

Integrated Pest Management Approach to Ectoparasite Management in Freshwater Aquaculture.

James Michael Forwood



Presented for the degree of Doctor of Philosophy

School of Biological Sciences

Faculty of Science and Engineering

Flinders University, South Australia

October 2014

Title page image: Sodium percarbonate treatment in an Australian freshwater aquaculture farm. Image
J. M. Forwood.

TABLE OF CONTENTS

TABLE OF CONTENTS	3
Summary	7
List of Figures	9
List of Tables	12
Declaration	14
Acknowledgements	15
Statement of Authorship	17
1. General Introduction	19
2. Literature Review	25
2.1 Ectoparasites in freshwater aquaculture	25
2.2 Integrated pest management	25
2.2.1 Integrated pest management in aquaculture.....	27
2.3 Australian freshwater aquaculture industry	27
2.3.1 Australian rainbow trout industry	28
2.3.2 Australian silver perch industry	29
2.4 Diseases of Australian freshwater aquaculture	30
2.4.1 <i>Ichthyophthirius multifiliis</i>	31
2.4.2 Monogeneans	34
2.5 Treatment application methods	36
2.5.1 Prolonged immersion.....	37
2.5.2 Short exposure bath	37
2.5.3 Constant flow treatments	38
2.6 Chemical treatments for external parasites	38
2.6.1 Formalin.....	38
2.6.2 Sodium chloride.....	39
2.6.3 Chloramine-T.....	40
2.6.4 Peracetic acid	41
2.6.5 Sodium percarbonate	43
2.7 Treatments specific to monogeneans	44
2.7.1 Trichlorfon.....	44
2.7.2 Praziquantel	45
2.8 Management practices for ectoparasitic infections in Australia	46
2.8.1 Monitoring of <i>Ichthyophthirius multifiliis</i> in Australia	47
2.8.2 Prevention strategies for <i>Ichthyophthirius multifiliis</i> in Australia.....	47
2.8.3 Current treatments for <i>Ichthyophthirius multifiliis</i>	48
2.8.4 Monitoring programs for <i>Lepidotrema bidyana</i> in Australia	49
2.8.5 Prevention strategies for <i>Lepidotrema bidyana</i> in Australia	50
2.8.6 Current treatments for <i>Lepidotrema bidyana</i>	51
2.9 Summary	52
3. Lifecycle and settlement of an Australian isolate of <i>Ichthyophthirius multifiliis</i> from rainbow trout.	53
3.1 Abstract	54
3.2 Introduction	55
3.3 Materials and methods	56
3.3.1 Culture of parasites	56
3.3.2 Isolation of trophonts	56
3.3.3 Temperature trials.....	57

3.3.4 Salinity trials	57
3.3.5 Detection of <i>I. multifiliis</i>	58
3.3.6 Statistical analysis	58
3.4 Results	59
3.4.1 Temperature trials	59
3.4.2 Salinity trials	60
3.4.3 Detection of <i>I. multifiliis</i>	61
3.5 Discussion	62
3.6 Acknowledgments	65
4. Minimum effective concentrations of formalin and sodium percarbonate on the free-living stages of an Australian isolate of <i>Ichthyophthirius multifiliis</i>.....	66
4.1 Abstract.....	67
4.2 Introduction	68
4.3 Materials and Methods	69
4.3.1 Culture of parasites	69
4.3.2 Experimental design	70
4.3.3 Treatment of theronts	70
4.3.4 Treatment of prototomonts	71
4.3.5 Treatment of tomocysts	71
4.3.6 Statistical analysis	72
4.4 Results	72
4.4.1 Dose response trials on theronts	72
4.4.2 <i>In vitro</i> effect on prototomonts	75
4.4.3 <i>In vitro</i> effect on tomocysts	77
4.4.4 Minimum effective concentrations	80
4.5 Discussion	81
4.6 Acknowledgements.....	83
5. Histological evaluation of sodium percarbonate exposure on the gills of rainbow trout.....	84
5.1 Abstract.....	85
5.2 Introduction	86
5.3 Materials and Methods	86
5.3.1 Experimental design	87
5.3.2 Dosing method and duration	87
5.3.3 Fish sampling	88
5.3.4 Histology processing and evaluation	88
5.3.5 Statistical analysis	90
5.4 Results	90
5.5 Discussion	93
5.6 Acknowledgments	93
6. Evaluation of treatment methods using sodium percarbonate and formalin on Australian rainbow trout farms	97
6.1 Abstract.....	98
6.2 Introduction	99
6.3 Materials and methods	100
6.3.1 Field trials	100
6.3.2 Fish Farm A	101
6.3.3 Fish Farm B	102
6.3.4 Fish Farm C	103
6.3.5 Fish Farm D	104
6.3.6 Chemical analyses.....	105
6.3.7 Statistical analyses	105
6.4 Results	106

6.4.1 Fish Farm A	106
6.4.2 Fish Farm B	107
6.4.3 Fish Farm C	109
6.4.4 Fish Farm D	110
6.4.5 Dissolved oxygen.....	111
6.5 Discussion.....	111
6.6 Acknowledgements.....	115
7. Surface features and attachment mechanism of the diplectanid monogenean, <i>Lepidotrema bidyana</i> Murray, 1931	116
7.1 Abstract.....	117
7.2 Introduction.....	118
7.3 Materials and methods	119
7.3.1 Source of parasites	119
7.3.2 Scanning electron microscope and histology processing	119
7.4 Results	120
7.4.1 Surface features of <i>L. bidyana</i>	120
7.4.2 Attachment by <i>L. bidyana</i>	121
7.5 Discussion.....	125
7.5 Acknowledgments	127
8. Validation of a rapid counting method for assessing treatment efficacy against <i>Lepidotrema bidyana</i> infecting silver perch (<i>Bidyanus bidyanus</i>)	129
8.1 Abstract.....	130
8.2 Introduction	131
8.3 Materials and methods	132
8.3.1 Source of fish and parasites	132
8.3.2 Experimental design	132
8.3.3 Validation of the counting methods.....	133
8.3.4 Statistical analysis.....	133
8.4 Results	133
8.5 Discussion.....	136
9. Efficacy of current and alternative bath treatments for <i>Lepidotrema bidyana</i> infecting silver perch (<i>Bidyanus bidyanus</i>)	138
9.1 Abstract.....	139
9.2 Introduction	140
9.3 Materials and methods	142
9.3.1 Source of fish and treatments.....	142
9.3.2 Source of parasites for the <i>in vitro</i> trial	142
9.3.3 <i>In vitro</i> trials.....	142
9.3.4 <i>In vivo</i> trials	143
9.3.5 Sampling procedure for <i>in vivo</i> trials.....	144
9.3.6 Statistical analysis.....	145
9.4 Results	146
9.4.1 <i>In vitro</i> trials.....	146
9.4.2 <i>In vivo</i> trials	148
9.5 Discussion.....	150
9.6 Conclusions	155
9.7 Acknowledgments	155
10. General Discussion.....	157
10.1 IPM plans specific to <i>I. multifiliis</i>	157
10.1.1 Development of monitoring programs.....	157
10.1.2 Strategic timing of treatment	158
10.2 IPM plans specific to <i>L. bidyana</i>	158

10.2.1 Development of monitoring programs.....	158
10.2.2 Setting action thresholds.....	159
10.2.3 Strategic timing of treatments.....	159
10.3 Treatment delivery	160
10.3.1 Minimum effective concentrations	160
10.3.2 Host safety	161
10.3.3 Treatment application methods.....	161
10.4 Limitations of the research.....	162
10.5 Future research	163
10.5.1 Future research for <i>I. multifiliis</i>	163
10.5.2 Future research for <i>L. bidyana</i>	165
10.6 Future management of external parasites on freshwater farms.....	166
Reference List.....	168

Summary

World aquaculture is dominated by the production of freshwater finfish. In comparison, Australia's dominant aquaculture production is marine, but there are abundant freshwater sites with high quality water available for the development of fish farms. Two fresh water finfish species have potential for aquaculture expansion in Australia: rainbow trout (*Oncorhynchus mykiss*) and silver perch (*Bidyanus bidyanus*). A factor limiting growth of the industry is the management of ectoparasitic diseases. Two problematic parasites in Australian freshwater aquaculture are the ciliate, *Ichthyophthirius multifiliis* and the monogenean *Lepidotrema bidyana*. To facilitate better on-farm management for these parasites information based on the lifecycles of these parasites, interactions between the parasites and hosts, epidemiology, minimum effective concentrations (MEC) for chemotherapeutants, histological changes in hosts exposed to chemotherapeutants and evaluated treatment application methods is required.

The effects of temperature and salinity on lifecycle duration of a temperate Australian isolate of *I. multifiliis* and the preferred settlement sites on rainbow trout are described. The Australian isolates reproduction was proportional to temperature and reproduced faster and had a greater sensitivity to salinity than other temperate isolates. Temperature-lifecycle information and identification of an optimal body region for skin scrapes for surveillance will aid in development of specific management plans for the Australian isolate of *I. multifiliis*, and facilitate strategic timing of treatments.

To improve treatment efficacy the MECs for formalin (FOR) and sodium percarbonate (SPC) were determined for *I. multifiliis*, this information is required on farms to set effective target doses. The MEC for SPC exceeded the current dose recommendations; therefore the structural damage to the gill in rainbow trout exposed to repeated higher doses of SPC was assessed based on the temperature-lifecycle information of *I. multifiliis*. There was minimal structural change in rainbow trout gills exposed to doses of SPC up to 150 mg/L

for 1 h indicating that SPC it is safe at this dose. A requirement of an efficacious treatment is delivering the target dose into the system for the desired time. To determine if current applications met this requirement, four SPC and two FOR application methods on four Australian trout farms with different flow and water quality characteristics were assessed. All methods resulted in under-dosing at various times and positions within the systems during the treatments, which can result in ineffective treatments. Applying the treatments as static baths or reducing flow limits system variables that can influence dose, and monitoring the dose throughout the treatment and adding additional product as required.

Attachment by *L. bidyana* to the gills of silver perch and resulting pathology was described using scanning electron microscopy (SEM) and histology. *Lepidotrema bidyana* attachment causes minor structural damage to the gills and presence is often associated with epitheliocystis. Treating juvenile fish in a short-term bath during grading is a way to economically reduce *L. bidyana* abundance, decreasing the need for repeated treatments in ponds. Efficacy of current treatments for *L. bidyana*: FOR, trichlorfon (DEP) and sodium chloride (NaCl) were assessed. Sodium chloride and FOR were effective *in vitro* but were ineffective at the current recommended doses *in vivo*, and DEP was ineffective *in vitro*. The current treatment's lack of efficacy highlighted the need for alternative treatments that can be administered in short-term baths. Five alternative treatments for *L. bidyana* were investigated. The only effective treatment was praziquantel (PZQ) but this was ineffective against juvenile parasites at the base of the secondary lamellae, suggesting a repeat bath or extended exposure is required to eliminate all parasites. To determine post treatment abundance a sub-sampling method used to count *L. bidyana* was validated.

Results from this research will aid in the development of integrated pest management (IPM) frameworks, enhance surveillance, inform when intervention is required, and improve efficacy when treatment is delivered through strategic timing of treatments, optimising dose and applying treatment using appropriate methods. This will improve management of these parasites on Australian freshwater aquaculture farms.

List of Figures

Figure 4.1: Survival of theronts exposed to different dose levels of sodium percarbonate (SPC) at 12°C (A) and 17°C (B) and formalin (FOR) at 12°C (C) and 17°C (D). Holm-Sidak estimates of the survival data show significant differences between treatment groups, which are represented by difference superscripts. CON = control. 74

Figure 4.2: Mean viability (%) of *Ichthyophthirius multifiliis* prototomonts (A) and tomocysts (B) exposed to formalin (37% formaldehyde); and prototomonts (C) and tomocysts (D) exposed to sodium percarbonate at 12°C and 17°C for 1 h at different concentrations. Different superscripts represent significant differences between doses using Tukey's analysis ($P < 0.05$). Error bars represent the SEM. CON = control..... 77

Figure 4.3: Mean number of theronts produced from viable *Ichthyophthirius multifiliis* prototomonts (A) and tomocysts (B) exposed to formalin (37 % formaldehyde); and prototomonts (C) and tomocysts (D) exposed to sodium percarbonate at 12°C and 17°C for 1 h. Different superscripts represent significant differences between doses using Tukey's analysis ($P < 0.05$). Error bars represent the SEM..... 80

Figure 5.1 Light microphotographs of histological sections of rainbow trout *Oncorhynchus mykiss* gills stained with haematoxylin and eosin (H & E). A) control group gills with normal function; B) fish exposed to 250 mg/L SPC displaying lamellar oedema (black arrow); C) fish exposed to 250 mg/L SPC displaying epithelial hyperplasia (black arrow); D) *Ichthyophthirius multifiliis* trophont (black arrow); E) fish exposed to 150 mg/L SPC displaying epithelial hyperplasia (black arrow); and F) fish exposed to 250 mg/L SPC displaying epithelial hyperplasia (black arrow) and lamellar oedema (white arrow). Scale bars = 50 µm. 92

Figure 5.2 Percentage change in lamellar oedema in rainbow trout exposed to sodium percarbonate on day 1, 2, 8 and 9 at 0 (CON = control), 50, 150 and 250 mg/L for 1 h. Fish were sampled immediately after treatment on day 2, day 3, day 7, and day 9 and on day 10, day 14 and day 18. Different superscript letters indicate significant differences between sample days and different numeric superscripts indicate significant difference between doses on sample day 4 ($P < 0.05$). Error bars represent the SEM. 93

Figure 6.1: Mean doses of hydrogen peroxide (HP) released from sodium percarbonate (SPC) (A) and formaldehyde (FA) from formalin (37% FA) (B) on Fish Farm A. Each trial was repeated three times. Error bars represent 95% CI. 107

Figure 6.2: Mean dose of hydrogen peroxide (HP) released from sodium percarbonate (SPC) administered by drip feed liquid application with one top up dose at 25 min (A); and by granular application in a static bath, with one top up dose at 45 min (B) on fish farm B. Each trial was repeated three times. Error bars represent 95% CI. .. 108

Figure 6.3: Mean doses of formaldehyde (FA) from formalin (37% FA) administered by one dose application into a static bath on Fish Farm C (A). Each trial was repeated three times. Error bars represent 95% CI..... 109

Figure 6.4: Mean doses of hydrogen peroxide (HP) released from sodium percarbonate (SPC) administered by granular application with one dose administered evenly throughout the raceway on Fish Farm D. Each trial was repeated three times. Error bars represent 95% CI. 110

Figure 7.1: SEM images of detached *Lepidotrema bidyana*: Lateral view (A); top view (B); dorsal view (C); and ventral view (D) of the haptor of *L. bidyana*. Scale bars: A, B and C = 12.5 μm ; D = 17.5 μm 121

Figure 7.2: SEM pictures of attached *Lepidotrema bidyana* from *Bidymanus bidyanus*: Two *L. bidyana*, one fully attached with the haptor penetrating the space between the two secondary lamellae (black arrow) and one semi-attached inbetween the secondary lamellae (white arrow) (A); *L. bidyana* attached to gill filament, with haptor penetrating the gill epithilium (white arrow), marginal hooks penetrating and tearing the gill epithilium (black arrow) and dorsal and ventral squamodiscs (blue arrows) (B); detail of the hamuli penetrating the gill epithelium (C); detail of the dorsal squamatodiscs aiding in attachment (white arrow) (D); Juvenile *L. bidyana* using hooklets to form an attachment in-between the secondary lamellae (E). Scale bars: A = 25 μm , B, C, D and E = 10 μm ,..... 122

Figure 7.3: *Lepidotrema bidyana* from *Bidymanus bidyanus*. A and B, H-E sections: longitudinal section of *L. bidyana* attached to the gill filament (A); detail of the haptor penetrating the lamellae during attachment (B). C-E, SEM images: *L. bidyana* haptor impression on the primary and secondary lamellae, with marked depressions at the base of the interlamellae space (white arrows) and epithelial swelling on each side of the secondary lamellae (black arrows) (C); D and E, details of Fig C, epithelial perforations produced by the 9 accessory spines (E), epithelial swelling produced by the marginal hooks (D). Scale Bars: A = 100 μm ; B = 10 μm ; C = 15 μm ; D-E = 7.5 μm 124

Figure 7.4: Epitheliocystis from *Bidymanus bidyanus*. H-E sections: longitudinal section of round-to-oval shaped granular basophilic cysts. Scale Bar = 10 μm 125

Figure 8.1: Relationship between the estimated remaining *Lepidotrema bidyana* abundance and the actual remaining *L. bidyana* abundance in silver perch (*Bidymanus bidyanus*) after treatment. *Significant relationships ($P < 0.05$) assessed by linear regression. (A) Bath treatment with fenbendazole (FBZ). (B) Oral treatment with FBZ. (C) Bath treatment with praziquantel (PZQ). (D) Oral treatment with PZQ.... 135

Figure 9 1: Effects of different treatments on *Lepidotrema bidyana*. (A) formalin, (B) trichlorfon, (C) sodium chloride, (D) peracetic acid, (E) chloramine-T, (F) praziquantel, (G) hydrogen peroxide and (H) sodium percarbonate. Values are means and standard error (SEM). Significant differences between treatment groups and the control group were made using a Mann-Whitney *U* Test and are indicated by * ($P < 0.001$). 10 worms in each well of a 24-well plate were used for each treatment dose

and exposed for 100 min. Three wells were used for each experimental dose and the experiment was performed three times using different batches of worms. 147

Figure 9.2: Mean intensity of *Lepidotrema bidyana* remaining after bath treatment with praziquantel for 60 min (A); hydrogen peroxide for 60 min (B); formalin for 30 min (C); sodium chloride for 60 min (D); sodium percarbonate for 60 min (E). Different super scripts represent significant differences between treatments analysed by Tukey's test ($P < 0.05$). Error bars represent the Standard Error of the Mean (SEM). CON = control..... 150

List of Tables

Table 2 1: Common freshwater external parasites present in Australia (adapted from Morrissy, 2002; Rowland et al., 2007).	31
Table 2.2: Treatment and dose rates used in the management of monogeneans infecting silver perch (<i>Bidyanus bidyanus</i>) (Read et al., 2007). DO = Dissolved Oxygen.....	51
Table 3. 1: Temperature-dependent development of <i>Ichthyophthirius multifiliis</i> tomonts (n = 24) at different water temperatures. Different superscripts indicate significant differences using Tukey's analysis ($P < 0.05$).	59
Table 3. 2: Salinity-dependent development of <i>Ichthyophthirius multifiliis</i> tomonts (n = 24) incubated at 12°C at different salinity levels. Different superscripts indicate significant differences using Tukey's analysis ($P < 0.05$).	60
Table 3. 3: Salinity-dependent development of <i>Ichthyophthirius multifiliis</i> tomonts (n = 24) incubated at 17°C at different salinity levels. Different superscripts indicate significant differences using Tukey's analysis ($P < 0.05$).	61
Table 3. 4: Mean (range) abundance of <i>Ichthyophthirius multifiliis</i> on different body regions of rainbow trout sampled from 5 farms. Different superscripts indicate significant differences using Tukey's analysis ($P < 0.05$).	62
Table 3 5: Range of time (h) for the development of <i>Ichthyophthirius multifiliis</i> from trophont to theront release. Comparison of the present results with literature data. Adapted from Aihua and Buchmann (2001).	62
Table 4.1: Treatments and dose rates (mg/L) administered to <i>Ichthyophthirius multifiliis</i> theronts and tomonts.....	72
Table 4.2: ANOVA interactions between treatment dose, water hardness and temperature on the treatment viability of prototomonts and theront production from viable prototomonts when exposed to formalin (A) and sodium percarbonate (B). – lack of viable prototomonts meant that insufficient data were available to test the term.	76
Table 4.3: ANOVA interactions between treatment dose, water hardness and temperature on the treatment viability of tomocysts and theront production from viable tomocysts when exposed to formalin (A) and sodium percarbonate (B). – lack of viable prototomonts meant that insufficient data were available to test the term. ..	79

Table 4.4: Minimum effective concentrations (mg/L) for different life-stages of <i>Ichthyophthirius multifiliis</i> using sodium percarbonate (SPC) and formalin (FOR) for 1 hour at different water temperatures.	81
Table 6.1 Description of the systems at Fish Farm A used in the validation of treatment methods using sodium percarbonate (SPC), measured as hydrogen peroxide (HP) and formalin (FOR) measured as formaldehyde (FA). N/A = Not applicable, N/D = Not determined.	102
Table 6.2 Description of the flow-through systems at Fish Farm B used in the validation of liquid and granular application methods of sodium percarbonate (SPC), measured as hydrogen peroxide (HP). R = Reduced flow, F = Full flow, N/A = Not applicable.	103
Table 6.3: Description of the flow-through systems at Fish Farm C used in the validation of application methods of formalin (FOR) measured as formaldehyde (FA).	104
Table 6.4: Description of the flow-through systems at Fish Farm D used in the validation of granular application of sodium percarbonate (SPC), measured as hydrogen peroxide (HP). N/A = Not applicable, N/D = Not determined.	105
Table 6.5: Mean \pm SD (range) dissolved oxygen levels during the treatment period using sodium percarbonate (SPC), measured as hydrogen peroxide (HP) and formalin (FOR) measured as formaldehyde (FA) on Australian trout farms.	111
Table 8.1: Actual and predicted prevalence; and actual intensity and predicted mean abundance of <i>Lepidotrema bidyana</i> infecting silver perch (<i>Bidyanus bidyanus</i>) ($n = 218$) after oral and bath treatment with praziquantel (PZQ) and fenbendazole (FBZ). CI = Confidence Interval and NT = No Treatment.	134
Table 9.1: Chemicals and designated concentrations for 100 minute <i>in vitro</i> exposure of <i>Lepidotrema bidyana</i> . – Higher dose not trialled.	143
Table 9.2: Designated dose and duration of chemical treatments and the weight of silver perch ($n = 120$) during <i>in vivo</i> trials.	144
Table 9.3: LC ₉₀ values (95% CI) in mg/L against <i>Lepidotrema bidyana</i> at various time points during a 100 min exposure period <i>in vitro</i> . FOR = formalin, DEP = trichlorfon, NaCl = sodium chloride PAA = peracetic acid, Cl-T = chloramine-T, PZQ = praziquantel, HP = hydrogen peroxide, and SPC = sodium percarbonate. - LC ₉₀ values were unable to be generated.	148

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text

James Michael Forwood

6/11/2014

Acknowledgements

I would first and foremost like to thank my supervisors, Dr Marty Deveney and Dr James Harris for taking a chance on me and allowing me to transition from my background in ecotourism into the new and changeling world of aquatic parasitology. I am forever in debt for my supervisors guidance, patience and encouragement that they provided me throughout my studies, and for putting up with my constant barrage of drafts or with my frequent drop in's with simple problems or questions.

My interest in the area all started with lectures in disease and immunology from James Harris, where the complex interactions between parasites and their hosts peaked my interest. This eventually led me into the field of aquaculture and a project developed by Marty on a troublesome little gill monogenean, *Lepidotrema bidyana*. I can clearly remember my first experience looking for monogenean eggs in sand for hours and hours under a microscope to no avail, it should of indicated how much time I would actually spend looking down a microscope, I should of ran then! I would also like to thank Professor Sonia Kliendorfer, one day I casually mentioned that I might like to do honours I suddenly found myself in a meeting with James Harris and a project, if it was not for Sonia I am sure I would be not writing this today.

I would also like to thank Dr Matt Landos, a field veterinarian, who was rarely seen during my studies but worked extremely hard behind the scenes with logistical work, providing a link back to the farmers, helping to develop project outlines and teaching me the importance of practical outcomes. Also to Professor Ian Whittington who provided valuable assistance in helping identify key aspects of monogenean biology and host interactions. Thanks to my fellow workers in the aquaculture lab; Georgia, Sam, Elise and Matt who made the time an enjoyable and a valuable experience. To my family, and in particular my mother, Jane, who provided me unwavering support and encouragement even with her infrequent inquires of if I might like to get a job soon.

I am very grateful to many people who helped me along the way to gain experience in various techniques used in the project and to Mark and Vicky Scifleet, who run the Pioneer Fish Farm for supplying silver perch and for allowing me access to their farm. To the Victorian Trout Growers Association (VGTA) and Snobs Creek Hatchery for also providing fish and allowing access to their facilities. Thankyou to

Andrew Clarke from the Victorian Department of Primary Industries and to the Australian Government Fisheries Research and Development Corporation (FRDC) for providing funding in chapters 3, 4, 5 and 6 (Project 211/255) awarded to my supervisor Dr Marty Deveney. I would also like to thank Flinders University and the bequest from AJ and IM Naylor for providing a generous honours and PhD scholarship.

Finally, to my father, Robin who instilled in me the drive and determination for hard work and the importance of resolve to achieve your goals.

Statement of Authorship

Chapters 1, 2 & 9: J.F.

Chapter 3:

Data collection: J.F.

Statistical analyses: J.F.

Manuscript writing: J.F., M.D., J.H., M.L.

Forwood, J., Harris, J.O. Landos M. and Deveney, M. (2014) Lifecycle and settlement of an Australian isolate of *Ichthyophthirius multifiliis* from rainbow trout. *Folia Parasitologica*.

Chapter 4:

Data collection: J.F.

Statistical analyses: J.F.

Manuscript writing: J.F., M.D., J.H., M.L.

Forwood, J., Harris, J.O. Landos M. and Deveney, M. (2014). Minimum effective concentrations of formalin and sodium percarbonate on the free-living stages of an Australian isolate of *Ichthyophthirius multifiliis*. *Parasitology Research*. 113 (9): 3251-3258.

Chapter 5:

Data collection: J.F.

Statistical analyses: J.F.

Manuscript writing: J.F., M.D., J.H., M.L.

Forwood, J., Harris, J.O. Landos M. and Deveney, M. (under review). Histological evaluation of sodium percarbonate exposure on the gills of rainbow trout. *Disease of Aquatic Organisms*.

Chapter 6:

Data collection: J.F., M.L.

Statistical analyses: J.F.

Manuscript writing: J.F., M.D., J.H., M.L.

Forwood, J., Harris, J.O., Landos, M., Deveney, M. (2014). Evaluation of treatment methods using sodium percarbonate and formalin on Australian rainbow trout farms. *Aquacultural Engineering*. 63: 9-15.

Chapter 7:

Data collection: J.F.

Data interpretation: M.D., J.F., I.W.

Manuscript writing: J.F., M.D., J.H. I.W.

Chapter 8:

Data collection: J.F.

Statistical analyses: J.F.

Manuscript writing: J.F., M.D., J.H.

Forwood, J., Harris, J.O. and Deveney, M. (2013). Validation of a rapid counting method for assessing treatment efficacy against *Lepidotrema bidyana* infecting silver perch *Bidyanus bidyanus*. *Diseases of Aquatic Organisms*, 105(3) pp. 253-257.

Chapter 9:

Data collection: J.F.

Statistical analyses: J.F.

Manuscript writing: J.F., M.D., J.H.

Forwood, J., Harris, J.O. and Deveney, M. (2013). Efficacy of current and alternative bath treatments for *Lepidotrema bidyana* infecting silver perch, *Bidyanus bidyanus*. *Aquaculture*, 416-417: 65-71.

Previous publications related to the PhD

Forwood, J., Harris, J.O. and Deveney, M. (2012). Host impact of monogenean *Lepidotrema bidyana* infection and intensity estimates for onsite monitoring. *Diseases of Aquatic Organisms*, 100(1): 51-57.

Forwood, J., Harris, J.O. and Deveney, M. (2013). Efficacy of bath and orally administered praziquantel and fenbendazole against *Lepidotrema bidyana* (Murray), a monogenean parasite of silver perch, *Bidyanus bidyanus* (Mitchell). *Journal of Fish Diseases*, 36: 939-947.