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A comparison of the palatability of racemic praziquantel and its two enantioseparated isomers in yellowtail kingfish *Seriola lalandi* (Valenciennes, 1833)

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Abstract

The bitterness of racemic praziquantel (*Rac*-PZQ) constrains its use as an in-feed treatment against monogenean flukes in finfish aquaculture. Evidence exists in mammals that the *R*-(−) enantiomer of PZQ is less bitter than the *S*-(+) enantiomer. If fish exhibit this same response then the recently described techniques for the large-scale resolution of *R*-(−)-PZQ from *Rac*-PZQ could facilitate the wide-spread application of this effective anthelmintic compound via feed.

The hypothesis that yellowtail kingfish *Seriola lalandi* would find *R*-(−)-PZQ more palatable than *Rac*-PZQ and *S*-(+)-PZQ was tested in four trials. During the first three trials, the palatability of diets top-coated with 10 g kg^{-1} of *Rac*-PZQ or its two enantioseparated isomers were compared in small (85 to 160 g) and large (1.2 kg) yellowtail kingfish. A fourth trial compared the palatability of *R*-(−)-PZQ and *Rac*-PZQ at dietary inclusion levels of 2.5, 5.0 and 10.0 g kg^{-1} in small yellowtail kingfish (170 grams).

Ingestion data showed *R*-(−)-PZQ to be no more palatable than either *Rac*-PZQ or *S*-(+)-PZQ to yellowtail kingfish, regardless of size. Indeed, evidence suggested the *S*-(+)-PZQ to be slightly more palatable than both *R*-(−)-PZQ and *Rac*-PZQ. From these data we hypothesise that the strong smell of *R*-(−)-PZQ (which was not present in *S*-(+)-PZQ) is an equally important determinant to palatability as taste in yellowtail kingfish. Results demonstrate that dietary inclusion level is a more important determinant to palatability than PZQ chirality, however administration of *R*-(−)-PZQ may still be advantageous if it is demonstrated to be the only enantiomer efficacious against monogeneans.

1. Introduction

Praziquantel (PZQ) is a broad spectrum anthelmintic compound that has been used in the treatment of trematode and cestode infections in human and veterinary medicine for over 40 years (Andrews, Thomas, Pohlke & Seubert 1983; Day, Bennett & Pax 1992). In aquaculture, PZQ has been demonstrated to be highly effective against a range of internal and external parasites in a wide range of fish species; mainly in the form of bath treatments at concentrations ranging from 2.5 to 1000 mg L^{-1} for periods of between 4 minutes and 48 hours (Sharp, Diggles, Poortenaar & Willis 2004; Tubbs & Tingle 2006). This method of administration, however, is expensive for sea cage operations and results in the discharge of large quantities of PZQ into the environment.

The oral application of PZQ in aquaculture has many advantages over bath treatments however its strong bitter taste is a major constraint to this delivery method (Hirazawa, Mitsuboshi, Hirata & Shirasu 2004; Williams, Ernst, Chambers & Whittington 2007). Traditional methods of taste masking, such as the addition of aromas or sugar, are ineffective in improving the taste of praziquantel (Watson 2009). Partridge, Michael & Thuillier (2014) demonstrated that microencapsulation of PZQ was effective in improving the palatability of PZQ in fish, however the microcapsules tested appeared to reduce bioavailability and palatability issues still remained at the high dietary inclusion levels required to achieve effective doses in large fish.

PZQ is synthesized as a racemate (*Rac*-PZQ); an equal combination of the *R*-(−) and *S*-(+) enantiomers (Woelfle, Seerden, de Gooijer, Pouwer, Olliaro & Todd 2011). Meyer, Sekljic, Fuchs, Bothe, Schollmeyer & Miculka (2009) found that the *S*-(+)-PZQ enantiomer is the main contributor to the bitter taste of the drug in humans and Oppel (2008) demonstrated

improved taste response in cats to *R*-(−)-PZQ during acceptance tests with medicated oral pastes. Furthermore, it has been demonstrated that the *R*-(−)-PZQ is the only enantiomer effective against the trematode *Schistosoma mansoni* (Staudt, Schmahl, Blaschke & Mehlhorn 1992). Despite the negative attributes of *S*-(+)-PZQ, PZQ has always been administered in the racemic form, as methods to directly synthesise pure *R*-(−)-PZQ or to resolve (enantioseparate) *Rac*-PZQ into its enantiomers on a commercial scale have not existed. Recently, however, two different techniques have been described for the large-scale resolution of *Rac*-PZQ into its enantiomers (Woelfle *et al.* 2011), potentially paving the way for the wide-scale administration of only *R*-(−)-PZQ.

Studies have not been conducted on whether fish find *R*-(−)-PZQ less bitter than *S*-(+)-PZQ or if the former is more efficacious than the latter against monogenean flukes. If fish show the same positive response to the taste of *R*-(−)-PZQ as mammals and if *R*-(−)-PZQ is the only enantiomer effective against monogenean flukes, then the constraints to the oral delivery of PZQ to fish infected with monogeneans will be overcome and the current administrative doses of *Rac*-PZQ could be reduced. Such advancements would see PZQ becoming the drug of choice against monogeneans.

The genus *Seriola* forms the largest true marine finfish aquaculture industry in the world, with over 150,000 tonnes per annum produced in Japan from three different species (*Seriola quinqueradiata* (Temminck & Schlegel), *Seriola dumerili* (Risso) *and Seriola lalandi* (Valenciennes)) (Nakada 2008). New *Seriola* industries are also developing in many other regions of the world including Australia and New Zealand, the Americas and Europe (Benetti 2008; Blanco Garcia, Partridge, Flik, Roques & Abbink 2014; Booth, Allan & Pirozzi 2010; Mylonas, Papandroulakis, Smboukis, Papadaki & Divanach 2004; Poortenaar, Hooker & Sharp 2001). All species of *Seriola* are susceptible to a range of monogenean parasites which affect the economic viability of their production (Hutson, Ernst & Whittington 2007). The

typical treatment for such parasites is the routine bathing of fish in hydrogen peroxide, a process which is labour intensive, logistically challenging and potentially dangerous to both the fish and operators if not properly executed. Furthermore, this management practice is expensive, with estimates that it contributes up to 20% of the cost of production (Ernst, Whittington, Corneillie & Talbot 2005). Praziquantel has been demonstrated to be effective against monogeneans infesting yellowtail kingfish *Seriola lalandi* (Valenciennes) (see Sharp *et al*. 2004, Tubbs & Tingle 2006), as too has the constraint of its bitterness to in-feed application (Partridge *et al.* 2014; Williams *et al.* 2007). The current study tested the hypothesis that enantioseparated *R*-(−) PZQ will be more palatable than *Rac*-PZQ, which will in turn be more palatable than *S*-(+)-PZQ to yellowtail kingfish.

2. Methods

2.1. Praziquantel

Racemic praziquantel (TNN Development Company, China) was obtained under an import consent permit issued by the Australian Pesticides and Veterinary Medicines Authority (APVMA) (Permit number KP40F37). It was separated into its two enantiomers based on methods described by Woelfle *et al*. (2011). The purity and enantiomeric ratios of all PZQ samples were independently assessed by the University of Sydney against certified standards. Purity was assessed using ^{1}H NMR Analysis (Bruker AVANCE 200 spectrometer, ^{1}H at 200.13 MHz and 300 K) following dissolution of 20 mg of sample into 0.5 mL of $CDCl_3$. The enantiomeric ratios of each sample were quantified using enantioselective normal phase HPLC analysis on a Waters 510 HPLC pump with a PDA detector. A Daicel Chiralcel OD-H analytical column (5 μm, 4.6 × 250 mm) was used with isopropanol/hexane/ TEA = 60:40:0.1

as the mobile phase at a flow rate of 0.5 mL min^{-1} and with retention times of *R*-(−)-PZQ and *S*-(+)-PZQ of 14 and 16 minutes, respectively

2.2. Experimental Design

Four palatability trials outlined in Table 1 were conducted at the Australian Centre for Applied Aquaculture Research (ACAAR) in Fremantle, Western Australia. The first three trials measured the ingestion of diets containing 10 g PZQ kg^{-1} by small (85 -160 g) and large (1.2 kg) fish. Whilst this inclusion level is much higher than required to deliver effective doses (50 – 150 mg PZQ kg body weight $(BW)^{-1}$) in the small fish, it was chosen on the basis that it will deliver effective doses across a wide range of fish sizes and therefore represents a useful model. An additional trial investigated the effect of varying dietary inclusion levels of *R*-(−) PZQ and *Rac*-PZQ on ingestion by small yellowtail kingfish.

In each trial, five yellowtail kingfish were stocked into replicate 180 L (Trials 1, 2 & 4) or 1,500 L (Trial 3) tanks with the number of replicates indicated in Table 1. Each trial used new fish with no previous exposure to PZQ. Each tank was supplied with flowing seawater (33 g L^{-1}) at exchange rates of 300% and 100% hr^{-1} in the small and large tanks, respectively. These exchange rates generated a tangential current which rapidly moved any uneaten food to a central pipe, where it was extracted into a collection cup on the outside of the tank. Each tank also had a single, central air stone to oxygenate the water and assist with the movement of waste across the tank floor towards the central pipe. All trials were conducted under a natural photoperiod and in water temperatures detailed in Table 1.

Each palatability trial ran for 5 days and was preceded by a period of 5 days acclimation to the experimental tanks, during which time the fish were fed the control diet described below. Prior to stocking, fish were anaesthetised (isoeugenol 20 mg L^{-1} (AQUI-S, Lower Hutt, New

Zealand)) and weighed to the nearest 1 g on a digital balance. During each trial, fish were fed daily at 0900 and 1500 on Nova ME (Skretting Australia™) pellets of 3mm (for small fish) or 9 mm (for large fish), top-coated with gelatine and the appropriate PZQ treatment, or the same diet coated only with gelatine (control). Each treatment diet was prepared in a 500 g batch by thoroughly hand-mixing the required amount and type of PZQ powder. Pellets were then top-coated with a 20% (w/v) gelatine solution at the rate of 50 mL kg^{-1} to adhere the PZQ to the surface (Partridge *et al*. 2014). Following the addition of gelatine the pellets were hand-mixed for a further 5 minutes to ensure a homogeneous coverage of gelatine and PZQ. Pellets were then spread in a thin layer and placed in a cool-room at 8°C to allow the gelatine to set. All pellets were prepared 1-2 days prior to the commencement of each trial and were stored in the cool-room prior to feeding.

During each trial, fish were offered a fixed ration based on their mean weight and water temperature using *Seriola* feed tables (Masumoto 2002). At each feeding period the fixed ration was offered slowly, allowing adequate time for the fish to encounter each pellet. Once encounters ceased, no further food was added to the tank. Five minutes after feeding ceased, rejected pellets were counted in the collection cup and converted to a weight of pellets based on their average dry weight. The weight of pellets eaten each day was summed from the morning and afternoon feeds and expressed as a percentage of the total ration offered according to the formula below, where 'offered' is the total weight of food in the daily ration, 'rejected' is the weight of pellets recovered in the collection cup and 'not offered' is the weight of pellets not fed into the tank.

$$\%\ of\ diet\ eaten = \frac{offered\ (g) - (rejected\ (g) + \ not\ offered\ (g))}{offered\ (g)} \times 100$$

These percentage data were arcsine transformed and compared between treatments by repeated measures analysis of variance, followed by post hoc Tukey's HSD tests on least

square means. Average daily ingestion data from Trial 4 were arcsine transformed then analysed by a two-way analysis of variance (with factors of dietary inclusion level and PZQ type), followed by Tukey's HSD tests. Statistical tests were performed on JMP (version 7, SAS, Cary, NC) and significance was accepted at $p < 0.05$.

3. Results

All batches of PZQ used in the trials had virtually identical ^{1}H NMR spectra to the pure commercial standard, demonstrating a very high purity. The *R*-(−)-PZQ used in Trial 1 was 97.8% *R*-(−)-PZQ, which was increased by further resolution to 99.6% in the batch used in all subsequent trials. The *S*-(+)-PZQ used in all trials contained 88.2% *S*-(+)-PZQ and 11.2% *R*-(−)-PZQ. It was observed that the characteristic smell of *Rac*-PZQ was more concentrated and acrid in the *R*-(−)-PZQ than in the *Rac*-PZQ, whilst the *S*-(+)-PZQ had very little smell.

3.1. Trial 1

Those fish fed the control diet ate 100% of their ration and were excluded from the data analysis. The percentage of medicated diets eaten on each day is shown in Figure 1. Repeated measures analysis of variance revealed no effect of time ($P = 0.21$), PZQ treatment ($P = 0.56$) or their interaction ($P = 0.08$) on the ingestion of medicated diets. The pooled average ingestion across time and medicated treatments was 32 ± 2%. While there was some indication that fish offered the diet medicated with *S*-(+)-PZQ ate more of their ration (54 ± 22%) than the other medicated treatments (23 ± 6%) on day 1, this difference was not significant.

3.2. Trial 2

Fish in the control treatment consumed 100% of their ration and were excluded from the data analysis. The percentage of diet eaten in each treatment on each day is shown in Figure 2.

Repeated measures analysis of variance revealed no significant effect of medicated treatment on ingestion rate ($P = 0.15$), but a significant effect of time ($P < 0.0001$), with no interaction between these factors ($P = 0.12$). Post hoc tests found no difference in ingestion between days 1 and 4, however all fish offered medicated diets ate significantly less food on day 5 (14.4 ± 5.0%) compared to previous days. The pooled average intake for all medicated diets across the 5 day feeding period was 38 ± 3%. There was no difference in ingestion between the *R*-(−)-PZQ with a purity of 97.8% (ingestion rate 32.3 ± 4.0%) and the more refined *R*-(−)-PZQ with a purity of 99.6% (ingestion rate 39.2 ± 4.0%). As in Trial 1, those fish offered the diet medicated with *S*-(+)-PZQ ate more food on day 1 (57.4 ± 7.4%) than the other medicated diets (35.6 ± 3.1%), but this difference was not significant.

3.3. Trial 3

The percentage of diet eaten by the large fish on each day is shown in Figure 3. While there was no control treatment included in this trial (due to a constraint on the number of tanks available) all fish ate 100% of their ration during the five day acclimation period and in the days immediately following the termination of the trial. Consumption of all medicated diets by the large fish in this trial was lower than seen in the previous two trials with small fish. Repeated measures analysis of variance revealed a significant effect of treatment ($P = 0.006$), time ($P = 0.001$) and their interaction ($P = 0.01$) on ingestion. Post hoc tests showed that those fish offered the diet medicated with *S*-(+)-PZQ ate significantly more (17.4 ± 1.3%) than those offered diets medicated with *R*-(−)-PZQ (2.5 ± 1.3%) and *Rac*-PZQ (2.3 ± 1.3%), which did not differ from each other. The difference between *S*-(+)-PZQ and the other two treatments was most pronounced on Day 1 when those fish offered *S*-(+)-PZQ ate 41.9% of their ration.

3.4. Trial 4

Fish in the control treatment consumed 100% of their ration and were excluded from the data analysis. Average daily ingestion of each medicated diet is shown in Figure 4. Two-way analysis of variance revealed a significant effect of PZQ dietary inclusion level on ingestion ($P = 0.002$), but no effect of PZQ type (*R*-(−)-PZQ or *Rac*-PZQ) ($P = 0.10$) and no interaction between these factors ($P = 0.25$). Post hoc tests revealed that ingestion of diets medicated with 2.5 g kg^{-1} (65 ± 3%) and 5 g kg^{-1} (57 ± 3%) were significantly higher than those medicated with 10 g kg^{-1} (39 ± 3%). Ingestion of diets containing 2.5 and 5.0 g kg^{-1} of *R*-(−)-PZQ were significantly higher than those containing 10.0 g kg^{-1} of *Rac*-PZQ.

Discussion

Our hypothesis that yellowtail kingfish would find *R*-(−)-PZQ more palatable than *Rac*-PZQ, which in turn would be more palatable than *S*-(+)-PZQ was rejected. Following the results of Trial 1 we hypothesised that the 2.2% of *S*-(+)-PZQ remaining within the enantioseparated *R*-(−)-PZQ may be been the cause of the poor palatability, however the results from Trial 2 demonstrated that this was not the case. Our findings are in contrast to studies in humans and cats and suggest that yellowtail kingfish may be more sensitive to the bitterness of PZQ. Oppel (2008) found that cats had better acceptance of an aqueous paste containing *R*-(−)-PZQ than the same paste containing *S*-(+)-PZQ, although acceptance of the former paste was not 100%. Meyer *et al.* (2009) reported that humans found *R*-(−)-PZQ to be significantly less bitter than *Rac*-PZQ, however the authors also noted a great deal of variation between members of the taste panel, with 2 out of 15 finding *R*-(−)-PZQ to be more bitter than *Rac*-PZQ. While these findings demonstrate that mammals find *R*-(−)-PZQ more appealing than *Rac*-PZQ and *S*-(+)-PZQ, they also highlight that it is not completely devoid of bitterness. Taste specificities vary greatly between fish species, but in general fish are considered to be more sensitive to taste than mammals (Kasumyan & Doving 2003). Channel catfish, *Ictalurus*

punctatus (Rafinesque), for example, have nearly 100 times more taste buds than that of an adult human (Finger, Drake, Kotrschal, Womble & Dockstader 1991). Fish have also been demonstrated to be highly sensitive to bitterness, with bitter compounds such as quinine, papaverine, sodium ricinolete, caffeine and theophylline all eliciting inhibitory feeding responses across a range of fish species (Kasumyan & Doving 2003).

In a previous study, we put forward the hypothesis that fish may become less sensitive to bitterness as they grow (Partridge *et al.* 2014), on the basis of reports of a decline in the number of taste buds in other fish species and animals as they age (Harvey & Batty 1998; Yamaguchi, Harada, Kanemaru & Kasahara 2001; Shin, Cong, Cai, Kim, Maudsley, Egan & Martin 2012). The current trials do not support this; 1.2 kg yellowtail kingfish consumed substantially less medicated feed than small fish at a PZQ inclusion level of 10 g kg^{-1}. This suggests that the larger yellowtail kingfish are more sensitive to the taste of PZQ than the smaller fish. Although there are no data on the ontogenetic change in taste bud density in *Seriola* spp., these results may indicate that density may increase to the sub-adult stage in yellowtail kingfish before declining. In Atlantic cod *Gadus morhua* (Linnaeus), which grow to ca.70 cm in length, taste bud density increases with fish size up to ca.10 cm before beginning to decline (Harvey & Batty 1998). Kohbara et al. (2000) found 3 kg wild Japanese yellowtail *Seriola quinqueradiata* (Temminck & Schlegel) to be less sensitive to five out of six chemicals tested than fish of ca. 40 grams, suggesting a decrease in taste sensitivity may occur at a large size in fish from this genus. Clearly further studies are needed on the taste sensitivity of yellowtail kingfish to PZQ over a range of age/size classes.

During Trials 1, 2 and 3, we saw evidence that the *S*-(+)-PZQ enantiomer, which is reported to be more bitter in mammals, was ingested to a higher degree than both *R*-(−)-PZQ and *Rac*-PZQ. While this difference was non-significant and limited to the first day of feeding in small fish, it was significant in the large fish. As previously reported, the *S*-(+)-PZQ was largely devoid of smell, whilst the characteristic smell of *Rac*-PZQ was concentrated and more acrid in the *R*-(−)-PZQ enantiomer. These factors suggest that smell may be an equal if not greater factor to the palatability of PZQ in yellowtail kingfish as taste. This is supported by other studies demonstrating that fish have a greater acuity for smell than taste. Channel catfish, for example, have the most taste buds of any fish species, yet are still 25 to 50 times more sensitive to the smell of the bitter compound quinine hydrochloride than its taste (Bardach & Atema 1971). In many fish species it has been demonstrated that the number and surface area of olfactory lamellae increases with age, suggesting an increase in sensitivity to smell (Hara 1994). Our data showing a reduced acceptance of the stronger smelling *R*-(−)-PZQ in larger fish compared with the smaller fish supports yellowtail kingfish conforming to this generalised model. Whilst there appear to be no data on the ontogenetic changes in the number or surface areas of the olfactory lamellae in *Seriola sp*., preliminary analysis conducted on the 1.2 kg fish used in this study revealed 26 lamellae on each olfactory bulb. In freshwater fish, the number of olfactory lamellae ranges from two in the three-spined stickleback *Gasterosteus aculeatus* (Linnaeus) to more than 60 in the European eel *Anguillia anguilla* (Linnaeus), which is renowned for its highly sensitive sense of smell (Hara 1993). On the basis of this simple comparison we suggest that yellowtail kingfish may have an intermediate sensitivity to smell.

Withholding food from fish for a few days prior to introducing diets medicated with PZQ has been suggested anecdotally as a method of encouraging fish to accept the diets. However, in

most cases our data do not show any improvement in ingestion over the few days of these trials, suggesting that hunger is not a sufficient driving force to encourage the fish to consume the unpalatable diets, at least over these relatively short time periods. Indeed, the small fish in Trial 2 ate significantly less of the fifth day of the trial that in the previous four days. The only fish to show an improvement in intake over time were the large fish offered the diet containing the *S*-(+)-PZQ enantiomer.

Despite the lack of evidence from this study that *R*-(−)-PZQ is more palatable than *S*-(+)-or *Rac*-PZQ, the incorporation of *R*-(−)-PZQ into diets may still be advantageous over the current practice of using *Rac*-PZQ. While the effectiveness of different PZQ enantiomers against monogenean parasites has yet to be determined, the *S*-(+)-PZQ enantiomer has virtually no antiparasitic effect against the trematode *Schistosoma* or cestodes (Andrews *et al.* 1983; Staudt *et al.* 1992). Our data demonstrate that dietary inclusion level is a more important factor in diet palatability than PZQ enantiomeric composition. Results from Trial 4, for example, demonstrated that the palatability of a diet containing 5 g kg^{-1} of *R*-(−)-PZQ was significantly greater than that containing 10 g kg^{-1} of *Rac*-PZQ, yet both diets would be equally efficacious if *R*-(−)-PZQ proves to be the only enantiomer effective against monogeneans. To the best of our knowledge, *R*-(−)-PZQ is not yet readily available on a large scale and therefore its commercial cost is unknown. It is safe to assume, however that the cost will be at least double that of racemic PZQ as the resolution process begins with racemic PZQ and retains at best only half of the starting material, with the S-(+)-PZQ being discarded.

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6. References:

Andrews P, Thomas H, Pohlke R, Seubert J (1983) Praziquantel. *Med. Res. Rev.,* **3**, 147-200.

Bardach J, Atema J (1971) The Sense of Taste in Fishes. In: *Taste* (ed by Beidler L). Springer Berlin Heidelberg, pp. 293-336.

Benetti D (2008) Review of *Seriola* spp. Aquaculture. In: *Proceedings of the International Workshop: Developing a Sustainable Aquaculture Industry in the Azores.* (eds Pham CK, Higgins RM, De Girolamo M, Isidro E). Arquipelago Life and Marine Sciences, Horta.

Blanco Garcia A, Partridge GJ, Flik G, Roques JAC, Abbink W (2014) Ambient salinity and osmoregulation, energy metabolism and growth in juvenile yellowtail kingfish (*Seriola lalandi* Valenciennes 1833) in a recirculating aquaculture system. *Aquac. Res.*, doi: 10.1111/are.12433.

Booth MA, Allan GA, Pirozzi I (2010) Estimation of digestible protein and energy requirements of yellowtail kingfish *Seriola lalandi* using a factorial approach. *Aquaculture*, **307**, 247-259.

Day TA, Bennett JL, Pax RA (1992) Praziquantel: The enigmatic antiparasitic. *Parasitol. Today*, **8**, 342-344.

Ernst I, Whittington ID, 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.

Finger TE, Drake SK, Kotrschal K, Womble M, Dockstader KC (1991) Postlarval growth of the peripheral gustatory system in the channel catfish, *Ictalurus punctatus*. *J. Comp. Neurol.,* **314**, 55-66.

Hara T (1993) Chemoreception. In: *The Physiology of Fishes* (ed by Evans).

Hara T (1994) The diversity of chemical stimulation in fish olfaction and gustation. *Rev. Fish Biol. Fish.,* **4**, 1-35.

Harvey R, Batty RS (1998) Cutaneous taste buds in cod. *J. Fish Biol.,* **53**, 138-149.

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.

Hutson KS, Ernst I, Whittington ID (2007) Risk assessment for metazoan parasites of yellowtail kingfish *Seriola lalandi* (Perciformes: Carangidae) in South Australian sea-cage aquaculture. *Aquaculture,* **271**, 85-99.

Kasumyan AO, Doving KB (2003) Taste preferences in fish. *Fish Fish.,* **4**, 289-347.

Kohbara J, Furukawa K, Taishaku H, Hidaka I (2000) Response changes with growth in the gustatory receptors of young yellowtails (*Seriola quinqueradiata*). *Fish. Sci. (Tokyo),* **66**, 1106-1114.

Masumoto T (2002) Yellowtail, *Seriola quinqueradiata*. In: *Nutrient Requirements and Feeding of Finfish for Aquaculture* (ed. by Webster CD, Lim C). CABI Publishing, New York, pp. 131-146.

Meyer T, Sekljic H, Fuchs S, Bothe H, Schollmeyer D, Miculka C (2009) Taste, A New Incentive to Switch to (*R*)-Praziquantel in Schistosomiasis Treatment. *PLoS Negl. Trop. Dis.*, **3**, e357.

Mylonas CC, Papandroulakis N, Smboukis A, Papadaki M, Divanach P (2004) Induction of spawning of cultured greater amberjack (*Seriola dumerili*) using GnRHa implants. *Aquaculture*, **237**, 141-154.

Nakada M (2008) Capture-based aquaculture of yellowtail. In: *Capture-based aquaculture. Global overview* (eds Lovatelli A, Holthus PF). FAO, Rome, pp. 199-215.

Oppel K (2008) Taste masking of an active pharmaceutical ingredient for veterinary application. University of Basel, Germany, pp. 137.

Partridge GJ, Michael RJ, Thuillier L (2014) Praziquantel form, dietary application method and dietary inclusion level affect palatability and efficacy against monogenean parasites in yellowtail kingfish. *Dis. Aquat. Org.,* **109**, 155-163.

Poortenaar CW, Hooker SH, Sharp N (2001) Assessment of yellowtail kingfish (*Seriola lalandi lalandi*) reproductive physiology, as a basis for aquaculture development. *Aquaculture*, **201**, 271-286.

Sharp NJ, Diggles BK, Poortenaar CW, Willis TJ (2004) Efficacy of Aqui-S, formalin and praziquantel against the monogeneans, *Benedenia seriolae* and *Zeuxapta seriolae*, infecting yellowtail kingfish *Seriola lalandi lalandi* in New Zealand. *Aquaculture,* **236**, 67-83.

Shin YK, Cong WN, Cai H, Kim W, Maudsley S, Egan JM, Martin B (2012) Age-related changes in mouse taste bud morphology, hormone expression, and taste responsivity. *J. Gerontol.*, **67**, 336-344.

Staudt U, Schmahl G, Blaschke G, Mehlhorn H (1992) Light and scanning electron microscopy studies on the effects of the enantiomers of praziquantel and its main metabolite on *Schistosoma mansoni* in vitro. *Parasitol. Res.,* **78**, 392-397.

Tubbs LA, Tingle MD (2006) Bioavailability and pharmacokinetics of a praziquantel bolus in kingfish *Seriola lalandi*. *Dis. Aquat. Org.*, **69**, 233-238.

382 Watson M (2009) Praziquantel. *J. Exot. Pet Med.*, **18**, 229-231.
383 Williams R, Ernst I, Chambers C, Whittington I (2007) Efficacy of orally administered
384 praziquantel against *Zeuxapta seriolae* and *Benedenia seriolae* (Monogenea) in
385 yellowtail kingfish *Seriola lalandi*. *Dis. Aquat. Org.*, **77**, 199-205.
386 Woelfle M, Seerden J-P, de Gooijer J, Pouwer K, Olliaro P, Todd MH (2011) Resolution of
387 Praziquantel. *PLoS Negl. Trop. Dis.*, **5**, e1260.
388 Yamaguchi K, Harada S, Kanemaru N, Kasahara Y (2001) Age-related alteration of taste bud
389 distribution in the common marmoset. *Chem. Senses*, **26**, 1-6.

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Table Captions:

Table 1: Treatments investigated in each trial. [1] = 97.8% *R*-(−)-PZQ, [2] = 99.6% *R*-(−)-PZQ, [*] = 88.2% *S*-(+)-PZQ.

Figure Captions:

Figure 1: Ingestion of diets medicated with different enantiomers of PZQ by small yellowtail kingfish over time in Trial 1.

Figure 2: Ingestion of diets medicated with different enantiomers of PZQ by small yellowtail kingfish over time in Trial 2.

Figure 3: Ingestion of diets medicated with different enantiomers of PZQ by large yellowtail kingfish over time.

Figure 4: Effect of dietary inclusion level on ingestion of diets medicated with different enantiomers of PZQ by small yellowtail kingfish. Columns sharing the same letter are not significantly different (P>0.05).

411 Table 1

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Trial	Treatment	Active PZQ Inclusion (g PZQ kg diet^{-1})	Fish size (g ± S.D)	Temperature (°C)
1	*R*-(−)-PZQ[1]	10	160 ± 45	23.1
(n = 3)	*S*-(+)-PZQ*	10		
	Rac-PZQ	10		
	Control	0		
2	*R*-(−)-PZQ[1]	10	85 ± 1	20.5
	R-(−)-PZQ[2]	10		
(n = 3)	*S*-(+)-PZQ*	10		
	Rac-PZQ	10		
	Control	0		
3	*R*-(−)-PZQ[2]	10	1175 ± 221	21.5
(n = 2)	*S*-(+)-PZQ*	10		
	Rac-PZQ	10		
4	*R*-(−)-PZQ[2]	2.5	170 ± 22	18.5
(n = 2)	*R*-(−)-PZQ[2]	5		
	R-(−)-PZQ[2]	10		
	Rac-PZQ	2.5		
	Rac-PZQ	5		
	Rac-PZQ	10		
	Control	0		

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