Vol. 121: 201–209, 2016
doi: 10.3354/dao03054

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published October 27

Praziquantel delivery via moist pellets to treat monogenean parasites of yellowtail kingfish *Seriola lalandi*: efficacy and feed acceptance

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ABSTRACT: Praziquantel (PZQ) is registered for oral use against *Benedenia seriolae* infecting *Seriola* spp. in Japan, but its bitter taste causes poor palatability. Incorporating PZQ in a moist pellet may help mask the flavor to improve intake. Altering delivery, however, may influence efficacy. We assessed the minimum effective concentrations of PZQ in moist pellets delivered by intubation for the monogeneans *Zeuxapta seriolae* and *B. seriolae* infecting yellowtail kingfish *Seriola lalandi* in flow-through tanks. The optimised dose was then evaluated in a sea-cage of *S. lalandi* to assess feed acceptance and efficacy. During intubation trials, efficacy was assessed as a percent reduction compared to control groups; in the field trial, efficacy was assessed by a percent reduction after treatment. PZQ delivered by intubation at 70 mg kg^{-1} body weight (BW) for 3 d was 99.7 and 81.7% effective against *Z. seriolae* and *B. seriolae*, respectively. Increasing the dose to 120 mg kg^{-1} BW for 3 d had a similar efficacy against *Z. seriolae* (98.4%) and increased efficacy against *B. seriolae* to 89.2%, but partial emesis of the medicated feed was sometimes noted. *S. lalandi* in a sea-cage at 17°C readily consumed PZQ administered daily in moist pellets at 70 mg kg^{-1} BW for 3 d (inclusion rate: 5.15 g kg^{-1}), which significantly reduced *Z. seriolae* and *B. seriolae* abundance with 99.4 and 81.6% efficacy, respectively. Juvenile *B. seriolae* were common on the eyes of fish post-treatment which indicates a strategically timed repeat treatment is necessary.

KEY WORDS: Abundance · Intubation · Oral · *Zeuxapta seriolae* · *Benedenia seriolae*

INTRODUCTION

Farming yellowtail kingfish *Seriola lalandi* (Carangidae) is a relatively new aquaculture industry in Australia, and Clean Seas Tuna Ltd. (CST) is the primary producer of this fish. A limitation to the growth of the industry is the significant cost in managing infestations of blood fluke *Paradeontacylix* spp. (Sanguinicolidae), skin fluke *Benedenia seriolae* (Capsalidae) and gill fluke *Z. seriolae* (Heteraxinidae), which can account for up to 22% of production costs (Ernst et al. 2002, Shinn et al. 2015). *Z. seriolae* and *B. seriolae* are managed by bathing fish in hydrogen peroxide (HP) (H_2O_2). Bathing fish, however, is operationally challenging, labour intensive, weather and tide dependant, and unless optimally managed, can be ineffective. Overdose, poorly managed HP exposure or lack of dissolved oxygen has been associated with fish mortalities during HP treatment. Improved production efficiency requires lower cost, weather-independent integrated fluke treatments. Of the available anthelminthics, orally administered prazi-

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quantel (PZQ) is effective against monogeneans in a variety of fish and has a high therapeutic index when administered orally in *S. lalandi* (see Forwood et al. 2016). There are, however, information gaps, palatability problems and regulatory issues that prevent broad commercial use of PZQ to treat flukes infecting *S. lalandi*.

PZQ has been administered orally by surface coating extruded feed (Kim & Cho 2000, Sitjà-Bobadilla et al. 2006, Williams et al. 2007, Forwood et al. 2013, Partridge et al. 2014), mixed into feed mash prior to extrusion (Hirazawa et al. 2000, 2004, 2013) or as a microencapsulated product that is surface coated or extruded in the pellet (Partridge et al. 2014). PZQ was effective against both Z. *seriolae* and *B. seriolae* when administered via intubation, but efficacy was reduced when combined with fish oil and surface coated on commercial feed at equivalent doses (Williams et al. 2007). Palatability of PZQ-medicated feed is poor in *S. lalandi*, resulting in fish often failing to receive an effective dose (Williams et al. 2007, Partridge et al. 2014). In an attempt to resolve palatability issues, microencapsulated PZQ was trialed, and Partridge et al. (2014) reported that a microencapsulated PZQ product at 47 mg kg^{-1} body weight (BW) d^{-1} for 7 d was 100% effective against *B. seriolae*. Palatability, however, remained poor at higher inclusion (25 g kg^{-1}) levels. Microencapsulation can lower the bioavailability of PZQ (Partridge et al. 2014) and is expensive, providing further disincentive for commercial use. Delivering PZQ in moist pellets, which contain ~50% fresh fish, may improve palatability and efficacy without the negative aspects of adopting a microencapsulated product.

This study aimed to provide further data to support regulatory approval to use PZQ as an oral treatment for *Z. seriolae* and *B. seriolae* infecting *S. lalandi* by determining the minimum effective dose and duration of delivery of PZQ in a moist pellet and assessing feed acceptance to improve treatment of *Z. seriolae* and *B. seriolae*.

MATERIALS AND METHODS

Experimental animals

Seriola lalandi (~1–1.5 kg) were transported from commercial cages off Port Lincoln, Australia, to holding tanks at the Lincoln Marine Science Centre (LMSC), Port Lincoln and held at 10–15 kg fish m^{-3}. Water temperature through the trial period was 17 ± 1°C, and fish were fed 6 times per week to satiation for 2 wk during acclimation to the system.

Establishing fluke abundance

Abundance (Bush et al. 1997) of *Zeuxapta seriolae* and *B. seriolae* were assessed following Williams et al. (2007) by placing fish into a seawater bath containing 5 ppm PZQ for 10 min to remove *Z. seriolae*, followed by 5 min immersion in dechlorinated municipal water to remove *B. seriolae*. The eyes and skin of the fish were visually inspected after freshwater immersion to count any *B. seriolae* that did not detach from the fish. The PZQ and freshwater were filtered through a 40 µm filter after the fish were removed. Flukes were collected in a 70 ml sample jar and counted under a dissection microscope. *Z. seriolae* maturity was assessed by the presence of the vitellarium (Tubbs et al. 2005, Mooney et al. 2006), and *B. seriolae* maturity was determined by possession of eggs in the ootype (Tubbs et al. 2005, Lackenby et al. 2007).

Manufacture of moist pellets

Moist pellets were made 24 h prior to the initiation of treatment. Pellets comprised 50% minced Australian sardine *Sardinops sagax* (Clupeidae) (Sardine Temptations) and 50% dry pre-pellet meal (Hayashikane Sangyo). PZQ (93.3–99.8% purity, Ninybo Samreal Chemical) was blended with the mash in a food processor, and then further blended with minced fish and processed through a TC22 meat mincer (Brice Australia) at a cutting speed of 200 rpm, using a 3 mm die. Moist pellets for the field trial were comprised of 50% sardine and 50% dry pre-pellet meal produced in a single screw custom-made commercial extruder (Taiwan Hung Kuo Industrial) with a hopper size of 300 kg using a 9 mm die. PZQ was blended with the mash in the hopper, and then further blended with minced fish and processed through the extruder to form the pellets.

Dose determination trial

To identify PZQ doses for the efficacy trials, 36 *S. lalandi* were transferred into 12 indoor 1000 l flow-through tanks at LMSC and administered PZQ in moist pellets by intubation daily, in a daily ration of 0.91% BW feed, for 3 d. Fish were fed 30, 45, 60, 75,

90, 150, 165, 180, 195, 210 and 225 mg PZQ kg^{-1} BW d^{-1}. Control fish were intubated with identical feed without PZQ. Fish were monitored for 1 h post intubation to assess if fish disgorged the feed. Parasite abundance of *Z. seriolae* and *B. seriolae* was assessed 24 h after administration of the final dose of PZQ.

Efficacy trials

A total of 120 *S. lalandi* were transferred into 12 indoor 1000 l flow-through tanks at LMSC and administered PZQ in moist pellets via intubation at a feed rate of 0.91% BW. Two efficacy trials were performed, outlined in Table 1. Fish were monitored for 1 h post delivery to assess emesis. Twenty-four h after administration of the final dose, the intensities of remaining *Z. seriolae* and *B. seriolae* were assessed. Each of the treatment and control groups was replicated 3 times.

Field trial

A farm-based feed acceptance and efficacy trial was conducted at CST's Fanny Point lease site (34° 43′ 45″ S, 135° 55′ 18″ E) in November 2014, in a 16 m diameter by 7 m side brass sea-cage containing 1990 *S. lalandi* (mean weight 2.1 kg) at a stocking density of 3 kg m^{-3}. Water temperature was 17 ± 1°C during the trial. All feed was provided manually. Fish were fed once per day with un-medicated moist pellets for 2 d, after which food was withheld for 48 h. The fish were then fed PZQ-medicated moist pellets to achieve 70 mg PZQ kg^{-1} BW d^{-1}, once daily for 3 d at a restricted ration of 0.85% BW d^{-1} (PZQ inclusion rate: 5.15 g kg^{-1}). During the 2 d acclimation period, 15 *S. lalandi* were sampled and the abundance of *Z. seriolae* and *B. seriolae* was determined. During delivery of the medication, feed acceptance was assessed by visual observation of fish response by staff above and in the cage. A feeding camera was also placed in the cage to determine if any unconsumed pellets sank through the cage or if emesis occurred following treatment. Forty-eight h after the treatment period, a further 15 *S. lalandi* were sampled to determine the post-treatment abundance of *Z. seriolae* and *B. seriolae*.

Table 1. Target dose, inclusion level and delivery time for praziquantel (PZQ) administered daily in moist pellets via intubation in 2 efficacy trials. BW: body weight

Target dose (mg PZQ kg^{-1} BW)	Inclusion level (mg g^{-1})	Time (d)
Trial 1		
50	5.5	1
50	5.5	3
70	7.7	3
Trial 2		
120	13.2	3
150	16.5	3
180	19.8	3

Statistical analysis

In efficacy trials the efficacy of each treatment group was assessed as a percentage according to the formula of Stone et al. (1999):

$$\% \text{ reduction} = 100 - \left(100 \times \frac{\text{Mean parasite abundance of each treated replicate}}{\text{Mean parasite abundance of the control replicates}}\right)$$

In field trials the efficacy of the treatment was assessed as a percent reduction of pre- and post-treatment abundance. Following APVMA (2007) guidelines for registration of a veterinary treatment, a clinical effect was judged at ≥90% efficacy. Normality of the data was tested using the Shapiro-Wilk test, and variances were tested using Levene's test. Data that did not conform to homoscedasticity was log (y + 1)-transformed, where y is parasite abundance, prior to analysis. Differences in treatment replicate efficacy between *Z. seriolae* and *B. seriolae* in the efficacy trials and pre- and post-treatment abundances in the field trial were analysed using an independent samples t-test. Differences in mean abundance between treatment groups in the efficacy trials was analysed using 1-way ANOVA. Where significant differences were detected, post-hoc comparisons were made via a Tukey's test. SPSS 20 statistical analysis software was used to analyse the data and significance for all tests was judged at $p < 0.05$.

RESULTS

Dose determination trial

Efficacy against *Zeuxapta seriolae* was 100% at all doses ≥45 mg PZQ kg^{-1} BW d^{-1} (Fig. 1A). *B. seriolae* abundance was reduced at doses of ≥75 mg kg^{-1} BW d^{-1}, and 165 and 210 mg PZQ kg^{-1} BW d^{-1} provided 100% efficacy (Fig. 1B).

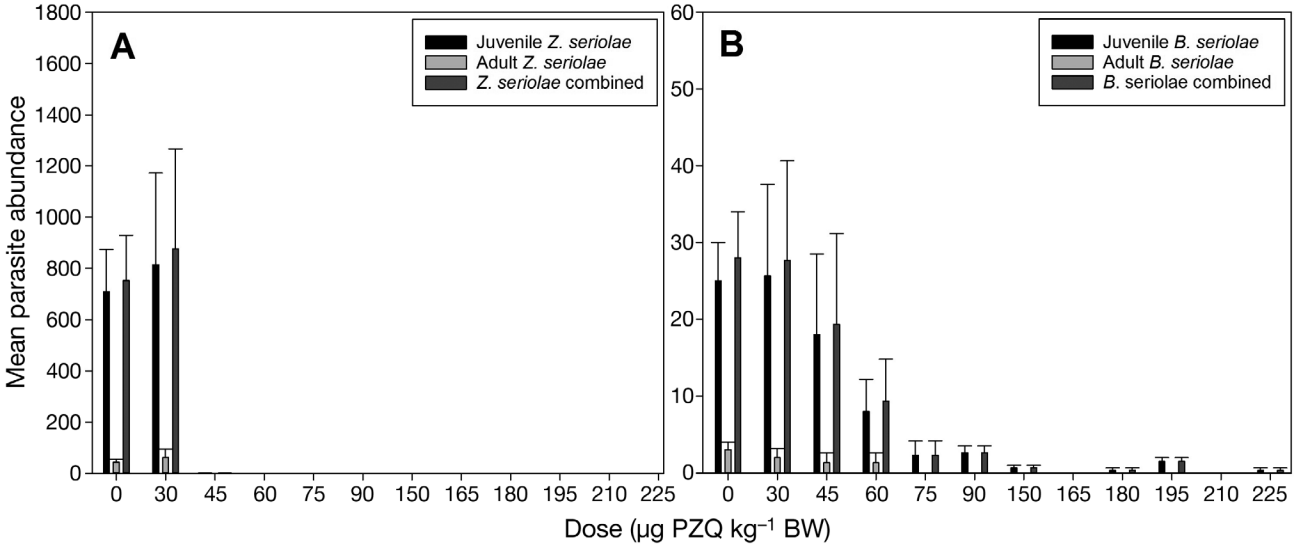

Fig. 1. Dose determination trial. Mean abundance (±SE) of (A) *Zeuxapta seriolae* and (B) *Benedenia seriolae* infecting *Seriola lalandi* after 3 d intubation of praziquantel (PZQ). Combined: juvenile and adult parasites combined; BW: body weight

Efficacy trials

PZQ was more effective against *Z. seriolae* than *B. seriolae*, but efficacies were not significantly different at 50 and 70 mg PZQ kg^{-1} BW d^{-1} for 3 d. A dose of 50 mg PZQ kg^{-1} BW d^{-1} for 1 d was significantly less effective against *B. seriolae* than *Z. seriolae* (Table 2).

Prevalence of *Z. seriolae* was 100% in the control groups, and mean abundance was 1056.7 ± 294.8 SE (264–4128). Prevalence of *Z. seriolae* in fish treated with 50 mg PZQ kg^{-1} BW d^{-1} for 1 and 3 d, and 70 mg PZQ kg^{-1} BW d^{-1} for 3 d was 92.9, 80 and 53.3%, respectively, and mean abundance was 28.2 ± 16.1 (0–208), 43.3 ± 33.3 (0–505) and 3.5 ± 1.6 SE (0–23), respectively, which was significantly different between groups (1-way ANOVA: $F_{3,52}$ = 51.524, p < 0.001). Fish treated with PZQ had a significantly lower mean abundance of *Z. seriolae* than control groups (Fig. 2A). Prevalence of *B. seriolae* in the control groups was 100%, and mean abundance was 42.2 ± 6.7 SE (15–88). Prevalence of *B. seriolae* in fish treated with 50 mg PZQ kg^{-1} BW d^{-1} for 1 and 3 d, and 70 mg PZQ kg^{-1} BW d^{-1} for 3 d was 100, 86.7 and 80%, respectively, and mean abundance was 27.8 ± 5.4 (9–82), 9.1 ± 2 (0–27) and 7.8 ± 2.3 SE (0–30), respectively, which was significantly different between groups (1-way ANOVA: $F_{3,52}$ = 16.513, p < 0.001). Fish treated with 50 and 70 mg PZQ kg^{-1} BW d^{-1} for 3 d had a significantly lower mean abundance of *B. seriolae* than fish treated with 50 mg PZQ kg^{-1} BW d^{-1} for 1 d and control groups (Fig. 2B).

Fish disgorged PZQ-medicated feed at higher doses (≥120 mg PZQ kg^{-1} BW), resulting in 3 fish from each of the 120 and 150 mg PZQ kg^{-1} BW groups retaining a dose of PZQ, which was probably below the target. Efficacy was higher against *Z. seriolae* than *B. seriolae*, but was not significantly different at 120 and 180 mg kg^{-1} BW d^{-1} for 3 d, but 150 mg kg^{-1}

Table 2. Efficacy Trial 1. Mean efficacy ± SE (%) (range) of praziquantel (PZQ) treatments (mg PZQ kg^{-1} body weight) against juvenile and adult *Zeuxapta seriolae* and *Benedenia seriolae* infecting *Seriola lalandi*. Significant differences (p < 0.05) in efficacy between *B. seriolae* and *Z. seriolae* are highlighted in **bold**

Treatment	*Z. seriolae*	*B. seriolae*	p
Adults			
50 for 1 d	99.9 ± 0.1 (99.6–100)	76.1 ± 10 (55.9–90.1)	**0.021**
50 for 3 d	99.4 ± 0.6 (98.2–100)	75.9 ± 8.2 (62.7–91)	**0.046**
70 for 3 d	100	72.9 ± 13.6 (45.8–87.6)	0.184
Juveniles			
50 for 1 d	96.7 ± 2.3 (92.2–99.3)	15.7 ± 12.7 (0–40.9)	**0.047**
50 for 3 d	94.5 ± 3.7 (87.2–98.3)	80.8 ± 10.7 (62.3–99.2)	0.289
70 for 3 d	99.5 ± 0.4 (98.7–99.9)	88.0 ± 3.0 (82.4–92.8)	**0.020**
Combined			
50 for 1 d	97.5 ± 1.7 (94.1–99.5)	35.6 ± 13.1 (18.6–61.3)	**0.040**
50 for 3 d	95.8 ± 2.9 (90.1–98.8)	78.8 ± 8.2 (62.5–88.8)	0.121
70 for 3 d	99.7 ± 0.3 (99.1–100)	81.7 ± 7.3 (67.2–90.6)	0.133

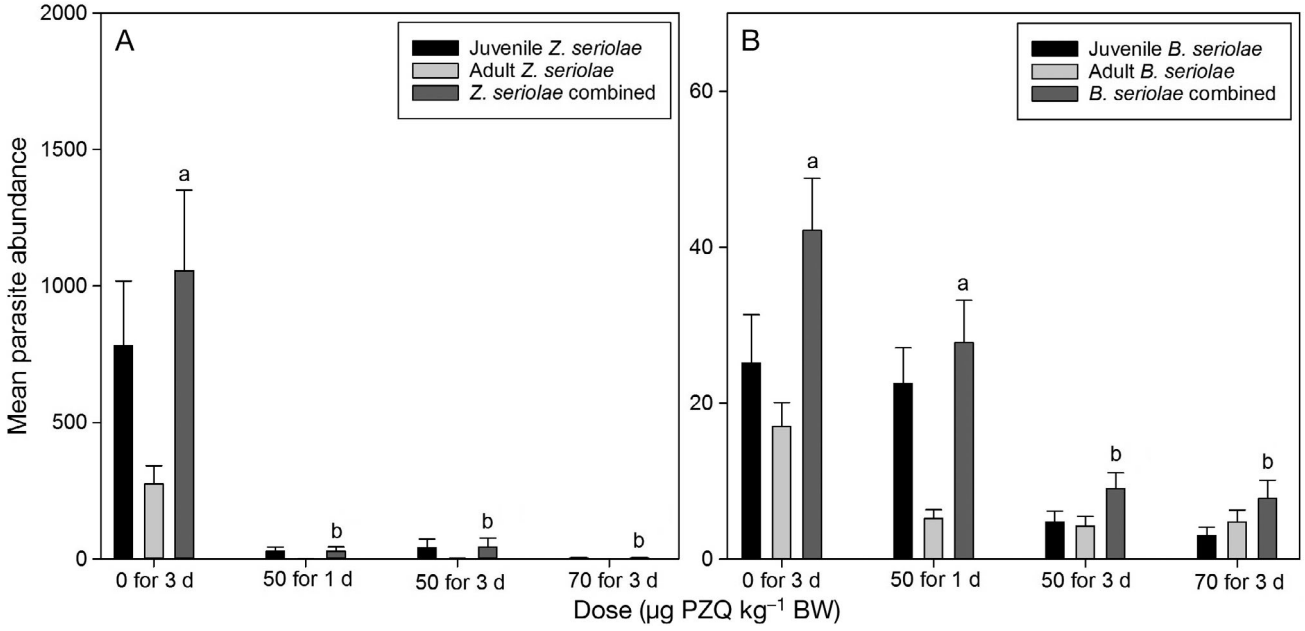

Fig. 2. Efficacy Trial 1. Mean abundance (+SE) of (A) *Zeuxapta seriolae* and (B) *Benedenia seriolae* infecting *Seriola lalandi* treated with 0, 50 and 70 mg praziquantel (PZQ) kg^{-1} body weight (BW) d^{-1}. Different superscript letters between treatments represent significant differences in combined parasite abundance between treatment groups ($p < 0.05$). Combined: juvenile and adult parasites combined

BW d^{-1} for 3 d was significantly less effective against *B. seriolae* (Table 3).

Prevalence of *Z. seriolae* in the control groups was 100%, and mean abundance was 1585.2 ± 314.5 SE (501–4282). Prevalence of *Z. seriolae* in fish treated with 120, 150 and 180 mg PZQ kg^{-1} BW d^{-1} for 3 d was 84.6, 78.5 and 8.3%, respectively, and mean abundance was 24.8 ± 12.9 (0–120), 20.1 ± 13.5 (0–193) and 0.4 ± 0.4 SE (0–4), respectively, which was significantly different between groups (1-way ANOVA: $F_{3,44} = 73.297$, $p < 0.001$). Fish treated with PZQ had significantly lower mean abundance of *Z. seriolae* than control groups (Fig. 3A). Prevalence of *B. seriolae* in the control groups was 100%, and mean abundance was 88.8 ± 19.8 SE (26–231). Prevalence of *B. seriolae* in fish treated with 120, 150 and 180 mg PZQ kg^{-1} BW d^{-1} for 3 d was 92.9, 92.9 and 66.7%, respectively, and mean abundance was 9.8 ± 2.5 (0–28), 12.1 ± 3.1 (0–38) and 2.6 ± 1.1 SE (0–11), respectively, which was significantly different between groups (1-way ANOVA: $F_{3,44} = 21.971$, $p < 0.001$). Fish treated with PZQ had a significantly lower mean abundance of *B. seriolae* than control groups (Fig. 3B).

Field trial

Feed response in *S. lalandi* was good, and no PZQ-medicated pellets dropped to the bottom of the cage, indicating fish consumed the ration offered. There was variability in acceptance of medicated feed between individual fish; some individuals were observed to have mouthed, and then disgorged PZQ-medicated pellets, which were then consumed by a different fish. Efficacy was

Table 3. Efficacy Trial 2. Mean efficacy ± SE (%) (range) for praziquantel (PZQ) treatments (mg PZQ kg^{-1} body weight for 3 d) against juvenile and *Benedenia seriolae* and *Zeuxapta seriolae* infecting *Seriola lalandi*. Significant differences ($p < 0.05$) in efficacy between *B. seriolae* and *Z. seriolae* are highlighted in **bold**

Treatment	*B. seriolae*	*Z. seriolae*	p
Adults			
120	88.9 ± 3.9 (83.9–96.6)	100	0.105
150	98.1 ± 1.2 (95.9–100)	99.9 ± 0.1 (99.8–100)	0.192
180	100	100	–
Juveniles			
120	89.3 ± 3.3 (85–95.8)	98.1 ± 1.6 (94.9–99.8)	0.074
150	84.8 ± 3.4 (78.6–90.2)	98.5 ± 0.9 (96.8–99.7)	**0.017**
180	96.0 ± 1.8 (93.6–99.5)	100	0.157
Combined			
120	89.2 ± 3.4 (84.8–95.9)	98.4 ± 1.4 (95.6–99.8)	0.067
150	86.9 ± 2.6 (82–91.1)	98.8 ± 0.7 (97.3–99.7)	**0.012**
180	96.6 ± 1.5 (94.6–99.6)	100	0.159

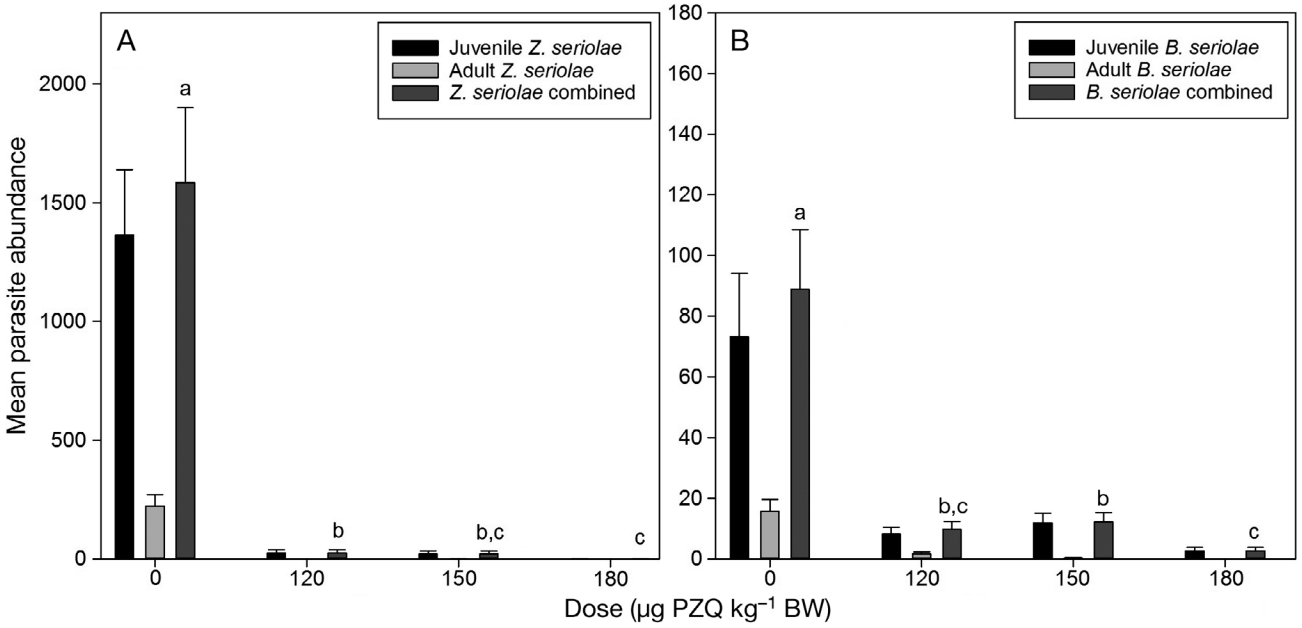

Fig. 3. Efficacy Trial 2. Mean abundance (+SE) of (A) *Zeuxapta seriolae* and (B) *Benedenia seriolae* infecting *Seriola lalandi* treated with 0, 120, 150 and 180 mg praziquantel (PZQ) kg^{-1} body weight (BW) d^{-1}. Different superscript letters between treatments represent significant differences in combined parasite abundance between treatment groups ($p < 0.05$). Combined: juvenile and adult parasites combined

higher against *Z. seriolae* (99.4%) than *B. seriolae* (81.6%) (Table 4). Pre-treatment prevalence was 100%, and mean abundance of *Z. seriolae* was 46.7 ± 16.7 SE (20–75) and *B. seriolae* was 8.3 ± 4.4 SE (2–16). Post-treatment prevalence of *Z. seriolae* and *B. seriolae* was 6.7 and 80%, and mean abundance was 0.3 ± 1 (0–4) and 1.5 ± 1.3 SE (0–4), respectively, which were significantly lower (*t*-test, $p \leq 0.009$) (Table 4). Of the *B. seriolae* that remained post-treatment 65% were found on the eyes of the fish.

Table 4. Field trial. Mean pre- and post-treatment abundances ± SE (range) of *Zeuxapta seriolae* and *Benedenia seriolae* infecting *Seriola lalandi* and efficacy of praziquantel (PZQ) administered in a moist pellet at 70 mg PZQ kg^{-1} body weight d^{-1} (inclusion rate of 5.15 g kg^{-1}) for 3 d

Parasite stage	Abundance		Efficiacy (%)
	Pre-treatment	Post-treatment	
B. seriolae			
Juveniles	7.1 ± 0.9 (1–15)	1.5 ± 0.3 (0–4)	79.2
Adults	1.3 ± 0.4 (0–5)	0.1 ± 0.1 (0–1)	94.7
Combined	8.3 ± 1.1 (2–16)	1.5 ± 0.3 (0–4)	81.6
Z. seriolae			
Juveniles	11.1 ± 1.7 (2–28)	0	100
Adults	36.5 ± 4.1 (14–65)	0.3 ± 0.3 (0–4)	99.3
Combined	47.6 ± 4.3 (20–75)	0.3 ± 0.3 (0–4)	99.4

DISCUSSION

The dose determination trial indicated that 50 mg kg^{-1} BW d^{-1} PZQ is required to treat *Zeuxapta seriolae*. This was validated in the efficacy trials where 50 mg kg^{-1} BW d^{-1} PZQ for 3 d provided 96% efficacy against *Z. seriolae*. Reducing the treatment duration to 1 d did not reduce efficacy (97.5%). This indicates that *Seriola lalandi* only need to consume the target dose on 1 of the 3 treatment days to achieve high efficacy against *Z. seriolae*. Increasing the dose to 70 mg kg^{-1} BW d^{-1} PZQ for 3 d, however, elevated efficacy against *Z. seriolae* to 99.7%.

PZQ was less effective against *B. seriolae* than *Z. seriolae*, and the dose determination trial indicated that ≥75 mg PZQ kg^{-1} BW d^{-1} for 3 d is required to effectively reduce the abundance of *B. seriolae*. In the efficacy trials, 50 mg kg^{-1} BW d^{-1} for 1 d did not significantly affect *B. seriolae* abundance. However, 50 or 70 mg PZQ kg^{-1} BW d^{-1} for 3 d significantly reduced *B. seriolae* abundance and provided efficacies of 79 and 82%, respectively, but these efficacies provide inadequate clinical effect. Increasing the dose to 120, 150 or 180 mg PZQ kg^{-1} BW d^{-1} improved efficacy against *B. seriolae* to 89, 86 and 97%, respectively, and parasites that remained post-treatment were predominantly juvenile *B. seriolae* on the eye of the fish. PZQ delivered orally is distributed

to the blood, skin and mucus of *S. lalandi* (see Tubbs & Tingle 2006a), but if it reaches the eye is unknown. The external surface of the eye, however, is poorly vascularized (Bill 1975), which would limit the transport of the product. Wistar rats orally administered PZQ had an eye PZQ concentration approximately 5 times lower than that of plasma (Steiner & Garbe 1976). Székely & Molnár (1991) reported that PZQ was 100% effective against *Diplostomum spathaceum* (Diplostomatidae) parasitizing the eye of grass carp *Ctenopharyngodon idella* (Cyprinidae) and silver carp *Hypophthalrnichthys molitrix* (Cyprinidae), but 330 mg PZQ kg^{-1} BW d^{-1} for 8 d was required to achieve this efficacy. These parasites, further, live in, rather than on, the eye (Palmieri et al. 1977) and are likely to be exposed to greater concentrations of PZQ than a parasite on the surface of the conjunctiva. It is likely that juvenile *B. seriolae* residing on the eye are unaffected by oral PZQ treatment because they are not exposed to an effective dose of the product.

The field trial showed good feed response to PZQ-medicated moist pellets, and *S. lalandi* consumed the entire ration, providing 99.4% efficacy and good clinical effect against *Z. seriolae* but was 81.6% effective against *B. seriolae*, which is inadequate for clinical effect. Commercial treatment of *B. seriolae* with oral PZQ is problematic primarily because high doses are required and these have poor palatability. For PZQ to reach the *B. seriolae*, it must be absorbed across the intestine by the fish, transported in blood, and via diffusion to the skin, incorporated into the epithelium or mucus in an active form. Only then could it be ingested or absorbed by *B. seriolae*. An equivalent dose provides a maximum PZQ concentration 3 times higher in the blood than in the skin of *S. lalandi*, but there may be a residual effect in the skin (Tubbs & Tingle 2006b) because of slower clearance of PZQ from the skin (Kim et al. 2001, Tubbs & Tingle 2006b). Tripling the initial dose of PZQ caused a significant 2.4-fold increase in the skin concentration of PZQ, but the same dose increase caused only an insignificant 1.5-fold increase in plasma concentration (Tubbs & Tingle 2006b). Increasing treatment duration significantly reduced *B. seriolae* abundance at 50 mg kg^{-1} BW d^{-1} PZQ, further indicating that skin concentrations of PZQ continue to increase with longer duration of treatment.

In fish, feed rejection rates of extruded diets surface coated with PZQ increase with increasing dose (Williams et al. 2007). Incorporating the PZQ in the mash prior to manufacturing feed improves palatability in *S. lalandi* when compared to PZQ surface coated onto the pellets at the same inclusion level (Partridge et al. 2014). *S. lalandi* can consume up to twice the ration of moist pellets than commercial extruded diets (authors' unpubl. data); therefore, the PZQ inclusion level in moist pellets can be lower. Incorporating PZQ in a pellet with high cohesion and low digestibility slowed PZQ uptake, and PZQ plasma concentrations did not reach an effective concentration (authors' unpubl. data), but homogenized pellets at 50 mg kg^{-1} BW d^{-1} delivered by intubation provide high efficacy (Williams et al. 2007). We found that *S. lalandi* readily consumed moist pellets containing 5.15 g PZQ kg^{-1} to deliver 70 mg PZQ kg^{-1} BW d^{-1}. This was effective against *Z. seriolae*, and although it did not produce an adequate clinical effect against *B. seriolae* it did significantly reduce abundance. Finalisation of the World Association for the Advancement of Veterinary Parasitology (WAAVP) 'Guidelines for testing the efficacy of ectoparasiticides for fish' (Sommerville et al. 2016) will provide a stable basis for the design and analysis of trials such as these and will improve comparability of results between trials. Developing an improved understanding of the effects of differing fluke-loads on *S. lalandi* will allow refinement of the efficacy required to achieve the desired clinical effect. Such developments would further facilitate the use of oral PZQ treatments as a component of an integrated management strategy, which would also incorporate preventative measures including net management and lease fallowing.

One limitation of our field trial was the small fish population (2000 *S. lalandi*) compared to a commercial cage (40 000 *S. lalandi*). It is likely that in smaller populations there is a lesser behavioral effect on feeding that can result in a lower average feed consumption for individual fish compared to individuals in a larger populations (Ellis et al. 2002). Such competitive feeding would aid all fish to receive the target dose of PZQ. The inclusion level we used was relatively high, but would need to be higher in some circumstances. For large *S. lalandi* (5 kg) in cool water (14°C), for example, 14 g kg^{-1} PZQ would be required to deliver 70 mg kg^{-1} BW d^{-1} PZQ. Low water temperatures (12°C) also prolong absorption of PZQ, causing lower peak concentrations in the skin than in warmer (18°C) water (Björklund & Bylund 1987). These temperature effects may further increase the required inclusion level of PZQ. On-farm observations indicate that *S. lalandi* may learn to avoid PZQ-medicated feed following repeated experimental exposure, leading to absolute feed rejection or reduced intake, even at previously accepted inclusion rates (authors' unpubl. data). Fur-

ther work to investigate the maximum acceptable inclusion rate for moist pellets and how much the delivered ration can be manipulated to achieve the required dose in larger populations of *S. lalandi* is required.

Oral treatment using PZQ is likely to be ineffective against juvenile *B. seriolae* on the eye of the fish. Managing these flukes will require a strategically timed second treatment with PZQ to target migrated flukes from the eye or an alternative treatment, such as hydrogen peroxide or freshwater, to achieve the efficacy required for strategic parasite management that breaks monogenean lifecycles to minimize fluke management costs.

Acknowledgements. This research was funded by the Australian Seafood Cooperative Research Centre (Project 2013/731 Improving fluke control in Yellowtail Kingfish culture) and all procedures were approved by the Flinders University Animal Welfare Committee (Permit E390). The authors thank Clean Seas Tuna Ltd. for significant contribution to the project.

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Editorial responsibility: Stephen Feist, Weymouth, UK

Submitted: May 9, 2016; Accepted: August 16, 2016
Proofs received from author(s): October 15, 2016