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Laboratory aquaculture of *Lecane inermis* (Bryce, 1842) isolated from the Hilla River in Babylon province-Iraq

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Abstract

The current study aimed to evaluate the environmental conditions necessary for cultivating the rotifer species (*Lecane inermis*) initially recorded in the Hilla River and their impact on its density. Using a zooplankton net, rotifer samples were collected in the early morning of winter 2024 from the Hilla River in Al-Mahawil district, Babylon province. The physical and chemical properties of the site were analyzed, including air temperature (6 °C), water temperature (9.5 °C), electrical conductivity (EC) (1090 µs/cm), total dissolved solids (TDS) (772 ppm), salinity (636 ppm), pH (8.6), dissolved oxygen (DO) (12.3 ppm) and turbidity (11.5 NTU). The cultured species *L. inermis* was isolated, identified and subsequently cultivated. Various factors, including temperature, salinity, dissolved oxygen and light versus dark, were then investigated to determine the effect on species density. The findings demonstrate that *L.inermis* exhibited higher density at low temperatures and revealed broad salinity tolerance, though it could only withstand lower salinity concentrations at high temperatures. Additionally, its density was greater under dark conditions than under light conditions. The highest density recorded was 585 ± 20 ind/L during the winter season.

Keywords

aquaculture; Hilla river; *Lecane inermis*; zooplankton density

Introduction

Zooplanktons are essential to the survival of a vast range of freshwater species, including fish. Gut content studies reveal that zooplankton constitutes a significant portion of the staple diet for several fish species in their natural habitats. Recognizing their importance in commercial aquaculture, several zooplankton species have been introduced and artificially cultivated. Zooplankton are considered excellent resources for live feed because of their high lipid and protein contents, which are crucial for the development and survival of fish larvae (1, 2). The availability of farmed zooplankton often facilitates a large culture of fish larvae. Even though commercially farmed fish species benefit significantly from zooplankton, their culture, and sufficient production must be financially viable. Regardless of the fish species, zooplankton culture is promoted because, in most cases, fish larvae obtain their entire or at least a significant portion of their nutrition from zooplankton food sources (3). Recent advances in zooplankton culture techniques have provided viable options for both small- and large-scale production. This study aims to evaluate the potential growth and survival of one zooplankton species (*L. inermis*) under artificial laboratory conditions, considering its nutritional benefits and ease of cultivation as it is known.

Aquaculture is one of the fastest-growing industries today. Fish require live food to survive. Although live food can be expensive, it is available in marketplaces. Therefore, culturing zooplankton is a cost-effective option for reducing production costs (4). Today, wastewater treatment facilities face significant problems due to the overgrowth of bacteria. *L. inermis* can effectively control the excessive growth of various bacteria (5). *L. inermis* is one of the most effective zooplankton species for treating wastewater. They are highly prolific rotifers, with populations dominated by females that lay an average of 20 eggs during their 9 days of lifespan. Successful growth of *Lecane* species depends upon precise feeding. Algae and bacteria serve as their primary source of food. *L. inermis* has already been identified as a possible control agent (6).

Many recent studies have highlighted the industrial importance of gradually replacing common live feed, such as brine shrimp and rotifer, with different zooplankton species, including Cladocera's like water fleas, either entirely or partially (7). Environmental factors play an important role in controlling zooplankton. Fish mostly regulate "top-down" processes for larger species, whereas "bottom-up" mechanisms primarily govern small species like rotifers and small Cladocera's (8).

Materials and Methods

Collection of samples

Water and zooplankton samples were collected in February 2024 from the Hilla River in Al-Mahaweel district in Babylon province. Zooplankton sampling was conducted in the early morning to minimize heat stress, using a zooplankton net with 50 µm mish size. Samples were collected from depths up to 2 m from the water's surface and then emptied into a zooplankton net to pass through 70 L of water. The net was then cleaned externally with a sprayer and the samples were transferred to the laboratory for isolation, diagnosis and culture (9). Water samples were collected in 5 L polyethene containers for physical and chemical analysis.

Physical and chemical parameters

Air and water temperatures were measured using a thermometer. Electrical conductivity, pH, TDS and salinity were measured using a HANNA multi-parameters device, DO was measured using a DO meter and Turbidity NTU (Nepheloetric Turbidity Unit) was measured using a Turbidimeter manufactured by the Lovibond company.

Enumeration of the zooplankton

To count the zooplankton per liter, the volume of water filtered through the net was calculated using the cylinder volume formula (10). Moreover, the method described by APHA was employed to calculate the number of individuals per liter, with all the results expressed as the number of individuals/liters (ind/L) (11). A Sedgewick-Rafter slide chamber was used for zooplankton counting.

Identification of rotifer Lecane inermis

For taxonomic identification, a live sample was placed on the slide, observed and then fixed using a formaldehyde solution of 4%. The *Lecane* specimens were examined under a light microscope and scanning electron microscope. The specimen was identified as *L. inermis*, a rotifer based on its size and morphological characteristics. Its identification was performed using the standard taxonomic keys (12-16).

The culture of algae and rotifer (Lecane inermis)

The culture of algae: The manufacturer (Reef Nutrition) provided a pure culture of *Nannochloropsis* sp., which was cultivated using the batch-culture technique. The cultivation conditions for the algae were established as per standard protocol (17). For rotifer growth under optimal laboratory conditions, a synthetic medium containing 96 mg $NaHCO_3$, 60 mg $CaSO_4.2H_2O$, 60 mg $MgSO_4$ and 4 mg KCl in 1 L of sterile deionized water was utilized and the right amount of algae was fed (17). The algae were harvested during the exponential phase, centrifuged at 2000 rpm for 8 min and concentrated to 1×10^8 cells/mL at 4 °C in a refrigerator.

The rotifer stock culture: The samples were screened in the laboratory through a 500 µm mesh net to remove fish and decapod larvae. Then, they were filtered through a 120 µm mesh to remove copepods, barnacles and nauplii. A Pasteur pipette and capillary tubes separated *L. inermis* from the remaining zooplankton to create a stock culture using a batch culture approach, beginning with a 25 mL conical flask. During cultivation, a photoperiod of 12 h of light and 12 h of darkness was maintained. The *Nannochloropsis* sp. was fed to the experimental rotifer (18). Aeration was necessary to prevent algae aggregation and enhance its rotifers accessibility (19). Algal cells were then supplied to the culture containers to maintain the target cell concentrations.

Results and Discussion

Table 1 shows the values of the physicochemical parameters during the sampling time. The air temperature was 6 °C and the water temperature was 9.5 °C, variations in air temperature reflected the general attitude of the geographical location and water temperature followed a similar trend, indicating a close relationship between the 2 (20, 21). This study's findings were consistent with those of previous Iraqi environmental studies. The current study observed a narrow pH value (8.6) attributed to the buffering capacity of carbonic acid and bicarbonate, which resist significant pH fluctuations (22). Additionally, numerous investigations have noticed a small pH range (15, 16, 23).

Dissolved salts influence EC in water, while salinity represents the total ion concentrations in the water. Hence, there is a strong correlation between EC, TDS and salinity.

Table 1. Physico-chemical parameters measured at the study site during sampling

Parameters	Mean value
Air temperature (°C)	6
Water temperature (°C)	9.5
pH	8.6
Electrical conductivity (EC) (µs/cm)	1090
Total Dissolved Solids (TDS) (mg/L)	772
Salinity (mg/L)	636
Dissolved Oxygen (DO) (mg/L)	12.3
Turbidity (NTU)	11.5

This study's EC, TDS and salinity values were 1090 µs/cm, 772 ppm and 636 ppm, respectively. DO is a critical factor for the survival of aquatic life (24). Dissolved oxygen levels were favorable during winter (12.3 ppm). This may be due to a decline in the ratios of consumption and decomposition associated with an increase in gas solubility. This might have changed due to rising temperatures, making gasses less water-soluble (25). Finally, the average density of rotifers recorded at the study station was 23.4 individuals per liter during the sampling time. Environmental factors also impact rotifer growth, but they are not independent of one another. For instance, a change in temperature influences other environmental factors like salinity and dissolved oxygen, which in turn impacts rotifer growth and density. Generally, the most crucial factor affecting the change is temperature.

Description of Lecane inermis

In the present study, one species of rotifers (*L. inermis*) belonging to the family Lecanidae was recorded for the first time in the Hilla River. The diagnostic features include the length of lorica (60-80 µm), width of lorica (36-48 µm), length of ventral plate (60-62 µm), width of ventral plate(39-40 µm), length of toe with claw (30-44 µm), length of claw only (10-12 µm), length of trophi (20 µm), width of trophi (25 µm), length of fulcrum (7.06 µm), length of rami (5.88 µm), length of incus (7.53 µm) and length of manubria (19.18-20.82 µm) as clear in Fig. 1-4.

Laboratory conditions of L. inermis aquaculture

The basic environmental factors recorded in the current study (Table 2) are crucial for the culture of *L. inermis*. The water temperature ranged from 7 °C to 26 °C during winter and summer, respectively, while the air temperature ranged from 10 °C to 30 °C. The difference in temperature between the water and air is because the water's high specific heat helps it retain its temperature and protect aquatic life from sudden temperature fluctuations (20, 21).

Temperature significantly influences zooplankton's distribution and determines the water's oxygen level (26). Numerous studies have demonstrated that rotifers population densities typically increase during the summer and decrease in the winter (27-30). However, the current study indicated that *L.inermis* reached its highest density (585 ± 20 ind/L) in the winter (Table 2). The species also exhibited a broad tolerance range for EC, salinity and TDS, with its highest density recorded at an elevated salinity level (Table 2).

A study reported that most rotifers require more than 1 ppm DO concentration (31). While some can survive briefly in anaerobic conditions or close to them, others can live for extended periods in low-oxygen environments. In our current study, *L. inermis* achieved its highest density at a DO level of 7.8 ppm (Table 2). However, this species was first observed at an oxygen concentration of less than 4 ppm. Research suggests that *L. inermis* may reproduce periodically in aerobic and anaerobic environments, tolerating DO concentrations as low as 1 ppm (32).

Zooplankton nutrition is influenced by light and darkness. Vertical migration plays a crucial role, with zooplankton ascending to the water's surface at night to feed and retreating to deeper layers during the day to evade predators (33). This behavior aligns with our findings, where *L. inermis* cultivated in the dark exhibited a higher density than those exposed to 200 Lux (Fig.5). The increased density in dark-cultured conditions may be attributed to more effective feeding than in light-cultured conditions.

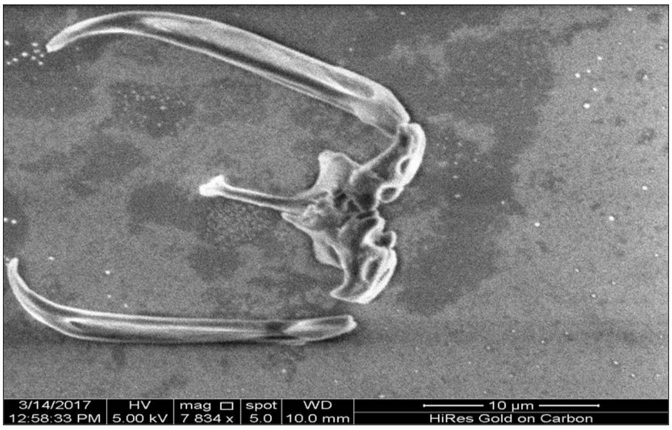

Fig. 1. Trophi of *L. inermis* observed under Scanning Electron Microscope (SEM).

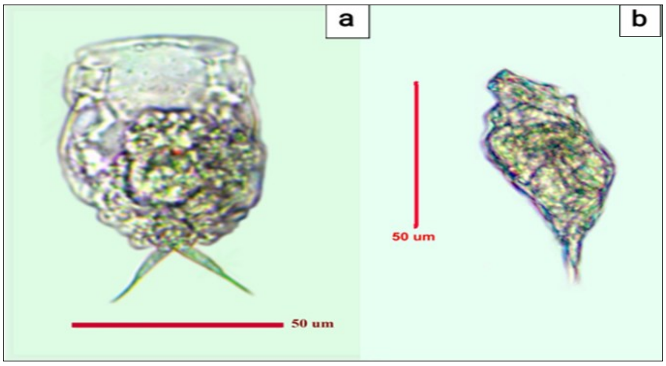

Fig. 2. *L. inermis* observed under a light microscope (a-front view, b-lateral view).

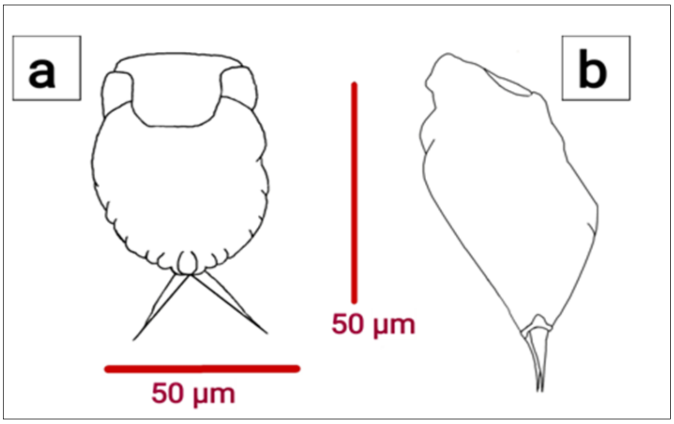

Fig. 3. Diagrammatic illustration of *L. inermis* (a - Front view, b - Lateral view).

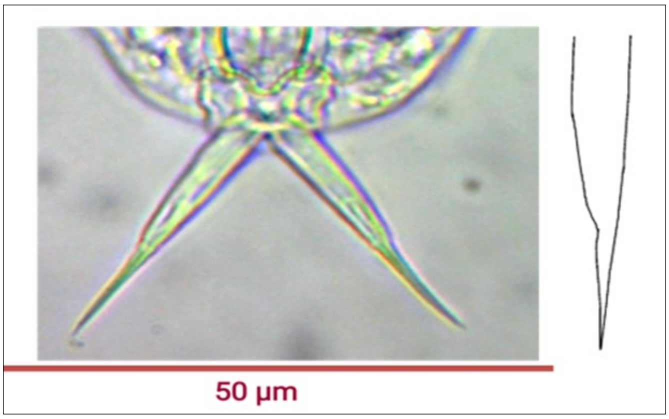

Fig. 4. Toe and claw of *L. inermis* observed under the light microscope.

Table 2. Laboratory conditions of *L. inermis* aquaculture

Parameters	Winter mean ± SD	Summer mean ± SD	Sig.
Water Temperature °C	7 ± 1	26 ± 2	0.00
Air Temperature °C	10 ± 1	30 ± 2	0.00
EC (mS)	9.6 ± 0.2	4 ± 0.1	0.00
Salinity ppt	6.1 ± 0.1	2.5 ± 0.1	0.00
TDS ppt	6.8 ± 0.1	2.4 ± 0.1	0.00
Do mg/L	7.8 ± 0.05	7.6 ± 0.1	0.055
pH	9.2 ± 0.01	9 ± 0.01	NS
Growth density (ind/L)	585 ± 20	137± 17	0.00

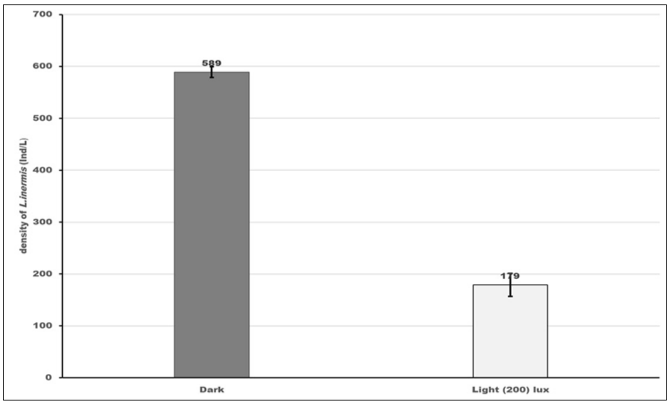

Fig. 5. Density of *L. inermis* in light and dark conditions.

Conclusion

The results of the current study demonstrate that *L. inermis* can be successfully cultivated in a high-density laboratory environment. The species exhibits a broad tolerance for salinity at low temperatures but is limited to lower salt concentrations at higher temperatures. Furthermore, its density is significantly higher in dark than light conditions.

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Authors' contributions

WAKAY and AJAAR designed and performed the experiment. AYWAK analyzed data and wrote the first draft of the manuscript; AARAJ performed statistical analysis and revised the manuscript. Both authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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