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Research Article

Effect of salinity and chemical treatments against egg and oncomiracidia larvae development of *Rhabdosynochus viridisi* in *Centropomus viridis* (Teleostei: Centropomidae)

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ABSTRACT. This study evaluated the effects of salinity and chemotherapeutic treatments on the embryonic development and hatching of *Rhabdosynochus viridisi*, a diplectanid monogenean ectoparasite of the Pacific white snook (*Centropomus viridis*). Egg morphology, oncomiracidial features, and larval development were also described. Naturally infected fish were collected during a parasitic outbreak and maintained in 500 L seawater tanks. Eggs were obtained using cotton threads and incubated to obtain oncomiracidia. Incubation time was 96 h at 35 ppt and 23°C, and larval longevity ranged from 96 to 120 h at 23°C. Three chemotherapeutic agents-Dermo-gard® AQUA (0.2 g L^{-1}), formalin (170 mg L^{-1}), and albendazole (20 mg L^{-1})-and six salinity levels (0, 7, 14, 21, 28, and 35 ppt) were tested. Treatments were applied to eggs and oncomiracidia in triplicate at 23°C. Formalin (170 mg L^{-1}) significantly inhibited embryonic development ($P < 0.001$) and eliminated oncomiracidia within 1 h ($P < 0.0002$). However, no development or survival occurred at 0 of salinity ($P < 0.000001$), indicating that freshwater exposure is more effective than chemical treatments in larvae after 15 min. These results provide baseline biological information for *R. viridisi* and support the use of low salinity as an effective strategy for controlling monogenean infections in aquaculture.

Keywords: *Centropomus viridis*; monogenean ectoparasites; *Rhabdosynochus viridisi* control; aquaculture

INTRODUCTION

Fish naturally harbor diverse parasite assemblages that rarely pose significant threats under wild conditions. In contrast, aquaculture environments characterized by confinement, high stocking densities, and suboptimal water quality promote the proliferation of protozoan and metazoan parasites, often resulting in disease outbreaks and mass mortality events (Bellay et al. 2015, Grano-Maldonado et al. 2018, Christaki 2020, Fahmy et al. 2022, Jeronimo et al. 2022).

Over the past decade, industrial marine aquaculture has undergone significant expansion, including notable advances in snook (*Centropomus* spp.) culture (Giovanni et al. 2022). In Mexico, two species are of particular commercial importance: *C. nigrescens* and *C. viridis*, the latter distinguished by its high market value and strong consumer demand (Martínez-Brown et al. 2021). The Pacific white snook, *C. viridis*, exhibits the widest distribution among congeners along the Pacific coast, inhabiting diverse aquatic environments characterized by salinities of 28-35 ppt and temperatures ranging from 26 to 28°C (Tapia-Varela et al. 2020).

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In addition to bacterial pathogens (Soto-Rodríguez 2025), monogenean parasites represent a critical limitation to aquaculture production due to their direct life cycle, rapid transmission, and pathogenic effects on cultured fish. Grano-Maldonado et al. (2015) demonstrated that the bullseye puffer, *Sphoeroides annulatus*, is highly susceptible to infection by the monogenean *Heterobothrium ecuadori*, leading to severe impacts on fish health and aquaculture performance. Similarly, Enríquez-Benavides et al. (2025) reported that *C. viridis* is highly vulnerable to infection by the gill monogenean *Rhabdosynochus viridisi*, which causes significant gill damage and elevated mortality in broodstock under culture conditions.

Monogeneans are ectoparasitic flatworms that primarily infect the gills, skin, and nasal cavities of fish. They possess a dorsoventrally flattened body and a specialized posterior attachment organ, the haptor, armed with sclerotized hook-like structures that enable firm attachment to host tissues (Trujillo-González et al. 2018). This attachment mechanism often leads to severe tissue damage, particularly under intensive aquaculture conditions (Grano-Maldonado et al. 2018). These infections are widespread in seawater aquaculture worldwide and are associated with significant mortality, economic losses, and emerging risks for the sustainability of aquaculture systems (Boerlage et al. 2020, Vanhove et al. 2024).

Monogeneans exhibit two main reproductive strategies: oviparity and viviparity. Viviparous monogeneans (e.g. gyrodactylids) are characterized by direct transmission, giving birth to fully developed juveniles that can immediately infect the same or nearby hosts, often leading to rapid population expansion under aquaculture conditions (Cable et al. 2002, Bakke et al. 2007). In contrast, oviparous monogeneans produce eggs that develop externally, and their life cycle comprises three stages: egg, free-swimming larva (oncomiracidium), and adult. Eggs are typically attached to substrates, whereas oncomiracidia disperse through the water column in search of a suitable host, to which they attach and subsequently develop into adults (Drago & Núñez 2017). Oviparous species are generally highly fecund, which complicates their control once infections are established (Dinh-Hoai & Hutson 2014, Chong 2022). In recent years, a wide range of chemical and natural treatments have been evaluated for monogenean control (Liu et al. 2021, López-Ceseña et al. 2024). Additionally, manipulation of environmental factors, particularly salinity, has proven effective because of the parasites' limited osmoregulatory capacity (Boylan et al. 2021, Tavares-Dias 2021, 2022). However, the diversity of reproductive strategies in monogeneans, including oviparity, viviparity, and self-fertilization, combined with incomplete knowledge of their life cycles, continues to delay the development of fully effective control protocols (Hutson et al. 2018).

In northwestern Mexico, *C. viridis* has emerged as a highly profitable species for aquaculture (Montoya-Ponce et al. 2024). However, under marine aquaculture facilities, *C. viridis* is susceptible to severe infestations by monogenean parasites (Enríquez-Benavides et al. 2025). Among these, *R. viridisi* has been identified as a major ectoparasite in farming systems. As a member of the Diplectanidae, this species attaches primarily to the gills, where it can induce significant histopathological damage (López-Moreno et al. 2024, Enríquez-Benavides et al. 2025). This parasite exhibits a direct life cycle involving a single host, leading to initial control efforts focused on the adult stage (Enríquez-Benavides et al. 2025). Nevertheless, recent epizootic outbreaks in marine aquaculture facilities, particularly during spring 2024, have highlighted *R. viridisi* as a critical threat to the sustainability of *C. viridis* production. These studies emphasize the need to expand control strategies beyond adults and target early developmental stages, which may exhibit greater resistance.

Currently, fundamental aspects of *R. viridisi* biology remain unknown, including egg incubation time and larval viability. This lack of baseline information limits the development of effective and sustainable management strategies. Previous studies have demonstrated that successful control of monogeneans depends on repeated chemotherapeutic treatments synchronized with the parasite's life cycle, particularly targeting the highly vulnerable oncomiracidium stage (Grano-Maldonado et al. 2015). However, information on the egg and larval phases of *R. viridisi* is null. Therefore, the present study aimed to evaluate the *in vitro* efficacy of chemotherapeutic treatments and salinity on the eggs and oncomiracidia of *R. viridisi*, as well as to determine egg incubation time and larval viability. In addition, key biological traits of diplectanid eggs and oncomiracidia are described for the first time, providing essential information to inform the development of effective control strategies in aquaculture systems.

MATERIALS AND METHODS

Fish and parasite collection

Five juveniles of *C. viridis* were obtained from a fish farm in Mazatlán, Sinaloa, Mexico (23°15'19.97"N, 106°24'43.2"W) in April 2024, during an infectious outbreak caused by the parasite *R. viridisi*, which

resulted in the mortality of 84 breeding fish. The fish were immediately transferred to one of the laboratories at the Facultad de Ciencias del Mar (FACIMAR) of the Universidad Autónoma de Sinaloa (UAS), using a 500 L plastic tank filled with seawater and ice to reduce the organisms' metabolism (29ºC). Upon arrival at the laboratory, the fish were placed in a 250 L plastic tank containing filtered seawater (50 µm) and maintained at 23 ± 1°C and a dissolved oxygen level of 5.1 ± 0.5 mg L^{-1} to initiate an acclimatization period. The fish were starved for two days, and from day 3 onward, they were fed fresh shrimp. During this acclimatization period, the fish exhibited lethargy and erratic swimming. After four days of acclimatization, cotton threads were placed in the tank aerator line to collect eggs (Grano-Maldonado et al. 2011). After 24 h, the threads were removed and transported in a Petri dish with seawater to the laboratory for observation under a stereomicroscope. Tetrahedral eggs were found, confirming the presence of monogenean adults on the gills of *C. viridis*. To confirm the presence of *R. viridisi* monogeneans, one host was euthanized in accordance with NOM-033-SAG/ZOO-2014. The gills were removed, and the adhering parasites were collected and relaxed using a near-boiling saline solution. For morphological characterization, specimens were stained with Gomori's trichrome, dehydrated through a graded ethanol series, and mounted on permanent slides using Canada balsam.

Experimental treatments

A common experimental protocol was applied for both chemical and salinity treatments, and for both eggs and oncomiracidia. Cotton threads were used for egg collection following the method of Grano-Maldonado et al. (2011), and the threads were removed after 1 h. Experimental units consisted of Petri dishes maintained at 23ºC, and experiments were conducted in triplicate.

Egg assays

Thirty eggs per treatment were randomly distributed into three Petri dishes (10 eggs per dish). Eggs were detached from the threads using insulin syringes (0.25 mL). Egg development was monitored every 24 h, and samples were photographed under an optical microscope (Leica ICC50HD) at 600× and 1000× magnification for up to 72 h, with both hatched and unhatched eggs recorded.

Oncomiracidia assays

Larvae were obtained from incubated eggs maintained at 23ºC and monitored after the initial 24 h until hatching to ensure the use of newly hatched individuals. For each treatment, 30 oncomiracidia were collected using a glass Pasteur pipette and distributed into three Petri dishes (10 ind per dish). Survival was recorded under a stereomicroscope at defined time intervals at 23°C, depending on the salinity treatment.

Chemical treatments

Three chemical treatments were evaluated against monogenean oncomiracidia: Dermo-gard® AQUA (0.2 g L^{-1}; AVIMEX; containing 56.90 ± 5 g ethylene-diamine dihydroiodide [EDDI] per 100 g of product), formalin (170 mg L^{-1}; 37% formaldehyde; JT Baker®), and albendazole (20 mg L^{-1}; 2 g 100 mL^{-1}; Alpharma), following the protocol of Enríquez-Benavides et al. (2025). Experimental units were prepared by combining 5 mL of seawater containing oncomiracidia with 5 mL of each treatment solution (1:1 dilution), yielding the final concentrations indicated above. Control groups consisted exclusively of seawater and were handled under the same experimental conditions. Oncomiracidia survival was monitored at 15-min intervals over a total experimental period of 165 min.

Salinity treatments

Six salinity treatments were established at 0, 7, 14, 21, 28, and 35 ppt (control). Solutions were prepared by diluting seawater and distilled water, and their concentrations were verified using a refractometer (Alefa ALF009), except for 0 ppt, which consisted solely of distilled water. Each Petri dish contained 10 mL of the corresponding salinity treatment. The 35 ppt treatment served as the control, representing natural seawater conditions. For oncomiracidia, survival was recorded every 15 min during the first hour, then at 2, 4, 8, 12, 14, and 24 h, and subsequently every 12 h until 96 h.

Statistical analyses

Survival analyses were conducted in R (version 4.4.3; R Core Team) using the survival and survminer packages. Kaplan-Meier survival curves were estimated using the survfit function, and differences among groups were assessed using the log-rank test (survdiff) and pairwise comparisons (pairwise_ survdiff). Survival data were defined by time-to-event and censoring status. Survival curves were generated using survminer. To assess differences in survival between NaCl treatments, Kaplan-Meier survival curves were compared in pairs using the Mantel-Cox (log-rank) test in GraphPad Prism 10.4.0 (GraphPad Software, San Diego, CA, USA).

Ethical procedures

The procedures carried out with the organisms adhered to the Mexican Official Standard NOM-062-ZOO-1999, which establishes the proper use and care of laboratory animals, covering topics ranging from facilities to feeding, and to NOM-033-SAG/ZOO-2014, which establishes the methods for humanely killing animals.

RESULTS

A single diplectanid monogenean species, *R. viridisi,* was found on the gill filaments of all fish examined in this study (Fig. 1). This monogenean produced eggs (Fig. 2) and larvae (Fig. 3), causing infection in *C. viridis*; this was achieved based on the morphological characteristics previously described by Montero-Rodríguez et al. (2021) and Enriquez-Benavides et al. (2025) for the structures of their copulatory organs and haptoral structures.

Viable eggs were ectolecithal, tetrahedral, and light brown, measuring 62.7 ± 3.5 × 55.2 ± 3.6 µm, and bearing a basal filament up to 660 ± 8.2 µm in length (Fig. 2a-c). Under standard conditions (35 ppt, 23ºC), the incubation period lasted approximately 96 h until the first swimming larvae hatched (Fig. 4-5). The oncomiracidia were dorsoventrally flattened, measuring 100.3 ± 9.2 × 40.2 ± 4.1 µm, and characterized by three ciliated regions and four ocelli (Fig 3). Larval longevity ranged from 96 to 120 h (35 ppt and 23°C). This study provides the first detailed morphological description of the oncomiracidium, offering a practical diagnostic reference for distinguishing this larval stage from other free-swimming organisms in aquaculture systems.

Chemical treatment of eggs

At 72 h, eggs treated with formalin (FORM) showed no hatching (0%), which was significantly lower than in the other treatments ($P < 0.001$), indicating the highest efficacy in inhibiting hatching. In contrast, hatching rates were 30% in albendazole-treated eggs (ALV) and 44% in Dermo-gard® (DG), the latter being the least effective treatment. At 48 h, embryonic development was evident in DG- and ALV-treated eggs, with larvae present. Ocelli and lipid reserves associated with oncomiracidium development were clearly distinguishable (Fig. 5a-b). In contrast, no larval development was observed in FORM-treated eggs, confirming its inhibitory effect on embryogenesis. These differences were statistically significant ($P < 0.001$).

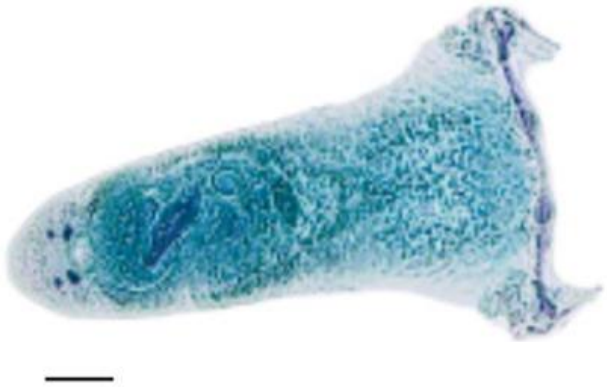

Figure 1. Juvenile *Rhabdosynochus viridisi* (Monogenea: Diplectanidae) is present in the gills of *Centropomus viridis*. Scale bar: 30 µm.

Chemical treatment of oncomiracidia

A comparative analysis of DG, FORM, and ALV treatments revealed distinct differences in larval tolerance. Kaplan-Meier survival curves showed that exposure to FORM resulted in the most rapid decline in larval survival, with a median survival time of 45 min (log-rank test, $P < 0.001$). DG exhibited an intermediate effect, whereas ALV resulted in the longest survival times, indicating a higher tolerance of oncomiracidia to this treatment. Overall, survival distributions differed significantly among treatments (log-rank test, $P < 0.05$) (Fig. 6). These results indicate that oncomiracidia are highly susceptible to formalin. At the same time, ALV shows comparatively lower efficacy, allowing prolonged larval survival.

Salinity treatment of eggs

Salinity treatments produced markedly different effects on egg hatching (Fig. 7). The 0 ppt treatment was the most effective at inhibiting hatching, with 0% hatch observed; eggs ruptured within 24 h of exposure, and this treatment differed significantly from all others ($P < 0.05$). At 7 ppt, hatching was low (20%) at 72 h, while 14 ppt showed a moderate increase (60%). In contrast, the 21, 28, and 35 ppt treatments exhibited high hatching success (84, 94, and 87%, respectively; Fig. 7), with no significant differences among them ($P > 0.05$), although they differed significantly from lower salinity treatments. In these higher salinity groups, hatching occurred earlier, beginning at approximately 48 h.

Overall, the effect of salinity on *R. viridisi* eggs was less pronounced than that observed for larvae. Kaplan-Meier survival curves indicated prolonged survival across treatments, with median survival times notably

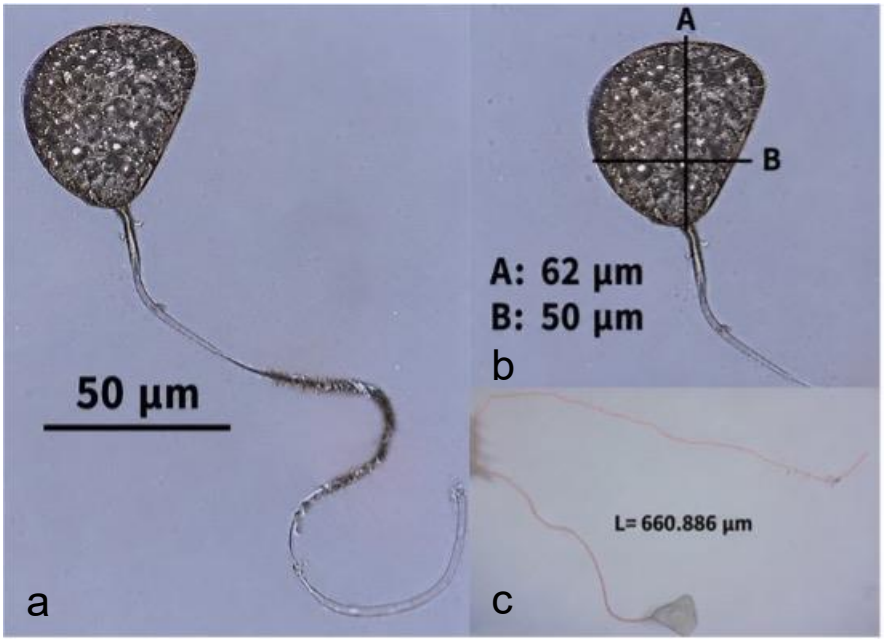

Figure 2. *R. viridisi* egg. a) Tetrahedral structure, b) egg dimensions, c) filament length (L).

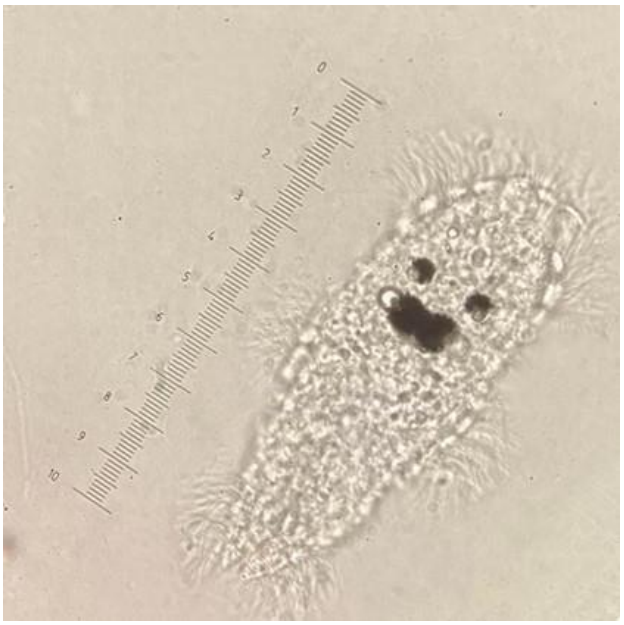

Figure 3. Oncomiracidium of *R. viridisi* newly hatched from the egg. Four eyespots can be observed, and cilia cover three sections of the body. Scale bar: 100 µm.

longer than those recorded for oncomiracidia under comparable conditions. Although some variation was observed among lower salinity treatments (0 and 7 ppt; $P < 0.001$), no significant differences were detected among higher salinities (21, 28, and 35 ppt; log-rank test, $P > 0.05$).

A photographic matrix (Fig. 8) was developed to illustrate embryonic development, hatching, and the effects of salinity treatments on eggs over time. Eggs exposed to 0 ppt were completely disrupted within 24 h, preventing embryonic development and indicating strong inhibitory effects. A similar pattern was observed at 7 ppt, where some eggs ruptured at 24 h; however, a proportion remained viable, showing embryonic development at 48 h and reaching 20% hatching at 72 h. At 14 ppt, partial egg rupture was also observed during the first 24 h, although viable embryos developed, resulting in 60% hatching. This pattern indicates that lower salinities (0-14 ppt) negatively affect egg integrity, but do not entirely prevent development at intermediate levels. In contrast, at 21 ppt, hatching began earlier, at approximately 48 h, compared with 7 and 14 ppt, where hatching was observed only at 72 h. By 72 h, the 21 ppt treatment reached an 84% hatching rate, although some eggs failed to develop, likely due to natural variability or non-viability. Overall, these observations suggest that salinity strongly influences both hatching timing and egg viability, with near-marine conditions favoring faster and more successful development at 23ºC.

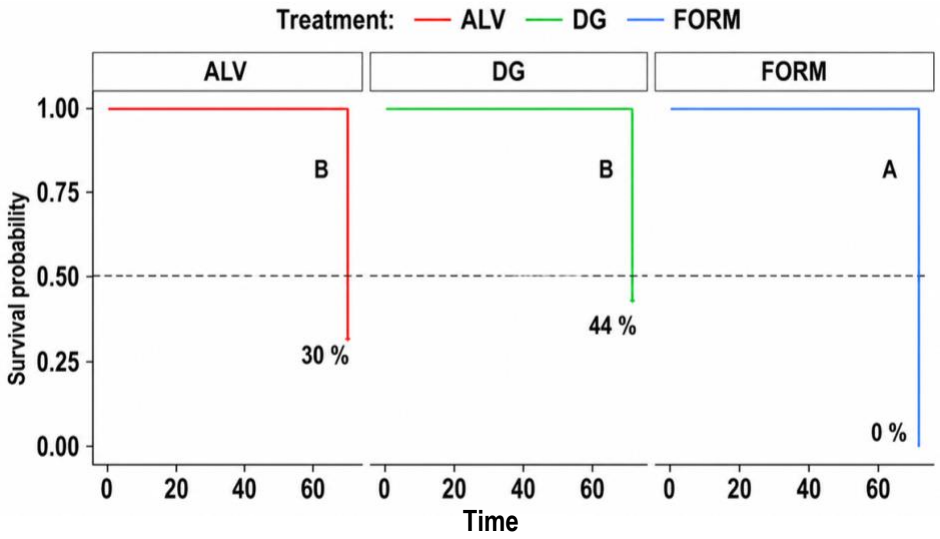

Figure 4. Kaplan-Meier curve depicting *Rhabdosynochus viridisi*'s egg survival after chemical treatments. ALV: albendazole 100 mL^{-1}, DG: Dermo-gard® AQUA 0.2 g L^{-1}, FORM: formalin 170 mg L^{-1}. Capital letters indicate significant differences between treatments ($P < 0.05$). Treatment was FORM ($P < 0.001$). Time is shown in minutes.

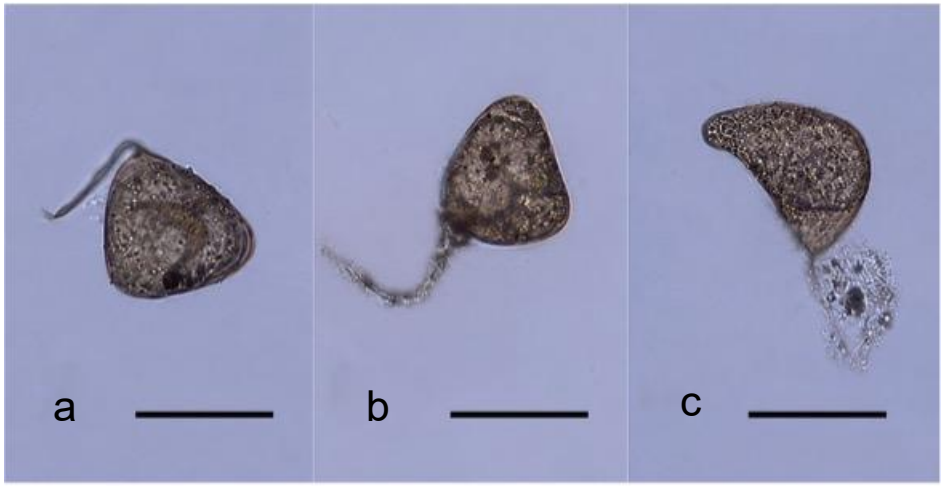

Figure 5. *R. viridisi* eggs 48 h after chemical treatment. a) Dermo-gard® AQUA 0.2 g L^{-1} treatment, b) albendazole 100 mL^{-1} treatment, c) formalin 170 mg L^{-1} treatment. Scale bar: 50 µm.

Salinity treatment of oncomiracidia

Salinity had a pronounced effect on the survival of *R. viridisi* oncomiracidia (Fig. 9). At 0 ppt, survival declined rapidly, with 50% of larvae remaining at 0.5 h and complete mortality (0%) observed by 1 h. This treatment differed significantly from all others ($P < 0.001$). A similar pattern was observed at 7 ppt, where 50% survival occurred at 0.5 h, followed by complete mortality by 8 h. Survival at 7 ppt differed significantly from that at 0 ppt (log-rank test, $P = 0.007$), indicating slightly greater tolerance at this salinity. At 14 ppt, survival also declined to 0%, although over a longer period compared to lower salinities, suggesting that intermediate salinity levels can extend larval viability. In contrast, no significant differences were detected among the 21, 28, and 35 ppt treatments ($P > 0.05$). In these groups, approximately 25% of oncomiracidia remained alive at 96 h, indicating that larvae can survive for extended periods under near-marine salinity conditions (23ºC). Overall, these results demonstrate that low salinity induces rapid mortality, whereas higher salinity levels promote prolonged survival. This

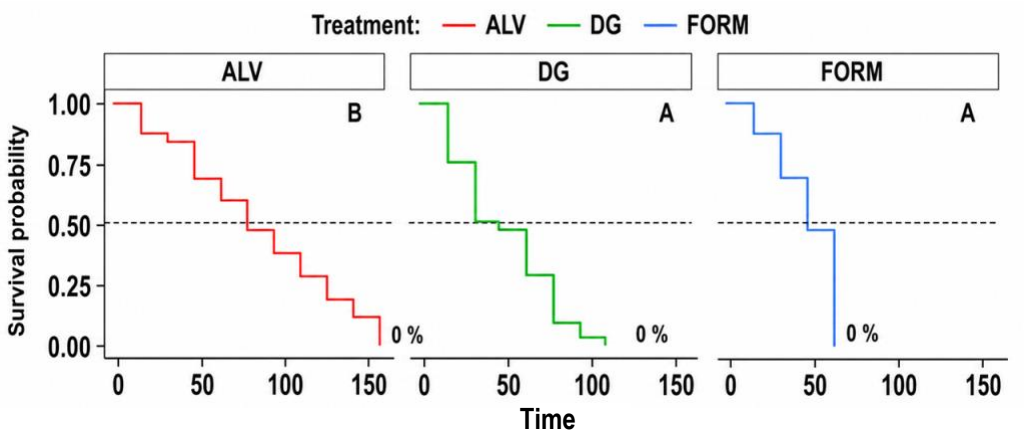

Figure 6. Kaplan-Meier curve depicting the survival of *R. viridisi's* oncomiracidia after chemical treatments. ALV: albendazole 100 mL^{-1}, DG: Dermo-gard® AQUA 0.2 g L^{-1}, FORM: formalin 170 mg L^{-1}. Time is shown in minutes. Capital letters indicate significant differences between treatments ($P < 0.05$).

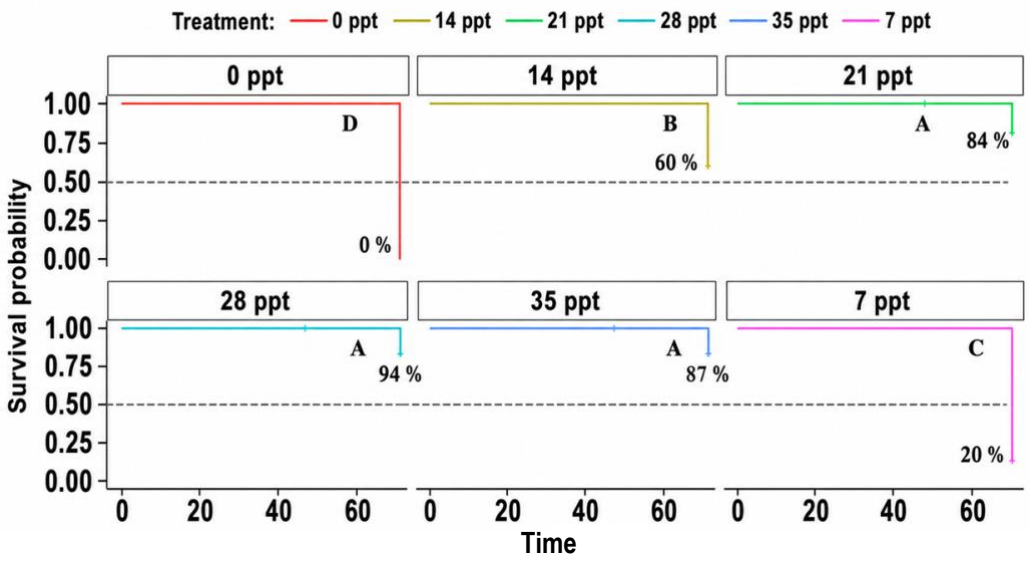

Figure 7. Kaplan-Meier curve depicting the survival of *R. viridisi* eggs after salinity treatments. Time is shown in hours. Capital letters indicate significant differences between treatments ($P < 0.05$).

pattern indicates that oncomiracidia have limited tolerance to abrupt osmotic stress. Accordingly, exposure to NaCl concentrations of 7-35 ppt significantly increased survival relative to the control (0 ppt), in which 100% mortality was observed within 72 h.

DISCUSSION

Monogenean infections pose a major constraint on the health and productivity of marine-cultured fish, emphasizing the need for rapid, effective control strategies. The acute outbreak of *R. viridisi* reported in 2024 in marine facilities culturing Pacific white snook (Enríquez-Benavides et al. 2025) underscores the urgency of understanding this parasite's early life stages and their susceptibility to control measures. In this context, chemical treatments evaluated in this study, FORM, showed the highest efficacy ($P < 0.05$), rapidly reducing both embryonic development and larval survival. In contrast, DG ($P < 0.05$) exhibited an intermediate effect, whereas ALV ($P > 0.05$) prolonged the survival of oncomiracidia, indicating lower efficacy

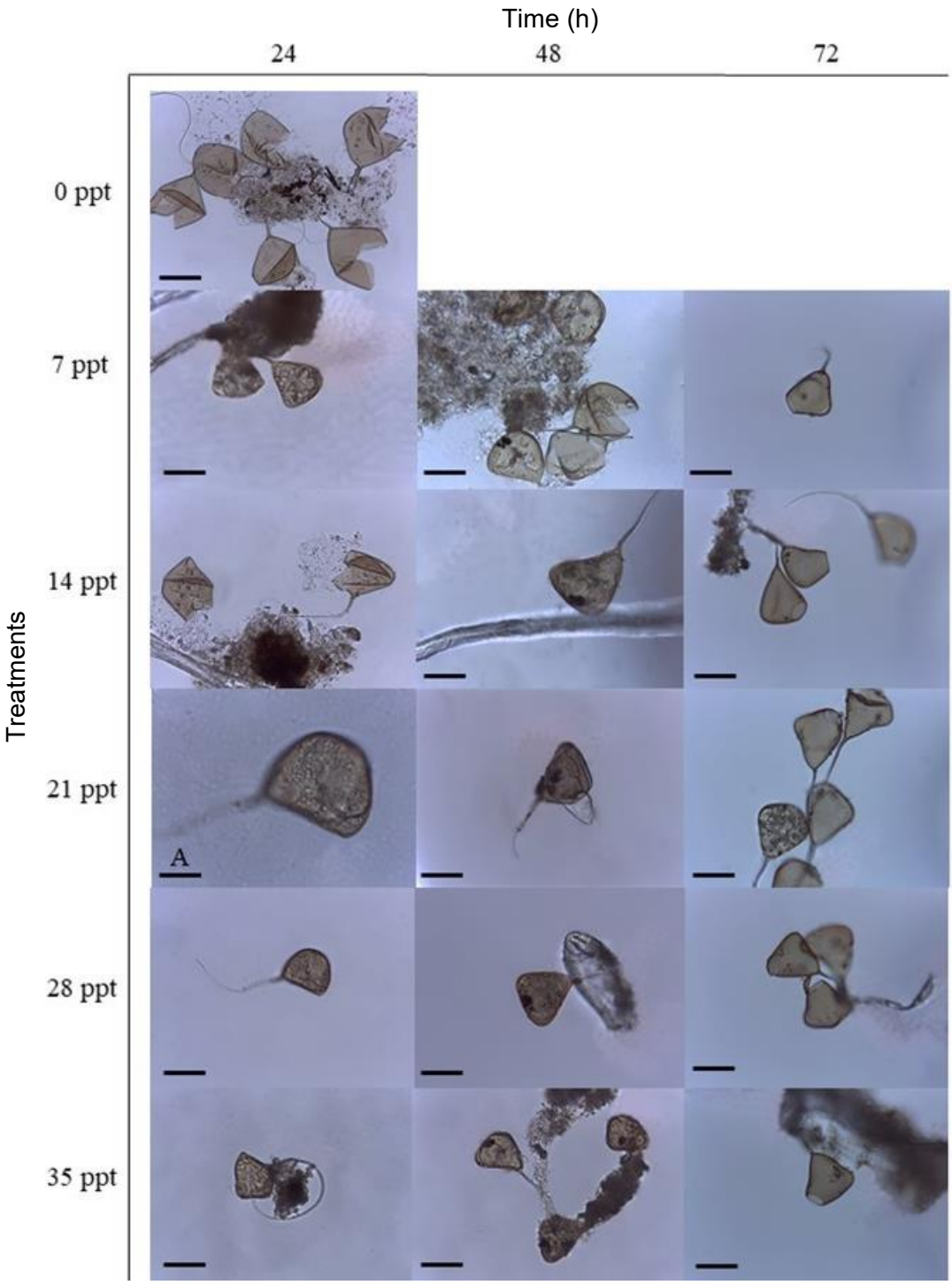

Figure 8. Photographic matrix of *R. viridisi* eggs treated at different salinities with respect to time. Scale bar: A: 20 µm, and the rest: 50 µm.

under the conditions tested. These differences highlight the importance of considering each compound's mode of action relative to the parasite's life stage.

Regarding chemical treatments, previous studies have reported variable efficacy depending on the compound, concentration, parasite species, and life stage. For

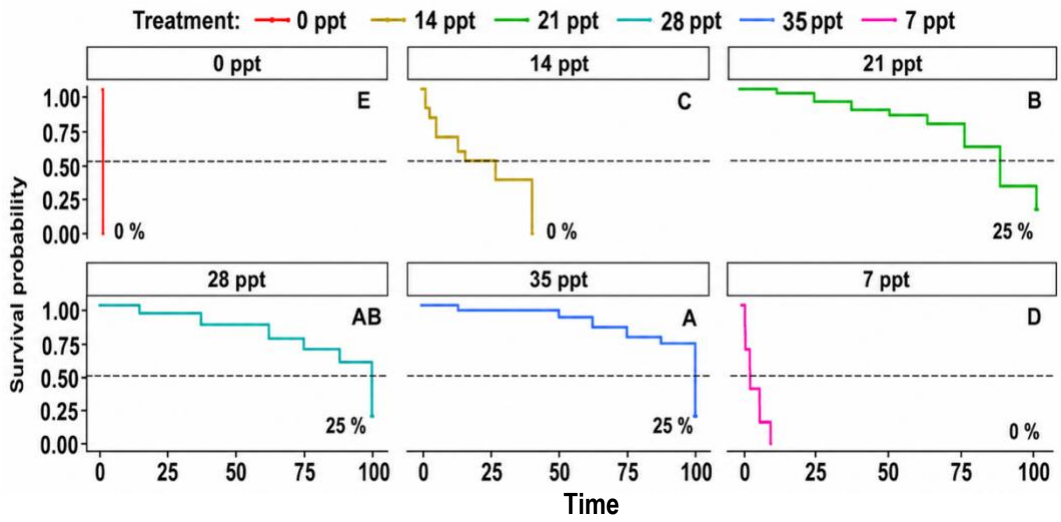

Figure 9. Kaplan-Meier curve depicting survival of *R. viridisi* oncomiracidia after salinity treatments. Albendazole. Statistically significant differences are indicated by an asterisk next to the survival percentage. Time is shown in hours.

instance, DG Aqua has been successfully used against adult monogeneans (*Gyrodactylus* sp. and *Cichlidogyrus* sp.) infecting *Oreochromis niloticus*, achieving up to 86% reduction at a lower concentration (0.1 g L^{-1}) than that used in the present study (López-Ceseña et al. 2024). Similarly, its efficacy has been evaluated against adult *R. viridisi* (Enríquez-Benavides et al. 2025), with outcomes differing from those reported here. These discrepancies highlight that the parasite's life stage strongly influences treatment effectiveness.

Beyond monogeneans, DG Aqua has also demonstrated high efficacy against crustacean parasites, such as *Argulus* sp. infecting *Dormitator latifrons*, achieving up to 90% removal at 0.2 g L^{-1} after 6 h of exposure (López-Ceseña et al. 2025). In contrast, although FORM is widely used and highly effective, its use is limited by known adverse effects on fish tissues, including damage to the gills and liver (Tavares-Dias 2021). In this context, our results emphasize the potential of salinity manipulation as a safer, cost-effective, and environmentally sustainable alternative for parasite control in aquaculture systems.

ALV exhibited the lowest efficacy, allowing longer survival of oncomiracidia, aligning with its mode of action as a benzimidazole, which mainly disrupts metabolic processes and is generally more effective against *R. viridisi* adults parasitizing the gills of *C. viridis* (Enríquez-Benavides et al. 2025) than against free-living ectoparasitic stages *(P* > 0.05). Therefore, its limited effect on *R. viridisi* larvae under the tested conditions suggests that it may not be suitable as a separate treatment to interrupt transmission in aquaculture systems.

Salinity showed a pronounced effect on egg viability and hatching success. Low salinity conditions (0-14 ppt) compromised egg integrity, often leading to rupture and reduced hatching rates, whereas near-marine conditions (21-35 ppt) promoted higher hatching success and faster development. These findings are consistent with previous studies in monogeneans, which show that exposure to freshwater or hyposaline conditions disrupts embryogenesis and reduces hatching success (Mueller et al. 1992, Grano-Maldonado et al. 2015). In contrast, oncomiracidia exhibited even higher sensitivity to osmotic stress, with rapid mortality at low salinities (0-7 ppt) and extended survival under near-marine conditions. This differential sensitivity between eggs and larvae reinforces the vulnerability of the free-swimming infective stage and its key role in transmission dynamics. This pattern highlights oncomiracidia's limited tolerance to osmotic stress and is consistent with their role as short-lived, free-swimming infective stages (Grano-Maldonado et al. 2015). Therefore, control strategies should prioritize interventions that rapidly reduce oncomiracidial survival to interrupt reinfection cycles in aquaculture systems.

The morphology and developmental patterns of *R. viridisi* eggs observed in this study are consistent with those described for related diplectanid species. However, some capsule features may be species-specific, as reported for *Diplectanum aequans* (Cecchini et al. 2010). Environmental drivers such as temperature and salinity are well known to regulate developmental rates in monogeneans, with hatching times ranging from a few days to over a week depending on conditions (Cecchini et al. 2010, Erazo-Pagador & Cruz-Lacierda 2010, Grano-Maldonado et al. 2015, Lewisch et al. 2021). This study provides novel observations on the embryonic development, hatching period, and longevity of the oncomiracidium of *R. viridisi* under experimental conditions. The egg morphology of *R. viridisi* is consistent with that reported for other species within the genus. However, certain features of the egg capsule may be species-specific, as described for *D. aequans*, a gill parasite of the sea bass *Dicentrarchus labrax* (Cecchini et al. 2010).

Previous studies have demonstrated that environmental factors, particularly temperature, play a key role in egg development and hatching dynamics in monogeneans. For example, the life cycles of *D. aequans* and *Pseudorhabdosynochus lantauensis* are strongly temperature-dependent, with eggs hatching within 6-7 days at 20 ± 2ºC and 2-6 days at 30ºC, respectively (Cecchini et al. 2010, Erazo-Pagador & Cruz-Lacierda 2010). In addition to temperature, salinity has been shown to critically influence embryonic development. Exposure to freshwater (0 ppt) or severely hyposaline conditions can inhibit hatching and lead to embryo mortality (Grano-Maldonado et al. 2015). Similarly, hatching success decreases with declining salinity and increasing exposure time, as demonstrated in *Neobenedenia melleni*, where no hatching occurred after 72-96 h of freshwater exposure (Mueller et al. 1992). Also, Maciel et al. (2017) described the influence of physical factors on egg hatching and oncomiracidia lifespan of *Dawestrema cycloancistrium*, a monogenean parasite on *Arapaima gigas*. Tubbs et al. (2005) proved the effects of temperature *in vitro*, egg hatching, and reproductive development of *Benedenia seriolae* and *Zeuxapta seriolae* (Monogenea) parasitic on yellowtail kingfish *Seriola lalandi*.

From an applied perspective, freshwater or hyposaline treatment exposure has been successfully employed as a control measure against monogenean infections in aquaculture, such as *Heterobothrium okamotoi* in *Takifugu rubripes* (Hirazawa et al. 2000), and *Heterobothrium ecuadori* in *Sphoeroides annulatus* (Grano-Maldonado et al. 2015), supporting its potential application as an environmentally based management strategy. These findings are consistent with the present study, which found that low-salinity treatments significantly reduced hatching success in *R. viridisi* ($P < 0.05$), while higher salinities favored successful development.

Treatment outcomes in aquaculture systems are shaped by multiple interacting factors, including host immune responses, water quality, stocking density, and parasite aggregation, all of which can modulate therapeutic efficacy against monogenean infections under field conditions (Buchmann & Lindenstrøm 2002, Boylan et al. 2021). Host immune competence is a key determinant of resistance to monogenean parasites. In contrast, environmental stressors such as poor water quality and high stocking densities can increase host susceptibility, facilitate parasite transmission, and intensify infection levels in cultured fish populations (López-Ceseña et al. 2024, Santos et al. 2024, Valles-Vega et al. 2024). In intensive aquaculture systems, these stress-related conditions may accelerate the proliferation and dispersion of monogeneans, given their direct life cycles and rapid reproductive capacity.

Although the osmotic regulation of *R. viridisi* egg development remains poorly understood, emerging genomic and transcriptomic resources offer a promising framework for elucidating the molecular mechanisms underlying parasite development and environmental tolerance. Previous studies have demonstrated that transmission dynamics in monogeneans are strongly linked to behavioral and physiological processes and that disruption of these pathways can significantly reduce infection success (Brooker et al. 2011). These authors noted that some chemical interventions, such as hyposaline exposure or fast-acting chemotherapeutics, may impair parasite motility and be particularly effective at limiting host colonization. In addition, conserved signaling pathways, such as G protein-coupled receptors, have been characterized in monogeneans (e.g. *Gyrodactylus bullatarudis*) and are known to regulate key processes, including locomotion and environmental sensing (Konczal et al. 2020). Complementarily, secretome analyses of *R. viridisi* have begun to reveal molecular mechanisms involved in host-parasite interactions (Mirabent-Casals et al. 2023). However, the regulatory pathways governing egg development and hatching remain largely unresolved.

These knowledge gaps, along with the current findings, highlight the need for integrated studies on the

osmotic regulation of early developmental stages and support the development of targeted, stage-specific control strategies for *R. viridisi* and other parasites in aquaculture systems.

CONCLUSION

Taken together, the bioassay results demonstrate that the developmental stage is a key determinant of susceptibility in *R. viridisi*. Oncomiracidia exhibited a strong salinity-dependent response, with rapid mortality at low salinities (0-7 ppt) and prolonged survival at higher salinities. In contrast, eggs showed greater tolerance to salinity, with less pronounced effects on hatching and survival, and in some cases, non-significant effects. These findings indicate that salinity alone may not be sufficient to fully disrupt the parasite's life cycle. Therefore, salinity-based control strategies are likely to be most effective when targeting the larval stage, which represents the most vulnerable phase. Under standard conditions (35 ppt, 23ºC), the incubation period of *R. viridisi* eggs was approximately 96 h, and larval viability extended up to 120 h. Overall, these results highlight the importance of stage-specific approaches and suggest that combining environmental and chemical treatments may improve the effectiveness of *R. viridisi* parasite control in marine aquaculture systems.

Credit author contribution

J.Á.G. López-Ceseña: conceptualization, validation, methodology, fish farming, formal analysis, data curation and drafting of the original manuscript; L.E. Enríquez-Benavides: data curation, fish farming, drafting and revision; E.A. Trillo-Hernández & M. Nieves-Soto: data curation and interpretation, drafting and revision; M.I. Grano-Maldonado: fundraising, project management, supervision, conceptualization, revision and editing. All authors have read and approved the final version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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