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Effects of the gill parasite *Zeuxapta seriolae* (Monogenea: Heteraxinidae) on the amberjack *Seriola dumerili* Risso (Teleostei: Carangidae)

Francisco E. Montero[a,*], Silvia Crespo[b,c], Francesc Padrós[b,c], Fernando De la Gándara[d], Antonio García[d], Juan Antonio Raga[a]

[a] *Departamento de Zoología and Instituto Cavanilles de Biodiversidad y Biología Evolutiva, Universitat de Valencia, P.O. Box 22085, Valencia 46071, Spain*
[b] *Departamento de Biologia Animal, Biologia Vegetal i Ecologia, Facultat de Veterinaria, Universitat Autónoma de Barcelona, 08193, Bellaterra, Barcelona, Spain*
[c] *Centre de Referència de Recerca i Desenvolupament en Aqüicultura de Catalunya, Spain*
[d] *Centro Oceanográfico de Murcia, Instituto Español de Oceanografía, Carretera de la Azohía s/n, Puerto de Mazarrón, Murcia, Spain*

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Abstract

Zeuxapta seriolae (Monogenea: Heteraxinidae) infection was associated with important mortalities of amberjacks (*Seriola dumerili*) reared in tanks in the experimental facilities of the Spanish Institute of Oceanography (Western Mediterranean) during the period 1998–2000. Fish infested by this parasite presented characteristics similar to those described for *Seriola quinqueradiata* parasitized by *Heteraxine heterocerca* in Japan. All dead amberjacks had high parasite abundance (mean abundance ± S.D. = 686.7 ± 125.4) and egg strings entangled in gills. The parasitological analysis of 17 live amberjacks collected from the infested tanks showed that 41.2% of fish harboured *Z. seriolae* with intensities ranging between 5 and 731 parasites per fish. No apparent relationship was observed between the abundance of the parasite and the condition factor ($r_S = 0.175$, $n = 17$, $p > 0.5$). However, the haematocrit values were significantly lower in the fish infested with monogeneans. The relationship between parasite abundance and haematocrit values was negative and statistically significant ($r_S = -0.625$, $n = 17$, $p < 0.01$). The light and scanning electron microscope studies of affected gills revealed that the parasites attached to the host grasping one or two lamellae with each clamp of the haptor, which led to lamellar synechiae, lamellar clubbing and disruption of epithelial and vascular structures. A mild to moderate epitheliocystis infection was also

* Corresponding author. Tel.: +34-96-354-36-85; fax: +34-96-354-36-70.
E-mail address: Francisco.E.Montero@uv.es (F.E. Montero).

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detected in gills. Combined effects of gill damage and parasite blood feeding could be related to the anaemia and, finally, the death of the fish.

Keywords: *Seriola dumerili*; Greater amberjack; *Zeuxapta seriolae*; Mortality; Gill damage; Anaemia; Epithelocystis

1. Introduction

Ectoparasites can easily multiply and disperse in confined areas, reaching very high intensities (Rohde, 1984; Thoney and Hargis, 1991). Many species of monogenea have been recorded to provoke important losses in fish cultures. Usually, blood-feeding polyopisthocotyleans have been considered less harmful to their hosts than monopisthocotyleans, which feed on mucus and epithelium (Thoney and Hargis, 1991; Cone, 1995), but outbreaks of mortality have also been reported in association with microcotylids (Faisal and Imam, 1990; Sanz, 1992) or heteraxinids (Kubota and Takakuwa, 1963; Eto et al., 1976; Egusa, 1992; Ogawa and Yokoyama, 1998).

Zeuxapta seriolae is a heteraxinid monogenean with a wide geographical distribution, which infests various species of the genus *Seriola* (Rohde, 1978). From 1998 to 2000, recurrent outbreaks of mortality occurred in greater amberjack (*Seriola dumerili*) reared in tanks of a pilot culturing project of the Spanish Institute of Oceanography (S.I.O.) in Puerto de Mazarrón (Murcia, Spain). The parasitological studies carried out on these fish and the tank substrata revealed the presence of *Z. seriolae* in high abundance. This paper provides the first description of Zeuxaptosis affecting the greater amberjack *S. dumerili* and evaluates related histopathological and haematological effects.

Table 1
Percentage mortality per tank of *S. dumerili* associated with the presence of *Z. seriolae* in Puerto de Mazarrón

	Date	Treatment	Age	*N*	Mortality (%)
Non-treated tanks					
1	March 1998	–	1+ and 2+	6	100
2	May/June 1998	–	0+ and 1+	20	45
3	July 1998	–	2+ and 3+	10	20
	Total			36	47.2
Treated tanks					
4	March 1999	*	3+	40	35
5	June/July 2000	**	2+	22	32
6	June/July 2000	**	2+	7	29
	Total			69	33.3
Total				105	38.1

Data for non-treated and treated with anthelmintic tanks are given separately. *N*, number of fish per tank (–, non-treated; *formalin-treated; **formalin and mebendazole).

2. Material and methods

Greater amberjacks (*S. dumerili* Risso) reared in tanks at the S.I.O. in Puerto de Mazarrón, Spain, were captured in August and September from Águilas (37°21′ 37°34′ N, 1°9′ –1°29′ W). Fish were controlled daily and alterations of their external aspect and behaviour were recorded. Dead fish were gathered, recorded and analysed macroscopically. During 1999 and 2000, the tanks were subjected to anthelmintic treatments which are usually used to exterminate monogeneans in aquaculture; solutions of formalin—300 ppm for 30 min—and/or mebendazole—0.4 ppm for 24 h (Thoney and Hargis, 1991). Parasites from a subsample of 23 amberjacks were collected, identified, and counted in June 2000. Samples of the substratum of each affected tank were also collected and examined for the presence of eggs. Parasites were identified as *Z. seriolae* according to the criteria of Rohde (1978).

A second subsample of 17 live individuals from the affected tanks was studied in order to observe possible physiological or anatomical effects in *S. dumerili* related to the presence of *Z. seriolae*. Fish were killed with a blow on the head, sized and weighed. The

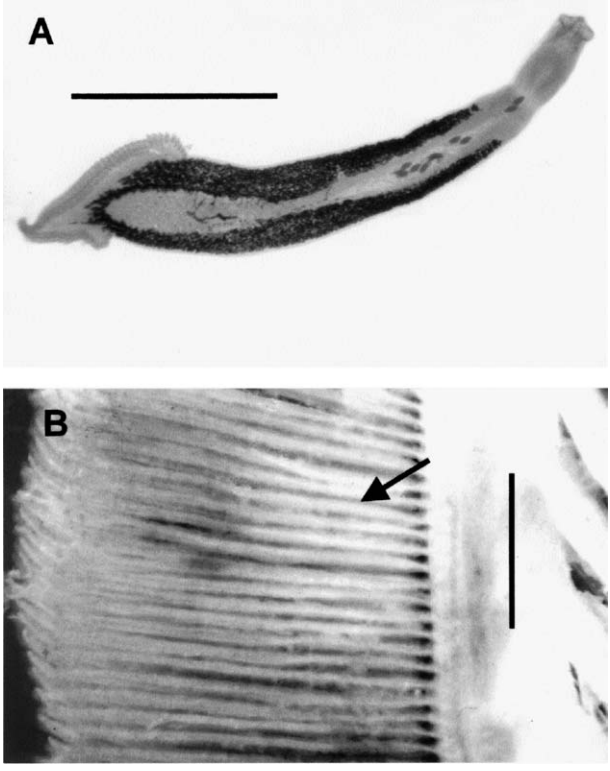

Fig. 1. *Z. seriolae* from *S. dumerili* in Puerto de Mazarrón, Spain. (A) Wet mount of *Z. seriolae*. (B) Gills of parasitized *S. dumerili*. Note in B the presence of whitish plaques (arrow) which appear in the zones of the filaments where the parasites were attached (bars = 2 mm).

condition factor of Fulton was calculated as (weight/length^3) × 100 (Lagler, 1978). Blood samples were taken and the haematocrit value of each individual was determined. Each amberjack was analysed (skin, gills, viscera and eyes) under a stereomicroscope. Samples of liver, spleen and the four left gill arches were collected and fixed in buffered 4% saline formalin. Intensity of *Z. seriolae* in individual fish was calculated by multiplying by 2 the number of parasites recorded on the four gill arches of the right side. The possible relation between the abundance of *Z. seriolae* and both the haematocrit value and the condition factor of each fish was studied using Mann–Whitney test and Spearman's correlation. Ecological terms followed the recommendations of Bush et al. (1997).

Samples of gills, spleen and liver were processed for paraffin wax sections, stained with haematoxylin and eosin and viewed under the light microscope (LM). A scanning electron microscope (SEM) study was performed on 4% buffered formalin-fixed gill samples which were then dehydrated in an ethanol series, critical-point dried with liquid CO_2, coated with gold and viewed in a Hitachi S570 SEM.

3. Results

A mortality rate of 38.1% was observed for a total of 105 amberjacks in the affected tanks, during Spring and Summer of 1998, 1999 and 2000. The mortality in non-treated fish was 47.2% while in the treated fish it was 33.3% (see Table 1). Most of the age classes were affected (0+ to 3+). The external aspect of the amberjacks was apparently healthy, with respect to both the coloration and weight. No anomalies in the behaviour of affected individuals were observed, except for some slow swimming. However, hypersecretion of

Table 2
Data on parasite abundance and host parameters assessed for killed *S. dumerili*, from tanks affected by Zeuxaptosis in Puerto de Mazarrón

	Parasite abundance	Fish length (cm)	Fish weight (g)	Condition factor	Haematocrit values (%)
Parasitized fish	537	32.0	432	1.32	12
	224	32.0	423	1.29	21
	731	36.5	557	1.20	22
	321	42.0	1023	1.38	23
	482	27.5	298	1.51	26
	5	31.0	452	1.52	30
	248	31.0	348	1.17	31
Non-parasitized fish	0	37.0	630	1.24	–
	0	32.5	481	1.50	25
	0	29.0	251	1.03	25
	0	42.0	926	1.25	30
	0	40.0	838	1.31	30
	0	38.0	752	1.37	30
	0	31.5	480	1.54	31
	0	36.0	543	1.16	38
	0	36.0	601	1.29	40
	0	35.0	452	1.10	42

mucus on gills, accompanied by a large quantity of parasites (Fig. 1A) and egg strings of *Z. seriolae* entangled in gill filaments, was observed in all dead fish. Egg strings were also very abundant in the substratum and the water of the tanks.

The mean intensity of *Z. seriolae* (Fig. 1A) in the subsample of 23 dead amberjacks was very high [± S.D. (confidence interval); 686.7 ± 125.4 (496–1401)]. The appearance of the viscera was normal, with the exception of a slightly paler tonality of the

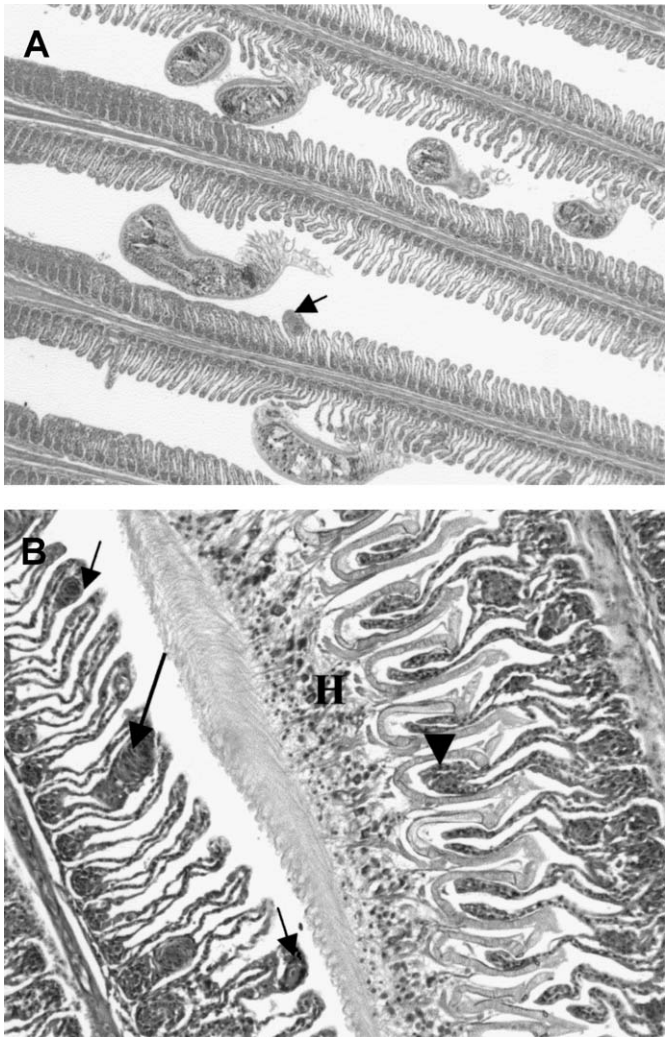

Fig. 2. *Z. seriolae* attaching to the gill lamellae of the host. See, in A (× 100), gill filaments of severely parasitized fish. The clamps of the haptor (H) grasped one or two lamellae, which led to lamellar synechia (arrowhead) [B (× 400)]. Note that parasitized fish were also affected by mild (A) to moderate (B) epitheliocystis infection (arrows).

spleen and, especially, of the liver of some fish, suggesting a possible anaemia condition. Whitish plaques were observed in the zones of the gills where the parasites were attached (Fig. 1B).

The parasitological analysis of the 17 live amberjacks collected from the infested tanks showed that 41.2% of fish harboured *Z. seriolae* with intensities ranging between 5 and 731 parasites per fish (Table 2). No apparent relation was observed between the abundance of the parasite and the condition factor ($r_S = 0.175$, $n = 17$, $p > 0.5$) (Table 2). However, the haematocrit values were significantly lower in the fish infested with monogeneans (Mann–Whitney $U = 11$, $p = 0.0156$, see also Table 2). In fact, the relationship between

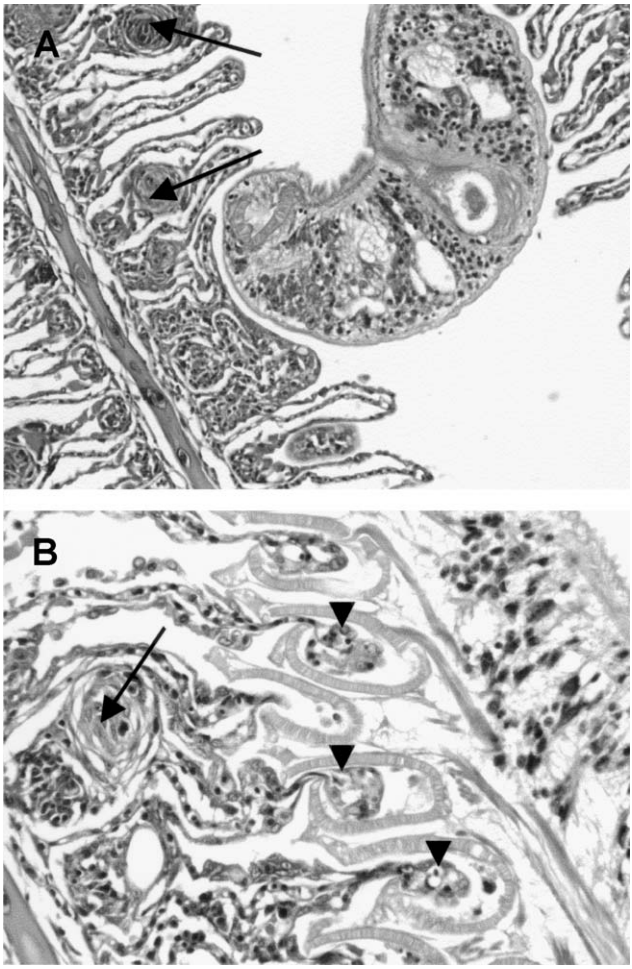

Fig. 3. Gill histological alterations caused by *Z. seriolae*. Note, in A (× 400), disruption of the lamellar structure and lamellar fusion in the vicinity of the attachment zone, and, in B (× 1000), lamellar clubbing (arrowheads) due to the compression of the tips of the lamellae by the clamp sclerites; the proliferative cell response to epitheliocystis infection is also shown (arrows).

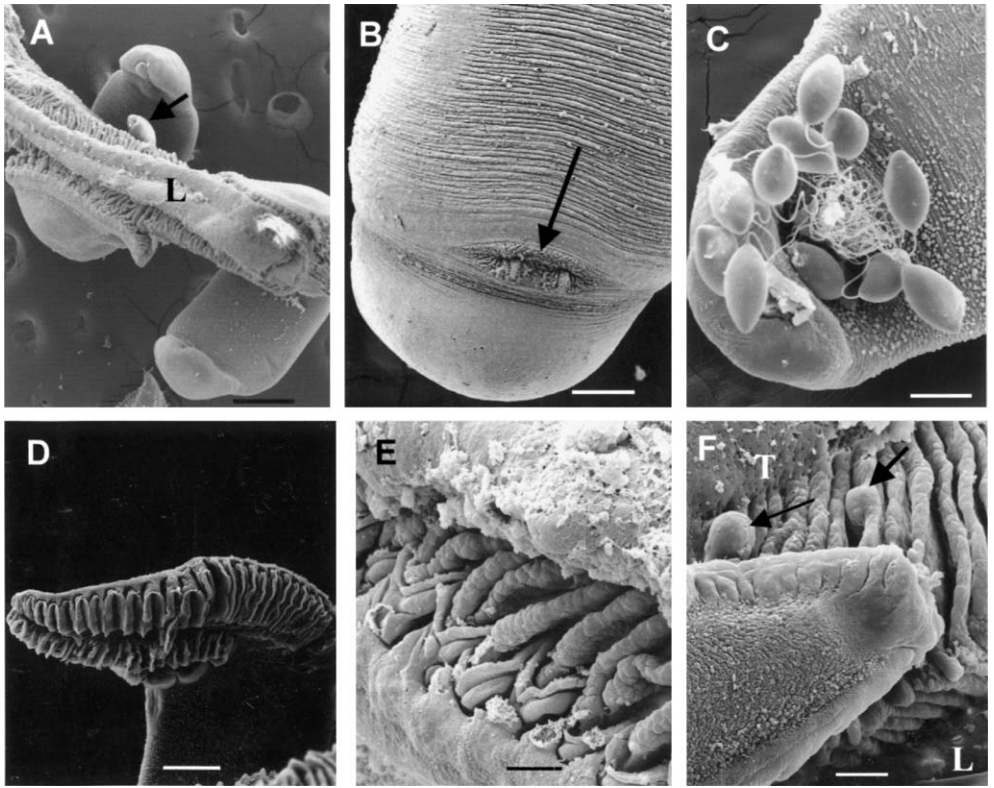

Fig. 4. SEM observations of *Z. seriolae*. Note, in A, two adult and one juvenile (arrow) parasites attached to the amberjack gill, the oral region of all of them facing the water current (L = leading edge of the gill filament; bar = 0.29 mm). The vaginal pore/atrium (arrow) of the parasite is shown in B (bar = 88 μm). Strings of eggs with a long polar filament are emerging from the genital atrium (C; bar = 76 μm). The asymmetrical haptor of the parasite exhibits two rows of clamps (D; bar = 0.15 mm). Note that clamps grasp lamellar tips, one or two lamellae being inserted into them like the teeth of a zip (E; bar = 37 μm). Note, in F, the presence of epitheliocystis (arrows) at the vicinity of the trailing (T) edge of the gill filament (L = leading edge; bar = 125 μm).

parasite abundance and haematocrit values was negative and statistically significant ($r_S = -0.625$, $n = 17$, $p < 0.01$).

The LM study did not reveal any apparent histopathological alterations in either the spleen or the liver of affected fish. Fig. 2A shows the gills heavily parasitized by *Z. seriolae*. Mild to moderate proliferative epitheliocystis was also evident (Figs. 2 and 3). Parasites were attached to the gill by means of their haptor clamps (Fig. 2B), with each clamp grasping one or two lamellae. Lamellar synechia of adjacent lamellae within a single clamp was often observed. Distortion of lamellar structure, synechiae and subsequent lamellar fusion commonly occurred in the vicinity of the attachment zone (Fig. 3A), although no severe proliferative cell response to the parasite was observed. The compression of the lamellar tips by the clamp sclerites induced lamellar clubbing and occasionally led to the rupture of distal epithelial and vascular structures, particularly those of the marginal vessel (Fig. 3B).

SEM findings coincided with LM observations. The frontal view of adult and juvenile parasites attached to a gill filament is shown in Fig. 4A. The surface morphology of their anterior region is shown in Fig. 4B (dorsal) and C (ventral). In all samples studied by SEM, the oral region of the parasite was observed facing the water current (Fig. 4A). The asymmetrical haptor of *Z. seriolae* exhibiting two rows of clamps is shown in Fig. 4D. When the parasite attached to the host, the clamps grasped the lamellar tips, with one or two lamellae being inserted into them like the teeth of a zip (Fig. 4E). Cysts corresponding to the proliferative cell response of epitheliocystis organisms were also observed at the vicinity of the trailing edge of the gill filament (Fig. 4F).

4. Discussion

There is only partial information about the sublethal effects caused by polyopisthocotyleans and even serious infestations with parasites of this group of monogenea have, apparently, few clinical signs (Noga, 1996). Relevant effects on fish hosts related to the presence of *Z. seriolae* have not been reported in cultures of *Seriola* spp. in either Japan or Australia (Ogawa and Fukudome, 1994; Ogawa and Yokoyama, 1998; Anshary and Ogawa, 2001; Ernst et al., 2002). However, important losses of greater amberjacks (*S. dumerili*), possibly associated with the presence of *Z. seriolae*, have also been recently observed in cultures at two different localities in the Western Mediterranean (June 1996, Messina, Italy, Di Cave, personal communication; January–March 2002, Majorca, Spain, Grau et al., submitted for publication). In the case of the greater amberjacks studied by us at Puerto de Mazarrón, despite the healthy external appearance of the fish, their gills showed general signs of severe monogenean infestation, such as hypersecretion of mucus or lesions of the lamellar gill epithelium and vascular structures. Hyperplasia of the gill epithelium with fusion of adjacent gill lamellae has also been observed in association with the presence of the polyopisthocotylean *Mycrocotyle* sp. in the sea bream, *Sparus aurata*, although this parasite only catches one lamella with each clamp (Padrós and Crespo, unpublished). Another polyopisthocotylean, *Kuhnia scombri*, can catch one or two lamellae with its clamps in the mackerel, *Scomber scomber* (Llewelyn, 1954). Most of the signs described in the present study (slow movement, hypersecretion of mucus and

appearance of whitish plaques in the gills) were also described in *S. quinqueradiata* parasitized by the heteraxinidae, *Heteraxine heterocerca*. Moreover, anaemia—indicated by the low haematocrit values—was also reported in this fish (Eto et al., 1976; Egusa, 1992). The great phylogenetic proximity between *H. heterocerca* and *Z. seriolae* (Unnithan, 1957; Mamaev, 1990; Montero et al., 2003) is consistent with the fact that both parasites have similar pathological effects on the host.

Lesions caused to *S. dumerili* gill tissue by *Z. seriolae* described in the present work, as well as mechanical obstruction of water fluxes between filaments due to the presence of the parasites might undoubtedly induce gill dysfunction. Nevertheless, the role played in respiratory and osmoregulatory impairment by epitheliocystis (see Crespo et al., 1990 and Grau and Crespo, 1991 for a detailed description of this pathological condition in the amberjack) ought not to be undervalued, and a mixed epitheliocystis/monogenean infestation might have been responsible for the outbreak of mortalities in the amberjack cultured at Puerto de Mazarrón as was previously described in gilthead sea bream *S. aurata* (Padrós and Crespo, 1995). The fact that haematocrit values were negatively correlated with the abundance of the parasites can be related to the blood-feeding activity of *Z. seriolae*. The polyopistocothyleans are essentially haematophagous and, when they are very numerous, can diminish considerably the volume of blood of the host (Llewelyn, 1954). Moreover, the rupture of the marginal vessels of gill lamellae caused by clamps, and the subsequent microhaemorrhages may have also contributed to the lower haematocrit values shown in *Z. seriolae*-infected amberjack in Puerto de Mazarrón. The synergic detrimental effect of the anaemic condition and the gill damage caused by the parasite might have seriously compromised both gas exchange and oxygen transport to tissues of diseased amberjack.

The large quantities of parasites and eggs revealed in both the tanks and the fish gills indicate a high rate of reproduction and easy transmission of *Z. seriolae* in aquaculture conditions. The mechanisms of transmission can be related to the specific traits of the eggs of *Z. seriolae* (Montero, 2001). Numerous eggs (up to 160) are released in long strings, attached to their long filaments. These strings can easily be entangled in the gills of the fish. Moreover, the strings can join together forming light masses of very numerous eggs with a wide surface area, which allows the eggs to stay in the water column and get hooked up to the handling nets used in aquaculture, thus spreading the infestation among tanks. Kearn et al. (1992) and Ogawa and Yokoyama (1998) observed similar patterns in strings of eggs of *H. heterocerca*. These traits, combined with the long survival time of *Z. seriolae* and the relatively high resistance of its eggs to desiccation (Montero, unpublished work), might facilitate the transmission of this parasite. Disinfection of the nets, tank surfaces and pipes in the aquaculture facilities is, therefore, strongly suggested.

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