A field evaluation of orally-administered praziquantel against the gill fluke *Sparicotyle chrysophrii* infecting gilthead seabream (*Sparus aurata*)

Use of PZQ against *S. chrysophrii* in gilthead seabream

Rigos George[1]*, Glaropoulos Alexis[2], Tzokas Kostantinos[2], Gourzioti Evgenia [3,4], Kogiannou Dimitra[1], Golomazou Eleni[5]

[1]*Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, 46.7 Athinon - Souniou ave, 19013 Anavyssos, Attiki, Greece*

[2]*Avramar Aquaculture SA, 19,3 km Markopoulou–Paiania Ave. 19002 Paiania, Greece*

[3] *Laboratory of Ichthyology and Fish diseases, Faculty of Veterinary Medicine, University of Thessaly, 43100 Karditsa, Greece*

[4] *Department of Aquaculture and Fish Diseases, Faculty of Veterinary Science, School of Health Sciences, University of Thessaly, Trikalon 224 str., 43100 Karditsa, Greece*

[5]*Department of Ichthyology and Aquatic Environment - Aquaculture Laboratory, School of Agricultural Sciences, University of Thessaly, Fytokou str., 38446, Volos, Greece*

Correspondence: grigos@hcmr.gr

Acknowledgments

The research was co-funded by Greece and the European Union under the Fisheries and Maritime Operational Program 2014-2020 (75 % EMFF contribution, 25 % National Contribution).

Keywords: Gilthead seabream; *Sparus aurata*; praziquantel; *Sparicotyle chrysophrii*; antiparasitics; efficacy

Data Availability Statement

We confirm the absence of shared data.

Introduction

Praziquantel (PZQ) is a synthetic broad-spectrum antiparasitic, widely used in veterinary and human medicine (Andrews et al., 1983). As a derivative of pyrazinoisoquinoline, it is highly effective against many species of helminths and cestodes. Although its mechanism of action lacks full understanding, PZQ seems to affect the integumental membrane of the parasite, disrupting the regulatory processes and inducing spastic paralysis (Staudt et al., 1992; Bais, Greenberg, 2018). Following a recent opinion of the Committee for Veterinary Medicinal Products (CVMP), PZQ was included in the group of 'allowed substances' (Annex to Commission Regulation No 37/2010), with a proposed maximum residue level (MRL) of 20 µg/kg in finfish muscle plus skin (EMA, 2022). PZQ however, has a long use in the Norwegian salmon farming industry against *Eubothrium* sp. infections (Lunestad et al., 2015) under specific veterinary prescription, and has been applied with success as aquatic medicine in several Asian countries (ASEAN, 2013) and in Australia.

There is a vast amount of literature on the control of fish platyhelminths by PZQ administered either by bath or via the feed (Bader et al., 2019). The drug has been tested against numerous fish parasites by applying various dietary treatment schedules with doses ranging from 5 to even 800 mg/kg/day. PZQ has shown remarkable efficacy as a fish anthelmintic in most cases (Bader et al., 2019; Norbury et al., 2022). These promising findings have triggered preliminary field attempts to combat helminths of Mediterranean-farmed fish with PZQ administered through the feed. In particular, PZQ administered at 150 mg/kg for three days showed ~80% reduction of the monogenean *Zeuxapta seriolae* in greater amberjack (*Seriola dumerili*) (Rigos et al., 2021).

The gill fluke *Sparicotyle chrysophrii* is undoubtedly the most severe pathogen of farmed gilthead seabream (Muniesa et al., 2020), causing considerable losses, reduced feed utilization, and growth retardation, predominantly at high (>20^{o}C) water temperatures (Sitjà-Bobadilla et al., 2009a). The parasite feeds on host tissue cells and blood, causing irritation, gill hyperplasia, overproduction of mucus and hemorrhages, and respiratory and osmoregulatory dysfunctions, leading to severe anaemic conditions and eventually to death (Sitjà-Bobadilla, Alvarez-Pellitero, 2009b; Henry et al., 2015).

Treatment of sparicotylosis in caged gilthead seabream is mainly based on formalin (37-40% formaldehyde) baths, the most commonly applied ectoparasitic measure in fish therapy (Boyd, McNevin, 2015). Although formalin is an effective anthelmintic solution, bath application in large pens is laborious, costly, weather dependent, and occasionally infeasible in open sea conditions. Formaldehyde can be also toxic to fish if improperly applied, while formalin baths are restricted in several European countries. Consequently, an effective dietary medicine such as PZQ would be an ideal solution to overcome the drawbacks associated with formalin applications in large cages. For this reason, this work aimed to evaluate the efficacy of PZQ against *S. chrysophrii* infections in caged gilthead seabream.

Materials and methods

Facilities and fish

The efficacy trial was carried out at a cage farming unit owned by Avramar in the Ionian Sea, Greece. A set-up of six small net cages (3m^3 total volume/ 2 m^3 clear net volume in water; 1x1x3m) was arranged in a floating collar to accommodate fish receiving or not PZQ feed treatment in triplicate. During the trial, the seawater temperature ranged at 23 ± 1^oC, salinity was 38 ppt, and oxygen saturation at approximately 80%.

S. chrysophrii-infected gilthead seabreams (62.7 ± 16.6 g) were obtained from an adjacent cage. When the number of parasites in the four outer gill arches (two from each side) of the fish reached approximately 8 parasites (adults and juveniles), 780 fish were transferred from the donor cage and randomly divided in each of the six experimental cages (4 kg/m^3). According to regular monitoring, this is a parasitic load capable of causing progression of the disease in caged gilthead seabream. The mortality was daily recorded during the experimentation.

Experimental diets, PZQ dosing, and feeding management

The experimental diets were produced in the facilities of the Hellenic Centre for Marine Research in Anavyssos, Attika). PZQ (50%) was supplied by Vethellas S.A. The formulation of the two experimental diets used for the PZQ trial (control vs 150 mg PZQ/kg fish) is provided in Table 1. Two pelleted feeds were coated externally with the drug and 3% fish oil as a feed attractant to mask the taste of PZQ. Krill meal was incorporated as a feed component into the diet formulation to also induce an additional masking effect (Oikawa, March, 1997; Querol et al., 2012). During the

104 experimentation, fish were fed by hand at two time -points at a daily feeding rate of
105 2%. The duration of each meal was 20-30 min. Fish served as control received the
106 experimental diet without PZQ, while treated fish were given a diet including 150 mg
107 PZQ/kg/day, for three consecutive days only in the first meal (1%) of the day, while
108 the remaining necessary daily feed quantity was completed by the control feed.
109 Feeding was carefully monitored to ensure complete diet consumption.

110

111 **Counting of parasitic load**

112 To assess parasitic prevalence and intensity, ten fish were sampled from each of the
113 six experimental cages. Sampling was carried out 24 hours before the first and after
114 the last medication. Sampled fish were killed using ice-slurry immersion and their four
115 outer gill arches were excised, placed in petri dishes with a few drops of filtered
116 seawater, and examined under a stereoscope for parasitic counting (adults and
117 juveniles). The aforementioned methodology has been previously considered a valid
118 and representative process of parasitic counting (Rigos et al., 2016).

119

120 **Statistical examination**

121 The non-parametric χ^2 test was applied to find statistical differences in parasitic load
122 between untreated and PZQ-treated groups. The level of significance was set at 95%.
123 The efficacy (%) of PZQ against *S. chrysophrii* in gilthead seabream or percentage
124 reduction in mean *S. chrysophrii*, relative to the control group was assessed as
125 previously suggested for parasites (Stone etal., 2000a,b), while mean prevalence,

mean abundance, and mean intensity were calculated according to Bush et al. (1997), as follows:

% Efficacy= 100- [100 * (mean number of parasites in PZQ-treated group) / (mean number of parasites in the control group)]

Mean prevalence= (number of hosts infected with parasites / total number of fish examined)

Mean abundance= (total number of parasites / total number of fish examined)

Mean intensity= (total number of parasites / total number of hosts infected with parasites).

Ethical statement

Procedures involving fish were performed according to the EU guidelines on the protection of animals used for scientific purposes (Directive 2010/63/EU). Avramar's facility is certified (Vet code: GR01FISH0004) and licensed for the rearing and use of fish for scientific purposes (EL 01 BIO exp 02). The experimental protocol was approved by the Ethics Committee of the competent authority (275108/1189/21-10-2020). Despite not being in full accordance with Directive 2010/63/EU, sampled fish were killed using ice-slurry immersion, a practice commonly accepted for Mediterranean fish species (Council Regulation No 1099/2009; Commission Report COM/2018/087 final). This aimed to avoid complications in the accurate measurement of gill monogeneans caused by immediate parasitic dislocation due to the use of anesthetics.

Results & Discussion

Although the efficacy of dietary PZQ has been sporadically evaluated in unpublished preliminary field attempts against fish helminths of Mediterranean farmed finfish species, the present study describes the first organized trial to assess the anthelminthic effects of the drug against *S. chrysophrii* in gilthead seabream.

The estimated prevalence, mean abundance, and mean intensity of *S. chrysophrii* in the sampled fish are provided in Table 2. Reduction of mean intensity in the PZQ-treated group was significant ($P<0.05$) when a comparison was carried out between two experimental groups of fish (2.6 ± 0.5 vs 8.0 ± 1.3), or between the same group before and after treatment. No mortalities were observed during the efficacy trial.

Reduced efficacy of PZQ may be attributed to its bitter taste, causing palatability problems and diminished consumption of the PZQ-medicated diets (Partridge et al., 2014). Reduced intake of PZQ-medicated diets necessitates the inclusion of masking agents (Pilmer, 2016) along with specific feed management to improve feed acceptance. The PZQ-medicated diet administered to gilthead seabream was well accepted herein, as was recently seen in *Z. seriolae*-infected greater amberjack (Rigos et al., 2021). The inclusion of krill meal in the dietary components in addition to the external coating with fish oil acted as effective masking agents in the medicated diets used. Perhaps, the dose of PZQ used in the present study approaches the palatability burden in this species since former laboratory attempts to feed PZQ to gilthead seabream at doses of 200-400 mg/kg for the treatment of *S. chrysophrii*, revealed that the effective dose ingested by the fish could not exceed 158 mg/kg for six days (Sitjà-Bobadilla et al., 2006).

171 The high (86%) anthelmintic efficacy of the PZQ treatment found in this study,
172 combined with the significant decrease in the mean parasitic intensity between
173 treated and control fish, meets the suggested criteria proposed by Somerville et al.
174 (2016). A similarly high PZQ efficacy has also been observed using also 150 mg/kg for
175 three days against *Z. seriolae* in greater amberjack (80.4%). The high anthelmintic
176 efficacy of PZQ seen in these two studies and elsewhere (Williams et al., 2007;
177 Yamamoto et al., 2011; Forwood et al., 2016), may be connected to its high
178 bioavailability values in farmed fish. Indeed, the absorption of PZQ seems to be
179 promising in gilthead seabream (Kogiannou et al, 2023) and yellowtail amberjack (*S.*
180 *lalandi*) (Tubbs, Tingle, 2006a), reaching values as high as 50%, exceeding those
181 estimated for terrestrial animals (3 to 32%) (Zeng et al., 1993; Cao et al., 2001; Giorgi
182 et al., 2001), perhaps due to considerable first-pass effect on absorbed PZQ on
183 livestock (Andrews et al., 1983).

184 Therapeutic strategies against flukes in aquaculture medicine necessitate consecutive
185 anthelmintic treatments, considering the incubation period of the parasitic eggs and
186 the survival of the infective swimming larvae (Villar-Torres et al., 2018). Based on the
187 current field knowledge and the pertinent literature (Villar-Torres et al., 2018; 2023),
188 three to four monthly treatments against *S. chrysophrii* are approximately advised
189 during the warm periods. Improved cage hygiene accomplished by several means (e.g.
190 ROVs), and frequent net replacement, may affect the viability of parasitic eggs and
191 thus, reduce the frequency of anthelmintic therapy. Another challenge when battling
192 monogeneans in aquatic medicine is the necessity to prevent reinfection at the farm
193 level which seems a considerable problem in Mediterranean aquaculture where farms
194 have several fish generations together. This causes a perpetual reinfection of

pathological agents and it is the main risk factor, not only for parasites, but for infectious diseases in general. This issue would require simultaneous therapy of all fish stocks maintained on the farm, which is virtually impossible, especially with chemical baths. Fortunately, the availability of dietary antiparasitics with evidenced anthelmintic efficacy as PZQ would allow simultaneous treatment of all the susceptible fish sizes in an infected population.

In conclusion, the findings of the present study indicate that oral PZQ treatments using specific feeding management can considerably control *S. chrysophrii* infections in farmed gilthead seabream. Dietary PZQ administration should also be assessed against other important helminths of Mediterranean finfish farmed species such as *Cardicola* spp. infections (Palacios-Abella et al., 2021), that may co-infect gilthead seabream.

References

Andrews, P., Thomas, H., Pohlke, R., Seubert, J., 1983. Praziquantel. Medicinal Research Reviews. 3, 147-200.

ASEAN, 2013. Association of Southeast Asian Nations, Guidelines for the Use of Chemicals in Aquaculture and Measures to Eliminate the Use of Harmful Chemicals. Jakarta, ASEAN Secretariat.

Bader, C., Starling, D.E., Jones, D.E., Brewer, M.T., 2019. Use of praziquantel to control platyhelminth parasites of fish. Journal of Veterinary Pharmacology and Therapeutics. 42, 139-153.

Bais, S, Greenberg, RM., 2018. TRP channels as potential targets for antischistosomals. Int J Parasitol Drugs. Drug Resistance. 8, 511-517.

Boyd, C.E., McNevin, A.A., 2015. Chemicals in Aquaculture, Aquaculture, Resource Use, and the Environment, pp. 173-210.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology Meets Ecology on Its Own Terms: Margolis et al. Revisited. The Journal of Parasitology. 83, 575-583.

Cao, J., Liu, E., Zhao, J., Li, K., Dou, S., 2001. Pharmacokinetics and bioavailability of praziquantel in cattle afteroral, intramuscular and intravenous administration. Chin J Vet Sci. 21, 612-614.

EMA, 2022. European Medicine Agency, Committee for Medicinal Products for Veterinary Use (9 September 2022). Praziquantel MRL summary opinion. EMA/CVMP/706481/2022.

Forwood, J.M., Bubner, E.J., Landos, M., Deveney, M.R., D□Antignana, T., 2016. Praziquantel delivery via moist pellets to treat monogenean parasites of yellowtail kingfish Seriola lalandi: efficacy and feed acceptance. Diseases of Aquatic Organisms. 121, 201-209.

Giorgi, M., Salvatori, A.P., Soldani, G., Giusiani, M., Longo, V., Gervasi, P.G., Mengozzi, G., 2001. Pharmacokinetics and microsomal oxidation of praziquantel and its effects on the P450 system in three-month-old lambs infested by Fasciola hepatica. Journal of Veterinary Pharmacology and Therapeutics. 24, 251-259.

Henry, M.A., Nikoloudaki, C., Tsigenopoulos, C., Rigos, G., 2015. Strong effect of long-term Sparicotyle chrysophrii infection on the cellular and innate immune responses of gilthead sea bream, Sparus aurata. Dev Comp Immunol. 51, 185-193.

Kogiannou, D., Nikoloudaki, C., Valsamidi, V., Kotsiri, M., Golomazou, E., Rigos, G., 2023. Pharmacokinetics of praziquantel in gilthead seabream (*Sparus aurata*) plasma and gills, Aquaculture, 739975.

Lunestad, B., Hannisdal, R., Samuelsen, O., 2015. Safety of medical feed additives in the food chain. in: DA, D. (Ed.), Feed and Feeding Practices in Aquaculture. Woodhead Publishing, Oxford, pp. 251–268.

Muniesa, A., Basurco, B., Aguilera, C., Furones, D., Reverté, C., Sanjuan-Vilaplana, A., Jansen, M.D., Brun, E., Tavornpanich, S., 2020. Mapping the knowledge of the main diseases affecting sea bass and sea bream in Mediterranean. Transboundary and Emerging Diseases. 67, 1089-1100.

Norbury, L.J., Shirakashi, S., Power, C., Nowak, B.F., Bott, N.J., 2022. Praziquantel use in aquaculture - Current status and emerging issues. International Journal for Parasitology. Drugs and drug resistance. 18, 87-102.

Oikawa, C.K., March, B.E., 1997. Communications: A method for assessment of the efficacy of feed attractants for fish. Progressive Fish-Culturist. 59, 213-217.

Palacios-Abella, J., Montero, F., Merella, P., Mele, S., Raga, J., Repullés-Albelda, A., 2021. Cardicola mediterraneus n. sp. (Trematoda, Aporocotylidae): a new species infecting the gilthead seabream, Sparus aurata L., from the Western Mediterranean Sea. Parasitology Research. 120, 1-15.

Partridge, G., Michael, R., Thuillier, L., 2014. Praziquantel form, dietary application method and dietary inclusion level affect palatability and efficacy against monogenean parasites in yellowtail kingfish. Diseases of Aquatic Organisms. 109, 155-163.

Pilmer, L., 2016. Novel methods of improving the palatability of feeds containing praziquantel for commercially cultured yellowtail kingfish, Honours thesis. Murdoch University.

Querol, P., Morillo-Velarde, P., Cerezo Valverde, J., Martínez-Llorens, S., Moñino, Jover, Tomás-Vidal, A., 2012. Inclusion of fish and krill meal in extruded diets for Octopus vulgaris (Cuvier, 1797): Assessment of acceptance. Aquaculture Research. 45.

Rigos, G., Kogiannou, D., Vasilaki, A., Kotsiri, M., 2021. Evaluation of Praziquantel Efficacy against Zeuxapta seriolae Infections in Greater Amberjack, Seriola dumerili. Applied Sciences. 11, 4656.

Rigos, G., Mladineo, I., Nikoloudaki, C., Vrbatovic, A., Kogiannou, D., 2016. Application of compound mixture of caprylic acid, iron and mannan oligosaccharide against Sparicotyle chrysophrii (Monogenea: Polyopisthocotylea) in gilthead sea bream, Sparus aurata. Folia Parasitol (Praha). 63.

Sitjà-Bobadilla, A., Alvarez-Pellitero, P., 2009b. Experimental transmission of Sparicotyle chrysophrii (Monogenea: Polyopisthocotylea) to gilthead seabream (Sparus aurata) and histopathology of the infection. Folia Parasitologica. 56, 143-151.

Sitjà-Bobadilla, A., de Felipe, M.C., Alvarez-Pellitero, P., 2006. In vivo and in vitro treatments against Sparicotyle chrysophrii (Monogenea: Microcotylidae) parasitizing the gills of gilthead sea bream (Sparus aurata L.). Aquaculture. 261, 856-864.

Sitjà-Bobadilla, A., Redondo, M., Alvarez-Pellitero, P., 2009a. Occurrence of Sparicotyle chrysophrii (Monogenea: Polyopisthocotylea) in gilthead sea bream (Sparus aurata L.) from different mariculture systems in Spain. Aquaculture Research - AQUAC RES. 41, 939-944.

Sommerville, C., Endris, R., Bell, T.A., Ogawa, K., Buchmann, K., Sweeney, D., 2016. World association for the advancement of veterinary parasitology (WAAVP) guideline for testing the efficacy of ectoparasiticides for fish. Veterinary Parasitology. 219, 84-99.

Staudt, U., Schmahl, G., Blaschke, G., Mehlhorn, H., 1992. Light and scanning electron microscopy studies on the effects of the enantiomers of praziquantel and its main metabolite onSchistosoma mansoni in vitro. Parasitology Research. 78, 392-397.

Stone, J., Sutherland, I.H., Sommerville, C., Richards, R.H., Varma, K.J., 2000a. Field trials to evaluate the efficacy of emamectin benzoate in the control of sea lice, Lepeophtheirus salmonis (Kroyer) and Caligus elongatus Nordmann, infestations in Atlantic salmon Salmo salar L. Aquaculture. 186, 205-219.

Stone, J., Sutherland, I.H., Sommerville, C., Richards, R.H., Varma, K.J., 2000b. Commercial trials using emamectin benzoate to control sea lice Lepeophtheirus salmonis infestations in Atlantic salmon *Salmo salar.* Diseases of Aquatic Organisms . 41, 141-149.

Tubbs, L.A., Tingle, M.D., 2006a. Bioavailability and pharmacokinetics of a praziquantel bolus in kingfish Seriola lalandi. Diseases of Aquatic Organisms. 69, 233-238.

Villar-Torres, M., Montero, F.E., Raga, J.A., Repullés-Albelda, A., 2018. Come rain or come shine: environmental effects on the infective stages of Sparicotyle chrysophrii, a key pathogen in Mediterranean aquaculture. Parasites & Vectors. 11, 558.

Villar-Torres, M., Montero, F.E., Raga, J.A., Repullés-Albelda, A., 2023. The influence of water temperature on the life-cycle of Sparicotyle chrysophrii (Monogenea: Microcotylidae), a common parasite in gilthead seabream aquaculture. Aquaculture. 565, 739103.

Williams, R., Ernst, I., Chambers, C., Whittington, I., 2007. Efficacy of orally administered praziquantel against Zeuxapta seriolae and Benedenia seriolae (Monogenea) in yellowtail kingfish Seriola lalandi. Diseases of Aquatic Organisms. 77, 199-205.

Yamamoto, S., Shirakashi, S., Morimoto, S., Ishimaru, K., Murata, O., 2011. Efficacy of oral praziquantel treatment against the skin fluke infection of cultured chub mackerel, Scomber japonicus. Aquaculture. 319, 53-57.

Zeng, Z., Chen, Z., Feng, Q., 1993. Pharmacokinetics and oral bioavailability of praziquantel in pigs following a single dose. Chin J Anim Vet Sci 24, 170-174.

350 **Table 1.** Formulation and composition of experimental diets (3mm pellets) for the

351 PZQ trial

Basic dietary components		
	Control (%)	**PZQ-treated (%)**
Fish meal	24.0	24.0
Krill meal	5.0	5.0
Poultry meal	13.0	13.0
Soy protein concentrate	4.4	4.4
Corn gluten	16.5	16.5
Soybean meal	10.0	10.0
Rapeseed meal	5.0	5.0
Wheat Flour	10.2	7.2
Fish oil	4.4	4.4
Salmon oil	6.0	6.0
Monocalcium phosphate	0.3	0.3
Phospholipid source	0.2	0.2
Premix of vitamins and minerals	1.0	1.0
Top coating components		
Fish oil	3.0	3.0
Praziquantel 50% (Vethellas)	-	3.0
Proximate composition	**g/100g**	
Crude protein	48	
Total carbohydrates	20	
Crude lipids	15	
Moisture	8	

Total ash	7
Crude fiber	2

352

353

354

355

356

357

358

359

360

361

362

363

364

365

366

Table 2. Prevalence, mean abundance and mean intensity of *S. chrysophrii* in gilthead seabream before and after PZQ treatment (dosed with 150 mg/kg for three days). Different letters (a, b) denote significant differences between groups (P<0.05). Data are presented as means ± st.dev.

	PZQ-treated	**Control**
	Prior to treatment	
Prevalence (%)	100 ± 0.0	96.3 ± 6.4
Mean abundance	8.2 ± 0.7	6.7 ± 1.3
Mean intensity	8.2 ± 0.7	7.0 ± 1.0
	After treatment	
Prevalence (%)	56.7 ± 28.9	100 ± 0.0
Mean abundance	1.5 ± 0.9 [a]	8.0 ± 1.3 [b]
Mean intensity	2.6 ± 0.5 [a]	8.0 ± 1.3[b]