellar oedema and some fish displayed slowed reaction time and a loss of equilibrium. Rainbow trout treated at 250 mg/L SPC for 1 h recovered quickly, however, and although there was no associated mortality is it likely that this dose is close to the maximum safe dose for rainbow trout. Fish exposed to 150 mg/L did not display any significant histological changes in the gills and did not display any adverse signs associated with treatment. Treatment at this dose at < 17° C is therefore likely to be safe. This information will be submitted to the APVMA to amend the current minor permit for SPC to increase the dose.

10.3.3 Treatment application methods

Chapter 6 evaluated delivery methods for FOR and SPC on Australian trout farms by assessing the dose throughout the system over the treatment period. No application method for either treatment completely achieved the target dose temporally or spatially. In trials where SPC was delivered dissolved in water consistent under-

dosing occurred, and was probably due to insufficient addition of the product by the delivery mechanism. Granular SPC trials often displayed significantly lower dose at the start of the treatment period, which is likely to be due to the gradual dissociation and release of HP from SPC. Higher flow also affected treatment distribution by flushing the product, resulting in a significantly lower dose at the inlet of the raceway. Under-dosing in FOR trials was probably due to an underestimation of the system volume combined with FA binding to organic material in the water.

Limiting the influence of these factors by decreasing or stopping flow to create a static bath during treatment provided the most effective treatment environment. Where this is not feasible, regular monitoring of dose throughout the treatment period and applying additional doses when required is recommended. Once the treatment dynamics are established, however, most methods provided a consistent dose-time relationship. The addition of mechanical devices that change flow can also help distribute and retain the product in systems where flow cannot be stopped.

10.4 Limitations of the research

This project provided information for the development of IPM programs for *I. multifiliis* and *L. bidyana*. Development of a complete IPM program is a substantial endeavour, requires regular revision as the host / parasite relationship changes, and such work is beyond the scope of a PhD project. As such there are information gaps for the IPM programs for both *I. multifiliis* and *L. bidyana*. The majority of the present work on the lifecycle of the Australian isolate of *I. multifiliis* in Chapter 3 was conducted in laboratory-based trials, which provides an indication about host-parasite dynamics in farming systems, but field validation is required. This is also the case for the MECs developed in Chapters 4 and 9, where field efficacy data is required. There appears to be a compounding effect of exposure to SPC that causes lamellar oedema

on rainbow trout gills (Chapter 5). The first two treatments did not cause significant changes, but after the second round of treatments there was a significant increase in lamellar oedema. A third round of treatments is required to investigate if the occurrence of oedema continued to increase.

Treatment application methods used on trout farms were investigated and recommendations made to improve delivery but the recommendations were not implemented and validated during this project due to time constraints. The methods will be trialled on farms during summer 2014-15. The colorimetric test strips used to assess FA concentrations during trials only differentiated increments of 10 mg/L; other methods are more precise but are more expensive and slower to implement. The colorimetric method was selected for trial because it was the only method available with a use-cost profile that would be acceptable to farmers for commercial use.

10.5 Future research

I researched ectoparasite and host biology to improve management of ectoparasites in freshwater aquaculture. Multiple factors contribute to the efficacy of a management program for a parasite, not just the treatment itself. Once adequate control measures are in place the focus should shift to the development of prevention strategies to reduce the abundance of the parasite in the aquaculture system. Future research to contribute to the IPM frameworks for *I. multifiliis* and *L. bidyana* developed in this study should focus on enhancing knowledge of parasite and host biology, development of prevention measures, investigating alternative treatments and introduction of new technologies.

10.5.1 Future research for *Ichthyophthirius multifiliis*

Parasite biology

The lifecycle of *I. multifiliis* is well described (Matthews, 2005) but differences in developmental period between isolates require development of programs specific to the target isolate. It is unknown what strain or strains of *I. multifiliis* are found in Australia; this could be resolved by molecular analysis. The development time of the trophont in the host epidermis at different temperatures is described for other isolates (Ewing et al., 1986) but is currently unknown if the Australian isolate fits this profile.

Prevention measures

Heinecke and Buchmann (2009) reported that filtration at 80 µm removes all tomonts, however, filtration to at least 30 µm would be required to prevent theronts from entering a system. Shinn et al. (2009) demonstrated that mechanical removal of the tomonts significantly reduced the abundance of *I. multifiliis*. Increasing the raceway water flow to > 85 cm/min and turnover to > 2.1/h eliminated mortality in juvenile channel catfish caused by *I. multifiliis* where 40 – 60% mortality had been recorded (Bodensteiner et al., 2000). The use of putative probiotics *Aermononas sobria* and *Brochothrix thermosphacta* in-feed, increases survival of rainbow trout, when challenged with *I. multifiliis* (Pieters et al., 2008) and vaccines have been investigated (Gaertig et al., 1999), with varying success (Matthews, 2005). These measures warrant further investigation for use on Australian trout farms although they can be expensive to develop and employ on farms.

New technologies

The development of alternative treatments to control *I. multifiliis* is required so that treatments use can be rotated to reduce the likelihood of the parasites developing resistance to treatments (Thoney and Hargis, 1991). There are other potential chemotherapeutants that that could be used to treat *I. multifiliis*, including a number of natural extracts (reviewed by Picón-Camacho et al., 2012b). Advances in filtration

may lower costs of implementing mechanical preventative measures on farms. Increasing water flow and turnover when designing new systems may help to reduce parasite settlement and lower infection rates. Xu et al. (2012) reported differences in susceptibility in three strains of blue catfish *I. furcatus* to *I. multifiliis,* indicating that selective breeding in trout may be beneficial for reducing the impact of *I. multifiliis*.

10.5.2 Future research for *Lepidotrema bidyana*

Parasite biology

The lifecycle of monogeneans from several genera has been investigated (Kearn, 1994), but the lifecycle of *L. bidyana* remains poorly defined. Short duration baths of PZQ have low efficacy against juvenile worms, therefore a second treatment needs to be strategically applied to coincide with parasite maturation prior to restocking in ponds. This requires knowledge of time to parasite maturation at different water temperatures to strategically time the second treatment to target worms large enough to be affected by the treatment but before the worm is sexually mature.

Prevention measures

Prevention of *L. bidyana* on farms will rely on improving farm biosecurity and increasing surveillance of quarantined translocated stock, and when infected, adequate treatment prior to being moved onto farms. Once established on a farm *L. bidyana* is almost impossible to eradicate. Infected fish can be treated in short-duration baths during grading in re-circulation tanks, before being moved to new, parasite free grow out ponds (Chapter 8). This would reduce the need for pond-based treatments that can be logistically difficult to implement, require large quantities of product, have greater environmental impact and fish undergoing treatment are unable to be removed if showing signs of distress.

New technologies

Lepidotrema bidyana was resistant to a number of broad-spectrum chemotheraputants (Chapter 8), however, fenbendazole (FBZ), a benzimidazole anthelmintic, has shown potential as a bath treatment as an alternative to PZQ (Forwood et al., 2013). Further efficacy studies on alternative anthelmintics are required. Anthelmintics can be delivered as in-feed oral treatments. Oral delivery of PZQ and FBZ has been trialled in silver perch, but showed low efficacy against juvenile *L. bidyana* for both anthelmintics and PZQ displayed poor palatability (Forwood et al., 2013). Refinement of oral delivery methods, an improved understanding of the metabolism and kinetics of these drugs in silver perch and development of MECs will improve efficacy provided by orally delivered anthelmintics and allow farmers to effectively treat fish infected with *L. bidyana* in earthen ponds.

10.6 Future management of ectoparasites in freshwater aquaculture.

Australian freshwater aquaculture is growing, but with industry expansion and higher production, there will be an increase in the incidence of disease. This requires a move away from the traditional 'crisis management' approach, where a chemotherapeutant is applied when it is assumed the parasite is resulting in substantial stock loss. A switch is required, adapting the principals of IPM to a more knowledge-based approach to freshwater fish parasite management. Developing strategies that prevent entry of the parasites onto farms and introducing effective monitoring programs for early detection should take highest priority. If the parasite is persistent and chemical intervention is justified, chemotherapeutants that are strategically timed to coincide with the parasite's lifecycle and effectively target multiple parasites at their minimum effective concentration should be employed. Chemotherapeutants should also have a wide safety margin for the host fish, high worker safety and low environmental

impact. The results presented in this thesis will aid in the future development of such plans for freshwater ectoparasites.