Pharmacokinetics of praziquantel in gilthead seabream (*Sparus aurata*) plasma and gills

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1 Abstract

The pharmacokinetics (PKs) of dietary administered praziquantel (PZQ) were determined in gilthead seabream (*Sparus aurata*), the most commercialized marine farmed fish species in the Mediterranean area. Gilthead seabreams weighting 52 g were given a single dietary dose of either 75 or 150 mg/kg fish at 21 ºC. The low dosing was also intravascularly (i.v) injected to assist the calculation of drug bioavailability (*F*). Plasma and gill samples were measured on 1, 2, 4, 6, 8, 12, 24, 48, 72, and 96 h post-administration. PZQ was rapidly absorbed in fish circulation as the maximum plasma concentration was achieved as early as 4 h (6.7 μg/mL) and 6 h post-administration (8.2 μg/mL), for the low and high dosing, respectively. The two-fold increase in dosing led to significant differences during the first six hours post administration, while the highest increase was less than 50%. The advantage of the higher dosing schedule was also apparent in PZQ measurements in gills, where maximum drug levels were measured to be 20.7 and 39.1 μg/g at 75 and 150 mg/kg fish, respectively. Albeit, plasma elimination half-life ($t_{1/2\beta}$) was shorter at high (14.4 h) compared to that calculated for the low PZQ dosing (25.7 h), depletion of PZQ from all gilthead seabream tissues examined was considered rapid at both dosing regimens. The *F* of PZQ was calculated to be 49%, confirming a high absorption in fish circulation. Due to the clearance pattern and the rapid removal of PZQ from fish circulation, daily dosing should be divided into two medicated meals, to ensure adequate drug circulatory levels during treatment. Overall, PZQ is readily bioavailable in gilthead seabream while the dosing of 150 mg/kg fish is preferable resulting in higher circulatory and gill levels compared to the low dose tested.

Keywords: Praziquantel, pharmacokinetics, plasma, gills, gilthead seabream, *Sparus aurata*

1. Introduction

As in all intensive animal production systems, disease may seriously threaten the well-being of aquaculture enterprises. The full assessment of the economic impact of disease on finfish production is rather unrealistic since is handicapped by occasionally incomplete information on mortalities, reduced growth, therapeutic expenses, and other related costs. Parasites are among the main limiting factors of the aquaculture industry, as they cause financial losses that accounted for about 20% of the total production value. Among them, it is estimated that the world annual grow-out loss due to parasites in finfish farming ranges from 1% to 10% of harvest size, with an annual cost that can exceed $10 billion (Shinn *et al.* 2015).

Aquaculture medicine is generally lacking vaccination strategies or other effective prevention measures against parasites, and on many occasions, the use of therapeutic approaches seems the single tool to battle the disease. Among parasitic pathogens, ectoparasites are of the most serious inducing severe impact on important European aquaculture activities, including the salmon industry and the Mediterranean marine finfish farming. The intensive use of sea cages in the Mediterranean region and elsewhere has allowed higher volumes of fish biomass but has contributed also to the dispersal of parasitic diseases. Poor net hygiene and other environmental upsets may stimulate the occurrence of parasitic epizootics which can be devastating to caged fish. Ectoparasitic infestations have acquired a severe role among the factors limiting the production of mainly caged gilthead seabream (*Sparus aurata*) and to a lesser extent European seabass (*Dicentrarchus labrax*) (Fioravanti *et al.* 2006), both attained the higher production volumes in Mediterranean mariculture. Indeed, *Sparicotyle chrysophrii*, a microcotylid blood-sucking monogenean, is undoubtedly the most serious pathogen for gilthead seabream farming

causing mortalities at a high prevalence and a notable growth reduction of the farmed stock partly due to emaciated and anemic survivors (Sitjà-Bobadilla *et al.* 2009).

Therapeutic attempts against ectoparasites are commonly based on bathing. Among the bath chemicals, formalin solution is undoubtedly the most commonly used therapeutic against ectoparasitic infections (Leal *et al.* 2018). Although formalin baths are usually very effective against fish ectoparasites, their use has been blamed for several issues including human, animal, and more importantly, environmental side effects (Leal *et al.* 2018). Formalin baths are moreover laborious, time-consuming and weather-dependent processes. Consequently, an effective dietary anthelmintic would be an ideal measure to overcome the drawbacks associated with bath applications in large cages.

Praziquantel (PZQ), a synthetic drug discovered earlier to battle human parasites, has been widely used in veterinary medicine and is considered an ideal antiparasitic compound against fish platyhelminths. Based on a recent opinion of the Committee for Veterinary Medicinal Products (CVMP), PZQ has been included in the group of 'allowed substances' with a proposed maximum residue level (MRL) of 20 μg/kg in finfish muscle plus skin (Commission Implementing Regulation (EU) 2023/981 amending Annex to Commission Regulation No 37/2010) (EMA, 2022). So far, the use of PZQ in Europe was feasible as fish medicine within the 'off-label' framework (Council Directive 90/676/EEC, Directive 2001/82/EC and Commission Regulation 37/2010) including its use in Norway against tapeworms of Salmonidae (Lunestad *et al.* 2015). Elsewhere, PZQ is registered as fish anthelminthic under specific conditions, having valid permits in Australia, Japan and several other Asian countries including Vietnam, Thailand, Malaysia, and the Philippines (ASEAN 2013).

Two recent and comprehensive reviews have provided wide evidence of the effective use of PZQ to control platyhelminth parasites (Bader *et al.* 2019; Norbury *et al.* 2022), covering additionally all relevant aspects of PZQ use in aquaculture (Norbury *et al.* 2022). Dietary PZQ preparations at numerous therapeutic schedules have been tested with success against helminths infecting a variety of cultured fish species (Kim *et al.* 2001; Tubbs & Tingle 2006a; Shirakashi *et al.* 2012). In Mediterranean farmed fish, a single study evaluating the efficacy of PZQ revealed promising findings against the gill fluke *Zeuxapta seriolae*, a severe pathogen of farmed greater amberjack (*Seriola dumerili*) (Rigos *et al.* 2021). To our knowledge, there is however, no literature assessing the pharmacokinetic (PKs) properties of PZQ in gilthead seabream, thus, this study aimed to determine the kinetic profile and bioavailability (*F*) of dietary administered PZQ following a single oral administration, as a first step to optimizing PZQ dosing regimens against *S. chrysophrii* infections in gilthead seabream.

2 Materials & methods

2.1 Ethical statement

The trials were carried out at the licensed facility (EL-43BIO/exp-01) of the Aquaculture Laboratory of the Department of Ichthyology and Aquatic Environment (DIAE), University of Thessaly, Greece. All the approvals required by the legislation were given by the Ethical Committee of the Department and competent authorities for the experimentation to be carried out under EU guidelines on the protection of animals used for scientific purposes (Directive 2010/63/EU, experimental protocol 114134/30-03-21). The '3Rs' and the ARRIVE guidelines endorsed for experiments using live animals, were applied during the experimental process. All

procedures involving fish were performed according to well-defined work protocols for the applied procedure based on standardized SOPs, under the supervision of FELASA-accredited scientists.

2.2 Chemicals

PZQ analytical standard was obtained from Sigma-Aldrich (USA). High-performance liquid chromatography (HPLC) chemicals were purchased from Fisher Scientific (USA). Other solvents and reagents of analytical grade were supplied by Fisher Scientific (USA). MS-222 was provided by Sigma-Aldrich Chemie GmbH (Germany). PZQ powder used for medicated administrations was obtained by Vethellas S.A. (Greece).

2.3 Fish & experimental design

Three hundred healthy gilthead seabreams (52±3.7 g), were obtained from a commercial fish farm in central-east Greece and transferred to the DIAE experimental aquarium facilities. Fish were equally randomized in three tanks (0.5 m^3) forming three fish groups (100 fish/group). Experimentation was preceded by an acclimatization period of two weeks, during which the fish fasted for the last 24 h. During the experimental procedure, fish were hand-fed once a day, assuring that the feed was consumed. Tanks were supplied with recirculating running and aerated artificial seawater. Experimental conditions were stable, with the water temperature maintained at 21±1 °C, pH at 8.0±0.3, salinity at 33±0.5 g/L, dissolved oxygen at >6.5 mg/L, total ammonia nitrogen at <0.1 mg/L, photoperiod at 12h light:12h darkness.

2.3.1 Oral administration

Oral administration of PZQ was applied in two fish groups (n=100/group) described as low and high, in which fish were fed with experimental diets supplemented with 75 or 150 mg PZQ/ kg fish, respectively (Table 1). The medicated fish feed was administered once, while fish were fed with commercial dry pellets in the following days.

2.3.2 Intravascular injection

To contribute to the estimation of the *F* of PZQ, fish were injected intravascularly (i.v) with the low drug dosing in the third fish group (n=100). In particular, fish were anaesthetized (buffered MS-222 (50 mg/L - pH 7) and received intravenously via the caudal vein a PZQ dosing of 75mg/kg fish dissolved in DMSO (0.5 mL/kg BW). The injection was applied with a 1 mL Terumo insulin syringe with a 27-gauge needle (Terumo Medical Corporation, Somerset, NJ, USA). After the injection, anaesthetized fish were transferred until recovery and sampling to the experimental tank. Fish were daily fed with commercial dry pellets.

2.3.3 Samplings

Ten fish per group were anesthetized (buffered MS-222, 150 mg/L - pH 7) and blood samples were collected 1, 2, 4, 6, 8, 12, 24, 48, 72, and 96 h post-treatment. Blood sampling was performed from the caudal vein, using a 2.5 mL Terumo syringe with a 23-gauge needle (Terumo Medical Corporation, Somerset, NJ, USA) coated with heparin (Merck KGaA, Germany). The blood was placed in Eppendorf tubes and it was centrifuged at 3000 *g* for 10 min (4 °C) to separate the plasma. Subsequently to blood collection, fish were then killed by an

overdose of anaesthetic (buffered MS-222 500 mg/L) and gill samples from fish that received the medicated diets were collected. All samples were kept at -20 °C until analyzed.

2.4 Chromatography

An HPLC apparatus, combining a Waters 600 Pump and a 600 Pump system Controller, a Waters 717 Plus Autosampler set at 10°C injection temperature, a Waters 2487 UV detector set at 210 and an Empower Chromatography Software (Waters, Milford, MA, USA) was used for drug analysis. PZQ separation was achieved using a reverse-phase chromatographic column (Luna-C18, 150 mm × 4.6 mm, 5 μm, Phenomenex, USA) equipped with a security guard cartridge (C18, 4 mm × 3.0 mm, Phenomenex, USA). As a mobile phase, an isocratic mixture of 35:65 v/v acetonitrile:water was used at a flow rate of 1.0 mL/min. Column temperature was maintained at 30 °C, the injected sample volume was 200 μL and the total run time was 25 min (PZQ retention time: 18.7 min).

2.4.1 Sample preparation

Plasma samples were prepared using a modified method (Kogiannou *et al.* 2021), based on a previous analytical method (Ridtitid *et al.* 2002). Briefly, in 0.5 mL plasma, 2 mL acetonitrile and 0.1 mL of zinc sulphate solution (0.2 M) were added. The mixture was then placed on a magnetic stirrer for 10 min. After centrifugation (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 (0.22 μm nylon filter) and injected into the HPLC.

159 Gill samples were prepared according to Tubbs and Tingle (2006b). Particularly, 1 g of ground
160 tissue sample was weighed into a centrifuge tube. Following the addition of 6 mL of ethyl
161 acetate, sample was homogenized for 30 s at16,000 rpm/min (IKA®-Werke GmbH & Co. KG,
162 Staufen, Germany). The mixture was agitated for 10 min and the supernatant was collected after
163 centrifugation (10,000 *g* for 10 min at 10 °C). Tissue PZQ extraction was repeated with 4 mL
164 ethyl acetate. The pooled extract was then evaporated to dryness at 45 °C under a nitrogen
165 stream. The dried residue was resuspended in 5 mL hexane and a clean-up step was followed as
166 described previously (Tubbs and Tingle, 2006b; Kogiannou *et al.*, 2021).

167 **2.4.2 Method validation**

168 Plasma and gill samples of untreated fish were spiked with PZQ standard solutions at final
169 concentrations ranged from 0.01 to 10 μg/mL in order to establish the calibration curves for
170 quantification of drug concentration in the examined tissues. Three replicates of spiked tissues
171 (0.5-5 μg/mL) were analyzed as described above, to evaluate the recovery rate of the methods.
172 The limits of detection (LOD) and the limits of quantification (LOQ) for PZQ analysis in both
173 examined tissues were calculated by 3.3*σ/S and 10*σ/S, respectively (σ = standard deviation of
174 the y-intercept of the regression line; S = slope of the calibration curve).

175 **2.5 Pharmacokinetics**

176 The plasma and gill levels obtained from low and high PZQ dosing after oral administration and
177 plasma levels after i.v injection, were analyzed for the best fit to a one-, two- or three-
178 compartment open pharmacokinetic models using non-linear regression analysis programs
179 (NLREG, P.H. Sherrod). The distribution ($t_{1/2\alpha}$) and the elimination half-lives ($t_{1/2\beta}$) were

estimated as follows: $t_{1/2\alpha}$=0.693/α and $t_{1/2\beta}$=0.693/β. The area under the concentration-time curve ($AUC_{0-\infty}$) was calculated using the trapezoidal rule and was extrapolated to infinity. The *F* was calculated according to:

$$F = \frac{AUC_{oral}}{AUC_{iv}}$$

2.6 Statistics

Results are presented as mean ± st.dev. The Student's t-test was used to evaluate statistical difference on mean PZQ concentrations in plasma and gills between the two oral dosing regimens. The levels of significance were set at $P<0.05$. The SPSS version 25.0 (International Business Machines Corporation, Armonk, NY, USA) was used for the statistical analysis.

3 Results

3.1 Calibration curve and recovery rates

A linear relationship for PZQ existed in the calibration curves over the range of 0.01-10 µg/mL or g of plasma and gill tissues, respectively. The coefficients of correlation were greater than 0.999 for both tissues examined. The average recovery rates of PZQ were 95% in plasma and 98% in gill and the limits of quantification (LOQ) were set at 0.03 µg/mL or µg/g in plasma and gill samples, respectively.

3.2 Oral administration

A one-compartmental model provided the best fit after oral PZQ treatment, in gilthead seabream plasma and gill tissues. The concentrations of PZQ found in plasma after oral administration are shown in Figure 1. Maximum PZQ plasma concentration in fish fed 75 mg/kg was achieved at 4 h post-feeding (6.7 μg/mL), while the corresponding value in the high-dose group was measured at 6h post-feeding (8.2 μg/mL) (Table 2). The two-fold increase in dosing led to significant differences between the mean PZQ plasma concentrations in sampling points up to 6 h, while the highest increase measured as mean plasma drug concentration was less than 50%. Moreover, $AUC_{0-\infty}$ revealed values 225.7 and 262.3 μg h/mL for fish fed the low and the high drug concentration, respectively (Table 2). Clearance of PZQ in gilthead seabream plasma did not follow a simple decay model at the low dosing regimen, as PZQ levels increased again 6 h post treatment. PZQ concentration declined relatively sharply at the 12 h sampling point (approximately 50%), however, minimum plasma concentration remained approximately at 1 μg/mL, 96 h post-treatment for both dosing regimens examined. The $t_{1/2\beta}$ of the drug from plasma was calculated to be 25.7 h and 14.1 h for low and high dosing regimens, respectively, reflecting a faster PZQ elimination at the high dosing (Table 2).

Gill concentrations of PZQ after oral administration are shown in Figure 2. Doubling PZQ dosing caused significant differences between the mean PZQ gill concentrations at sampling points up to 8 h post-treatment. Maximum PZQ values in fish fed 75 and 150 mg/kg were achieved at 4 h post-feeding (20.7 for the low and 39.1 μg/g, for the high dosing) and were three- and five-fold higher compared to corresponding plasma values, 4 h post drug administration,

respectively. The two-fold increase in drug dosing led to a significant dose-dependent effect in the relative exposure to PZQ in the target tissue of gilthead seabream since $AUC_{0-\infty}$ revealed values of 186.3 and 378.9 μg h/mL for fish fed the low and the high dosing, respectively. Regarding drug elimination from gills, a different pattern between the two dosing regimens was observed. The $t_{1/2\beta}$ of PZQ in the gills of the low dosed group was shorter compared to that calculated for the high-dose group and ranged between 8.9-11.7 h, reflecting a faster PZQ elimination. Moreover, the elimination of PZQ from gills was rapid as its concentration diminished to 0.35 μg/g at 72 h post treatment, for the low-, and to 0.52 μg/g at 96 h post-treatment for the high-dose group.

3.3 i.v. injection

PZQ concentrations, after i.v. administration, are shown in Figure 3. The best fit of plasma PZQ concentrations following i.v injection was obtained by a three-compartment open pharmacokinetic model. The estimated PKs of PZQ delivered by i.v. are given in Table 3. The $t_{1/2\alpha}$ and the $t_{1/2\beta}$ of PZQ were measured to be 3.4 and 28.8 h, respectively. The apparent volume of distribution of the drug at steady-state (V_{dss}) was found to be 6.1 L/kg. Using an $AUC_{0-\infty}$ of 455.6 μg h/mL, the F of PZQ was calculated as high as 49%.

4 Discussion

In aquatic medicine, therapeutic attempts to newly treated fish species with established fish antimicrobials is traditionally implemented by extrapolation of existing treatment regimens from other fish species or animals. This approach however can create inefficiencies in several aspects

connected to drug use including drug acceptance, absorption, removal, and efficacy and so on. New knowledge nevertheless, produced by assessing drug PKs in the targeted farmed organism, can optimize treatment schedules by increasing efficacy and reducing cost and duration, while at the same time, enhancing environmental welfare. This practice maximizes prudent drug use and perhaps delays the potential development of antimicrobial resistance, which in some cases with PZQ use in fish, has been associated with extended exposure to subcurative treatments (Tubbs & Tingle 2006a).

Medication with PZQ is perhaps the most promising dietary anthelmintic and an ideal solution for formalin alternative in Mediterranean fish farming and elsewhere, as evidenced by the high efficacy of the drug against a wide range of parasitic helminths affecting numerous farmed fish species (Bader *et al.* 2019; Norbury *et al.* 2022). The present study is however the first attempt to investigate the PKs of dietary-administered PZQ in gilthead seabream, which substantially suffers from monogeneans infections (Sitjà-Bobadilla *et al.* 2009), although its depletion profile from the edible tissues has been previously studied (Baralla *et al.* 2020; Kogiannou & Rigos 2021).

A one-compartmental model provided the best fit after oral PZQ treatment, in gilthead seabream herein, which agrees to the findings of Xu *et al.* (2016) and Kogiannou *et al.* (2021) in rice field eels (*Monopterus albus*) and greater amberjack, respectively. On the contrary, in the study of Xie *et al.* (2015) a two-compartmental model best described the PKs of PZQ in grass carp (*Ctenopharyngodon idellus*) after oral administration, while Tubbs and Tingle (2006a) reported a non-compartmental model to best fit the data from oral treatment in yellowtail kingfish (*Seriola*

lalandi). These inter-studies discrepancies could be attributed to varied factors such as fish species, size and experimental conditions (Rigos *et al.* 2002) among the comparable studies.

The high oral dosing of PZQ was readily accepted by gilthead seabream and appeared superior to low dosing, leading to significant differences in mean PZQ plasma concentrations, mostly in the first sampling period. Significant higher plasma concentrations of PZQ (approximately 50%) were also detected for yellowtail kingfish fed 150 vs 50 mg/kg 1.5 h post-treatment, while the three-fold increase in dosing resulted in smaller differences in PZQ levels between the two doses 24 h post drug administration (Tubbs & Tingle 2006b). Similar to the present study absorption profile was also evident in greater amberjack following a single oral administration of 30 and 60 mg/kg (Kogiannou *et al.* 2021). Maximum plasma concentrations of PZQ were measured early in both fish groups (T_{max} of 4 and 6h in fish fed 75 and 150 mg/kg, respectively), indicating that PZQ is readily absorbed in the plasma of gilthead seabream, while its clearance was found to be relatively sharp for both examined dosing regimens. Rapid absorption of orally administered PZQ preparations has been also demonstrated in other fish species. In *Seriola spp.* the time to reach peak concentrations in plasma ranged from 2-8 h after oral administration of PZQ (Tubbs & Tingle 2006a; Partridge *et al.* 2019; Kogiannou & Rigos 2021). Fast absorption has been also reported for rockfish (*Sebastes schlegeli*) (T_{max} of 9; Kim *et al.*, 2001) and rice field eels plasma (Xu *et al.* 2016), fed with PZQ-medicated diets.

The maximum plasma concentration value of PZQ in gilthead seabream plasma was found 6.7 μg/mL in fish fed 75 and 8.2 μg/mL in fish fed 150 mg/kg, while long exposure duration was evident (AUC: 225.7 and 267.4 μg h/mL for low and high dosing regimens, respectively). Interestingly, shorter exposure time to PZQ was determined in yellowtail kingfish (100-159 μg

h/ml), albeit similar C_{max} were achieved (5.5-10.6 μg/mL) (Partridge *et al.*, 2019, Tubbs and Tingle, 2006). This trend in AUC plasma values is expected when smaller dosing regimens are offered (40-50 vs 75-150 mg/kg). The higher metabolic rates characterizing *Seriola spp*. may additonally interpret the aforementioned discrepancies in PK parameters. Furthermore, the concentrations that Kim *et al.* (2001) reported in rockfish were similar to those achieved in the high dosing examined in the present study, even though a considerably higher dose was administered (400 mg PZQ/kg fish). On the contrary, Kogiannou *et al.*(2021) reported lower C_{max} and $AUC_{0-\infty}$ plasma values (3.0 and 4.2 μg/mL; $AUC_{0-\infty}$: 50.3 and 66.3 μg h/mL), when lower PZQ dosing regimen was delivered in greater amberjack (30 and 60 PZQ/kg). In agreement with that, lower C_{max} and $AUC_{0-\infty}$ plasma values were reported in rice field eel (0.4 μg/mL and 6.1 μg h/mL, respectively), after receiving an oral dosing of 10 mg PZQ /kg fish (Xu *et al.* 2016).

The $t_{1/2\beta}$ of PZQ in gilthead seabream plasma was calculated to be 25.7 h for the low and 14.1 h for the high PZQ dosing group, reflecting a faster drug elimination in fish circulation when the PZQ dose is increased. This finding was also observed in the study of Kogiannou *et al.* (2021) and Tubbs and Tingle (2006b), who examined the PKs of PZQ following dietary treatments in greater amberjack and yellowtail kingfish, respectively. It was hypothesized that these PZQ dose-dependent differences may be due to the enzymatic auto-induction effect, interpreted as a dose-dependent process in which the elimination clearance of a drug increases following multiple doses and the increase in clearance is greater after a high than after a low dose (Lin, 1994; Kogiannou *et al.*, 2021). In numerous other fish species such as rockfish, yellowtail kingfish, rice field eel, grass carp, Pacific bluefin tuna (*Thunnus orientalis*) and greater

amberjack, the estimated $t_{1/2\beta}$ of PZQ in plasma or serum ranged from 5.4 to 120 h, after oral administrations.(Kim *et al.* 2001; Tubbs & Tingle 2006b; Ishimaru *et al.* 2013; Xu *et al.* 2016; Partridge *et al.* 2019; Kogiannou & Rigos 2021). Such differences can can be attributed to the varied experimental setup between different PK studies.

It is worth mentioning that a bimodal concentration-time profile of orally administered PZQ in gilthead seabream plasma fed 75 mg/kg was revealed herein. Specifically, PZQ levels in fish-fed low dosing reached the first peak at 4 h, declined at 6 h and increased again at 8 h post-adminitration. A double peak phenomenon was also apparent in greater amberjack (Kogiannou *et al.* 2021), yellowtail kingfish (Tubbs & Tingle 2006b), rockfish (Kim *et al.* 2001) and Pacific bluefin tuna (Ishimaru *et al.* 2013). This pattern has previously been attributed to enterohepatic circulation where bile fluid, containing the drug, is evacuated into the intestine and subsequently partly reabsorbed into the fish circulation (Björklund & Bylund 1987). This could be an additional explanation of the significant differences in $t_{1/2\beta}$ values between the two dosing regimens (25.7 h and 14.1 h for the low and high PZQ dosing, respectively). Considering the sharp decrease in PZQ plasma levels after 8 h in gilthead seabream, a daily typical therapeutic dosing should be divided into two medicated meals, to ensure adequate drug circulatory levels during treatment.

The present study also includes the first attempt to determine the PKs of PZQ in gilthead seabream gills. Interestingly, some discrepancies in the kinetic profiles of PZQ in fish plasma versus gills were observed. Maximum PZQ values in gills of fish fed 75 and 150 mg/kg were three- and five-fold higher compared to the corresponding plasma values, 4 h post-drug

administration, respectively. Additionally, a dose-dependent effect in relative exposure to PZQ was observed in gills, while this was not distinguishable in plasma (Table 2). Furthermore, the $t_{1/2\beta}$ of PZQ was calculated to be shorter in gills. Gills represent an important site of xenobiotics elimination in fish (Hansen *et al.* 2001; Sun *et al.*, 2010), probably resulting in a lack of resemblance in the PK profile of antimicrobials between plasma and gills found herein and elsewhere (Mallik *et al.*, 2023). However, these assumptions have yet to be verified.

A three-compartmental model best described the PKs of PZQ in gilthead seabream after i.v. injection. This finding is in agreement with the results of Xu *et al.* (2016) in rice field eels after intravenous administration. On the contrary, a non-compartmental model was found to best fit the data from intravenous treatment in yellowtail kingfish (Tubbs & Tingle 2006a). Once again, factors related to differences in fish species used and experimental conditions can explain the reported discrepancies. Intravenous administration of PZQ in gilthead seabream produced PZQ blood levels almost twice as high as compared to oral delivery, as reflected by the maximum plasma levels and the calculated $AUC_{0-\infty}$. Similar observations have been evident in yellowtail kingfish (Tubbs & Tingle 2006a). The estimated $t_{1/2\alpha}$ and $t_{1/2\beta}$ of PZQ in gilthead seabream were slower compared to those calculated in ice field eels (Xu *et al.* 2016) (0.54 and 17.10 h, respectively).

The *F* of PZQ in gilthead seabream after oral administration was estimated to be as high as 49%. The *F* of an antimicrobial is apparently influenced by the delivery mode in the targeted animal. Admittedly, drug deliveries by injection and intubation produce high drug absorption, however, they are considered rather impractical to farmed fish, therefore, for the veterinarian, dietary administration and hence the absorption properties of a compound following in-feed

administrations are of primary importance. A high *F* of PZQ has also been reported in yellowtail kingfish (51%) (Tubbs & Tingle 2006a), while a lower value was estimated (21%) in rice field eels (Xu *et al.* 2016). The diversity in *F* values observed between the three studies may be attributed to the factors described earlier. On terrestrial food animals, corresponding values have been found lower, ranging from 3 to 32% (Zeng *et al.* 1993; Cao *et al.* 2001; Giorgi *et al.* 2001; Sun & Bu 2012), perhaps due to the considerable first-pass effect on absorbed PZQ on livestock (Andrews *et al.* 1983).These finding may be due to the rapid metabolism of the drug in the liver of mammals, to mono- and dihydroxy derivatives (Schepmann & Blaschke 2001). On the other hand in fish, at least 7 mono- or dihydroxylated derivatives of the parent compound were identified in yellowtail kingfish, with the major metabolite being different from that found in mammals (Tubbs *et al.* 2008). Whether hydroxylation of PZQ in farmed fish creates metabolites with possible anthelmintic activity requires further investigation. Attempts to increase the bioavailability, and hence efficacy, of PZQ in fish have also been carried out. Cimetidine co-administration of PZQ led to higher drug levels in the blood of rockfish and increased treatment efficacy against *Microcotyle sebastis* (Kim & Kim 2002). Alternatively, drug delivery technologies e.g. nanobioparticles consist a promising alternative strategy to increase PZQ efficacy against fish endoparasites (Madrid *et al.* 2021, Mathews *et al.* 2021), although lack of improvement in *F* of PZQ was evident in yellowtail kingfish after oral administration of PZQ incorporations into solid-lipid nanoparticles which was attributed to the particles size (Partridge *et al.* 2019).

In conclusion, the results of the present study indicate that PZQ is readily absorbed in gilthead seabream circulation, while its clearance was found to be relatively sharp, at both

examined dosing regimens. Based on the information obtained from the PZQ analysis in fish plasma, there is an apparent benefit from the 150 mg/kg dosing regimen, as confirmed by the significantly higher drug levels compared to low dosing. The advantage of the higher dosing schedule was also apparent in PZQ measurements in gills. Considering the sharp decrease in PZQ concentration in gilthead seabream plasma after oral administration, a daily drug administration should be divided into two medicated meals, to ensure adequate drug circulatory levels during treatment. Further field trials using dietary PZQ against *S. chrysophrii* are required to verify that the suggested dosing schedules are also the most effective anthelmintic schemes.

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478

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

Ingredient	g/100g
Fish meal 68	60.00
Krill meal	12.00
Wheat meal	18.25
Wheat gluten	3.00
Fish oil	5.00
Vitamins	0.27
Shrimp-based attractant	1.50
PZQ low	0.75
PZQ high	1.50

480 **Table 2.** Pharmacokinetic parameters of PZQ following two single oral PZQ doses (low=75
481 mg/kg, high=150 mg/kg) in gilthead seabream plasma at 21 ºC

Parameters	**low**	**high**	**low**	**high**
Tissue	plasma		gills	

β	0.027	0.049	0.078	0.059
C_{max} (µg/mL)	6.7	8.2	20.7	39.1
T_{max} (h)	4	6	4	4
$t_{1/2\beta}$ (h)	25.7	14.1	8.9	11.7
$AUC_{0-\infty}$ (µg h/mL)	225.7	267.4	186.3	378.9

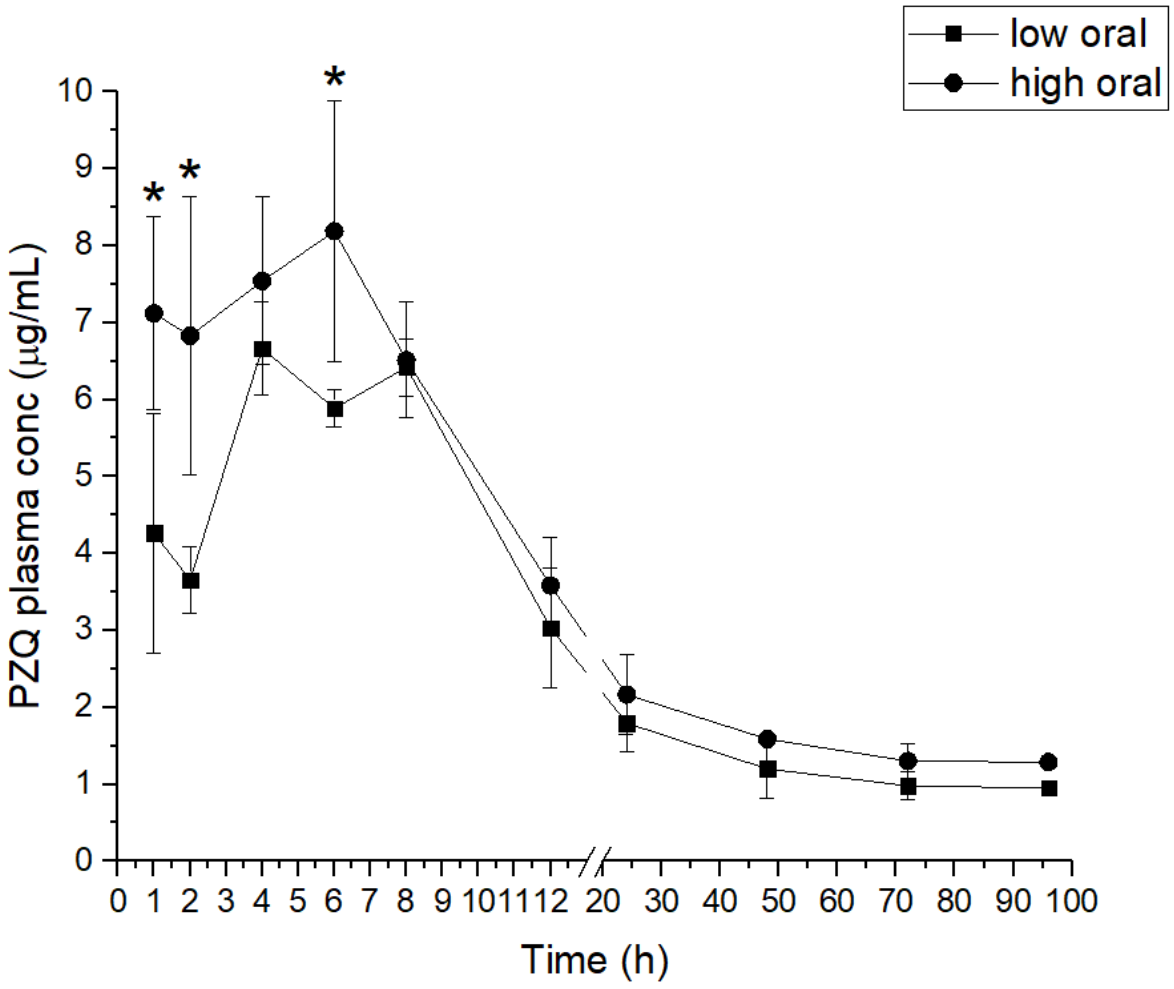
482 *β:slope;* $t_{1/2\beta}$: elimination half-life of the drug; $AUC_{0-\infty}$: area under the drug concentration curve
483 extrapolated to infinity; C_{max}: maximum plasma/gill concentration; T_{max}: time of maximum
484 plasma/gill concentration

485 **Table 3.** Pharmacokinetic parameters of PZQ following i.v (75 mg/kg) in gilthead seabream
486 plasma at 21 °C

Parameters	**i.v**
Dose	75 mg/kg
β	0.024
$t_{1/2\alpha}$ (h)	3.4
$t_{1/2\beta}$ (h)	28.8

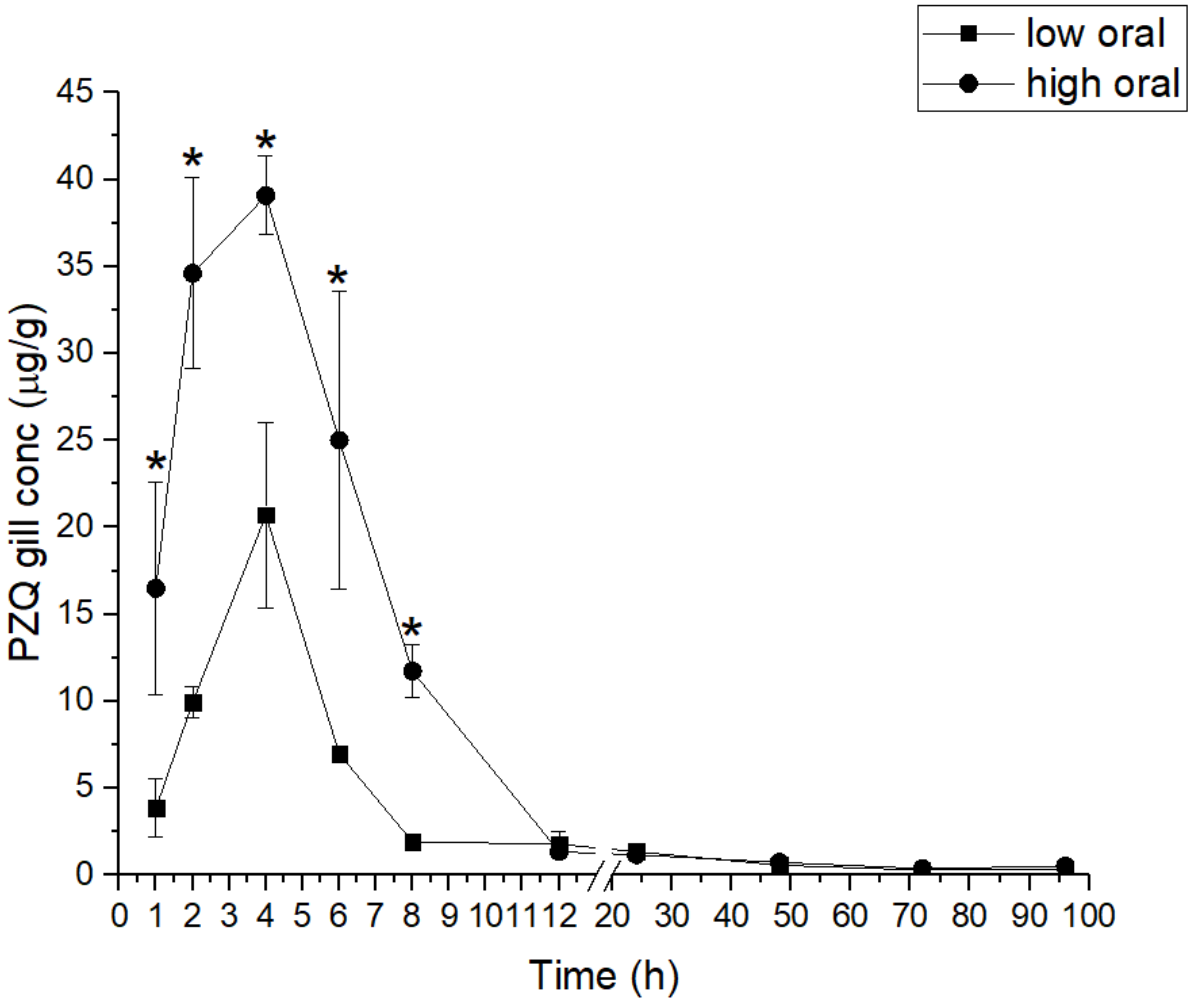
V_{dss} (L/kg)	6.1
$AUC_{0-\infty}$ (μg h/mL)	455.6
F (%)	49

487



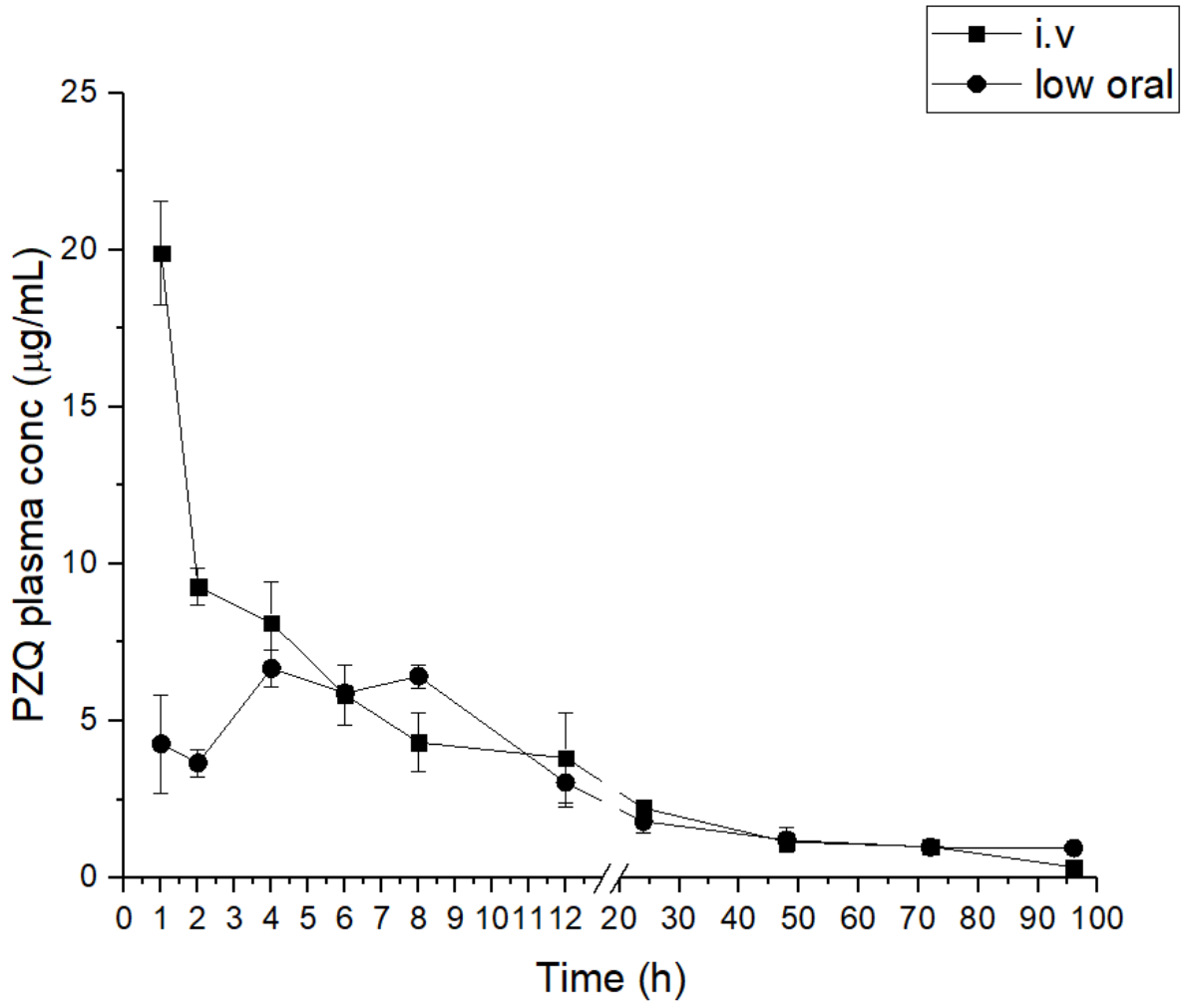
488

489 **Figure 1.** PZQ concentration (μg/mL) in gilthead seabream plasma after two single oral doses
490 (low=75 mg/kg, high=150 mg/kg). Values shown are mean±stdev (N=10). * indicates a
491 statistically significant difference ($P<0.05$).



492

493 **Figure 2.** PZQ concentration (µg/g) in gilthead seabream gills after two single oral doses
494 (low=75 mg/kg, high=150 mg/kg). Values shown are mean±stdev (N=5). * indicates a
495 statistically significant difference ($P<0.05$).



496

497 **Figure 3.** PZQ concentration (µg/mL) in gilthead seabream plasma following a single oral and

498 intravenous administration (75 mg/kg). Values shown are mean±stdev (N=10).