

Research Article

Mixed parasitism induced experimentally in yellowtail, *Seriola dorsalis* reared in RAS: intensity and spatial distribution on the skin and gills

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ABSTRACT. Fish mariculture from the genus *Seriola* spp. can present high mortalities due to parasites. In Mexico, the mortality yellowtail *Seriola dorsalis* caused by parasites is low, and there are no reports of massive mortality events. However, as the aquaculture activity of yellowtail intensifies, parasites will be increasingly conspicuous. Therefore, the present study is an aim to know *S. dorsalis*'s susceptibility to a monogenean infection mixed under RAS experimental conditions and their intensity and spatial distribution on the skin and gills. In this study, a parasitic mix induction was performed using two monogenean species such as *Zeuxapta seriolae* and *Benedenia seriolae* besides the *Amyloodinium ocellatum*, a dinoflagellate that was naturally presented. At the end of the experiment after 45 days, *B. seriolae* prevalence was 100% with a mean intensity of 122 parasites and showed a preference for the head and body of the fish. *Zeuxapta seriolae* prevalence was 100% with a mean intensity of 40 parasites preferentially found in the second branchial arch. In the case of *A. ocellatum* prevalence was of 100% with a mean intensity greater than 200 trophozoites per fish. The fish mortality reached 90% at the end of the experiment. During the infection, the fish weight decreased a 14% at the end of the experiment. Therefore, it will be necessary to carry out prophylactic and control strategies, to reduce the impact of these parasites under culture conditions.

Keywords: *Seriola dorsalis*; *Benedenia seriolae*; *Zeuxapta seriolae*; *Amyloodinium ocellatum*; parasites; aquaculture

INTRODUCTION

The yellowtail, *Seriola dorsalis* Gill, 1863 distribution is in North America, the United States of America and Mexico. The yellowtail is a pelagic marine fish of great commercial importance, mainly in Asian countries where it is commonly sold as hamachi or hiramasa in the seafood industry (Martínez-Takeshita *et al.*, 2015). The yellowtail shows a high potential for expansion for its cultivation due to the high commercial value in the market and its wide adaptation to captive conditions, rapid growth, and resistance to management (Poortenaar *et al.*, 2001; Moran *et al.*, 2009).

Fish mariculture of the genus *Seriola* spp. at high densities can present health problems to the production

(Nakada, 2002). One of the parasites that have the greatest impact on the marine fish culture is the dinoflagellate protozoan *Amyloodinium ocellatum* Brown, 1931, due to the high mortalities that it causes (Fajer-Ávila *et al.*, 2012). However, the most frequent parasites in the culture of *Seriola* have been the monogeneans ectoparasites (Ernst *et al.*, 2002; Montero *et al.*, 2004; Mansell *et al.*, 2005; Williams *et al.*, 2007). Protozoan and monogeneans are parasites with a direct life cycle. These parasites only need a host to reproduce which allows them to increase their population in a few days in culture conditions (Fajer-Ávila *et al.*, 2012). In yellowtail, *Zeuxapta seriolae* (Meserve, 1938) commonly attached to the gills while *Benedenia seriolae* (Yamaguti, 1934) to the fish body epithelial zone.

Z. seriolae is a blood-feeding parasite that can cause hyperplasia, lamellar fusion, and destruction of the gill tissue (Grau *et al.*, 2003; Umeda *et al.*, 2006). These injuries and the presence of parasites in gills impede the water flow, causing a decrease in the gases exchange, which in extreme cases can cause anemia and death of hosts (Grau *et al.*, 2003; Lu *et al.*, 2012). Parasitized fish with *B. seriolae* have erratic behavior. Commonly, parasitized organisms rub against the surfaces of culture systems, causing lesions and hemorrhages in the skin, with possible secondary infections (Ernst *et al.*, 2002).

Sea-cage aquaculture of yellowtail *S. dorsalis* is an emerging industry in the Baja California Peninsula, México; parasitic events have occurred due to monogeneans infection with mortalities of less than 5% of total production (Avilés-Quevedo & Castelló-Orvay, 2004). Although mass mortality has not been reported, as the aquaculture activity of yellowtail intensifies, parasites will be increasingly conspicuous. The recirculation aquaculture systems (RAS) are using to produce yellowtail often focuses on hatchery operations. RAS offer fish producers a variety of important advantages over others culture systems such as maximize production on a limited supply of water and land, improved opportunities for waste management and nutrient recycling, complete environmental control to maximize fish growth year-round, for a better hygiene and for a quick and effective disease control (Martins *et al.*, 2010). Therefore, identifying species that pose a potential threat to the yellowtail culture, and the microhabitats in which they prefer to live, can aid in the development of effective quarantine procedures and disease management strategies (Sharp *et al.*, 2003). Thus, the present study is an aim to know *S. dorsalis*'s susceptibility to a parasitic infection mixed under a closed recirculating aquaculture system. Also, the spatial distribution of the monogeneans *B. seriolae* and *Z. seriolae* on the skin and gills of *S. dorsalis* was determined.

MATERIALS AND METHODS

Seriola dorsalis (n = 300) were transported to our facilities at the Universidad Autónoma de Baja California (UABC). Experimental parasitic induction was conducted under a closed recirculating aquaculture systems, with 12,500 L tanks, connected to a polygeiser biofilter (6-cu. Ft). The filtered seawater passed through a 40-watt ultraviolet (UV) light. The RAS was equipped with an 1,100 L water reservoir with a 6,000 W titanium heater. Seawater was delivered to the tanks with a 1/3 HP water pump with a water renewal of 5% daily.

Subsequently, 60 healthy yellowtails were randomly placed in the 12 tanks (5 fish per tank). A diet was formulated to contain 46% crude protein and 16% lipids as previously tested (Guerra-Olvera & Viana, 2015; Rombenso *et al.*, 2016). This diet was manufactured in the Laboratorio de Investigación y Desarrollo de Alimentos para Acuicultura (LINDEAACUA-UABC) under an extrusion process for floating feed (3 mm) using an Extrutech E325 extruder and dried in a horizontal air dryer (Extrutech, USA). Fish were hand fed three times a day (8:00, 13:00 and 18:00 h) until apparent satiation during the trial. Before starting the essay, the fish were acclimated 10 days in the system at 19°C. After the period of acclimatization, the organisms were exposed to a temperature like the maximum found during the summer under the conditions of production. The temperature in the system was increased (1°C per day), until reaching a temperature of $24 \pm 1^\circ\text{C}$ with the finality to make them more susceptible to the parasites. During the essay, water dissolved oxygen was maintained at $6.5 \pm 0.5 \text{ mg L}^{-1}$, salinity $34.6 \pm 0.4 \text{ g L}^{-1}$, and pH 7 ± 0.3 .

To induce the parasite's infection in healthy fish was realized the method proposed by Grano-Maldonado *et al.* (2011), which consisted of the use of egg collectors of monogeneans (*B. seriolae* and *Z. seriolae*) of other parasitized fish. In this experiment, the parasitized fish (donors) were in another tank separated from the experimental system. 12 nylon collectors of 40 cm in length were attached to the aeration tubes for 24 h to collect the parasite's eggs. The collectors were examined with a stereoscopic microscope, and the number of eggs of the monogeneans was quantified in the 12 collectors. Subsequently, each collector was placed in the 12 tanks of the experimental system. This activity was carried out for three days. The average number was of 206 ± 141 monogenean eggs.

A parallel bioassay was performed *in vitro* for seven days, in order to follow the development of the parasites. Eggs (n = 410) attached to the nylon collector were incubated at 24°C in two Petri dishes under natural photoperiod 12:12 h with sterilized and aerated seawater until larvae hatched. The larvae began hatched on the fourth day of incubation. From this time and for another three days more the recirculation system not had water exchange, to facilitate that the larvae will find the fish (cero time). After 15 days of the experimental infection, nylon collectors were placed again in the tanks, to confirm the presence of monogeneans by its eggs production. When verifying the presence of the eggs of the parasites, the first sampling of the fish was carried out. The infection by *Amyloodinium ocellatum* was naturally presented in the RAS. The sample was taken every 15 days or when fish dying were found. A

fish was taken from each tank; it was sacrificed and weighed to count the parasites later.

All fishes were examined under the microscope (Zeiss, Stemi 2000-C) to locate ectoparasites on the different body parts, *i.e.* head, gills, caudal fin, pectoral fin, dorsal fin, pelvic fin, and anal fin. The extraction and processing (fixation, staining and assembly) of monogeneans was carried out with the technique described by Pérez-Ponce de León *et al.* (1999). In the case of *A. ocellatum* only the right-side gills of each fish were sampled, after removal, the gills were placed into Petri dishes with sea water, and parasites were counted. The parasites found were determined taxonomically for monogeneans (Whittington *et al.*, 2001; Montero *et al.*, 2003), and *A. ocellatum* (Brown, 1934; Lom & Dykova, 1992). Prevalence (%) and mean intensity of infection were determined as defined by Bush *et al.* (1997). Mortality was calculated as the accumulated percentage of dead fish concerning the total number of live fishes.

Data are expressed as means \pm standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA) or by Kruskal-Wallis one-way analysis of variance on ranks, using Sigma stat 4 software (Systat Software, Inc., San Jose, CA, USA) to determine the significance of the mean intensity of infection and the fish weight. When a significant effect was revealed, the Holm-Sidak test or the Dunn's test were used for a multiple comparison procedure. Alpha level of 0.05 was used to determine the significance ($P < 0.05$).

RESULTS

Three parasites species were collected from *S. dorsalis*. Two species of monogeneans were found *Z. seriolae* in the gills and *B. seriolae* in the skin of hosts. In addition, a protozoan *A. ocellatum* was recorded. This protozoan naturally presents in the recirculation system after 15 days of experimentation. The prevalence of *A. ocellatum* in the gills of the yellowtail from day 15 after the infestation, until the end of the experiment, was greater than 90% (Fig. 1), with a mean intensity greater than 200 trophozoites per fish (Fig. 2a).

In the case of *B. seriolae*, its prevalence from day 15 to 45 increased from 53 to 100% (Fig. 1), with a mean intensity that increased from 5 to 122 parasites per fish (Fig 2b). The presence of the gill parasite *Z. seriolae* was registered until day 30 with a prevalence of 41% (Fig. 1) and a mean intensity of six parasites per fish, which was increased to 40 by the day 45 (Fig. 2c).

Concerning the fixation, sites preferred by the parasites in the different body parts of the yellowtail; in

the case of *B. seriolae* showed a preference for the head, followed by the body and caudal fin (Fig. 3). *Zeuxapta seriolae* showed a preference to be attached on the second left gill arch, followed by the first gill arch (Fig. 4). The protozoan *A. ocellatum* was found indiscriminately in skin and gills (Data not shown).

Mortality in juveniles of *S. dorsalis* increased significantly through the time (Fig. 5). Fish mortality (20%) at day 15 after the experimental infestation began and continued until reaching 90% on the last day of the experiment (Fig. 5).

Fish weight was affected by the parasitic infestation (Fig. 6). The weight of yellowtail decreased significantly after 15 days of the beginning of the infestation and continued until the end of the experiment reaching a decrease of 14% (Fig. 6).

DISCUSSION

In this study, it was possible to induce parasitism at the experimental level. At the beginning of the experiment, only monogeneans were present, but naturally, an outbreak of *A. ocellatum* was developed. The entry of *A. ocellatum* into of the RAS could be due to the parasite density low and it no was detected in the fish sample taken previously to the experiment. Fish maintained in RAS, are constrained to an area, providing ideal conditions to favor the development of the infection by *A. ocellatum*. Roberts-Thomson *et al.* (2006) cite that *A. ocellatum* and other infections can bypass even the strictest biosecurity protocols, which focus typically on transmission through either contaminated seawater, equipment or staff. Also, they found dinospores of *A. ocellatum* could travel in aerosol droplets (up to 440 mm in a static system and up to 3 min a dynamic one). So, factors such as density, water quality, but above all an increase in water temperature favor the growth rates of parasitic populations (Brown, 1934). At the world level, in the commercial culture of yellowtail *Seriola* spp., the presence of monogenean and protozoan parasites has been reported, affecting both wild and cultivated organisms (Aiello & D'Alba, 1986; Montero *et al.*, 2004; Repullés-Albelda *et al.*, 2013). In the environment, monogeneans such as *Z. seriolae* have been reported in *Seriola dumerili* of the Balearic Islands. When a seasonal change occurs, towards the beginning of summer (April-June), an increase in water temperature can occur until reaching 27°C, and there is also an increase in *Z. seriolae* populations with a parasitic abundance of more than 200 parasites per fish (Repullés-Albelda *et al.*, 2013). Mansell *et al.* (2005) infect by the method of cohabitation to *S. lalandi* with *Z. seriolae* achieving a peak of parasitic intensity at four weeks, registering a

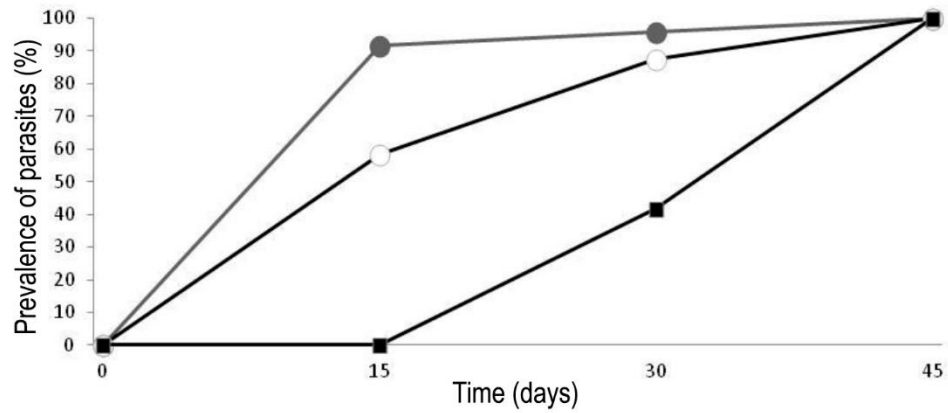


Figure 1. Prevalence of *Amyloodinium ocellatum* (●), *Benedenia seriolae* (○), and *Zeuxapta seriolae* (■) in juvenile *Seriola dorsalis* during 45 days of experimentation.

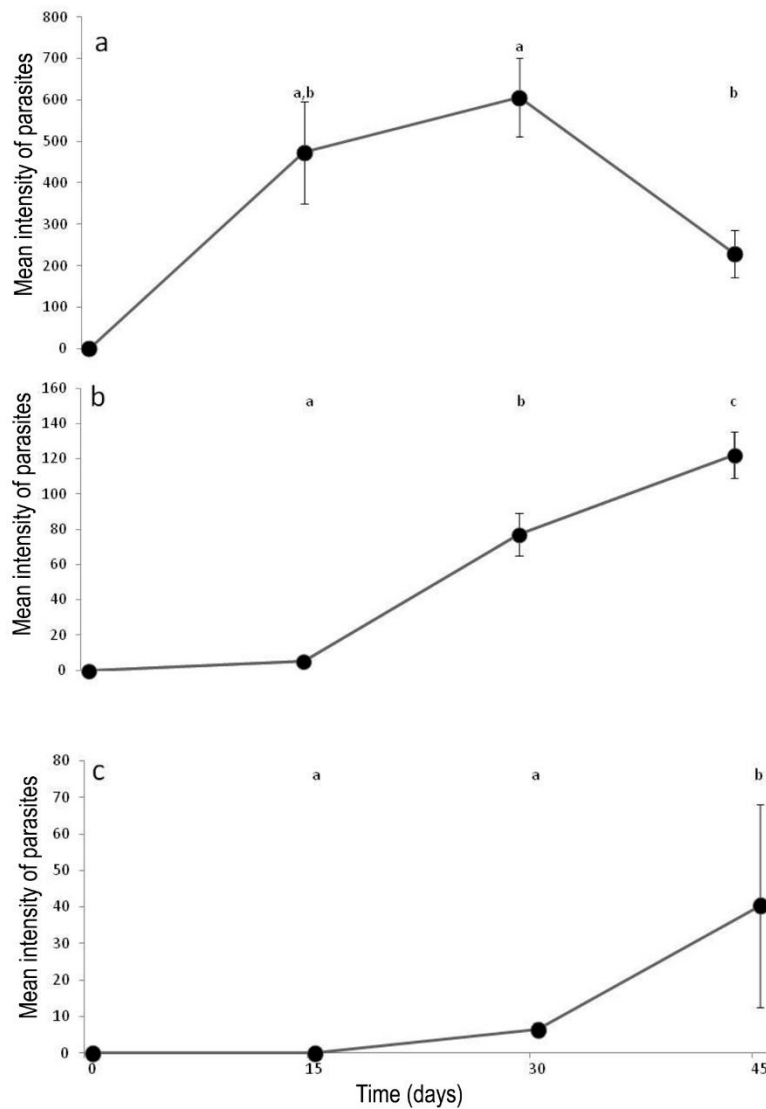


Figure 2. Mean intensity of a) *Amyloodinium ocellatum*, b) *Benedenia seriolae* and c) *Zeuxapta seriolae* in juvenile *Seriola dorsalis* during 45 days of experimentation. Values represent means and standard errors, resulting from one-way ANOVA tests; means with common letter labels are not significantly different ($P > 0.05$).

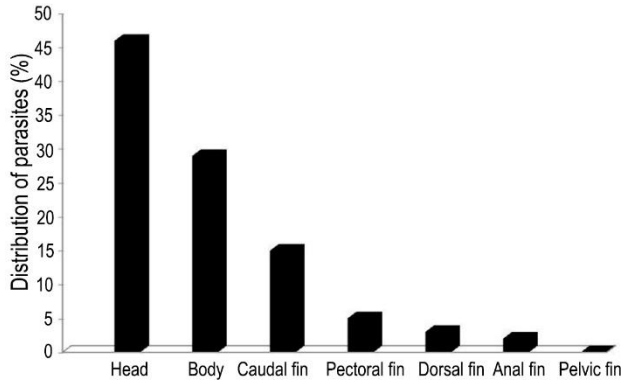


Figure 3. Distribution of *Benedenia seriolae* collected in juvenile of *Seriola dorsalis*.

mean of 565.9 parasites per fish. The intensity was higher than that revealed in this study with *S. dorsalis* (40 parasites). The difference of the parasitic intensity between these two studies can be due at factors such as the culture system conditions, the method of infection and the weight of the organisms used. The weight of *S. lalandi* (289 g) was larger compared with *S. dorsalis* (161 g), and it is known that bigger fishes resist greater parasitic loads. On the other hand, Williams *et al.* (2007) reported in *S. lalandi* maintained in sea-cage aquaculture, similar levels of infection found in our study with *S. dorsalis* for the case of *Z. seriolae*, but in the case of *B. seriolae* the parasitic intensities were lower than those reported in our study with *S. dorsalis*.

The decrease in weight of the *S. dorsalis* in this study was observed in the fish parasitized. Similar weight loss was reported by Mansell *et al.* (2005) in *S. lalandi* highly infected by *Z. seriolae*. These authors suggest that the decrease in weight is due to the metabolic cost due to stress caused by the infections in the fish. Also, if it is considered that under these circumstances the fish stop eating; this also affects the performance of the fish.

The mortality was associated to the infections of monogeneans and the high number of *A. ocellatum* trophozoites recorded in the gills of *S. dorsalis*, which is like that reported in other marine fish species (Kuperman & Matey, 1999; Reyes-Becerril *et al.*, 2008). Monogenean parasites in fish culture can be deadly, since the behavior of the fish changes. Fish stop feeding, swim erratically with the mouth open and rub themselves causing infections in the skin. When fish are severely affected, die by asphyxia as a result of an excess mucus secretion of the gills causing a deficient oxygen exchange (Ernst *et al.*, 2002; Stephens *et al.*, 2003; Fajer-Ávila *et al.*, 2012). Besides, if their co-infection of parasites species, the situation worsens, as is the case of the dinoflagellate *A. ocellatum* that can cause high mortalities in a short time in the farming systems (Fajer-Ávila *et al.*, 2012). It has been reported that *A. ocellatum* not only parasitizes the fish species, but also other previously established parasites as happens with *Neobenedenia melleni* what it shows the ability of *A. ocellatum* to exploit alternative host phyla A characteristic that should be considered for the control of this parasite (Colorni, 1994).

Parasites distribution in the different parts of the body of the *S. dorsalis* showed that in the case of *B. seriolae* there is a preference for the head of the fish, followed by the body and caudal fin, while *Z. seriolae* showed a preference by the second gill arch. Sharp *et al.* (2003) record in *S. lalandi* captured in the coast of New Zealand, that *B. seriolae* prefers the sides of the body, just behind the dorsal fin. However, *Z. seriolae* showed a preference for the second gill arch. The above coincides with the reported in this work with *Z. seriolae* but differ with *B. seriolae* that prefer attachment on the fish head followed by the body zone. These results show that parasite site preferences such as *B. seriolae* can change as environmental conditions and culture conditions change. Several hypotheses suggest infesta-

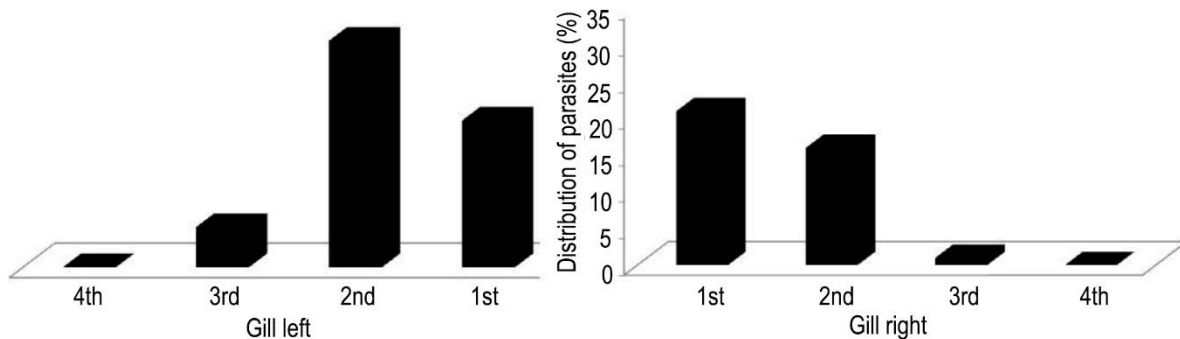


Figure 4. Distribution of *Zeuxapta seriolae* collected in the gills of juvenile *Seriola dorsalis*.

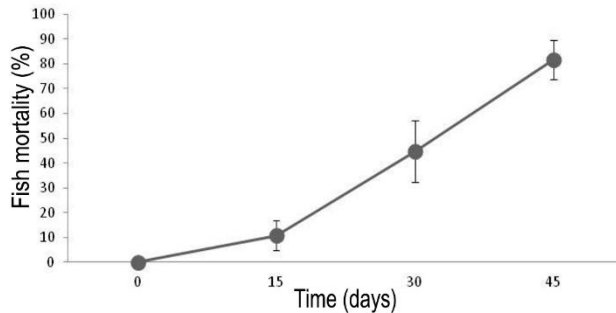


Figure 5. Accumulative mortality of juvenile *Seriola dorsalis* due to *Zeuxapta seriolae*, *Benedenia seriolae* and *Amyloodinium ocellatum*.

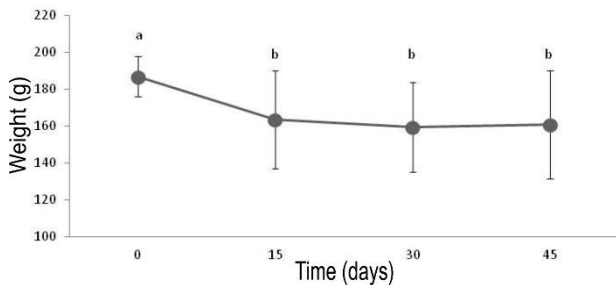


Figure 6. The average weight of juvenile *Seriola dorsalis*, after 45 days of infection by *Zeuxapta seriolae*, *Benedenia seriolae* and *Amyloodinium ocellatum*. Values represent means and standard errors, resulting from Kruskal-Wallis One Way Analysis of Variance on Ranks. Common letter labels are not significantly different ($P > 0.05$).

tion preferences where a parasite as *B. seriolae* prefers the sites where its development phase occurs. Young parasites prefer different sites to adults since a spatial separation can offer different sources of food and in some way reduce some intraspecific competition (Whittington & Ernst, 2002). Alternatively, due to the morphological change suffered by the parasites from young to adult, with a change towards a more flattened shape as it matures, a change that offers a lower resistance to water.

Similarly, the anterior hamuli continue to develop, allowing the parasite to attach more efficiently to the fish epidermis (Whittington & Ernst, 2002). Another hypothesis arises towards the susceptibility of each organism to be parasitized, which could have an immunological basis (Whittington & Ernst, 2002). In the case of gill parasites such as *Z. seriolae*, coupled with those mentioned above, could be due to physical and chemical factors such as water flow and oxygen levels (Sharp *et al.*, 2003).

In this work with *S. dorsalis* was possible to induce mixed parasitism caused by two species of monogeneans (*Z. seriolae* and *B. seriolae*) and one protozoan

(*A. ocellatum*) that occurred naturally in the system. The preceding demonstrates the high susceptibility of this fish to be simultaneously infected by these three parasites. That can represent a problem in Mexico as its cultivation intensifies and will be necessary to develop and carry out various prophylactic and control strategies to reduce the impacts that these parasite species may cause in *S. dorsalis*.

ACKNOWLEDGMENTS

Our thanks to Dr. Alejandro Cabello Pasini for reviewing the manuscript and to Director Roberto Flores Aguilar of Ocean Baja Labs for donating the fish used in this study. The authors thank the reviewers for their comments to improve the manuscript. This project was financed by PRODEP UABC-PTC-560.

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Received: 16 April 2018; Accepted: 29 October 2018