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Research Article

Effective control and treatment of *Rhabdosynochus viridisi* (Monogenea: Diplectanidae) in *Centropomus viridis* (Teleostei: Centropomidae) in marine aquaculture

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ABSTRACT. The prevalence of infection by the monogenean *Rhabdosynochus viridisi* in the Pacific white snook (*Centropomus viridis*) is a significant threat to the species' long-term viability in aquaculture. This study assessed the efficacy of five treatments: freshwater (FW), formalin (FOR), dermo-gard® AQUA (DG), praziquantel + fenbendazole + pyrantel pamoate (PZQ), and albendazole (ALB). Two experiments were conducted *in vitro*: 1) a detached-parasite assay, where individual adult parasites were exposed directly to chemical treatments, and 2) an attached-parasite assay, where tests were carried out with parasites on the gills of fish maintained under controlled conditions. In the first experiment, PZQ treatment resulted in 100% parasite mortality within 15 min of exposure. The DG, FOR, and ALB treatments exhibited promising results when administered 3 h after the initial exposure. The results of the attached parasite assay indicated that the ALB, DG, FOR and PZQ treatments dislodged and eventually killed 100% of the parasites on the gills after 1 h. In contrast, the PZQ and FW treatments did not dislodge parasites from the gills. Thus, when considering the efficacy of treatments under fish-farm conditions, it is essential to evaluate their effectiveness and implied economic cost when applied to farmed fish stocks.

Keywords: *Rhabdosynochus viridisi*; *Centropomus viridis*; monogenean; ectoparasite; treatments; Sinaloa; Mexico

INTRODUCTION

Substantial developments have been made in industrial marine aquaculture (Davies et al. 2019) in the last decade, including shrimp (Chang et al. 2020), oysters (Ridlon et al. 2021), and fish aquaculture. Advancements in fish aquaculture have included tuna (de la Gándara et al. 2016, Gürses et al. 2019), salmon (Moe-Føre et al. 2022), and snook aquaculture (Giovanni et al. 2022).

Marine aquaculture involves different challenges from those in freshwater aquaculture (Knapp & Rubino 2016), and one of the most important ones is the lack of proper husbandry methods and health protocols. One of the reasons is that the cultured organisms are usually directly exposed to the environment (i.e. the open sea or coastal lagoons) (Lester et al. 2022), exposing them to parasite infections, which occur worldwide in organisms cultured in seawater, resulting in mortality

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and significant economic losses (Mohamad et al. 2019, Boerlage et al. 2020). Some parasite species represent potential risks to aquaculture practice. For instance, in Mexico, Grano-Maldonado et al. (2010) showed that the bullseye puffer, *Sphoeroides annulatus*, is affected by the monogenean *Heterobothrium ecuadori* Meserve, 1938, which compromises the production of this species under aquaculture conditions.

The Pacific white snook, *Centropomus viridis*, is a tropical species commonly found in different aquatic environments with large salinity ranges (Tapia-Varela et al. 2020). *C. viridis* exhibits the most widespread distribution range among species of the genus along the Pacific coast. Its range extends southwards from Asuncion Island, Baja California Sur, and the Gulf of California (including Guaymas, Sonora, and Conception Bay) in Baja California, Mexico, to Paita, Peru, and the Galapagos Islands (Rivas 1986). Centropomids have a tolerated salinity range of 28-35 and a temperature range of 26-28°C (Álvarez-Lajonchere & Tsusuki 2008).

Two species of *Centropomus* have commercial importance: *C. viridis* and *C. nigrescens* (Martínez-Brown et al. 2021). Both species have significant commercial value for fisheries in Mexico. In 2021, production reached 8,355 t, the 13th highest (CONAPESCA 2021). Additionally, the *C. viridis* has high market value and demand for consumption. This species has potential for aquaculture (Martínez-Brown et al. 2021), and several publications have outlined protocols to standardize and optimize its culture in seawater (Hernández-López et al. 2021, Giovanni et al. 2022).

However, *C. viridis* is susceptible to infections by bacteria such as *Vibrio* sp., which cause histopathological damage, a specific immune response, and death (Soto-Rodriguez et al. 2019, Lozano-Olvera et al. 2020). Recently, López-Moreno et al. (2024), assessed the histological and immunological changes of the monogenean *Rhabdosynochus viridisi* on the gills of *C. viridis*. Monogeneans are mostly parasites of fish and have a direct life cycle (Bakke et al. 2007). They mainly parasitize the gills, skin, and nostrils of fish. They have a flat, elongated body with a haptor organ with sclerotized structures resembling hooks. The haptor allows the parasite to attach to the host's gills (Trujillo-González et al. 2018) and causes severe damage, especially in aquacultured fish (Grano-Maldonado et al. 2018a).

The Diplectanidae family of monogeneans can be found on the gills of freshwater and marine fish (Chaabane et al. 2016). They can reach up to 1 mm in length, and the larval stage uses cilia to swim and reach the fish hosts (Alghamdi et al. 2023). Several species of diplectanids have been reported on fish along the Pacific coast of Mexico, including *Centropomus* spp. For example, *Cornutohaptor nigrescensi* has been reported on the gills of the black snook (*Centropomus nigrescens*) (Mendoza-Franco et al. 2006); *Rhabdosynochus alterinstitus* has been reported on *C. nigrescens*; *Rhabdosynochus lituparvus*, *R. volucris* and *R. siliquaus* have been reported on *Centropomus robalito* (Mendoza-Franco et al. 2008) and *R. viridisi* is a specific parasite of *C. viridis* (Montero-Rodríguez et al. 2021).

Despite the economic importance and the damage caused, there is very little information on treatments to control *R. viridisi* infections of *C. viridis*. Morales-Serna et al. (2020) tested the efficacy of formalin against *Rhabdosynochus* sp. on juvenile *C. viridis* and *in vitro*.

Given the commercial importance of this fish, more effective alternatives to controlling this parasite species must be identified. Therefore, this study evaluated different commercial chemical compounds to determine the most effective treatment in a marine culture system.

MATERIALS AND METHODS

Fish and parasites

Seven juvenile specimens of *C. viridis* were obtained from an aquaculture fish farm in Mazatlán, Sinaloa (23°15'19.97"N, 106°24'43.2"W) in the eastern tropical Pacific in April and May 2024. A recent infestation of monogeneans led to high mortalities in snook broodstock (~84 ind), which allowed us to test different treatments to control the infection. According to the fish-farm manager, the fish were lethargic and presented erratic swimming and skin discoloration. Upon arrival at the fish farm, cotton threads were suspended in the aeration tube of the tank to collect monogenean eggs and to confirm the presence of the parasites according to reported protocols (Grano-Maldonado et al. 2010).

After 1 h, the threads were removed, placed in a Petri dish containing tank water, and observed under a stereomicroscope *in situ*. Observations revealed the presence of tetrahedral eggs attached to the threads, confirming the presence of the parasites. The water parameters were as follows: a dissolved oxygen level of 5.2 mg L^{-1}, 83% saturation, a temperature of 29.2°C, a salinity of 35, a conductivity of 5,083 µS cm^{-1}, a pH of 8.2, an ammonia level of 0.5 ppm, and an absence of nitrites and nitrates.

Five parasitized fish were transported in seawater in a 500-L plastic tank without any chemicals added to avoid interference with later treatments. Only ice was used to reduce the fish's metabolism (29°C) during transport to the Facultad de Ciencias del Mar facilities at the Universidad Autónoma de Sinaloa in Mazatlán, Mexico. Upon arrival, the fish were acclimated and placed in a 250-L circular plastic tank with a supply of filtered seawater (50 µm) with constant aeration. After 2 days, the fish were fed a commercial diet, fresh filleted fish, and live shrimp.

Chemical treatments

Five different treatments were employed. The first was formalin (37% formaldehyde; JT Baker®), and the second was dermo-gard® AQUA (200 g bag^{-1}; AVIMEX). Each 100 g of dermo-gard® AQUA contains 56.90 ± 5 g of ethylenediamine dihydroiodide (EDDI). The third treatment was Alben Max 10® (Mederi Lab), which contains praziquantel (50 mg), fenbendazole (150 mg), and pyrantel pamoate (150 mg L^{-1}). Before the administration, the tablets were pulverized using a mortar and pestle. The fourth treatment was a liquid emulsion of albendazole (2 g albendazole 100 mL^{-1}; Alpharma), and the fifth was freshwater.

Detached-parasite test

Two fish were separated from the initial group and kept in a 75-L tank with constant aeration. To obtain blood samples, the fish were sedated with 0.01 M 2-phenoxyethanol (Grano-Maldonado et al. 2018b). Using a heparinized syringe, 500 µL of blood was withdrawn by caudal puncture and stored in a microtainer at 4°C for analysis. Blood biometry was conducted using fluorescence flow cytometry. The fish were sacrificed by deep anesthesia with 0.01 M (2-phenoxyethanol-MERCK-Germany) by following procedures approved by NOM 033 DOF (2024), which did not cause alterations to the parasites (Grano-Maldonado & Palaiokostas 2015). The gills were removed, placed in 10×1 cm Petri dishes with 10 mL of filtered seawater (35 of salinity), and observed for adult *R. viridisi* under a stereomicroscope (LEICA MZ9.5).

For histopathological studies, two gill arches with adult parasites were separated and fixed in 10% formalin. Eighty-seven parasites were separated from the gills with dissecting needles, and 18 ind were distributed to five Petri dishes with fresh water (salinity at 0) and monitored every 15 min for 3 h. Monitoring was then carried out 12 and 24 h later according to reported protocols (Grano-Maldonado et al. 2015, Montero-Rodríguez et al. 2021). The recovered parasites were relaxed with near-boiling saline and preserved in 96% ethanol. The parasites were stained with Mayer's paracarmine stain or Gömöri trichrome stain for morphological analyses and mounted on permanent slides with Canada balsam. The remaining 69 ind were tested for resistance to chemical treatments: freshwater (T1); formalin (170 mg L^{-1}) (T2); ethylenediamine dihydroiodide (0.2 g L^{-1}; dermo-gard® AQUA) (T3); Alben Max 10® praziquantel (50 mg L^{-1}) + fenbendazole (150 mg L^{-1}) + pyrantel pamoate (150 mg L^{-1}) (T4); and albendazole (20 mg L^{-1}) (T5).

Individual wells were observed at 15, 30, 60, and 180 min. Both trials included a control treatment with seawater (salinity of 35). The results of these tests were documented with microphotographs at 600 and 1,000x on a Leica ICC50HD optical microscope with a camera attached. The parasites were identified according to the literature (Mendoza-Franco et al. 2006, 2008, Montero-Rodríguez et al. 2021). The specimens were identified as *R. viridisi* based on morphological traits (haptoral and copulatory structures) (Mendoza-Franco et al. 2006, 2008, Montero-Rodríguez et al. 2021). This identification was important for determining an effective treatment aligned with industry best practices.

Attached-parasite test

For the attached parasite test, fish were placed in a tank under the same conditions as those used for the previous test. The same procedures outlined above were carried out for sedation, euthanasia, and blood collection. Gills parasitized with *R. viridisi* were separated and cut into ~5 to 10 mm fractions. Each gill fraction was placed in a Petri dish containing one of the five treatments to test the parasite's effects on the gills. Each treatment was replicated three times. A control treatment was also conducted using seawater with 35 of salinity.

Ethical procedures

All experiments followed the Mexican laws for the euthanasia of animals and scientific procedures (NOM-033-ZOO-1995).

Histological analysis

Four branchial arches were used for histopathological analyses. The samples were fixed in 10% neutralized formalin and processed as reported by Tonguthai et al. (1999). Thin sections of gill tissue (5 µm) were obtained with a rotation microtome (Leica RM2125 RT) and stained with hematoxylin-eosin-phloxin stain

(Luna 1968). The histological preparations were analyzed with an optical microscope (Olympus CX41).

Statistical analyses

The log-rank test was used to test the equality of the survival curves in the treatment groups. The probability of survival was examined using a Kaplan-Meier survival curve. Statistical analyses were conducted to evaluate the success rates (when the parasite died) and failure rates (when it remained alive). Censored data referred to instances where the parasite was lost during the experiment, died from causes unrelated to the treatment, or survived throughout the entire experiment. In this experiment, no parasites were lost or died from causes unrelated to the treatment. The data were analyzed using the statistical software R.

RESULTS

Macroscopic examination and histology

A single diplectanid monogenean species, *R. viridisi*, parasitized all fish on the gills with a prevalence of 100% (Fig. 1). The approximate number of parasites per fish was ~4,320, which were attached to the gills and caused hypoxia and tissue damage (Fig. 2). *R. viridisi* was present on the gill filaments (Figs. 2a,c-d) at the base of the secondary lamellae at different heights of the primary lamella. The gill tissues also showed other pathologies, such as hyperplasia with lamellar fusion, telangiectasia (Fig. 2b), an increased number of ionocytes (Fig. 2c), and inflammation (Fig. 2d).

Detached-parasite assay

At the beginning of the detached-parasite assay, the probability of survival was 1 (100% of the parasites were alive). The median survival time was 720 min (when survival is 50%). The probability of survival of detached parasites was 72.2% after 180 min, and 27.8% of the individuals died within the first 180 min. The probability of survival beyond 720 min was 33.3% (Fig. 3).

Monitoring parasites exposed to freshwater allowed us to reduce the influence of censored data, which could affect the interpretation of survival treatment effects. Long-term monitoring suggested that the parasites may die in the absence of a host, as observed in the initial monitoring, where individuals exhibited an overall decline after 12 h (Fig. 3). Therefore, treatments were determined to last up to 180 min. A significant difference in overall survival was observed between groups ($P < 0.05$). The treatments with dermo-gard® AQUA, albendazole, formalin, and freshwater showed significantly different results from those of the control group (Fig. 4), and praziquantel treatment showed the most significant differences. Physical tissue damage to parasites was evident due to the treatments (Fig. 5).

Figure 1. Specimen of *Rhabdosynochus viridisi* (Monogenea: Diplectanidae) from the gills of *Centropomus viridis.* Scale bar: 200 µm.

Attached-parasite assay

For parasites attached to the gills, the median survival time after treatments was 60 min (Fig. 6). The overall mean detachment rate at 30 min was 72.2%, and complete detachment occurred at 60 min for the dermo-gard® AQUA and formalin treatments (95% confidence interval CI). Albendazole demonstrated enhanced efficacy, inducing gill detachment and parasite death at 30 min. However, the praziquantel, freshwater, and control treatments exhibited no discernible impact on parasite attachment or mortality during the 60-min observation period (95% CI).

Hematological parameters

The presence of the parasite compromised the fish's blood chemistry, as evidenced by the differences observed between healthy and sick individuals in red blood cell count, total hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet count, white cell count, and neutrophil percentage (Table 1).

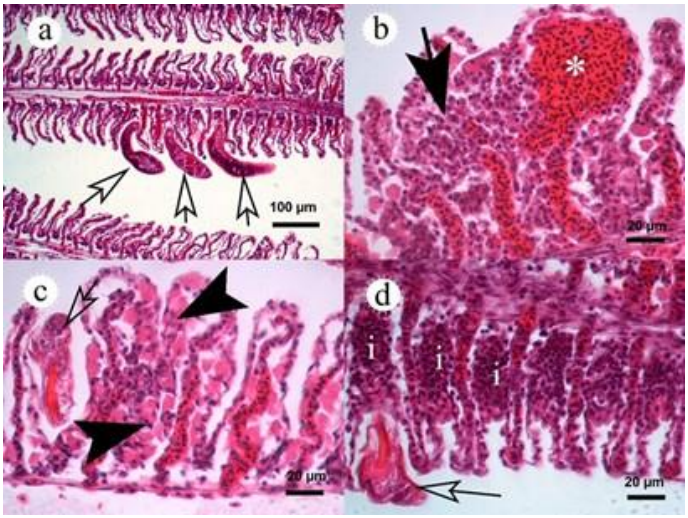

Figure 2. Photomicrograph of gill filaments of *Centropomus viridis* from a fish farm in Mazatlan, Mexico. a) *Rhabdosynochus viridisi* fixed between the secondary gill filaments (empty arrows); b) detail of the gill section showing hyperplasia (arrow) and telangiectasia (asterisk); c) presence of ionocytes (arrowhead) between simple plane epithelium and the pilar cells of the secondary filaments; d) inflammation between secondary filaments (i = inflammation). Haematoxylin eosin-phloxin stain.

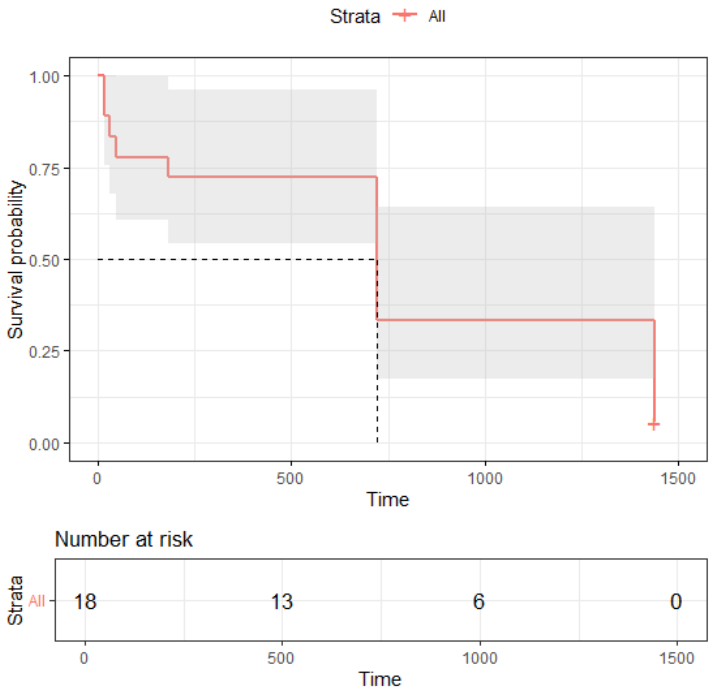

Figure 3. *Rhabdosynochus viridisi* exposed to freshwater (detached parasites). The red line represents the Kaplan-Meier survival curve. The time point of 1,440 min is denoted by (+) on the survival curve, indicating a censored observation. Time is displayed in minutes.

DISCUSSION

Microscopic examinations of *C. viridis* specimens revealed infection by *R. viridisi*. The haptor has tegumental scales, and the parasite features a copulatory complex, a broad cephalic margin, four eyes, and a sub-spherical pharynx exhibited similar dimensions and morphology to those in previous reports

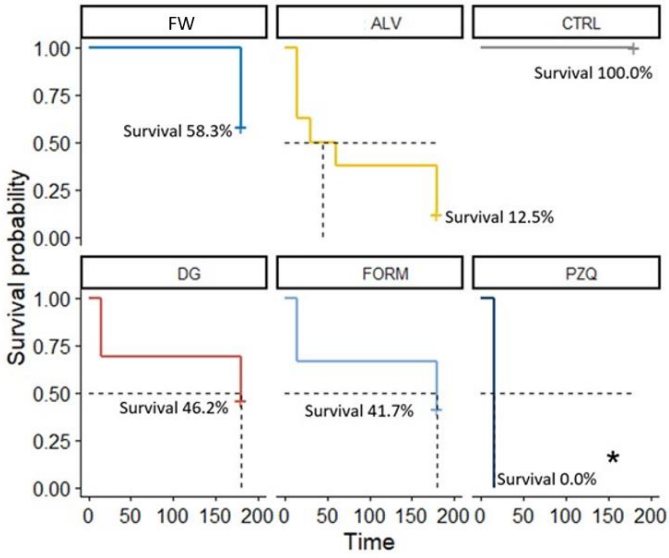

Figure 4. Kaplan-Meier curve represented the survival of parasites like *Rhabdosynochus viridisi* to chemical treatments (detached parasites). FW: fresh water; ALB: albendazole; CTRL: control; DG: Dermo-gard® AQUA; FORM: formalin; PZQ: praziquantel + fenbendazole + pyrantel pamoate. Significant statistical differences are indicated by an asterisk next to the percentage of the survival rate. Time is displayed in minutes.

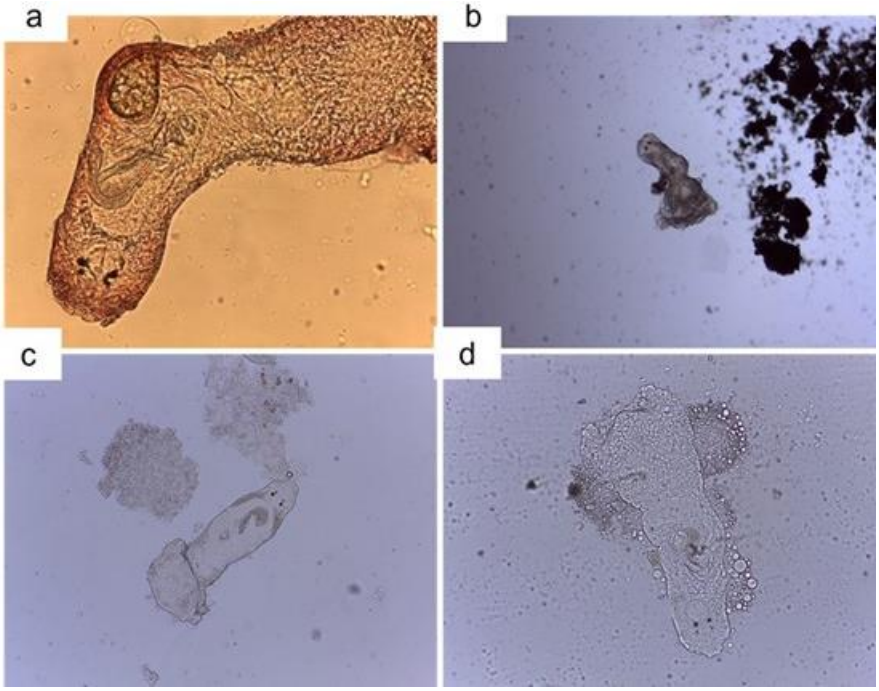

Figure 5. Microphotography of the physical damages at 30 min chemical treatment exposures in the parasite *Rhabdosynochus viridisi*. a) Dermo-gard® AQUA (100x); b) albendazole (10x); c) formalin (40x); d) praziquantel + fenbendazole + pyrantel pamoate (40x).

(Mendoza-Franco et al. 2006, 2008, Montero-Rodríguez et al. 2021) (Fig. 1). After treatments, there were distortions in the parasite morphology. Notable alterations in the parasite's overall structure, including losing its defining shape and internal tissue organization are shown (Fig. 5). The presence of vacuoles in the cytoplasm suggests that cellular processes such as digestion and metabolism were al-

Table 1. Blood parameters of immunocompromised infected by *Rhabdosynochus viridisi vs.* non-infected white snook *Centropomus viridis* juveniles.

Hematological parameter	Parasitized	Non parasitized
Red blood cells	1.44×10^6 µL^{-1}	2.69×10^6 µL^{-1}
Total hemoglobin	3.8 g dL^{-1}	8.0 g dL^{-1}
Hematocrit	15.60%	51.80%
Mean corpuscular volume	108.6 fL	192.6 fL
Mean corpuscular hemoglobin	26.4 pg	29.7 pg
Mean corpuscular hemoglobin concentration	24.4 g dL^{-1}	15.4 g dL^{-1}
Red blood cell distribution width	15.40%	10.90%
Platelets	172×10^3 µL^{-1}	12×10^3 µL^{-1}
Mean platelet volume	9.9µm^3	11.0 µm^3
White blood cells	21×10^3 µL^{-1}	16.2×10^3 µL^{-1}
Neutrophils	3%	0.40%
Lymphocytes	81%	93.50%
Monocytes	13%	0.10%
Eosinophils	3%	0.10%
Basophils	0%	5.80%

tered. It can be inferred that some parasites had burst because of massive lysis of the cells, which is likely attributable to the action of the treatments (Fig. 5d). According to Chan et al. (2013), anthelmintics disrupt Ca_2+ homeostasis, cause muscle immobility, are effective against parasitic flatworms, and cause death (Xiao et al. 2018).

Many parasites were found in the *C. viridis* samples (~4,320 parasites), which aligns with their potential to cause significant fish mortality due to confinement and their direct life cycle. High mortality rates from the monogenean genus *Gyrodactylus* have also been observed in fish farms (Grano-Maldonado et al. 2018a, López-Ceseña et al. 2024). The presence of monogenean infections has significant implications for the health and survival of fish species in culture, and prompt and effective treatment is essential; this is particularly relevant given this fish species' potential for aquaculture and their significance in the coastal lagoon systems of the Mexican Pacific (Abdo-de la Parra et al. 2020, 2023, Giovanni et al. 2022).

The first experiment produced statistically significant outcomes and indicated a notable distinction in the efficacy of the praziquantel treatment on detached parasites compared to the other treatments. Praziquantel has been tested to control infections by different parasite species. Examples include *Benedenia seriolae* and *Zeuxapta seriolae* in yellowtail amberjack (Williams et al. 2007, Forwood et al. 2016), *Benedenia seriolae* in yellowtail kingfish (Forwood et al. 2016), *Lepidotrema bidyana* in silver perch (Forwood et al. 2013), *Heterobothrium okamotoi* in Japanese pufferfish (Hirazawa et al. 2000), *Neobenedenia melleni* in ornamental reef fish (Cardoso et al. 2019), and *Microcotyle sebastis* in rockfish (Kim & Cho 2000). Praziquantel has also been proven efficient in treating fish infections by cestodes and trematodes. This product exhibits minimal toxicity in fish and requires a 100 mg L^{-1} bath treatment or oral doses above 500 mg kg^{-1} before adverse effects are reported in most species (Bader et al. 2019). Nevertheless, despite the evidence, it was unexpected that praziquantel demonstrated no effect in gill-attached parasites in the present study, having only affected detached parasites.

Dermo-gard® AQUA, albendazole, and formalin demonstrated encouraging outcomes concerning mortality and monogenean gill detachment. These treatments exhibited notable distinctions in comparison to the control group. In all cases, the probability of parasite survival exceeded 50% with a median survival time of 180 min. In the second experiment, albendazole exhibited a more rapid effect on parasite clearance and achieved detachment at 30 min. An albendazole concentration of 500 mg L^{-1} in 24 h baths was highly effective, with a success rate of 94.9% parasite mortality in the freshwater fish *Colossoma macropomum* (Baia et al. 2024).

In the second experiment with dermo-gard® AQUA and formalin, the median overall survival time was 60 min, and 72.2% parasite detachment was observed at 30 min with all treatments. Praziquantel exhibited the most effective outcome in the first experiment, but not the

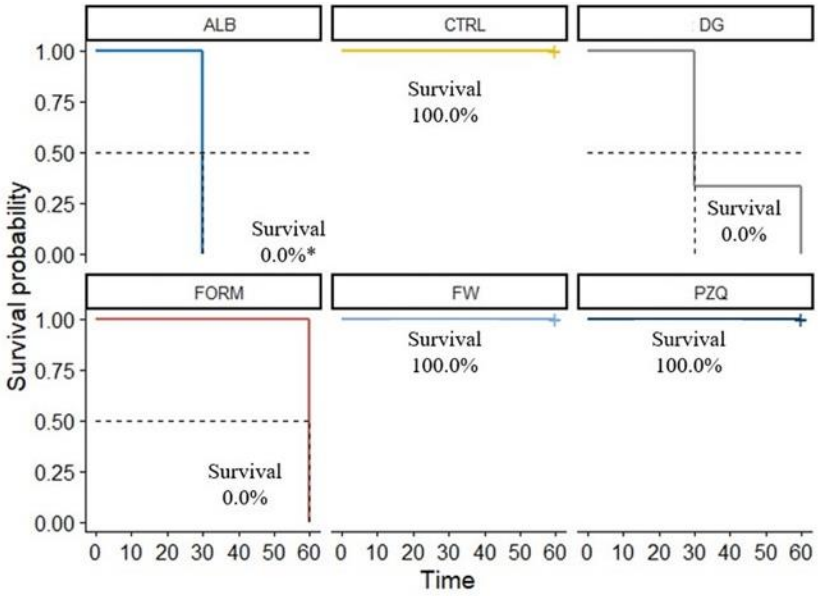

Figure 6. Kaplan-Meier curve represented survival of parasites like *Rhabdosynochus viridisi* to chemical treatments (gill-attached parasites). FW: fresh water; ALB: albendazole; CTRL: control; DG: dermo-gard® AQUA; FORM: formalin; PZQ: praziquantel + fenbendazole + pyrantel pamoate. Significant statistical differences are indicated by an asterisk next to the percentage of the survival rate. Time is displayed in minutes.

most effective in the second. Morales-Serna et al. (2020) used formalin concentrations of 110 and 218 mg L^{-1} for 1 h on *R. viridisi*, and their results showed that 110 mg L^{-1} was ineffective, but 218 mg L^{-1} led to high parasite mortality. In our study, the formalin concentration was 170 mg L^{-1} (an average concentration used in a previous study), effective after 3 h of treatment.

It is important to consider additional factors when evaluating farm conditions. Freshwater is a more cost-effective and readily available option in fish farming. Our findings indicate that freshwater is as efficacious as dermo-gard® AQUA, albendazole, and formalin. Based on these results, we recommend the prolonged use of freshwater as the most cost-effective alternative treatment option, even though the most efficacious treatment for eradicating parasites was praziquantel, fenbendazole, and pyrantel pamoate for 15 min (50, 150, and 150 mg, respectively). Nevertheless, it is essential to emphasize that this treatment is prohibitively expensive in bath formulations but may be viable in oral feed formulations.

Furthermore, despite the efficacy shown by albendazole, it is similarly limited by economic considerations in farm settings and has the same cost challenges as praziquantel. It is essential to acknowledge that large-scale aquaculture operations require significant volumes of water (approximately 384 m³ in this instance) and could potentially present a substantial economic challenge.

We tested dermo-gard® AQUA because the active ingredient, EDDI, is generally recognized as a safe additive by the United States Food and Drug Administration (FDA 2019), and a commercial line has been designed explicitly for aquaculture. This pharmaceutical product acts as a skin protector, and skin protectors have been reported to be successful as expectorant treatments (Van Van et al. 2021, López-Ceseña et al. 2024). It promotes the production of skin and gill mucus, thereby regenerating the fish's natural mucus barrier.

In our study, the use of Dermo-gard® AQUA for 1 h resulted in more parasites being released from the gills than the other treatments which suggests that dermo-gard® AQUA (US$44, 200 g) may be an appropriate alternative to Alben Max 10® (Mederi Lab), which contains praziquantel (50 mg), fenbendazole (150 mg), and pyrantel pamoate (150 mg L^{-1}) (US$30, 60 g). Similar studies have shown that comparable commercial veterinary products, such as Drontal® Plus, exhibit the same antiparasitic results (Grano-Maldonado et al. 2010, 2011).

Van Van et al. (2021) used Dermo-gard® AQUA in carp feed at a concentration of 0.2-0.4 g kg^{-1} for 7 days, which achieved better outcomes than immersion baths.

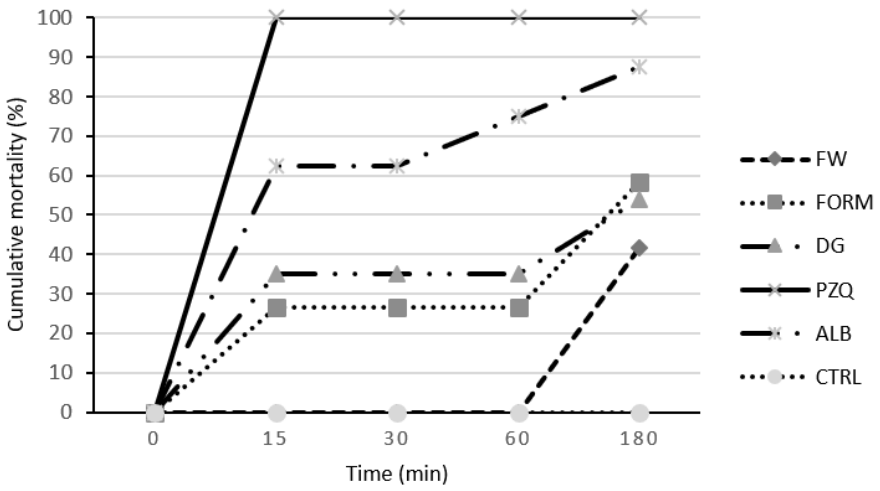

Figure 7. Cumulative mortality of parasites *Rhabdosynochus viridisi* to chemical treatments (detached parasites). FW: fresh water; ALB: albendazole; CTRL: control; DG: Dermo-gard® AQUA; FORM: formalin; PZQ: praziquantel + fenbendazole + pyrantel pamoate.

The efficacy of dermo-gard® AQUA was evaluated in bath treatments for tilapia infected with two parasites: *Cichlidogyrus* sp. on the gills and *Gyrodactylus* sp. on the skin (López-Ceseña et al. 2024): and also to control *Argulus* sp. (Crustacea) in the Pacific fat sleeper *Dormitator latifrons* (Eleotridae) in México (López-Ceseña et al. 2025). The most effective treatment time using 0.1 g L^{-1} was 6 h, which resulted in a statistically significant reduction in parasite load of up to 89% ($P < 0.05$). In our study, we employed 0.2 g L^{-1} on the detached parasites, with which the most effective treatment time was 3 h (53.8% mortality) (Fig. 7). In contrast, the most effective time for attached parasites on the gills was 1 h (100% mortality).

A pronounced decline was observed in several hematological parameters of interest (Table 1). The discrepancy between the red and white cell values in infected and uninfected *C. viridis* is noteworthy. The number of hematological studies conducted on *C. viridis* is limited. The reference value for this species indicates that the average hematocrit level is 49.56% (Abdo-de la Parra et al. 2023). For this study, the hematocrit of a parasitized fish was less than the average value (15.6%) and less than the reference percentage determined for this study (51.8%).

Similar findings were obtained with the parasite *Heterobothrium okamotoi*, which infects the gills of the tiger puffer, causing inflammation and tissue damage to the fish. This damage hinders the fish's ability to breathe and leads to suffocation and death (Ogawa 1996). Additionally, these parasites feed on the fish's blood and cause a decrease in red blood cells and hemoglobin, resulting in anemia (Ogawa & Inouye 1997).

When selecting appropriate treatments, economic factors such as cost and ease of application must be evaluated, in addition to considering the treatment's efficacy in mortality and parasite detachment (Buchmann 2022). Furthermore, the potential adverse effects on the fish and the surrounding environment, as well as the probability of parasite resistance development, must be assessed.

An effective control strategy could entail the combination of different treatments, the development of more sustainable alternatives, and continuous monitoring of fish health. Primarily, however, a clear understanding of the complete life cycle of the parasite is essential for combating it on several fronts, including the eggs, oncomiracidia, and adults. A preventive and proactive approach must be adopted, including the implementation of biosecurity measures and the establishment of improved management practices on aquaculture farms. For example, cotton threads could help confirm the parasites' presence (Grano-Maldonado et al. 2010, 2011, 2015) and quantify the infestation, which could help standardize protocols.

Research regarding managing this centropomid fish is still in its early stages. For this reason, further studies are needed to investigate more natural treatments. Additionally, therapeutic methods that are less toxic to the environment should be considered, and formalin should be avoided.

The continuous global development of new fish farming methods has promoted the inappropriate overuse of toxic drugs to control parasites, which may trigger the development of genetic resistance to therapeutic agents that can be transmitted to new generations of parasites. This can increase the drug doses needed and, most importantly, harm the environment. Aquaculture in Mexico must be conducted sustainably. It is recommended that wild fish be subjected to quarantine before their incorporation into the culture system to prevent the introduction of monogeneans into facilities.

Credit author contribution

L.E. Enriquez-Benavides: conceptualization, validation, methodology, fish husbandry, formal analysis, data curation, writing-original draft; J.A.G. López-Ceseña: methodology, writing and review; G.A. Rodríguez-Montes de Oca: funding acquisition, review and editing; S.M. Abad-Rosales: methodology, review and editing; D.A. Maciel-Ibarra: methodology; E.A. Rodríguez-Vázquez: methodology and fish husbandry; Z. Ibarra-Zatarain: funding acquisition, review and editing; M.I. Grano-Maldonado: funding acquisition, project administration, supervision, writing-conceptualization, review and editing. All authors have read and accepted the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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