

Case Report

Control of *Neobenedenia* sp. Infestations in the Ocean Tank at Gran Acuario Mazatlán

Francisco Neptalí Morales-Serna ^{1,*}, Dania López-Moreno ², José Antonio Velázquez Garay ³
and Luis Antonio Rendón-Martínez ³

¹ Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Mazatlán 82040, Sinaloa, Mexico

² Facultad de Ciencias del Mar, Universidad Autónoma de Sinaloa, Mazatlán 82000, Sinaloa, Mexico

³ Gran Acuario Mazatlán, Mazatlán 82017, Sinaloa, Mexico

* Correspondence: neptali@ola.icmyl.unam.mx

Abstract: The Gran Acuario Mazatlán (GAM), Mexico's largest aquarium, is located at the entrance of the Gulf of California. In April 2023, fish in the Ocean Tank (OT) began exhibiting disease symptoms, followed by significant mortality, primarily affecting *Caranx caninus*, *Chaetodipterus zonatus*, and *Lutjanus colorado*. Parasitological examinations identified heavy infestations of *Neobenedenia* sp. (Monogenea: Capsalidae), with parasites extensively covering body surfaces and gills of some individuals. To control the outbreak, an environmental management strategy was implemented, involving a gradual reduction in water temperature (from 26 °C to 22 °C) and salinity (from 35 ppt to 26 ppt), increased cleaning frequency, and parasite egg abundance monitoring. Over time, egg abundance declined, and by July, fish behavior and feeding rates had returned to normal, with parasite-associated mortality dropping below 2%. Preventive measures, including strict quarantine protocols for new fish, have been implemented to maintain optimal conditions in the OT. This case highlights the challenges of managing parasite outbreaks in large, multi-species aquaria and underscores the importance of environmental control strategies in marine animal care.



Academic Editor: David Carmena

Received: 26 February 2025

Revised: 27 March 2025

Accepted: 31 March 2025

Published: 2 April 2025

Citation: Morales-Serna, F.N.; López-Moreno, D.; Velázquez Garay, J.A.; Rendón-Martínez, L.A. Control of *Neobenedenia* sp. Infestations in the Ocean Tank at Gran Acuario Mazatlán. *Parasitologia* **2025**, *5*, 16. <https://doi.org/10.3390/parasitologia5020016>

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Keywords: fish; health; parasite; outbreak; disease; Capsalidae; Monogenea; control; Mexico; Pacific; aquarium

1. Introduction

The Gran Acuario Mazatlán (GAM) is the largest aquarium in Mexico and one of the most important in Latin America. It opened to the public on 6 May 2023, and is located in Mazatlán City on the Mexican Pacific coast, at the entrance of the Gulf of California (also known as the Sea of Cortez), a marine ecosystem renowned for its rich biodiversity. This region is home to approximately 900 species of teleost and cartilaginous fish, over 150 species of birds, 115 species of reptiles, 36 species of mammals, 31 species of cetaceans, 5 of the world's 7 sea turtle species, and 5000 species of macroinvertebrates [1]. The GAM is dedicated to enhancing public knowledge of Sea of Cortez biodiversity through engaging exhibitions as well as educational, research, and outreach activities.

The primary attraction at GAM is its Ocean Tank (OT), which spans 427 m², reaches a depth of 7.6 m, and holds 2,583,000 L of water. The tank houses a wide diversity of fish, ranging from small pelagic species to sharks, while maintaining high water quality standards. However, in 2023, an outbreak of parasite-associated fish mortality occurred. This article describes the incident and the control measures implemented. While fish parasite

infestations are a known challenge in aquariums, published reports on their management remain scarce. By detailing this outbreak and the implemented control measures, this study aims to contribute to existing knowledge and aid aquarium professionals in improving parasite management strategies.

2. Case Report

At GAM, water quality in each tank is maintained within optimal parameters by its own dedicated life support system. However, in April 2023, a few days before GAM's official opening, fish in the OT began exhibiting disease symptoms, such as reduced appetite, cloudy eyes, erratic swimming, lethargy, loss of vertical orientation, and scale loss, followed by mortality. At that time, the OT housed 754 specimens from 19 fish species (Table 1). By 16 May, mortality had risen to 15%, primarily affecting three species: *Caranx caninus*, *Chaetodipterus zonatus*, and *Lutjanus colorado*. Additionally, complete mortality was observed in a smaller tank (24,300 L) containing 204 specimens of *Acanthurus xanthopterus*. During the outbreak, average water conditions were as follows: temperature 26 °C, salinity 35 ppt, dissolved oxygen 6.5 mg/L, pH 7.9, and nitrite 0.05 mg/L.

Table 1. Fish species present in the Ocean Tank at Gran Acuario Mazatlán during the *Neobenedenia* sp. outbreak.

Fish Species	Fish Family	Common Name	Number of Individuals
<i>Diodon hystrix</i>	Diodontidae	Spot-fin porcupine fish	3
<i>Anisotremus interruptus</i>	Haemulidae	Bacoco	11
<i>Caranx caballus</i>	Carangidae	Green jack	15
<i>Caranx caninus</i>	Carangidae	Pacific crevalle jack	31
<i>Chaetodipterus zonatus</i>	Ephippidae	Pacific spadefish	127
<i>Trachinotus rhodopus</i>	Carangidae	Gafftopsail pompano	3
<i>Lutjanus argentiventris</i>	Lutjanidae	Yellow snapper	427
<i>Hoplopagrus guentherii</i>	Lutjanidae	Barred snapper	10
<i>Lutjanus novemfasciatus</i>	Lutjanidae	Pacific dog snapper	11
<i>Lutjanus colorado</i>	Lutjanidae	Colorado snapper	65
<i>Diodon holocanthus</i>	Diodontidae	Long-spined porcupinefish	27
<i>Pteroplatytrygon violacea</i>	Dasyatidae	Pelagic Stingray	1
<i>Aetobatus narinari</i>	Aetobatidae	Spotted eagle ray	2
<i>Carcharhinus altimus</i>	Carcharhinidae	Bignose shark	5
<i>Ginglymostoma cirratum</i>	Ginglymostomatidae	Nurse shark	9
<i>Negaprion brevirostris</i>	Carcharhinidae	Lemon shark	2
<i>Bodianus diplotaenia</i>	Labridae	Mexican hogfish	3
<i>Hyporthodus acanthistius</i>	Epinephelidae	Rooster hind	1
<i>Trachinotus</i> sp.	Carangidae	Pompano	1

Parasitological examinations of deceased and moribund fish revealed heavy infestations of *Neobenedenia* sp. (Monogenea: Capsalidae) (Figure 1), with parasites completely covering body surfaces and gills of some individuals. Identification was conducted by one of us (F.N.M.S.) based on morphological characteristics, following descriptions of *Neobenedenia melleni* and *N. girellae* [2,3]. Species-level identification was not possible, as it requires molecular analysis for accuracy, which was beyond the scope of this study. A total of twenty specimens of *L. colorado*, five of *A. xanthopterus*, and five of *C. zonatus* were visually examined. Initially, fish body surfaces were inspected under good illumination. Although the monogeneans are translucent, their movements produced a flashing effect that facilitated detection. Subsequently, smears of skin, fins, and gills were obtained using a scalpel and examined under a light microscope at 100× and 400× magnifications. Based on this diagnostic evaluation, immediate action was taken to mitigate the issue.

Fish exhibiting disease symptoms were removed from the exhibition tank and placed in a 1000 L tank, where they received a 3 min freshwater bath, followed by a 50 min formalin bath (0.17 mL/L) the next day. However, this treatment did not resolve the problem, as the parasite remained in the exhibition tanks. Since chemotherapeutic methods were not feasible in that environment, the staff opted for environmental management strategies to eradicate the monogeneans.

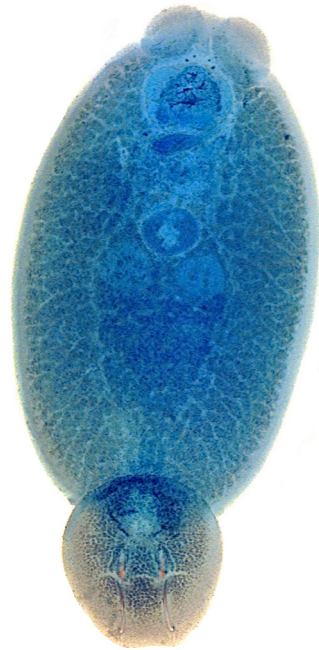


Figure 1. *Neobenedenia* sp. (Monogenea: Diplectanidae) found on *Lutjanus colorado*. Specimen stained with Gomori's trichrome.

The control method involved gradually reducing both water temperature and salinity. Temperature was lowered from 26 °C to 22 °C over five days (18–22 May), while salinity was decreased from 35 ppt to 26 ppt over one month (18 May–17 June). Additionally, tank cleaning frequency was increased, with backwashing performed for 25 min once daily and siphoning twice daily (morning and evening). Monogenean infestation levels were monitored indirectly by estimating the abundance of monogenean eggs in the water. To collect the eggs, new 60 cm long cotton threads were placed in the tanks every two days, as *Neobenedenia* produces eggs with filaments that become entangled in the threads. These threads were examined under a stereomicroscope 48 h after placement. This cleaning regimen and egg monitoring protocol began on 18 May and continued for two months. In the second month, however, the threads were replaced and examined weekly.

Egg abundance decreased over time. Initially, each cotton thread was nearly covered with eggs, but after one month, only about one-third of each thread was covered. By July, conditions returned to normal, as indicated by typical fish behavior, increased feeding rates, and rare egg detection. During June and July, parasite-associated mortality was less than 2%. To prevent another outbreak, the cleaning regimen has been maintained, with backwashing reduced to 15 min. Water temperature and salinity have been kept at an average of 23 °C and 31 ppt, respectively, while egg monitoring continues on a monthly basis. Prior to introducing new fish into the exhibition tanks, they are quarantined for three to four weeks and treated with a 3 min freshwater bath followed by three 50 min formalin baths (0.17 mL/L), with two days of rest between treatments. These control measures have ensured that the OT and other tanks continue to operate under optimal conditions.

3. Discussion

Addressing a mortality event like the one that occurred at GAM requires immediate action to minimize both animal and economic losses. However, applying antiparasitic treatments in oceanic tanks is challenging due to the large water volume and high species diversity. Moreover, *Neobenedenia* has biological traits that complicate control efforts. This monogenean follows a direct life cycle, is hermaphroditic, and capable of self-fertilization; a single adult can produce over 3000 eggs within days and develop from egg to adult in about two weeks, allowing a rapid outbreak [4]. Additionally, controlling capsalid monogenean infections is further complicated by the resilience of its eggs, which float in the water column and are more tolerant to treatments than juvenile or adult stages [5,6].

The monogenean control method implemented at GAM was based on previous studies demonstrating that lowering both temperature and salinity can significantly slow parasite development [7–9]. Additionally, its application was inspired by a prior report suggesting that hyposalinity is an effective strategy for controlling *Neobenedenia* in large, multi-species display aquaria [10]. This report highlights the case of the South Carolina Aquarium, where staff successfully eradicated *Neobenedenia* infestations by implementing prolonged hyposalinity, replacing the repeated use of copper and organophosphate treatments.

The case reported here is not uncommon, as monogeneans have long been recognized as a significant threat to fish in captivity [11]. The detrimental effects of monogeneans, including *Neobenedenia* spp., on fish include excessive mucus secretion, hemorrhage, tissue damage from feeding activities, and inflammatory reactions caused by the parasite's attachment hooks, all of which can impair physiological processes and lead to disease outbreaks and mass mortalities in cases of heavy infestations [12–15].

The species of *Neobenedenia* responsible for the outbreak at GAM has yet to be identified and requires molecular analysis. Among the species known to occur in the region, *N. melleni* and *N.girellae* have been reported as serious pathogens affecting farmed marine fish in the southern Gulf of California [9,16]. Notably, *N. girellae* is a cosmopolitan parasite capable of infecting a wide range of fish species, including wild, farmed, and aquarium fish [15,17]. For instance, *N. girellae* has been detected in 40 fish species from 12 families of imported marine ornamental fish at a public aquarium in the Mexican state of Yucatán [18]. This suggests that *Neobenedenia* remains a persistent threat to animal welfare at GAM, emphasizing the need for continued preventive strategies.

Author Contributions: Conceptualization, F.N.M.-S.; methodology, D.L.-M. and J.A.V.G.; resources, F.N.M.-S. and L.A.R.-M.; writing—original draft preparation, F.N.M.-S.; writing—review and editing, D.L.-M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This work was exempted from ethical review and approval as no fish were suppressed for this case report.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study.

Conflicts of Interest: The authors declare no conflicts of interest.

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