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EFFECT OF TEMPERATURE ON PRODUCTION OF *Moina brachiate* AS LIVE FEED FOR *Hypophthalmichthys molitrix* FRY

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ABSTRACT

The lack of mass culture techniques for *Moina brachiate* limits its use as a sustainable live feed in aquaculture. Present study was conducted to culture *M. brachiate* under indoor conditions at different temperatures and use it as live feed for *Hypophthalmichthys molitrix* fry. The isolated *M. brachiate* was cultured in the laboratory for 10 days in glass aquaria under two experimental treatments in which water temperature was maintained either at 28.5° ± 1.5 °C (T1) or 23.5° ± 1.5 °C (T2). *Chlorella vulgaris* was used to feed *M. brachiate* daily and the feed consumption was regularly monitored. Chemical quality parameters of the water were regularly monitored and kept within suitable range for propagation of *Moina*. At the end of the culture period, the maximum density of *M. brachiate* was found in T2 (5.92 individuals/ ml). In T1 (83.05 ± 1.39%) a decrease in density of *M. brachiate* density was observed compared to its initial count. Consumption of *C. vulgaris* by *M. brachiate* was significantly high in T2 compared to T1 (90.54 ± 3.69% and 69.32 ± 11.81% respectively). Cysts of *M. brachiate* were observed to be formed under drastic environmental conditions with hatching time of 7-8 days under optimum treatment. In the second phase of the experiment, *M. brachiate* was used to feed *H. molitrix* fry reared in glass aquaria and resulted in 86.0% survival of fry. The findings demonstrate the significant influence of temperature on *M. brachiate* propagation and suggest that its culture at 23.5° ± 1.5 °C water temperature with daily feeding of *Chlorella vulgaris***,** can be a viable live feed option for *H. molitrix* fry in aquaculture.

Keywords: Live feed, Feed Consumption, Temperature, Survival rate, *C. vulgaris.*

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INTRODUCTION

In aquaculture, the successful rearing of fish fry depends on the availability of appropriate live feed that provides essential nutrition during their early developmental stages (Shipton, 2021). Post-hatch fish larvae, during their early developmental stages, possess distinct characteristics that necessitate careful consideration when designing their feeding strategies. Firstly, their digestive systems are underdeveloped, making it challenging for them to efficiently process complex or artificial feeds (Lucas *et al*., 2019). The challenge of low survival rates of fish larvae further highlights the importance of investigating suitable live feed (Lahnsteiner *et al*., 2023). Additionally, these larvae show preference for live feed sources compared to artificial feeds (Estévez *et al.*, 2019). The use of formulated diets fortified with antioxidants and other functional ingredients has been extensively investigated to enhance the growth and immune responses of fish fingerlings (Ahmad *et al*., 2022; Hussain *et al*., 2023, Shahzad *et al*., 2023). However, the use of live feed for fish fry is an area of ongoing research with special focus on their potential to improve larval survival rates.

For optimal nourishment, the live feed chosen for post-hatch fish larvae should match its mouth size to ensure that they can be captured and consumed without excessive effort. Moreover, the ability of live food to exhibit active swimming behavior is crucial, as it triggers the larvae's predatory instincts and encourages feeding behaviors (Arevalo *et al.*, 2023). Due to nutritional variability of the fish fry, live feed organisms can also be enriched with highly nutritious feed to improve their efficacy (Suhaimi *et al.*, 2022). The mass production techniques must be investigated for the selected live food organisms to ensure a sustainable and cost-effective feed supply for larval rearing (Conceição *et al.*, 2010, Carter and Codabaccus, 2022).).

Cladocerans are the second major group of crustaceans with *Moina* and *Daphnia* being the significant genera with regard to live fish feed (Melaku *et al.*, 2022). *Moina* with its ideal size, movement pattern

and nutritional value serves as an ideal live fish feed and is preferred over brine shrimp and *Daphnia* for feeding freshwater fish fry and shrimp postlarvae (Manklinniam *et al*., 2018). While its significance as a live feed in aquaculture is undeniable, its mass production is still a challenge due to lack of practical mass culture methods. Understanding the optimal conditions for *Moina* cultivation can have significant implications for sustainable aquaculture and the production of live feed for various aquatic organisms.

The temperature necessary for optimal growth of *Moina* varies depending on the species (Neri *et al.*, 2020). Understanding the temperature dependence of *Moina sp*. is crucial for optimizing the cultivation techniques and ensuring a consistent supply of high-quality live feed for aquaculture systems. Culture techniques for a number of *Moina sp*. including *Moina macrocopa* and *Moina micrura* have been investigated as high nutritional value live feed for fish fry (Samat *et al.*, 2021, Joshua *et al.*, 2022;; Suhaimi *et al.*, 2022). However, literature about potential of *M. brachiate* as live feed and its culture techniques is rather meager. Moreover, the optimum temperature for culture of *M. brachiate* and its potential as live feed for fish fry has not been investigated yet according to the best of our knowledge. Therefore, the objective of the present study was to optimize the culture conditions for *M. brachiate*, with special focus on the influence of temperature, and to evaluate its suitability as live feed for fish fry in aquaculture systems.

MATERIALS AND METHODS

Preliminary trials for *Moina* culture and experiments to investigate effect of temperature on feed consumption and propagation of *M. brachiate* were conducted at Biology & Ecology Laboratory at Fisheries Research and Training Institute (FRTI), Lahore, Pakistan (31.589435° N, 74.465944° E) in 2021.

Collection and Isolation of *M. brachiate*: In order to collect *M. brachiate.,* an intensive survey of nineteen (19) water bodies in Lahore, Mianwali and Chakwal district was conducted from January-February, 2021. Concentrate of mixed plankton from each site was obtained with the help of 47µm "Wisconsin plankton net" and passed separately through sieves of 600µm, 210µm, 177µm, 125µm, 75µm and 38µm mesh size for the size fractionation of target species of *Moina*. Fractionated samples were subjected to microscopic examination to investigate the presence of *Moina.* Cladoceran found in fractionated samples was identified as *M. brachiate* using taxonomic keys (Błędzki and Rybak, 2016).

Preliminary trials: Preliminary trials were conducted to culture isolated *M. brachiate* in glass aquaria under laboratory conditions. The inoculum of *M. brachiate* was cultured in glass aquaria (each containing 40 L water) and fed with *C. vulgaris* or yeast to observe the effect of feed on *Moina* propagation. Water temperature in glass aquaria was maintained at 24.0 °C ± 1.0 °C. Aerators were installed in the aquaria to maintain adequate dissolved oxygen level in water. It was observed through these trials that density of *M. brachiate* increased enormously when fed with *C. vulgaris.* Use of yeast as feed led to collapse of *Moina* culture due to reduction in water dissolved oxygen to critical levels (<0.4 mg/L). On the basis of several initial experiments, *C. vulgaris* was selected to be used as feed in subsequent trials. In cases where *Moina* culture collapsed, its cysts were found at the bottom of the aquaria that were collected through 75µm sieve. Collected cysts were kept in glass jars with 200 mL tap water and regularly fed with *C. vulgaris*. Time required for hatching of cysts was recorded.

Experimental design: In order to determine the effect of temperature on the propagation of *M. brachiate*, a single factor experimental design was used with two levels for water temperature. Consumption of *C. vulgaris by M. brachiate* and change in the density of *M. brachiate* were set as the dependent variables. Two treatments (T1 and 2) each with two replicates were used for cultivation of *M. brachiate* in which water temperature was either maintained at 28.5 °C ± 1.5 °C or 23.5 °C ± 1.5 °C respectively using thermostats. Adequate dissolved oxygen level in water was maintained through aerators. Experimental trial for cultivation of *M. brachiate* lasted for 10 days.

Culture of *C. vulgaris:* Our research group has successfully optimized the culture technique for *C. vulgaris* in earlier investigations (Ashraf *et al.*, 2011). Semi-continuous culture system was used for production and stocking of *C. vulgaris* to be used as *Moina* feed. *C. Vulgaris* was isolated from plankton rich pond water using a plating method. Starter culture of *C. vulgaris* was prepared with its isolated colonies in 1000 mL conical flasks using nutritive media under indoor conditions (1000-1500 lux light, 25.0 °C ± 2.0 °C). After the exponential growth phase, stocks of *C. vulgaris* in aspirators were refrigerated at 5 °C ± 1.0 °C until used as *Moina* feed.

Cultivation of *M. brachiate:* Adult *M. brachiate,* acclimated under laboratory conditions*,* were stocked in two sets of glass aquaria at the rate of 0.3 individual/ mL in each aquarium containing 40 L water. *Moina* cultivated under both experimental treatments was fed daily with *C. vulgaris* at 10:00. About 1.0 L of preserved concentrated stock *C. vulgaris* was added in each aquarium as *M. brachiate* feed. Immediately after feeding, density of *C. vulgaris* in water of each aquarium was determined using Neubauer counting chamber. Density of *C. vulgaris* in each aquarium was again determined the next day at 9:00 before feeding.

Difference in density of *Chlorella* was used to find rate of its consumption by *M. brachiate.* Dissolved oxygen and pH of water in experimental aquaria were monitored on daily basis using electronic meters and maintained within suitable range for propagation of *Moina sp.* At the end of 10 days experimental trial, density of *M. brachiate* in each replicate of both experimental treatments was determined using Sedgewick Rafter counting chamber.

***M. brachiate* as live feed for *H. molitrix* fry:** In the next phase of the experiment, *M. brachiate* cultured in the laboratory was used as live feed for *H. molitrix* fry. *H. molitrix* fry at the age of 3 days were procured from a private fish hatchery (Muridke, Punjab), and shifted to the Laboratory at FRTI, Lahore. To determine the effect of *Moina* as live feed, *H. molitrix* fry were stocked in a set of aquaria with stocking density of 42 fry per L water. Water (500 mL) containing 5 individuals of *Moina*/ mL *was* added in each aquarium to feed *H. molitrix* fry two times a day for 10 days. The survival rate of *H. molitrix* fry in each aquarium was determined after 10 days.

Statistical Analysis: Two sample t-test was used to find significant differences in consumption of *C. vulgaris* by *M. brachiate* cultivated under two treatments. The same test was also used to find any significant variation in water dissolved oxygen and pH monitored in two treatments. All statistical analysis was two tailed and carried out using SPSS (version 26) at $P<0.05$.

RESULTS AND DISCUSSION

Identification of Moina sp.: Figure 1 describes the morphological features of isolated cladoceran that led to its identification as *M. brachiate* while Figure 2 shows the microscopic view of *M. brachiate*.

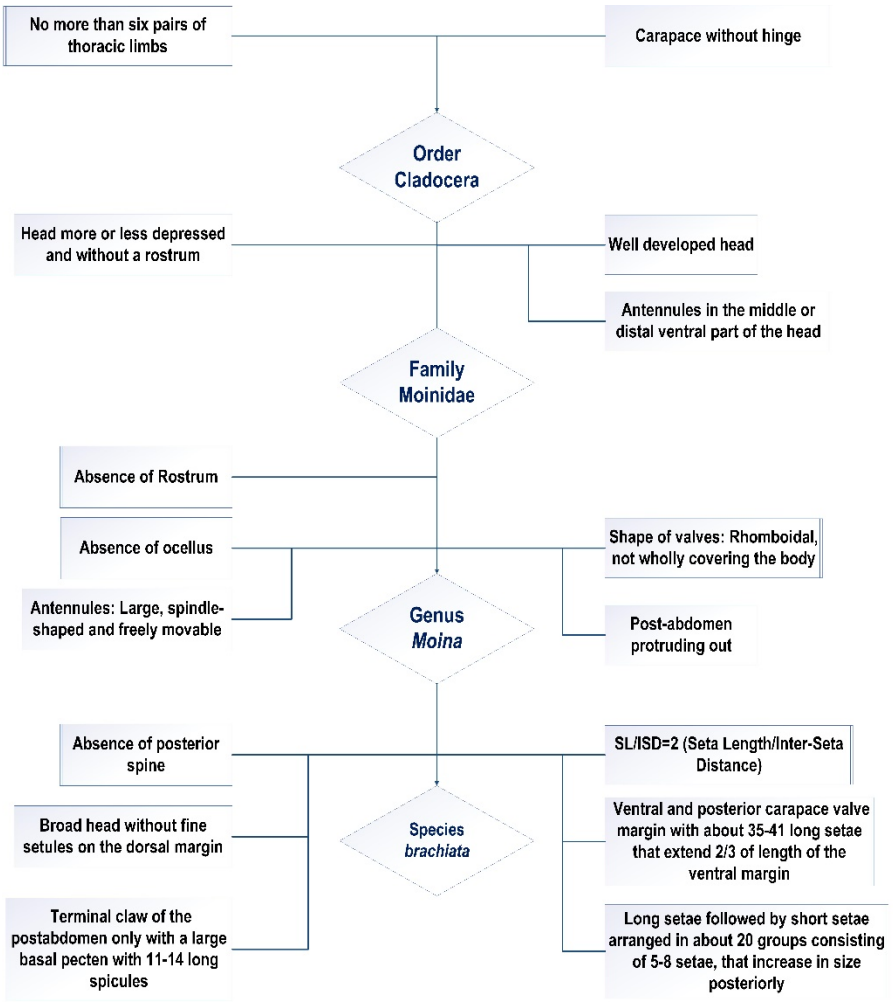

Figure 1: Identification of *M. brachiate*

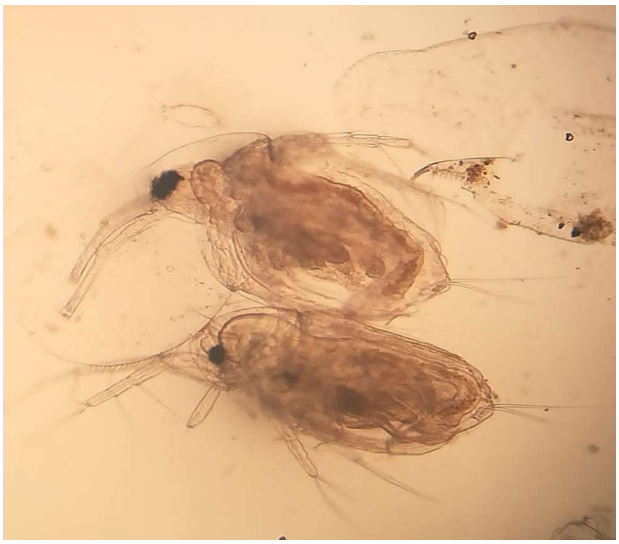

Figure 2. Microscopic view of *M. brachiate*

Effect of temperature on *M. brachiate* culture: Daily consumption of *C. vulgaris* by *M. brachiate* in the two experimental treatments is presented in Figure 3. Consumption of *C. vulgaris* in T1 was significantly low ($P<0.05$) compared to that observed in T2. Following Day 3, feed consumption remained higher than 90% in T2 till the end of culture period. In T1, feed consumption was higher at initiation of the culture period (78.23% - 85.85%) but decreased to 49.84% on Day 4 and remained <75.0% in subsequent days.

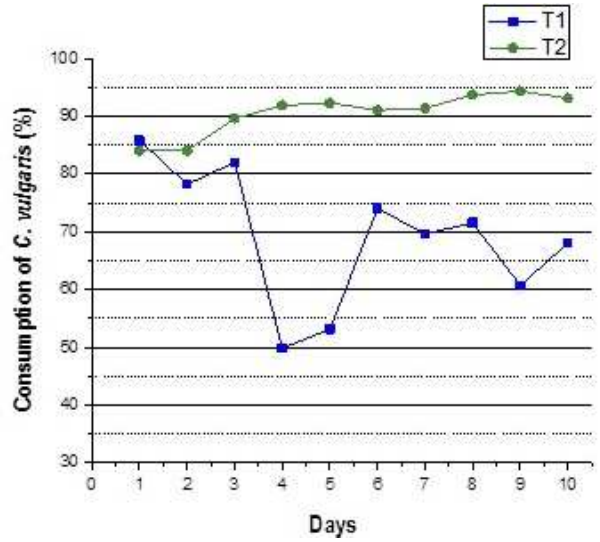

Figure 3: Consumption of *C. vulgaris* by *M. brachiate* in two experimental treatments

The results showed that *M. brachiate* could not propagate in T1 where water temperature was maintained at 28.0 °C ± 1.5 °C and die. There was 83.05 ± 1.39% decrease in the final density of *M. brachiate* compared to its initial count in T1 (Figure 4). Feed consumption was also significantly low ($P<0.05$) at 28.0 °C ± 1.5 °C compared to that observed at 23.0 °C ± 1.5 °C. Our results agree with earlier studies that reported negative effect of increase in temperature on *Moina* growth and survival. Benider *et al.* (2002) reported that optimal growth of *M. macrocopa* was observed at 20- 25 °C and its mortality started between 8th and 12th day of culture and increased progressively when temperature was maintained at 15 °C, 18 °C and 20 °C. The authors reported that at higher temperatures (25 °C and 30 °C), mortality of *Moina* started at the 6th and 7th day of culture. Complete extinction of all individuals occurred at day 14 at 25 °C and day 9 at 30 °C. Yoon *et al*. (2000) also reported reduced growth and reproductive rate of *M. macrocopa* at 25 - 28 °C compared to that observed at 20 - 25 °C. However, the effect of temperature on growth, reproductive output and longevity of *Moina* individuals seems to be species specific. Samat *et al.* (2022) reported that maximum productivity of *M. micrura* occurred at 30 °C compared to that of 15 °C, 20 °C and 25 °C. The lifespan of *Moina* individuals was, however, shorter at 30°C compared to lower temperatures. According to Engert *et al*. (2013), increase in temperature can enhance the growth rate of *Moina* sp., however, the longevity of individuals will decrease. At the end of the culture period, the maximum average density of *M. brachiate* was found in T2 (5.95 individuals/mL) compared to T1 (0.05 individual/ mL, Figure 4).

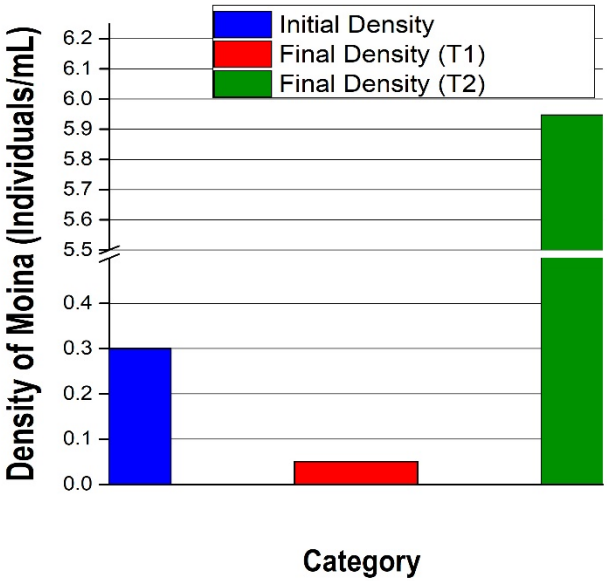

Figure 4: Change in density of *M. brachiate* in two treatments

Water quality parameters: Physico-chemical parameters of water used for *M. brachiate* culture under two treatments are presented in Table 2. In T1, water dissolved oxygen ranged from 2.65 ± 0.07 mg/L to 3.05 ±

0.21 mg/L. In T2, dissolved oxygen of water remained within 2.75 ± 0.07 mg/L to 3.0 ± 0.28 mg/L. Water pH ranged from 8.60 ± 0.0 to 8.80 ± 0.14 in T1 and 8.65 ± 0.07 to 8.85 ± 0.07 in T2. No significant difference in water dissolved oxygen and pH in two treatments was found for 10 days culture period (P>0.05).

Table 1: Water quality parameters observed during the culture of *M. brachiate.*

Days	DO (mg/L)		pH	
	T1	T2	T1	T2
1	2.85 ± 0.07	2.95 ± 0.21	8.75 ± 0.07	8.75 ± 0.21
2	2.75 ± 0.07	3.0 ± 0.14	8.80 ± 0.14	8.70 ± 0.28
3	2.90 ± 0.14	3.0 ± 0.28	8.70 ± 0.14	8.80 ± 0.0
4	2.75 ± 0.21	2.85 ± 0.07	8.70 ± 0.14	8.65 ± 0.07
5	2.65 ± 0.07	2.8 ± 0.14	8.90 ± 0	8.75 ± 0.21
6	2.75 ± 0.07	3.0 ± 0.14	8.60 ± 0	8.65 ± 0.21
7	2.80 ± 0	2.75 ± 0.07	8.80 ± 0.14	8.75 ± 0.21
8	3.0 ± 0.14	2.75 ± 0.07	8.70 ± 0.28	8.85 ± 0.07
9	3.05 ± 0.21	2.85 ± 0.07	8.80 ± 0	8.85 ± 0.07
10	2.8 ± 0.14	2.8 ± 0.28	8.60 ± 0	8.65 ± 0.07

Hatching of *M. brachiate* cysts: Cysts of *M. brachiate* took 7-8 days for hatching under optimum conditions of feeding and water quality. Figure 5 shows the microscopic view of *M. brachiate* cysts. Nevertheless, dormant stages of daphnia have been reported earlier (Cambronero and Orsini, 2018) , prior studies on formation of *Moina* cysts are limited. Our study, therefore, contributed to the broader understanding of *Moina* biology by conducting a comprehensive examination of cyst formation and hatching times.

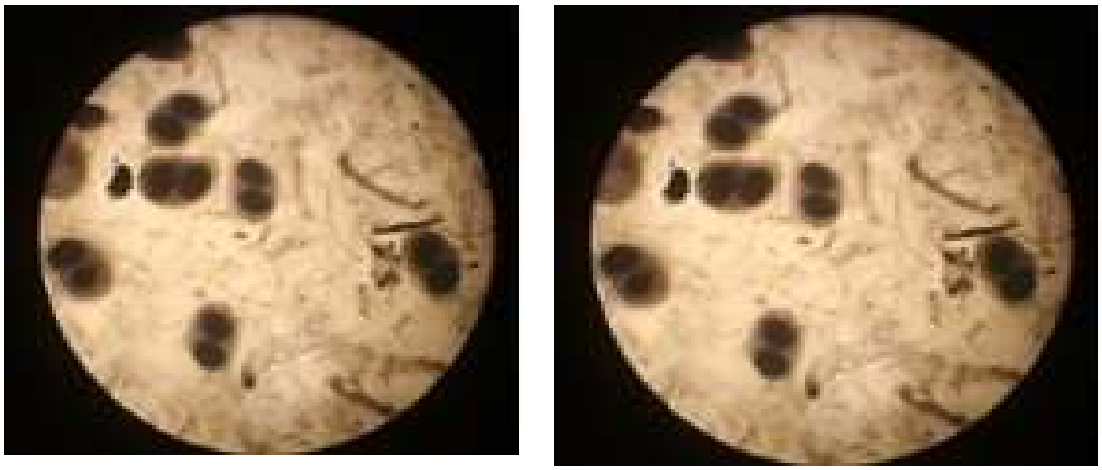

Figure 5: Cysts of *M. brachiate* observed under microscope

Use of *Chlorella* as *M. brachiate* feed: Besides temperature, food quality is also the most important factors affecting *Moina* growth and propagation. Improved nutritional composition in *Moina sp.* through utilization of appropriate feed can indirectly lead to enhanced fish growth and survival fed with this live feed (Suhaimi *et al.*, 2022). *Chlorella* is considered nutritious and viable food source for *Moina* culture (Nugroho andEkasari *et al*. 2021). Neri *et al*., (2020) reported that highly unsaturated fatty acid (HUFA) content (18:2n-6 and 18:3n-3) was high in *M. macrocopa* fed with *Chlorella sp.* compared to *Moina* fed with *Erythrobactor* sp. or *Saccharomyces cerevisiae.* Therefore, in present study, we used pure culture of *C. vulgaris* as *M. brachiate* feed.

Use of *M. brachiate* as live feed for *H. molitrix* fry:In present study, use of *M. brachiate* as feed of *H. molitrix* fry resulted in mean fish survival of 86.11%. Live food is preferred by fish larvae and fry than micropellet based feed because of the digestibility and attractiveness of live food behavior (i.e. jerky movement). A number of earlier studies have reported the potential of different *Moina sp*. as live feed for fish fry. Islam *et al.* (2017) and Suhaimi *et al.* (2022) found that feeding *M. macrocopa* resulted in 91.5% and 86.67% survival of *Oreochromis sp*. fry, respectively. Samat *et al.* (2021) reported that feeding

Oreochromis sp. fry with probiont enriched *M. micrura* resulted in 77.0% survival rate of the fry. A comparison of the efficacy of *Thermocyclops decipiens, M. micrura* and their combination as live feed for *Catla catla* fry showed the fry survival rate to be 82.0%, 82.67% and 85.07% respectively (Kadhar *et al.*, 2014). A number of studies also compared the efficiency of *Moina sp.* with *Artemia nauplii* as live feed for fish fry and reported similar results (Kotani *et al.*, 2016, Aruho *et al.*, 2020). Neverthless, use of *M. brachiate,* as live feed for fish, has not been investigated yet according to the best of our knowledge.

***M. brachiate*: Insights from historical and contemporary studies:** Reported in 1820 by Louis Jurine in his French book "History of the Monocles, which are located near Geneva," *M. brachiate* is a widely distributed freshwater species found in river floodplains, oxbow lakes, ponds, and hypertrophic water bodies. It was reported in natural water bodies of Lahore, Punjab, Pakistan by G.I. Arora in 1930 (Arora, 1931). *M. brachiate* displays a brief egg development period and a high rate of egg production. This species exhibits adaptability to low dissolved oxygen concentration, surviving at levels as low as 1.2 mg/L. In the present study, water dissolved oxygen was maintained in the range of 2.65-3.05 mg/L. Notably, *M. brachiate* can also tolerate saltwater conditions. It shows a preference for alkaline environments, thriving in waters with pH levels between 7.5 and 11.0. This is in line with the findings of present study values for pH 8.60-8.85. While capable of reaching high densities exceeding 10.0 individuals/mL, the maximum average density recorded in the present study is approximately 5.95 individuals/mL in freshwater and agrees with previous research (Illyová and Matečný, 2014; Tóth *et al.*, 2014, Błędzki and Rybak, 2016).

Reports on its population structure, morphological features and distribution of *M. brachiate* are available in literature (Lieberman, 1970; Nédli *et al.*, 2014; Tackx *et al.*, 2004) and a few studies based on feeding behavior of gamefishes common in North America and Europe have also targeted this species (Barkoh, 1984; Barkoh and Modde, 1987; Wickstrom, 1984). However, culture techniques for optimum production of *M. brachiate* and its use as live feed for fish fry have not been investigated yet. Results of present study will therefore aid in ongoing research on potential and viable live feed for freshwater fish fry.

Conclusion: In conclusion, the results of the present study demonstrate an indoor culture technique of *M. brachiate* that can be used as a sustainable live feed source in aquaculture. In the future, research should be conducted to investigate the effects of culture conditions on the nutritional composition of *M. brachiate*, as well as its enrichment using viable feed ingredients. Moreover, determining the potential of *M. brachiate* as live feed for growth and survival of commercially important fish and shrimps during their early life stages is imperative to determine the full potential of this locally available cladoceran.

Authors Contribution: Zahid Sharif Mirza and Tariq Rashid conceptualized the study, conducted experimental work, wrote and reviewed the manuscript. Javairia Shafi helped in data interpretation and writing of manuscript. Ahmed Shakeeb helped in experimental work. Abida Saeed helped in writing the manuscript.

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