Oral treatments for monogenean parasites of farmed yellowtails, *Seriola* spp. (Carangidae)

Rissa E. Williams

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Rissa Williams
30 November 2009

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DEDICATION

To my parents, Daisy and Terry Williams

You taught me how to watch, listen and learn. You gave me the freedom to grow and be independent and a loving home to come back to. Thank you for believing in me.

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TABLE OF CONTENTS

PUBLICATIONS ARISING FROM THIS PHD

Williams, R.E., Ernst, I., Chambers, C.B., Whittington, I.D., 2007. Efficacy of orally administered praziquantel against *Zeuxapta seriolae* and *Benedenia seriolae* (Monogenea) in yellowtail kingfish *Seriola lalandi*. Diseases of Aquatic Organisms 77, 199-205. doi: 10.3354/dao01824

ABSTRACT

Japanese yellowtail *Seriola quinqueradiata* has been commercially farmed in Japan since the 1940s. In comparison, sea-cage farming of yellowtail kingfish *Seriola lalandi* in Australia is still developing, with commercial production commencing in 1998. In Australia, *S. lalandi* is parasitised by *Zeuxapta seriolae* and *Benedenia seriolae*. In Japan, *S. quinqueradiata* is parasitised by *Heteraxine heterocerca* and *B. seriolae*. These monogeneans affect industries in both countries and management of these parasites is required to prevent impacts on fish health and commercial losses.

I investigated efficacy (% reduction of mean parasite abundance) for orally administered praziquantel, fenbendzole and oxfendazole against *Z. seriolae* and *B. seriolae* on *S. lalandi* and the efficacy of orally administered praziquantel and febantel against *H. heterocerca* and *B. seriolae* on *S. quinqueradiata*. Medications were administered to fish by surface coating feed pellets or via direct intubation of the stomach. *Seriola lalandi* administered fenbendazole and oxfendazole by surface coating of feed had lower abundance of the gill parasite *Z. seriolae*. *Seriola quinqueradiata* intubated with febantel had lower abundance of the gill parasite *H. heterocerca*. Neither fenbendazole nor oxfendazole administered to *S. lalandi* in Australia, nor febantel administered to *S. quinqueradiata* in Japan resulted in a lower abundance of the skin parasite *B. seriolae*.

Praziquantel was first administered to *S. lalandi* by surface coating of feed. Fish rejected medicated feed, suggesting praziquantel affected its palatability. Fish treated with feed medicated with praziquantel had fewer *Z. seriolae* and *B. seriolae* than untreated fish. Praziquantel administered to *S. lalandi* by intubation allowed a more accurate dose to be tested without differential feeding or reduced palatability obstructing results, and resulted in fewer *Z. seriolae* (99.5-100 % reduction) and *B. seriolae* (91 – 97.7 % reduction). Intubated praziquantel also led to fewer recruitment life stages of *Z. seriolae* and *B. seriolae*, even at low doses, but did not completely eliminate them from *S. lalandi*. Praziquantel administered to *S. lalandi* alone and combined with cimetidine had high efficacy (>99%) against *Z. seriolae*. In comparison, praziquantel administered alone resulted in fewer *B. seriola*e (68.3 –

9

69.7 % reduction) than the same doses of praziquantel combined with cimetidine (36.9 – 40.9 % reduction). A 90.4 -100 % reduction in *H. heterocerca* was achieved when praziquantel was administered by intubation to *S. quinqueradiata* in Japan but there was only a 22-77.8 % reduction in *B. seriolae*. The dose of PZQ (150 mg kg^{-1} body weight day^{-1} for 3 days) on the label of a commercially available product used to treat *B. seriolae* in Japanese aquaculture resulted in a 50.9% reduction against *B. seriolae*, but completely eliminated *H. heterocerca.*

In trials against *Z. seriolae* and *B. seriolae* on *S. lalandi* in South Australia, I also screened 27 other anthelmintics and antiparasitics from the chemical groups: amprolium derivatives, benzimidazoles, benzyl ureas, diphosphate salts, imidazothiazoles, macrocyclic lactones, nitromidazoles, organophosphates, piperazines, salicylanilides, substituted phenols and tetrahydropyrimidines. Of these, only the benzimidazole, albendazole, was effective against *Z. seriolae* and none appeared to have an effect against *B. seriolae*.

ACKNOWLEDGEMENTS

The reason I am where I am today is because I decided to follow the less conventional field of Marine Parasitology. For this I must thank my supervisors for inspiring me (and sometimes, towards the end, having to shove me a little!) down this path. From the formative stages of this thesis, to the final draft, I am immensely in debt to Ian Whittington and Marty Deveney. I am forever grateful for your careful guidance, tireless enthusiasm and encouragement. I thank you for your steady belief that I would get there in the end.

It all began with Associate Professor Ian Whittington, during his memorable undergraduate lectures at The University of Queensland, which stirred my interest to pursue more parasitology courses and eventually Honours and PhD degrees. He turned me down for an Honours project - and I almost turned to Marine Botany - but I wouldn't take no for an answer! Eventually I landed this PhD project with him and Dr Ingo Ernst in Adelaide. I acknowledge the formative input into my project, particularly for experimental design and fieldwork, from my co-supervisor Dr Ingo Ernst, and thank him for the invaluable opportunities to work with the aquaculture industry in South Australia, Northern Territory and Japan. I would also like to embarrass my other co-supervisor, Dr Marty Deveney. I will always remember the day he helped me find my first monogenean on the tail of a whiting at Moreton Bay Research Station.

Next I would like to show my gratitude to my comrades in the Marine Parasitology Laboratory – Allan Mooney, Kate Hutson, David Schmarr and Vanessa Glennon. Some of my best memories are of working with you in Adelaide. And to Lizzie Perkins – a special mention of gratitude for not only keeping me sane but most of all for sharing in the insanity! To my family – Daisy my mum and Terry my dad, and my "little" brother James – I am indebted to you for your unwavering love, belief and pride, which never fails to lift me up at the lowest of times.

I would also like to acknowledge the continued support of my employer, the New Zealand Ministry of Agriculture and Forestry, Biosecurity New Zealand, and particularly the Incursion Investigation (Animals and Marine) Team. Thank you for your patience during this testing time and kind words of encouragement.

CHAPTER 1 - GENERAL INTRODUCTION

Chapter 1: General Introduction

Many of the world's aquatic resources have been diminished by overfishing. As a consequence there has been an increased need for aquaculture to contribute seafood to the world market. In 2006, aquaculture contributed 47% of the world's seafood supplies (Anonymous, 2008). While growth in wild capture fisheries stopped in the mid 1980s, the annual average growth in aquaculture has been steady at approximately 8.7%, and was 6.1% between 2004 and 2006 (Anonymous, 2008).

The Japanese yellowtail *Seriola quinqueradiata* Temminck and Schlegel (Carangidae) is a highly regarded table fish that has been commercially farmed in Japan for more than 60 years. In 2003, *S. quinqueradiata* represented approximately 57% of the total finfish production in Japan, and in 2004 production exceeded 150000 tonnes worth an estimated $US 1.27 billion (Dhihendra, 2009). Compared with Japan, sea-cage farming of yellowtail kingfish *S. lalandi* Valenciennes in Australia is still a developing industry. Commercial production commenced in 1998 (Hernen and Hutchinson, 2003) and was expected to reach 2 500 tonnes in 2007 (Norwood, 2008).

Sea-cage aquaculture provides a suitable environment for proliferation of parasitic organisms. Fish numbers and stocking densities in aquaculture are often high, allowing for increased transmission success by parasites compared with fish in the natural environment, which are at much lower densities and populations are more widely dispersed. Numerous factors contribute to the growth of parasite populations in sea-cage aquaculture. Sea-cages are open to the ocean and farmed fish are exposed to wild fish swimming past, which transmit pathogens to the farmed fish. This is difficult to prevent with current technologies and practices. Poor water quality and frequent crowding or handling of fish (e.g. for grading or health management) may stress them (Conte, 2004), leaving them more susceptible to pathogens (Barton and Iwama, 1991). Parasites with a single host lifecycle such as caligid sea lice (Arthropoda) and monogeneans (Platyhelminthes) may become a problem as they can parasitise fish directly and proliferate in culture conditions with high stocking densities (Roy et al., 2000). While they can cause direct losses due to mortality, these parasites also affect fish appetite, growth, behaviour and marketability (Ogawa, 1996; Scholz, 1999).

14

Sea-cage aquaculture of *Seriola* spp. in Japan and *S. lalandi* in Australia has demonstrated vulnerability to monogenean parasites (Anonymous, 2002a) and is a limiting factor to the development of this potentially lucrative industry in Australasia. In Australia, *S. lalandi* is parasitised by the polyopisthocotylean *Zeuxapta seriolae* (Meserve) Price (Heteraxinidae) (see Sharp et al., 2001) and the monopisthocotylean *Benedenia seriolae* (Yamaguti) Meserve (Capsalidae) (see Whittington, 1996). These parasite species are also found on species of *Seriola* cultured in Japan, along with the polyopisthocotylean *Heteraxine heterocerca* (Goto) Yamaguti (Heteraxinidae) (see Egusa, 1983) and the monopisthocotylean *Neobenedenia* sp. (see Ogawa and Yokoyama, 1998). *Heteraxine heterocerca* and *Neobenedenia* sp. have not been reported on wild or cultured *S. lalandi* in Australia. The heteraxinids *Z. seriolae* and *H. heterocerca* attach to the gills of fish and feed on blood (Whittington and Chisholm, 2008) and heavy infestations by one or the other of these monogenean species have been associated with anaemia and mortality in *S. lalandi* in Australia (Sharp et al., 2001), in *S. quinqueradiata* in Japan (Egusa, 1983; Ogawa and Yokoyama, 1998) and *S. dumerili* (Risso) in the western Mediterranean (Grau et al., 2003; Montero et al., 2004). Heavily infested fish appear lethargic and have reduced appetite. The capsalid flukes *B. seriolae* and *Neobenedenia* sp. inhabit the skin and fins of their hosts where they feed on mucus and epithelial cells. Serious infestations by *B. seriolae* on *S. quinqueradiata* in Japan and on *S. lalandi* in Australia have been associated with mortality, reduced growth rates and increased rubbing behaviour (sometimes termed "flashing") of fish (Egusa, 1983; Ernst et al., 2002; Williams et al., 2007; Whittington and Chisholm, 2008).

In Japan, attempts to manage monogenean parasites in *Seriola* spp. sea-cage aquaculture have occurred since the 1960s (Whittington et al., 2001), where management of *B. seriolae* has been estimated to contribute up to 22% of the production costs (Ernst et al., 2002). In Japan, *H. heterocerca* and *B. seriolae* are treated by bathing with freshwater or hydrogen peroxide (Dhihendra, 2009). Bathing is labour-intensive, time-consuming, weather-dependent and stressful to fish (Tojo and Santamarina, 1998b; Stone et al., 1999; Kim and Cho, 2000). The Japanese industry has developed considerable expertise in bathing techniques, but fish mortality may still occur during bathing due to anoxia or physical damage from crowding fish. In 2000, Hadaclean[®] (Bayer, Japan), an in-feed treatment, became commercially available to Japanese farmers for the treatment of *B. seriolae* (see

Stephens et al., 2003; Mansell et al., 2005; Tubbs and Tingle, 2006a; Williams et al., 2007).

In South Australia, management of *Z. seriolae* and *B. seriolae* on *S. lalandi* is currently dependent on bathing fish in hydrogen peroxide (Chambers and Ernst, 2005). The therapeutic margin of hydrogen peroxide is narrow, especially at water temperatures above 18°C, which can make it difficult to use this treatment in summer (Rae, 1999). Fish mortality may occur due to stress, miscalculation of bath solution, physical damage from crowding and/or anoxia. No oral medications are registered for the treatment of *Z. seriolae* and *B. seriolae* in Australia. The continuing growth of the kingfish farming industry in South Australia has generated interest in development of in-feed treatments for *Z. seriolae* and *B. seriolae* on *S. lalandi* to provide an alternative to bath treatments. Unlike bath treatments, oral treatments require little extra labour or infrastructure and do not stress fish through handling or crowding. There are many drugs registered for livestock and veterinary applications that are effective against parasitic platyhelminths, but few have been tested against monogenean parasites of fish. The aim of my PhD research was to investigate a number of veterinary medicines with potential as oral treatments against *Z. seriolae* and *B. seriolae* infesting *S. lalandi* in sea-cage aquaculture in South Australia and against *H. heterocerca* and *B. seriolae* infesting *S. quinqueradiata* in Japanese sea-cage culture.

Oral treatments are not intended to be a "silver bullet". When combined with effective on-farm husbandry practices and knowledge of parasite biology, these treatments contribute to more efficient management of monogeneans in *Seriola* spp. aquaculture through an Integrated Parasite Management (IPM) approach and provide a cost effective alternative to bath treatments. My research, therefore, is directly relevant to the Australian and Japanese marine finfish aquaculture industries.

Most experimental trials in my study were conducted on *S. lalandi* from South Australian sea-cage aquaculture where the following compounds administered orally were tested against *Z. seriolae* and *B. seriolae*: fenbendazole and oxfendazole (Chapter 3), praziquantel alone (Chapter 4) and specifically against invasion by larvae and their persistence after attachment (Chapter 7) and praziquantel in combination with cimetidine (Chapter 6). Other experimental trials assessed febantel (Chapter 3) and praziquantel (Chapter 5) administered orally against *H. heterocerca* and *B. seriolae* on *S. quinqueradiata* in Japanese sea-cage culture. From the

16

literature, I also identified several other compounds as prospective oral treatments for monogenean parasites of *S. lalandi* and tested for activity against *Z. seriolae* and *B. seriolae* in South Australia in short, one-off screening trials (Chapter 8).

Notes on chapter style

      Chapter 4 is already published and therefore conforms precisely to the style of the journal *Diseases of Aquatic Organisms*. Copyright consent from *Diseases of Aquatic Organisms* is provided in Appendix 1. A statement of authorship declaration detailing publication information and co-author contributions precedes Chapter 4 and a reprint is provided in Appendix 2. I intend to submit each of my other data chapters for publication. The text, therefore, also reflects multiple authors who made similar contributions to those outlined for Chapter 4 in securing funding, project supervision, assistance in the field and commenting on chapter drafts during the preparation of my thesis. Specifically I use the pronoun "we" in those chapters that have been produced with input from others, and the pronoun "I" in Chapters 1-2 and Chapter 9, which I wrote independently.

      To provide consistency in presentation and format, data chapters 3 and 5-8 are prepared using the current "Guide for Authors" for the journal *Aquaculture*. My thesis complies with the "Specifications for Thesis 2009" provided by the Adelaide Graduate Centre at The University of Adelaide.

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CHAPTER 2 – LITERATURE REVIEW

Chapter 2: Literature Review

1. Parasites of finfish in aquaculture

Parasites are ubiquitous, although they generally do not cause disease in wildlife (Scholz, 1999). Parasites do, however, cause disease when animals are kept intensively. This applies equally to terrestrial and aquatic animals. In culture conditions, fish, crustaceans and shellfish become stressed if they are crowded or handled often, or if water quality is reduced (Conte, 2004), which may leave them more vulnerable to disease (Barton and Iwama, 1991). Cultured fish are often kept at high densities and the close proximity of individuals may also contribute to rapid parasite transmission (Ogawa, 1996). The inability of the fish to move away from sources of parasites to other areas exacerbates this enhanced transmission.

Fish or shellfish that are grown in semi-open systems (e.g. sea-cages) are exposed to parasite transmission from wild fish, which is impossible to eliminate with current technology. Temperature and salinity vary seasonally and cannot be controlled or manipulated and these may affect the vulnerability of fish to parasites and intensify parasite reproduction and transmission. Treatments to control parasites are more difficult to administer in semi-open systems. Treatment application technologies are often labour intensive, dependent on weather conditions, and recruitment by new parasites often occurs immediately after treatment.

2. Monogenean parasites

The Monogenea is a class of parasitic flatworms (platyhelminths). Monogeneans are commercially significant parasites in aquaculture, reducing production efficiency and survival of fish (Thoney and Hargis, 1991; Whittington and Chisholm, 2008). They principally attach to the external surfaces of their fish hosts, including the skin, fins, eyes, buccal cavity and gills. Based on their attachment organ (the haptor) and diet they can be divided into two sub-classes: the Monopisthocotylea and the Polyopisthocotylea. Monopisthocotyleans have a relatively simple haptor and feed on host mucus and epithelial cells. They are predominantly found on the skin and fins of their hosts but may also attach to the gills. Polyopisthocotyleans have a more complex haptor, comprising several

20

attachment units. These parasites feed on host blood and are almost exclusively found on the gills. Monogeneans are not normally pathogenic to their hosts in the wild but can become pathogenic in aquaria or aquaculture (Whittington and Chisholm, 2008) (see Table 1). They have a direct lifecycle, requiring only one host, which allows them to reproduce rapidly under a variety of environmental conditions (Thoney and Hargis, 1991).

Table 1

Selected monogenean parasites affecting sea-caged finfish. The four species that are of concern to sea-caged *Seriola* spp. in Australia and/or Japan are in bold.

Monogenean species	Host species	Site on host	Source
Monopisthocotylea			
Benedenia seriolae	*Seriola lalandi*	Skin and body surfaces	Williams et al. (2007)
	S. quinqueradiata		Ogawa and Yokoyama (1998)
	S. dumerili		Ogawa and Yokoyama (1998)
Diplectanum aequans	*Dicentrarchus labrax*	Gill lamellae	Whittington and Chisholm (2008)
Laticola paralatesi	*Lates calcarifer*	Gill lamellae	Tingbao et al. (2006)
Gyrodactylus salaris	*Salmo salar*	Skin and body surfaces	Whittington and Chisholm (2008)
Neobenedenia sp.	*Seriola quinqueradiata*	Skin and body surfaces	Ogawa and Yokoyama (1998)
			Ogawa and Yokoyama (1998)
	S. dumerili		Deveney et al. (2001)
	L. calcarifer		Leong (1997)
	Epinephelus spp.		
Polyopisthocotylea			
Heteraxine heterocerca	*S. lalandi*	Gill lamellae	Ogawa and Yokoyama (1998)
	S. quinqueradiata		Egusa (1983)
	S. dumerili		Ogawa and Yokoyama (1998)
Heterobothrium okamotoi	*Takifugu rubripes*	Gill lamellae	Ogawa et al. (2005)
Microcotyle sebastis	*Sebastes schlegeli*	Gill lamellae	Kim and Choi (1998)
Zeuxapta seriolae	*Seriola lalandi*	Gill lamellae	Williams et al. (2007)
	S. quinqueradiata		Ogawa and Yokoyama (1998)
	S. dumerili		Ogawa and Yokoyama (1998)

My research focussed on monogenean parasites of sea-caged *Seriola* spp. in Australia and Japan. I will review the biology of these parasites because the site and mode of attachment and diet preference of these monogeneans are factors likely to influence the efficacy of chemical compounds aiming to target and treat these parasites.

3. Monogenean parasites of *Seriola* spp.

Four monogenean parasite species commonly parasitise *Seriola* spp. in Australia and/or Japan: *Benedenia seriolae, Neobenedenia* sp., *Heteraxine heterocerca* and *Zeuxapta seriolae* (see species in bold, Table 1). These species require management to prevent impacts on fish health and commercial losses. Both *H. heterocerca* and *Z. seriolae* (Heteraxinidae) are polyopisthocotylean monogeneans that attach to gill lamellae using numerous haptoral clamps and feed on blood (Whittington and Chisholm, 2008). Heavy infestations of blood-feeding monogeneans may cause anaemia, which can kill fish directly (Kim and Choi, 1998). In Japan, heavy burdens of *H. heterocerca* have been associated with anaemia and mortality (see Figs. 1a, b) in cultured *S. quinqueradiata* since the 1960s (Egusa, 1983; Ogawa and Yokoyama, 1998). Infestations by *Z. seriolae* have similarly caused anaemia, reduced appetite and decreased growth of cultured *S. lalandi* in New Zealand and Australia (Sharp et al., 2001) and mortality in cultured amberjack *S. dumerili* in the western Mediterranean (Grau et al., 2003; Montero et al., 2004). A similar polyopisthocotylean *Heterobothrium okamotoi* (Didiclophoridae) increased the amount of blood it fed upon from its host as it grew in size (Ogawa et al., 2005). It is likely that *Heteraxine heterocerca* and *Z. seriolae* do the same, but how much blood they consume and how regularly these parasites feed is unknown (Whittington and Chisholm, 2008).

Benedenia seriolae (Capsalidae) is a monopisthocotylean monogenean that attaches to the skin, fins and eyes of *Seriola* sp. using a sucker-like haptor armed with hooked sclerites (Whittington, 1996). Attachment appears to cause little damage to fish, but feeding wounds in heavy infestations may penetrate the epidermis deeply (Whittington and Chisholm, 2008) and are likely to compromise osmotic control. Serious infestations by *B. seriolae* on *S. quinqueradiata* in Japan and *S. lalandi* in Australia have been associated with mortality, reduced growth rates and increased rubbing behaviour (sometimes termed "flashing") of fish (Egusa, 1983; Ernst et al., 2002; Williams et al., 2007; Whittington and Chisholm, 2008). Aggravated epidermal wounds affect the appearance of fish, reducing their value and marketability. They may also lead to secondary bacterial, viral or fungal infections (Thoney and Hargis, 1991).

Image: C. Chambers

Fig. 1a: Gills of a normal *Seriola quinqueradiata*.

Image: C. Chambers

Fig. 1b: Gills of a dead specimen of *S. quinqueradiata* with apparent anaemia caused by heavy infestation by *Heteraxine heterocerca*, which was a likely cause of death.

4. Sea-cage aquaculture of *Seriola* spp.

World aquaculture of *Seriola* spp. includes three key species: S. *quinqueradiata, S. lalandi* and *S. dumerili*. These fish are demersal predatory fish in the Carangidae, which includes the trevallies and amberjacks. They are found globally in warm temperate to tropical waters. Japan is the leading producer of *Seriola* spp. worldwide. Their rapid growth (reaching 1 kg in their first year), schooling behaviour, ready adaptation to culture conditions and acceptance of artificial diets make *Seriola* spp. ideal candidates for intensive aquaculture (García and Díaz, 1995; Mazzola et al., 2000; Skaramuca et al., 2001).

In Japan, three species of *Seriola* are cultured: *S. quinqueradiata, S. lalandi* and *S. dumerili*. Collectively, they comprise one of the most important wild fishery resources (Mushiake, 1999), are the most widely cultured marine finfish in Japan and is the most valuable sea-caged finfish industry in a single country worldwide (Anonymous, 2002b). Culture is mainly concentrated around Shikoku and Kyushu (Ogawa and Fukudome, 1994). *Seriola quinqueradiata* was the first marine fish to be cultured in Japan, in 1930 (Ogawa, 1996). Initially, *S. quinqueradiata* were kept in tidal enclosures. In the 1960s, offshore sea-cage culture was introduced, which led to improved productivity and management and the rapid expansion of the industry (Ogawa, 1996). *Seriola* spp. fingerlings are captured from the wild and transferred to sea-cages where they are grown to harvest size. Amberjack, *S. dumerili*, is a high-quality and sought-after product for the sashimi market and attracts higher prices in Japan than *S. quinqueradiata* (see Ogawa and Fukudome, 1994; Nakada 2002), which also makes them appealing for aquaculture (Grau et al., 1999). Yellowtail kingfish, *S. lalandi*, is the only *Seriola* sp. cultured in Australia and is a relatively young industry that commenced in 1998 (Hernen and Hutchinson, 2003). It is also relatively small and commercial production occurs only in the Spencer Gulf in South Australia. Juvenile fish are raised to approximately 5 g in land-based hatcheries before transfer to sea-cages for growth to market size (Love and Langenkamp, 2003). In Australia, local demand for kingfish is small (Anonymous, 2002b). Australian farmers market *S. lalandi* under the Japanese name *hiramasa* and focus their product on the sashimi market in Japan and USA (Love and Langenkamp, 2003) and recently Russia.

5. Management of monogeneans in sea-cage aquaculture: current practises

5.1 Bathing

The most common method of treating sea-caged fish infested with monogeneans is by bathing with hydrogen peroxide (H_2O_2) or fresh water. To bathe fish effectively and to allow accurate calculation of bath solution they should be briefly contained in a closed environment. This is achieved by completely enclosing the sea-cage using a tarpaulin (Fig. 2; H_2O_2 only) or by physically removing fish from their cage and placing them in a bath solution. In South Australia, a 300 ppm H_2O_2 solution is used to treat *S. lalandi* (see Mansell et al., 2005), while in Japan, both fresh water and H_2O_2 bath treatments are used to treat *Seriola* spp. for monogeneans.

Fig. 2: Bath treatment of *Seriola lalandi* in South Australia with H_2O_2.

Bathing is labour-intensive, time-consuming, weather dependent and presents a risk to fish because therapeutic margins for these treatments are typically low. Good farm management aims to minimise losses, but it is not unusual for fish to die due to treatment miscalculation through estimation of the bath volume, from lack of oxygen, or from being crushed while manoeuvring the tarpaulin or nets. Following bath treatment, fish are stressed and often have reduced appetite, which diminishes growth rates. Bathing is costly, e.g. in Tasmania the cost of bathing to treat amoebic gill disease contributes up to 20% of the production costs of sea-caged Atlantic salmon, *Salmo salar* (see Munday and Zilberg, 2003).

5.2 Farm practises and fish stress

Stress is known to increase the susceptibility of fish to disease (Barton and Iwama, 1991) and has been linked to increase the vulnerability of salmonids to the monogenean *Gyrodactylus salaris* Malmberg 1957 (see Harris et al., 2000). It also impairs fish growth and reproduction and therefore stress to fish needs to be managed in any aquaculture situation (Davis, 2006). Stress can be induced by environmental factors, so farms should be sited in regions with environmental conditions suited to the fish species under culture (Conte, 2004). In particular water temperature and salinity should be within acceptable limits for the species, and the site should have adequate depth and water flow. Nets and cages should be regularly cleaned of fouling organisms to maintain water flow which can affect dissolved oxygen concentrations. Furthermore, the eggs of *Z. seriolae* and *B. seriolae* (see Chambers and Ernst, 2005) and *H. heterocerca* (see Mooney et al., 2008) can entangle and collect on submerged structures such as sea-cages. Regular removal of fouling from nets and cage structures may also, therefore, reduce sources of larval parasites.

Many everyday farm practises such as handling, grading or transport are sources of stress (Barton and Iwama, 1991, Davis, 2006). It is important, therefore, to carefully manage these processes to reduce stress where possible. Repeated exposure to predators is also a source of stress, which can lead to increased disease occurrence (Nash et al., 2000). If achievable, exclusion of predators from marine farms is desirable. Correct nutrition and proper feed management is important as this can improve fish coping with and recovering from exposure to stress factors (Barton,

2002). Other practises, such as strategic treatments based on knowledge of parasite lifecycles and fallowing of sites between harvests and re-stocking directly target parasites. A surveillance program to monitor fish health is also essential because accurate and early detection of a parasite infestation is essential for correct timing of treatments and to prevent mortalities and production losses (Grant, 1993).

5.3 Integrated Parasite Management

Parasite treatment in sea-cage aquaculture has often been reactive and unplanned. Haphazard application of medicines is inefficient, uneconomical and also promotes resistance. Integrated Parasite Management (IPM) is used extensively in livestock and crop production for the effective control of parasites and other pests. IPM promotes more efficient and effective chemical use, which leads to less product being required and diminishes development of parasite resistance. Effective IPM leads to consistently lower parasite levels, which are easier to manage and in the long term, are cheaper than less coordinated treatments. A thorough knowledge of the parasites' lifecycles, transmission and epizootiology is essential in order to coordinate management (Scholz, 1999). Individual management units need to be identified to effectively implement IPM (Chambers and Ernst, 2005). Treatments coordinated both spatially and temporally ensure the parasite lifecycle is disrupted during treatment. Husbandry practises such as separation of age classes and fallowing of sites also contribute towards reducing parasite numbers (Denholm et al., 2002). It is important that once an IPM strategy is developed, all producers in a management unit coordinate and follow this approach.

5.3.1 Case study: IPM for sea lice in Scotland

Sea lice *Lepeophtheirus salmonis* and *Caligus elongatus* are parasitic copepods of substantial importance to the sea-caged salmon industry in the Northern Hemisphere (Tully and Nolan, 2002). They cause significant economic losses through mortality, lost growth and management and it was estimated that sea lice cost Scottish salmon farmers £13 million each year (Rae, 2002). There has been an enormous amount of research invested into the control of these pests. Initial efforts concentrated on the development of chemicals for bath treatments, followed by oral treatments. In Scotland emamectin benzoate and teflubenzuron are registered oral treatments against sea lice for Atlantic salmon (Rae, 2002). With the onset of sea

lice resistance to treatments and the increase of consumer awareness regarding use of chemicals, it was soon realised that a new approach was required. New strategies incorporating coordinated treatment, improved farm practises and an appreciation of parasite biology were developed (Pike and Wadsworth, 1999). Cooperation between farmers, veterinarians, researchers, treatment manufacturers and governing bodies led to a National Treatment Strategy for the Control of Sealice being implemented in salmon sea-cage culture in Scotland in 1998 (Rae, 2002).

5.4 Advantages of oral treatments over bath treatments

Oral administration of medications to sea-caged fish has advantages over conventional bath treatments (Table 2). Oral treatment requires less labour than bath treatments, as fish can be treated as part of their normal feeding regime (Grant, 2002). During bathing, fish are frequently stressed from crowding and reductions in dissolved oxygen can cause mortalities due to anoxia and/or overdose of the bath chemical (Grant, 2002), or from physical injury. Oral, in-feed medications have wider safety margins and do not require crowding. All cages on a farm can be treated quickly (Stone et al., 1999; Duston and Cusack, 2002) reducing the chance of recruitment of new parasites from nearby untreated cages and increasing the time between required treatments. Bath treatments often end with the medicine being released into the surrounding environment, which may impact on non-target organisms (Grant, 2002). Medicines administered orally still enter the environment through faeces, urine and uneaten feed, but are released more gradually and at a much lower concentration than from bath treatments (Ramstad et al., 2002). Stone et al. (2002) found that treatment of salmon smolts with orally administered emamectin benzoate after their transfer to seawater was effective in delaying initial infestation by sea lice. It would also be advantageous if feeding juvenile *Seriola lalandi* an oral treatment prior to their transfer from hatcheries to sea-cages had a prophylactic effect, i.e. if it provided a period of protection from recruitment of parasites.

Oral treatments do have some disadvantages. Medicines may reduce the palatability of feed, leading to reduction in feeding rates and reduced growth. Competitive feeding and differences in fish size within a cage may mean that the dose of drug may not be delivered uniformly (Duston and Cusack, 2002). Variation between farms in the application and/or delivery of an oral treatment among

28

individual fish, cages, sites and seasons may result in exposure of parasites to subtherapeutic doses (Berg and Horsberg, 2009). If fish are given a subtherapeutic dose, resistance to the drug will be promoted in the parasite population (Kim and Choi, 1998). Conversely, giving more than the required dose is wasteful. Careful feeding practises and regular grading of fish should ensure delivery of an effective and safe dose (Sakai, 1999; Stone et al., 2002). Stone et al. (2000) found that despite differences in fish weights and feeding competition amongst Atlantic salmon, fish treated orally with emamectin benzoate had fewer parasites than untreated fish.

Table 2

Comparison of bath treatment versus oral treatment.

	Bath treatment	**Oral treatment**
Effect on fish		
Handling	Extensive	Nil
Stress	Considerable	Very low
Mortalities	Frequent	Nil
Feeding and growth	Maybe temporarily reduced post-treatment	Very low unless oral treatment reduces palatability of feed
Industry Operations		
Cost	Chemical/freshwater	Chemical cost and application to feed
Equipment/infrastructure	Extra needed e.g. tarpaulins	
Labour	Labour intensive	No extra labour/equipment
Weather	Weather-dependent	Generally independent of weather
Effect on Environment	Depends on chemical	Depends on chemical
	All of bath usually released into environment	Waste depends on feed uptake and assimilation by fish

6. Suitability of compounds as oral treatments

In addition to efficacy, there are other criteria that must be considered when assessing the suitability of oral treatments for fish. Medications should be cost-effective and easily acquired. The chemical must be absorbed by fish and expressed in tissues where the parasite feeds, e.g. blood, skin or mucus (Grant, 2002). Compounds should have minimal effects on non-target organisms. Palatability of feed should not be affected (Stone et al., 1999). In Australia, farmed *S. lalandi* are fed extruded pellets and this type of feed is also becoming more common in Japan. The pressure, humidity and high temperatures involved in extrusion are potentially destructive to medicines (Broz et al., 1997; Vertommen and Kinget, 1998) and it would be an advantage if the compound is tolerant to this process and can be

incorporated in commercial feed with no loss of activity. Medications are classed as being prophylactic, i.e. they prevent infection occurring, and/or are therapeutic, i.e. they treat an existing infection. I investigated most compounds for their therapeutic effect on existing monogenean infestations, but it is possible that some may inhibit further recruitment to some degree (prophylaxis).

7. Candidate anthelmintics for oral treatment of monogenean parasites of *Seriola* spp.

The majority of my PhD research has focussed on praziquantel and the benzimidazoles. Other compounds are reviewed briefly in the respective data chapters where they appear.

7.1 Benzimidazoles

Benzimidazoles act by binding with helminth-specific tubulin, thus interfering with cell functions such as mitosis, cellular transport and mobility. This causes paralysis of the worm, which loses its ability to remain attached to the host (Kohler, 2001). Tojo et al. (1992) tested several benzimidazoles *in vitro* and *in vivo* and reported that fenbendazole, mebendazole and triclabendazole were effective *in vivo* against a monogenean, *Gyrodactylus* sp. on rainbow trout, *Oncorhynchus mykiss*. Albendazole and oxfendazole are other benzimidazoles that have been identified as prospective oral treatments for monogeneans. Further review of albendazole, mebendazole, thiabendazole and triclabenedazole is provided in Chapter 8.

7.1.1 Fenbendazole

Fenbendazole is used for the treatment of gastrointestinal nematodes and tapeworms in terrestrial animals and has been used commercially in aquaculture (Short et al., 1988; Iosifidou et al., 1997). Fenbendazole is metabolised to oxfendazole (see below), which is also an anthelmintic and may extend the duration of fenbendazole's effectiveness. Fenbendazole reaches the muscle and skin of fish after being absorbed by the intestines, making it a good candidate for oral use (Iosifidou et al., 1997). Iosifidou et al. (1997) also found that levels of fenbendazole

were higher in the skin of rainbow trout than in the muscle after oral treatment, which suggests it could be effective against skin-dwelling monogeneans such as *B. seriolae* and *Neobenedenia* spp. Fenbendazole was effective against *Z. seriolae* on *S. lalandi* in preliminary oral trials (I. Ernst and C.B. Chambers, unpublished data), therefore further evaluation of dose and efficacy was carried out as described in Chapter 3.

7.1.2 Oxfendazole

Oxfendazole is a sulphoxide metabolite of fenbendazole; it is likely that most of the anthelmintic activity of fenbendazole is due to oxfendazole (Reinemeyer and Courtney, 2001). Like fenbendazole, oxfendazole was effective against *Z. seriolae* on *S. lalandi* in preliminary oral trials (I. Ernst and C.B. Chambers, unpublished data), therefore further evaluation of dose and efficacy was carried out as described in Chapter 3.

7.1.3 Febantel

Febantel was developed for the treatment of terrestrial gastrointestinal nematodes and tapeworms, but is also used routinely as an oral treatment for the gill monogenean *Heterobothrium okamotoi* (Didiclidophoridae) parasitising farmed tiger puffer *Takifugu rubripes* in Japan (Ogawa et al., 2005), where it is available commercially to farmers as Marinban® at a labelled dose of 25 mg kg^{-1} body weight (BW) day^{-1} for 5 days. Febantel must be metabolized *in vivo* to fenbendazole and oxfendazole before becoming active. In view of its use in Japan against *H. okamotoi*, I evaluated dose and efficacy of febantel against *Heteraxine heterocerca* and *B. seriolae* on *S. quinqueradiata* in Japan in Chapter 3.

7.2 Praziquantel

Praziquantel is a pyrazinoisoquinoline anthelmintic that was released to the human medical market in 1975 specifically to target platyhelminths (Harder, 2002). Praziquantel is used to treat a variety of livestock, veterinary and human flatworm infections (Day et al., 1992). Although an enduring and thoroughly researched anthelmintic, the mode in which praziquantel acts is still to be fully elucidated (Day et al., 1992; Kohler, 2001). It appears to interfere with the calcium flux across the

worm's tegumental membranes, causing vacuolisation and muscle spasms (Greenberg, 2005). While parasites may not be killed directly, they ultimately lose their ability to attach. As virtually all adult monogeneans are unable to swim, they cannot reattach to their host. Praziquantel has mainly been used as a bath treatment for finfish, but Kim et al. (1998) reported that praziquantel administered orally treated gill monogeneans of cultured rockfish, *Sebastes schlegeli*, effectively. Praziquantel is commercially available to Japanese aquaculturalists as Hadaclean® (Bayer Japan) for the treatment of *B. seriolae* at 150 mg kg^{-1} BW day^{-1} for 3 days (the dose recommended on the product label) (Stephens et al., 2003; Tubbs and Tingle, 2006b). Orally administered praziquantel is also effective against polyopisthocotylean monogeneans, including *Microcotyle sebastis* parasitising *S. schlegeli* in Korea (Kim et al., 1998, Kim and Cho, 2000, Kim et al., 2001a, Kim and Kim, 2002), *Heterobothrium okamotoi* parasitising *Takifugu rubripes* in Japan (Hirazawa et al., 2000), *Zeuxapta seriolae* parasitising farmed *Seriola lalandi* in New Zealand (Tubbs and Tingle, 2006a) and Australia (Williams et al., 2007) and *Sparicotyle chrysophrii* parasitising *Sparus auratus* in the Mediterranean (Sitja-Bobadilla et al., 2006).

8. Pharmacology

In order for a compound to be an effective oral treatment, it must be absorbed and distributed by the host, reach the target tissues where the parasite resides in an active form at a sufficient dose and it must be taken up by the parasite. Little is known about the uptake, pharmacokinetics and bioavailability of oral anthelmintics in fish, compared with terrestrial animals.

Tubbs and Tingle (2006a) determined that orally administered praziquantel is eliminated from *Seriola lalandi* quickly, and removal was likely to be much faster than for *Oncorhynchus mykiss* and *Sebastes schlegeli*. Tubbs and Tingle (2006b) reported that less praziquantel is metabolised and distributed to the skin of *Seriola lalandi* compared to the plasma. Kim and Kim (2002) found that praziquantel administered in combination with the histamine H2-receptor antagonist cimetidine, provided higher levels of plasma praziquantel in *Sebastes schlegeli*. Cimetidine inhibits the production of stomach acid and Kim and Kim (2002) related the addition of cimetidine to increased efficacy of praziquantel against the gill monogenean

Microcotyle sebastis. The effect of cimetidine on the bioavailability and clearance time of praziquantel in *Seriola* spp., however, is unknown.

9. Case study of a registered oral treatment for parasites of sea-caged finfish: Calicide®

Investigating the effectiveness of a compound against a parasite requires significant planning, design, experimentation, sampling, parasite enumeration and statistical analysis of data. If a compound shows promise, a great deal of research is still required before an application can be made to register it for commercial use in aquaculture, including (but not limited to) commercial field trials to confirm efficacy, target animal safety studies to determine treatment safety margins, assessment of ecotoxicty (toxicity to non-target organisms), environmental and tissue residues and depletion times to determine withholding periods. This requires investment of time and large amounts of funds. Teflubenzuron is an insect growth regulator that interferes with the synthesis of chitin in sea lice (Branson et al., 2000). It is effective at removing feeding lifestages of *Lepeophtheirus salmonis* when administered to *Salmo salar* at 10 mg kg^{-1} BW day^{-1} for 7 consecutive days (Anonymous, 1999; Ritchie et al., 2002). Teflubenzuron was developed for commercialisation by Nutreco as Calicide® and is registered as an oral treatment against sea lice (Copepoda) *Caligus elongatus* and *L. salmonis* in Scotland, Norway, Canada, Chile and Ireland (Anonymous 1999). The development of Calicide® cost Nutreco approximately £3.4 million, of which about £900,000 was spent on ecotoxicological research (Anonymous, 1999).

10. Summary

While efficacy of an anthelmintic against the target parasite is often the focus in anthelmintic treatment trials, the absolute dose, required treatment duration and the effect on feed palatability also need to be taken into account when designing an optimal treatment program (Ogawa and Yokoyama, 1998). Other factors such as parasite biology, compound pharmacology and the physiology of host species should also be considered as they influence efficacy. Integrated Parasite Management (IPM) should be employed to minimise chemical use, and by combining good

husbandry and farm practices, efficacy can be maximized while the conditions which facilitate the development of parasite resistance are minimized (Berg and Horsberg, 2009).

CHAPTER 3 – EFFICACY OF ORALLY ADMINISTERED FENBENDAZOLE, OXFENDAZOLE AND FEBANTEL AGAINST *ZEUXAPTA SERIOLAE*, *HETERAXINE HETEROCERCA* AND *BENEDENIA SERIOLAE*, MONOGENEAN PARASITES OF FARMED *SERIOLA* SPP.

Chapter 3: Efficacy of orally administered fenbendazole, oxfendazole and febantel against *Zeuxapta seriolae, Heteraxine heterocerca* and *Benedenia seriolae*, monogenean parasites of farmed *Seriola* spp.

Abstract

We investigated the efficacies of fenbendazole (FBZ) and oxfendazole (OXF) as oral treatments for the monogeneans *Zeuxapta seriolae* and *Benedenia seriolae* parasitising yellowtail kingfish *Seriola lalandi* farmed in South Australia, and the efficacy of febantel (FEB) as an oral treatment for the monogeneans *Heteraxine heterocerca* and *B. seriolae* parasitising Japanese yellowtail *S. quinqueradiata* in sea-cage aquaculture in Japan. In Trial 1, FBZ and OXF were surface coated onto commercial feed pellets and administered to *S. lalandi* at four daily doses: 50 and 75 mg kg^{-1} body weight (BW) day^{-1} for 6 days and 100 and 150 mg kg^{-1} BW day^{-1} for 3 days. In Trial 2, FEB was administered to *S. quinqueradiata* by intubation at 25, 50, 75 and 100 mg kg^{-1} BW day^{-1} for 6 days and 50, 100, 150 and 200 mg kg^{-1} BW day^{-1} for 3 days. Mean parasite abundance was compared between treated fish and control fish. In Trial 1, efficacy (expressed as a % reduction of mean parasite abundance) against the gill parasite *Z. seriolae* ranged from 63.7 – 92.3% for FBZ and 77.2 – 87.4% for OXF. In Trial 2, FEB was found to be a very effective oral treatment against the gill parasite *H. heterocerca* with 99.8-100% efficacy. Neither FBZ nor OXF administered to *S. lalandi* in Australia, nor FEB administered to *S. quinqueradiata* in Japan appeared to have an effect against the skin parasite *B. seriolae*.

1. Introduction

Japanese yellowtail *Seriola quinqueradiata* farmed in Japan and yellowtail kingfish *Seriola lalandi* farmed in South Australia are impacted by monogenean parasites: in South Australia, *S. lalandi* is parasitised by *Zeuxapta seriolae* (Heteraxinidae) and *Benedenia seriolae* (Capsalidae) and in Japan, *S. quinqueradiata* is parasitised by *Heteraxine heterocerca* (Heteraxindae) as well as *B. seriolae*. The specific effects and associated health issues caused by these Monogenea as well as

current management techniques to control these parasites in South Australia and Japan and the issues that arise using current bathing methods with freshwater and hydrogen peroxid were reviewed in Chapters 1 and 2. In-feed oral treatments against monogeneans present advantages over bathing (see Table 2, Chapter 2). For example, it requires no extra labour or expensive infrastructure and does not stress fish through increased handling or crowding. Many anthelmintic medications are available for use in livestock industries, but relatively few have been explored as treatments for monogenean parasites of finfish.

Fenbendazole (FBZ), oxfendazole (OXF) and febantel (FEB) are benzimidazole anthelmintics, which act by binding with helminth-specific tubulin (Kohler, 2001). This interferes with helminth cell functions such as mitosis, cellular transport mobility and thus the parasite's attachment to the host (Kohler, 2001). FBZ is a broad-spectrum veterinary anthelmintic used to treat nematode and tapeworm parasites of cattle, sheep and horses. It has been used in-feed to treat adult tapeworms in salmon farming in Norway (Nordmo, 1993; Wall, 1993). OXF is a major sulphoxide metabolite of FBZ (Short et al., 1988), and Reinemeyer and Courtney (2001) suggested most of the anthelmintic activity of FEB and FBZ can be attributed to their metabolism to OXF. FEB is also used for the treatment of gastrointestinal nematodes and tapeworms, but must be metabolized *in vivo* to FEB and OXF before becoming active. FEB is used routinely as an oral treatment for the gill monogenean *Heterobothrium okamotoi* (Didiclidophoridae) parasitising farmed tiger puffer *Takifugu rubripes* in Japan (Ogawa et al., 2005). According to Iosifidou et al. (1997), FBZ and OXF reach and accumulate in skin of fish after oral adminstration to rainbow trout *Oncorhynchus mykiss,* providing these compounds with potential to reach skin dwelling monogenean parasites of fish, such as *B. seriolae*.

Kimura et al. (2006) found FEB, which is metabolised to FBZ after oral administration, had high efficacy against *H. okamotoi*, which, like *Z. seriolae* and *Heteraxine heterocerca,* is a blood-feeding gill monogenean. FBZ and OXF administered orally at a dose of 150 mg kg^{-1} BW day^{-1} for two days demonstrated potential to remove existing populations of *Z. seriolae* parasitising *S. lalandi* in preliminary oral trials (I. Ernst and C.B. Chambers, unpublished data). Here we present further experimentation carried out to explore different doses and treatment strategies of FBZ and its closely related oxidative metabolite OXF, as oral treatments

against *Z. seriolae* and *B. seriolae* infecting *S. lalandi* in Australia, and FEB as an oral treatment against *H. heterocerca* and *B. seriolae* parasitising *S. quinqueradiata* in Japan.

2. Methods

2.1 Trial 1 – FBZ and OXF treatment of *Z. seriolae* and *B. seriolae* infestations of *S. lalandi*

The trial design and methods following Williams et al. (2007) were used in separate trials to test FBZ and OXF (each purchased from Sigma Aldrich, Sydney, Australia) as oral treatments against *Z. seriolae* and *B. seriolae* parasitising the gills and skin, respectively, of *S. lalandi.* This research was conducted under APVMA Minor Use Permit 7250 for small-scale trials of Agvet chemicals (APVMA, 2004).

2.1.1 Source of fish and parasites

A commercial hatchery in upper Spencer Gulf, South Australia provided hatchery-reared *S. lalandi* and tank facilities for experiments during July 2003. Water temperature during the trial was 13-14 °C and salinity was 45 ppt. Despite salinity being naturally high in upper Spencer Gulf, *Z. seriolae* and *B. seriolae* appear unaffected and parasitise wild and farmed kingfish in this region. The mean body weight of 180 fish was 0.1 (0.052-0.157) kg and 0.092 (0.036-0.163) kg for the FBZ and OXF trials, respectively. Experimental fish were naïve (i.e. not infested with monogeneans previously) and infestations of *Z. seriolae* and *B. seriolae* were achieved by exposing them in 12 m^3 tanks to naturally infested fish. Infested source fish originated from a sea-cage farm and concurrent infestations by *Z. seriolae* and *B. seriolae* were previously confirmed using the parasite sampling method detailed below.

The prevalence of monogeneans on experimental fish was confirmed by examining a subsample of 10 fish from the same population before the trials. Parasite sampling was a two stage process and involved bathing fish individually in 60 L plastic bins, first in dechlorinated freshwater for 5 min, where any remaining dead *B. seriolae* still attached to fish were manually removed (Chambers and Ernst, 2005; Williams et al., 2007). This was followed by a bath in 5 ppm praziquantel

38

(purchased from MP Biomedicals) in seawater for 10 min to remove *Z. seriolae* (see Mooney et al., 2006). Dislodged parasites were collected by filtering bath water from each fish through 75 μm mesh. Filtrate was preserved in 2% formalin solution and parasites were counted using a dissecting stereomicroscope. This subsample of fish was also used to determine mean body weight (BW) and thus feed ration in % kg BW in kg of feed (% kg BW) and anthelmintic dose to administer during the trial in mg per kg BW (mg kg^{-1} BW).

Fifteen circular cages 1.5 m in diameter constructed from 12 mm plastic mesh were used to partition three tanks 12 m^3 in volume, so that five cages in each tank represented each of four treatment groups and the control. Each mesh cage contained 10 randomly selected fish for each treatment, except control cages which contained 20 randomly selected fish (i.e. double the number). This number represented 10 control fish for the 3 day experiment and 10 control fish for the 6 day experiment because lack of tank space did not permit a separate cage for each of the control groups).

2.1.2 Pellet preparation

Four daily dose treatments of FBZ and OXF were tested: 50 and 75 mg kg^{-1} BW day^{-1} administered for 6 days and 100 and 150 mg kg^{-1} BW day^{-1} administered for 3 days. Each dose of FBZ and OXF, calculated from the mean weight of fish, was dissolved in 2 mL of absolute ethanol and sprayed evenly onto the ration of commercial feed pellets (4 mm Classic HS, Skretting Australia) using a household trigger-style spray bottle. As these sprayers often leave a small amount of liquid behind in the bottle, this was measured prior to the trials and compensated for. Pellets were prepared the day before and allowed to dry overnight. Control fish were fed the same pellets but without medication. Treatments are summarised in Tables 1a and b for FBZ and OXF, respectively.

During each anthelmintic trial, respective doses were administered for 3 days and 6 days simultaneously. Fish in each cage were observed carefully for signs of feed rejection or toxicity (e.g. abnormal behaviour, darkening of skin) that may have been caused by medicated food. Iosifidou et al. (1997) found that FBZ and its sulfoxide metabolite OXF were largely depleted from *O. mykiss* 4 days following oral administration. As no pharmacokinetic data for *S. lalandi* is available, treatment

was withdrawn for 5 days based on this information, to allow metabolism of the anthelmintic to occur. Fish were then sampled individually to determine parasite abundance using the method detailed previously. Due to the length of this trial, the water temperature and the physical set-up of the tanks, new recruitment of parasites was impossible to prevent. When *Z. seriolae* and *B. seriolae* were counted, they were therefore divided into three life stages: adults, juveniles and recruits (parasites that had settled during the trial). Sizes for each life stage were estimated from water temperature-related parasite growth based on data in Lackenby et al. (2007) for *B. seriolae* and unpublished data from A.J. Mooney for *Z. seriolae*.

2.1.3 Statistical analysis

SPSS 15.0 software was used for statistical analyses (SPSS Inc., Chicago IL, USA). Data from individual fish were pooled for each cage. Treatments administered for 3 days were analysed separately from those administered for 6 days. Mean parasite abundance (the total number of individuals of a particular parasite species, divided by the total number of hosts examined, whether infested or not, see Bush et al., 1997) was calculated for each monogenean species. A one-way ANOVA was used to compare mean parasite abundance from treatment fish with controls and $P \leq 0.05$ was considered significant. The Levene's statistic was calculated as an indication of equal variance, and where considered significant ($P \leq 0.05$) Games-Howell *post-hoc* multiple comparisons were carried out. Efficacy for each treatment was calculated as a % reduction of the mean parasite abundance using the formula below (adapted from Stone et al., 2000):

$$\% \text{ efficacy} = 100 - (100 \text{ x } \frac{\text{mean parasite abundance of treatment group}}{\text{mean parasite abundance of control group}}).$$

2.2 Trial 2 – FEB treatment of *H. heterocerca* and *B. seriolae* infestations of *S. quinqueradiata*

2.2.1 Source of fish and parasites

The trial was conducted in a disused rectangular floating sea-cage in Saiki Harbour, Oita Prefecture, Kyushu, Japan, during August 2004. Water temperature

was 25±1 ºC and the salinity was 35 ppt. Specimens of *S. quinqueradiata* were obtained from a commercial sea-cage farm. Presence of *H. heterocerca* and *B. seriolae* was confirmed by routine monitoring of fish in the same cohort the experimental fish were taken from, prior to trial. Parasite sampling was a two stage process and followed the same methods outlined in Trial 1 (see 2.1.1). Fish had acquired *H. heterocerca* and *B. seriolae* infections by exposure to parasites on the farm. One hundred and ten parasitised fish of mean weight 530 (510-540) g were randomly distributed among 11 square cages 3.375 m^3 suspended in a larger floating sea-cage, so that there were 10 fish per cage. Batches of 10 fish were weighed prior to assignment to experimental cages, so that FEB dose to administer during the trial in mg kg^{-1} BW could be determined for each cage. Fish were not acclimated prior to trial and the experiment commenced the day following transfer of fish to cages.

2.2.2 Administration of FEB

FEB in the form of Marinbantel (Meiji Seika Kaisha Ltd., Japan), was delivered orally to fish in a paste made from pre-pellet meal (provided by Yamaha Nutreco Aquatech), fish oil and tapwater. FEB was administered to *S. quinqueradiata* by intubation at 25, 50, 75 and 100 mg kg^{-1} BW day^{-1} for 6 days and 50, 100, 150 and 200 mg kg^{-1} BW day^{-1} for 3 days, summarised in Table 1c. To administer the treatments, each fish was anaesthetised in a 60 L plastic tub containing a solution of 130 ppm FA-100 aquatic anaesthetic (4-allyl-2-methoxyphenol) (Tanabe Pharmaceutical Company Ltd, Osaka, Japan) in seawater until it had lost its reflex activity (Stage 4 anaesthesia, see Hikasa et al., 1986). Paste containing the respective dose of FEB was intubated into their stomach directly using a 10 mL syringe fitted with a 10 cm extension of soft air hose. Fish were placed into a 60 L tub of clean seawater to recover and monitored for regurgitation before being returned to their respective cage. A control cage was maintained for each treatment duration (3 days and 6 days) and control fish underwent an identical procedure as treatment fish but received unmedicated paste. As in Trial 1, fish were held for 5 days after treatment to allow absorption of medication and then sampled using method described in 2.1.1 to determine the parasite abundance remaining on fish.

The duration of the trial and location of experimental cages in the ocean meant that it was impossible to prevent exposure to parasite recruitment (from

infested wild fish or proximity of nearby farmed *S. quinqueradiata*). When *H. heterocerca* and *B. seriolae* were counted, they were therefore divided into three life stages: adults, juveniles and recruits (parasites that had settled during the trial). Sizes for each life stage were estimated from water temperature-related parasite growth based on data in Lackenby et al. (2007) for *B. seriolae* and Mooney et al. (2008) for *H. heterocerca*.

2.2.3 Statistical analysis

SPSS 15.0 software was used for statistical analyses for Trial 2. In Trial 2, each fish was intubated individually, therefore each fish was considered a replicate and data were not pooled. Each treatment period (3 days and 6 days) was analysed separately. *Heteraxine heterocerca* were analysed using a *T*-test for unequal variance. *Benedenia seriolae* data, as well as recruitment data for both *H. heterocerca* and *B. seriolae*, were analysed using one-way ANOVA followed up with Games-Howell *post-hoc* multiple comparisons test where the Levene's statistic was considered significant ($P \leq 0.05$) to account for unequal sample sizes and unequal variance. Efficacy for each treatment was calculated as a % reduction according to the formula given previously (see 2.1.3).

Table 1

Summary of daily dose and treatment duration during Trial 1 for (a) fenbendazole (FBZ), and (b) oxfendazole (OXF) (administered by surface coating of feed), and Trial 2 for (c) febantel (FEB) (administered by intubation). For Trial 2, additional data are presented on sample size and % mortality of fish after typhoon.

(a)

Target daily dose (mg kg^{-1} BW day^{-1})	Actual daily dose (mg kg^{-1} BW day^{-1})	Treatment duration (days)	Total target dose (mg kg^{-1} BW)	Total dose delivered (mg kg^{-1} BW)
0	0	3, 6	0	0
50	55	6	300	330
75	84	6	450	504
100	110	3	300	330
150	180	3	450	540

(b)

Target daily dose (mg kg^{-1} BW day^{-1})	Actual daily dose (mg kg^{-1} BW day^{-1})	Treatment duration (days)	Total target dose (mg kg^{-1} BW)	Total dose delivered (mg kg^{-1} BW)
0	0	3, 6	0	0
50	54	6	300	324
75	81	6	450	486
100	114	3	300	342
150	162	3	450	486

(c)

Target daily dose (mg kg^{-1} BW day^{-1})	Treatment duration (days)	Total target dose (mg kg^{-1} BW)	Sample size (no. fish)	Mortality (%)
0 (controls)	3, 6	0	20	0
25	6	150	10	10
50	6	300	10	50
75	6	450	1	90
100	6	600	0	100
50	3	150	10	10
100	3	300	6	0
150	3	450	10	40
200	3	600	10	30

3. Results

3.1 Trial 1 – FBZ and OXF treatment of *Z. seriolae* and *B. seriolae* infestations of *S. lalandi*

During each trial of FBZ and OXF, a mean daily ration of 3.6% BW was offered to each treatment group. Actual daily dose delivered was calculated from mean fish weights for individual treatment groups during sampling at the end of the trial, and are provided in Tables 1a and 1b for FBZ and OXF, respectively. All actual daily doses were higher than the nominal target daily dose, ensuring that an adequate dose was tested. Neither FBZ nor OXF appeared to affect palatability of feed and no signs of toxicity (e.g. skin darkening, erratic swimming, loss of equilibrium) was observed in fish, irrespective of the dose administered. The prevalence (the number of hosts infested with one or more individuals of a particular parasite species, see Bush et al., 1997) at the start of the trial was 100 % for *Z. seriolae* and 90 % for *B. seriolae*.

3.1.1 FBZ trial

One-way ANOVA indicated that mean abundance of *Z. seriolae* on fish administered FBZ at 50 and 75 mg kg^{-1} BW day^{-1} for 6 days was significantly less than control fish (Table 2a). One-way ANOVA also indicated that mean abundance of *Z. seriolae* on fish administered FBZ at 100 and 150 mg kg^{-1} BW day^{-1} for 3 days was significantly less than control fish (Table 2b), however the Levene's statistic indicated unequal variance in *Z. seriolae* data from fish administered 100 and 150 mg kg^{-1} BW day^{-1} for 3 days ($P < 0.05$). For this reason, Games-Howell *post-hoc* multiple comparisons were conducted to ensure this difference was not a Type 1 error. This analysis found that only the mean abundance of *Z. seriolae* from fish treated with 100 mg kg^{-1} BW day^{-1} of FBZ for 3 days was at the limit of being significantly different to control fish, and that the mean abundance of *Z. seriolae* of fish treated with 150 mg kg^{-1} BW day^{-1} of FBZ for 3 days was not significantly different from the control (Table 2c). One-way ANOVA found no significant differences between mean abundance of *B. seriolae* from fish treated with any dose of FBZ compared with control fish (Tables 2a and 2b).

44

Table 2

Comparison of mean abundance using one-way ANOVA of *Zeuxapta seriolae* and *Benedenia seriolae* after oral treatment by surface coating with FBZ for (a) 6 days and (b) 3 days. (c) *Post-hoc* Games-Howell multiple comparsions of *Z. seriolae* data from fish that received, following unequal variances as indicated by Levene's statistic ($P < 0.05$). df = degrees of freedom. F = F-value. $P \leq 0.05$ was considered significant.

(a)

Species	Sum of Squares	df	Mean Square	F	*P*-value
Zeuxapta seriolae	3141.4	2	1570.70	19.46	**0.002**
Benedenia seriolae	0.11	2	0.05	0.065	0.938

 (b)

Species	Sum of Squares	df	Mean Square	F	*P*-value
Zeuxapta seriolae	5302.55	2	2651.27	23.41	**0.001**
Benedenia seriolae	0.1	2	0.05	0.19	0.83

(c)

Treatment duratiion	Treatment (mg kg^{-1} BW day^{-1})	*n*	Mean	Std. Deviation	Std. Error	*P*-value
3 days	control	3	62.9	17.37	-	-
	100	3	4.83	2.36	10.11	**0.049**
	150	3	22.8	5.70	10.55	0.087

Efficacy was only calculated for *Z. seriolae* (Table 3a) as FBZ appeared to have no effect on *B. seriolae*. The highest efficacy for FBZ against *Z. seriolae* was achieved by 100 mg kg^{-1} BW day^{-1} administered for 3 days (92.3 % reduction of *Z. seriolae*).

Table 3

Efficacy, calculated as a percentage reduction of mean parasite abundance of *Zeuxapta seriolae* from the control mean (adapted from Stone et al., 2000) of (a) FBZ and (b) OXF; delivered by surface coating at two total doses for two durations.

(a)

Target dose (mg kg^{-1} BW day^{-1}), duration (days)	Efficacy (% reduction)
50 (6)	82.9
75 (6)	70.8
100 (3)	92.3
150 (3)	63.7

(b)

Target dose (mg kg^{-1} BW day^{-1}), duration (days)	Efficacy (% reduction)
50 (6)	87.4
75 (6)	78.9
100 (3)	77.2
150 (3)	77.2

3.1.2 OXF trial

One-way ANOVA indicated that the mean abundance of *Z. seriolae* on fish administered OXF at 50 and 75 mg kg^{-1} BW day^{-1} for 6 days and 100 and 150 mg kg^{-1} BW day^{-1} for 3 days was significantly less than control fish (Tables 4a and 4b) ($P <$ 0.05) . The Levene's statistic was not considered significant ($P > 0.05$) therefore no further *post-hoc* tests were carried out. One-way ANOVA found no significant differences between mean abundance of *B. seriolae* from fish treated with any dose of OXF compared with control fish (Tables 4a and 4b).

Efficacy was only calculated for *Z. seriolae* (Table 3b) as OXF appeared to have no effect on *B. seriolae*. The highest efficacy for OXF against *Z. seriolae* was achieved by 50 mg kg^{-1} BW day^{-1} treatment, administered for 6 days (87.4% reduction of *Z. seriolae*). Efficacy was not calculated for any OXF treatment against *B. seriolae* because mean abundance of this species on treated fish did not differ significantly from control fish.

46

Table 4

Comparison of mean abundance using one-way ANOVA of *Zeuxapta seriolae* and *Benedenia seriolae* after oral treatment by surface coating with OXF for (a) 6 days and (b) 3 days. df = degrees of freedom. F = F-value. $P \leq 0.05$ was considered significant.

(a)

Species	Sum of Squares	df	Mean Square	F	*P*-value
Zeuxapta seriolae	5056.7	2	2528.36	22.99	**0.002**
Benedenia seriolae	2.6	2	1.298	2.774	0.14

(b)

Species	Sum of Squares	df	Mean Square	F	*P*-value
Zeuxapta seriolae	4099.05	2	2049.52	142.34	**0.000**
Benedenia seriolae	0.036	2	0.018	0.03	0.971

3.2 Trial 2 – FEB treatment of *H. heterocerca* and *B. seriolae* infestations of *S. quinqueradiata*

Calculation of dose was based on the mean weight of each treatment group taken at the start of the trial. No regurgitation of medicated paste or signs of toxicity (e.g. skin darkening, erratic swimming, loss of equilibrium) was observed in fish, irrespective of the dose administered. Severe weather (Typhoon 2004-21 *Meari*) occurred during the trial which stressed fish and led to bacterial septicaemia and mortality in several treatment groups (see Table 1c). Nearby farms also incurred similar mortality and were undergoing antibiotic treatment, however experimental fish could not be treated during the trial in case it confounded the results. The prevalence (as defined previously in 3.1) was 100 % for *Z. seriolae* and *B. seriolae* at the start of the trial.

3.2.1 *Heteraxine heterocerca*

Levene's test statistic indicated unequal variance in *H. heterocerca* data ($P \leq$ 0.05) therefore results of independent *T*-tests for unequal variance were used (Table 5a and 5b). The mean abundance of all life stages of *H. heterocerca* for FEB

administered for 6 days and 3 days were found to be significantly different from control fish, and efficacy was very high, (see Tables 6a and 6b). FEB administered at 100, 150 and 200 mg kg[1] BW day[-1] for 3 days achieved 100% efficacy against all life stages of *H. heterocerca*, and 50 mg kg[1] BW day[-1] administered for 3 days achieved 99.8-100% reduction of mean parasite abundance (Table 6a). Similarly, FEB administered at 50 and 75 mg kg[1] BWday[-1] for 6 days achieved 100% efficacy against all life stages of *H. heterocerca*, and 25 mg kg[1] BW day[-1] administered for 6 days achieved 99.4-100% efficacy (Table 6b). Results of one-way ANOVA found mean abundance of recruited *H. heterocerca* in 3 day but not 6 day FEB treatments were significantly different from the control fish (Table 7a). The Levene's statistic was significant for recruited *H. heterocerca* data ($P < 0.05$), therefore *post-hoc* Games-Howell multiple comparisons were also conducted to ensure this result was not due to a Type 1 error. These confirmed that the mean abundance of recruited *H. heterocerca* in treatments were significant from the control in 3 day but not in 6 day FEB treatments (Table 7b). Efficacy against recruited *H. heterocerca* was therefore only calculated for 3 day doses, and was also found to be high (96.3-98.9% reduction, Table 6a).

Table 5

Results of *T*-test comparison of mean abundance of *Heteraxine heterocerca* after oral treatment by intubation with FEB for (a) 3 days, and (b) 6 days. Equal variances not assumed. $P \leq 0.05$ was considered significant, t = t-value, df = degrees of freedom

(a)

Life stage	Treatment (mg kg^{-1} BW day^{-1})	*n*	Mean	Std. Deviation	Std. Error Mean	t	df	*P*-value
Adults	control	10	17.10	16.536	5.229	-	-	-
	50	9	0.00	0.000	0.000	3.270	9.0	**0.010**
	100	10	0.00	0.000	0.000	3.270	9.0	**0.010**
	150	6	0.00	0.000	0.000	3.270	9.0	**0.010**
	200	7	0.00	0.000	0.000	3.270	9.0	**0.010**
Juveniles	control	10	48.00	27.146	8.584	-	-	**-**
	50	9	0.11	0.333	0.111	5.578	9.0	**0.000**
	100	10	0.00	0.000	0.000	5.592	9.0	**0.000**
	150	6	0.00	0.000	0.000	5.592	9.0	**0.000**
	200	7	0.00	0.000	0.000	5.592	9.0	**0.000**
Total	control	10	65.10	40.962	12.953	-	-	**-**
	50	9	0.11	0.333	0.111	5.017	9.0	**0.001**
	100	10	0.00	0.000	0.000	5.026	9.0	**0.001**
	150	6	0.00	0.000	0.000	5.026	9.0	**0.001**
	200	7	0.00	0.000	0.000	5.026	9.0	**0.001**

(b)

Life stage	Treatment (mg kg^{-1} BW day^{-1})	*n*	Mean	Std. Deviation	Std. Error Mean	t	df	*P*-value
Adults	control	11	15.64	11.826	3.566	-	-	-
	25	9	0.11	0.333	0.111	4.352	10.0	**0.001**
	50	5	0.00	0.000	0.000	4.385	10.0	**0.001**
Juveniles	control	11	62.82	53.671	16.182	-	-	**-**
	25	9	0.00	0.000	0.000	3.882	10.0	**0.003**
	50	5	0.00	0.000	0.000	3.882	10.0	**0.003**
Total	control	11	78.45	64.013	19.301	-	-	**-**
	25	9	0.11	0.333	0.111	4.059	10.0	**0.002**
	50	5	0.00	0.000	0.000	4.065	10.0	**0.002**

Table 6

Efficacy (calculated as a percentage reduction of mean abundance of *Heteraxine heterocerca* from the control mean, adapted from Stone et al., 2000) after intubation with FEB for (a) 3 days, and (b) 6 days.

(a)

Daily dose (3 days)	Adults	Juveniles	Total	Recruitment
50 mg kg^{-1} BW	100	99.8	99.8	98.9
100 mg kg^{-1} BW	100	100	100	98.8
150 mg kg^{-1} BW	100	100	100	96.3
200 mg kg^{-1} BW	100	100	100	97.3

(b)

Daily dose (6 days)	Adults	Juveniles	Total	Recruitment
25 mg kg^{-1} BW	99.4	100	99.9	-45.0
50 mg kg^{-1} BW	100	100	100	37.5
75 mg kg^{-1} BW	100	100	100	-119.2

Table 7

Results of (a) comparison of mean abundance of recruited *Heteraxine heterocerca* after oral treatment by intubation with FEB, and (b) Results of Games-Howell *post-hoc* multiple comparisons following unequal variances as indicated by Levene's statistic ($P < 0.05$). df = degrees of freedom. F = F-value. $P \leq 0.05$ was considered significant.

(a)

Treatment	Sum of Squares	df	Mean Square	F	*P*-value
3 days	126859.406	4	31714.852	28.939	**<0.000**
6 days	1972.978	2	986.489	2.232	0.131

(b)

Treatment	Treatment (mg kg^{-1} BW day^{-1})	*n*	Mean	Std. Deviation	Std. Error	*P*-value
3 days	control	10	131.60	66.850	-	-
	50	9	1.44	1.878	21.149	**0.001**
	100	10	1.60	1.506	21.145	**0.001**
	150	6	4.83	6.969	21.330	**0.001**
	200	7	3.57	2.507	21.161	**0.001**
6 days	control	11	33.3	21.55	-	**-**
	25	9	48.3	24.11	10.337	0.633
	50	5	20.8	10.33	7.973	0.174

3.2.2 *Benedenia seriolae*

The mean abundance of adult *B. seriolae* on fish treated with FEB for either 3 days or 6 days was not significantly different from control fish (Tables 8a and 8b); however significant differences were detected between the mean abundance of all other life stages and the controls (Tables 8a and 8b). The Levene's test statistic indicated unequal variance in these data ($P < 0.05$). *Post-hoc* Games-Howell multiple comparisons were conducted and this confirmed that the mean abundance of adult *B. seriolae* from fish treated with FEB was not significantly different from control fish (Tables 9a and 9b). A closer examination of the data showed that mean abundance of juvenile, total and recruited *B. seriolae* were *greater* than that of control fish (Tables 9a and 9b) and calculating efficacy was therefore inappropriate.

Table 8

Results of comparison of mean abundance of *Benedenia seriolae* using one-way
ANOVA after oral treatment by intubation with FEB for (a) 3 days, and (b) 6 days.
df = degrees of freedom. F = F-value. $P \leq 0.05$ (indicated by bold type) was
considered significant.

(a)

Life stage	Sum of Squares	df	Mean Square	F	*P*-value
Adults	3204.143	4	801.036	.734	0.574
Juveniles	179782.405	4	44945.601	5.902	**0.001**
Total	156879.071	4	39219.768	5.454	**0.001**
Recruitment	217407.071	4	54351.768	2.993	**0.031**

(b)

Life stage	Sum of Squares	df	Mean Square	F	*P*-value
Adults	1269.375	2	634.688	.647	0.533
Juveniles	91053.564	2	45526.782	9.908	**0.001**
Total	110823.836	2	55411.918	7.850	**0.003**
Recruitment	80975.829	2	40487.914	11.125	**0.000**

Table 9

Results of Games-Howell *post-hoc* multiple comparisons of *Benedenia seriolae* after oral treatment by intubation with FEB for (a) 3 days, and (b) 6 days. df = degrees of freedom. F = F-value. $P \leq 0.05$ (indicated by bold type) was considered significant.

(a)

Life stage	Treatment (mg kg^{-1} BW day^{-1})	n	Mean	Std. Deviation	Std. Error	*P*-value
Adults	control	10	64.10	42.375	-	-
	50	9	69.00	34.315	17.618	0.999
	100	10	53.70	26.234	15.760	0.962
	150	6	55.33	20.186	15.731	0.979
	200	7	43.29	33.014	18.310	0.785
Juveniles	control	10	87.70	48.037	-	**-**
	50	9	177.67	119.280	38.388	0.167
	100	10	195.40	82.848	30.879	**0.046**
	150	6	294.50	96.660	44.519	**0.021**
	200	7	225.71	80.313	34.439	**0.047**
Total	control	10	151.80	49.398		
	50	9	246.67	105.196	0.167	-218.81
	100	10	249.10	84.233	0.046	-193.04
	150	6	349.83	102.116	0.021	-361.35
	200	7	269.00	81.206	0.047	-232.72
Recruitment	control	10	176.20	131.428	-	**-**
	50	9	364.00	196.022	77.438	0.166
	100	10	320.10	119.409	56.153	0.121
	150	6	334.50	70.253	50.497	**0.049**
	200	7	237.29	96.721	55.351	0.802

(b)

Life stage	Treatment (mg kg^{-1} BW day^{-1})	n	Mean	Std. Deviation	Std. Error	P-value
Adults	control	11	56.09	41.558	-	-
	25	9	70.78	17.288	13.792	0.550
	50	5	69.80	21.982	15.926	0.673
Juveniles	control	11	62.18	37.778		**-**
	25	9	141.67	65.517	24.631	**0.018**
	50	5	220.20	114.537	52.474	0.075
Total	control	11	118.27	71.765	-	**-**
	25	9	212.44	72.762	32.503	**0.026**
	50	5	290.00	123.925	59.495	0.071
Recruitment	control	11	39.91	23.428	-	**-**
	25	9	161.56	93.983	32.114	**0.011**
	50	5	138.60	31.278	15.670	**0.002**

4. Discussion

Our oral treatment trials show that FBZ and OXF surface coated onto feed (Trial 1) are effective against the gill monogenean *Z. seriolae* parasitising *S. lalandi* and that FEB administered by intubation (Trial 2) is effective against *H. heterocerca* parasitising the gills of *S. quinqueradiata*. The highest efficacy against *Z. seriolae* in Trial 1 was achieved by 100 mg kg^{-1} BW day^{-1} of FBZ for 3 days (92.3% reduction; Table 3a) and 50 mg kg^{-1} BW day^{-1} of OXF administered for 6 days (87.4%; Table 3b). In Trial 2, complete elimination (100% reduction) of *H. heterocerca* was achieved by FEB administered at 100, 150 and 200 mg kg^{-1} BW day^{-1} for 3 days (Table 6a) and at 50 and 75 mg kg^{-1} BW day^{-1} for 6 days (Table 6b), although remaining doses of FEB also recorded high efficacy (99.4-99.8%) against *H. heterocerca*. No dose of FBZ, OXF and FEB, however, appeared to have a therapeutic effect against *B. seriolae* parasitising *S. lalandi* in Australia or *S. quinqueradiata* in Japan.

In Trial 1, actual daily doses of each anthelmintic tested were similar for both compounds (see Tables 1a, b). The daily dose of FBZ that produced the highest efficacy against *Z. seriolae* (92.3% reduction in mean parasite abundance) was 100 mg kg^{-1} BW day^{-1} for 3 days (Table 3a). The daily dose of OXF that produced the

54

highest efficacy against *Z. seriolae* (87.4% reduction in mean parasite abundance) was 50 mg kg^{-1} BW day^{-1} for 6 days (Table 3b).

Our findings from Trial 2 demonstrate that FEB, registered in Japan for oral treatment of *Heterobothrium okamotoi* parasitising *T. rubripes*, is also a very effective oral treatment against the gill monogenean *Heteraxine heterocerca* parasitising *S. quinqueradiata*. All but two doses tested resulted in complete elimination of total *H. heterocerca* and those doses that did not completely eliminate the parasites nonetheless achieved high efficacy (99.8-99.9%) (Tables 6a and 6b). As found in Trial 1 with FBZ and OXF, in Trial 2 FEB did not demonstrate any efficacy against the skin parasite *B. seriolae*. Some treated fish in Trial 2 had a greater number of *B. seriolae* than controls (Tables 9a and 9b). These results are puzzling and seem unlikely to be related to treatments with FEB. It is more likely that fish used in this trial were from different cohorts, e.g. fish may have been taken from a cage that had been recently graded. It also may have occurred by random chance, i.e. that fish with higher abundanceies of *B. seriolae* were inadvertently assigned to control treatments. Differences in *B. seriolae* abundance are unlikely to be related to treatment with FEB. Despite this, FEB still appeared to have a strong effect on *H. heterocerca*. These unusual results mean it would be beneficial to conduct *in vitro* trials to clarify what effect (if any) the anthemintics FBZ, OXF and FEB have against *B. seriolae* and if the product is present in the skin of the host, before further pursuing further *in vivo* oral treatment trials.

FBZ, OXF and FEB might be expected to have similar efficacies against related blood feeding polyopisthocotyleans such as *Z. seriolae* and *H. heterocerca*, however many aspects of the biology of monogeneans are undocumented (Whittington and Chisholm, 2008). There may be differences between these parasite species in feeding or other characteristics. Differences in feeding between individual fish may also have led to differences in the resulting efficacy. In Trial 1, FBZ and OXF were administered by surface coating of pellets, whereas FEB was administered by direct intubation in Trial 2. Differential feeding between individual fish in Trial 1 may mean that different fish received varying doses of medicine, and resulted in varying efficacy, whereas administration of FEB by intubation gave more exact control over the dose delivered in Trial 2.

Kimura et al. (2006) reported that FEB was more effective against *Heterobothrium okamotoi* infestations of *T. rubripes* when FEB was administered

consecutively at 50 mg kg^{-1} BW day^{-1} for 2 days, rather than in a single one-off dose of 25, 50 or 100 mg kg^{-1} BW. Kimura et al. (2007), furthermore, found that the number of *H. okamotoi* did not decrease until 3 days into a 5-day course of FEB at 25 mg kg^{-1} BW day^{-1}. While we found FEB to be very effective against *Heteraxine heterocerca* parasitising *S. quinqueradiata* in Trial 2, the mean parasite abundance of *Z. seriolae* parasitising *S. lalandi* in Trial 1 was not significantly reduced after 3 days of oral treatment with FBZ at 150 mg kg^{-1} BW day^{-1} (Table 2c). FBZ administered for 6 days at 50 and 75 mg kg^{-1} BW day^{-1} did result in significantly less *Z. seriolae*, and it is possible that FBZ also needs to be administered consecutively for >3 days to have a significant effect against *Z. seriolae* parasitising *S. lalandi*.

The uptake and tissue distribution of these benzimidazoles in *Seriola* spp., as well as the bioavailability of active metabolites may well explain the efficacy observed in these trials against monogeneans. The pharmacokinetics of FBZ, OXF and FEB are unknown in *Seriola* spp (Carangidae), but in mammals and avians, FBZ and OXF are metabolized from FEB *in vivo* (Reinemeyer and Courtney 2001) and this occurs in *Takifugu rubripes* (Tetraodontidae) after oral administration (Kimura et al., 2006). Much of the anthelmintic activity of FBZ and FEB is attributed to OXF (Reinemeyer and Courtney, 2001), and it would be useful to conduct a comparative study of orally administered FBZ, OXF and FEB in *Seriola* spp. to see if one has greater efficacy than the others at removing gill monogeneans such as *Z. seriolae* and *H. heterocerca*. It would also be useful to examine the pharmacokinetics of FBZ, OXF and FEB to determine the bioavailability and distribution of the active metabolites following oral administration. This information would be useful in optimising dosing strategies for treatment of monogeneans, and may explain why efficacy is observed against *Z. seriolae* and *H. heterocerca* in our trials, but not *B. seriolae*. It also may clarify whether FBZ, OXF and FEB accumulate in the tissues, and therefore confirm whether consecutive dosing is required to achieve efficacy against monogeneans.

The results of our experiments indicate that the benzimidazoles FBZ, OXF and FEB are effective oral treatments for the blood-feeding monogeneans *Z. seriolae* parasitising the gills of *S. lalandi* and *H. heterocerca* parasitising the gills of *S. quinqueradiata*. FBZ and OXF are the active metabolites of FEB (Reinemeyer and Courtney, 2001). It is likely then that FBZ, OXF and FEB will also have an effect on *Z. seriolae* parasitising *S. dumerili* (see Grau et al., 2003; Montero et al., 2004). In

Australia FBZ and OXF are not registered and there is no Minor Use Permit for their use as oral treatment for monogeneans of *S. lalandi*, but in Japan FEB is already routinely used as an oral treatment for *Heterobothrium okamotoi* from the gills of cultured tiger puffer *T. rubripes* (see Ogawa et. al., 2005). It would be worth exploring what is required for farmers to use FEB as an oral treatment for *Heteraxine heterocerca* and *Z. seriolae* infestations of *Seriola* spp. culture in Japan. Despite the apparent absence of activity against *B. seriolae*, our results suggest that FBZ, OXF and FEB warrants further investigation for other fish species in aquaculture infected by blood-feeding gill monogeneans.

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CHAPTER 4 – EFFICACY OF ORALLY ADMINISTERED PRAZIQUANTEL AGAINST *ZEUXAPTA SERIOLAE* AND *BENEDENIA SERIOLAE* (MONOGENEA) IN YELLOWTAIL KINGFISH *SERIOLA LALANDI*

Statement of Authorship

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I give consent for R.E. Williams to include this paper for examination towards the degree of Doctor of Philosophy.

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Signed: Date 4 June 2009

Chapter 4: Efficacy of orally administered praziquantel against *Zeuxapta seriolae* and *Benedenia seriolae* (Monogenea) in yellowtail kingfish *Seriola lalandi*
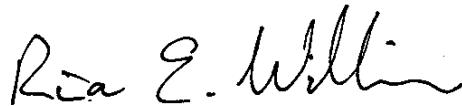
ABSTRACT: We investigated the efficacy of praziquantel (PZQ) administered orally to yellowtail kingfish (*Seriola lalandi* in sea-cage aquaculture in South Australia) against the monogeneans *Zeuxapta seriolae* and *Benedenia seriolae* infesting gills and skin, respectively. PZQ was administered to fish by surface-coating feed pellets (Trial 1) or by direct intubation of the stomach (Trial 2). In both trials 4 daily doses were administered: 50 and 75 mg kg^{-1} body weight (BW) d^{-1} for 6 d, and 100 and 150 mg kg^{-1} BW d^{-1} for 3 d. Mean parasite intensity was compared between medicated fish and control fish. In Trial 1, fish fed lower daily doses of PZQ for 6 d (50 and 75 mg kg^{-1} BW d^{-1}) had fewer *Z. seriolae* and *B. seriolae* than fish fed higher daily doses for 3 d (100 and 150 mg kg^{-1} BW d^{-1}). Fish rejected feed pellets surface-coated with PZQ, suggesting PZQ affected palatability of feed, and may explain differences in efficacy between treatments. In Trial 2, where PZQ was administered by intubation, there were fewer *Z. seriolae* and *B. seriolae* in medicated fish than control fish. Intubated PZQ was also effective against newly recruited *Z. seriolae* and *B. seriolae*. PZQ could be developed as a useful treatment for *Z. seriolae* and *B. seriolae* parasitising *S. lalandi* in sea-cage aquaculture if suspected palatability problems are resolved.

KEY WORDS: Monogenea · Sea-cage aquaculture · Oral treatment · Anthelmintic · *Seriola lalandi*

INTRODUCTION

Yellowtail kingfish *Seriola lalandi* (Carangidae) farmed in sea-cages in Spencer Gulf, South Australia are parasitised by the monogeneans *Zeuxapta seriolae* (Heteraxinidae) and *Benedenia seriolae* (Capsalidae). *Zeuxapta seriolae,* a polyopisthocotylean, attaches to gill lamellae by haptoral clamps. This species feeds on blood and heavy infestations have been associated with anaemia and mortality in aquaculture of *Seriola* spp. in Japan (Ogawa and Yokoyama 1998), in *S. lalandi* in

Australia (Ernst et al. 2002) and New Zealand (Sharp et al. 2003), and in *S. dumerili* in the Mediterranean (Grau et al. 2003, Montero et al. 2004). *Benedenia seriolae*, a monopisthocotylean, attaches to the skin, fins and eyes of fish using a sucker-like haptor armed with sclerites (Whittington 1996). Although attachment appears to cause little damage to fish, *B. seriolae* feeds on epithelial cells and heavy infestations can result in wounds that penetrate the epidermis deeply, and may be associated with increased rubbing behaviour of infested fish. Aggravated wounds may provide entry for secondary bacterial, fungal or viral infections (Paperna, 1991, Thoney and Hargis 1991) and affect the appearance of fish, therefore reducing their value and marketability.

Management of *Zeuxapta seriolae* and *Benedenia seriolae* in South Australian *Seriola lalandi* aquaculture relies on bathing cages of fish in a hydrogen peroxide solution. Bath treatments of sea-caged *S. lalandi* are labour-intensive, time-consuming, weather-dependent and stressful to fish. Although the industry has developed considerable expertise in bath treatments, some mortalities may still occur due to difficulties in calculating bath solution, physical damage to fish from crowding, or lack of oxygen. A treatment effective against *Z. seriolae* and *B. seriolae* administered in-feed would be a practical alternative to bath treatment, because it requires no extra labour or infrastructure and does not stress fish through handling or crowding.

Praziquantel (PZQ), a synthetic anthelmintic, was developed to treat internal platyhelminths in livestock, domestic animals and humans (Day et al. 1992). Recent studies have investigated its effect on the polyopisthocotyleans *Microcotyle sebastis* (Kim et al. 1998, 2001a, Kim and Cho 2000, Kim and Kim 2002), *Heterobothrium okamotoi* (Hirazawa et al. 2000) and *Sparicotyle chysophrii* (Sitjà-Bobadilla et al. 2006), as well as the monopisthocotyleans *Gyrodactylus* sp. (Tojo and Santamarina 1998b) and *Neobenedenia* sp. (Hirazawa et al. 2004). PZQ is used as an oral treatment for tapeworm in rainbow trout *Onchorhynchus mykiss* and Atlantic salmon *Salmo salar* farmed in Norway (Hormazabel and Yndestad 1995), and it is contained in Hadaclean[®] (Bayer), an oral treatment for *Benedenia seriolae* parasitising sea-caged Japanese yellowtail *Seriola quinqueradiata* in Japan. However, PZQ has not been tested as an oral treatment for monogeneans parasitising *S. lalandi* farmed in Australia. This study investigated the efficacy of PZQ, administered orally either in-feed or by direct intubation, against *Z. seriolae* and *B. seriolae* parasitising *S. lalandi*.

MATERIALS AND METHODS

Source of fish and parasites. Yellowtail kingfish *Seriola lalandi* were obtained from a commercial sea-cage aquaculture farm in Spencer Gulf, South Australia, in May 2003 and March 2004 for Trial 1 and Trial 2, respectively, and maintained in a 12 m^3 tank in a commercial fish hatchery prior to experiments. Fish became infested with *Zeuxapta seriolae* and *Benedenia seriolae* while in sea-cages. Before each trial, 10 fish were sampled from the cohort to confirm presence of *Z. seriolae* and *B. seriolae*. Parasite sampling involved bathing each fish individually in 2 separate bath solutions. A bath in dechlorinated tap water for 5 min killed all *B. seriolae* and any remaining attached to fish were manually removed (Chambers and Ernst, 2005). This was followed by a seawater bath containing 5 ppm PZQ for 10 min to remove *Z. seriolae* (Mooney et al. 2006). Parasites were collected by filtering bathwater from each fish through 75 μm mesh. The filtrate was preserved in 2% formalin solution and parasites were counted using a stereoscopic dissection microscope. PZQ was purchased from MP Biomedicals.

Trial 1—PZQ delivered by surface-coating of feed. Trial 1 was conducted in a rectangular floating sea-cage in the warm-water outlet channel of a power station during June and July 2003. Temperature ranged from 19 to 21°C and the salinity was 48 psu. The salinity is naturally high in the upper Spencer Gulf, but despite this, both wild and farmed kingfish carry viable infestations of both *Zeuxapta seriolae* and *Benedenia seriolae*. Ten fish from the same cohort were sampled before the trial to confirm presence of parasites, according to methods detailed above. The mean weight of these fish was used to determine the ration of feed (in percentage BW [body weight, kg]) and the actual PZQ dose (mg kg^{-1} BW) to administer during the trial. A total of 163 fish with mean weight 0.32 kg (range 0.12 to 0.51 kg) were randomly distributed among 15 cages, each 1.5 m in diameter and constructed from 12 mm plastic mesh. Fish were acclimated for 7 d prior to experimentation and fed to satiation with 5 mm Skretting Classic HS pellets (Skretting Australia). The mean daily ration consumed during the acclimation period was calculated and used as the ration during the trial. Medicated feed was prepared by surface-coating pellets with PZQ. Each dose, calculated from mean weight of fish, was dissolved in 2 ml of absolute ethanol and sprayed evenly onto the ration of pellets using a household

trigger-style spray bottle. As these sprayers often leave a small amount of liquid behind in the bottle, this was measured prior to the trial and compensated for. Fish oil (2 ml) was also applied in an effort to mask the PZQ 'flavour' on medicated feed. Pellets were prepared the day before use and allowed to dry overnight. Control fish were fed regular pellets (unmedicated without fish oil). Four daily doses were administered: 50 and 75 mg kg^{-1} BW d^{-1} for 6 d, and 100 and 150 mg kg^{-1} BW day^{-1} for 3 d. Three replicates were used for each of the 4 treatments and 1 control. Respective doses were administered over 3 d and 6 d simultaneously. Each cage was provided with feed, and fish were observed carefully for rejection of pellets, or signs of toxicity such as darkened skin or loss of equilibrium caused by medicated feed. Fish were held for 4 d following completion of treatment to allow PZQ to be completely absorbed and metabolised. The fish were then sampled using the method described earlier, to determine remaining parasite intensity.

Trial 2—PZQ administered by intubation. Trial 2 was conducted in a 12 m^3 tank at a commercial hatchery in the Spencer Gulf region of South Australia in May 2004. A total of 50 fish averaging 1.25 (0.88 to 1.5) kg in weight were distributed randomly among 5 cages identical to those used in Trial 1. Water temperature ranged from 17 to 18°C and salinity was 48 psu. Presence of monogeneans was confirmed on a sample of fish from the same cohort using the method detailed above. Mean weight of fish for each cage was determined to calculate actual PZQ dose (mg kg^{-1} BW). The experiment commenced the day following transfer of fish to cages. PZQ was delivered in a paste made from 18 g of pre-pellet meal and 35 ml of dechlorinated tap water. Fish were anaesthetised in a 60 l tub containing a bath solution of 25 ppm clove oil in seawater until they were unresponsive to touch, then 4 ml of paste was intubated into their stomach directly using a 60 ml cathether syringe fitted with an extension of soft silicon hose 4 mm in diameter. Fish were placed in a 60 l tub of clean seawater to recover, monitored for 5 min to ensure no medication was regurgitated, then returned to their cage. Control fish underwent an identical procedure, but were administered 4 ml of paste without PZQ. Four daily doses were administered: 50 and 75 mg kg^{-1} BW d^{-1} for 6 d, and 100 and 150 mg kg^{-1} BW d^{-1} for 3 d. Fish were sampled using the method as described previously. Monogeneans have a direct lifecycle allowing them to reproduce rapidly in a closed environment (Thoney and Hargis 1991). The primary focus of this trial was to measure the ability of PZQ to remove existing parasites.

Due to the length of this trial, the water temperature and the physical set-up of the tanks, new parasite recruitment was impossible to prevent; therefore *Zeuxapta seriolae* and *Benedenia seriolae* <1.2 mm in total length (I. Ernst and I. D. Whittington, unpubl. data) were considered to have parasitised fish after treatment, and were counted separately from the remaining parasites.

Statistical analyses. Genstat Release 6.1 and SPSS 11.0 statistical software were used to analyse the data. The efficacy for each treatment was calculated as a percentage according to the formula of Stone et al. (2000):

$$\% \text{ efficacy} = 100 - (100 \text{ x } \frac{\text{mean parasite intensity of treatment}}{\text{mean parasite intensity of control}}).$$
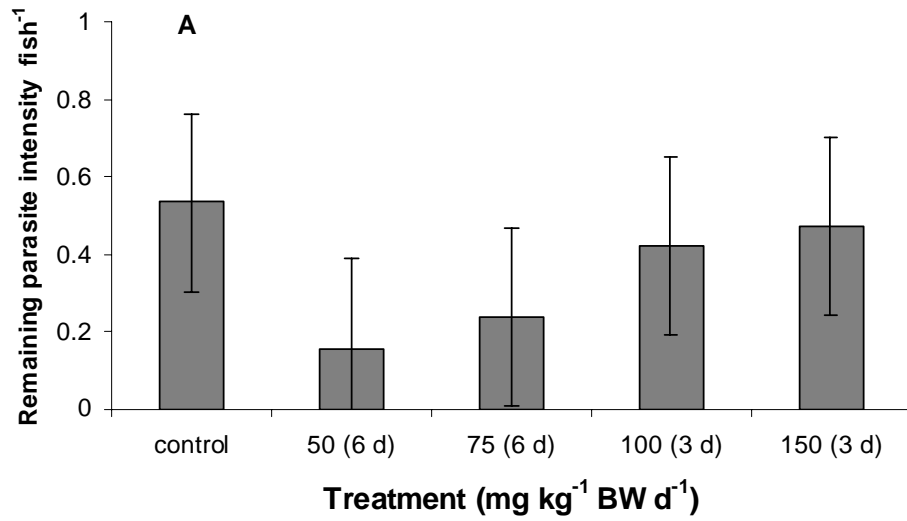
Fig 1A
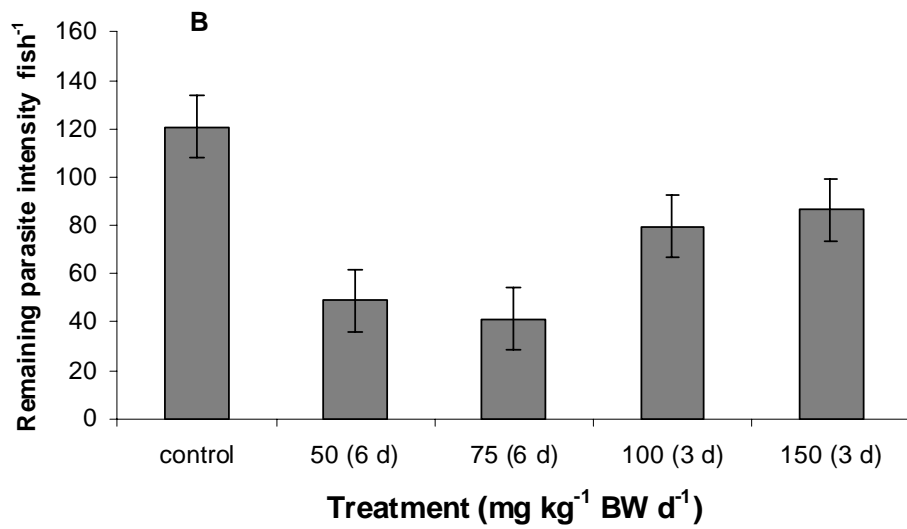
Fig 1B

Fig. 1: Mean intensity of (A) *Zeuxapta seriolae* and (B) *Benedenia seriolae* remaining on fish after Trial 1 (surface-coated feed treatment with PZQ [praziquantel]) at 4 daily doses and 2 durations: 50 and 75 mg kg–1 BW d–1 over 6 d and 100 and 150 mg kg–1 BW d–1 over 3 d. Error bars represent 95% confidence intervals of the means. *Z. seriolae* data have been $\log10(y+1)$ transformed. BW = body weight of fish

Fig 2A

Fig 2B

Fig. 2: Fig. 2. Mean intensity of (A) *Zeuxapta seriolae* and (B) *Benedenia seriolae* remaining on fish after Trial 2 (treatment by intubation with PZQ) at 4 daily doses and 2 durations: 50 and 75 mg kg–1 BW d–1 over 6 d and 100 and 150 mg kg–1 BW d–1 over 3 d. Error bars represent 95% confidence intervals of the means. *B. seriolae* data have been sqrt(y+ 0.5)-transformed. See Fig. 1 legend for definitions of acronyms
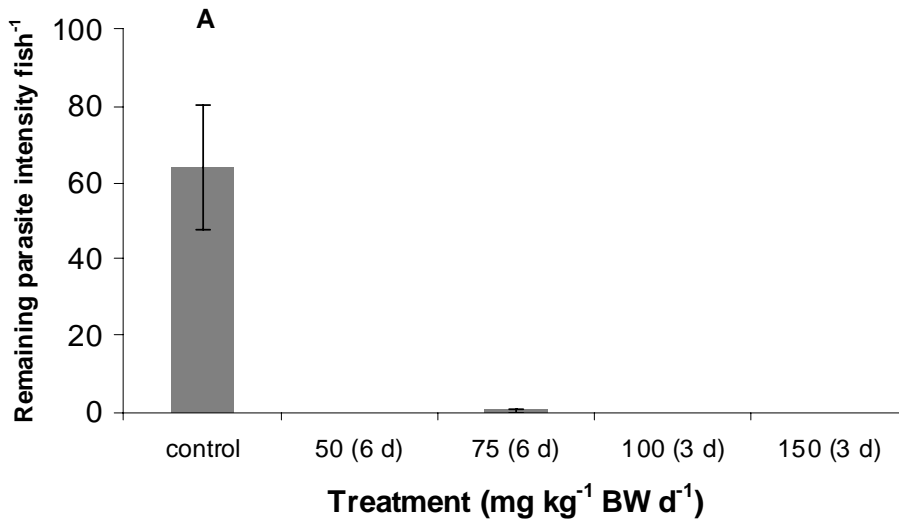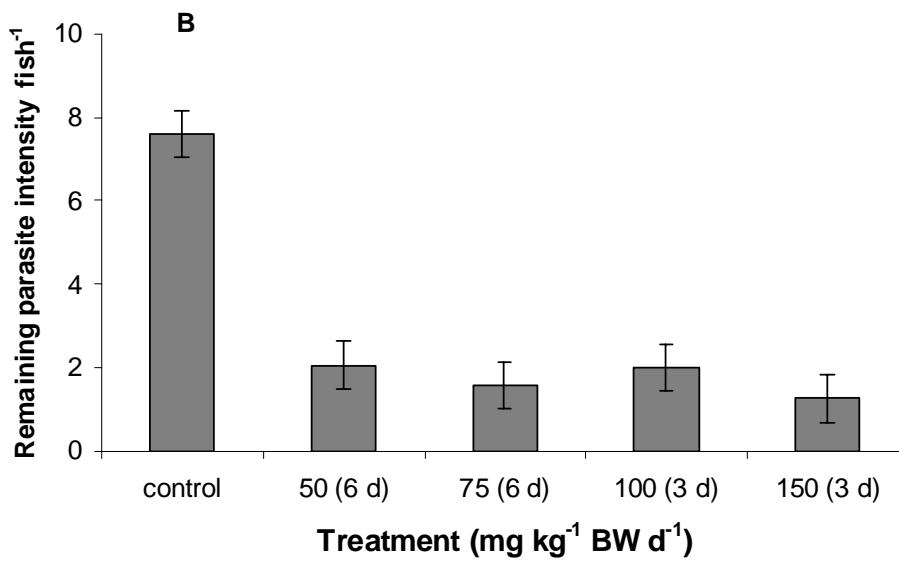
In Trial 1, parasite intensity data for individual fish were pooled for each cage. To satisfy the assumption of equal variances, data for *Zeuxapta seriolae* were $\log_{10}(y+1)$-transformed, where y is the number of *Z. seriolae* (Fig. 1A), before performing a 1-way ANOVA. No transformation was required for *Benedenia seriolae* data (Fig. 1B); therefore a 1-way ANOVA was performed on raw (untransformed) data. Where significant differences were detected, the efficacy of each treatment was calculated as a percentage, according to the formula in Stone et al. (2000) (see formula given above). Linear contrasts were carried out on data for *Z. seriolae* and *B. seriolae* to explore (1) whether the mean parasite intensity for control fish was significantly different from the mean parasite intensities for the treatments, (2) whether the mean parasite intensities for fish fed the lower daily PZQ concentrations (50 and 75 mg kg^{-1} BW d^{-1}) were significantly different from the mean parasite intensities of fish fed higher daily concentrations (100 and 150 mg kg^{-1} BW d^{-1}), and (3) whether fish fed the lowest daily concentration of PZQ (50 mg kg^{-1} BW d^{-1}) had significantly higher mean parasite intensity than fish fed the highest daily concentration (150 mg kg^{-1} BW d^{-1}).

In Trial 2, parasite intensity data for individual fish were not pooled as each fish was intubated individually and therefore each was considered a replicate. The mean number of *Zeuxapta seriolae* remaining on fish in the 50, 100 and 150 mg kg^{-1} BW d^{-1} treatments was zero (Fig. 2A); therefore a 1-tailed 1-sample *t*-test was carried out to determine whether the control mean parasite intensity differed from the mean parasite intensity of *Z. seriolae* on fish fed 50, 100 and 150 mg kg^{-1} BW d^{-1}. A 1-tailed independent-sample *t*-test was performed between the 75 mg kg^{-1} BW d^{-1} treatment (the only treatment with a mean parasite intensity >0, see Fig. 2A) and the control. To satisfy the assumption of equal variance, data for *Benedenia seriolae* remaining on fish (Fig. 2B) were $\sqrt{(y+1)}$-transformed, where y is the number of parasites. This was followed by a 1-way ANOVA, and where significant differences were detected (p < 0.05), linear contrasts were carried out (1) if the mean parasite intensity for control fish was significantly different from that for the treatments, (2) if the mean parasite intensities for fish fed the lower daily concentrations of PZQ (50 and 75 mg kg^{-1} BW d^{-1}) were significantly different from fish fed higher daily concentrations of PZQ (100 and 150 mg kg^{-1} BW day^{-1}), and (3) if fish fed the lowest daily concentration of PZQ (50 mg kg^{-1} BW d^{-1}) had significantly different

mean parasite intensity from fish fed the highest daily concentration of PZQ (150 mg kg^{-1} BW d^{-1}).

RESULTS

Trial 1—PZQ delivered by surface-coating of feed

A mean daily ration of 1.3% BW was offered to each treatment group during the feed trial. Fish were observed to reject pellets surface-coated with PZQ at all treatment doses, but especially at the higher daily doses (100 and 150 mg kg^{-1} BW d^{-1}), suggesting PZQ reduced palatability of feed. The amount of pellets consumed was difficult to quantify because rejected pellets fell through cage mesh and were lost. Therefore, doses assigned to each treatment group are approximate at best, and the actual dose delivered is likely to be less than the target total dose. No behavioural abnormalities nor adverse physical signs of toxicity (e.g. skin darkening or loss of equilibrium) were observed in any fish at any of the treatment doses.

Fish fed pellets medicated with PZQ had fewer *Zeuxapta seriolae* and *Benedenia seriolae* remaining compared with control fish, which received unmedicated pellets (Fig. 1A and B, respectively). Significant differences were detected between the mean intensity of *Z. seriolae* remaining on control fish and the mean intensities remaining on fish that received doses of PZQ (1-way ANOVA, $p = 0.044$). Significant differences were also detected between the mean intensity of *B. seriolae* remaining on control fish and the mean intensities remaining on fish that received doses of PZQ (1-way ANOVA, $p < 0.001$). Efficacy of PZQ against *Z. seriolae* and *B. seriolae* was greater with lower daily doses (50 and 75 mg kg^{-1} BW d^{-1}) than with higher daily doses (100 and 150 mg kg^{-1} BW d^{-1}) (Table 1A). This is reflected by the significant differences obtained in all 3 linear contrasts performed (Table 2A).

70

Table 1: Efficacy, calculated as percentage reduction of mean parasite intensities from the control mean (Stone et al. 2000), of (A) PZQ (praziquantel) delivered in feed (Trial 1) and (B) PZQ delivered by intubation (Trial 2) at 2 total doses and durations. BW = body weight

	Target daily dose (mg kg^{-1} BW d^{-1}), duration (d)			
	50 (6)	75(6)	100 (3)	150 (3)
(A)				
Zeuxapta seriolae	81.4	70.8	35.8	14.7
Benedenia seriola	58.1	66.4	30/9	21.6
(B)				
Zeuxapta seriolae	100	99.5	100	100
Benedenia seriolae	92.5	95.5	91	97.7

Table 2: Linear contrasts performed on intensities of (A) *Zeuxapta seriolae* and *Benedenia seriolae* remaining on treatment fish after PZQ (praziquantel) was delivered by surface-coating feed (Trial 1); (B) *B. seriolae* remaining on treatment fish after PZQ was delivered by intubation (Trial 2). BW = body weight

Contrast	*Zeuxapta seriolae*	*Benedenia seriolae*
(A)		
Control vs. all treatments	$p = 0.040$	$p < 0.001$
50 and 75 vs 100 and 150 mg kg^{-1} BW d^{-1}	$p = 0.014$	$p < 0.001$
50 vs 150 mg kg^{-1} BW d^{-1}	$p = 0.024$	$p = 0.002$
(B)		
Control vs. all treatments	-	$p < 0.001$
50 and 75 vs 100 and 150 mg kg^{-1} BW d^{-1}	-	$p < 0.001$
50 vs 150 mg kg^{-1} BW d^{-1}	-	$p = 0.054$

Trial 2—PZQ administered by intubation

Calculation of dose was based on mean weight of each treatment group taken at the start of the trial. No regurgitation of medicated paste nor adverse physical signs associated with the treatment were observed. PZQ administered by direct intubation at 50, 100, and 150 mg kg^{-1} BW d^{-1} resulted in complete elimination of existing *Zeuxapta seriolae* infestation and 75 mg kg^{-1} BW d^{-1} PZQ resulted in an efficacy of 99.5% (Table 1B; Fig. 2A). A 1-tailed 1-sample *t*-test suggested the mean parasite intensity of *Z. seriolae* remaining on control fish was >0. This indicated that the mean parasite intensity of *Z. seriolae* on control fish was likely to be greater than the mean parasite intensities remaining on fish in the treatment groups 50, 100 and 150 mg kg^{-1} BW d^{-1} (p > 0.001), which were also zero. The 75 mg kg^{-1} BW d^{-1} PZQ treatment had a lower mean intensity of *Z. seriolae* than the control (1-tailed independent-sample *t*-test, p > 0.001).

Efficacy of PZQ administered by intubation against *Benedenia seriolae* was >90% on all treatment fish compared with control fish (Table 1B; Fig. 2B). Mean parasite intensities of *B. seriolae* remaining on treated fish were found to be significantly different from control fish (1-way ANOVA, p > 0.001). Linear contrasts indicated that the higher daily doses for shorter treatment periods (100 and 150 mg kg^{-1} BW d^{-1} delivered over 3 d) of PZQ were not significantly different from the lower daily doses for longer treatment periods (50 and 75 mg kg^{-1} BW d^{-1} delivered over 6 d) (p = 0.549, see Table 2B). Linear contrasts also suggested that the mean intensity of *B. seriolae* remaining on fish fed the lowest daily dose (50 mg kg^{-1} BW d^{-1}) was not significantly different from fish fed the highest daily dose (150 mg kg^{-1} BW d^{-1}; p = 0.054), but overall, found that fish in all 4 treatment doses had significantly different numbers of *B. seriolae* remaining on them than the control fish (p < 0.001) (Table 2B).

Mean parasite intensities of newly recruited *Zeuxapta seriolae* and *Benedenia seriolae* in Trial 2 (i.e. infesting fish after treatment) appeared lower in fish from all treatment doses when compared with control fish. While treatments were not considered independent, and statistical analysis was not performed, the raw data are presented for discussion (Fig. 3).
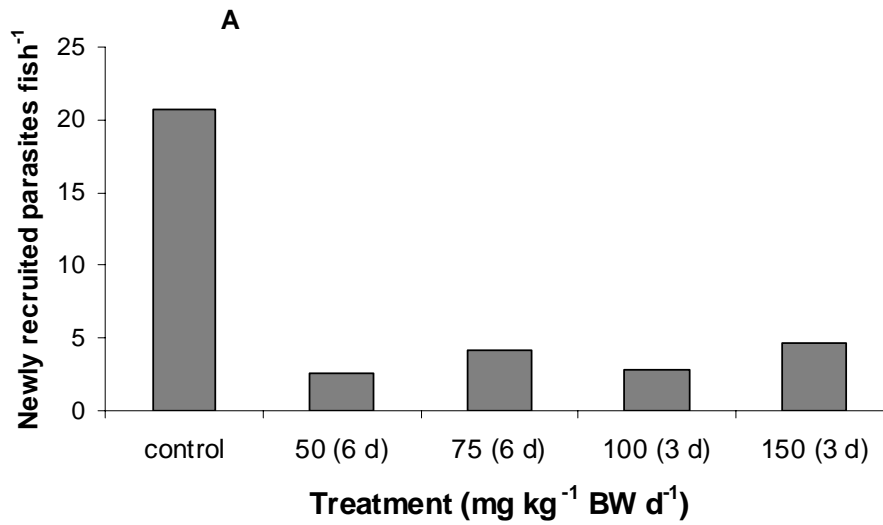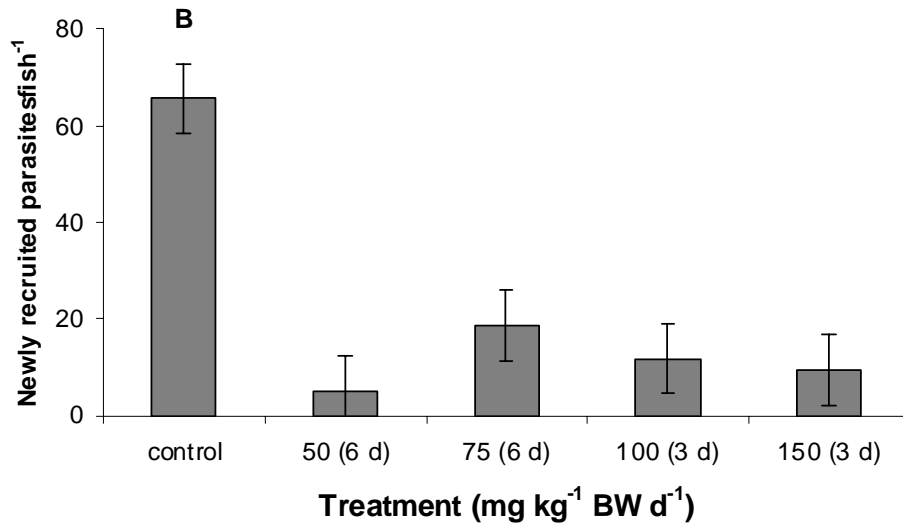
Fig 3A

Fig 3B

Fig. 3: Mean intensity of newly recruited (A) *Zeuxapta seriolae* and (B) *Benedenia seriolae* after Trial 2 (treatment by intubation with PZQ) at 4 daily doses and 2 durations: 50 and 75 mg kg–1 BW d^{-1} over 6 d and 100 and 150 mg kg^{-1} BW d^1 over 3 d. See Fig. 1 legend for definitions of acronyms

DISCUSSION

Fish treated with PZQ by surface coating of feed pellets (Trial 1) had fewer *Zeuxapta seriolae* and *Benedenia seriolae* than control fish that received unmedicated pellets (Fig. 1). However, higher daily doses (100 and 150 mg kg^{-1} BW d^{-1}) had lower efficacy than lower daily doses (50 and 75 mg kg^{-1} BW d^{-1}) for treating infections of *Z. seriolae* and *B. seriolae* (Table 1A). While we expected higher daily doses to have the same or higher efficacy in removing parasites from fish, this result could be explained by fish in the higher daily dose treatments consuming fewer feed pellets than fish fed the lower daily dose treatments. Fish were observed to reject pellets at all doses administered, especially at higher daily doses. This indicated that surface-coating pellets with PZQ reduced the palatability of pellets to *Seriola lalandi*. Hirazawa et al. (2004) also observed reduced appetite in spotted halibut *Verasper variegatus* fed pellets medicated with PZQ at a dose of 150 mg kg^{-1} BW d^{-1} and noted that Japanese yellowtail *Seriola quinqueradiata* and amberjack *Seriola dumerili* in commercial aquaculture in Japan may reject pellets medicated with PZQ. Sitjà-Bobadilla et al. (2006) also encountered suspected palatability problems with gilthead sea bream *Sparus auratus*, demonstrating reduced appetence towards PZQ-medicated feed. An oral treatment that reduces feed palatability makes it difficult for farmers to administer the required dose accurately. Furthermore, uneaten food and therefore unassimilated medication is wasteful and expensive, and a prolonged reduction in feeding may lead to slower fish growth, which is highly undesirable for farmers. If PZQ were administered to commercially farmed *Seriola lalandi* by surface coating of feed, other strategies may be required, such as the use of agents to mask the 'flavour' of the medicated feed, e.g. microencapsulation of PZQ prior to incorporation into feed or fasting fish for a period prior to treatment. However, microencapsulation may be too expensive, and fasting fish for any period may still be undesirable for farmers trying to achieve maximum growth from their fish. Alternatively, a lower, more palatable daily dose over a longer duration may be required to ensure fish do actually consume the required dose to treat *Z. seriolae* and *B. seriolae*, while retaining their appetite.

A number of studies investigating the use of PZQ as an orally administered anthelmintic have not recorded this fish rejecting feed medicated with PZQ; however those studies delivered the medication incorporated within the feed ration. For

example, Hirazawa et al. (2004) incorporated PZQ into a pre-pellet mixture before pellets were passed through a disc pelleter and dried. Kim et al. (2003) delivered PZQ by a moist pelleted feed (a feed normally manufactured on-site, immediately prior to feeding, with no drying process). In light of these studies, the occurrence of pellet rejection with surface-coated feed suggests that incorporating PZQ homogenously within the feed may overcome suspected palatability issues for some fish species. In South Australia, fish are fed commercially available pellets made through an extrusion process and it is possible that medicated diets incorporating PZQ could be commercially manufactured. However, extrusion does involve potentially destructive processes for medication such as pressure, humidity and high temperatures (Broz et al. 1997, Vertommen and Kinget 1998). Although the use of extruded feed pellets has been widely adopted in Japanese finfish aquaculture, incorporation of medications at the point of manufacture is not permitted. Consequently, farmers must apply medications themselves, usually by surface-coating feed, and this may exacerbate suspected palatability problems, depending on how refined their method of application is. Further experimentation is required to ensure extrusion processes do not reduce the activity of PZQ before a commercial medicated extruded pellet feed can be developed.

Three treatment doses (50, 100 and 150 mg kg^{-1} BW d^{-1}) in Trial 2 resulted in complete elimination of existing *Zeuxapta seriolae* infestations (Fig. 2A) and the 4th treatment dose (75 mg kg^{-1} BW day^{-1}) had an efficacy of 99.5% (Table 1B). Treatment fish also had fewer newly recruited *Z. seriolae* than control fish (Fig. 3), suggesting that all 4 daily doses of PZQ may prevent *Z. seriolae* recruiting to fish. As the treatments were not independent, a separate trial would be required to investigate this apparent prophylactic effect further.

These results demonstrate that PZQ is an excellent candidate for oral treatment against *Zeuxapta seriolae*. Through strategic application, by timing treatments based on knowledge of the parasite life cycle at different water temperatures, PZQ could be a useful tool in the management of *Z. seriolae*. PZQ has the potential for wide application, as *Z. seriolae* not only parasitises *Seriola lalandi* farmed in South Australia, but also farmed *S. lalandi* in New Zealand, farmed *S. dumerili* in the Mediterranean and farmed *S. lalandi* and *S. dumerili* in Japan. There are no published reports of the efficacy of orally administered PZQ for treatment of *Heteraxine heterocerca* (a blood-feeding gill monogenean) on farmed *S.*

quinqueradiata. However, considering the results reported here for *Z. seriolae*, and prior reports of the efficacy of PZQ for treating other polyopisthocotylean Monogenea, it is likely that PZQ will be effective against *H. heterocerca* in Japan.

Intubation by hand of individual fish is labour intensive. Administration of any treatment to an entire cage of fish or to an entire farm stock using this method is not practical. However, this method allowed exact doses of PZQ to be tested without suspected palatability problems confounding results. It also requires fewer fish as each is treated individually, and therefore can be treated as a replicate, while still retaining a statistically robust experimental design. Using intubation, we have demonstrated that orally administered PZQ can achieve high efficacy against *Zeuxapta seriolae* and *Benedenia seriolae* parasitising *Seriola lalandi*, if suspected palatability problems can be resolved.

CHAPTER 5 – EFFICACY OF ORALLY ADMINISTERED PRAZIQUANTEL AGAINST *HETERAXINE HETEROCERCA* AND *BENEDENIA SERIOLAE*, MONOGENEAN PARASITES OF JAPANESE YELLOWTAIL *SERIOLA QUINQUERADIATA*

Chapter 5: Efficacy of orally administered praziquantel against *Heteraxine heterocerca* and *Benedenia seriolae*, monogenean parasites of Japanese yellowtail *Seriola quinqueradiata*

Abstract

We investigated the efficacy of orally administered praziquantel (PZQ) delivered by intubation against the monogenean parasites *Heteraxine heterocerca* and *Benedenia seriolae* parasitising the gills and skin respectively of Japanese yellowtail (*Seriola quinqueradiata*) in sea-cage aquaculture off Kyushu, Japan. PZQ was administered at 50 and 75 mg kg^{-1} BW day^{-1} for 3 days and for 6 days, 100 and 150 mg kg^{-1} BW day^{-1} for 3 days, and at 300 and 450 mg kg^{-1} BW as single doses. Comparisons of mean parasite abundance were made between treated fish and control fish. Complete elimination of *H. heterocerca* was achieved by PZQ administered for 3 days at 50, 100 and 150 mg kg^{-1} BW day^{-1}. For all other doses of PZQ trialled, efficacy was high (90.4 - 99.6%) against infestations of *H. heterocerca*. Efficacy of PZQ against *B. seriolae* at all doses administered and treatment durations was low. The highest efficacy (77.8%) against *B. seriolae* was achieved by administration of a single 450 mg kg^{-1} BW dose of PZQ. In our experiment, the dose of PZQ (150 mg kg^{-1} BW day^{-1} for 3 days) as prescribed on the label of a commercially available product used to treat *B. seriolae* in aquaculture in Japan resulted in a 50.9% efficacy against *B. seriolae*, but completely eliminated the gill monogenean *H. heterocerca.*

1. Introduction

Seriola quinqueradiata (Carangidae) in sea-cage aquaculture in Japan is parasitised by the monogeneans *Heteraxine heterocerca* (Polyopisthocotylea: Heteraxinidae) and *Benedenia seriolae* (Monopisthocotylea: Capsalidae) (see Egusa, 1983). Details about attachment, feeding, pathology and health problems caused by these Monogenea species and attempts to manage them in Japanese aquaculture using freshwater or hydrogen peroxide baths were given in Chapters 1 and 2. Although the Japanese industry has developed considerable expertise with these bathing techniques, mortality may still occur. Use of in-feed medications avoids these problems, but incorporation of veterinary medicines into stock feed at the point of manufacture is not permitted in Japan, so farmers are required to add medications

to the feed. PZQ is commercially available to Japanese farmers as Hadaclean[®] (Bayer, Japan) to treat *B. seriolae* with the product label prescribing a dose of 150 mg kg^{-1} day^{-1} PZQ for 3 days (Stephens et al., 2003; Tubbs and Tingle, 2006a). No oral medications are currently registered in Japan to treat *H. heterocerca*, but Mooney et al. (2008) used a 15 ppm bath treatment of PZQ in experiments to successfully remove *H. heterocerca* from *S. quinqueradiata*. Previous studies have reported that orally administered PZQ is effective against other polyopisthocotyleans, including *Microcotyle sebastis* on *Sebastes schlegeli* in Korea (Kim et al., 1998; Kim and Cho, 2000; Kim et al., 2001a; Kim and Kim, 2002), *Heterobothrium okamotoi* on *Takifugu rubripes* in Japan (Hirazawa et al., 2000), *Sparicotyle chysophrii* on *Sparus auratus* in the Mediterranean (Sitja-Bobadilla et al., 2006) and *Zeuxapta seriolae* on *Seriola lalandi* in New Zealand (Tubbs and Tingle, 2006a) and Australia (Williams et al., 2007). There are no published reports on the efficacy of orally administered PZQ against *Heteraxine heterocerca*. Our aims were to explore whether orally administered PZQ is effective against *H. heterocerca* and simultaneously review its efficacy against *B. seriolae* parasitising *S. quinqueradiata* in Japan.

2. Methods

2.1 Source of fish and parasites

The trial was conducted in a disused floating sea-cage in Saiki Harbour, Oita Prefecture, Kyushu, Japan, during August 2004. Water temperature was 25±1 ºC and the salinity was 35 ppt. Specimens of *S. quinqueradiata* were obtained from a commercial sea-cage farm (South Sea Food, Oita Prefecture, Kyushu, Japan). Infestations of *H. heterocerca* and *B. seriolae* were confirmed by routine monitoring of fish from the same cohort that trial fish were taken from. Parasite sampling was a two stage process following methods detailed in Chambers and Ernst (2005) and Williams et al. (2007). Briefly, fish were first individually bathed in 60 L plastic bins for 5 min, in dechlorinated freshwater, which kills skin flukes, and any dead *B. seriolae* that remained attached were manually removed. This was followed by a bath in 15 ppm PZQ in seawater for 10 min to remove *H. heterocerca* (Mooney et al., 2008). Parasites were collected by filtering the bath solutions from each fish through 75 µm mesh. Filtrate was preserved in 5% formalin solution and parasites

79

were counted using a dissecting stereomicroscope. One hundred and ten parasitised fish of mean weight 265 g (range 91-358 g) were randomly distributed among 11 square cages 3.375 m^3 suspended in a larger floating sea-cage, so that there were 10 fish per cage. Fish were individually weighed so that the PZQ dose to administer during the trial in mg per kg body weight (mg kg^{-1} BW) could be determined for each cage. Fish were not acclimated prior to trial and the experiment commenced the day following their transfer to cages.

Table 1

Summary of PZQ doses tested, treatment duration and sample sizes used during trial.

Daily dose (mg kg^{-1} BW day^{-1})	Treatment duration (days)	Total dose (mg kg^{-1} BW)	Sample size of fish
0 (controls)	1, 3, 6	0	30
50	3	150	10
50	6	300	10
75	3	225	10
75	6	450	10
100	3	300	10
150	3	450	10
300	1	300	10
450	1	450	10

2.2 Administration of PZQ

A paste containing each daily dose of PZQ as Hadaclean® (Bayer Japan, Tokyo, Japan), pre-pellet meal (Yamaha Nutreco Aquatec), fish oil and tap water was prepared daily for intubation into fish. A summary of the doses of PZQ and treatment durations is given in Table 1. To administer the treatments, each fish was anaesthetised in a 60 L plastic tub containing a solution of 130 ppm FA-100 aquatic anaesthetic (4-allyl-2-methoxyphenol) (Tanabe Pharmaceutical Company Ltd, Osaka, Japan) in seawater until it had lost its reflex activity (Stage 4 anaesthesia, see Hikasa et al., 1986). Then paste was intubated into the stomach of each fish directly using a 10 mL syringe fitted with a 10 cm extension of 4 mm diameter soft silicon tube. Fish were placed into a 60 L bin of clean seawater to recover and were monitored for signs of regurgitation before being returned to their respective cage. A control cage was maintained for each treatment group (single dose, 3 days and 6

days, see Table 1) and each control fish underwent an identical procedure as treatment fish but received unmedicated paste by intubation. Kim et al. (2003) found that PZQ was detected in the skin of *Sebastes schlegeli* (Sebastidae) for 3 days after the end of oral treatment at 200 mg kg^{-1} BW day^{-1} for 3 days. For this reason, fish were held for 4 days after the last treatment to ensure that the parasites were recovered and counted after full exposure to the medication. Fish were then sampled using the method described in 2.1 to determine mean parasite abundance. The primary focus of this trial was to measure the efficacy of PZQ against existing parasite infestations. The duration of the trial and location of experimental cages in the ocean meant that it was impossible to prevent exposure to parasite recruitment (from infested wild fish or proximity of nearby farmed *S. quinqueradiata*). When *H. heterocerca* and *B. seriolae* were counted, they were therefore divided into three life stages: adults, juveniles and recruits (parasites that had settled during the trial). Sizes for each life stage were estimated from water temperature-related parasite growth based on data in Lackenby et al. (2007) for *B. seriolae* and Mooney et al. (2008) for *H. heterocerca*.

2.3 Statistical analysis

SPSS 15.0 software was used for statistical analyses (SPSS Inc., Chicago IL, USA). Each treatment period (single dose, 3 days and 6 days) and parasite species was analysed separately. Mean parasite abundance (the total number of individuals of a particular parasite species, divided by the total number of hosts examined, whether infested or not, see Bush et al., 1997) was calculated for each monogenean species. Comparisons of mean abundance of adult, juvenile and total (adults + juveniles) *H. heterocerca* were made between treatment and controls using independent *T*-tests for unequal variance. Comparisons of mean abundance of recruited *H. heterocerca* and all life stages (adults, juveniles, total and recruited) of *B. seriolae* were made between treatments and controls using a one-way ANOVA. A *P*-value ≤ 0.05 was considered significant. Where the Levene's statistic indicated unequal variance (*P* ≤ 0.05), Games-Howell *post-hoc* multiple comparisons were carried out.

Efficacy for each treatment was calculated as a % reduction of the mean parasite abundance using the formula below (adapted from Stone et al., 2000):

$$\% \text{ efficacy} = 100 - (100 \text{ x} \frac{\text{mean parasite abundance of treatment group}}{\text{mean parasite abundance of control group}}).$$

3. Results

Calculation of dose was based on the mean fish weight of each treatment group at the start of the trial. No regurgitation of medicated paste or signs of toxicity (e.g. skin darkening, erratic swimming, and loss of equilibrium) was observed in fish, irrespective of the dose administered. The prevalence at the start of the trial (the number of host infested with one or more individuals of a particular parasite species, see Bush et al., 1997) was 100 % for *H. heterocerca* and *B. seriolae*.

3.1 Efficacy of PZQ against *Heteraxine heterocerca*

The mean abundance of adult, juvenile and total *H. heterocerca* on fish treated with PZQ at all doses and treatment durations were significantly different from control fish (see results of independent *T*-tests, Tables 2a, 2b and 2c). Efficacy against existing *H. heterocerca* infestations was high for all PZQ doses administered, ranging from 90.4-100% (Tables 3a, 3b and 3c). Complete elimination of adult *H. heterocerca* was achieved by PZQ administered as a single dose of 450 mg kg^{-1} BW (Table 3a), 50, 100 and 150 mg kg^{-1} BW day^{-1} for 3 days (Table 3b) and 50 mg kg^{-1} BW day^{-1} for 6 days (Table 3c). Complete elimination of juvenile and total *H. heterocerca* was also achieved by PZQ administered at 50, 100 and 150 mg kg^{-1} BW day^{-1} for 3 days (Table 3b). The dose of PZQ which achieved the highest efficacy (66.9%) against recruited *H. heterocerca* was a single dose of 300 mg kg^{-1} BW (Table 3a).

One-way ANOVA found significant differences between the mean abundance of recruited *H. heterocerca* on fish receiving PZQ treatments administered as a single dose and for 3 days, but not for 6 days (Table 4a). The Levene statistic indicated unequal variance in recruited *H. heterocerca* from the fish receiving PZQ for 3 days (Table 4a), therefore one-way ANOVA was followed by Games-Howell *post-hoc* multiple comparisons of treatments to the control to ensure the observed significant difference in the one-way ANOVA was not the result of a Type I error (Table 4b). The Games-Howell *post-hoc* test found that the mean

abundance of recruited *H. heterocerca* on fish receiving PZQ at 50 and 75 mg kg^{-1} BW day^{-1} for 3 days differed significantly from the controls (Table 4b). However the mean abundance of recruited *H. heterocerca* on fish that received 100 and 150 mg kg^{-1} BW day^{-1} for 3 days did not differ significantly from the controls (Table 4b).

Table 2

Results of independent *T*-tests for unequal variance to compare mean abundance of adult, juvenile and total (adult + juvenile) *Heteraxine heterocerca* remaining on fish after PZQ was orally administered by intubation at (a) 300 and 450 mg kg^{-1} BW day^{-1} as single doses, (b) 50, 75, 100 and 150 mg kg^{-1} BW day^{-1} for 3 days, and (c) 50 and 75 mg kg^{-1} BW day^{-1} for 6 days. L = Levene's statistic, *P*-value of ≤ 0.05 was considered significant, t = t-value, df = degrees of freedom (Note: df varied from 9-10 depending on whether unequal or equal variance was assumed).

(a)

Treatment (compared with control)			
	Adults	Juveniles	Total (adults + juveniles)
300 mg kg^{-1} BW day^{-1}	L < 0.001, *P* = **0.006**, t = 3.42, df = 10	L = 0.01, *P* = **0.001**, t = 4.55, df = 9	L < 0.001, *P* = **0.001**, t = 4.12, df = 9
450 mg kg^{-1} BW day^{-1}	L < 0.001, *P* =**0.004**, t = 3.92, df = 9	L = 0.01; *P* = **0.001**, t = 4.63, df = 9	L < 0.001, *P* = **0.002**, t = 4.42, df = 9

(b)

Treatment (compared with control)			
	Adults	Juveniles	Total (adults + juveniles)
50 mg kg^{-1} BW day^{-1}	L = 0.003, *P* = **0.001**, t = 4.74, df = 10	L = 0.002, *P* = **0.001**, t = 4.50, df = 10	L = 0.004, *P* = **0.001**, t = 4.81, df = 10
75 mg kg^{-1} BW day^{-1}	L = 0.003, *P* = **0.001**, t = 4.71, df = 10	L = 0.002, *P* = **0.001**, t = 4.50, df = 10	L = 0.004, *P* = **0.001**, t = 4.79, df = 10
100 mg kg^{-1} BW day^{-1}	L = 0.003, *P* = **0.001**, t = 4.74, df = 10	L = 0.002, *P* = **0.001**, t = 4.50, df = 10	L = 0.004, *P* = **0.001**, t = 4.81, d.f = 10
150 mg kg^{-1} BW day^{-1}	L = 0.003, *P* = **0.001**, t = 4.74, df = 10	L = 0.002, *P* = **0.001**, t = 4.50, df = 10	L = 0.004, *P* = **0.001**, t = 4.81, df = 10

(c)

Treatment (compared with control)			
	Adults	Juveniles	Total (adults + juveniles)
50 mg kg^{-1} BW day^{-1}	L = 0.03, **P = 0.033**, t = 2.52, df = 9	L = 0.034, **P = 0.015**, t = 2.00, df = 9	L = 0.039, **P = 0.018**, t = 2.88, df = 9
75 mg kg^{-1} BW day^{-1}	L = 0.036, **P = 0.035**, t = 2.48, df = 9	L = 0.031, **P = 0.015**, t = 3.02, df = 9	L = 0.039, **P = 0.018**, t = 2.88, df = 9

Table 3

Mean parasite abundance and efficacy (in bold, calculated as a percentage reduction of mean parasite abundance from the control mean, see Stone et al., 2000) after oral treatment by intubation with PZQ (a) as a single dose, (b) for 3 days, and (c) for 6 days.

(a)

	Heteraxine heterocerca				*Benedenia seriolae*			
Single dose	Adults	Juveniles	Total	Recruits	Adults	Juveniles	Total	Recruits
0 (controls)	7.3	8.7	16	91.5	10.1	10.2	20.3	445.2
300 mg kg^{-1} BW day^{-1}	0.7	0.20	0.9	30.3	4.4	3.8	8.4	228.9
Efficacy	**90.4**	**97.7**	**94.4**	**66.9**	**56.4**	**62.7**	**58.4**	**48.6**
450 mg kg^{-1} BW day^{-1}	0	0.1	0.1	49.2	2.9	1.6	4.5	324.9
Efficacy	**100**	**98.8**	**99.4**	**46.2**	**71.3**	**84.3**	**77.8**	**27**

84

(b)

	Heteraxine heterocerca				Benedenia seriolae			
Daily dose (3 days)	Adults	Juveniles	Total	Recruits	Adults	Juveniles	Total	Recruits
0 (controls)	15.1	12.6	27.7	77.6	13.5	117	130.5	387.1
50 mg kg^{-1} BW day^{-1}	0	0	0	40.9	4.8	49	53.8	317
Efficacy	**100**	**100**	**100**	**47.3**	**64.6**	**58.1**	**58.8**	**18.1**
75 mg kg^{-1} BW day^{-1}	0.1	0	0.1	34.8	5.1	56	61.1	412.5
Efficacy	**99.3**	**100**	**99.6**	**55.2**	**62.3**	**52.1**	**53.2**	**-6.6**
100 mg kg^{-1} BW day^{-1}	0	0	0	46.6	5.9	54.6	60.5	413.7
Efficacy	**100**	**100**	**100**	**40**	**56.4**	**53.3**	**53.6**	**-6.9**
*150 mg kg^{-1} BW day^{-1}	0	0	0	55.9	4	60.1	64.1	564.2
Efficacy	**100**	**100**	**100**	**28**	**70.5**	**48.6**	**50.9***	**-45.7**

* Dose on label of Hadaclean ® for treatment of *B. seriolae* in Japan.

(c)

	Heteraxine heterocerca				Benedenia seriolae			
Daily dose (6 days)	Adults	Juveniles	Total	Recruits	Adults	Juveniles	Total	Recruits
0 (controls)	6.6	18.2	24.8	99	13	322.2	335.2	48.8
50 mg kg^{-1} BW day^{-1}	0	0.1	0.1	78.4	2.8	258.8	261.6	73.3
Efficacy	**100**	**99.4**	**99.6**	**20.8**	**78.5**	**19.7**	**22**	**-50.2**
75 mg kg^{-1} BW day^{-1}	0.1	0	0.1	71.7	3	149.2	152.2	44.5
Efficacy	**98.5**	**100**	**99.6**	**27.6**	**76.9**	**53.7**	**54.6**	**8.8**

Table 4

(a) Results of one-way ANOVA comparison of mean abundance of recruited *Heteraxine heterocerca* after PZQ was orally administered by intubation. *Indicates Levene's statistic was significant ($P \leq 0.05$). (b) Results of Games-Howell *post-hoc* multiple comparisons of recruited *H. heterocerca* data for 3-day treatments. $P \leq 0.05$ was considered significant (see bold figures), df = degrees of freedom, F = F-value.

(a)

Treatment group	Levene's statistic	Sum of Squares	df	Mean Square	F	*P*-value
300 and 450 mg kg⁻¹ BW day⁻¹ for as a single dose	0.431	19639.8	2	9819.9	13.36	**<0.001**
50, 75, 100 and 150 mg kg⁻¹ BW day⁻¹ for 3 days*	0.36	11858.64	4	2964.66	3.16	**0.023**
50 and 75 mg kg⁻¹ BW day⁻¹ for 6 days	0.188	4048.47	2	2024.23	0.82	0.450

(b)

Treatment (mg kg⁻¹ BW day⁻¹)	n	Mean	Std. Deviation	Std. Error	*P*-value
control	10	77.64	31.99	-	-
50	10	40.9	23.86	12.25	**0.052**
75	10	34.8	16.96	11.03	**0.010**
100	10	46.6	36.43	15.03	0.276
150	10	55.9	38.49	15.53	0.636

3.2 Efficacy of PZQ against *Benedenia seriolae*

Mean abundance of adult, juvenile and total *B. seriolae* on fish treated with PZQ at all doses and treatment durations were significantly different from control fish (Tables 5a, 5b and 5c). The Levene's statistic indicated unequal variance in juvenile and total *B. seriolae* data for fish admininistered PZQ as single doses of 300 and 450 mg kg⁻¹ BW and in adult *B. seriolae* for fish administered PZQ at 50 and 75 mg kg⁻¹ BW day⁻¹ for 6 days. For this reason, Games-Howell *post-hoc* multiple comparisons were performed between treatments and the controls to ensure the observed significant difference in the one-way ANOVA was not the result of a Type I error (Table 6a and 6b). The Games-Howell *post-hoc* test confirmed that mean abundance of *B. seriolae* on the treatment fish were significantly different from the

controls (Tables 6a and 6b). The highest efficacy against adult *B. seriolae* was 78.5% achieved in fish administered a dose of 50 mg kg^{-1} BW day^{-1} for 6 days (Table 3c). The highest efficacy against juvenile (84.3%) and total (77.8%) *B. seriolae* was achieved by PZQ administered in a single 450 mg kg^{-1} BW dose (Table 3a). The highest efficacy against recruited *B. seriolae* (48.6%) was achieved by a single dose of 300 mg kg^{-1} BW of PZQ (Table 3a).

Table 5

Results of one-way ANOVA comparison of mean abundance of *Benedenia seriolae* remaining on fish after PZQ was orally administered by intubation at (a) 300 and 450 mg kg^{-1} BW day^{-1} as single doses, (b) 50, 75, 100 and 150 mg kg^{-1} BW day^{-1} for 3 days, and (c) 50 and 75 mg kg^{-1} BW day^{-1} for 6 days. $P \leq 0.05$ was considered significant (see bold figures), df = degrees of freedom, F= F-value.

(a)

Life stage	Levene's statistic	Sum of Squares	df	Mean Square	F	*P*-value
Adults	0.088	288.6	2	144.3	6.96	**0.004**
Juveniles	**<0.001**	399.2	2	199.6	13.49	**<0.001**
Total	**0.002**	1345.32	2	672.3	17.3	**<0.001**
Recruitment	0.807	234912.6	2	117456.3	6.55	**0.005**

(b)

Life stage	Levene's statistic	Sum of Squares	df	Mean Square	F	*P*-value
Adults	0.186	655.9	4	163.98	9.5	**<0.001**
Juveniles	0.147	33875.68	4	8468.92	7.8	**<0.001**
Total	0.19o	43654.67	4	10913.67	9.8	**<0.001**
Recruitment	0.499	326747	4	81686.76	7.7	**<0.001**

(c)

Life stage	Levene's statistic	Sum of Squares	df	Mean Square	F	*P*-value
Adults	**<0.001**	680.27	2	340.13	10.99	**0.001**
Juveniles	0.859	153202.4	2	76601.2	7.46	**0.003**
Total	0.882	169581.07	2	84790.53	7.86	**0.002**
Recruitment	0.849	4827.27	2	2413.63	6.63	**0.005**

Table 6

Results of Games-Howell *post-hoc* multiple comparisons of *Benedenia seriolae* data, where Levene's statistic was significant ($P \leq 0.05$; see bold figures) for fish administered PZQ at (a) 50, 75, 100 and 150 mg kg^{-1} BW day^{-1} for 3 days, and (b) 50 and 75 mg kg^{-1} BW day^{-1} for 6 days. $P \leq 0.05$ was considered significant (see bold figures).

(a)

Life stage	Treatment (mg kg^{-1} BW day^{-1})	n	Mean	Std. Deviation	Std. Error	P-value
Juveniles	control	10	10.2	5.96	-	-
	300	10	3.8	2.44	2.04	**0.02**
	450	10	1.6	1.71	1.96	**0.003**
Total	control	10	20.3	9.44	-	**-**
	300	10	8.44	3.54	3.2	**0.008**
	450	10	4.5	3.47	3.18	**0.001**

(b)

Life stage	Treatment (mg kg^{-1} BW day^{-1})	n	Mean	Std. Deviation	Std. Error	P-value
Adults	control	10	13	8.61	-	-
	50	10	2.8	3.08	2.89	**0.012**
	75	10	3	3.02	2.89	**0.013**

4. Discussion

PZQ administered by intubation to *S. quinqueradiata* had very high efficacy against the gill fluke *H. heterocerca*. Complete elimination of total (adult + juvenile) *H. heterocerca* was achieved by PZQ administered for 3 days at 50, 100 and 150 mg kg^{-1} BW day^{-1} (see Table 3b) and 94.4 - 99.6% efficacy was observed for all other doses against total *H. heterocerca* (Tables 3a, 3b and 3c). Efficacy of PZQ against total *B. seriolae* at all doses and treatment durations was considerably lower. The highest efficacy (77.8%) against total *B. seriolae* was achieved by PZQ administered at 450 mg kg^{-1} BW day^{-1} as a single dose (Table 3a).

We found the dose of PZQ labelled on the product registered in Japan for treatment of *B. seriolae* (150 mg kg^{-1} BW day^{-1} for 3 days) had an efficacy of 50.9% against total *B. seriolae* when intubated to *S. quinqueradiata* (Table 3b). Williams et

88

al. (2007) found that the same dose of PZQ, administered by intubation to farmed *S. lalandi* in Australia, produced an efficacy of 97.7% against *B. seriolae*. While PZQ was administered by intubation at the same doses in both trials, differences between the experiments including water temperature, paste components and formulation of PZQ may have influenced the different results in medicine availability, uptake, incorporation and efficacy against the same parasite species but on different host species. Williams et al. (2007) used no fish oil in the paste delivered to the fish but in the present study, we used a small amount of fish oil to simulate the composition of a feed pellet. It is possible the addition of fish oil to our paste may have affected the availability of PZQ and its resulting efficacy against *B. seriolae*.

The dose with highest efficacy (77.8%) against total *B. seriolae* (450 mg kg^{-1} BW day^{-1}) on *S quinqueradiata* also had high efficacy (99.4%) against *H. heterocerca*. Such doses of PZQ may not, however, be practical for oral administration because of adverse effects on palatability of fish feed (see Chapter 9 – General Discussion).

The relatively low efficacy (50.9%) of the dose of PZQ intubated at the labelled dose for treatment of *B. seriolae* in Japan observed, suggests that parasites are being exposed to subtherapeutic doses of PZQ. This may be compounded by the likely variation in the application of Hadaclean® between farms to feed pellets and the likely variation in distribution of medicated feed among individual fish, cages, sites and seasons, as observed by Berg and Horsberg (2009) when *Salmo salar* were orally administered emamectin benzoate in Norway. It is widely recognised that the exposure of parasites to subtherapeutic doses of medicine promotes the development of parasite resistance (Thoney and Hargis, 1991). PZQ has been approved for use in aquaculture in Japan for nearly nine years (Anonymous, 2000). It would be pertinent to review the use of PZQ over that time and assess if resistance to PZQ is emerging in *B. seriolae* in Japan. This information should be used to determine if reviews of the label dose and practises associated with administration of PZQ for treatment of *B. seriolae* are required.

We acknowledge that the experimental design of our trial had limitations. Only one replicate of 10 fish for each dose was used in this trial. Therefore if there had been any differences in parasite loads due, for example, to positioning of cages, these may have confounded the effect of the treatment. Space constraints were a restriction of working with the industry and we made the most of fish and facilities

that were available to us at the time. As described in section 2.2 of this chapter, it was also acknowledged that the duration of the trial and location of experimental cages meant that it was impossible to prevent exposure of fish to parasite recruitment. This recruitment was accounted for by separating parasites counted at the end of trial into life stages based on knowledge of temperature-related parasite growth (see Lackenby et al., 2007 for *B. seriolae* and Mooney et. al., 2008 for *H. heterocerca*). Ideally, the experiment should have been conducted in a closed system, not influenced by external factors such as currents and weather, with a completely randomised experimental design as used later in Chapters 6 and 7.

Our results demonstrate that PZQ administered by intubation is effective against *H. heterocerca* and *B. seriolae* parasitising sea-caged *S. quinqueradiata* in Japan. It is also further evidence that PZQ is more effective against blood-feeding polyopisthocotylean monogeneans than monopisthocotyleans which feed on mucus and epithelial cells. Through strategic application by timing treatments based on knowledge of the parasite lifecycles at different water temperatures, PZQ could, however, be a useful tool in the management of these monogeneans, but the variations in efficacy reported against *B. seriolae* need to be investigated and addressed.

CHAPTER 6 - EFFECT OF ORALLY ADMINISTERED PRAZIQUANTEL COMBINED WITH CIMETIDINE AGAINST *ZEUXAPTA SERIOLAE* AND *BENEDENIA SERIOLAE*, MONOGENEAN PARASITES YELLOWTAIL KINGFISH *SERIOLA LALANDI*

Chapter 6: Effect of orally administered praziquantel combined with cimetidine against *Zeuxapta seriolae* and *Benedenia seriolae*, monogeneans parasites of yellowtail kingfish *Seriola lalandi*

Abstract

Two doses of praziquantel (PZQ) administered with and without cimetidine (CIM) were investigated as oral treatments for *Zeuxapta seriolae* and *Benedenia seriola*e, monogenean parasites of yellowtail kingfish *Seriola lalandi*. Five treatments were tested: 200 mg kg^{-1} body weight (BW) day^{-1} of CIM alone, 75 and 150 mg kg^{-1} BW day^{-1} of PZQ alone and 75 and 150 mg kg^{-1} BW day^{-1} of PZQ combined with 200 mg kg^{-1} BW day^{1} of CIM, all administered by intubation for 3 consecutive days. Comparisons of parasite abundance were made between fish fed a medicated diet and control fish fed an unmedicated diet. All doses of PZQ, both alone and combined with CIM had very high efficacy (>99%) against *Z. seriolae*. However PZQ administered alone appeared to have greater efficacy against *B. seriola*e than the same doses of PZQ combined with 200 mg kg^{-1} BW day^{-1} of CIM. CIM administered alone at 200 mg kg^{-1} BW day^{-1} did not appear to have any effect against *Z. seriolae* and *B. seriola*e.

1. Introduction

Yellowtail kingfish, *Seriola lalandi* (Carangidae), farmed in sea-cages in Spencer Gulf, South Australia, are affected by two monogenean species, *Zeuxapta seriolae* (Polyopisthocotylea: Heteraxinidae) and *Benedenia seriolae* (Monopisthocotylea: Capsalidae). Aspects of the different biologies of these parasites is given by Whittington and Chisholm (2008) and in Chapters 1 and 2. Management of *Z. seriolae* and *B. seriolae* in South Australian *Seriola lalandi* aquaculture relies on bathing cages of fish in a hydrogen peroxide solution but the concept of an in-feed treatment effective against these parasites as a practical alternative to bathing has been explored as a useful alternative. Previous studies have reported that orally administered PZQ is effective against other monogeneans, including *Microcotyle sebastis* on *Sebastes schlegeli* in Korea (Kim and Cho, 2000; Kim et al., 2001a; Kim et al., 1998). In an attempt to increase efficacy against *M. sebastis*, co-administration of PZQ with cimetidine (CIM) was investigated (Kim et

al., 2001b; Kim and Kim 2002). CIM is a histamine H2-receptor antagonist that inhibits the production of acid in the stomach, is commonly used to treat gastric hyperacidity in humans, but has also been reported to increase the levels of PZQ in plasma in mammals (Dachman et al., 1994; Jung et al., 1997) and fish (Kim and Kim, 2002; Kim et al., 2001b). Kim et al. (2001b) reported that PZQ administered orally in combination with CIM resulted in higher efficacy against *M. sebastis* than PZQ alone and suggested that CIM may suppress the metabolism of PZQ in *S. schlegeli* resulting in longer retention of PZQ in the plasma. Kim and Kim (2002) confirmed that when PZQ was coadministered with CIM, higher levels of PZQ were found in the plasma of *S. schlegeli* which may explain the higher efficacy observed against *M. sebastis*. Tubbs and Tingle (2006a) suggested that PZQ may be eliminated faster from the tissues of *Seriola lalandi* than other fish species such as *Sebastis schlegeli* and *Oncorhynchus mykiss*. This may lead to a short window of time for parasites such as *Z. seriolae* and *B. seriolae* to be exposed to PZQ before it is eliminated from *Seriola lalandi*.

In humans, PZQ undergoes metabolism in the liver by two cytochrome P-450 isoenzymes (Ridtitid et al., 2002). In mammals, CIM increases plasma PZQ because it inhibits cytochrome P450 oxidase, which is responsible for metabolism of PZQ (Levine et al., 1998). Tubbs et al. (2008) suggested that it would be useful to explore combining PZQ with enzyme inhibitors to see what effect it had on the residence and clearance time of the active metabolites. This trial aimed to compare the efficacy of oral PZQ administered with and without CIM against *Z. seriolae* and *B. seriolae* on *S. lalandi*.

2.1 Methods

2.1 Source of fish and parasites

Yellowtail kingfish, *S. lalandi,* were obtained from a commercial sea-cage aquaculture farm in Spencer Gulf, South Australia, in April 2005 and maintained in a concrete raceway at a landbased facility with a seawater supply. During the experiment, the temperature range was $18\pm1°C$ and salinity was 40 ppt. A total of 60 fish of mean weight 591 (429-819) g was randomly removed from the cohort. Presence of *Z. seriolae* and *B. seriolae* was confirmed by routine monitoring of fish

in the same cohort that the experimental fish were taken from, prior to trial. Parasite sampling was carried out as specified previously (see Chapters 3 and 4).

2.2 Administration of PZQ and CIM

This research was conducted under APVMA Minor Use Permit 7250 for small-scale trials of Agvet chemicals (APVMA, 2004). PZQ and CIM were purchased from MP Biomedicals, Sydney, Australia. Five daily treatments were administered: CIM only at 200 mg kg^{-1} body weight (BW) day^{-1}, PZQ only at 75 and 150 mg kg^{-1} BW day^{-1}, PZQ + CIM at 75 and 200 mg kg^{-1} BW day^{-1}, respectively and PZQ + CIM at 150 and 200 mg kg^{-1} BW day^{-1}, respectively (Table 1). Treatments were delivered in a paste made from pre-pellet meal (provided by Skretting Australia), fish oil and dechlorinated tap water. At the beginning of the trial, fish were individually weighed so that the treatment dose in mg kg^{-1} BW day^{-1} could be calculated. Fish were anaesthetised in a 60 L tub containing a a bath solution of 33 ppm AQUI-S™ aquatic anaesthetic in seawater until they were unresponsive to touch, then 4 mL of paste was intubated into their stomach using a 30 mL cathether syringe fitted with a 10 cm extension of soft silicon hose 4 mm in diameter. Fish were tagged to indicate which treatment group they belonged to. This involved taking a small clip off the tip of either the pectoral or pelvic fin while the fish was anaesthetised, and did not appear to affect the mobility of the fish. Fish were placed into a 60 L tub of clean seawater to recover and monitored for 5 min to ensure no paste was regurgitated and then returned to the raceway. Control fish underwent an identical procedure but were administered paste without PZQ. Apart from intubation, fish were not fed during the trial. Treatments were administered for 3 consecutive days after which fish were withdrawn from treatment for 4 days before being sampled for parasites as described previously. Due to the trial length, water temperature and the physical set-up of the tanks, new parasite recruitment was impossible to prevent. Therefore, when *Z. seriolae* and *B. seriolae* were counted, they were divided into three life stages: adults, juveniles and recruits (parasites that had settled during the trial). Sizes for each life stage were estimated from water temperature-related parasite growth based on data in Lackenby et al. (2007) for *B. seriolae* and unpublished data from A.J. Mooney for *Z. seriolae*.

Table 1

Summary of doses of PZQ and CIM administered by intubation and number of fish per treatment. All doses were administered for 3 consecutive days.

PZQ (mg kg^{-1} BW day^{-1})	CIM (mg kg^{-1} BW day^{-1})	No. of fish
Control (0)	Control (0)	10
0	200	10
75	0	10
75	200	10
150	0	10
150	200	10

2.3 Statistical analysis

SPSS 15.0 software was used for statistical analyses (SPSS Inc., Chicago IL, USA). Comparisons of mean parasite abundance (the total number of individuals of a particular parasite species, divided by the total number of hosts examined, whether infested or not, see Bush et al., 1997) of all life stages (adults, juveniles, total and recruited) of *Z. seriolae* were made between treatment and control groups using independent *T*-tests. Comparisons of mean abundance of all life stages (adults, juveniles, total and recruited) of *B. seriolae* were made between treatments and control groups using a one-way ANOVA. A *P*-value ≤ 0.05 was considered significant. The Levene's statistic was calculated and where significant ($P \leq 0.05$), unequal variance was assumed.

Efficacy for each treatment was calculated as a % reduction of the mean parasite abundance using the the formula below (adapted from Stone et al., 2000):

$$\% \text{ efficacy} = 100 - (100 \text{ x } \frac{\text{mean parasite abundance of treatment group}}{\text{mean parasite abundance of control group}}).$$

3. Results

Calculation of dose was based on the mean weight of each treatment group at the start of the trial. No regurgitation of medicated paste or signs of toxicity associated with any treatment (e.g. skin darkening, erratic swimming, and loss of equilibrium) was observed in fish, irrespective of the dose administered. The prevalence at the start of the trial (the number of hosts infested with one or more

individuals of a particular parasite species, see Bush et al., 1997) was 100 % for *H. heterocerca* and *B. seriolae*.

3.1 Efficacy of PZQ and CIM against *Zeuxapta seriolae*

Mean abundance of adult, juvenile, total (adult + juvenile) and recruited *Z. seriolae* on fish treated with PZQ at all doses, with or without CIM were significantly different from control fish (Table 2). However CIM administered alone at 200 mg kg^{-1} BW day^{-1} appeared to have no effect on any life stage of *Z. seriolae* (Table 2). Efficacy against *Z. seriolae* was high for all PZQ doses administered and when combined with CIM (Table 3). Complete elimination of adult *Z. seriolae* was achieved by all doses of PZQ, with and without CIM. PZQ administered alone at 150 mg kg^{-1} BW day^{-1} also completely eliminated juvenile and total *Z. seriolae*. PZQ administered alone at 75 mg kg^{-1} BW day^{-1} and at 75 and 150 mg kg^{-1} BW day^{-1} combined with CIM achieved > 99% reduction against juvenile and total *Z. seriolae* (Table 3). Efficacy against recruited *Z. seriolae* was higher when PZQ was administered without CIM at 75 and 150 mg kg^{-1} BW day^{-1} than when combined with CIM.

Table 2

Results of independent T-tests to compare mean abundance of adult, juvenile, total (adult + juvenile) and recruited *Zeuxapta seriolae* remaining on fish after oral treatment by intubation with PZQ and CIM. *P*-values in bold are significantly different from controls, t = t-value, df = degrees of freedom.

Treatment (compared with control)	Adults	Juveniles	Total (adults + juveniles)	Recruitment
CIM 200	*P* = 0.84, t = -0.2, df = 18	*P* = 0.44, t = -0.79, df = 18	*P* = 0.46, t = -0.75, df = 18	*P* = 0..26, t = -1.2, df = 18
PZQ 150	***P* = 0.019**, t = 2.85, df = 9	***P* = 0.02**, t =2.83, df = 9	***P* = 0.019**, t = 2.85, df = 9	***P* = 0.009**, t =3.28 , df = 9.1
PZQ 150 + CIM 200	***P* = 0.019**, t = 2.85, df = 9	***P* = 0.02**, t = 2.83, df = 9	***P* = 0.019**, t = 2.85, df = 9	***P* = 0.02**, t = 2.76, df = 10.2
PZQ 75	***P* = 0.019**, t = 2.85, df = 9	***P* = 0.02**, t = 2.83, df = 9	***P* = 0.019**, t = 2.85, df = 9	***P* = 0.011**, t = 3.17, df = 9.5
PZQ 75 + CIM 200	***P* = 0.019**, t = 2.85, df = 9	***P* = 0.02**, t = 2.80, df = 9	***P* = 0.02**, t = 2.83, df = 9	***P* = 0.045**, t = 2.26, df = 10.8

Table 3

Efficacy, calculated as a % reduction of mean parasite abundance from the control mean (adapted from Stone et al., 2000) after intubation with PZQ and/or CIM.

Dose (mg kg^{-1} BW day^{-1})	*Zeuxapta seriolae*				*Benedenia seriolae*			
	adults	juveniles	total	recruitment	adults	juveniles	total	recruitment
CIM 200	-11.2	-56.2	-67.7	-52.4	-3.9	6.2	4.3	-31.6
PZQ 150	100	100	100	92.9	90.6	64.8	69.7	69.7
PZQ 150 + CIM 200	100	99.8	99.8	80.4	62.6	30.9	36.9	-3.5
PZQ 75	100	99.9	99.9	90.9	78.0	66.1	68.3	5.3
PZQ 75 + CIM 200	100	99.1	99.2	67.0	57.1	37.1	40.9	45.6

3.2 Efficacy of PZQ and CIM against *Benedenia seriolae*

Mean abundance of adult, juvenile and total (adult + juvenile) *B. seriolae* was significantly greater on control fish than on fish treated with PZQ at all doses, with or without CIM (one-way ANOVA, Table 4). The Levene's statistic indicated unequal variance, therefore Games-Howell *post-hoc* multiple comparisons were conducted to confirm differences were not due to a Type I error. Games-Howell *post-hoc* multiple comparisons found that the mean abundance of all life stages of *B. seriolae* from fish treated with 200 mg kg $^{-1}$ BW day $^{-1}$ of CIM alone were not significantly different to control fish (Table 5). The mean abundance of adult *B. seriolae* was found to be significantly different from control fish for all doses of PZQ, administered with and without CIM (Table 5). However, only fish that received PZQ alone (75 and 150 mg kg $^{-1}$ BW day $^{-1}$) had significantly different mean abundance of juvenile and total *B. seriolae* compared with control fish. No significant differences were detected between the mean abundance of juvenile and total *B. seriolae* on fish that received PZQ combined with CIM when compared with controls (Table 5). No significant differences were detected between mean abundance of *B. seriolae* recruits on treated fish compared with controls (Table 4).

Table 4

Results of one-way ANOVA comparison of mean abundance of *Benedenia seriolae* remaining on fish after oral treatment by intubation with PZQ and CIM. $P \leq 0.05$ was considered significant (see bold figures), df = degrees of freedom, F = F-value.

Life stage	Levene's statistic	Sum of Squares	df	Mean Square	F	*P*-value
Adults	**0.003**	487831	5	97566	28.97	**<0.001**
Juveniles	**0.03**	4476596	5	895319	6.17	**<0.001**
Total	**0.022**	7776007	5	1555201	8.67	**<0.001**
Recruitment	0.156	101.93	5	20.39	1.93	0.105

Table 5

Results of Games-Howell *post-hoc* multiple comparisons of *B. seriolae* data, where Levene's statistic was significant ($P \leq 0.05$, see Table 4).

Life stage	Treatment (mg kg^{-1} BW day^{-1})	n	Mean	Std. Deviation	Std. Error	*P*-value
Adults	Control	10	246.8	27.2	-	-
	CIM 200	10	256.8	25.37	37.42	1.000
	PZQ 150	10	23.2	6.70	28.31	**<0.001**
	PZQ 150 + CIM 200	10	92.3	11.87	29.96.	**0.002**
	PZQ 75	10	54.3	16.48	32.07	**<0.001**
	PZQ 75 + CIM 200	10	105.9	12.75	30.32	**0.005**
Juveniles	Control	10	1067.7	489.81	-	-
	CIM 200	10	1001.2	488.32	218.72	1.000
	PZQ 150	10	375.5	303.60	181.23	**0.018**
	PZQ 150 + CIM 200	10	737.4	458.62	212.19	0.635
	PZQ 75	10	362.0	159.49	162.90	**0.012**
	PZQ 75 + CIM 200	10	671.2	254.23	174.51	0.269
Total (adults + juveniles)	Control	10	1314.5	566.32	-	-
	CIM 200	10	1257.7	550.82	249.82	1.000
	PZQ 150	10	398.7	312.01	204.47	**0.006**
	PZQ 150 + CIM 200	10	829.7	485.56	235.90	0.353
	PZQ 75	10	416.3	197.62	189.68	**0.006**
	PZQ 75 + CIM 200	10	777.1	282.19	200.09	0.143

4. Discussion

In this experiment, we attempted to explore whether the efficacy of PZQ against *Z. seriolae* and *B. seriolae* on *S. lalandi* could be improved by co-administration with CIM. All doses of PZQ, alone and combined with CIM, had very high efficacy (>99%) against *Z. seriolae*. PZQ administered alone, however, appeared to have greater efficacy against *B. seriola*e than the same doses of PZQ combined with 200 mg kg^{-1} BW day^{-1} of CIM (Table 3). The mean abundance of adult *B. seriolae* were found to be significantly different from control fish for all doses of PZQ, administered with and without CIM (Table 5). Fish that received PZQ alone (75 and 150 mg kg $^{-1}$ BW day $^{-1}$) had significantly different mean abundance of juvenile and total *B. seriolae* to control fish, but no significant differences were detected between the mean abundance of juvenile and total *B. seriolae* on fish that received PZQ combined with CIM when compared with controls (Table 5). CIM administered alone at 200 mg kg^{-1} BW day^{-1} did not appear to have any effect on *Z. seriolae* and *B. seriola*e.

Previous investigations into the use of CIM in combination with PZQ were made against the polyopisthocotylean *Microcotyle sebastis* on the sebastid, *Sebastes schlegeli* (see Kim et al., 2001b; Kim and Kim, 2002). Kim and Kim (2002) found higher levels of PZQ in the plasma of *S. schlegeli* when it was coadministered with CIM, than when PZQ was administered alone. We did not measure plasma levels of PZQ in this trial, therefore we do not know whether CIM had the same effect on plasma levels of PZQ in *Seriola lalandi* as it does in mammals and the fish *Sebastes schlegeli*. It is also possible that the carangid *Seriola lalandi* has a different metabolism to *Sebastes schlegeli*, and the biology of *Z. seriolae* and especially the monopisthocotylean, *B. seriolae,* are different to *M. sebastis*. These factors, which are explored in further detail in the General Discussion (Chapter 9), may also explain why our results differed from previous investigations.

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CHAPTER 7 - EFFECT OF ORALLY ADMINISTERED PRAZIQUANTEL ON RECRUITMENT OF THE MONOGENEAN PARASITES *ZEUXAPTA SERIOLAE* AND *BENEDENIA SERIOLAE* TO YELLOWTAIL KINGFISH *SERIOLA LALANDI*

Chapter 7: Effect of orally administered praziquantel on recruitment of the monogenean parasites *Zeuxapta seriolae* and *Benedenia seriolae* to yellowtail kingfish *Seriola lalandi*

Abstract

The effect of orally administered praziquantel (PZQ) on recruitment of the monogenean parasites *Zeuxapta seriolae* and *Benedenia seriolae* to yellowtail kingfish (*Seriola lalandi*) in sea-cage aquaculture in South Australia was investigated. PZQ was administered to fish via direct intubation of the stomach at four doses: 6.25, 12.5, 25 and 50 mg kg^{-1} BW day^{-1} for 10 days. Mean parasite abundance was compared between medicated fish and control fish. All fish treated with PZQ had significantly fewer *Z. seriolae* and *B. seriolae* recruits when compared with control fish, however complete elimination of recruits was not achieved. The highest % reduction of *Z.seriolae* (95.7 %) and *B. seriolae* (88.3 %) recruits was achieved by the highest dose of PZQ administered (50 mg kg^{-1} BW day^{-1}) over 10 days.

1. Introduction

Two monogenean species, *Zeuxapta seriolae* (Polyopisthocotylea: Heteraxinidae) and *Benedenia seriolae* (Monopisthocotylea: Capsalidae) infect and affect farmed yellowtail kingfish, *Seriola lalandi* (Carangidae), in sea-cages in Spencer Gulf, South Australia. Management of these parasites relies on bathing caged fish using a hydrogen peroxide solution but praziquantel (PZQ) as an in-feed treatment has been investigated as a useful alternative. PZQ has been used as an oral treatment for tapeworm infections in rainbow trout, *Onchorhynchus mykiss* and Atlantic salmon, *Salmo salar* farmed in Norway (Hormazabal and Yndestad, 1995) and is the active ingredient in Hadaclean® (Bayer), an oral treatment approved for *B. seriolae* parasitising sea-caged Japanese yellowtail *Seriola quinqueradiata* in Japan (Stephens et al., 2003; Tubbs and Tingle 2006b). Its efficacy orally against polyopisthocotyleans has been well documented (e.g. Kim et al., 1998; Hirazawa et al., 2000; Kim and Cho 2000; Kim et al., 2001a; Kim and Kim 2002; Sitja-Bobadilla et al., 2006) but there is limited information about its efficacy against monopisthocotyleans (e.g. Tojo and Santamarina, 1998b; Hirazawa et al., 2004;

Williams et al., 2007). Research presented elsewhere in my thesis has identified PZQ as a candidate for in-feed treatment of *S. lalandi* against *Z. seriolae* and *B. seriolae* in South Australia (see Williams et al., 2007) and against *Heteraxine heterocerca* and *B. seriolae* on *S. quinqueradiata* in Japan (see Chapter 5). These studies focused on the effect of PZQ on *existing* monogenean infestations (i.e. those already established on fish, see Williams et al., 2007) but in Chapter 5, I reported that PZQ appeared to have an effect on *recruited* monogeneans during the trial. Recruitment is defined as invasion by larval stages and their persistence during the trial. In this study, I investigated the effect of four oral doses of PZQ specifically against *Z. seriolae* and *B. seriolae* recruiting to *S. lalandi*.

2. Material and methods

2.1 Source of fish and parasites

Yellowtail kingfish, *Seriola lalandi,* obtained from a commercial sea-cage farm in Spencer Gulf, South Australia in April 2005, were maintained in a concrete raceway at a land based facility with seawater supply. During the experiment, the water temperature was 18±1˚C and salinity was 40 ppt. A total of 50 fish of mean weight 584 (417-741) g was randomly selected from a cohort of sea-caged fish and any infestations of *Z. seriolae* and *B. seriolae* acquired while in sea-cages were removed by bathing each fish in two separate solutions as detailed in Chapters 3, 4 and 6. The experiment was conducted in the same raceway that had previously held the fish. The raceway was partitioned with plastic mesh, which separated experimental fish from infested fish. The plastic partition also served to collect eggs of *Z. seriolae* and *B. seriolae* from infested fish, adapted from methods outlined in Kearn et al. (1992) and Ernst and Whittington (1996). Presence of larval *Z. seriolae* and *B. seriolae* in the raceway was confirmed by examining the plastic mesh for recently hatched monogenean eggs. No acclimation occurred after parasite infestations had been removed from experimental fish, and the trial began on the same day parasite-free fish were transferred into the partitioned raceway.

2.2 Administration of PZQ

This research was conducted under APVMA Minor Use Permit 7250 for small-scale trials of Agvet chemicals (APVMA, 2004). PZQ was purchased from MP Biomedicals, Sydney, Australia. The medicine was delivered to fish in a paste made from pre-pellet meal (provided by Skretting Australia), fish oil and dechlorinated tap water. At the beginning of the trial, fish were individually weighed, so that the dose of PZQ in mg kg^{-1} BW day^{-1} could be calculated. Fish were anaesthetised in a 60 L tub containing a bath solution of 33 ppm AQUI-S™ aquatic anaesthetic in seawater until they lost their reflex activity (Stage 4 anaesthesia, see Hikasa et al., 1986) when 4 mL of paste was intubated into their stomach using a 30 mL catheter syringe fitted with a 10 cm extension of soft silicon hose 4 mm in diameter. Fish were tagged while anaesthetised to indicate which treatment group they belonged to by taking a small clip from the tip of either the pectoral or pelvic fin. This procedure did not appear to affect the mobility of the fish. Fish were placed into a 60 L tub of clean seawater to recover and were monitored for 5 min to ensure no medication was regurgitated and then returned to the raceway. Control fish underwent an identical procedure but were administered paste without PZQ. Four daily doses of PZQ were administered: 6.25, 12.5, 25 and 50 mg kg^{-1} BW day^{-1} for 10 consecutive days. The 10-day trial duration was based on the time taken for *Z. seriolae* and *B. seriolae* eggs to embryonate and hatch at 18±1°C to ensure fish were exposed to larval monogeneans (Tubbs et al., 2005; Ernst et al., 2005). Other than daily intubation, fish were not fed during the trial. Fish were sampled for parasites the day after the final treatment was administered, using the same method described previously and counted using a dissecting stereomicroscope.

2.3 Statistical analysis

SPSS 15.0 software was used for statistical analyses (SPSS Inc., Chicago IL, USA). One-way ANOVA was used to compare mean parasite abundance (the total number of individuals of a particular parasite species, divided by the total number of hosts examined, whether infested or not, see Bush et al., 1997) from treatment fish with controls and a P value ≤ 0.05 was considered significant. The Levene's statistic was calculated as an indication of equal variance, and where considered significant ($P \leq 0.05$) Games-Howell *post-hoc* multiple comparisons were carried out. The % reduction of the mean parasite abundance was calculated using the the formula below (adapted from Stone et al., 2000):

$$\% \text{ reduction} = 100 - (100 \text{ x} \frac{\text{mean parasite abundance of treatment group}}{\text{mean parasite abundance of control group}}).$$

3. Results

Calculation of dose was based on the mean weight of each treatment group taken at the start of the trial, and the range of actual doses delivered is given in Table 1. Two fish administered with 12.5 mg kg^{-1} BW day^{-1} PZQ showed clinical signs of bacterial septicaemia and died and were excluded from the analysis. One-way ANOVA indicated that fish administered PZQ had significantly different mean abundance of *Z. seriolae* and *B. seriolae* compared with control fish (Table 2). The Levene's statistic was significant ($P < 0.001$) for *Z. seriolae* and *B. seriolae* indicating unequal variance. For this reason, Games-Howell *post-hoc* multiple comparisons were performed between treatments and the controls to ensure the observed significant difference in the one-way ANOVA was not the result of a Type I error (Table 3). This verified that fish treated with each PZQ dose tested had significantly different mean abundance of *Z. seriolae* and *B. seriolae* compared with controls (Table 3).

Table 1

Summary of PZQ doses administered by intubation for 10 days (target and range of actual dose of PZQ delivered). Reduction of the mean abundance of parasites on treated fish compared with control fish is expressed as a percentage (adapted from Stone et al., 2000).

Target daily dose (mg kg^{-1} BW day^{-1})	Actual daily dose (mg kg^{-1} BW day^{-1})	Total target dose (mg kg^{-1} BW)	Total dose delivered (mg kg^{-1} BW)	Reduction of the mean abundance of *Z. seriolae*	Reduction of the mean abundance of *B. seriolae*
0	0	0	0	-	-
6.25	5 – 8	62.5	50 - 80	73.7	57.2
12.5	10 -16	125	100 -160	92.9	68.5
25	22 – 33	250	220 - 330	95.0	83.8
50	41 – 61	500	410 - 610	95.7	88.3

Table 2

Comparison of mean abundance of *Zeuxapta seriolae* and *Benedenia seriolae* using one-way ANOVA after oral treatment by intubation with PZQ at 6.25, 12.5, 25 and 50 mg kg^{-1} BW day^{-1} for 10 days. $P \leq 0.05$ was considered significant (see bold figures), df = degrees of freedom, F = F-value.

Species	Sum of Squares	df	Mean Square	F	*P*-value
Zeuxapta seriolae	728054	4	182013	53.45	**<0.001**
Benedenia seriolae	1.3E +008	4	32136126	29.97	**<0.001**

Table 3

Results of Games-Howell *post-hoc* multiple comparisons of mean abundance of *Z. seriolae* and *B. seriolae* following unequal variances as indicated by Levene's statistic ($P < 0.05$). $P \leq 0.05$ was considered significant and is indicated in bold type.

Species	Treatment (mg kg^{-1} BW day^{-1})	n	Mean	Std. Dev.	Std. Error	*P*-value
Zeuxapta seriolae	control	10	331.7	100.9	-	-
	6.25	10	87.2	71.1	39.0	**<0.001**
	12.5	8	23.6	28.7	33.5	**<0.001**
	25	10	16.6	15.7	32.3	**<0.001**
	50	10	14.2	11.9	32.1	**<0.001**
Benedenia seriolae	control	10	5054.7	2154.5	-	-
	6.25	10	2158.9	799.4	726.7	**0.014**
	12.5	8	1589.5	705.3	725.5	**0.004**
	25	10	819.3	383.9	692.1	**0.001**
	50	10	590.3	310.2	688.3	**0.001**

No dose of PZQ administered in this trial completely prevented recruitment of either *Z. seriolae* or *B. seriolae*. Reduction of recruited *Z. seriolae* ranged from 73.7% for the lowest dose of PZQ (6.25 mg kg^{-1} BW day^{-1}) to 95.7% for the highest dose of PZQ (50 mg kg^{-1} BW day^{-1}, see Table 1). Similarly, reduction of recruited *B. seriolae* ranged from 57.2% for the lowest dose of PZQ (6.25 mg kg^{-1} BW day^{-1}) to 88.3% for the highest dose of PZQ (50 mg kg^{-1} BW day^{-1}, see Table 1).

4. Discussion

Numerous interactions occur between monogeneans and their hosts, and most of the requirements for a larval monogenean to successfully locate, settle on and finally establish and survive on a suitable host fish are unknown (Buchmann and Lindenstrøm, 2002). Such interactions are not documented for *Z. seriolae* and *B. seriolae*. We could not determine whether PZQ either prevented initial settlement by larval monogeneans or affected them once they had attached to fish. Nevertheless, our results show that fish treated orally with PZQ have significantly fewer *Z. seriolae* and *B. seriolae* recruits than control fish.

Previously trials have shown that PZQ has efficacy against existing infestations (i.e. parasites present on fish at the start of the trial) of *Z. seriolae* and *B. seriolae* on *S. lalandi* (see Williams et al., 2007), but this is the first experiment to specifically investigate its effect on recruiting life stages. The highest % reduction of *Z. seriolae* recruits (95.7%) and *B. seriolae* recruits (88.3%) was achieved by the highest dose of PZQ administered by intubation (50 mg kg^{-1} BW day^{-1} for 10 days, see Table 1). While all doses of PZQ administered resulted in significantly fewer *Z. seriolae* and *B. seriolae* recruits compared with controls (Table 2), no dose completely eliminated recruiting life stages of either species. Williams et al. (2007) also found that higher oral doses of PZQ did not completely eliminate recruits of *Z. seriolae* and *B. seriolae*. PZQ also did not completely prevent or eliminate *Heteraxine heterocerca* (a relative of *Z. seriolae*) or *B. seriolae* recruits on *S. quinqueradiata* (see Chapter 5). It is plausible that PZQ only affects *Z. seriolae* and *B. seriolae* once they have settled and established on fish rather than preventing them from settling.

Host immunity against parasite infestation must also be considered when viewing the results of this trial. Fish have been reported to exhibit acquired immune responses against monogeneans. Bondad-Reantaso et al. (1995) reported that "primed" (fish that had pre-existing monogeneans infestations that had been removed) *Paralichthys olivaceus* had significantly fewer *Neobenedenia* sp. than naïve control fish. This was also reported by Ohno et al. (2008), who observed that primed *S. dumerili* had significantly less *Neobenedenia* sp. than naïve *S. dumerili*. Fish used in this trial were also primed, i.e. they had pre-existing infestations of *Z. seriolae* and *B. seriolae* removed prior to the experiment. There are no published reports on whether *S. lalandi* parasitised by *Z. seriolae* or *B. seriolae* develop acquired immunity but this may have been a confounding factor in this experiment. It would have been beneficial to use naïve fish in this trial, but these were unavailable from the local industry at the time.

It is recognised that intubation used to deliver treatment in this trial was likely to be stressful to the fish and may have confounded the results of this trial. Crowding, handling and netting of fish are common aquaculture practises known to cause stress (Barton and Iwama, 1991) and are generally minimised for this reason. However, in our trial, daily crowding and netting of fish occurred over 10 consecutive days. Efforts were made to minimise stress to fish, including careful

handling, use of soft, non-abrasive nets, anaesthesia and reducing the amount of time fish were removed from the raceway. Stress however could not be eliminated completely from experimental methods and is known to suppress the immune function of fish (Harris et al., 2000) and increase their susceptibility to disease (Barton and Iwama, 1991). Nash et al. (2000) demonstrated that artificial immunosuppression of salmonid species increased their susceptibility to the monogenean *Gyrodactylus salaris*. It is possible, therefore, that fish in our trial experienced immunosuppression as a result of stress induced by the intubation procedure, and this may have altered their normal responses to challenge by larval monogeneans. Importantly, control fish underwent an identical procedure as fish treated with PZQ and a significant and measurable difference in numbers of recruited *Z. seriolae* and *B. seriolae* was still detectable. Intubation, employed here to allow accurate delivery of PZQ doses and to avoid the apparent reduction in palatability of PZQ-treated feed, is not considered to be a practical everyday treatment method on a commercial scale.

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CHAPTER 8 - SCREENING OF NOVEL COMPOUNDS FOR ACTIVITY AGAINST *ZEUXAPTA SERIOLAE* AND *BENEDENIA SERIOLAE*, MONOGENEAN PARASITES OF YELLOWTAIL KINGFISH *SERIOLA LALANDI*

Chapter 8: Screening of novel compounds for activity against *Zeuxapta seriolae* and *Benedenia seriolae,* monogenean parasites of yellowtail kingfish *Seriola lalandi*

Abstract

A review of published literature identified 27 anthelmintics and antiparasitic compounds in 12 chemical families that may have efficacy as oral treatments against the monogenean parasites *Zeuxapta seriolae* and *Benedenia seriolae* on yellowtail kingfish, *Seriola lalandi,* in sea-cage aquaculture in South Australia. A brief review of the broad chemical groups is presented. Some trials were conducted by surface coating of pellets (four benzimidazoles: albendazole, mebendazole, thiabendazole and triclabendazole; two macrocyclic lactones, emamectin benzoate and abamectin). Other trials were perfomed by direct intubation to the stomach (two amprolium derivatives, amprolium hydrochloride and ethopabate; one benzyl urea, diflubenzuron; two diphosphate salts, chloroquine and quinine hydrochloride; one imidazothiazole, levamisole;three nitromidazoles, dimetridazole, metranidazole and ronidazole; one organophosphate, trichlorfon; two piperazines, diethylcarbamazine citrate and piperazine; three salicylanilides, 5-chlorosalicylanilide, niclosamide and salicylanilide; two substituted phenols, bithionol and hexachlorophene; four tetrahydropyrimidines, morantel citrate, oxantel pamoate, pyrantel citrate and pyrantel pamoate). Of the 27 compounds screened, only the benzimidazole, albendazole, was effective against *Z. seriolae* and no compound screened here was effective against *B. seriolae*.

1. Introduction

Compared with veterinary medicines for terrestrial animals, relatively few oral medications have been tested against parasites of fish; especially monogeneans (see Table 1). For this reason, screening trials were conducted to evaluate the potential of several anthelmintics and antiparasitic compounds as oral treatments against *Zeuxapta seriolae* and *Benedenia seriolae* parasitising *Seriola lalandi*. Candidates were identified from the literature that may be effective against these monogenean species. The cost of the chemicals, ease of application to feed pellets,

112

palatability, potential toxicity to fish, the likely residence time of the medicine in the tissues (based on their currently approved applications), and potential for environmental toxicity were factors that were considered. Some chemicals screened here have demonstrated activity against other flatworm parasites such as trematodes in terrestrial animals, but others were included because their utility against flatworm parasites was unknown. The previous chapters have dealt with fenbendazole, oxfendazole, febantel and praziquantel, which had already demonstrated potential against monogeneans. In this chapter I report on compounds that have not been tested previously against *Z. seriolae* and *B. seriolae*. Chemicals from several groups of antiparasitic compounds were chosen from those screened against the monopisthocotylean *Gyrodactylus* sp. by Tojo and Santamarina (1998b). The broad chemical groups are introduced briefly here.

Table 1

Examples of oral medications from the literature already tested against parasites of finfish. Febantel, fenbendazole, oxfendazole and praziquantel are omitted from this table as they are reviewed in detail in previous chapters.

Compound	Parasite	Host	Treatment doses	Effective	Reference
1, 3-di-quinolylurea	*Ichthyobodo necator* (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
Albendazole	*Benedenia seriolae* (Monogenea)	*Seriola lalandi*	458 mg kg^{-1} BW total dose, 2 days	No	I. Ernst and C.B. Chambers, unpublished data
	Hexamita salmonis (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	Yes	Tojo and Santamarina (1998a)
	Zeuxapta seriolae (Monogenea)	*Seriola lalandi*	458 mg kg^{-1} BW total dose, 2 days	Yes	I. Ernst and C.B. Chambers, unpublished data
Aminosidine	*Gyrodactylus* sp. (Monogenea)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998b)
	Hexamita salmonis (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998a)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
Amprolium	*Gyrodactylus* sp. (Monogenea)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998b)
	Hexamita salmonis (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998a)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
Benznidazole	*Gyrodactylus* sp. (Monogenea)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998b)
	Hexamita salmonis (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	Yes	Tojo and Santamarina (1998a)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
Bithionol	*Gyrodactylus* sp. (Monogenea)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998b)
	Hexamita salmonis (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998a)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
	Microcotyle sebastis (Monogenea)	*Sebastes schlegeli*	100 or 200 mg kg^{-1} BW single dose	Yes	Kim and Choi (1998)
	*Neoparamoeba perurans (Protozoa)	*Salmo salar*	25 mg kg^{-1} BW day^{-1}, 14 days	Prophylactic only	Florent et al. (2007)
Caprylic acid	*Benedenia seriolae* (Monogenea)	*Seriola lalandi*	400 mg kg^{-1} day^{-1} BW, 15 days	No	Divett (2003)
	Cryptocaryon irritans (Protozoan)	*Pagrus major*	75 mg kg^{-1} BW day^{-1}, 5 days	Yes	Hirazawa et al. (2001a)
	Heterobothrium okamotoi (Monogenea)	*Takifugu rubripes*	50, 75 and 100 mg kg^{-1} day^{-1} BW, 15 days	Yes	Hirazawa et al. (2001b)
	Zeuxapta seriolae (Monogenea)	*Seriola lalandi*	400 mg kg^{-1} day^{-1} BW, 15 days	Prophylactic only	Divett (2003)

Table 1 (continued)

Compound	Parasite	Host	Treatment doses	Effective	Reference
Chloroquine	*Gyrodactylus* sp. (Monogenea)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998b)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
Diethylcarbamazine	*Gyrodactylus* sp. (Monogenea)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998b)
	Hexamita salmonis (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	Yes	Tojo and Santamarina (1998a)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
Dimetridazole	*Ichthyobodo necator* (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
Diminazene	*Ichthyobodo necator* (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
Emamectin benzoate	*Caligus elongatus* (Copepoda)	*Salmo salar*	50 µg kg^{-1} BW day^{-1}, 14 days	Yes	Stone et al. (2000)
	Lepeophtheirus salmonis (Copepoda)	*Salmo salar*	50 µg kg^{-1} BW day^{-1}, 14 days	Yes	Stone et al. (2000)
Flubendazole	*Gyrodactylus* sp. (Monogenea)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998b)
	Hexamita salmonis (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998a)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
Ivermectin	*Caligus elongatus* (Copepoda)	*Salmo salar*	200 µg kg^{-1} BW day^{-1}, 14 days	Yes	Palmer et al. (1987)
	Lepeophtheirus salmonis (Copepoda)	*Salmo salar*	200 µg kg^{-1} BW day^{-1}, 14 days	Yes	Palmer et al. (1987)
	Z. seriolae	*Seriola lalandi*	100 µg kg^{-1} BW, 1 day	No, toxic to fish	I. Ernst and C.B. Chambers, unpublished data
	B. seriolae	*Seriola lalandi*	100 µg kg^{-1} BW, 1 day	No, toxic to fish	I. Ernst and C.B. Chambers, unpublished data
Ketoconazole	*Ichthyobodo necator* (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
Levamisole	*Gyrodactylus* sp. (Monogenea)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998b)
	Hexamita salmonis (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998a)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
	*Neoparamoeba perurans (Protozoa)	*Salmo salar*	5 mg kg^{-1} BW day^{-1}, 10 days	No	Zilberg et al. (2000)
Metronidazole	*Gyrodactylus sp.* (Monogenea)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998b)
	Hexamita salmonis (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	Yes	Tojo and Santamarina (1998a)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	Yes	Tojo and Santamarina (1998c)

Table 1 (continued)

Compound	Parasite	Host	Treatment doses	Effective	Reference
Mebendazole	*Benedenia seriolae* (Monogenea)	*Seriola lalandi*	926 mg kg^{-1} BW total dose	No	I. Ernst and C.B. Chambers, unpublished data
	Bothriocephalus scorpii (Cestoda)	*Scophthalmus maximus*	150 mg kg^{-1} BW	Yes	Sanmartín Duran et al. (1989)
	Gyrodactylus sp. (Monogenea)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998b)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
	Microcotyle sebastis (Monogenea)	*Sebastes schlegeli*	50 mg kg^{-1} BW sigle dose	Yes	Kim and Choi (1998)
	Zeuxapta seriolae (Monogenea)	*Seriola lalandi*	926 mg kg^{-1} BW total dose	No	I. Ernst and C.B. Chambers, unpublished data
Niclosamide	*Hexamita salmonis* (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998a)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
Nitroscanate	*Gyrodactylus* sp. (Monogenea)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	Yes	Tojo and Santamarina (1998b)
	Hexamita salmonis (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	Yes	Tojo and Santamarina (1998a)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
Nitroxynil	*Gyrodactylus* sp. (Monogenea)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998b)
	Hexamita salmonis (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998a)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
Oxibendazole	*Gyrodactylus* sp. (Monogenea)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998b)
	Hexamita salmonis (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998a)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
Parbendazole	*Gyrodactylus* sp. (Monogenea)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998b)
	Hexamita salmonis (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998a)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
Piperazine	*Gyrodactylus* sp. (Monogenea)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998b)
	Hexamita salmonis (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998a)
Ronidazole	*Gyrodactylus* sp. (Monogenean)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998b)
	Hexamita salmonis (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	Yes	Tojo and Santamarina (1998a)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)

Table 1 (continued)

Compound	Parasite	Host	Treatment doses	Effective	Reference
Secnidazole	*Gyrodactylus* sp. (Monogenean	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998b)
	Hexamita salmonis (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	Yes	Tojo and Santamarina (1998a)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	Yes	Tojo and Santamarina (1998c)
Sulphaquinoxaline	*Ichthyobodo necator* (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
Teflubenzuron	*Caligus elongatus* (Copepoda)	*Salmo salar*	10 mg kg^{-1} BW day^{-1}, 7 days	Yes	Anonymous (1999)
	Lepeophtheirus salmonis (Copepoda)	*Salmo salar*	10 mg kg^{-1} BW day^{-1}, 7 days	Yes	Anonymous (1999)
Tetramisole	*Gyrodactylus* sp. (Monogenea)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998b)
	Hexamita salmonis (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998a)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
Thiophanate	*Gyrodactylus* sp. (Monogenea)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998b)
	Hexamita salmonis (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998a)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
Toltrazuril	*Gyrodactylus* sp. (Monogenea)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998b)
	Hexamita salmonis (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998a)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)
Trichlorfon	*Gyrodactylus* sp. (Monogenea)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998b)
	Hexamita salmonis (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998a)
Triclabendazole	*Benedenia seriolae* (Monogenea)	*Seriola lalandi*	405 mg kg^{-1} BW total dose	No	I. Ernst and C.B. Chambers, unpublished data
	Gyrodactylus sp. (Monogenea)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	Yes	Tojo and Santamarina (1998b)
	Hexamita salmonis (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998a)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	Yes	Tojo and Santamarina (1998c)
	Zeuxapta seriolae (Monogenea)	*Seriola lalandi*	405 mg kg^{-1} BW total dose	No	I. Ernst and C.B. Chambers, unpublished data
Undecylenic acid	*Benedenia seriolae* (Monogenea)	*Seriola lalandi*	400 mg kg^{-1} day^{-1} BW, 15 days	No	Divett (2003)
	Zeuxapta seriolae (Monogenea)	*Seriola lalandi*	400 mg kg^{-1} day^{-1} BW, 15 days	No	Divett (2003)
	Ichthyobodo necator (Protozoa)	*Onchorhynchus mykiss*	800 mg kg^{-1} BW day^{-1}, 10 days	No	Tojo and Santamarina (1998c)

Neoparamoeba perurans was recently identified as the aetiological agent of amoebic gill disease, previously ascribed to *P. pemaquidensis* (see Young et al., 2000).

1.1 Amprolium derivatives

Amprolium is a structural derivative of thiamine or vitamin B1, used for the treatment of coccidian (protozoan) parasites in terrestrial animals including cattle, swine, sheep, goats and birds (Plumb, 1991). It acts by competitively inhibiting thiamine utilisation of the parasite (Plumb, 1991). Amprolium hydrochloride and ethopabate were screened here for activity against *Z. seriolae* and *B. seriolae*.

1.2 Benzimidazoles

Benzimidazoles act by binding with helminth-specific tubulin, thus interfering with cell functions such as mitosis, cellular transport and mobility. This causes paralysis of the worm, which loses its ability to remain attached to the host (Kohler, 2001). Albendazole, mebendazole and triclabendazole have all demonstrated some activity against monogenean species in preliminary, previously unpublished trials (Table 1) and therefore were screened for activity against *Z. seriolae* and *B. seriolae*. As febantel, fenbendazole and oxfendazole were reviewed in Chapter 3, they are not considered further here.

1.2.1 Albendazole

Albendazole was developed for the treatment of nematodes and trematodes in livestock and is the treatment of choice for neurocysticercosis in humans. Tojo and Santamarina (1998a) reported albendazole as being effective at eliminating the flagellate parasite *Hexamita salmonis* from *Oncorhynchus mykiss*. In preliminary screening trials, albendazole significantly reduced total numbers of *Z. seriolae* on *S. lalandi* at a total dose of 458 mg kg^{-1} BW over 2 days (I. Ernst and C.B. Chambers, unpublished data). This unpublished trial involved small numbers of fish and therefore it was screened again to see if the result could be repeated against *Z. seriolae* and *B. seriolae*.

1.2.2 Mebendazole

Mebendazole is used to treat nematodes and tapeworms in humans. Sanmartín Duran et al. (1989) reported that mebendazole was effective at removing

118

the tapeworm *Bothriocephalus scorpii* in turbot (*Scophthalmus maximus*) at 150 mg kg^{-1} body weight (BW) administered by intubation. A single, orally administered dose of mebendazole was reported to be effective against the monogenean *Microcotyle sebastis* infecting sea-caged rockfish, *Sebastes schlegeli* (see Kim and Choi, 1998). Preliminary oral trials of mebendazole at a total dose of 926 mg kg^{-1} BW over 2 days have shown it is ineffective at removing *Z. seriolae* and *B. seriolae* parasitising *Seriola lalandi* (see I. Ernst and C.B. Chambers, unpublished data). It was screened again here at a higher dose to confirm this result.

1.2.3 Thiabendazole

Thiabendazole has been used to treat nematodes in sheep, goats, horses, cattle and swine and is active against adult and some juvenile nematodes. It has also been reported to inhibit embryonation of nematode eggs (Lynn, 2008) and has been used as an antifungal agent for the treatment of plant diseases. There are no data reporting whether it is effective against monogeneans of fish and therefore it was decided to screen thiabendazole against *Z. seriolae* and *B. seriolae* on *S. lalandi*.

1.2.4 Triclabendazole

Triclabendazole is an anthelmintic developed to treat liver flukes in sheep, goats and cattle. Tojo and Santamarina (1998b) found that it was 100% effective at treating *Gyrodactylus* sp. infections on *O. mykiss* and treated fish showed no signs of toxicity. Triclabendazole is poorly absorbed by the gut due to its low hydrosolubility which reduces its efficacy (Luzardo-Alvarez et al., 2003). It can also reduce the palatability of fish feed because *O. mykiss* occasionally rejected feed that included triclabendazole (Luzardo-Alvarez et al., 2003). Preliminary oral trials of triclabendazole at a total dose of 405 mg kg^{-1} BW over 2 days have shown it is ineffective at removing *B. seriolae* and *Z. seriolae* infecting *S. lalandi* (see I. Ernst and C.B. Chambers, unpublished data). Like mebendazole, it was screened again here to see if a higher dose had any effect on *Z. seriolae* and *B. seriolae*.

1.3 Benzyl ureas

Benzyl ureas are insect growth regulators that build up in body fat when orally administered, and are slowly released into the bloodstream and excreted largely unchanged (Anonymous, 2005). Diflubenzuron is used for the prevention of fly strike in sheep. Teflubenzuron interferes with the synthesis of chitin in sea lice, *Lepeotheirus salmonis* (see Branson et al., 2000). Teflubenzuron is effective at removing feeding lifestages of *L. salmonis* when administered to *Salmo salar* at 10 mg kg BW^{-1} day^{-1} for 7 consecutive days (Anonymous 1999; Ritchie et al., 2002). Teflubenzuron is registered as an oral treatment Calicide$^{®}$ for sea lice *Caligus elongatus* and *L. salmonis* in Scotland, Norway, Canada, Chile and Ireland (Anonymous, 1999), however was not available during the time of the trial, therefore diflubenzuron was screened here instead to see if it had activity against *Z. seriolae* and *B. seriolae*.

1.4 Diphosphate salts

Quinine and chloroquine are diphosphate salts commonly used to treat the malaria parasite *Plasmodium falciparum* in humans, although resistance to these compounds is now widespread. Chloroquine has also been used to treat *Plasmodium* spp. in birds (Anonymous, 2005) and has been reported to be an effective bath treatment against the dinoflagellate parasite of fish *Amyloodinium* (see Noga and Levy, 2006). Quinine hydrochloride and chloroquine were screened here to see if they had activity against *Z. seriolae* and *B. seriolae*.

1.5 Macrocyclic lactones

The macrocyclic lactones or avermectin group of anthelmintics interfere with glutamate-gated chloride channels in the parasite tegument. They are produced by culture of the soil bacterium *Streptomyces avermilitus* and have strong insecticidal and anthelmintic properties (Stone et al., 1999). They work by interrupting transmission of signals in the nervous system (Davies and Rodger, 2000), resulting in flaccid muscle paralysis which causes parasites to lose their ability to move, feed or attach to their host (Kohler, 2001).

1.5.1 Abamectin

Abamectin is a result of natural fermentation of the avermectin product of the bacterium and is used to control insect and mite pests of plants (Lankas and Gordon, 1989). Abamectin was screened here to see if it had activity against *Z. seriolae* and *B. seriolae*.

1.5.2 Ivermectin

Ivermectin is a chemically-modified derivative of avermectin that was developed to treat nematodes in horses, sheep, cattle, pigs and dogs (Stone et al., 1999). When administered orally, ivermectin is poorly absorbed by fish and most chemical is excreted in the faeces (Davies and Rodger, 2000). Ivermectin has been used in salmon culture to treat sea lice *L. salmonis* and *C. elongatus*, but it has a narrow therapeutic margin (Stone et al., 2002) and accumulates in sediments (Davies and Rodger, 2000), making it a poor candidate as an oral treatment of sea-caged finfish. Furthermore, preliminary trials in which a single ivermectin dose of 100 μg kg^{-1} BW was administered orally to *Seriola lalandi* showed marked toxicity to the host (I. Ernst and C.B. Chambers, unpublished data) and did not appear to be effective against *Z seriolae* and *B. seriolae*. For these reasons, ivermectin was not considered further here.

1.5.2 Emamectin benzoate

Emamectin benzoate is an insecticide that is registered as an oral treatment for sea lice (*L. salmonis* and *C. elongatus*) in Europe (Roy et al., 2000). It is administered to salmon as a premix coated onto feed pellets (Grant, 2002; Willis and Ling, 2003). Compared with ivermectin, emamectin benzoate appears to have a wider therapeutic margin (Roy et al., 2000; Willis and Ling, 2003). It does not appear to reduce the palatability of the feed when administered orally to *Salmo salar* to treat sea lice (Grant, 2002; Stone et al., 1999). Willis and Ling (2003) found that the levels of emamectin benzoate entering the environment after sea lice treatments were unlikely to be detrimental to free-living planktonic copepods. The dose of emamectin benzoate effective against sea lice is very low (50 μg kg^{-1} BW day^{-1} over 14 days) and it can remain effective against sea lice for up to 55 days after initial

treatment (Stone et al., 2000) making it a very cost effective treatment. Therefore emamectin benzoate not only has the potential to reduce existing parasite burdens (therapeutic effect), but also the potential to inhibit recruitment of new parasites (prophylactic effect) (Stone et al., 2000). There are no published reports of emamectin benzoate being trialled against monogeneans and therefore it was screened against *Z. seriolae* and *B. seriolae* on *Seriola lalandi*.

1.6 Organophosphates

Organophosphates are a large group of compounds which have been used to treat animal and plant pests. They act by binding irreversibly with acetylcholinesterase, which blocks nerve transmission in invertebrates, resulting in paralysis. Trichlorfon is an organophosphate that has been used orally to treat nematodes in horses and cattle (Plumb, 1991). Tojo and Santamarina (1998a, b, c) reported that orally administered trichlorfon had no effect on *Hexamita salmonis*, *Gyrodactylus* sp. or *Ichthyobodo necator*, respectively, on *O. mykiss* but it has been used as a bath treatment for the monogeneans *Dactylogyrus* sp. (see Noga, 2000). Trichlorfon was screened here to see if it had activity against *Z. seriolae* and *B. seriolae* as an oral treatment.

1.7 Piperazines

Piperazines are largely effective against ascarid nematodes in animals and humans (Anonymous, 2005). They act by blocking neuromuscular transmission in the parasite causing paralysis (Anonymous, 2005). Tojo and Santamarina (1998a) reported that the piperazine derivative diethylcarbamazine administered orally to *O. mykiss* was effective against the protozoan parasite *Hexamita salmonis* (see Table 1). Diethylcarbamazine citrate and piperazine were screened here to see if they had activity against *Z. seriolae* and *B. seriolae*.

1.8 Nitroimidazoles

Nitroimidazoles are the only oral pharmacological treatments for *Hexamita salmonis* (see Tojo and Santamarina, 1998a). Tojo and Santamarina (1998b) reported that metronidazole and ronidazole had no effect against the monogenean

122

Gyrodactylus sp. on *O. mykiss.* Metridazole, metronidazole and ronidazole were screened here against *Z. seriolae* and *B. seriolae* on *Seriola lalandi* because these host and parasite species may show differences in responses to these compounds.

1.9 Salicylanilides and substituted phenols

The salicylanilides and substituted phenols are active against adult liver flukes in livestock and are used extensively for the treatment of fasciolosis and haemonchosis in sheep and cattle (Anonymous, 2005). Orally administered bithionol has reported efficacy against the monogenean *M. sebastis* on *Sebastes schlegeli* (see Kim and Choi, 1998) but not against *Gyrodactylus* sp. on *O. mykiss* (see Tojo and Santamarina, 1998b) (see Table 1). The salicylanilides 5-chlorosalicylanilide, niclosamide and salicylanilide and the substituted phenols bithionol and hexachlorophene were screened here to see if they had activity against *Z. seriolae* and *B. seriolae*.

1.10 Tetrahydropyrimidines

The tetrahydropyrimidines are antinematodal compounds used predominantly to treat livestock. Pyrantel pamoate has activity against cestode parasites of horses (Plumb, 1991). They are an agonist of the acetylcholine receptors of the muscle cells of nematodes, causing paralysis of the parasite (Plumb, 1991). They have unknown activity against monogenean parasites and therefore two pyrantel formulations, pyrantel citrate and pyrantel pamoate, plus morantel citrate and oxantel pamoate were screened here.

1.11 Immunostimulants

Complement factors, enzymes and enzyme inhibitors are chemical defence mechanisms produced by specialised cells in fish skin and mucosal membranes to combat parasites. Immunostimulants have been used to enhance these non-specific immune responses of fish in aquaculture. Immunostimulants such as certain beta-glucans produced by yeast stimulate these cells and therefore may enhance the fish's natural defence to parasite invasion (Raa, 2000). Immunostimulants are appealing because nutritional supplements do not require registration and they are more

acceptable in markets that are increasingly conscious of food safety and chemical use in food production. While they do not confer permanent immunity and the effect of immunostimulants is shortlived, they may assist in management of bacterial pathogens such as *Vibrio* spp. and parasites such as *Ichthyophthirius multifilis* (see Sakai, 1999). Glucan, chitin, levamisole and lactoferrin are already incorporated into some fish feed (Sakai, 1999). It is possible that an immunostimulant and an anthelmintic may be used in concert in fish feed to not only treat existing parasitic infections (therapeutic), but also to stimulate the fish's immune system, which may help fish resist secondary bacterial infections and withstand recruitment of new monogeneans (a prophylactic effect). Until the fish immune system and how it is modulated are fully understood, especially the way in which it responds to parasites, the full potential of immunostimulants may not be attained.

1.11.1 Levamisole

Levamisole is a recognised immunostimulant in mammals (Findlay et al., 2000) and is grouped with the nicotinic agonist family of anthelmintics (Kohler 2001). Findlay and Munday (2000) reported that levamisole administered in a freshwater bath is effective in enhancing the non-specific immune response of *Salmo salar* and enhanced resistance to amoebic gill disease (AGD). However, Zilberg et al. (2000) found levamisole administered orally did not reduce the mortality of *S. salar* exposed to AGD. Levamisole was screened here to see if it had activity against *Z. seriolae* and *B. seriolae* on *Seriola lalandi*.

1.11.2 Medium-chain fatty acids

Caprylic acid (octanoic acid) is a medium-chain fatty acid derived from coconut oil, peanut oil and other edible oils (Hirazawa et al., 2000). Its exact mode of action is unknown but it is used in human medicine to treat yeast infections. Hirazawa et al. (2001a, b) found caprylic acid had a harmful effect on ectoparasites such as *Cryptocaryon irritans* on red sea bream (*Pagrus major*) and *Heterobothrium okamotoi* on tiger puffer (*Takifugu rubripes)*, respectively. Undecylenic acid is a naturally occurring compound found in human sweat. Commercially it is derived from distilling castor bean oil. Undecylenic acid is apparently six times more

124

effective than caprylic acid as an antifungal agent (Anonymous, 2002c). Divett (2003) examined the effect of caprylic acid and undecylenic acid against *Z. seriolae* and *B. seriolae* infecting *Seriola lalandi* in sea-cage aquaculture in South Australia. Caprylic acid administered orally significantly reduced the recruitment of *B. seriolae* larvae, but had no effect on existing infections. Undecylenic acid administered orally significantly reduced the recruitment of *Z. seriolae* larvae but had no effect on existing infections (Divett, 2003). Based on these results, medium-chain fatty acids were not considered further.

2. Methods

The methodology used to screen compounds was refined over time. In Trials 1 and 2, medications were surface coated onto pellets and fed to fish. In Trial 3, compounds were intubated directly to fish, which allowed high doses to be tested accurately without differential feeding and/or potential palatability issues creating variability in doses of medication received between individuals. A chief disadvantage of using intubation to screen anthelmintics is that whether the anthelmintic affects the palatability of feed to fish is not assessed. This, however, is no different to *in vitro* trials, and because the purpose of these screening trials was to initially identify compounds with potential to treat monogeneans, it was anticipated that factors such as palatability may be addressed in future follow up assessments for medications that showed potential against monogeneans.

Time held after treatment (last column, Table 2) was an approximate determination by reference to the tissue residence time of each compound in their more common applications e.g. in terrestrial animals. This research was conducted under APVMA Minor Use Permit 7250 for small-scale trials of Agvet chemicals (APVMA, 2004)

Table 2

Summary of anthelmintics tested by surface coating of feed (Trials 1 and 2) and direct intubation (Trials 3a - 3d).

Trial	Chemical group	Anthelmintic	Daily dose,	Duration (days)	Time between treatment and sampling (days)
Trial 1	Benzimidazoles	Albendazole	500 mg kg^{-1} BW day^{-1}	2	7
	Benzimidazoles	Mebendazole	500 mg kg^{-1} BW day^{-1}	2	7
	Benzimidazoles	Thiabendazole	250 mg kg^{-1} BW day^{-1}	2	7
	Benzimidazoles	Triclabendazole	500 mg kg^{-1} BW day^{-1}	2	7
Trial 2	Macrocylic lactones	Emamectin benzoate	50 μg kg^{-1} BW day^{-1}	7	14
	Macrocylic lactones	Emamectin benzoate	250 μg kg^{-1} BW day^{-1}	7	14
Trial 3a	Salicylanilides	5-Chlorosalicylanilide	25 mg kg^{-1} BW day^{-1}	1	14
	Macrocylic lactones	Abamectin	1 mg kg^{-1} BW day^{-1}	1	14
	Salicylanilides	Niclosamide	25 mg kg^{-1} BW day^{-1}	1	14
	Salicylanilides	Salicylanilide	25 mg kg^{-1} BW day^{-1}	1	14
Trial 3b	Benzyl ureas	Diflubenzuron	30 mg kg^{-1} BW day^{-1}	3	7
	Nitroimidazoles	Dimetridazole	800 mg kg^{-1} BW day^{-1}	3	7
	Imidazothiazoles	Levamisole	1000 mg kg^{-1} BW day^{-1}	3	7
	Nitroimidazoles	Metronidazole	800 mg kg^{-1} BW day^{-1}	3	7
	Nitroimidazoles	Ronidazole	473 mg kg^{-1} BW day^{-1}	3	7
	Organophosphates	Trichlorfon	30 mg kg^{-1} BW day^{-1}	3	7
Trial 3c	Amprolium derivatives	Amprolium hydrochloride	50 mg kg^{-1} BW day^{-1}	3	7
	Substituted phenols	Bithionol	500 mg kg^{-1} BW day^{-1}	3	7
	Diphosphate salts	Chloroquine	50 mg kg^{-1} BW day^{-1}	3	7
	Amprolium derivatives	Ethopabate	50 mg kg^{-1} BW day^{-1}	3	7
	Substituted phenols	Hexachlorophene	60 mg kg^{-1} BW day^{-1}	3	7
	Diphosphate salts	Quinine hydrochloride	300 mg kg^{-1} BW day^{-1}	3	7
Trial 3d	Piperazines	Diethylcarbamazine citrate	60 mg kg^{-1} BW day^{-1}	3	3
	Tetrahydropyrimidines	Morantel citrate	30 mg kg^{-1} BW day^{-1}	3	3
	Tetrahydropyrimidines	Oxantel pamoate	30 mg kg^{-1} BW day^{-1}	3	3
	Piperazines	Piperazine	300 mg kg^{-1} BW day^{-1}	3	3
	Tetrahydropyrimidines	Pyrantel citrate	60 mg kg^{-1} BW day^{-1}	3	3
	Tetrahydropyrimidines	Pyrantel pamoate	50 mg kg^{-1} BW day^{-1}	3	3

2.1 Source of fish and parasites

Fish for all screening trials were sourced from commercial *S. lalandi* farms in the Spencer Gulf, South Australia. With the exception of those fish that were administered albendazole, mebendazole, thiabendazole and triclabendazole (benzimdazoles – Trial 1), fish had acquired monogenean parasites naturally while in sea-cages during growout, prior to their relocation to land-based tank facilities. Fish used in the benzimidazole trial acquired parasites by co-habitation with infested fish in land-based tanks. Methods used to sample monogeneans are detailed in Chapter 3. Compounds for all trials were purchased from Sigma-Aldrich, Sydney, Australia.

2.2 Compounds administered by surface coating of feed

Four benzimidazoles (Trial 1) and emamectin benzoate (Trial 2) were screened by surface coating of feed using methods detailed in Chapters 3 and 4.

2.3 Compounds administered by intubation

Compounds screened by intubation were delivered in a paste made from pre-pellet meal (provided by Skretting Australia), fish oil and dechlorinated tap water. At the beginning of the trial, fish were individually weighed and the mean weight for 10 fish in each treatment group was used to calculate the treatment dose in mg per kg body weight per day (mg kg^{-1} BW day^{-1}). Fish were anaesthetised in a 60 L tub containing a bath solution of 33 ppm AQUI-S™ aquatic anaesthetic in seawater until they lost their reflex activity (Stage 4 anaesthesia, see Hikasa et al., 1986). Paste was then intubated into their stomach using a 30 mL catheter syringe fitted with a 10 cm extension of soft silicon hose 4 mm in diameter. Fish were placed into a 60 L tub of clean seawater to recover and were monitored for 5 min to ensure no medication was regurgitated before they were returned to their cage (see details of experimental setup below). Control fish underwent an identical procedure but were administered paste without medication. Apart from intubation, fish were not fed during the trials. The treatment duration and time between treatment and sampling for each compound is listed in Table 2. Fish were sampled for remaining monogeneans using methods detailed previously.

2.4 Trials (see Table 2)

The benzimidazoles (Trial 1) albendazole, mebendazole, thiabendazole and triclabendazole were administered to fish by surface coating of feed pellets, using methods detailed in Chapter 3. Treatment groups of fish were separated into circular cages 1.5 m in diameter constructed from 12 mm plastic mesh and maintained in a circular tank (volume, 12 m^3) in at a landbased facility with flow-through seawater supply. Each cage contained 10 randomly selected fish for each treatment. One cage per treatment was used. Each compound was administered at 500 mg kg^{-1} BW day^{-1}, calculated from mean weight of fish, except for thiabendazole, which was administered at 250 mg kg^{-1} BW day^{-1} (Table 2). High target doses were chosen, based on results of previous screening trials (I. Ernst and C.B. Chambers, unpublished data) and the literature, in order to detect whether the compound had any activity at all against monogeneans. Control fish were fed regular (unmedicated) pellets daily. The treatments were administered for 2 consecutive days and then fish were held for 7 days (Table 2) before being sampled for parasites (see Chapter 3). Fish were observed for signs of toxicity throughout treatment (e.g. abnormal behaviour, darkening of skin) that may have been caused by medication. Trial 1 for albendazole, mebendazole and triclabendazole was conducted in July 2003 [water temperature, 13.5 ˚C (no range recorded); salinity, 48 ppt; mean weight of experimental fish, 96.3 (59 - 133) g]. Due to the initial unavailability of thiabendazole, this compound was screened separately using the same methods in March 2004 [water temperature, 21.5 ˚C (no range recorded); salinity, 48 ppt; mean weight of experimental fish, 1.1 (0.95 – 1.33) kg].). For all benzimidazoles screened in Trial 1, fish were held for 7 days before being sampled for parasites.

Emamectin benzoate (Trial 2) was screened by surface coating of pellets fed to treatment groups of fish using the same experimental cages used in Trial 1. A dose of 50 μg kg^{-1} BW day^{-1} was administered in April 2004 [water temperature, 21.5 ˚C (no range recorded); salinity, 48 ppt; mean weight of experimental fish, 1.2 (0.65 – 1.5) kg]. A dose of 250μg kg^{-1} BW day^{-1} was administered in May 2005 (water temperature, 18 ˚C (no range recorded); salinity, 40 ppt; mean weight of experimental fish, 1.25 (0.86 – 1.54) kg]. These treatments were administered for 7 days and then fish were held for 14 days (Table 2) before being sampled for parasites (see Chapter 3 for sampling methods).

128

All remaining compounds were screened by intubation in Trial 3 in February and March 2005. Treatment groups of fish were separated into the same experimental cages used in Trials 1 and 2 so that there were 10 fish per cage maintained in a concrete raceway at a landbased facility with flow-through seawater supply. Water temperature was 20 ± 2 °C and salinity was 40 ppt. Doses, duration of treatment and time to assessment of efficacy from treatment are summarised in Table 2. The following numbers of fish were used: Trial 3a, a total of 50 fish of mean weight 563 (197-783) g; Trial 3b, a total of 70 fish of mean weight 550 (215-742) g; Trial 3c, a total of 70 fish of mean weight 556 (271-747) g; Trial 3d, a total of 70 fish of mean weight 552 (271-747) g. Each compound was administered to 10 fish (ne cage per treatment) and 10 fish were maintained as untreated controls. Fish were obseved for signs of toxicity throughout treatment. Presence of *Z. seriolae* and *B. seriolae* was confirmed by routine monitoring of fish in the same cohort in the raceway that the experiment fish were taken from, prior to trial using parasite sampling methods for *Z. seriolae* and *B. seriolae* detailed in Chapter 3.

2.5 Statistical analysis

SPSS 15.0 software was used for statistical analyses (SPSS Inc., Chicago IL, USA). The efficacy for treatments found to have be significantly different from the control was calculated as a percentage according to the formula presented in previous chapters adapted from Stone et al. (2000). Comparisons of mean abundance (as defined by Bush et al., 1997) of *Z. seriolae* and *B. seriolae* were made between treatment and control groups using independent *T*-tests for Trials 1 and 2 and one-way ANOVAs for Trials 3a, 3b, 3c and 3d. A *P*-value of ≤ 0.05 was considered significant. The Levene's statistic was calculated and where significant ($P \leq 0.05$), unequal variance was assumed and Games Howell *post-hoc* comparisons were conducted.

3. Results

3.1 Trials 1 and 2: Benzimidazoles and emamectin benzoate administered by surface coating of feed

In Trials 1 and 2, a mean daily ration of 3.6% BW was offered to each treatment group. The benzimidazoles did not appear to produce signs of toxicity such as a dark appearance in treated fish or loss of equilibrium. Palatability of feed medicated with albendazole, thiabendazole and triclabendazole appeared to be reduced, and the actual dose delivered was less than the target dose. Actual doses delivered for albendazole were 379 and 113 mg kg^{-1} BW day^{-1} for Days 1 and 2, respectively for a total dose of 492 mg kg^{-1} BW. Actual doses delivered for thiabendazole were 150 and 100 mg kg^{-1} BW day^{-1} for Days 1 and 2, respectively for a total dose of 250 mg kg^{-1} BW. Actual doses delivered for triclabendazole were 341 and 96 mg kg^{-1} BW day^{-1} for Days 1 and 2, respectively for a total dose of 437 mg kg^{-1} BW. Mebendazole did not appear to affect feed palatability and the actual total dose delivered was calculated as 926 mg kg^{-1} BW (target dose, 1000 mg kg^{-1} BW). In Trial 2, 3 fish fed emamectin benzoate at the highest dose (250 μg kg^{-1} BW day^{-1} for 7 days) developed a dark appearance, which may indicate toxicity. No further signs of toxicity such as loss of appetite, loss of equilibrium or mortality were observed in treated fish.

From independent *T*-tests, fish fed pellets medicated with , had significantly different mean abundance of *Z. seriolae* compared with controls (Table 3a). Efficacy of albendazole (expressed as a reduction of the mean abundance) was 77.65 % against *Z. seriolae*. However, independent *T*-tests did not detect any significant differences between mean abundance of *Z. seriolae* and *B. seriolae* on fish fed pellets medicated with mebendazole, triclabendazole (Table 3a), thiabendazole (Table 3b) and emamectin benzoate (Table 4a and 4b) compared to controls.

130

Table 3

Results of independent *T*-test comparisons of mean abundance of *Zeuxapta seriolae* and *Benedenia seriolae,* after oral treatment by surface-coating of feed with (a) albendazole, mebendazole and triclabendazole at 500 mg kg^{-1} BW day^{-1} for 2 days (trial conducted in July 2003) and (b) thiabendazole at 250 mg kg^{-1} BW day^{-1} for 2 days (trial conducted in March 2004) . $P \leq 0.05$ was considered significant **(see bold figures)**, t = t-value, df = degrees of freedom.

(a)

Compound	Species	Mean	Std. Deviation	Std. Error Mean	Levene's statistic	t	df	*P*-value
Control	*Z. seriolae*	53.7	31.86	10.08	-	-	-	**-**
	B. seriolae	3.1	1.29	0.40	-	-	-	**-**
Albendazole	*Z. seriolae*	12.0	8.55	2.17	**0.02**	3.00	10	**0.002**
	B. seriolae	1.9	1.45	0.46	0.59	1.95	18	0.066
Mebendazole	*Z. seriolae*	64.4	25.15	7.95	0.69	-0.83	18	0.415
	B. seriolae	2.8	1.48	0.47	0.73	0.49	18	0.634
Triclabendazole	*Z. seriolae*	59.2	29.53	9.34	0.89	-0.40	18	0.694
	B. seriolae	3.0	1.41	0.47	0.44	0.17	18	0.870

(b)

Compound	Species	Mean	Std. Deviation	Std. Error Mean	Levene's statistic	t	df	*P*-value
Control	*Z. seriolae*	67.33	20.88	6.96	-	-	-	**-**
	B. seriolae	89.44	41.23	13.74	-	-	-	**-**
Thiabendazole	*Z. seriolae*	59.80	27.09	8.57	0.77	0.67	17	0.510
	B. seriolae	67.20	36.14	11.43	0.54	1.25	17	0.227

Table 4

Results of independent *T*-test comparisons of mean abundance of *Zeuxapta seriolae* and *Benedenia seriolae,* after oral treatment by surface-coating of feed with emamectin benzoate at (a) 50 μg kg^{-1} BW day^{-1} for 7 days in April 2004, and (b) 250 μg kg^{-1} BW day^{-1} for 7 days in May 2005. $P \leq 0.05$ was considered significant, t = t-value, df = degrees of freedom.

(a)

Compound	Species	Mean	Std. Deviation	Std. Error Mean	Levene's statistic	t	df	*P*-value
Control	*Z. seriolae*	69.88	33.52	11.85	-	-	-	-
	B. seriolae	102.38	38.08	13.46	-	-	-	-
Emamectin benzoate	*Z. seriolae*	56.25	15.45	5.46	0.58	-0.01	18	0.990
	B. seriolae	95.00	34.40	12.16	0.60	0.41	14	0.691

(b)

Compound	Species	Mean	Std. Deviation	Std. Error Mean	Levene's statistic	t	df	*P*-value
Control	*Z. seriolae*	366.50	190.33	60.19	-	-	-	-
	B. seriolae	162.70	73.05	23.10	-	-	-	-
Emamectin benzoate	*Z. seriolae*	251.40	99.32	31.41	0.18	1.69	18	0.107
	B. seriolae	175.10	58.44	18.48	0.83	-0.42	18	0.680

3.2 Trial 3: Various compounds intubated to fish

Doses calculated for each compound were based on the mean fish weight for each treatment group at the start of the trial. Unequal sample sizes in Trial 3a were due to 2 fish from the control group, 2 fish from the salicylanilide treatment group and 1 fish from the niclosamide treatment group jumping from their cages. This was probably due to a 'fright' event (e.g. lights being switched on inadvertently at night), and was not thought to be associated with treatment because control fish were also affected. No signs of toxicity were observed in any treatment group, except those intubated with diethylcarbamazine citrate (Trial 3d), in which 50% mortality occurred within 24 h of the treatment being administered, indicating that this compound was toxic to *S. lalandi* at 60 mg kg^{-1} BW day^{-1}.

One-way ANOVAs did not detect any significant differences between mean abundance of *Z. seriolae* or *B. seriolae* on fish intubated with any of the compounds screened in Trials 3a, 3b, 3c or 3d compared to controls (see Tables 5-8).

Table 5

(a) Comparison of mean abundance using one-way ANOVA of *Zeuxapta seriolae* and *Benedenia seriolae* after oral treatment for 1 day by intubation with the salicylanilides 5-chlorosalicylanilide, niclosamide and salicylanilide and the macrocyclic lactone, abamectin in February/March 2005 (Trial 3a). (b) Results of Games-Howell *post-hoc* multiple comparisons of mean abundance of *Z. seriolae* following unequal variances as indicated by Levene's statistic (Table 5a). $P \leq 0.05$ was considered significant, df = degrees of freedom, F = F-value.

(a)

Species	Levene's statistic	Sum of Squares	df	Mean Square	F	*P*-value
Z. seriolae	**0.002**	285220.49	4	71305.12	0.714	0.587
B. seriolae	0.673	8.036	4	2.009	0.178	0.949

(b)

Treatment	*n*	Mean	Std. Deviation	Std. Error Mean	*P*-value
Control	8	501	496.85	175.66	-
5-chlorosalicylanilide	10	407.2	260.72	194.05	0.987
Abamectin	10	482.0	314.59	201.88	1.00
Niclosamide	9	302.2	256.33	195.34	0.842
Salicylanilide	8	320.5	184.32	187.36	0.865

Table 6

Comparison of mean abundance using one-way ANOVA of *Zeuxapta seriolae* and *Benedenia seriolae* after oral treatment by intubation with diflubenzuron, dimetridazole, levamisole, metronidazole, ronidazole and trichlorfon for 3 consecutive days (Trial 3b) in February/March 2005. $P \le 0.05$ was considered significant, df = degrees of freedom, F = F-value.

Species	Levene's statistic	Sum of Squares	df	Mean Square	F	*P*-value
Z. seriolae	0.318	117560.79	6	19593.47	2.242	0.051
B. seriolae	0.777	69.59	6	11.60	1.796	0.115

Table 7

Comparison of mean abundance using one-way ANOVA of *Zeuxapta seriolae* and *Benedenia seriolae* after oral treatment by intubation with amprolium hydrochloride, bithionol, chloroquine, ethopabate, hexachlorophene and quinine hydrochloride for 3 consecutive days (Trial 3c) in February/March 2005. $P \le 0.05$ was considered significant, df = degrees of freedom, F = F-value.

Species	Levene's statistic	Sum of Squares	df	Mean Square	F	*P*-value
Z. seriolae	0.198	158942.95	6	26490	0.526	0.786
B. seriolae	0.158	149.07	6	24.84	0.434	0.853

Table 8

a) Comparison of mean abundance using one-way ANOVA of *Zeuxapta seriolae* and *Benedenia seriolae* after oral treatment by intubation with diethylcarbamazine citrate, morantel citrate, oxantel pamoate, piperazine, pyrantel citrate and pyrantel pamoate for 3 consecutive days (Trial 3d) in February/March 2005. (b) Results of Games-Howell *post-hoc* multiple comparisons of mean abundance of *Z. seriolae* following unequal variances as indicated by Levene's statistic (Table 8a). $P \leq 0.05$ was considered significant, df = degrees of freedom, F = F-value.

(a)

Species	Levene's statistic	Sum of Squares	df	Mean Square	F	*P*-value
Z. seriolae	**<0.001**	10240.85	6	17080.14	1.63	0.154
B. seriolae	0.339	22.75	6	3.79	0.56	0.760

(b)

Treatment	*n*	Mean	Std. Deviation	Std. Error Mean	*P*-value
Control	10	169.30	108.41	34.28	-
Diethylcarbamazine citrate	5	88.00	35.57	37.79	0.384
Morantel pamoate	10	116.30	60.11	39.20	0.817
Oxantel pamoate	10	88.90	62.91	39.64	0.439
Piperazine	10	194.30	168.69	63.41	1.000
Pyrantel citrate	10	95.30	74.08	41.52	0.577
Pyrantel pamoate	10	155.70	116.28	50.27	1.000

4. Discussion

Of the 27 compounds I screened, only the benzimidazole albendazole appeared to have efficacy (77.65 % reduction) against the gill fluke *Z. seriolae* (Table 3a) and none appeared to have an effect against the skin fluke *B. seriolae*. In Chapter 3, the benzimidazoles fenbendazole and oxfendazole administered by surface coating of feed to *S. lalandi* were also found to be only effective against *Z. seriolae* and had no significant effect against *B. seriolae*. The benzimidazole febantel was similarly very effective against the blood-feeding *H. heterocerca* when administered by intubation to *S. quinqueradiata* but did not appear to affect *B. seriolae* (see Chapter 3).

Some compounds I screened have been reported to have activity against monogeneans in previous studies (Table 1). Differences between fish hosts and parasite species, methods of medication administration and trial conditions (e.g. water temperature) may influence the effect of these compounds against *Z. seriolae* and *B. serolae*. Similarly, differences between the biology, specifically the feeding habits of *Z. seriolae*, a blood feeder, and *B. seriolae*, an epithelial grazer, may explain why albendazole only had an effect against *Z. seriolae*.

Two other benzimidazoles, mebendazole and triclabendazole, have been reported to have efficacy against other monogeneans (Table 1). In particular, Tojo and Santamarina (1998b) reported triclabendazole to be effective against the epithelial feeder *Gyrodactylus* sp. on *O. mykiss* when administered at 800 mg kg^{-1} BW day^{-1} for 10 days, and Kim and Cho (1998) found that mebendazole was effective against *Microcotyle sebastis* parasitising *Sebastes schlegeli* when administered a single dose of 50 mg kg^{-1} BW. No effect against *Z. seriolae* and *B. seriolae* on *S. lalandi* was detected for mebendazole or triclabendazole when administered over 2 consecutive days in this study (Table 3a). Tojo and Santamarina (1998b) also highlighted that triclabendazole did not appear to have an effect against *Gyrodactylus* sp. until after 5 consecutive days of a 10-day treatment. It is possible, therefore, that benzimidazoles need to be administered for a longer duration in order to achieve efficacy against monogeneans on *S. lalandi*.

While many of the compounds screened in this study had not previously been reported to have activity against flatworm parasites, it is possible that some may have been screened at least against flatworm parasites of terrestrial animals. It is generally more accepted to report positive results in which a compound has efficacy and it is unfortunate that negative results are rarely published. This, however, emphasises the importance of publishing negative results in order to build a more complete picture for future research so that others are aware of the details about what compounds have been investigated and under what conditions.

It is possible that some, if not most, of the compounds screened here are not active against monogeneans, or do not reach the monogeneans at high enough doses to achieve therapy following oral administration to fish. Some compounds may not have been screened at a sufficient dose to achieve efficacy, or that peak tissue levels of active metabolites occur longer at a longer time interval than tested. Higher doses than those screened, however, are likely to be uneconomical for farmers, and a

long time interval from administration of a treatment to when it takes effect would make it difficult to time treatments precisely.

The efficacy of albendazole as a treatment against *Z. seriolae* is worth further investigation. As with other benzimidazoles, this compound may work better when administered for a longer duration (i.e. a greater number of consecutive days). While administration of some compounds by intubation allowed the target dose to be administered more precisely, any apparent reduction in palatability of feed medicated with albendazole would not have been detected had it been screened in this way. As demonstrated for praziquantel in Chapter 4, reduction of feed palatability is undesirable in an oral treatment and makes it difficult to deliver the target dose and achieve adequate levels of efficacy against target parasites. Further work to assess in detail how much albendazole may affect feed palatability is also required.

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CHAPTER 9 – GENERAL DISCUSSION

Chapter 9: General Discussion

There are several aspects to consider when evaluating efficacy of a medication against target parasite(s). Method of delivery and palatability of medicated feed may strongly influence results of efficacy trials. Uptake of compounds, distribution to tissues, metabolic rate and elimination times, parasite size and parasite and host physiology have been linked to the efficacy of antiparasitic medications (Treves-Brown, 2000). There is a paucity of information, however, about oral delivery of treatments for fish parasites and these knowledege gaps are also discussed.

For treatments to be used legally in commercial aquaculture, registration with the relevant agency governing the use of veterinary chemicals is required. In Australia this is the Australian Pesticides and Veterinary Medicines Authority (APVMA). My project concentrated on determining the efficacy of praziquantel (PZQ) and the benzimidazoles febantel (FEB), fenbendazole (FBZ) and oxfendazole (OXF) against skin and gill flukes infecting *Seriola* spp. The purpose of my trials was to determine the optimal dose required to treat or manage the parasites, in this case the monogeneans *Zeuxapta seriolae* on *S. lalandi*, *Heteraxine heterocerca* on *S. quinqueradiata* and *Benedenia seriolae*, a parasite of both *S. lalandi* and *S. quinqueradiata*. While fulfilling all the information required to attain the registration of a veterinary medicine was well beyond the scope of my PhD project, a better understanding of some aspects would help explain the results of my oral treatment trials.

1. Delivery of oral treatments

I used two methods to deliver medications to fish: surface coating of feed pellets and direct intubation. Surface coating of feed pellets was the original method in my trials to deliver compounds to fish and was used in Chapter 3 to deliver FBZ and OXF, in Chapter 4 to deliver PZQ and in Chapter 8 to screen other benzimidazoles and emamectin benzoate. Difficulties with apparent reduced palatability of feed observed for PZQ (see below) and reduced feeding activity of fish during periods of low water temperature led me to consider other methods. Intubation directly into the stomach allowed administration of target doses of compounds when feeding activity (whether related to water temperature or

140

palatability) was otherwise reduced. It also avoided differential feeding behaviours that can lead to variations between doses received by individual experimental fish which may confound results. For intubation, compounds at a known dose were delivered in a paste of pre-pellet meal to simulate how compounds would be delivered in pellet form. A disadvantage of intubation is that it did not test palatability, but I assumed that if the compound showed potential as an effective treatment against monogeneans, palatability would be addressed in future studies before refinement of doses and field trials. Intubation is also labour intensive and the repeated anaesthesia and excessive handling is probably stressful to fish. It is worth noting that combining medications into a paste had an unknown effect on the bioavailability of the active compound; if it binds to feed components such as ions or lipids, the amount of active compound may decrease.

Two other methods to screen compounds for potential as treatments are *in vitro* procedures, i.e. applying a compound directly to parasites contained in artifical conditions, and the incorporation of medication into either moist pellets (a feed normally manufactured by the farm, immediately before feeding with no drying process) or extruded pellets during manufacture. As my research focused on oral treatments of fish, I ruled out *in vitro* trials because I wanted to test each compound's activity against target monogeneans after it had been digested, absorbed and metabolised by fish. The differences in feeding habits of *Z. seriolae* and *H. heterocerca* (blood-feeders) and *B. seriolae* (a mucus and epithelial grazer) also mean that they are likely to have very different exposure to and uptake of treatment compounds. These aspects of oral treatment against ectoparasites are not encompassed by *in vitro* trials, which are more suited for screening topical treatments where the parasite is exposed directly to the compound, e.g. bath treatments. The second method, incorporation of medication into feed, was dismissed because I had no access to equipment to make moist feed pellets. Furthermore, unlike parts of Asia, including Japan, moist feed pellets are not used in the South Australian *S. lalandi* industry. Similarly, I did not have access to feed mill equipment to incorporate different compounds into small batches of extruded feed pellets and it was not economical to employ a commercial feed manufacturer to produce small quantities of pellets on their equipment. These innovations may occur further into the development process of an effective oral treatment, for example, once approval

141

has been sought and granted to conduct full-scale commercial feed trials for a compound that shows promise against target parasites.

2. Palatability

A treatment that reduces feed palatability is especially problematic for oral delivery because it is difficult to accurately administer the required dose. Uneaten food and unassimilated medication is wasteful, expensive and potentially environmentally deleterious. A prolonged reduction in feeding may lead to slower fish growth, which is highly undesirable. In Chapter 4, specimens of *S. lalandi* were observed to reject pellets surface coated with PZQ at all doses administered (Trial 1), especially at higher daily doses. In that trial, higher daily doses (100 and 150 mg kg^{-1} BW day^{-1}) had lower efficacy than lower daily doses (50 and 75 mg kg^{-1} BW day^{-1}) against *Z. seriolae* and *B. seriolae*. While higher daily doses were expected to have the same or higher efficacy in removing parasites from fish, the results were consistent with fish in the higher daily dose treatment groups consuming fewer feed pellets and less PZQ than fish in the lower daily dose treatment groups. If data on plasma, muscle and skin concentrations of PZQ had been collected during the trial, these results would be better understood. For example, pharmacokinetic data would indicate whether fish receiving lower daily doses of PZQ actually had higher plasma, muscle and skin concentrations of PZQ than fish receiving higher less palatable daily doses, explaining the higher efficacy observed. Hirazawa et al. (2004) also observed reduced appetite in spotted halibut *Verasper variegatus* (Pleuronectidae) fed extruded pellets medicated with PZQ at a dose of 150 mg kg^{-1} BW day^{-1} but not at 40 mg kg^{-1} BW day^{-1}. They also observed that Japanese yellowtail *S. quinqueradiata* and amberjack *S. dumerili* in commercial aquaculture in Japan may reject pellets medicated with PZQ. Sitjà-Bobadilla et al. (2006) encountered suspected palatability problems in gilthead sea bream *Sparus auratus* (Sparidae), which demonstrated reduced appetence when offered PZQ-medicated feed. If PZQ was to be administered to commercially farmed *Seriola lalandi* by top coating of feed, strategies to mask the "flavour" of the medicated feed, including microencapsulation of PZQ prior to incorporation into feed, fasting fish for a period prior to treatment, or addition of flavour masking agents or attractants, may be required to improve feed palatability. It is important to note that the cost of microencapsulation may be high,

and fasting fish for any period is undesirable for farmers whose primary goal is to achieve maximum growth from their fish. Incorporating PZQ homogenously in the feed may overcome suspected palatability issues for some fish species. Hirazawa et al. (2004) incorporated PZQ into a pre-pellet mixture before pellets were passed through a disc pelleter and dried, but still observed reduced palatability in *V. variegatus* at higher doses. Kim et al. (2003) delivered PZQ via a moist pelleted feed to *Sebastes schlegeli* but did not report any reduction in palatability. In South Australia, *Seriola lalandi* are fed commercially produced extruded pellets. It is possible that medicated diets incorporating PZQ could be commercially manufactured. Extrusion, however, involves potentially destructive processes to medication such as pressure, humidity and high temperatures (Broz et al., 1997, Vertommen and Kinget, 1998). The need to clean extrusion equipment between production of medicated and non-medicated feeds is problematic. The risk of contamination of feed that was not meant to be medicated with PZQ would need to be thoroughly addressed. Further tests and experimentation are required to ensure that extrusion processes do not affect the activity of PZQ and that such a feed can be produced without contaminating commercial feed mills before a commercially medicated extruded pellet feed is developed. Incorporation of medications at the point of manufacture is not permitted in Japan. Consequently, farmers must apply medications themselves, usually by surface coating feed. PZQ is available to farmers in Japan as Hadaclean® to treat *B. seriolae* infestations of *S. quinqueradiata*. The Hadaclean® I used in Chapter 5 was not labelled with explicit instructions on how to prepare the medicated feed, but its package warns against attempting to feed fish at a dose higher than that recommended ($150 \text{ mg kg}^{-1} \text{ BW day}^{-1}$) because the product may affect the "taste" of the diet. In Chapter 5, a single dose of PZQ at 450 mg kg^{-1} BW day^{-1} administered by intubation achieved the highest efficacy against *B. seriolae* in my experimental trial. However it may not be possible to administer this dose in practice due to palatability issues.

A lower, more palatable daily dose of PZQ administered over a longer duration could be explored as an alternative to ensure fish do consume the required dose. In Chapter 7, the highest total dose ($50 \text{ mg kg}^{-1} \text{ BW day}^{-1}$) administered in a trial over 10 consecutive days equated to a total target dose of 500 mg kg^{-1} BW (see Chapter 7, Table 1). This is a higher total dose than administering PZQ at 150 mg kg^{-1} BW day^{-1} for 3 days, the recommended dose to treat *B. seriolae* in Japan with

Hadaclean®. In this example, administering a lower daily dose over a longer duration may allow the same or even a higher *total* dose of PZQ to be administered, without encountering palatability issues. In Chapter 7, as this dose gave the highest efficacy against recruited parasites, it should be investigated further to see if it has comparable efficacy against juvenile and adult *Z. seriolae* and *B. seriolae*.

3. Limitations to research

This project benefited from close collaboration with *S. lalandi* farms in Australia and an *S. quinqueradiata* farm in Japan. Working closely with industries in each locality allowed me to gain a better understanding of how an oral treatment might be utilised. Industry partners also provided access to fish and tank facilities to carry out field work. In Australia, trials were conducted in land-based hatcheries in between fingerling production seasons, typically between February and June. Fish were also sourced from sea-cages nearby which had naturally-acquired monogenean infestations. The access to large flow-through tank facilities and numbers of kingfish for trials would be difficult to procure through other means. It was not always possible to get the number of fish required for each season of field work due to availability of fish in the sea-cages or space in tanks at the hatchery. To minimise disruption to farm operations, often there was only one opportunity to transfer fish from sea-cages to land-based facilities, which limited the number of fish that I was able to acquire for trials.

There were also limitations to the numbers of fish that could be managed and sampled for parasites at any one time by one to two people. These factors contributed to the lack of replication of treatments in some trials. Only one replicate of 10 fish for each treatment was used in some trials (Trial 2 in Chapter 4, Chapter 5, and Chapter 8) and therefore if there had been any differences in parasite loads due to, for example, positioning of cages, these may have confounded the effect of the treatment. The duration of some trials, water temperature and location of experimental cages meant that it was impossible to prevent exposure of some experimental fish to parasite recruitment. This recruitment was accounted for by separating parasites counted at the end of the trial into life stages based on knowledge of temperature-related parasite growth (see Lackenby et al., 2007 for *B. seriolae* and Mooney et. al., 2008 for *H. heterocerca*). Ideally, all trials would have

been conducted in a closed system, not influenced by external factors such as currents, water temperature, weather and infestation pressure from external sources, with a completely randomised experimental design as used later in Chapter 6 and Chapter 7. Constraints, however, precluded this.

The dependence of my project on naturally infested fish also introduced variability to trials. In an ideal world, one would experimentally infest fish with a known number of parasites and then experimentally treat these fish. It would also be advantageous to have naïve fish infested with one generation of parasites, which would simplify quantification, and reduce the possible effect of not only muiltiple generations, but mixed species infections (in this case, co-infestation by either *Z. seriolae* and *B. seriolae* or *H. heterocerca* and *B. seriolae*). Monogeneans cannot be cultured *in vitro* and then transplanted to host fish. While other researchers have experimentally infested fish by exposing them to a known number of oncomiracidia, there is no guarantee that these larvae will survive, find a host, settle and then successfully parasitise all experimental fish uniformly. One might also argue that such a challenge model and stochastic procedure may itself introduce more complexity and possible confounding errors to the experiments. The advantage of using my approach of naturally infested fish allowed treatments to be tested against infections that occur in the farm environment.

As flow-through water systems in land-based facilities were used, water temperature could not be controlled and low water temperatures were experienced during some trials. Fish had reduced appetite at lower water temperatures and it was difficult at times to administer in-feed oral treatments, which contributed to my investigations into administering treatments by intubation. In Japan, trials were conducted in small cages attached to floating pontoons on a sea-cage farm. Trials were limited by availability of parasitised fish of suitable size, disease events occurring farm-wide and severe weather, especially a number of typhoons that occurred during the experimental period.

4. Current knowledge and future research

The close collaboration and sponsorship of this project by the industry also meant that its scope was focussed on evaluating the efficacy of oral treatments against monogeneans. A range of factors can influence treatment efficacy, not just

the choice of compound or dose. Many of these areas need additional research which will contribute to further development of oral treatments. This includes understanding why some compounds may work and why others do not, and evaluation of the optimal conditions in which they have efficacy and identifying new approaches for delivery. Much of this research will rest on an enhanced knowledge of parasite and host biology, physiology and metabolism.

4.1 Pharmacology of oral treatments

For an orally delivered compound to be effective against ectoparasites of fish, it must reach the parasite after ingestion by the fish host. For this to occur, it must be absorbed, sometimes metabolised by the host, and then reach the target tissues where the parasite resides in an active form at a sufficient dose. Compared with terrestrial animals, little is known about the bioavailability, uptake, assimilation and pharmacokinetics of oral anthelmintics in fish. I found that the benzimidazoles albendazole, FBZ and OXF had activity against *Z. seriolae* and FEB against *H. heterocerca*, but none was effective against *B. seriolae*. Elucidation of the tissue distribution of benzimidazoles in *Seriola* spp. may shed more light on whether this result is due to the lack of activity of these compounds against *B. seriolae*, or a failure of the compound to reach the external body surfaces where *B. seriolae* lives and feeds in a sufficient dose to be effective.

After oral administration to *S. lalandi*, PZQ is eliminated from the fish quickly, and less of the active compound is distributed to the skin of the fish compared to the plasma (Tubbs and Tingle, 2006a, b). These properties of PZQ may explain why the efficacy of all doses against the blood feeding polyopisthocotylean monogeneans *Z. seriolae* (Chapter 4) and *H. heterocerca* (Chapter 5) is high. Orally administered PZQ is effective against other polyopisthocotylean monogeneans (see Table 1a). Williams et al. (2007) found that oral PZQ had high efficacy (99.5-100%) against total *Z. seriolae* when intubated to *S. lalandi* in Australia. Efficacy against the epithelial feeding monopisthocotylean *B. seriolae*, however, is generally low.

Tubbs and Tingle (2006b) found that PZQ accumulation in the skin of *S. lalandi* is limited, probably due to the rapid clearance of the compound. If PZQ is metabolised too quickly for broad tissue distribution to occur, parasites may not be exposed to a sufficient dose for enough time for the compound to take effect,

146

contributing to the low efficacy of PZQ against *B. seriolae*. To overcome this, Tubbs and Tingle (2006b) suggested administering PZQ at shorter intervals than 24 h (i.e. administering oral treatment twice a day or more). It would be worthwhile to test whether this dosing strategy improves the efficacy of PZQ against *B. seriolae*. A greater understanding of the proportional distribution of PZQ in the skin and mucus of fish after oral administration would contribute to our understanding of the efficacy of PZQ against skin dwelling and feeding monogeneans such as *B. seriolae* and against monopisthocotyleans that live on gills and feed on gill epithelium such as dactylogyrids and diplectanids.

The pharmacokinetics of orally administered compounds in fish is likely to be influenced by water temperature. For example, Roy et al. (2006) found that the depletion of emamectin residues was faster at 15 ℃ in rainbow trout (*Oncorhynchus mykiss*) than at 6 ℃. Changes in residence time of a compound in a fish may change the duration a parasite is exposed to treatment. This may result in the same treatment regime displaying different efficacy at different water temperatures, therefore further work is required to evaluate the effect of water temperature on the efficacy of PZQ and benzimidazoles against monogeneans.

Table 1

Review of oral treatments using PZQ against (a) polyopisthocotylean, and (b) monopisthocotylean monogeneans. Note where multiple doses were reported, the dose with the highest efficacy is given.

(a)

Monogenean species	Host species	Dose of oral PZQ in mg kg^{-1} BW day^{-1} (days)	Method	Efficacy (% reduction)	Reference
Microcotyle sebastes	*Sebastes schlegeli*	200 (1)	Intubation	100	Kim et al. (1998)
Heterobothrium okamotoi	*Takfugu rubripes*	40 (20)	In feed	67.2	Hirazawa et al. (2000)
Sparicotyle chrysophrii	*Sparus auratus*	158 (6)	In feed	Reduction in *prevalence* from 90% to 40%. Efficacy not stated	Sitja-Bobadilla et al. (2006)
Zeuxapta seriolae	*Seriola lalandi*	50 (8)	Not stated	Not stated, but reports "elimination", implying 100%	Tubbs and Tingle (2006a)
Zeuxapta seriolae	*Seriola lalandi*	150 (3)	Intubation	100	Williams et al. (2007)
Heteraxine heterocerca	*Seriola quinqueradiata*	50, 100 & 150 (3)	Intubation	100	Williams (unpublished, see Chapter 5)
Zeuxapta seriolae	*Seriola lalandi*	150 (3) (co-administered with cimetidine 200 mg kg^{-1} BW day^{-1})	Intubation	99.8	Williams (unpublished, see Chapter 6)
Zeuxapta seriolae (recruits)	*Seriola lalandi*	50 (10)	Intubation	95.7	Williams (unpublished, see Chapter 7)

148

(b)

Monogenean species	Host species	Dose of oral PZQ in mg kg^{-1} BW day^{-1} (days)	Method	Efficacy (% reduction)	Reference(s)
Gyrodactylus sp.	*Oncorhynchus mykiss*	800 (10)	In feed	Efficacy not stated, but no effect reported	Tojo et al. (1992)
*Clemacotyle australis**	*Aetobatus narinari*	10-40 (1)	In feed	Efficacy not stated, but no effect reported	Janse and Borgsteed (2003)
Neobenedenia sp.	*Verasper variegatus*	40 (11)	In feed	66	Hirazawa et al. (2004)
Benedenia seriolae	*Seriola lalandi*	150 (3)	Intubation	97.7	Williams et al. (2007)
Benedenia seriolae	*Seriola quinqueradiata*	450 (1)	Intubation	77.8	Williams (unpublished, see Chapter 5)
Benedenia seriolae	*Seriola lalandi*	75 (3) (co-administered with cimetidine 200 mg kg^{-1} BW day^{-1})	Intubation	40.9	Williams (unpublished, see Chapter 6)
Benedenia seriolae (recruits)	*Seriola lalandi*	50 (10)	Intubation	88.3	Williams (unpublished, see Chapter 7)

*Named in this paper as *Clemacotyle australis* but probably misidentified and likely

to be *Dendromonocotyle torosa* (see Whittington and Chisholm, 2008).

Kim and Kim (2002) found that when PZQ was administered in combination with cimetidine (CIM) to *Sebastes schlegeli*, higher levels of PZQ were found in the plasma in comparison to an identical dose of PZQ alone and related the addition of CIM to an observed increase in efficacy of PZQ against the polyopisthocotylean *Microcotyle sebastis*. In Chapter 6, I found that PZQ had very high efficacy against the polyopisthocotylean *Z. seriolae,* regardless of whether it was administered with or without CIM. In Chapter 6, I found that when PZQ was administered with CIM, it was less effective against the skin fluke *B. seriolae*. The effect of CIM on the bioavailability and clearance time of PZQ in fish skin is unknown and research is required because it may help explain these unexpected results.

Due to the short half-life of PZQ observed in *Seriola lalandi*, Tubbs and Tingle (2006a) suggested that the clearance time of PZQ in *S. lalandi* was likely to be much faster than in other fish species such as *Oncorhynchus mykiss* (Salmonidae) or *Sebastes schlegeli* (Sebastidae) due to differences in physiology and metabolism.

Seriola lalandi (Carangidae) is a pelagic predator whereas *Sebastes schlegeli* is a nearshore demersal species and their physiologies are likely to differ.

Although *Seriola lalandi* and *S. quinqueradiata* are closely related and likely to have similar physiology, there may be differences between the two species that affect their uptake and metabolism of PZQ. This may have contributed to differences in efficacy of PZQ observed against *B. seriolae* on each host species in Chapters 4 and 5. In Japan, S. *quinqueradiata* are wild-caught as juveniles before transfer to sea-cages in Japan, whereas in Australia *S. lalandi* are hatchery-reared before transfer to sea-cages. Japanese yellowtail juveniles are, therefore, likely to have been exposed to infestation by *B. seriolae* and *H. heterocerca* prior to capture, unlike hatchery-reared kingfish in Australia. Whether this difference contributed towards the observed differences in efficacy of PZQ against *B. seriolae* from each *Seriola* species is unknown. While we can presume that the uptake and pharmacokinetics of PZQ in *S. quinqueradiata* is similar to *S. lalandi*, experimentation is required to confirm this. There were also likely to be differences in paste formulations, which may have affected the bioavailability of PZQ.

As proposed by Tubbs and Tingle (2006b) for PZQ, further research is required to determine whether a threshold of exposure (the actual "dose") or cumulative exposure (administration over a consecutive number of days) is needed for each anthelmintic to achieve efficacy against monogenean parasites. In Chapter 4, the mean parasite abundance of *Z. seriolae* parasitising *S. lalandi* treated for 3 days with orally administered fenbendazole at 150 mg kg^{-1} BW day^{-1} in Trial 1 was not significantly less than control fish (Table 2c), but significantly fewer *Z. seriolae* were found on *S. lalandi* administered fenbendazole for 6 days at 50 and 75 mg kg^{-1} BW day^{-1}. Kimura et al. (2006) reported that febantel was more effective against *Heterobothrium okamotoi* infestations of *Takifugu rubripes* (Tetradontidae) when administered consecutively at 50 mg kg^{-1} BW day^{-1} for 2 days, rather than in a single one-off dose of 25, 50 or 100 mg kg^{-1} BW. Kimura et al. (2007), furthermore, found that the number of *H. okamotoi* did not decrease until 3 days into a 5-day course of febantel at 25 mg kg^{-1} BW day^{-1}. Tojo and Santamarina (1998b) reported that triclabendazole did not appear to have an effect against *Gyrodactylus* sp. until after 5 consecutive days of a 10-day treatment. In Chapter 8, I reported that triclabendazole did not appear to have activity against *Z. seriolae* or *B. seriolae*, but it was only administered for 2 days. It is possible that some benzimdazoles need to be

administered consecutively for a number of days to have a significant effect against monogeneans. It is unclear, however, if the efficacy was improved from accumulation of product to a therapeutic threshold or lengthened exposure duration. This information is essential when refining an optimal dosing strategy, e.g. a one-off dose compared with consecutive dosing. Consecutive dosing may also allow the required treatment dose to be administered when higher daily doses are rejected by fish, e.g. if medication affects feed palatability at higher doses.

4.2 Monogenean biology

While we have a thorough knowledge of the biology of one species of monopisthocotylean monogenean *Entobdella soleae* (see Kearn, 2002), the feeding habits of most other monogenean species are poorly documented (Whittington and Chisholm, 2008). It is very likely that differences between the blood-feeding polyopisthocotyleans *Z. seriolae* and *Heteraxine heterocerca* and the mucus and epithelial grazer *B. seriolae* also contribute to observed differences in efficacy of treatments fed to fish against these parasites.

The quantity of host tissue that monogenean parasites consume has not been well documented; it is likely, however, that it is related to parasite size (Whittington and Chisholm, 2008). Monogeneans are thought to begin feeding on their host soon after settling (Whittington and Chisholm, 2008). Ogawa et al. (2005) found that the polyopisthocotylean *Heterobothrium okamotoi* increased the amount of blood it consumed as it developed, and it is likely that this also occurs in *Heteraxine heterocerca* and *Z. seriolae*. Larval parasites recruiting to the fish may, therefore, have to reach a certain size before they ingest enough anthelmintic fed to their fish host to receive a sufficient dose of medicine to have an effect. This may explain the low efficacy of PZQ against recruited *H. heterocerca* and *B. seriolae* in Chapter 5 (Tables 3a, 3b and 3c), why PZQ was not completely effective against recruited life stages of *Z. seriolae* and *B. seriolae* in Chapter 7 and why no treatment of PZQ (whether with or without CIM) appeared to be effective against recruited *B. seriolae* in Chapter 6. The frequency of feeding by *Z. seriolae*, *H. heterocerca* and *B. seriolae* is also unknown, but it is thought that they do not feed continuously (Whittington and Chisholm, 2008). This information, combined with

pharmacokinetic data, would also help us understand efficacy results and improve treatment design.

While no studies have documented what life stages of *B. seriolae* reside on the eyes of *Seriola* spp., newly-settled *B. seriolae* seem to appear first on the eyes of *S. lalandi* and *S. quinqueradiata* (pers. obs.). This may be because small *B. seriolae* are easier to detect on the eyes than on other body surfaces. The eyes of fish (like mammals), however, are thought to be a region where host barriers prevent a host immune response (Sitja-Bobadilla, 2008). Furthermore the cornea does not have mucous cells (Kearn, 1999) or a blood supply, both of which are immunologically active (Buchmann, 1999; Whittington and Chisholm, 2008). This may mean the eyes of fish have limited immunologically activity compared with other sites (Llewellyn, 1957). By settling on or migrating to the eyes, larval *B. seriolae* may be able to establish and develop while avoiding the fish's immune system (as well as escaping exposure to orally administered treatments such as PZQ), unless they migrate onto the body surfaces, where mucous cells are immunologically active. Takuro and Hitoshi (2000) reported that *B. seriolae* could not be "exterminated" from the eyes of *S. quinqueradiata* following oral treatment with PZQ (as Hadaclean[®]) and suggested that this product may only be used as a "subsidiary" anthelmintic. It is unknown how much PZQ, if any, reaches the surfaces of fish eyes after oral administration. These data, in addition to knowledge of the type, amount and rate of tissues consumed when *Z. seriolae* and *B. seriolae* are feeding, would help us understand the observed efficacy of PZQ against recruits.

4.3 New technologies

Like *B. seriolae*, the capsalid monogenean *Neobenedenia* sp. has been identified as a serious pathogen of *Seriola* spp. in Japan (Ogawa and Yokoyama, 1998) and can be treated with freshwater bathing (Leong, 1997) or by orally administered praziquantel at 150 mg kg^{-1} BW day^{-1} (Hirazawa et al., 2006). Problems with these treatments, however, have directed research into alternatives to chemotherapeutants. Methods to interrupt key processes in parasite biology such as reproduction and egg-laying have been investigated. Hirazawa et al. (2006) characterised some serine proteases of *Neobenedenia* sp. and found that by applying a proteinase inhibitor, egg laying in mature worms was suspended and swimming of

hatched oncomiracidia was affected. Ohashi et al. (2007) demonstrated that double-stranded RNA interference of *vasa* (*vas*)-related genes in germ cells of *Neobenedenia* sp. resulted in interference of gametogenesis producing sterile worms. The severity of amoebic gill disease (AGD) in *Salmo salar* varies with major histocompatibility polymorphisms (Wynne et al., 2008), suggesting some fish are genetically more resistant to AGD. The same may occur with *Seriola* spp. and monogenean parasites, and selective breeding for fish that are naturally resistant would have long term benefits. Ohno et al. (2008) found that *Seriola dumerili* was more susceptible to *Neobenedenia* sp. than its close relative *S. quinqueradiata*. It is not known whether differences in susceptibility to *Z. seriolae*, *H. heterocerca* and *B. seriolae* exist within and between different *Seriola* spp., and this topic should be explored. If a genetic component can be identified, then selective breeding or genetic manipulation can be pursued to improve natural resistance of cultured stocks.

While these technologies are still in their early phases of development, these methods could also be considered for *Z. seriolae*, *H. heterocerca* and particularly *B. seriolae*, a capsalid monogenean that like *Neobenedenia* sp., has proven difficult to treat by other methods. Selective breeding and genetic manipulation for host resistance, anthelmintic vaccines and the identification of "neutraceutical" plants with anthelmintic activity that can be fed to animals are just some approaches being investigated to helminth parasites of animals (Waller, 2006). This research should be closely monitored to identify technologies that may also be developed for application to aquaculture.

5. Registration

Much information is required when applying for registration of a veterinary medication. The APVMA Veterinary Manual of Requirements and Guidelines (VetMORAG, 2009) provides information on data requirements and guidelines for applications to register or approve veterinary chemical products, labels, active constituents and issue of permits in Australia. My research fits primarily into the category of dose optimisation of PZQ and the benzimidazoles FEB, FBZ and OXF. The next steps towards the registration of these compounds would be confirmation of the optimum dose for treatment, then field trials to confirm efficacy under 'real' (= sea-cage) conditions. Target animal safety must also be addressed, with a focus on

calculating the margin of safety or the difference between the recommended dose and the minimum dose that produces toxicity in target animals. Any effects by factors such as sex, age, nutritional status and animal husbandry that may affect the safety of medication use also need to be investigated. The environmental safety and toxicity (ecotoxicty) to non-target organisms also needs careful consideration, including the evaluation of environmental residues. The pharmacology of medications in fish is mostly unknown (see above) and further research would not only aid in optimising treatment efficacy but is necessary to determine depletion of medication residues in fish tissues and the appropriate treatment withdrawal time.

6. The future of treatment for monogeneans parasites in the culture of *Seriola* spp.

Worldwide, consumers of high-end and luxury seafood products have become increasingly informed and aware of their product selections and are often willing to pay more for products that are seen as "environmentally friendly". For this reason, it has been identified as a goal for the South Australian *S. lalandi* industry to be recognised internationally as "clean and green" (Hernen and Hutchinson, 2003). Careful consideration must be given, therefore, to the use of chemicals in the production of *S. lalandi* to uphold potential market advantage and trends in consumer choice should be monitored.

The development of oral treatments for monogenean parasites of sea-caged *Seriola* spp. could greatly assist the development of the industry, but chemicals alone will not solve the problem of monogeneans in aquaculture and should not be seen as a "silver bullet". Although oral treatments are often promoted as an alternative to bathing, it is likely that they will be used to augment bath treatments, or *vice versa*. An information brochure for Benesal® (Kyowa Hakko, Japan) (a commercial oral formula of praziquantel equivalent to Hadaclean® released in Japan for the treatment of *B. seriolae*) instructs users to maintain "traditional" management practices including net changes and freshwater bathing, while administering Benesal® as an oral treatment. It is also important to recognise that the information required to apply for registration of a treatment is time consuming and costly to obtain, and there are no guarantees that the application for registration will be accepted. The development

of Calicide[®], an oral treatment for sea lice, for example, cost approximately £3.4 million (see Chapter 2).

Regardless of the method of treatment used, efficient management of monogeneans will only be achieved through the informed use of treatments. Aspects of monogenean biology including an intimate knowledge of their lifecycles and transmission dynamics is required. The pharmacology of compounds in *Seriola* spp. is another area of research that requires further attention. Only through cooperation between industry, regulatory authorities, manufacturers of treatments and researchers will effective management of monogeneans be achieved (Denholm et al., 2002). Periodic review of these practices must be conducted to ensure that efficacy has been maintained and that conditions for use are being adhered to, and the development of new technologies to aid parasite control, especially those that do not rely solely upon chemical treatments, should be closely followed.

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REFERENCES

Anonymous, 1999. Calicide (Teflubenzuron) - Authorisation for use as an in-feed sea lice treatment in marine cage salmon farms. Risk Assessment, EQS and Recommendations. Scottish Environment Protection Agency Fish Farming Advisory Group, pp. 15.

Anonymous, 2000. Bayer and Kyowa Hakko announce development of oral ectoparasiticide for aquacultural use. Kyowa Hakko Kirin Co., Ltd. http://www.kyowa-kirin.co.jp/english/news/kyowa/er000307.html Accessed 24 January 2009

Anonymous, 2002a. State of World Fisheries and Aquaculture. FAO Fisheries and Aquaculture Department, Rome.

Anonymous, 2002b. Yellowtail kingfish aquaculture in SA. PIRSA. http://www.pir.sa.gov.au/pages/aquaculture/finfish/yellowtail_fs.pdf Accessed 4 March 2003

Anonymous, 2002c. Undecylenic acid. Alternative Medicine Review 7, 68-70.

Anonymous, 2005. The Merck Veterinary Manual. Merck and Co., Inc. http://www.merckvetmanual.com/mvm/index.jsp. Accessed 4 June 2009

Anonymous, 2008. The State of the World Fisheries and Aquaculture. FAO Fisheries and Aquaculture Department, Rome, pp. 196.

APVMA, 2004. Permit to allow the conduct of small-scale trials with Agvet chemicals. Australian Pesticides and Veterinary Medicines Authority, permit number 7250.

Barton, B.A., Iwama, G.K., 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Ann. Rev. Fish Dis. 1, 3-26.

Barton, B.A., 2002. Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. Integ. Comp. Biol. 42, 517-525.

Berg, A.-G.T., Horsberg, T.E., 2009. Plasma concentrations of emamectin benzoate after Slice[TM] treatments of Atlantic salmon (*Salmo salar*): Differences between fish, cages, sites and seasons. Aquaculture 288, 22-26.

Bondad-Reantaso, M.G., Ogawa, K., Yoshinaga, T., Wakabayashi, H., 1995. Acquired protection against *Neobenedenia girellae* in Japanese Flounder. Fish Pathol. 30, 233-238.

Branson, E.J., Ronsberg, S.S., Ritchie, G., 2000. Efficacy of teflubenzuron (Calicide®) for the treatment of sea lice, *Lepeophtheirus salmonis* (Kroyer 1838), infestations of farmed Atlantic salmon (*Salmo salar* L.). Aquac. Res. 31, 861-867.

Broz J, Schai E., Gadient M., 1997. Micronutrient stability in feed processing. American Soybean Association Technical Bulletin. Report No. FT42-1997

Buchmann, K., 1999. Immune mechanisms in fish against monogeneans - a model. Folia Parasitol. 46, 1-9.

Buchmann, K., Lindenstrom, T., 2002. Interactions between monogenean parasites and their fish hosts. Int. J. Parasit. 32, 309-319.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis *et al.* revisited. J. Parasitol. 83, 575-583.

Chambers, C.B., Ernst, I., 2005. Dispersal of the skin fluke *Benedenia seriolae* (Monogenea: Capsalidae) by tidal currents and implications for sea-cage farming of *Seriola* spp. Aquaculture 250, 60-69.

Conte, F.S., 2004. Stress and the welfare of cultured fish. Appl. Anim. Behav. Sci. 86, 205-223.

Dachman, W.D., Adubofour, K.O., Bikin, D.S., Johnson, C.H., Mullin, P.D., Winograd, M., 1994. Cimetidine-induced rise in the praziquantel levels in a patient with neurocysticercosis being treated with anticonvulsants. J. Infect. Dis. 169, 689-691.

Davies, I.M., Rodger, G.K., 2000. A review of the use of ivermectin as a treatment for sea lice [*Lepeophtheirus salmonis* (Kroyer) and *Caligus elongatus* Nordmann] infestation in farmed Atlantic salmon (*Salmo salar* L.)]. Aquac. Res. 31, 869-883.

Davis, K.B., 2006. Management of physiological stress in finfish aquaculture. N. Am. J. Aquac. 68, 116-121.

Day, T.A., Bennett, J.L., Pax, R.A., 1992. Praziquantel: The enigmatic antiparasitic. Parasitol. Today 8, 342-344.

Denholm, I., Devine, G.J., Horsberg, T.E., Sevatdal, S., Fallang, A., Nolan, D.V., Powell, R., 2002. Analysis and management of resistance to

158

chemotherapeutants in salmon lice, *Lepeoptheirus salmonis* (Copepoda: Caligidae). Pest Manag. Sci. 58, 528-536.

Deveney, M.R., Chisholm, L.A., Whittington, I.D., 2001. First published record of the pathogenic monogenean parasite *Neobenedenia melleni* (Capsalidae) from Australia. Dis. Aquat. Org. 46, 79-82.

Dhihendra, P.T., 2009. *Seriola quinqueradiata* (Temminck and Schlegel, 1845). FAO Fisheries and Aquaculture Department (online). http://www.fao.org/fishery/culturedspecies/Seriola_quinqueradiata/en Accessed 15 January 2009

Divett, B., 2003. Medium chain fatty acids: A natural remedy for monogenean parasite infestations in the culture of yellowtail kingfish (*Seriola lalandi*)? Honours report. Environmental Biology, The University of Adelaide.

Duston, J., Cusack, R.R., 2002. Emamectin benzoate: an effective in-feed treatment against the gill parasite *Salmincola edwardsii* on brook trout. Aquaculture 207, 1-9.

Egusa, S., 1983. Disease problems in Japanese yellowtail, *Seriola quinqueradiata*, culture: a reveiw. Rapp. P.-v Ren. Cons. int Explor. Mer 182, 10-18.

Ernst, I., Whittington, I.D., 1996. Hatching rhythms in the capsalid monogeneans *Benedenia lutjani* from the skin and *B. rohdei* from the gills of *Lutjanus carponotatus* at Heron Island, Queensland, Australia. Int. J. Parasit. 26, 1191-1204.

Ernst, I., Whittington, I.D., Corneillie, S., Talbot, C., 2002. Monogenean parasites in sea-cage aquaculture. Austasia Aquacult., 46-48.

Ernst, I., Whittington, I.D., Corneillie, S., Talbot, C., 2005. Effects of temperature, salinity, desiccation and chemical treatments on egg embryonation and hatching success of *Benedenia seriolae* (Monogenea: Capsalidae), a parasite of farmed *Seriola* spp. J. Fish Dis. 28, 157-164.

Findlay, V.L., Munday, B.L., 2000. The immunomodulatory effects of levamisole on the nonspecific immune system of Atlantic salmon, *Salmo salar* L. J. Fish Dis. 23, 369-378.

Findlay, V.L., Zilberg, D., Munday, B.L., 2000. Evaluation of levamisole as a treatment for amoebic gill disease of Atlantic salmon, *Salmo salar* L. J. Fish Dis. 23, 193-198.

Florent, R.L., Baker, J.A., Powell, M.D., 2007. Efficacy of bithionol as an oral treatment for amoebic gill disease in Atlantic salmon *Salmo salar* (L) (2007). Aquaculture 270, 15-22.

García, A., Díaz, M.V., 1995. Culture of *Seriola dumerilii*, Seminar of the CIHEAM network on technology of aquaculture in the Mediterranean (TECAM) Cypress. CIHEAM - Options Méditerranéennes, Zaragosa, Spain, pp. 103-114.

Grant, A.N., 1993. Basic husbandry on fish farms. In: Brown, L.A. (Ed.), Aquaculture for veterinarians, fish husbandry and medicine. Pergamon Press, Oxford, pp. 31-41.

Grant, A.N., 2002. Medicines for sea lice. Pest Manag. Sci. 58, 521-527.

Grau, A., Riera, F., Carbonell, E., 1999. Some protozoan and metazoan parasites of the amberjack from the Balearic sea (western Mediterranean). Aquac. Int. 7, 307-317.

Grau, A., Crespo, S., Pastor, E., Gonzalez, P., Carbonell, E., 2003. High infection by *Zeuxapta seriolae* (Monogenea: Heteraxinidae) associated with mass mortalities of amberjack *Seriola dumerili* Risso reared in sea cages in the Balearic Islands (western Mediterranean). Bull. Eur. Assoc. Fish Pathol. 23, 139-142.

Greenberg R.M. 2005. Are Ca2+ channels targets of praziquantel action? Int. J. Parasitol. 35:1-9

Harder, A., 2002. Milestones of helmintic research at Bayer. Parasitol. Res. 88, 477-480.

Harris, P.D., Soleng, A., Bakke, T.A., 2000. Increased susceptibility of salmonids to the monogenean *Gyrodactylus salaris* following the administration of hydrocortisone acetate. Parasitology 120, 57-64.

Hernen, M., Hutchinson, W., 2003. Yellowtail kingfish strategic research and development plan 2003-2008. Report prepared for the Fisheries Research and Development Corporation and South Australian Marine Finfish Farmers Association Incorporated.

Hikasa, Y., Takase, K., Ogasawara, T., Ogasawara, S., 1986. Anesthesia and recovery with tricaine methanesulfonate, eugenol and thiopental sodium in the carp, *Cyprinus carpio*. Japanese Journal of Veterinary Research 48, 341-351.

Hirazawa N., Ohtaka T., Hata K., 2000. Challenge trials on the anthelmintic effect of drugs and natural agents against the monogenean *Heterobothrium okamotoi* in the tiger puffer *Takifugu rubripes*. Aquaculture 188:1-13

Hirazawa, N., Oshima, S., Hara, T., Mitsuboshi, T., Hata, K., 2001a. Antiparasitic effect of medium-chain fatty acids against the ciliate *Cryptocaryon irritans* infestation in the red sea bream *Pagrus major*. Aquaculture 198, 219-228.

Hirazawa, N., Oshima, S., Mitsuboshi, T., Hata, K., 2001b. The anthelmintic effect of medium-chain fatty acids against the monogenean *Heterobothrium okamotoi* in the tiger puffer *Takifugu rubripes*: evaluation of doses of caprylic acid at different water temperatures. Aquaculture 195, 211-223.

Hirazawa N., Mitsuboshi T., Hirata T., Shirasu K., 2004. Susceptibility of spotted halibut *Verasper variegatus* (Pleuronectidae) to infection by the monogenean *Neobenedenia girellae* (Capsalidae) and oral therapy trials using praziquantel. Aquaculture 238:83-95

Hirazawa, N., Umeda, N., Hatanaka, A., Kuroda, A., 2006. Characterization of serine proteases on the monogenean *Neobenedenia girellae*. Aquaculture 255, 188-195.

Hormazabal V., Yndestad M., 1995. Determination of praziquantel in medicated fish feed and sediment by HPLC. J Liq Chromatogr 18:1231-1238

Iosifidou, E.G., Haagsma, N., Tanck, M.W.T., Boon, J.H., Olling, M., 1997. Depletion study of fenbendazole in rainbow trout (*Oncorhynchus mykiss*) after oral and bath treatment. Aquaculture 154, 191-199.

Janse, M., Borgsteede, F.H.M., 2003. Praziquantel treatment of captive white-spotted eagle rays (*Aetobatus narinari*) infested with monogean trematodes. Bull. Eur. Assoc. Fish Pathol. 23, 152-156.

Jung, H., Medina, R., Castro, N., Corona, T., Sotelo, J., 1997. Pharmacokinetic study of praziquantel administered alone and in combination with cimetidine in a single-day therapeutic regimen. Antimicrob. Agents Chemother. 41, 1256-1259.

Kearn, G.C., Ogawa, K., Maeno, Y., 1992. Egg production, the oncomiracidium and larval development of *Benedenia seriolae*, a skin parasite of the yellowtail, *Seriola quinqueradiata*, in Japan. Publ. Seto Mar. Biol. Lab. 35, 351-362.

Kearn, G.C., 1999. The survival of monogenean (platyhelminth) parasites on fish skin. Parasitology 119, S57-S88.

Kearn, G., 2002. *Entobdella soleae* - pointers to the future. Int. J. Parasit. 32, 367-372.

Kim, K.H., Choi, E.S., 1998. Treatment of *Microcotyle sebastis* (Monogenea) on the gills of cultured rockfish (*Sebastes schlegeli*) with oral administration of mebendazole and bithionol. Aquaculture 167, 115-121.

Kim K.H., Park S.I., Jee B.Y., 1998. Efficacy of oral administration of praziquantel and mebendazole against *Microcotyle sebastis* (Monogenea) infestation of cultured rockfish (*Sebastes schlegeli*). Fish Pathol 33:467-471

Kim K.H., Cho J.B., 2000. Treatment of *Microcotyle sebastis* (Monogenea: Polyopisthocotylea) infestation with praziquantel in an experimental cage simulating commercial rockfish *Sebastes schlegeli* culture conditions. Dis Aquat Org 40:229-231

Kim, K.H., Kim, C.S., Kim, J.W., 2001a. Depletion of praziquantel in plasma and muscle tissue of cultured rockfish *Sebastes schlegeli* after oral and bath treatment. Dis. Aquat. Org. 45, 203-207.

Kim KH, Lee EH, Kwon SR, Cho JB 2001b Treatment of *Microcotyle sebastis* infestation in cultured rockfish *Sebastes schlegeli* by oral administration of praziquantel in combination with cimetidine. Dis Aquat Org 44:133-136

Kim K.H., Kim C.S., 2002. Cimetidine enhances the plasma praziquantel concentration and treatment efficacy against *Microcotyle sebastis* in cultured rockfish *Sebastes schlegeli*. Dis Aquat Org 49:45-49

Kim C.S., Cho J.B., Ahn K.J., Lee J.I., Kim K.H., 2003. Depletion of praziquantel in muscle tissue and skin of cultured rockfish (*Sebastes schlegeli*) under the commercial culture conditions. Aquaculture 219:1-7

Kimura, T., Sameshima, M., Nomura, Y., Morita, J., Mizoguchi, H., Ishihara, M., 2006. Efficacy of orally administered febantel against monogenean *Heterobothrium okamotoi* infection of cultured tiger puffer *Takifugu rubripes*. Fish Pathol. 41, 147-151.

Kimura, T., Morita, J., Nomura, Y., Enomoto, J., Mizoguchi, H., Ishihara, M., 2007. Concentration-time profile of febantel in cultured tiger puffer *Takifugu rubripes*. Aquaculture Science 55, 279-284.

Kohler, P., 2001. The biochemical basis of anthelmintic action and resistance. Int. J. Parasit. 31, 336-345. Lankas, G.R., Gordon, L.R. Toxicology. In: Campbell, W.C. (Ed.), Ivermectin and Abamectin. Springer-Verlag, NY.

Lackenby, J.A., Chambers, C.B., Ernst, I., Whittington, I.D., 2007. Effect of water temperature on reproductive development of *Benedenia seriolae* (Monogenea: Capsalidae) from *Seriola lalandi* in Australia. Dis. Aquat. Org. 74, 235-242.

Lankas, G.R., Gordon, L.R. Toxicology. In: Campbell, W.C. (Ed.), Ivermectin and Abamectin. Springer-Verlag, NY.

Leong, T.S., 1997. Control of parasites in cultured marine finfishes in Southeast Asia - an overview. Int. J. Parasit. 27, 1177-1184.

Levine, M., Law, E.Y., Bandiera, S.M., Chang, T.K., Bellward, G.J., 1998. *In vivo* cimetidine inhibits hepatic CYP2C6 and CYP2C11 but not CYP1A1 in adult male rats. J. Pharmacol. Exp. Ther. 284, 493-499.

Llewellyn, J., 1957. Host-specificity in monogenetic trematodes. In: Baer, J.G. (Ed.), First Symposium on Host-Specificity Among Parasites of Invertebrates, Neuchatel, Switzerland, pp. 199-212.

Love, G., Langenkamp, D., 2003. Australian Aquaculture: Industry Profiles for Related Species. ABARE E-Report 03.8, prepared for the Fisheries Research Fund, Canberra

Luzardo-Alvarez, A., Martinez-Mazagatos, J., Santamarina-Fernadez, M.T., Otero-Espinar, F.J., Blanco-Mendez, J., 2003. Oral pharcmacological treatments for ichthyopthiriosis of rainbow trout *(Onchorhynchus mykiss)*. Aquaculture 220, 15-25.

Lynn, R.C., 2008. Antiparasitic drugs. In: Bowman, D.D. (Ed.), Georgis' Parasitology for Veterinarians. Elsevier Science, Ithaca.

Mansell, B., Powell, M.D., Ernst, I., Nowak, B.F., 2005. Effects of the gill monogenean *Zeuxapta seriolae* (Meserve, 1938) and treatment with hydrogen peroxide on pathophysiology of kingfish, *Seriola lalandi* Valenciennes,1833. J. Fish Dis. 28, 253-262.

Mazzola, A., Favaloro, E., Sara, G., 2000. Cultivation of the Mediterranean amberjack, *Seriola dumerili* (Risso, 1810), in submerged cages in the Western Mediterranean Sea. Aquaculture 181, 257-268.

Montero, F.E., Crespo, S., Padros, F., De la Gandara, F., Garcia, A., Raga, J.A., 2004. Effects of the gill parasite *Zeuxapta seriolae* (Monogenea : Heteraxinidae) on the amberjack *Seriola dumerili* Risso (Teleostei : Carangidae). Aquaculture 232, 153-163.

Mooney, A.J., Ernst, I., Whittington, I.D., 2006. An egg-laying rhythm in *Zeuxapta seriolae* (Monogenea: Heteraxinidae), a gill parasite of yellowtail kingfish (*Seriola lalandi*). Aquaculture 253, 10-16.

Mooney, A.J., Ernst, I., Whittington, I.D., 2008. Egg-laying patterns and *in vivo* egg production in the monogenean parasites *Heteraxine heterocerca* and *Benedenia seriolae* from Japanese yellowtail *Seriola quinqueradiata*. Parasitology 135, 1295-1302.

Munday, B.L., Zilberg, D., 2003. Efficacy of, and toxicity associated with, the use of levamisole in seawater to treat amoebic gill disease. Bull. Eur. Assoc. Fish Pathol. 23, 3-6.

Mushiake, K., 1999. Achieving advanced maturation and spawning in yellowtail *Seriola quinqueradiata* by the manipulation of photoperiod and water temperature. In: Tamaru, C. (Ed.), UJNR Aquaculture, Kihei, Hawaii.

Nakada, M., 2002. Yellowtail cutlure development and solutions for the future. Reviews in Fisheries Science 10, 559-575.

Nash, C.E., Iwamoto, R.N., Mahnken, C.V.W., 2000. Aquaculture risk management and marine mammal interactions in the Pacific Northwest. Aquaculture 183, 307-323.

Noga, E.J., 2000. Fish disease: Diagnosis and treatment. Iowa State University Press, Ames, USA, 367 pp.

Noga, E.J., Levy, M.G., 2006. Protozoan and Metazoan Infections. In: Woo, P.T.K. (Ed.), Fish Disease and Disorders. CABI Publishing, Oxfordshire, UK, pp. 16-45.

Nordmo, R., 1993. The Veterinary approach to salmon farming in Norway. In: Brown, L.A. (Ed.), Aquaculture for veterinarians, fish husbandry and medicine. Pergamon Press, Oxford, pp. 179-191.

Norwood, C., 2008. Science combats kingfish jaw deformity. FISH: Fisheries Research and Development Corporation News 16, 31.

Ohashi, H., Umeda, N., Hirazawa, N., Ozaki, Y., Miura, C., Miura, T., 2007. Expression of *vasa* (*vas*)-related genes in germ cells and specific interference with gene functions by double-stranded RNA in the monogenean, *Neobenedenia girellae*. Int. J. Parasit. 37, 515-523.

Ohno, Y., Kawano, F., Hirazawa, N., 2008. Susceptibility by amberjack (*Seriola dumerili*), yellowtail (*S. quinqueradiata*) and Japanese flounder (*Paralichthys*

olivaceus) to *Neobenedenia girellae* (Monogenea) infection and their acquired protection. Aquaculture 274, 30-35.

Ogawa, K., Fukudome, M., 1994. Mass mortality caused by blood fluke (*Paradeontacylix*) among amberjack (*Seriola dumerili*) imported to Japan. Fish Pathol. 29, 265-269.

Ogawa, K., 1996. Marine parasitology with special reference to Japanese fisheries and mariculture. Vet. Parasitol. 64, 95-105.

Ogawa, K., Yokoyama, H., 1998. Parasitic diseases of cultured marine fish in Japan. Fish Pathol. 33, 303-309.

Ogawa, K., Yamabata, N., Yoshinaga, T., 2005. Egg-laying of the monogenean *Heterobothrium okamotoi* on experimentally infected Tiger puffer *Takifugu rubripes*. Fish Pathol. 40, 111 - 118.

Palmer, R., Rodger, H., Drinan, E., Dwyer, C., Smith, P.R., 1987. Preliminary trials on the efficacy of Ivermectin against parasitic copepods of Atlantic salmon. Bull. Eur. Assoc. Fish Pathol. 7, 47-54.

Paperna, I., 1991. Diseases caused by parasites in the aquaculture of warm water fish. Ann. Rev. Fish Dis. 1, 155-194.

Pike, A.W., Wadsworth, S.L., 1999. Sealice on salmonids: Their biology and control. In: Baker, J.R., Muller, R., Rollinson, D. (Eds.), Advances in Parasitology. Academic Press, London, pp. 233-337.

Plumb, D.C., 1991. Veterinary Drug Handbook. Pharmvet Publishing, White Bear Lake, Minnesota, 688 pp.

Raa, J., 2000. The use of immune-stimulants in fish and shellfish feeds. In: Cruz-Suarez, L.E., Ricque-Marie, D., Tapia-Salszar, M., Olvera-Novoa, M.A., Civera-Cerecedo, R. (Eds.), Avances en Nutrición Acuicola V: Memorias del V Simposium Internacional de Nutrición Acuícola Nutrición Acuicola, Mérida, Yucatan, México.

Rae, G.H., 1999. Sea lice, medicines and a natural strategy for control. Fish Veterinary Journal 3, 46-51.

Rae, G.H., 2002. Sea louse control in Scotland, past and present. Pest Manag. Sci. 58, 515-520.

Ramstad, A., Colquhoun, D.J., Nordmo, R., Sutherland, I.H., Simmons, R., 2002. Field trials in Norway with SLICE[(R)] (0.2% emamectin benzoate) for the oral

treatment of sea lice infestation in farmed Atlantic salmon *Salmo salar*. Dis. Aquat. Org. 50, 29-33.

Ridtitid W., Wongnawa M., Mahatthanatrakul W., Panyo J., Sunbhainch M.,. Rifampin markedly decreases plasma concentrations of praziquantel in healthy volunteers. Clin. Pharm. Therap., 2002, 72(5), 505-513

Ritchie, G., Ronsberg, S.S., Hoff, K.A., Branson, E.J., 2002. Clinical efficacy of teflubenzuron (Calicide[(R)]) for the treatement of *Lepeophtheirus salmonis* infestations of farmed Atlantic salmon *Salmo salar* at low water temperatures. Dis. Aquat. Org. 51, 101-106.

Reinemeyer, C.R., Courtney, C.H., 2001. Anticestodal and Antinematodal Drugs. In: Adams, H.R. (Ed.), Veterinary Pharmacology and Therapeutics. Blackwell Publishing, Ames, Iowa, pp. 980-991.

Roy, W.J., Sutherland, I.H., Rodger, H.D.M., Varma, K.J., 2000. Tolerance of Atlantic salmon, *Salmo salar* L., and rainbow trout, *Oncorhynchus mykiss* (Walbaum), to emamectin benzoate, a new orally administered treatment for sea lice. Aquaculture 184, 19-29.

Roy, W.J., Gillan, N., Crouch, L.S., Parker, R., Rodger, H., Endris, R., 2006. Depletion of emamectin residues following oral administration to rainbow trout, *Oncorhynchus mykiss*. Aquaculture 259, 6-16.

Sakai, M., 1999. Current research status of fish immunostimulants. Aquaculture 172, 63-92.

Sanmartín Duran, M.L., Caamano-García, F., Fernández Casal, J., Leiro, J., Ubeira, F.M., 1989. Anthelminthic activity of praziquantel, niclosamide, netobimin and mebendazole against *Bothriocephalus scorpii* naturally infecting turbot (*Scophthalmus maximus*). Aquaculture 76, 199-210.

Scholz, T., 1999. Parasites in cultured and feral fish. Vet. Parasitol. 84, 317-335.

Sharp, N., Poortenaar, C., Diggles, B., 2001. Monogenean parasites on NZ kingfish. Fish Farm. Int. 32.

Sharp N.J., Poortenaar C.W., Diggles B.K., Willis T.J., 2003. Metazoan parasites of yellowtail kingfish, *Seriola lalandi lalandi*, in New Zealand: prevalence, intensity and site preference. NZ J. Mar. Fresh. Res. 37:273-282

Short, C.R., Flory, W., Hsieh, L.C., Barker, S.A., 1988. The oxidative metabolism of fenbendazole: a comparative study. J. Vet. Pharmacol. Ther. 11, 50-55.

Sitjà-Bobadilla A., Conde de Felipe M., Alvarez-Pellitero P., 2006. *In vivo* and *in vitro* treatments against *Sparicotyle chrysophrii* (Monogenea: Microtylidae) parasitizing the gills of gilthead sea bream (*Sparus auratus* L.). Aquaculture 261: 856-864

Sitja-Bobadilla, A., 2008. Living off fish: A trade-off between parasites and the immune system. Fish. Shellfish. Immunol. 25, 358-372.

Skaramuca, B., Kozul, V., Teskeredzic, Z., Bolotin, J., Onofri, V., 2001. Growth rate of tank-reared Mediterranean amberjack, Seriola dumerili (Risso 1810) fed on three different diets. J. Appl. Ichthyol. 17, 130-133.

Stephens, F.J., Cleary, J.J., Jenkins, G., Jones, J.B., Raidal, S.R., Thomas, J.B., 2003. Treatments to control *Haliotrema abaddon* in the West Australian dhufish, *Glaucosoma hebraicum*. Aquaculture 215, 1-10.

Stone, J., Sutherland, I.H., Sommerville, C.S., Richards, R.H., Varma, K.J., 1999. The efficacy of emamectin benzoate as an oral treatment of sea lice, *Lepeophtheirus salmonis* (Kroyer), infestations in Atlantic salmon, *Salmo salar* L. J. Fish Dis. 22, 261-270.

Stone, J., Sutherland, I.H., Sommerville, C., Richards, R.H., Endris, R.G., 2000. The duration of efficacy following oral treatment with emamectin benzoate against infestations of sea lice, *Lepeophtheirus salmonis* (Kroyer), in Atlantic salmon *Salmo salar* L. J. Fish Dis. 23, 185-192.

Stone, J., Roy, W.J., Sutherland, I.H., Ferguson, H.W., Sommerville, C., Endris, R., 2002. Safety and efficacy of emamectin benzoate administered in-feed to Atlantic salmon, *Salmo salar* L., smolts in freshwater, as a preventative treatment against infestations of sea lice, *Lepeophtheirus salmonis* (Kroyer). Aquaculture 210, 21-34.

Takuro, H., Hitoshi, I., 2000. Development of management skill for good parent fish (*Seriola*, striped horse mackerel). Nippon Saibai Gyogyo Kyokai Jigyo Nenpo, 246-250.

Thoney, D.A., Hargis, W.J., Jr., 1991. Monogenea (Platyhelminthes) as hazards for fish in confinement. Annual Review of Fish Diseases 1, 133-153.

Tingbao, Y., Kritsky, D.C., Yuan, S., Jianying, Z., Suhua, S., Agrawal, N., 2006. Diplectanids infesting the gills of the barramundi *Lates calcarifer* (Bloch) (Perciformes: Centropomidae), with the proposal of *Laticola* n. g. (Monogenoidea: Diplectanidae). Syst. Parasitol. 63, 127-141.

Tojo, J.L., Santamarina, M.T., Ubeira, F.M., Estevez, J., Sanmartin, M.L., 1992. Anthelmintic activity of benzimidazoles against *Gyrodactylus* sp. infecting rainbow trout *Onchorhynchus mykiss*. Dis. Aquat. Org. 12, 185-189.

Tojo, J.L., Santamarina, M.T., 1998a. Oral pharmacological treatments for parasitic diseases of rainbow trout *Oncorhynchus mykiss*. I: *Hexamita salmonis*. Dis. Aquat. Org. 33, 51-56.

Tojo JL, Santamarina M.T., 1998b. Oral pharmacological treatments for parasitic diseases of rainbow trout *Oncorhynchus mykiss*. II: *Gyrodactylus* sp. Dis Aquat Org 33:187-193

Tojo, J.L., Santamarina, M.T., 1998c. Oral pharmacological treatments for parasitic diseases of rainbow trout *Oncorhynchus mykiss*. III: *Ichthyobodo necator*. Dis. Aquat. Org. 33, 195-199.

Treves-Brown, K.M., 2000. Applied Fish Pharmacology. Kluwer Academic Publishers, Dordrecht, 309 pp

Tubbs, L.A., Poortenaar, C.W., Sewell, M.A., Diggles, B.K., 2005. Effects of temperature on fecundity in vitro, egg hatching and reproductive development of *Benedenia seriolae* and *Zeuxapta seriolae* (Monogenea) pa

Tubbs L.A., Tingle M.D., 2006a. Bioavailability and pharmacokinetics of a praziquantel bolus in kingfish *Seriola lalandi*. Dis Aquat Org 69:233-238

Tubbs, L.A., Tingle, M.D., 2006b. Effect of dose escalation on multiple dose pharmacokinetics of orally administered praziquantel in kingfish *Seriola lalandi*. Aquaculture 69, 233-238.

Tubbs, L., Mathieson, T., Tingle, M., 2008. Metabolism of praziquantel in kingfish *Seriola lalandi*. Dis. Aquat. Org. 78, 225-233.

Tully, O., Nolan, D.T., 2002. A review of the population biology and host-parasite interactions of the sea louse *Lepeophtheirus salmonis* (Copepoda : Caligidae). Parasitology 124, S165-S182.

Vertommen J., Kinget R., 1998. Pellets as a dosage form for drugs in aquaculture: technological aspects. J Appl Ichthyol 14:259-264

Veterinary Manual of Requirements and Guidelines. The Australian Pest and Veterinary Medicine Authority. http://www.apvma.gov.au/MORAG_vet/MORAG_vet_home.shtml. Accessed 12-06-2009

Wall, T., 1993. The veterinary approach to salmon farming in Scotland. In: Brown, L.A. (Ed.), Aquaculture for veterinarians, fish husbandry and medicine. Pergamon Press, Oxford, pp. 193-221.

Waller, P.J., 2006. From discovery to development: Current industry perspectives for the development of novel methods of helminth control in livestock. Vet. Parasitol. 139, 1-14.

Whittington, I.D., 1996. Benedeniine capsalid monogeneans from Australian fishes: Pathogenic species, site-specificity and camouflage. J. Helminthol. 70, 177-184.

Whittington, I.D., Corneillie, S., Talbot, C., Morgan, J.A.T., Adlard, R.D., 2001. Infections of *Seriola quinqueradiata* Temminck and Schlegel and S- dumerili (Risso) in Japan by *Benedenia seriolae* (Monogenea) confirmed by morphology and 28S ribosomal DNA analysis. J. Fish Dis. 24, 421-425.

Whittington, I.D., Chisholm, L.A., 2008. Diseases caused by Monogenea. In: Eiras, J.C., Segner, H., Wahli, T., Kapoor, B.G. (Eds.), Fish Diseases. Science Publishers, Enfield (NH), pp. 684-816.

Williams, R.E., Ernst, I., Chambers, C.B., Whittington, I.D., 2007. Efficacy of orally administered praziquantelagainst *Zeuxapta seriolae* and *Benedenia seriolae* (Monogenea) in yellowtail kingfish *Seriola lalandi*. Dis. Aquat. Org. 77, 199-205.

Willis, K.J., Ling, N., 2003. The toxicity of emamectin benzoate, an aquaculture pesticide, to planktonic marine copepods. Aquaculture 221, 289-297.

Wynne, J.W., O'Sullivan, M.G., Cook, M.T., Stone, G., Nowak, B.F., Lovell, D.R., Elliot, N.G., 2008. Transciptome analyses of amoebic gill disease-affected Atlantic salmon (*Salmo salar*) tissues reeal localised gene suppression. Marine Biotechnology 10, 388-403.

Young, N.D., Crosbie, P.B.B., Adams, M.B., Nowak, B.F., Morrison, R.N., 2007. *Neoparamoeba perurans* n. sp., an agent of amoebic gill disease of Atlantic salmon (*Salmo salar*). Int. J. Parasit. 37, 1469-1481.

Zilberg, D., Findlay, V.L., Girling, P., Munday, B.L., 2000. Effects of treatment with levamisole and glucans on mortality rates in Atlantic salmon (*Salmo salar* L.) suffering from amoebic gill disease. Bull. Eur. Assoc. Fish Pathol. 20, 23-27.

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