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

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17 **Running Title:** Palatability of praziquantel and its isomers

18
19 **Key Words:** praziquantel; enantiomers; isomers, yellowtail kingfish; palatability;
20 monogeneans

22 **Abstract**

23

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

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

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

44

45 1. Introduction

46

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

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

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

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

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

85 The genus *Seriola* forms the largest true marine finfish aquaculture industry in the world,
86 with over 150,000 tonnes per annum produced in Japan from three different species (*Seriola*
87 *quinqueradiata* (Temminck & Schlegel), *Seriola dumerili* (Risso) and *Seriola lalandi*
88 (Valenciennes)) (Nakada 2008). New *Seriola* industries are also developing in many other
89 regions of the world including Australia and New Zealand, the Americas and Europe (Benetti
90 2008; Blanco Garcia, Partridge, Flik, Roques & Abbink 2014; Booth, Allan & Pirozzi 2010;
91 Mylonas, Papandroulakis, Smboukis, Papadaki & Divanach 2004; Poortenaar, Hooker &
92 Sharp 2001). All species of *Seriola* are susceptible to a range of monogenean parasites which
93 affect the economic viability of their production (Hutson, Ernst & Whittington 2007). The

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

104

105 **2. Methods**

106 **2.1. Praziquantel**

107

108 Racemic praziquantel (TNN Development Company, China) was obtained under an import
109 consent permit issued by the Australian Pesticides and Veterinary Medicines Authority
110 (APVMA) (Permit number KP40F37). It was separated into its two enantiomers based on
111 methods described by Woelfle *et al.* (2011). The purity and enantiomeric ratios of all PZQ
112 samples were independently assessed by the University of Sydney against certified standards.
113 Purity was assessed using ¹H NMR Analysis (Bruker AVANCE 200 spectrometer, ¹H at
114 200.13 MHz and 300 K) following dissolution of 20 mg of sample into 0.5 mL of CDCl₃.
115 The enantiomeric ratios of each sample were quantified using enantioselective normal phase
116 HPLC analysis on a Waters 510 HPLC pump with a PDA detector. A Daicel Chiralcel OD-H
117 analytical column (5 μm, 4.6 × 250 mm) was used with isopropanol/hexane/ TEA = 60:40:0.1

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

120 **2.2. Experimental Design**

121

122 Four palatability trials outlined in Table 1 were conducted at the Australian Centre for
123 Applied Aquaculture Research (ACAAR) in Fremantle, Western Australia. The first three
124 trials measured the ingestion of diets containing 10 g PZQ kg^{-1} by small (85 -160 g) and large
125 (1.2 kg) fish. Whilst this inclusion level is much higher than required to deliver effective
126 doses ($50 - 150 \text{ mg PZQ kg body weight (BW)}^{-1}$) in the small fish, it was chosen on the basis
127 that it will deliver effective doses across a wide range of fish sizes and therefore represents a
128 useful model. An additional trial investigated the effect of varying dietary inclusion levels of
129 *R*-(-) PZQ and *Rac*-PZQ on ingestion by small yellowtail kingfish.

130 In each trial, five yellowtail kingfish were stocked into replicate 180 L (Trials 1, 2 & 4) or
131 1,500 L (Trial 3) tanks with the number of replicates indicated in Table 1. Each trial used
132 new fish with no previous exposure to PZQ. Each tank was supplied with flowing seawater
133 (33 g L^{-1}) at exchange rates of 300% and 100% hr^{-1} in the small and large tanks, respectively.
134 These exchange rates generated a tangential current which rapidly moved any uneaten food to
135 a central pipe, where it was extracted into a collection cup on the outside of the tank. Each
136 tank also had a single, central air stone to oxygenate the water and assist with the movement
137 of waste across the tank floor towards the central pipe. All trials were conducted under a
138 natural photoperiod and in water temperatures detailed in Table 1.

139 Each palatability trial ran for 5 days and was preceded by a period of 5 days acclimation to
140 the experimental tanks, during which time the fish were fed the control diet described below.

141 Prior to stocking, fish were anaesthetised (isoeugenol 20 mg L^{-1} (AQUI-S, Lower Hutt, New

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

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

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

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

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

169 **3. Results**

170 All batches of PZQ used in the trials had virtually identical ^1H NMR spectra to the pure
171 commercial standard, demonstrating a very high purity. The *R*-(-)-PZQ used in Trial 1 was
172 97.8% *R*-(-)-PZQ, which was increased by further resolution to 99.6% in the batch used in
173 all subsequent trials. The *S*-(+)-PZQ used in all trials contained 88.2% *S*-(+)-PZQ and 11.2%
174 *R*-(-)-PZQ. It was observed that the characteristic smell of *Rac*-PZQ was more concentrated
175 and acrid in the *R*-(-)-PZQ than in the *Rac*-PZQ, whilst the *S*-(+)-PZQ had very little smell.

176 **3.1. Trial 1**

177 Those fish fed the control diet ate 100% of their ration and were excluded from the data
178 analysis. The percentage of medicated diets eaten on each day is shown in Figure 1. Repeated
179 measures analysis of variance revealed no effect of time ($P = 0.21$), PZQ treatment ($P = 0.56$)
180 or their interaction ($P = 0.08$) on the ingestion of medicated diets. The pooled average
181 ingestion across time and medicated treatments was $32 \pm 2\%$. While there was some
182 indication that fish offered the diet medicated with *S*-(+)-PZQ ate more of their ration ($54 \pm$
183 22%) than the other medicated treatments ($23 \pm 6\%$) on day 1, this difference was not
184 significant.

185 **3.2. Trial 2**

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

188 Repeated measures analysis of variance revealed no significant effect of medicated treatment
189 on ingestion rate ($P = 0.15$), but a significant effect of time ($P < 0.0001$), with no interaction
190 between these factors ($P = 0.12$). Post hoc tests found no difference in ingestion between days
191 1 and 4, however all fish offered medicated diets ate significantly less food on day 5 ($14.4 \pm$
192 5.0%) compared to previous days. The pooled average intake for all medicated diets across
193 the 5 day feeding period was $38 \pm 3\%$. There was no difference in ingestion between the *R*-
194 $(-)$ -PZQ with a purity of 97.8% (ingestion rate $32.3 \pm 4.0\%$) and the more refined *R*- $(-)$ -PZQ
195 with a purity of 99.6% (ingestion rate $39.2 \pm 4.0\%$). As in Trial 1, those fish offered the diet
196 medicated with *S*- $(+)$ -PZQ ate more food on day 1 ($57.4 \pm 7.4\%$) than the other medicated
197 diets ($35.6 \pm 3.1\%$), but this difference was not significant.

198 **3.3. Trial 3**

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

211 3.4. Trial 4

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

220 Discussion

221 Our hypothesis that yellowtail kingfish would find *R*-(-)-PZQ more palatable than *Rac*-PZQ,
222 which in turn would be more palatable than *S*-(+)-PZQ was rejected. Following the results of
223 Trial 1 we hypothesised that the 2.2% of *S*-(+)-PZQ remaining within the enantioseparated *R*-
224 (-)-PZQ may be been the cause of the poor palatability, however the results from Trial 2
225 demonstrated that this was not the case. Our findings are in contrast to studies in humans and
226 cats and suggest that yellowtail kingfish may be more sensitive to the bitterness of PZQ.
227 Oppel (2008) found that cats had better acceptance of an aqueous paste containing *R*-(-)-PZQ
228 than the same paste containing *S*-(+)-PZQ, although acceptance of the former paste was not
229 100%. Meyer *et al.* (2009) reported that humans found *R*-(-)-PZQ to be significantly less
230 bitter than *Rac*-PZQ, however the authors also noted a great deal of variation between
231 members of the taste panel, with 2 out of 15 finding *R*-(-)-PZQ to be more bitter than *Rac*-
232 PZQ. While these findings demonstrate that mammals find *R*-(-)-PZQ more appealing than
233 *Rac*-PZQ and *S*-(+)-PZQ, they also highlight that it is not completely devoid of bitterness.
234 Taste specificities vary greatly between fish species, but in general fish are considered to be
235 more sensitive to taste than mammals (Kasumyan & Doving 2003). Channel catfish, *Ictalurus*

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

241

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

258

259 During Trials 1, 2 and 3, we saw evidence that the *S*-(+)-PZQ enantiomer, which is reported
260 to be more bitter in mammals, was ingested to a higher degree than both *R*-(-)-PZQ and *Rac*-
261 PZQ. While this difference was non-significant and limited to the first day of feeding in small
262 fish, it was significant in the large fish. As previously reported, the *S*-(+)-PZQ was largely
263 devoid of smell, whilst the characteristic smell of *Rac*-PZQ was concentrated and more acrid
264 in the *R*-(-)-PZQ enantiomer. These factors suggest that smell may be an equal if not greater
265 factor to the palatability of PZQ in yellowtail kingfish as taste. This is supported by other
266 studies demonstrating that fish have a greater acuity for smell than taste. Channel catfish, for
267 example, have the most taste buds of any fish species, yet are still 25 to 50 times more
268 sensitive to the smell of the bitter compound quinine hydrochloride than its taste (Bardach &
269 Atema 1971). In many fish species it has been demonstrated that the number and surface area
270 of olfactory lamellae increases with age, suggesting an increase in sensitivity to smell (Hara
271 1994). Our data showing a reduced acceptance of the stronger smelling *R*-(-)-PZQ in larger
272 fish compared with the smaller fish supports yellowtail kingfish conforming to this
273 generalised model. Whilst there appear to be no data on the ontogenetic changes in the
274 number or surface areas of the olfactory lamellae in *Seriola sp.*, preliminary analysis
275 conducted on the 1.2 kg fish used in this study revealed 26 lamellae on each olfactory bulb.
276 In freshwater fish, the number of olfactory lamellae ranges from two in the three-spined
277 stickleback *Gasterosteus aculeatus* (Linnaeus) to more than 60 in the European eel *Anguilla*
278 *anguilla* (Linnaeus), which is renowned for its highly sensitive sense of smell (Hara 1993).
279 On the basis of this simple comparison we suggest that yellowtail kingfish may have an
280 intermediate sensitivity to smell.

281

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

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

290

291 Despite the lack of evidence from this study that *R*-(-)-PZQ is more palatable than *S*-(+)-or
292 *Rac*-PZQ, the incorporation of *R*-(-)-PZQ into diets may still be advantageous over the
293 current practice of using *Rac*-PZQ. While the effectiveness of different PZQ enantiomers
294 against monogenean parasites has yet to be determined, the *S*-(+)-PZQ enantiomer has
295 virtually no antiparasitic effect against the trematode *Schistosoma* or cestodes (Andrews *et al.*
296 1983; Staudt *et al.* 1992). Our data demonstrate that dietary inclusion level is a more
297 important factor in diet palatability than PZQ enantiomeric composition. Results from Trial 4,
298 for example, demonstrated that the palatability of a diet containing 5 g kg⁻¹ of *R*-(-)-PZQ was
299 significantly greater than that containing 10 g kg⁻¹ of *Rac*-PZQ, yet both diets would be
300 equally efficacious if *R*-(-)-PZQ proves to be the only enantiomer effective against
301 monogeneans. To the best of our knowledge, *R*-(-)-PZQ is not yet readily available on a
302 large scale and therefore its commercial cost is unknown. It is safe to assume, however that
303 the cost will be at least double that of racemic PZQ as the resolution process begins with
304 racemic PZQ and retains at best only half of the starting material, with the *S*-(+)-PZQ being
305 discarded.

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307

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310

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312

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392 **Table Captions:**

393 Table 1: Treatments investigated in each trial. ¹ = 97.8% *R*-(-)-PZQ, ² = 99.6% *R*-(-)-
394 PZQ, ^{*} = 88.2% *S*-(+)-PZQ.

395

396 **Figure Captions:**

397

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

400

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

403

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

406

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

410

411 Table 1

412

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

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