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Absorption and depletion of dietary administered praziquantel in greater amberjack, *Seriola dumerili*

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ABSTRACT

The absorption and depletion of dietary administered praziquantel (PZQ) were explored in greater amberjack, *Seriola dumerili*, a promising farmed fish species in the Mediterranean area and elsewhere. Greater amberjack weighing 84 g were fed once daily for 5 days on diets containing either 1.5 or 3.0 g/kg of PZQ to deliver dose rates of 30 or 60 mg/kg fish, respectively. During the first day, plasma samples were measured for PZQ concentration at 2, 4, 8 and 24 h post-feeding. On the other treatment days, blood (plasma), muscle plus skin and gills were collected daily 24 h post-administration. Praziquantel was rapidly absorbed into fish circulation as the maximum plasma concentration (C_{max}) was achieved at 8 h (3.0 μg/ml) and 2 h post administration (4.3 μg/ml) for the low and high dosing, respectively. The two-fold increase in dosing led to a significant dose-dependent effect between the mean PZQ concentrations of the two dosing regimens in most selected sampling points such as 2, 4, 24, 120 h. While the elimination half-life ($t_{1/2b}$) was shorter in high PZQ dosing (8.2 h) compared to that calculated for the low (15.1 h), depletion of PZQ from greater amberjack plasma was considered rapid on both trials, as its concentration fell to 0.31 (low) and 0.56 (high) μg/ml 24 h post-treatment. Elimination of PZQ was also rapid from the gills and muscle plus skin, with drug levels measured below the limit of quantification as early as 72 and 96 h post treatment, for the low and the high dosing, respectively. Overall, the high dosing regimen is preferable over the low dosing schedule since it was readily accepted and resulted in higher circulatory levels in greater amberjack. Moreover, the rapid elimination of PZQ from the body compartment of greater amberjack suggested a twice a day administration of medicated meals to ensure adequate drug circulatory levels.

1. Introduction

Praziquantel (PZQ) is a synthetic drug that was discovered in the 1970s and approved for medical use a decade later (Andrews et al. 1983). Praziquantel is remarkably effective against a broad range of gastrointestinal and external parasites including those that cause schistosomiasis, clonorchiasis, opisthorchiasis, tapeworm infections, cysticercosis and hydatid disease in humans (WHO, 2016). The compound disrupts the parasite's integument, inducing spastic muscular paralysis (Staudt et al. 1992). Paralysis is possibly accompanied by a rapid Ca^{2+} influx into the parasite as the Ca^{2+} channels appeared the molecular target of PZQ (Doenhoff et al. 2009). The synergistic action of PZQ and host specific immune response has also been demonstrated in human diseases (Ribeiro et al. 2004). In addition to its universal prescription as a human antiparasitic, PZQ has also been widely used in veterinary medicine (Eom et al. 1988), but generally lacks registration in aquaculture.

Currently, the only compound registered to confront fish parasites in most European countries, the United States and elsewhere is formalin. Selection of other compounds such as PZQ is however feasible in fish medicine within the 'off-label' framework described as the Cascade Principle. Indeed, at a European level, the Council Directive 90/676/EEC, the Directive 2001/82/EC and the Commission Regulation 37/2010, provide legal details about the prescribing cascade system to support the use of drugs authorized for other farmed animals, when no suitable registered compound is available to treat diseases in fish. In such cases, a standard withdrawal time (WT) of 500 degree days (dd) is imposed to ensure consumer safety. For production animals where the use of PZQ is approved (i.e. 'labeled'), the Committee for Veterinary Medicinal Products (CVMP) has not established a maximum residue level (MRL) because the compound is extensively metabolised and rapidly excreted and thus amended its entry in Annex II of Council Regulation (EEC) No. 2377/90 (EMEA 1988).

A wide and comprehensive review, on bath or orally-administered

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PZQ to control platyhelminth parasites of fish has been recently published (Bader et al. 2019). Praziquantel has been proven as a very effective chemotherapeutic against monogeneans infecting gills, skin and branchial cavities (Hirazawa et al. 2004; Sharp et al. 2004). Orally administered PZQ preparations at doses of 10 to 500 mg/kg BW daily for up to 20 d have been tested in a variety of cultured fish species (see Björklund and Bylund 1987; Kim et al. 2001, 2003; Tubbs and Tingle 2006a, 2006b; Williams et al. 2007; Tubbs et al. 2008; Shirakashi et al. 2012; Hirazawa et al. 2013; Ishimaru et al. 2013; Partridge et al. 2014; Xu et al. 2016; Partridge et al. 2019).

Typically, an empirical approach is used to design treatment schedules in aquatic medicine which is commonly based on extrapolation from other, in most cases, related farmed species. However, species and environmental-specific pharmacokinetic differences are clearly apparent in aquatic chemotherapy (Rigos and Troisi 2005). Circulatory and tissue (especially at the site of activity) concentrations of a selected drug in a targeted animal are thus paramount to design an effective treatment schedule. However, to our knowledge, there is no literature examining the absorption properties of PZQ in greater amberjack, *Seriola dumerili*, a promising emerging farmed finfish species for the Mediterranean area (Mazzola et al. 2000; Yilmaz and Sereflisan 2011). Greater amberjack, however can suffer from severe infestations such as those caused by the gill fluke *Zeuxapta seriolae* (Montero et al. 2004; Lu et al. 2012). Mortalities due to this monogenean can exceed 70% and are therefore a substantial obstacle for the further expansion of greater amberjack in the area (Montero et al. 2004). Baths with hydrogen peroxide (100 ppm for an hour) appear very effective against *Z. seriolae* infections (Mansell et al. 2005), however, a dietary medicine would solve the practical issues associated with applications of such baths in large cages such as laborious processes, stressful and potentially toxic to fish and inability to apply in adverse weather conditions.

Thus, the aim of this study was to compare the absorption and depletion profiles of two different doses of dietary administered PZQ in greater amberjack as a first step to optimize PZQ dosing regimens for this species. In addition to PZQ measurements in greater amberjack plasma, drug levels were also determined in gills, the target site of *Z. seriolae* activity and in muscle plus skin to calculate removal of the drug from edible tissues.

2. Materials & methods

2.1. Experimental fish

Three hundred and twelve clinically healthy greater amberjack averaging 84 ± 12 g were obtained from a local fish farm (Argosaronikos Fish Farms S.A). Fish were separated into two treatment groups (low and high dose) and distributed into four 1 m^3 cages, located within a 50 m^3 cement tank (78 fish per cage). Water was supplied by open flow (60 l/min) and oxygen was provided continuously by bubbling air. Water temperature and salinity were 24 °C and 38‰, respectively. Fish were allowed to acclimate for 10 days prior to experimentation and fed a drug-free commercial diet at 2% BW/day in a single feed. To ensure the same nutritional status, fish were starved for 24 h prior to administering the medicated diets. Management of experimental animals followed the EU legislation "on the protection of animals used for scientific purposes" according to the EC Council Directive 2010/63/ EU (EU, 2010).

2.2. Medicated feed and drug administration

Fish received one of two medicated diets containing different amounts of PZQ (Bayer Ltd.) (Table 1). One batch of 2 kg for each of the two distinct experimental diets was made by mixing the active substance (1.5 g PZQ/kg feed and 3 g PZQ/kg feed) homogeneously with all the dietary ingredients prior to the cold pelleting process with a Hobart food pellet mill (Arlington, USA). Fish of the low dosing group were fed the 1.5 g PZQ/kg feed experimental diet, while fish of the high dosing group were fed the 3 g PZQ/kg feed experimental diet. Feeding was performed once per day for 5 consecutive days at 2% BW/day. To evaluate the medicated feed acceptance, fish behaviour was monitored (macroscopically) during feeding and for 30 min after the completion of feeding. Moreover, underwater cameras recorded fish behaviour throughout the duration of the trial. The amount of feed offered was calculated on a daily basis, depending on the number of fish remaining in the tanks. The doses of administered PZQ received by the fish were estimated to be 30 (low) and 60 mg/kg fish (high) based on the dietary inclusion levels and feed rates.

Table 1
Ingredient inclusion (%) in the experimental diets.

Ingredient (g/100 g)	Low inclusion	High inclusion
Fish meal 68	60.00	60.00
Krill meal	12.00	12.00
Wheat meal	18.25	18.25
Wheat gluten	3.00	3.00
Fish oil	5.00	5.00
Vitamins	0.27	0.27
Shrimp-based attractant	1.50	1.50
PZQ	0.15	0.30

2.3. Sampling

Fish sampling was performed at predetermined time points after anesthetization with clove oil (1 ppm). After sampling, fish were killed by a blow to the head. During the first day, blood samples were collected at 0, 2, 4, 8 and 24 h post-feeding. On the other intervention days during therapy, blood was collected daily 24 h post- administration until day 5. In order to minimise stress effects, fish sampling was carried out sequentially between the duplicate experimental cages. Approximately, 2 ml of blood were drawn with a needle (Microlance 23G 11/4 0.6 × 30, Becton Dickinson, Zaragoza, Spain) from the caudal vein of 7 individuals and transferred into heparinised test tubes. Plasma was separated from blood samples by centrifugation at 14,000 rpm for 10 min at 4 °C. Gills as well as muscle plus skin (approximately 5 g) from the anterior dorsal region were collected on days 1, 2, 3, 4 and 6 post-treatment from 5 and 10 individuals, respectively. All prepared tissue samples were immediately frozen and stored at −20 °C until analysis.

2.4. Chemicals and reagents

Praziquantel analytical standard was obtained from Sigma-Aldrich (USA). High performance liquid chromatography (HPLC) grade ethyl acetate, hexane, acetone, diethyl ether and HPLC gradient grade acetonitrile were purchased from Fisher Scientific (USA). Other solvents and reagents of analytical grade were supplied by Fisher Scientific (USA), while heparin (5000 U·I/ml) was obtained from Merck KGaA (Germany). Stock solution of 100 μg/ml PZQ was prepared by dissolving PZQ in acetonitrile and stored at −20 °C, while working solution (10 μg/ml) was prepared before use with acetonitrile:water (35:65 *v*/v).

2.5. Sample preparation

A modified method of Ridtitid et al. (2002) was used for the extraction of PZQ in plasma samples. Briefly, in 0.5 ml of plasma, 0.1 ml of zinc sulphate solution (0.2 M) were added drop-wise and mixed for 30 s on a vortex mixer. Two milliliters of acetonitrile were then added also drop-wise and shaken thoroughly on a vortex mixer. The sample was placed on a magnetic stirrer for 10 min, and then was centrifuged at 10000 *g* for 10 min at 10 °C.The supernatant was collected and transferred to a 15 ml polypropylene centrifuge tube, and the extraction step was repeated. The combined extract was then evaporated to dryness at 45 °C under a gentle stream of nitrogen. The dry residue was reconstituted by 1 ml of mobile phase solution, filtered using 0.22 μm nylon

filter and an aliquot (200 μl) injected into the HPLC.

Gill and muscle plus skin samples were prepared according to Tubbs and Tingle (2006b). Briefly, tissue sample was sheared, and subsequently 1 g of ground sample was weighed into a 50 ml centrifuge tube. Six ml of ethyl acetate were added and the mixture was homogenized with an IKA Ultra-Turrax T25 Disperser (IKA®-Werke GmbH & Co. KG, Staufen, Germany) for 30 s at16,000 rpm/min. The mixture was agitated for 10 min and then was centrifuged at 10,000 *g* for 10 min at 10 °C. The supernatant was transferred to a 15 ml tube and liquid extraction was repeated with 4 ml ethyl acetate before being subjected to vortex mixing. The combined extract was then evaporated to dryness at 45 °C under nitrogen stream. The dried residue was resuspended in 5 ml hexane and loaded onto an activated silica column (Isolute 500 mg SI-IST, UK) which was rinsed with 5 ml of 15% *v*/v diethyl ether-hexane where drug elution was performed with 5 ml 70% acetone in hexane. Finally, the solvent was evaporated to dryness at 45 °C under nitrogen stream and the dry residue was reconstituted by 1 ml of mobile phase solution, filtered using 0.22 μm nylon filter and injected (200 μl) into the HPLC apparatus.

2.6. Chromatographic method

Chromatographic separation of PZQ was carried out in a HPLC apparatus combining a Waters 600 Pump and a 600 Pump system Controller (Milford, MA, USA, a Waters 717 Plus Autosampler (Milford, MA, USA) set at 10 °C injection temperature, a 150 mm × 4.6 mm Luna-C18 column packed with 5 μm particle size equipped with a 4 mm × 3.0 mm C18 security guard cartridge (both from Phenomenex, USA), a 2487 UV detector set at 210 and an Empower Chromatography Software (both from Waters, Milford, MA, USA). An isocratic mixture of 35:65 v/v acetonitrile:water was used as a mobile phase. The flow rate was constantly maintained at 1.0 ml/min, column temperature was maintained at 30 °C and was rinsed for 20 min with 100% acetonitrile between injections.

To establish the calibration curves and the recovery rates of PZQ in plasma, gill and muscle plus skin samples, drug standards were spiked into blank greater amberjack tissues at final concentrations of 0.01–10 μg/ml or μg/g and analysed as described above. A linear relationship for PZQ existed in the calibration curves over the range of 0.01–10 μg/ml of plasma, gill and muscle plus skin tissues ($R = 0.9999$, $R = 0.9998$ and $R = 0.9997$, respectively). The average recovery rates of PZQ are presented in Table 2. The limits of quantification (LOQ) were set to 0.03 μg/ml or μg/g in plasma and gill samples and 0.04 μg/g in muscle plus skin tissues.

2.7. Pharmacokinetic parameters

To calculate the elimination half-life of the drug ($t_{1/2b}$), data was analysed for the best fit to a compartment pharmacokinetic (PK) model using non-linear regression analysis programs (NLREG 1991–2010, P.H. Sherrod). The area under the concentration–time curve ($AUC_{0-\infty}$) was determined using trapezoidal method and was extrapolated to infinity. The observed maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (T_{max}) were measured directly from the individual plasma drug concentration versus time profiles.

Table 2
Average recovery rates (%) of PZQ in spiked plasma, muscle plus skin and gills.

PZQ added (μg/ml or g)	Average recovery rates		
	Plasma	Muscle	Gills
0.05	93.5	98.5	104.3
0.5	101.6	78.1	105.0
2	88.5	82.8	90.7
5	94.1	78.6	92.5
Average	94.6	84.5	98.1

2.8. Statistical analysis

Results are presented as mean ± st.dev. Mean PZQ concentrations of each intervention day and dosing regimen were separately analysed using one-way analysis of variance (ANOVA), while levels of significance were set at $P < 0.05$. The effects of dose and day interval were evaluated by conducting a two-way ANOVA (Univariate General Linear Model-GLM) with dose and day as the fixed factors. Distributions of data sets were checked for homogeneity and homoscedasticity, and since they were found homogenous the means of PZQ concentration were compared by Tukey test with levels of significance $P = 0.05$. The SPSS version 25.0 (International Business Machines Corporation, Armonk, NY, USA) was used for the statistical analysis.

3. Results

3.1. Pharmacokinetics of PZQ in plasma

Medicated diets were regularly consumed by greater amberjack with only little signs of feed rejection seen in both doses examined and subsequently the compound was readily detected in all plasma samples. The mean concentrations of PZQ in greater amberjack plasma during the first 24 h for both dosing regimens are shown in Fig. 1. Mean plasma concentrations of PZQ for fish receiving 30 mg/kg fish were measured to be 2.4 and 2.3 μg/ml at 2 and 4 h post treatment, respectively, while the C_{max} of PZQ was achieved at 8 h post feeding (3.0 μg/ml), indicating that the absorption of PZQ in low dosing regimen followed a bimodal concentration-time profile (Table 3, Fig. 1). On the other hand, the C_{max} of PZQ of fish receiving 60 mg/kg fish was evident at 2 h following delivery (4.3 μg/ml) (Fig. 1). Notably, a two-fold increase in the administered dose led to an almost 76% increase in the mean plasma concentration of PZQ at 2 h post-treatment, 40% in C_{max} and 32% in the total drug exposure in $AUC_{0-\infty}$ (Table 3). Significant differences ($P < 0.05$) were found between the mean PZQ concentrations of the two doses examined in all selected sampling points, except for the post-8 h sampling.

Clearance of PZQ from plasma occurred rapidly in greater amberjack as its concentrations diminished significantly 24 h after feeding in both dosing regimens. Mean plasma concentrations of PZQ at the 24 h sampling intervals for both dosing regimens examined are presented in Fig. 2. Values ranged from 0.15–0.44 μg/ml and 0.54–0.93 μg/ml for

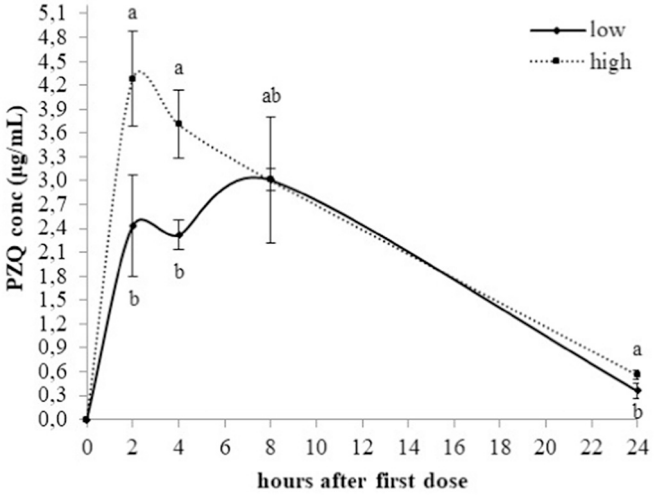

Fig. 1. Mean plasma concentrations of two PZQ doses (low: 30 mg/kg fish, high: 60 mg/kg fish) following single oral administration (the first experimental day) in greater amberjack held at 24 °C. Different letters among mean plasma concentrations denote significant differences among treatments. Data present mean ± st.dev., $n = 7$.

Table 3
Pharmacokinetic parameters of PZQ after single oral administration at 30 mg/kg/day (low) and 60 mg/kg/day (high) in greater amberjack held at 24 °C.

Parameter	Low	High
C_{max} (μg/ml)	3.0	4.3
T_{max} (h)	8.0	2.0
$t_{1/2b}$ (h)	15.1	8.2
$AUC_{0-\infty}$ (μg h/ml)	50.3	66.3

C_{max}: maximum plasma concentration.
T_{max}: time to reach maximum plasma concentration.
$t_{1/2b}$: elimination half-life of the drug.
$AUC_{0-\infty}$: area under the drug concentration curve extrapolated to infinity.

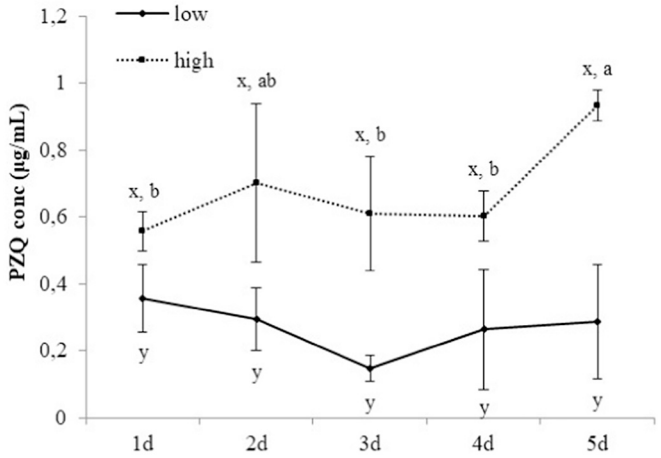

Fig. 2. Mean plasma concentrations of two PZQ doses (low: 30 mg/kg fish, high: 60 mg/kg fish) following multiple oral administration at 24 h intervals in greater amberjack held at 24 °C. Different letters among mean plasma concentrations denote significant differences for dosing regimens (x, y) and interval days (a, b, c). Data present mean ± st.dev., n = 7.

low and high dosing, respectively (Fig. 2). The $t_{1/2b}$ of the drug was calculated to be 15.1 h and 8.2 h for low and high dosing regimen, respectively, reflecting a faster PZQ elimination at the high dosing (Table 3). No significant differences were found between the residual plasma PZQ concentrations of either the 30 mg/kg or 60 mg/kg dose over the first four days of treatment, however, as shown in Fig. 2, mean plasma concentrations of PZQ were significantly higher ($P < 0.05$) on the fifth day of treatment compared to the other intervention days for high dosing regimen, indicating that some accumulation of the drug occurred. Finally, factorial analysis revealed that both dosing regimen ($F = 173.39$, $P = 0.000$) and interval days ($F = 7.25$, $P = 0.000$) as well as their interaction (dose *x* day) ($F = 4.75$, $P = 0.005$) significantly affected residual PZQ concentrations.

3.2. Depletion of PZQ in muscle plus skin and gills

Means and standard deviations of PZQ in muscle plus skin and gill samples are presented in Tables 4 and 5, respectively. The elimination profile of PZQ appeared similar in both muscle plus skin and gill tissues of greater amberjack. Doubling PZQ dosing caused an insignificant 2-fold increase in the muscle plus skin and gill concentrations of PZQ, 24 h post-treatment. Moreover, depletion of PZQ from muscle plus skin was rapid, as its concentrations declined below the LOQ on the third and fourth day post treatment, for low and high dosing regimen, respectively (Table 4). Fast removal of PZQ was also apparent in gills (Table 5), as its concentration diminished to 0.06 μg/g at 48 h post treatment, for low and to 0.04 μg/g at 72 h post-treatment, for high dosing.

Table 4
Muscle plus skin concentrations of PZQ after multiple oral administrations at 30 mg/kg/day (low) and 60 mg/kg/day (high) for 5 consecutive days in greater amberjack held at 24 °C. Data present mean ± st.dev., $n = 10$.

Sampling time (days after treatment)	Muscle (μg/g)	
	Low	High
1	0.10 ± 0.10	0.19 ± 0.06
2	0.04 ± 0.03	0.06 ± 0.02
3	< LOQ	0.03 ± 0.01
4	n.d	< LOQ
5	n.d	n.d
7	n.d	n.d

n.d: not detected.

Table 5
Gill concentrations of PZQ after multiple oral administrations at 30 mg/kg/day (low) and 60 mg/kg/day (high) for 5 consecutive days in greater amberjack held at 24 °C. Data present mean ± st.dev., $n = 5$.

Sampling time (days after treatment)	Gills (μg/g)	
	Low	High
1	0.12 ± 0.01	0.19 ± 0.05
2	0.06 ± 0.04	0.10 ± 0.02
3	< LOQ	0.04 ± 0.01
4	n.d	< LOQ
5	n.d	n.d
7	n.d	n.d

n.d: not detected.

4. Discussion

Typically, an empirical approach is used to implement treatment schedules in aquatic medicine however, with uncertain efficiency. On the other hand, pharmacokinetic studies provide useful knowledge to optimize drug dosing regimens for targeted fish species. Particularly, in the case of dietary administrated PZQ, these insights would be beneficial in order to reduce the cost of the medicated feed and chemical release in the environment, as well as to minimise the potential development of anthelmintic resistance caused by prolonged exposure of parasites to subcurative doses (Tubbs and Tingle 2006b). Medication with PZQ is perhaps the most commonly used dietary measure to battle monogeneans of farmed *Seriola spp.*, with dosing regimens ranging from 30 to 225 mg/kg for 3 to 8 consecutive days (Tubbs and Tingle 2006a, 2006b; Williams et al. 2007; Tubbs et al. 2008; Hirazawa et al. 2013; Partridge et al. 2014, 2019).

As in all dietary-administered drugs in aquaculture medicine, PZQ levels in fish diets are generally determined by the target dose rate and the fish size (Partridge et al. 2014). Considering that smaller fish have more voracious appetite, food intake is regularly decreased by increased fish size, thus resulting to the need for higher dietary inclusion levels of PZQ, when larger fish are to be medicated. This necessity is apparently beneficial when attempting to overcome palatability issues in PZQ-medicated smaller fish. In the current study, very low signs of inappetance were evident, without affecting thought the overall consumption of the medicated feed, partly due to small fish used and to the low dietary inclusion levels used in the trial (1.5 and 3 g/kg). Hirazawa et al. (2013) also observed that similar sized greater amberjack (77 g) to those of the current study consumed the entire quantity of a diet medicated with 7.5 g/kg, while larger individuals (>200 g) rejected or vomited a diet containing 10 g/kg. These observations indicate that fish size should be taken into account when a PZQ treatment is proposed for farmed fish species.

Rejection of PZQ oil-coated diets (20 and 40 g PZQ/kg diet) given for 6 days was previously observed in gilthead sea bream, *Sparus aurata* (Sitjà-Bobadilla et al. 2006). Likewise, Partridge et al. (2014) reported a signifigant reduction in feed intake in yellowtail kingfish fed gelatin-

coated diets with PZQ (16 g PZQ/kg for 7 days). Hirazawa et al. (2004) also noticed an appetite suppression in spotted halibut, *Verasper variegates* when fed a high PZQ dose for a short period (15 g PZQ/kg for 3 days), as opposed to a long-term low dose-treatment (4 g PZQ /kg for 11 days). The same study stated that Japanese yellowtail, *S. quinqueradiata* and greater amberjack had both shown signs of reduced appetite when offered PZQ-supplemented diets. Similar palatability issues were encountered by Williams et al. (2007) investigating the efficacy of orally administered PZQ (3.8 and 5.8 g PZQ g/kg for 6 days, and 7.7 and 11.5 g PZQ /kg for 3 days) against monogeneans parasitising yellowtail kingfish. Notably, some of the above studies demonstrated that fluke elimination was even more pronounced at the lower tested dosing, which was attributed to the poor acceptance of the higher treatment regimens (Hirazawa et al. 2004; Williams et al. 2007).

Few trials have been carried out to enhance the palatability of PZQ experimental diets with masking agents. Partridge et al. (2014) and Hirazawa et al. (2004) unsuccessfully attempted to mask the bitterness of the drug by incorporating the drug into microcapsules and by mixing it with all the dietary ingredients, though without the inclusion of attractants. Incorporation of PZQ into solid-lipid nanoparticles also failed to increase palatability of medicated diet in yellowtail kingfish (Partridge et al. 2019). On the contrary, effective preparations to enhance the palatability of the PZQ-medicated diets included frozen krill coated with PZQ and PZQ-medicated moist pellets (Yamamoto et al. 2011; Forwood et al. 2016). However, these preparations require specific maintenance conditions including storage of frozen of conventional or semi-moist feeds, which may not be an available option in all commercial fish farms. Admittedly, masking with certain additives/attractants seems the best solution to overcome the low acceptance of PZQ-medicated diets (Partridge et al. 2014; Pilmer, 2016) and consequently, the shrimp-based attractant and krill meal that were included in the medicated feed production along with the low levels of PZQ inclusion, could explain the low signs of inappetence evidenced herein.

The absorption profile of PZQ in the present study showed that dietary administered PZQ is rapidly distributed in greater amberjack circulation, as reflected by the measured T_{max} of 2 and 8 h in fish fed 30 and 60 mg/kg fish, respectively. Moreover, no statistical differences were detected between PZQ plasma concentrations at 2 and 8 h post-treatment for high dosing regimen due to the large variance, and this could probably explain the discrepancy among the two treatments. In agreement to the findings of this study, the T_{max} of PZQ ranged from 4 to 9 h in rainbow trout, *Oncorhynchus mykiss* serum (Björklund and Bylund 1987; Rogstad et al. 1987), rockfish, *Sebastes schlegeli* plasma (Kim et al. 2001) and yellowtail kingfish plasma (Tubbs and Tingle 2006b; Partridge et al. 2019), fed PZQ-medicated diets. Interestingly, our measurements revealed a bimodal concentration-time profile of orally administered PZQ in greater amberjack plasma, which is in agreement with previous trials in yellowtail kingfish (Tubbs and Tingle 2006b), rockfish (Kim et al. 2001) and Pacific bluefin tuna, *Thunnus orientalis* (Ishimaru et al. 2013). The plasma concentration of PZQ in fish receiving the low dose reached the first peak at 2 h, experienced a small decline at 4 h and increased again at 8 h post-treatment. Similar drug patterns were seen in other PZQ-fed species with the first and second peaks being reached at 1.5 and 6 h in yellowtail kingfish plasma or at 0.5 and 1.5 h in Pacific bluefin tuna serum (Tubbs and Tingle 2006b; Ishimaru et al. 2013). A proposed hypothesis to interpret the two peaks in PZQ absorption profile might be the fact that the first peak represents a first-pass metabolism of the drug passing the liver and the second the result of a possible active reabsorbion of the drug from bile fluid via enterohepatic recirculation (Björklund and Bylund 1987). These assumptions remain to be scientifically verified.

Praziquantel seemed to be adequately absorbed in greater amberjack circulation as indicated by the measured C_{max} (3.0 μg/ml and 4.2 μg/ml for low and high dosing regimen, respectively) but the exposure duration was found to be short ($AUC_{0-\infty}$: 50.3 μg h/ml and 66.3 μg h/ml for low and high dosing regimen, respectively). It is worth mentioning that in the current study medicated feed was offered by hand instead of oral gavage or intubation used in other studies to ensure identical feed amount and subsequently, drug intake (Kim et al. 2001; Tubbs and Tingle 2006b; Xu et al. 2016; Partridge et al. 2019). Tubbs and Tingle (2006b) reported much higher C_{max} and AUC_{0-24} plasma values (10.6 μg/ml and 101.1 μg/ml, respectively) in yellowtail kingfish received a dosing of 40 mg PZQ/kg fish. Higher also C_{max} and $AUC_{0-\infty}$ plasma values (5.5–9.6 μg/ml and 100–159 μg h/ml, respectively) were observed for the same species after receiving a single PZQ oral dosing of 50 mg/kg (Partridge et al. 2019). Additionally, the C_{max} that Kim et al. (2001) reported in rockfish was higher than that achieved in our study (8.6 vs 3.0 and 4.2 μg/ml), probably due to the administration of considerably higher dose (400 mg PZQ/kg fish). On the contrary, Ishimaru et al. (2013) reported lower C_{max} (2.0 μg/ml), when smaller dosing regimen of orally administered PZQ was delivered in pacific bluefin tuna (15 mg PZQ/kg fish). In agreement to that, lower C_{max} and $AUC_{0-\infty}$ plasma values were reported (0.4 μg/ml and 6.1 μg h/ml, respectively) in rice field eel, *Monopterus albus* after receiving an oral dosing of 10 mg PZQ /kg fish (Xu et al. 2016). Moreover, studies have shown that apart from dosing regimen, the size of fish may also affect the absorption of PZQ. Specifically, Partridge et al. (2019) examined PZQ pharmacokinetic profile on both large (4100 g) and small (~300 g) yellowtail kingfish and found that smaller fish had higher C_{max} and $AUC_{0-\infty}$ (9.6 vs 5.5 μg/ml and 100 vs 159 μg h/ml, respectively) than larger fish. On the contrary, Hirazawa et al. (2013) demonstrated that oral PZQ treatment against both flukes i.e. *Benedenia seriolae* and *Neobenedenia girellae* was more effective in large *Seriola* spp. studied rather than in smaller fish. Overall, the factors that affect the pharmacokinetic parameters and could interpet the aformentioned variations in C_{max} and $AUC_{0-\infty}$ include differences in fish species, size and age as well as differences in experimental set up i.e. drug dosage, frequency of drug administration, water temperature and administration route (Hayton, 1999). Specifically, forced administration of medicated diet or solution which attempts to individualise dose schedules favours intragroup variation and avoids several constraints, commonly occurring in the natural delivery of a medicated diet, such as reduced feed palatability and drug leaching. These observations confirm that the absorption profiles of PZQ in greater amberjack obtained herein are very different compared to those obtained in other fish species, even belonging to the same genus. Consequently, any attempt to extrapolate effective PZQ dose rates against monogeneans from other species, might be erroneous for pure use in anthelminthic therapy concerning greater amberjack. Trials investigating the relationship of different PZQ dosing schedules in greater amberjack with PZQ in situ effectiveness against *Z. seriolae*, are therefore of primary importance.

The $t_{1/2b}$ of PZQ was calculated to be 15.1 h for low and 8.2 h for high PZQ dosing, reflecting a faster drug elimination in plasma at the high dosing of the present study. This finding can be attributed to the auto-induction effect, interpreted previously as a dose-dependent process in which the elimination clearance of a drug increases following multiple doses and moreover, the increase in clearance is greater after a high dose compared to a lower dose (Lin 1994). Admittedly, due to the limited number of sample points available in the elimination phase, a concern mentioned also by Tubbs and Tingle (2006b), the accuracy in the determination of the half-life of PZQ in greater amberjack should be relatively questioned. Faster elimination profile following a 150 compared to 50 mg/kg fish dosing regimen for 3 consecutive days was also reported by Tubbs and Tingle (2006a) in yellowtail kingfish plasma. Similar $t_{1/2b}$ values were observed in other farmed fish such as yellowtail kingfish (ranged from 5.4–11.4 h) (Tubbs and Tingle 2006b; Partridge et al. 2019) and rice field eel (6.7 h) (Xu et al. 2016). Moreover, the elimination time of PZQ was found to be 24 h in Pacific bluefin tuna serum fed 15 mg/kg fish and 120 h in rockfish plasma fed 400 mg/kg fish (Kim et al. 2001; Ishimaru et al. 2013).

Some signs of PZQ accumulation were apparent in greater amberjack plasma, as reflected by its significantly higher plasma concentrations on

the fifth day of treatment compared to the other intervention days in the high dosing regimen. Weak evidence of drug accumulation has been also observed in yellowtail kingfish plasma after receiving multiple oral doses of 50 mg/kg for 8 days (Tubbs and Tingle 2006a). The authors attributed the lack of stronger accumulation to the rapid clearance of the drug, either via hepatic metabolism or renal excretion, rather than poor absorption.

Additionally, PZQ concentrations were measured in gills as an attempt to evaluate the depletion pattern of the drug in the targeted tissue for *Z. seriolae* infestations in our study. The elimination profile of PZQ observed in greater amberjack gills was consistent with that seen in the other examined tissues, revealing a clear dose-dependent effect. Its concentration rapidly declined below the level of quantification at 96 h post-therapy in both dosing regimens. Rapid elimination leads to low drug availability at a daily level, therefore mutliple dietary administration of PZQ at 12 h intervals may be more effective to eliminate the parasites of greater amberjack.

Legal administration of PZQ in some production animals has no established MRL due to the fast excretion and lack of accumulation of the compound (EMEA 1988), however its possible 'off label' use (Council Directive 90/676/EEC) should follow the cascade system imposed by the 500 dd withdrawal. Depletion of PZQ from muscle plus skin of greater amberjack was indeed very rapid in this study confirming the lack of MRL necessity if the cascade system is to be applied. Specifically, the PZQ muscle residual concentrations declined to below the LOQ on the third and fourth day post treatment, for the low and the high dosing, respectively. Inevitably, doubling the PZQ dose caused an increase in the muscle plus skin PZQ concentrations and subsequently an increased elimination time by one day. Pharmacokinetic studies in fish have revealed that residual time of PZQ in skin is longer than muscle tissues (Kim et al. 2003; Xu et al. 2016). The mechanism underlying this phenomenon is probably the extremely lipophilic nature of PZQ, which may cause the drug to partition more readily into the fatty tissues under hypodermis (Tubbs and Tingle 2006a). Muscle plus skin represents the edible part in fish, thus residual drug concentrations should be estimated in both tissues when consumer's safety is concerned. Unfortunately, there are no studies examining the specific residual time of PZQ in fish edible tissues, although Kim et al. (2003) reported an elimination time of 2 and 4 days in rockfish muscle and skin tissues, respectively, after receiving a multiple oral dosing of 200 mg/kg for 3 days. Similar to what was found in the present study, elimination time increased in rockfish muscle and skin tissues by 4 and 3 days, respectively, when a double PZQ dose was administrated (Kim et al. 2003). In agreement to the depletion profile of PZQ in edible tissues tested in the present study, PZQ concentrations in muscle and skin tissues of rice field eel were undetectable 72 and 96 h post-treatment, respectively, following oral administration of 10 mg/kg fish for 3 consecutive days (Xu et al. 2016).

In conclusion, based on the information obtained from the PZQ analysis in greater amberjack plasma, there is an apparent benefit from the double dosing regimen (60 mg/kg fish for 5 days), as confirmed by the significantly higher drug levels compared to low dosing. An in situ trial against *Z. seriolae* is required to verify that the high dosing schedule suggested herein is also the most effective medicated scheme.

The rapid elimination of PZQ from the body compartment of greater amberjack suggested at least a twice a day administration of medicated meals to maintain adequate drug levels in the systemic circulation and in gills which ideally coincides with the feeding strategy (2–3 daily feedings) for greater amberjack at high water temperatures. The treatment duration should be carefully considered when an anthelmintic treatment is proposed, as an increase in PZQ muscle elimination time may occur, however, the cascade principle is more than adequate, since removal of PZQ from the edible tissues of greater amberjack was very rapid.

CRediT authorship contribution statement

Dimitra Kogiannou: Conceptualization, Methodology, Validation, Formal analysis, Writing - review & editing. **Chrysanthi Nikoloudaki:** Resources. **George Rigos:** Conceptualization, Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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