



Fauna Associated with *Artemia salina* Used to Feed Larvae of *Macrobrachium rosenbergii* (Decapoda: Palaemonidae) in Culture

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Abstract

Macrobrachium rosenbergii, the main species of the shrimp genus that is traded worldwide, commonly cultivated in China, India and Thailand, was introduced to Mexico in 1973. It is susceptible to infectious diseases associated with high mortality during its eleven larval stages, resulting in severe economic losses. To infer the source of contamination by protozoans during two larval development cycles of the shrimp in the aquaculture farm "Santa Fe" in Santa Fe, Veracruz, Mexico in 2021 was one of the aims of the study. The shrimp larvae (stages I-XI) *in vivo*, the culture medium and the food (*Artemia salina*) supplied for the larvae were examined using light microscopy, based on records, albeit rare, where food was contaminated by protozoans. The Hill numbers (N0, N1 and N2), the Shannon-Wiener diversity index, the Simpson's dominance and the Pielou's evenness indices were determined. Mortality rate in both cycles was similar: 89% in cycle 1 and 90% in cycle 2. The results showed that the mortality of larvae was not caused by the protozoan contaminants in the culture medium in which ten protozoan (nine free-living and an epizoic) and two rotifer taxa (*Rotaria rotatoria* and *Keratella* sp.) were identified. Taxonomic protozoan diversity was the lowest in the first shrimp larval stages and was higher in the last stages. During the two cycles, Hymenostomatida gen. sp. was the most abundant, and Litostomatea gen. sp. was the least abundant. The results suggested the food (*A. salina*) as a probable source of contamination of the shrimp culture by protozoans.

Keywords: *Artemia*; Cultivation; Mexico; *Macrobrachium rosenbergii*; Protozoans

Introduction

Macrobrachium rosenbergii De Man 1879 is a tropical crustacean, native to southern and southeastern Asia, northern Oceania and the western Pacific islands [1]. It is one of the representatives of the genus *Macrobrachium* Spence Bate, which includes approximately 238 species [2]. Some of the species in this genus are highly valued because

of their flavor, high protein content and visual appeal [3]. The main species of the genus that is traded worldwide is *M. rosenbergii*, commonly cultivated in China, India and Thailand. China holds 90% of the patents worldwide for the genus *Macrobrachium* [4]. This species was introduced to Mexico in 1973 [5]. However, to date the expected results have not been obtained in terms of the scarce development of its cultivation at a commercial level due to the scarce

supply of postlarvae. *M. rosenbergii* is susceptible to infectious diseases associated with high mortality during its eleven larval stages, resulting in severe economic losses and diminishing investments for the aquaculture sector [6-8].

Diseases that affect *M. rosenbergii* are of two types: non-infectious and infectious. Non-infectious diseases are subdivided into those caused by nutritional deficiencies and those caused by the effect of water quality. This last factor, in addition to subjecting the cultured organisms to stressful conditions such as variations in temperature, salinity and pH, can favor the development of infectious diseases because poor water quality causes the organisms to reduce their cleaning capacity, become incapable of molting or even develop physical lesions, making them vulnerable to opportunistic pathogens. Among the infectious diseases that affect *M. rosenbergii* is infestation by Protozoa [9-11], and this may be a cause of the high mortality rate during the larval stages of the crustacean.

When cultured crustaceans are colonized by epibionts, their continuous reproduction takes place on the host as time passes. Thus, the crustacean acquires an increasing load of epibionts in two possible ways. One is that the loss of the exoskeleton provides relief to the host during molting [12]. The other possibility is that the crustacean dies due to the problems caused by hindrance of locomotion, obstruction of the ocular organs or interference in gas exchange [13]. However, the specific causes of excessive colonization are not completely understood; apparently, healthy individuals are not excessively colonized by epibionts [14].

The term "protozoan" is assigned to unicellular heterotrophic eukaryotic organisms, which include four types: amoeboids, ciliates, flagellates and parasitic sporozoans [15]. Currently, there are ca. 8000 described ciliate species, of which ca. 4500 species are free-living [16]. Ciliates can be found in terrestrial and aquatic environments, in fresh, brackish and seawater and in wastewater treatment systems [17,18]. Protozoa, in general, feed heterotrophically; however, mixotrophy has been documented in some species [19-21]. They feed on bacteria, microalgae and zooplankton, depending on their size [22,23]. Ciliates are capable of establishing a wide variety of symbioses such as phoresis, parasitism, mutualism and commensalism.

At the global level, there are few studies on the presence of epibiontic Protozoa in crustaceans. In Mexico, these studies began in the mid-1960s; however, they are relatively scarce because of lack of interest in biodiversity studies [24,25]. In studies on epibiosis in crustaceans, it was observed that Protozoa do not feed directly on the crustacean to which they adhere, but rather use it as a means to obtain their food [26] without becoming sick. However, the severity of protozoan

infestation for crustaceans depends on the number of epibionts that colonize the organs [13]. In contrast, as the cropping/culture cycle progresses, effects of epibiontic Protozoa become more severe.

Furthermore, although scarce, there are records that food (*Artemia* sp.) can serve as a vehicle for disseminating Protozoa due to their encystment capacity [27]. Therefore, in the present study, both the food (*Artemia salina*) supplied to the larvae of *M. rosenbergii*, as well as the culture medium and the eleven crustacean larval stages were analyzed to reveal the species composition and possible sources of infestation.

Material and Methods

Sampling. Two culture cycles (a cycle includes eleven larval stages, or 22-28 days) of the *M. rosenbergii* larvae were monitored using the "Clear Water Model". This model is characterized by using closed systems with water recirculation, offering a more efficient and controlled environment for organisms. The cultures were located at the "Santa Fe" aquaculture farm located in the State of Veracruz, southeastern Mexico. The first cropping cycle (C1) was developed in April 2021 and the second cycle (C2) in June 2023. In addition to monitoring the cultures of the *M. rosenbergii* larvae, the food supplied to the cultures of the crustacean was also monitored.

Samples of both *M. rosenbergii* larvae and the food were collected in their respective culture medium (brackish water: 15-18, temperature: 29-31°C). Sampling was carried out for each stage of the shrimp larval development (from stage I to stage XI). At the time of sample collection, the main water quality parameters were also measured: water temperature, salinity, dissolved oxygen (DO) and pH. In each cycle, 20 larvae per stage of *M. rosenbergii* were collected in a 25 µm mesh-sized sieve. Simultaneously with sampling the larvae, water from the bottom of the pond was stirred to ensure the homogeneity of the sample. The larvae were placed in a sterile plastic clinical sample bottle with a capacity of 120 mL. At each larval sampling, a sample of the food was collected in a sterile jar.

Laboratory analyses. Samples were transferred in a thermos at room temperature to preserve the temperature conditions of the collected samples and analyzed immediately upon arrival at the laboratory.

Samples were examined *in vivo*. A larva in 1 mL of culture water (henceforth called the larval sample) was placed in a modified Sedgwick-Rafter chamber of 2 mL capacity and examined for confirmation of protozoan adhesion under normal conditions. Subsequently, Protozoa present in the environment were examined for shape, swimming

movements and habits. A half mL of 0.5 g/L chlorphenamine syrup was added to slow down the movement of Protozoa for accurate counts. Richness and abundance of protozoan and rotifer taxa were estimated. Twenty replicates were analyzed for each larval stage of *M. rosenbergii*, using a Motic BA300 compound microscope, equipped with 40x/0.65 and 100x/1.25 objectives in bright field.

For the *in vivo* food, the volume analyzed in the Sedgwick-Rafter chamber was 1 mL, and the same procedure was followed as for each larval sample with three replicates for each shrimp stage. The taxonomic identification was based on the guides of Foissner, et al. [28] and Fontaneto, et al. [29].

Statistical analysis. To analyze the diversity of ciliates, the series of Hill numbers was used [30]. Similarly, the Shannon-Wiener index estimated from \ln and \log_2 was also determined. In addition, Simpson's dominance index (Dsi) and Pielou's evenness index (J') were determined.

Using the data on the abundances of the protozoan taxa present in the food and the larval sample, a database in MS Excel, version 97-2003, was created. The Wilcoxon signed rank test was performed with a $p < 0.05$ to compare the similarity between the abundances in both the food and the larval sample. Abundance data that did not meet the assumptions of normality and homoscedasticity were expressed as median and interquartile range. All calculations reported in the present study were performed using the R program, version 4.1.1.

Results

Taxonomic composition and abundance. In total, ten protozoan taxa were identified, and six of them were observed in the food (*A. salina*). Nine taxa were observed in the larval sample, including ciliates and amoeboids during both cycles. In addition, two species of rotifers (*Rotaria rotatoria* (Pallas) and *Keratella* sp.) were identified in both the food and in the larval sample; these were included in the estimations of richness and abundance. No protozoans attached to the larvae were observed.

The species with the greatest abundance were the ciliates (Ciliophora) *Hypotracha* gen. sp. with 340 individuals (36, 1935; median (interquartile range) and *Hymenostomatida* gen. sp. with 212 individuals (7, 1182). The maximum values of both species were recorded in the food of cycle 2. *Hymenostomatida* gen. sp. was the most abundant species for both shrimp larval cycles, both in the food and in the larval sample.

The taxa in which significant differences were found, using the Wilcoxon signed rank test between the abundances observed in the food and the larval sample were *Hypotracha* gen. sp. of cycle 2 ($p < 0.001$), *Hymenostomatida* gen. sp. from cycle 2 ($p < 0.001$) and *Disematostoma* sp. from cycle 2 ($p < 0.001$). For these species the abundances were higher in food by one to two orders of magnitude (Figure 1).

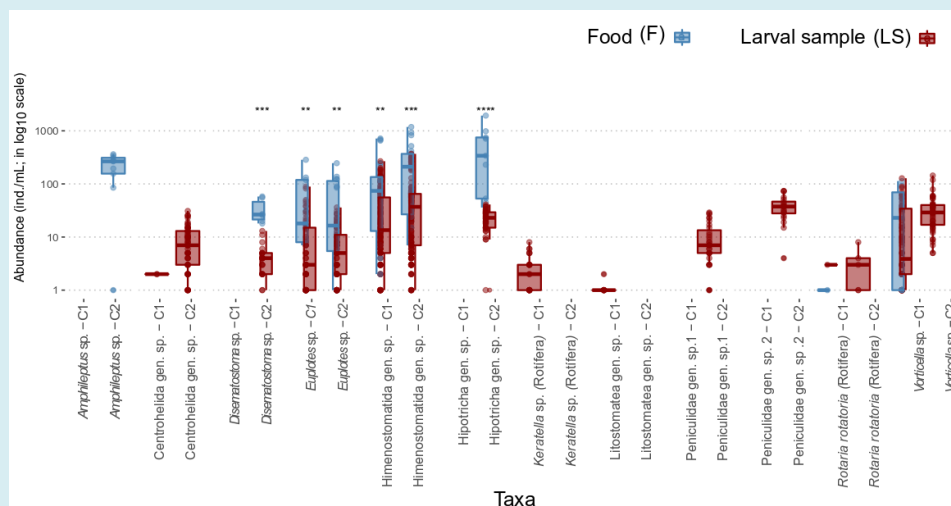


Figure 1: Comparison of the abundances of organisms associated with the cultured *Macrobrachium rosenbergii* per cycle (C1 and C2) and per substrate: food (F), larval sample (LS). Central vertical lines show the median, boxplots show the interquartile range, and whiskers show the minimum and maximum values, except for atypical values (shown by circles). The p value represents significant differences between substrates: ns = $p > 0.05$; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; **** = $p \leq 0.0001$.

Diversity indices. During cycle 1 in food, the highest Hill diversity indices, the highest Pielou's evenness (J') and the highest Shannon-Wiener index occurred in stage VII ($N_0 = 4$, $N_1 = 3$, $N_2 = 2$; $J' = 0.72$; $H' \ln = 1.0$; $H' \log 2 = 1.45$), while the Simpson's dominance index was highest in larval stages II, III, V, VI and VIII ($\lambda = 1.0$). The lowest diversity indices for cycle 1 food, in general, occurred during stages I and XI, where protozoans were not observed (Table 1).

During cycle 2 in food, the highest Hill diversity indices occurred in stages IX and X: stage IX ($N_0 = 4$, $N_1 = 2$, $N_2 =$

2); stage X ($N_0 = 4$, $N_1 = 3$, $N_2 = 2$). The highest Shannon-Wiener index occurred in stage X ($H' \ln = 1.03$; $H' \log 2 = 1.48$). The Hill and Shannon-Wiener diversity indices were lowest in stages I, II, V, VI, VII and XI, in which only one protozoan taxon was found ($N_0 = 1$, $N_1 = 1$, $N_2 = 1$; $H' \ln = 0$; $H' \log 2 = 0$). In contrast, Simpson's dominance index was highest in stages I, II, V, VI, VII and XI ($\lambda = 1.0$). The greatest Pielou's evenness in cycle 2 food was observed in stage X ($J' = 0.74$) (Table 1).

Stage and cycle	N_0 (S)	N_1	N_2	Shannon_In	Shannon_2	Simpson's dominance	Pielou's evenness	<i>Amphileptus</i> sp.	Centrohelida gen.sp.	<i>Disematostoma</i> sp.	<i>Euplotes</i> sp.	Hymenostomatida gen.sp.	Hypotricha gen.sp.	<i>Keratella</i> sp. (Rotifera)	Litostomatea gen.sp.	Peniculidae gen.sp.1	Peniculidae gen.sp.2	<i>Rotaria rotatoria</i> (Rotifera)	<i>Vorticella</i> sp.
C1																			
I	0	1	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
II	1	1	1	0	0	1	—	0	0	0	0	13	0	0	0	0	0	0	0
III	1	1	1	0	0	1	—	0	0	0	0	18	0	0	0	0	0	0	0
IV	2	1.22	1.1	0.2	0.29	0.91	0.3	0	0	0	0	19	0	0	0	0	0	0	1
V	1	1	1	0	0	1	—	0	0	0	0	244	0	0	0	0	0	0	0
VI	1	1	1	0	0	1	—	0	0	0	0	717	0	0	0	0	0	0	0
VII	4	2.73	2.45	1	1.45	0.41	0.7	0	0	0	285	111	0	0	0	0	0	1	113
VIII	1	1	1	0	0	1	—	0	0	0	0	7	0	0	0	0	0	0	0
IX	3	1.82	1.46	0.6	0.86	0.69	0.5	0	0	0	32	247	0	0	0	0	0	0	23
X	2	1.3	1.15	0.26	0.37	0.87	0.4	0	0	0	9	116	0	0	0	0	0	0	0
XI	0	1	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Median	1	1	1.1	0	0	0.9	—	0	0	0	0	19	0	0	0	0	0	0	0
Minimum	0	1	1	0	0	0	—	0	0	0	0	0	0	0	0	0	0	0	0
Maximum	4	2.7	2.5	1	1.5	1	—	0	0	0	285	717	0	0	0	0	0	1	113
C2																			
I	1	1	1	0	0	1	—	0	0	0	0	47	0	0	0	0	0	0	0
II	1	1	1	0	0	1	—	0	0	0	0	15	0	0	0	0	0	0	0
III	2	1.14	1.06	0.13	0.19	0.94	0.2	0	0	0	7	237	0	0	0	0	0	0	0
IV	2	1.2	1.1	0.19	0.27	0.91	0.3	0	0	0	18	374	0	0	0	0	0	0	0
V	1	1	1	0	0	1	—	0	0	0	0	517	0	0	0	0	0	0	0
VI	1	1	1	0	0	1	—	0	0	0	0	1182	0	0	0	0	0	0	0

VII	1	1	1	0	0	1	—	0	0	0	0	43	0	0	0	0	0	0
VIII	3	1.74	1.53	0.55	0.8	0.66	0.5	195	0	0	5	0	711	0	0	0	0	0
IX	4	2.01	1.54	0.7	1.01	0.65	0.5	311	0	58	137	0	1935	0	0	0	0	0
X	4	2.79	2.42	1.03	1.48	0.41	0.7	362	0	26	246	0	53	0	0	0	0	0
XI	1	1	1	0	0	1	—	0	0	0	0	38	0	0	0	0	0	0
Median	1	1	1	0	0	1	—	0	0	0	0	43	0	0	0	0	0	0
Minimum	1	1	1	0	0	0.4	—	0	0	0	0	0	0	0	0	0	0	0
Maximum	4	2.8	2.4	1	1.5	1	—	362	0	58	246	1182	1935	0	0	0	0	0

Table 1: Ecological indices and abundances in individuals per milliliter (ind./mL) of the taxa present in the food of eleven larval stages (n = 3) of *M. rosenbergii* during two life-cycles (C1 and C2).

During cycle 1 in larval samples, the highest Hill and Shannon-Wiener diversity indices occurred in stage XI (N0 = 5, N1 = 3, N2 = 2, H'ln = 1.01, H'log2 = 1.46); the Pielou evenness was

highest in stage VI (J' = 0.94). Simpson's dominance index was highest in stage II ($\lambda = 1.0$). The lowest values occurred in stage III, where protozoa were not observed (Table 2).

Stage and cycle	N0 (S)	N1	N2	Shannon_In	Shannon_2	Simpson's_dominance	Pielou's evenness	<i>Amphileptus</i> sp.	<i>Centrohelida</i> gen.sp.	<i>Disematostoma</i> sp.	<i>Euplotes</i> sp.	Hymenostomatida gen.sp.	Hypotricha gen.sp.	<i>Keratella</i> sp. (Rotifera)	Litostomatea gen.sp.	Peniculidae gen.sp.1	Peniculidae gen.sp.2	<i>Rotaria rotatoria</i> (Rotifera)	<i>Vorticella</i> sp.
C1																			
I	3	1.9	1.5	0.64	0.92	0.66	0.58	0	0	0	0	6	0	0	0	0	0	3	35
II	1	1	1	0	0	1	—	0	0	0	0	1	0	0	0	0	0	0	0
III	0	1	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IV	2	1.8	1.6	0.56	0.81	0.63	0.81	0	0	0	0	9	0	0	0	0	0	0	3
V	3	1.9	1.5	0.63	0.91	0.65	0.58	0	0	0	1	15	0	0	0	0	0	0	3
VI	2	1.9	1.9	0.65	0.94	0.54	0.94	0	0	0	0	18	0	0	0	0	0	0	10
VII	3	2.1	1.7	0.76	1.09	0.58	0.69	0	0	0	8	48	0	0	0	0	0	0	9
VIII	3	1.4	1.2	0.33	0.48	0.84	0.3	0	2	0	0	10	0	0	0	0	0	0	128
IX	5	1.5	1.2	0.38	0.54	0.85	0.23	0	2	0	5	268	0	6	0	0	0	0	10
X	4	2.3	2.1	0.83	1.19	0.47	0.6	0	0	0	89	93	0	5	1	0	0	0	0
XI	5	2.8	2.2	1.01	1.46	0.46	0.63	0	0	0	7	37	0	8	2	0	0	0	89
Median	3	1.9	1.6	0.63	0.91	0.62	—	0	0	0	0	15	0	0	0	0	0	0	9
Minimum	0	1	1	0	0	0	—	0	0	0	0	0	0	0	0	0	0	0	0
Maximum	5	2.8	2.2	1.01	1.46	1	—	0	2	0	89	268	0	8	2	0	0	3	128
C2																			
I	3	2.6	2.4	0.93	1.35	0.42	0.85	0	2	0	10	10	0	0	0	0	0	0	0
II	5	2.1	1.5	0.73	1.06	0.67	0.46	0	11	0	3	12	0	0	0	0	0	8	144
III	4	3.2	3	1.17	1.69	0.34	0.85	0	7	0	36	58	0	0	0	0	0	0	79

IV	4	3.4	3	1.22	1.77	0.33	0.88	0	17	0	31	89	0	0	0	0	0	0	60
V	1	1	1	0	0	1	—	0	0	0	0	7	0	0	0	0	0	0	0
VI	2	1.3	1.2	0.27	0.39	0.86	0.39	0	0	0	6	73	0	0	0	0	0	0	0
VII	2	1.1	1.1	0.13	0.18	0.95	0.18	0	2	0	0	71	0	0	0	0	0	0	0
VIII	3	1.5	1.2	0.4	0.57	0.81	0.36	0	31	0	12	368	0	0	0	0	0	0	0
IX	4	2.8	2.4	1.02	1.47	0.41	0.74	0	4	0	0	129	0	0	0	29	74	0	0
X	5	4.1	3.3	1.4	2.03	0.3	0.87	0	0	13	9	10	41	0	0	12	0	0	0
XI	4	2.8	2.3	1.03	1.49	0.44	0.75	0	0	5	3	13	32	0	0	0	0	0	0
Median	4	2.6	2.3	0.93	1.35	0.44	—	0	2	0	6	58	0	0	0	0	0	0	0
Minimum	1	1	1	0	0	0.3	—	0	0	0	0	7	0	0	0	0	0	0	0
Maximum	5	4.1	3.3	1.4	2.03	1	—	0	31	13	36	368	41	0	0	29	74	8	144

Table 2: Ecological indices and abundances in individuals per milliliter (ind./mL) of the taxa present in the sample (culture medium) for eleven larval stages (n = 20) of *M. rosenbergii* during two life-cycles (C1 and C2).

During cycle 2 in larval samples, the highest values of the Hill and Shannon-Wiener diversity indices occurred in stage X ($N_0 = 5$, $N_1 = 4$, $N_2 = 3$, $H'_n = 1.40$, $H'_{log2} = 2.03$). Pielou's evenness index was higher in stage IV ($J' = 0.88$), and Simpson's dominance index was higher in stage V ($\lambda = 1.0$). The lowest diversity indices were observed in stage V, where only one protozoan taxon was found ($N_0 = 1$, $N_1 = 1$, $N_2 = 1$, $H'_n = 0.0$, $H'_{log2} = 0.0$) (Table 2).

Variation in protozoan abundances associated with the culture of *M. rosenbergii*. In cycle 1 the abundances of protozoans decreased dramatically in the transition from

stage I to stage II when carrying out the change from the hatching tub (stage I) to the growth tub (stage II). From stage III onward, the abundances of Protozoa in larval samples began to increase as the crop aged and then decreased in stage X where the food "flan" was administered (semi-moist preparation based on egg, rice flour, vitamins and minerals) instead of *A. salina*. In turn, in general, the abundances in food of cycle 1 were greater than in larval samples (stages II, III, IV, V, VI, VII, IX and X). The *A. salina* monitored during stage XI was observed to have been recently hatched, and Protozoa were absent (Figure 2).

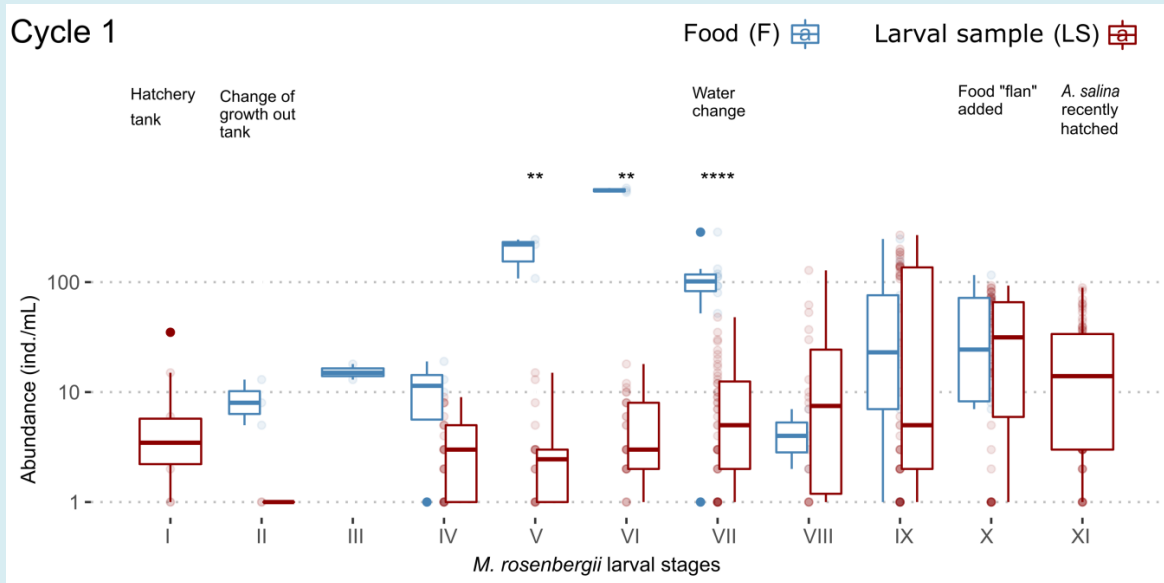


Figure 2: Variation in the total abundance of organisms associated with the cultured *Macrobrachium rosenbergii* during cycle 1 in food (F) and larval sample (LS).

During cycle 2, the abundances of Protozoa increased as the larval stages of *M. rosenbergii* advanced within the hatching tub until reaching stage IV. Upon reaching this stage, the larvae were transferred to the growth tank, where the abundances of protozoans decreased dramatically during stage V and increased during stage VI. During stage VII, the water was changed, and the abundances remained stable; abundances increased during stage VIII and decreased during

stage IX. In stage X, the food “flan” was administered to the crop, decreasing the protozoan abundances with respect to the previous stage. Finally, in stage XI a slight decrease in the protozoan abundances was observed with respect to stage X. In the food monitored during cycle 2, in general, protozoan abundances were higher than in the larval samples, except in stage VII (Figure 3).

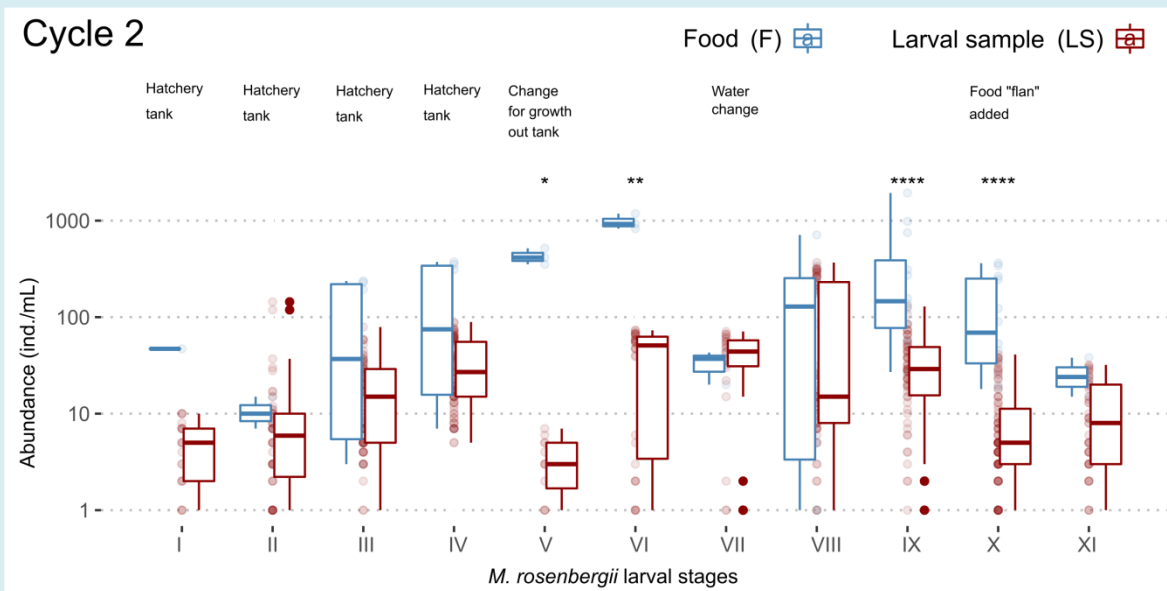


Figure 3: Variation in the total abundance of organisms associated with the cultured *Macrobrachium rosenbergii* during cycle 2 in food (F) and larval sample (LS).

Mortality: The mortality rate in both cycles was similar: in cycle 1 it was 89%, and in cycle 2 it was 90%. Both cycles started with 70 larvae/L.

Discussion

The protozoans in the culture medium of *M. rosenbergii* did not show any affinity for adhering to healthy larvae. However, some cells had a preference for adhering to detritus and mainly to the cysts of *A. salina* used as a food in the culture of the shrimp, while other protozoans were free-living. Feeding types of ciliates include saprozoic and bacterivorous forms [31-33]. Consequently, the protozoan abundance and diversity in the larval sample was higher in stages in which a greater amount of organic matter accumulated over time.

The protozoans identified in the present study were mostly free-living ciliates (nine taxa). This differs from that reported by Rodríguez, et al. [6], who found peritrichous (Ciliophora: Peritricha) and suctorian (Ciliophora: Suctoria) ciliates on the *M. rosenbergii* larvae. These protozoans adhere to a substrate and/or vector (crustacean), unlike

free-swimming ciliates. This could be due to the region from which the *A. salina* came, the season in which the study was carried out, or even the place where the culture of the *M. rosenbergii* larvae was developed, as has been reported in the literature [34,35].

Additionally, two species of rotifers (*Rotaria rotatoria* and *Keratella* sp.) were identified in both the food and the larval samples. Rotifers serve as a food for larvae of fish, crustaceans and mollusks [36], although both species are also considered as indicators of water quality [37]. Probably, due to the latter, the rotifers were most abundant in the stages in which the crop water was used longer (stages IX, X and XI); however, there are no studies of this to date.

Among the protozoans studied in the shrimp culture, Hypotricha gen. sp. (340 cells; 36, 1935; median (interquartile range) and Hymenostomatida gen. sp. (212 cells; 7, 1182) were highest in abundance (Figure 1). Both taxa have in common that they are mostly free-living saprozoic or bacterivorous organisms and, generally, are

natural inhabitants of freshwater bodies [38]. It can be noted that due to the high adaptability of these taxa, they were found in higher abundances in most stages in both cycles where there was a greater amount of organic matter.

The taxonomic diversity of protozoans was lower in the early stages of the cultured shrimp and higher in the later stages (Table 2). This could be due to the water exchange that was made at the beginning of the cycle and to different incubation periods of different taxa, so that as the culture duration increased, new taxa appeared [39]. In general, as the larval stages of *M. rosenbergii* progressed, the diversity of protozoans in the culture medium increased. In the case of food, maximum diversity values occurred at different points in the larval development cycle of *M. rosenbergii* because *A. salina* was prepared and added to the shrimp culture periodically (Table 1).

In contrast, the Simpson's index was higher in the food (*A. salina*) than in the larval samples (crop water). In the larval samples, the index values were higher in the stages where the taxonomic diversity of the studied fauna was lower in both cycles (Tables 1 & 2). In all cases, the dominant taxon was Hymenostomatida gen. sp., which has been reported as one of the abundant taxa in wastewater [40,41]. It is likely that because this taxon is quite common, it was the only one that occurred in stages where there was a recent water change.

Equitability (J' de Pielou) in the sample was generally higher during the intermediate stages, while in the food, the index reached its highest values at different points of both cycles (cycle 1: stage VII; cycle 2: stage X) (Table 2). This could reflect that most of the protozoan species reached their incubation period in intermediate stages of the *M. rosenbergii* culture and, as the stages progress, certain species tend to be more abundant [42]. This trend was not observed in the food (Table 1), probably because of the frequency of preparation.

Monitoring the different larval stages of *M. rosenbergii* during the two cycles demonstrated that the control of culture conditions influences the protozoan abundance (Figures 2 & 3). According to Sherr, et al. [43] and Ponce-Palafox, et al. [9] the quality of crop water influences the protozoan abundance and the presence of diseases in cultured organisms. This could explain why the protozoan abundances were zero or low when the change of water in the culture medium was more recent.

Our study revealed that the food (*A. salina*) was a source of protozoan contamination in the culture medium of *M. rosenbergii*. This agrees with what was reported by Méndez-Hermida, et al. [27], who demonstrated that *A. franciscana* Kellogg can serve as a disseminating vehicle for protozoans

due to its encystment capability. However, the protozoans found in the larval sample of *M. rosenbergii* were not observed attached to the larvae of the shrimp, but rather were free-living forms (nine taxa), which do not affect the shrimp's mortality. This coincides with Ramírez-Ballesteros, et al. [13], who observed an impairment of the respiratory rate of the crayfish *Cambarellus montezumae* (de Saussure) only after the crustacean's gills were colonized by 50 or more suctorians. This is supported by Morales-Covarrubias, et al. [10], who point out that protozoans, in general, are cosmopolitan organisms that can be found naturally in the crustacean culture ponds, and that there may even be a certain number of epibionts on gills, appendages and cuticle of various parts of the body of the hosts without causing sickness.

Six protozoan taxa were found exclusively in the food (*A. salina*), and a total of ten protozoan taxa (nine free-living ciliates and a peritrich) were found in the food and the larval samples during both cycles. The protozoans in the larval samples of *M. rosenbergii* were not observed attached to the shrimp larvae, with the exception of the peritrich *Vorticella* sp., which was found both in the food and in the larval samples, and it was present attached to detritus. Despite the presence of protozoans in the larval samples of *M. rosenbergii*, they did not affect the mortality of the shrimp larvae during the monitored two culture cycles. However, the protozoan diversity associated with the culture of *M. rosenbergii* was higher during the stages in which the culture water was used longer. Finally, it was concluded that *A. salina* was a probable source of the protozoan contamination for the culture water of the shrimp [44].

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