1. General Introduction

Freshwater finfish is the largest sector in world aquaculture, accounting for 60% of production in 2008 (Bostock et al., 2010) but in Australia mariculture is the dominant form of aquaculture (ABARE, 2012). This focus on mariculture is due to abundant availability of marine sites for aquaculture. This has left potential for the development of a sustainable freshwater aquaculture industry in Australia (Rowland, 2009). Two freshwater finfish species in particular have potential for aquaculture production to increase in Australia. Rainbow trout (*Oncorhynchus mykiss*) is a highly palatable species that is cultured worldwide and its production has seen exponential growth since the 1950s (FAO, 2012). Culture of rainbow trout is an established industry in southern Australia but is still a developing industry in comparison to other trout producing countries. The native warm temperate silver perch (*Bidyanus bidyanus*) has been identified as an ideal candidate for culture, and there is a developing industry in Australia (Rowland 2009). Rainbow trout are mainly cultivated in flow-through concrete or earthen raceways. Silver perch are predominantly cultivated in earthen ponds but production in other systems is developing.

Disease is a major limitation on the development of aquaculture industries (Myer, 1991). Australia's isolation and restrictions on imports of animals and biological material have ensured historical freedom from many important diseases (Morrissy, 2002). There are, however, numerous viral, bacterial, fungal, protozoan and metazoan pathogens endemic (Callinan et al., 1986) and introduced (Whittington and Chong, 2007) in Australia. Parasitic protozoa and metazoa are prevalent groups in Australian freshwater aquaculture and can reside within (endoparasites) or outside (ectoparasites) the host. Ectoparasites that have a direct lifecycle can proliferate rapidly once introduced to aquaculture systems (Thoney and Hargis, 1991). High intensity infections are aided by fish in culture being stressed by handling, crowding and suboptimal water quality (Conte, 2004), which leaves them more susceptible to disease (Barton and Iwama, 1991). In Australian freshwater aquaculture, management of parasitic diseases is often reactive, with treatment initiated when the operator believes the parasite is the cause of significant disease or loss of stock.

The concept of Integrated Pest Management (IPM) can readily be applied to the management problems associated with *I. multifiliis* and *L. bidyana*. Integrated pest management utilises detailed knowledge of the pest's biology and ecology to develop management strategies and is used extensively in terrestrial agricultural industries (Kogan 1998). Effective management is achieved by understanding interactions between the host and pathogen, developing monitoring programs based of the pest's lifecycle, investigating natural biological controlling factors and when these measures are inadequate, and applying strategic targeted chemical intervention (Kogan, 1998).

Two important parasites of Australian freshwater aquaculture are the ciliate *I. multifiliis* and the gill dwelling monogenean, *Lepidotrema bidyana*. Treatment of *I. multifiliis* on Australian rainbow trout *Oncorhynchus mykiss* farms involves repeated 1 hour exposure to either formalin (FOR) or sodium percarbonate (SPC) (M. Landos, pers. comm.), although there have been reports of failed treatments (E. Meggit pers. comm.). Silver perch *Bidyanus bidyanus* farms use long term dissipative baths of FOR or copper sulphate ($CuSO_4$) (Read et al., 2007), which have also been ineffective in controlling *I. multifiliis* (M. Landos, pers. comm.). Outbreaks of *I. multifiliis* are particularly problematic in naïve rainbow trout fingerlings at high stocking density when water temperature rises to 17°C and on silver perch farms when water temperature drops to 15°C (Rowland et al., 2007). Optimising management of *I.*

multifiliis is a priority for both the rainbow trout and silver perch aquaculture industries in Australia. Improved information would facilitate better management in both industries and inform whether information sharing between the sectors would provide mutually improved production outcomes.

Silver perch farmers identified a lack of knowledge about *L. bidyana,* therefore, improvement of management stratergies should be a research priority. When *L. bidyana* is treated on farms FOR or the organophosphate trichlorfon (DEP) is applied directly to the pond as a dissipative bath. Treating ponds requires large amounts of product, which is expensive and logistically difficult to administer. There are also significant disadvantages with the use of these chemicals (Forwood et al. 2013). Combined with a low information base to justify treatments, the parasite was often left unmanaged.

Effective monitoring of the parasite is an integral part of IPM programs to determine parasite intensity to assess when intervention is required. The current 'trigger' for treating *I. multifiliis* on trout farms is the presence of the parasite, combined with a rise in water temperature to 17°C (E. Meggit pers. comm.). To facilitate early detection of *I. multifiliis* on Australian rainbow trout farms an understanding of the preferred settlement site of *I. multifiliis* on rainbow trout was required (Chapter 3). Routine monitoring of *L. bidyana* is required to identify increased abundance that indicate a need for intervention. Forwood et al. (2012) developed a method for using subsamples to determine the abundance of *L. bidyana* on an individual host. It was unknown if this method could estimate post treatment abundance, when prevalence and intensity are low and parasites may have been killed differentially on different gills arches. The validity of this method for post-treatment fish was required to rapidly evaluate the efficacy of treatments and is outlined in

Chapter 8. Routine monitoring is used in conjunction with an ‘action threshold’, the point where the parasite causes significant economic loss and at which treatment is advised. An understanding of the pathology of *L. bidyana* on silver perch gills provided information that contributes to the development of an ‘action threshold’ for *L. bidyana* (Chapter 7).

Effective treatment programs require detailed knowledge of the parasite lifecycle and the influence that environmental parameters have on it, so that treatments are timed to disrupt the lifecycle and reduce re-infection rates (Tubbs et al., 2005). There are two developmental phases to consider for *I. multifiliis*: development of the trophont, the stage that resides on the host, and the development of the tomont, one of the free-living stages (Chapter 3). In IPM programs, if the use of a chemotherapeutant is required, the lowest effective concentration is used (Kogan, 1998). To apply this principle to trout and silver perch aquaculture, minimum effective concentrations (MEC) of FOR and SPC for *I. multifiliis* were required to effectively treat the parasite were assessed and are outlined in Chapter 4. Due to reports of low efficacy, treatments for *L. bidyana* required evaluation, and if ineffective, a range of alternatives and their MECs were needed. These were determined and are outlined in Chapter 9. The use of broad-spectrum chemotherapeutants can facilitate the treatment of mixed infections in aquaculture systems (Ostland et al., 1995; Rach et al., 2000), therefore, the use of FOR and SPC were trailed against both parasites.

SPC and FOR are applied in aquaculture using several methods. The major influences on the efficacy of bath treatments are the distribution of the product in the system and achieving and maintaining the minimum effective concentration for the treatment period (Rach et al., 1997a). The distribution of the dose in aquaculture systems is affected by application method, flow, mixing, degradation rate, environmental and water conditions (Rach and Ramsey, 2000). Understanding how these conditions influence the treatment is critical for achieving the target dose (Chapter 6). Treatments, particularly broad-spectrum oxidising and reducing agents, have a range of effects on fish. The effect of FOR (Speare et al., 1997) and hydrogen peroxide (HP) (Speare et al., 1999; Tort et al., 2002) on the gills of rainbow trout is well defined. Physio-chemical water properties such as alkalinity and pH, could affect the efficacy and host toxicity of HP treatment (Tort et al., 2002). Sodium percarbonate slowly releases HP over time and increases the alkalinity of the water, the effect that this has on the tolerance of rainbow trout was unknown and is described in Chapter 5.

Thesis scope and objectives

The chapters of this thesis examine key areas of parasite management and investigate the potential causes of a reported ineffectiveness of current treatments, which results in diminished fish welfare and lost production. This information is integral to the development of IPM programs for *I. multifiliis* and *L. bidyana* and provides a framework for other external parasites in freshwater aquaculture.

The specific aims of the study were to:

1. Characterise the lifecycle of *I. multifiliis* under different salinities and temperatures.
2. Identify the preferred settlement site of *I. multifiliis* on rainbow trout.
3. Identify the MECs of FOR and SPC against *I. multifiliis*.

4. Describe the histological changes in the gills of rainbow trout repeatedly exposed to SPC.

5. Evaluate current treatment application methods for SPC and FOR used on Australian trout farms.

6. Describe the attachment of *L. bidyana* to the gills of silver perch and the resulting pathology.

7. Determine a method for accurately determining post-treatment abundance of *L. bidyana*.

8. Evaluate the efficacy of the current recommended treatments for *L. bidyana*.

9. Investigate the efficacy of potential alternative treatments for *L. bidyana*.

Notes on thesis style

The thesis is presented as a series of manuscripts that are either published or submitted for publication in peer-reviewed, scientific journals. The thesis is comprised of published papers (Chapters 3, 4, 6, 8 and 9), one paper that is submitted and "in review" (Chapter 5), and one paper that is 'in prep' (Chapter 7). For continuity the chapters are presented following the "guide for authors" for the journal Aquaculture. A general discussion of the main findings of this research, limitation of the research and suggestions for future work is included at the end of the research chapters.