

Efficacy of orally administered praziquantel against *Zeuxapta seriolae* and *Benedenia seriolae* (Monogenea) in yellowtail kingfish *Seriola lalandi*

Rissa E. Williams^{1,3,*}, Ingo Ernst^{1,4}, Clinton B. Chambers^{1,5}, Ian D. Whittington^{1,2}

¹School of Earth and Environmental Sciences, Darling Building, DP418, The University of Adelaide, Adelaide, South Australia 5005, Australia

²Parasitology Section, The South Australian Museum, North Terrace, Adelaide, South Australia 5000, Australia

³Present address: Investigation and Diagnostic Centre–Wallaceville, Biosecurity New Zealand, Ministry of Agriculture and Forestry, PO Box 40742, Ward Street, Upper Hutt 5018, New Zealand

⁴Present address: Aquatic Animal Health Unit, Product Integrity, Animal and Plant Health, Australian Government Department of Agriculture, Fisheries and Forestry, GPO Box 858, Canberra, Australia Capital Territory 2601, Australia

⁵Present address: Worley Parsons Ltd., Level 3, QV1 Building, 250 St. Georges Terrace, Perth, Western Australia 6000, Australia

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 unmedicated 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*

Resale or republication not permitted without written consent of the publisher

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). *Z. seriolae*, a polyopisthocotylean, attaches to gill lamellae by haptor clamps. This species feeds on blood and heavy infestations have been associated with anaemia and mortality in aquaculture of *Seriola*

spp. in Japan (Ogawa & 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

*Email: rissa.williams@adelaide.edu.au

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 & 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, weatherdependent 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, 2001, Kim & Cho 2000, Kim & Kim 2002), *Heterobothrium okamatoi* (Hirazawa et al. 2000) and *Sparicotyle chysophrii* (Sitjà-Bobadilla et al. 2006), as well as the monopisthocotyleans *Gyrodactylus* sp. (Tojo & Santamarina 1998) and *Neobenedenia girellae* (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 & 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 m³ 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 & 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 (Lot no. 95758).

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 dark-

ened 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 m³ 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 catheter 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 life-cycle allowing them to reproduce rapidly in a closed environment (Thoney & 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 & 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. (1999):

$$\% \text{ efficacy} = 100 - \left(100 \times \frac{\text{mean parasite intensity of treatment}}{\text{mean parasite intensity of control}} \right)$$

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

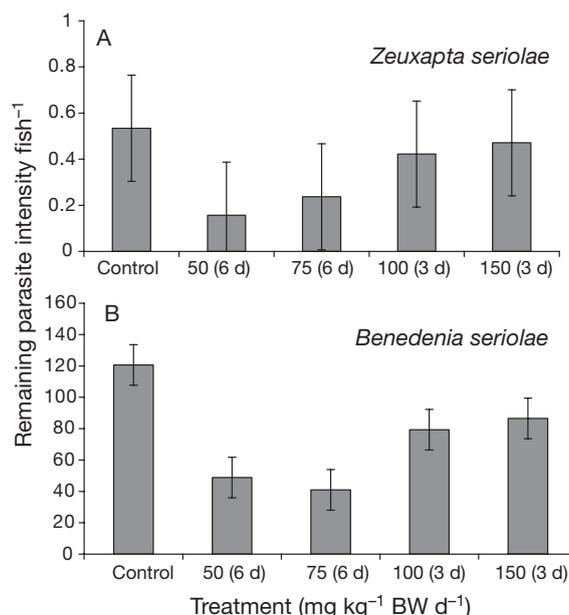


Fig. 1. Mean intensity of (A) *Zeuxapta seriolae* and (B) *Benedenia seriolae* remaining on fish after Trial 1 (surface-coated feed treatment with PZQ [praziquantel]) at 4 daily doses and 2 durations: 50 and 75 mg kg⁻¹ BW d⁻¹ over 6 d and 100 and 150 mg kg⁻¹ BW d⁻¹ over 3 d. Error bars represent 95%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

log₁₀(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. (1999) (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

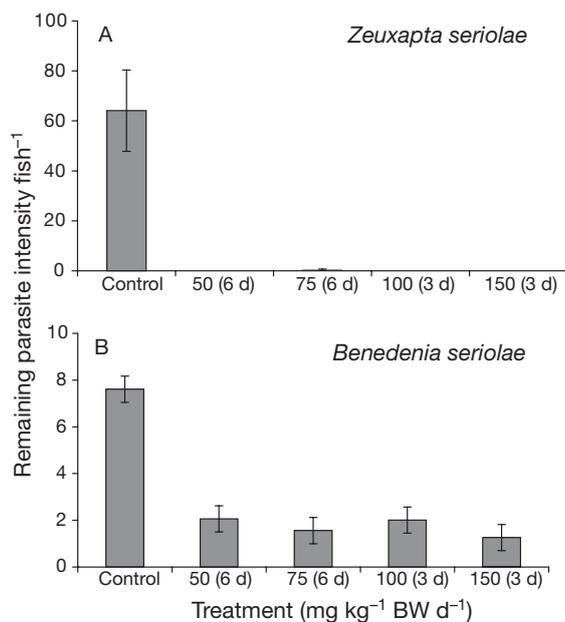


Fig. 2. Mean intensity of (A) *Zeuxapta seriolae* and (B) *Benedenia seriolae* remaining on fish after Trial 2 (treatment by intubation with PZQ) at 4 daily doses and 2 durations: 50 and 75 mg kg⁻¹ BW d⁻¹ over 6 d and 100 and 150 mg kg⁻¹ BW d⁻¹ over 3 d. Error bars represent 95% confidence intervals of the means. *B. seriolae* data have been $\sqrt{(y+0.5)}$ -transformed. See Fig. 1 legend for definitions of acronyms

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 $\sqrt{(y+0.5)}$ -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 d⁻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 of 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 remaining *Zeuxapta seriolae* and *Benedenia seriolae* compared with control fish, which received unmedicated pellets (Figs. 1A and B, respectively). Significant

Table 1. Efficacy, calculated as percentage reduction of mean parasite intensities from the control mean (Stone et al. 1999), 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)				
<i>Zeuxapta seriolae</i>	81.4	70.8	35.8	14.7
<i>Benedenia seriolae</i>	58.1	66.4	30.9	21.6
(B)				
<i>Zeuxapta seriolae</i>	100	99.5	100	100
<i>Benedenia seriolae</i>	92.5	95.5	91.0	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	<i>Zeuxapta seriolae</i>	<i>Benedenia seriolae</i>
(A)		
Control vs. all treatments	<i>p</i> = 0.040	<i>p</i> < 0.001
50 and 75 vs. 100 and 150 mg kg ⁻¹ BW d ⁻¹	<i>p</i> = 0.014	<i>p</i> < 0.001
50 vs. 150 mg kg ⁻¹ BW d ⁻¹	<i>p</i> = 0.024	<i>p</i> = 0.002
(B)		
Control vs. all treatments		<i>p</i> < 0.001
50 and 75 vs. 100 and 150 mg kg ⁻¹ BW d ⁻¹		<i>p</i> = 0.549
50 vs. 150 mg kg ⁻¹ BW d ⁻¹		<i>p</i> = 0.054

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, $p = 0.040$, $F = 2.58$, $df = 4$). 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, $p < 0.001$, $F = 23.40$, $df = 4$). 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).

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, $p > 0.001$, $F = 86.28$, $df = 4$). 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).

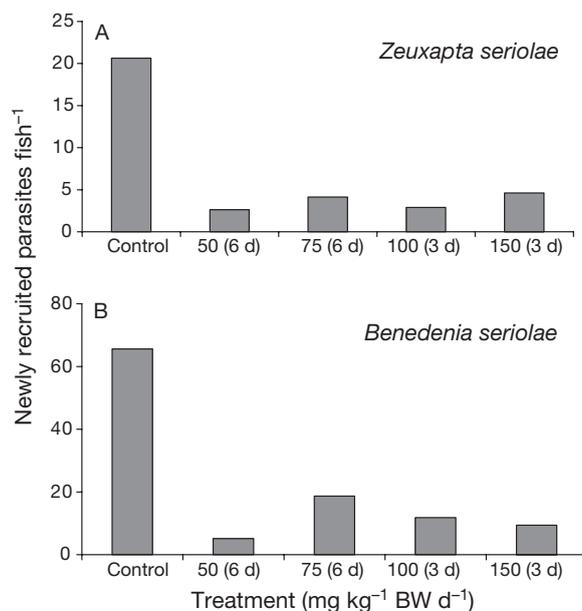


Fig. 3. Mean intensity of newly recruited (A) *Zeuxapta seriolae* and (B) *Benedenia seriolae* after Trial 2 (treatment by intubation with PZQ) at 4 daily doses and 2 durations: 50 and 75 mg kg⁻¹ BW d⁻¹ over 6 d and 100 and 150 mg kg⁻¹ BW d⁻¹ over 3 d. See Fig. 1 legend for definitions of acronyms

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).

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 hal-

ibut *Verasper variegates* 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 appetite 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 rejection by fish of 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 pelletter 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 homogeneously 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 & 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 prob-

lems, 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 fourth treatment dose (75 mg kg⁻¹ BW d⁻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* from 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.

Acknowledgements. We are grateful to South Australian Aquaculture Management for providing fish, access to their hatchery facilities, and other helpful assistance during the course of this study. We acknowledge The University of Adelaide for providing a PhD scholarship for R.E.W. and the generous contributions from our industry partners: Yamaha Nutreco Aquatech, South Australian Marine Finfish Farmers

Association, and Skretting Australia. Funding was provided by an Australian Research Council Linkage grant (LP0211375) awarded to I.D.W. and I.E.

LITERATURE CITED

- Broz J, Schai E, Gadiant M (1997) Micronutrient stability in feed processing. Report No. FT42-1997. American Soybean Association, Singapore
- Chambers CB, 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
- Day TA, Bennett JL, Pax RA (1992) Praziquantel: the enigmatic antiparasitic. *Parasitol Today* 8:342–344
- Ernst I, Whittington ID, Corneillie S, Talbot C (2002) Monogenean parasites in sea-cage aquaculture. *Austasia Aquacult* (February-March 2002):46–48
- 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
- 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, 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
- Hormazabal V, Yndestad M (1995) Determination of praziquantel in medicated fish feed and sediment by HPLC. *J Liq Chromatogr* 18:1231–1238
- Kim KH, Cho JB (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 KH, Kim CS (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 KH, Park SI, Jee BY (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 KH, Lee EH, Kwon SR, Cho JB (2001) 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 CS, Cho JB, Ahn KJ, Lee JI, Kim KH (2003) Depletion of praziquantel in muscle tissue and skin of cultured rockfish (*Sebastes schlegeli*) under the commercial culture conditions. *Aquaculture* 219:1–7
- Montero FE, Crespo S, Padros F, De la Gandara F, Garcia A, Raga JA (2004) Effects of the gill parasite *Zeuxapta seriolae* (Monogenea: Heteraxinidae) on the amberjack *Seriola dumerili* Risso (Teleostei: Carangidae). *Aquaculture* 232:153–163
- Mooney AJ, Ernst I, Whittington ID (2006) An egg-laying rhythm in *Zeuxapta seriolae* (Monogenea: Heteraxinidae), a gill parasite of yellowtail kingfish (*Seriola lalandi*). *Aquaculture* 253:10–16
- Ogawa K, Yokoyama H (1998) Parasitic diseases of cultured marine fish in Japan. *Fish Pathol* 33:303–309
- Paperna I (1991) Diseases caused by parasites in the aquaculture of warm water fish. *Ann Rev Fish Dis* 1:155–194
- Sharp NJ, Poortenaar CW, Diggles BK, Willis TJ (2003) Meta-zoan parasites of yellowtail kingfish, *Seriola lalandi*, in New Zealand: prevalence, intensity and site preference. *NZ J Mar Freshw Res* 37:273–282
- 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
- Stone J, Sutherland IH, Sommerville CS, Richards RH, Varma KJ (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
- Thoney DA, Hargis WJ Jr (1991) Monogenea (Platyhelminthes) as hazards for fish in confinement. *Ann Rev Fish Dis* 1:133–153
- Tojo JL, Santamarina MT (1998) Oral pharmacological treatments for parasitic diseases of rainbow trout *Oncorhynchus mykiss*. II: *Gyrodactylus* sp. *Dis Aquat Org* 33:187–193
- Vertommen J, Kinget R (1998) Pellets as a dosage form for drugs in aquaculture: technological aspects. *J Appl Ichthyol* 14:259–264
- Whittington ID (1996) Benedeniine capsalid monogeneans from Australian fishes: pathogenic species, site-specificity and camouflage. *J Helminthol* 70:177–184

Editorial responsibility: Robin Overstreet, Ocean Springs, Mississippi, USA

Submitted: May 22, 2006; Accepted: May 19, 2007
Proofs received from author(s): September 11, 2007