



Original Research Paper

Influence of Different Water Sources on Population density, Intrinsic Growth Rate and Culture Performance of *Daphnia pulex* and *Moina micrura*

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Key Words Abstract

Aquaculture, Baker's yeast, Cladocera, Live feed, Rainwater, Reverse osmosis water.

Water quality is a critical factor influencing the mass culture of freshwater cladocerans used as live feed in aquaculture. The present study evaluated the effects of three water sources such as borewell water, rainwater and reverse osmosis (RO) water on the population growth of *Daphnia pulex* and *Moina micrura* cultured under laboratory conditions using dried baker's yeast as the sole feed. Triplicate cultures were maintained for 10 days, and population density was determined daily using a Sedgwick–Rafter counting chamber. The intrinsic population growth rate (r) was calculated during the exponential growth phase, while one-way and two-way ANOVA were performed to evaluate the effects of water source and culture duration. Rainwater consistently supported the highest population density in both species. *Daphnia pulex* attained a maximum population density of 107.0 ± 6.48 ind. mL^{-1} on Day 6, whereas *Moina micrura* reached 119.0 ± 2.33 ind. mL^{-1} on Day 7. RO water produced the second-highest population densities, while borewell water consistently resulted in the lowest production. The intrinsic population growth rate of *D. pulex* was highest in rainwater (0.480 ± 0.055 day^{-1}), whereas *M. micrura* exhibited the highest growth rate in RO water (0.410 ± 0.041 day^{-1}). One-way ANOVA revealed significant differences in mean population density among water sources for both *D. pulex* ($F_{2, 27} = 6.365$, $P = 0.005$) and *M. micrura* ($F_{2, 27} = 4.740$, $P = 0.017$). Two-way ANOVA further demonstrated that culture day, water source and their interaction significantly affected population density ($P < 0.001$) in both species. The findings indicate that rainwater is the most suitable water source for the laboratory culture of *D. pulex* and *M. micrura*, while RO water represents a practical alternative where rainwater is unavailable. These results provide useful baseline information for optimizing freshwater cladoceran production as live feed for aquaculture.

Introduction

Aquaculture has become one of the fastest growing food production sectors worldwide and is expected to play an increasingly important role in meeting the rising global demand for high quality animal protein. According to the Food and Agriculture Organization, aquaculture now contributes more than half of the aquatic animals consumed globally, highlighting its importance in ensuring food security, nutritional sustainability, and economic development. However, the rapid expansion of aquaculture depends largely on improving larval survival and juvenile production, as the early developmental stages of fish and crustaceans remain highly vulnerable to nutritional deficiencies, environmental stress, and disease. Consequently, optimizing larval rearing practices has become one of the primary objectives of modern aquaculture research (Ashaaria et al., 2024). The success of larviculture is strongly influenced by the availability

of nutritionally balanced live feed during the first-feeding stage. Newly hatched larvae possess an incompletely developed digestive system and exhibit limited feeding efficiency, making them highly dependent on live food organisms that are easily captured, digested, and assimilated (Samat et al., 2020). Although substantial progress has been achieved in the development of formulated microdiets, artificial feeds are still unable to completely replace live feed because they often lack appropriate particle size, movement, digestive enzymes, and certain essential nutrients required for normal larval growth and development (Lavens and Sorgeloos, 1996; Lahnsteiner et al., 2023). Live food organisms not only provide balanced nutrition but also stimulate natural feeding behaviour, thereby enhancing feed acceptance, digestive enzyme activity, nutrient utilization, growth performance and survival of cultured aquatic species (Mona et al., 2017). Among the diverse live food organisms used in aquaculture, freshwater zooplankton plays a fundamental ecological and nutritional role. More than 75%

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of freshwater fish species depend on planktonic organisms during at least one stage of their life cycle, emphasizing the significance of zooplankton in both natural ecosystems and hatchery production systems (Rahman et al., 2023). Zooplankton acts as a vital intermediary between primary producers and higher trophic levels by converting phytoplankton and microbial biomass into highly digestible animal protein, thereby facilitating energy transfer through aquatic food webs (Sultana et al., 2023; Rasdi et al., 2020). In addition to supporting larval nutrition, zooplankton contributes to nutrient recycling, maintenance of water quality, and overall ecosystem functioning. Consequently, efficient production of high-quality zooplankton has become an important component of sustainable aquaculture practices. Live food organisms are frequently referred to as "living nutritional capsules" because they naturally contain an optimal balance of proteins, lipids, carbohydrates, vitamins, minerals, digestive enzymes, essential amino acids, and highly unsaturated fatty acids required for the normal growth and physiological development of aquatic organisms (Mona et al., 2017). Compared with formulated feeds, live feed exhibits superior digestibility, greater palatability, and enhanced nutrient bioavailability, resulting in improved larval growth, survival, stress tolerance, and resistance to disease (Lomartire et al., 2021). Furthermore, the integration of live-feed production into aquaculture systems can reduce dependence on expensive commercial diets, lower production costs, and contribute to environmentally sustainable aquaculture by improving feed utilization efficiency and minimizing nutrient losses (Kashem et al., 2023). Among the various groups of freshwater zooplankton used as live feed, cladocerans have attracted considerable attention because of their rapid reproductive capacity, short life cycle, ease of mass culture, and excellent nutritional quality. These characteristics make them one of the most suitable live food organisms for freshwater hatcheries, ornamental fish production, and aquaculture research. Ecologically, cladocerans occupy an important position in freshwater food webs by grazing on phytoplankton, bacteria, and suspended organic matter, thereby transferring energy from primary producers to higher trophic levels while contributing to nutrient cycling and ecosystem stability (Sultana et al., 2023; Rasdi et al., 2020). Their ability to efficiently convert low-cost feed resources into nutrient-rich biomass further enhances their value in sustainable aquaculture systems. Among freshwater cladocerans, *Daphnia pulex* and *M. micrura* are widely recognized as two of the most important live-feed organisms because of their favourable biological characteristics and high nutritional value (Ortega-Salas and Reyes-Bustamante, 1998; Rodríguez et al., 2013). Both species reproduce predominantly through cyclical parthenogenesis, enabling rapid population growth under favourable environmental conditions without the need for fertilization. Their short generation time, early maturity, high fecundity, and continuous production of neonates allow the maintenance of dense cultures capable of supplying large quantities of live feed throughout the larval rearing period (Lampert, 2011). These characteristics make them particularly attractive

for hatcheries that require a reliable and continuous supply of live food. The nutritional composition of cladocerans further contributes to their widespread application in aquaculture. They are rich in high-quality proteins, essential amino acids, lipids, vitamins, minerals, digestive enzymes, and polyunsaturated fatty acids, all of which are indispensable for larval growth and development (Samat et al., 2020b). In particular, *M. micrura* has been reported to contain high crude protein levels together with essential amino acids such as lysine, making it a valuable dietary component for freshwater fish larvae (Ovie and Ovie, 2006). Moreover, because cladocerans readily accumulate nutrients from their culture medium, they can be enriched with essential fatty acids, vitamins, probiotics, pigments, or immunostimulants before feeding, thereby improving the nutritional quality of live feed and enhancing larval performance (Lavens and Sorgeloos, 1996; Dhert, 1996). Beyond their role as live feed, these two species also serve as important model organisms in ecological, toxicological, and physiological research. *Daphnia pulex* is extensively used as a freshwater bioindicator because of its high sensitivity to changes in water chemistry, pollutants, and environmental stressors (Abdullahi et al., 2022). In contrast, *M. micrura* exhibits comparatively greater tolerance to environmental fluctuations, allowing it to survive and reproduce successfully under a wider range of physicochemical conditions (Oh and Choi, 2012). These contrasting ecological characteristics provide an excellent opportunity to compare species-specific responses to different environmental conditions and identify suitable culture strategies for aquaculture applications. As non-selective filter feeders, both *D. pulex* and *M. micrura* continuously ingest suspended microorganisms, microalgae, bacteria, yeast cells, and detrital organic particles from the surrounding water (Otero et al., 2013; Rodríguez et al., 2013). Consequently, their growth, survival, fecundity, and population density are influenced not only by food quality but also by the physicochemical characteristics of the culture medium. Environmental variables such as pH, dissolved oxygen, hardness, electrical conductivity, alkalinity, and dissolved mineral composition regulate physiological processes including filtration efficiency, osmoregulation, metabolism, moulting, and embryonic development (Lampert, 2011; Dodson and Frey, 2001). Therefore, identifying suitable environmental conditions is fundamental for achieving stable and high-yield cladoceran cultures for freshwater aquaculture. The productivity of cladoceran cultures is determined by the combined influence of nutritional and environmental factors. Among these, diet is one of the principal determinants of growth, reproduction, and population development because it directly affects energy acquisition, nutrient assimilation, and reproductive output. Numerous studies have demonstrated that microalgae, yeast, bacteria, agricultural by-products, and mixed diets can support cladoceran growth; however, their effectiveness varies depending on the nutritional composition of the feed and the environmental conditions under which the organisms are cultured (Samat et al., 2020b; Ricci et al., 2003). Among the various feeds used in laboratory and hatchery cultures, baker's yeast (*Saccharomyces cerevisiae*) has gained considerable attention because of its high protein content, vitamin richness, ease of preparation, low cost, year round

availability, and ability to support rapid population growth under controlled conditions (Ricci et al., 2003). Furthermore, yeast can be readily standardized as a diet, minimizing variations associated with algal cultures and making it particularly suitable for comparative laboratory experiments. Although diet provides the nutrients required for growth and reproduction, its utilization depends largely on the physicochemical characteristics of the culture medium. Water quality directly influences physiological processes such as filtration efficiency, osmoregulation, respiration, nutrient assimilation, enzymatic activity, moulting, embryonic development, and parthenogenetic reproduction in freshwater cladocerans (Lampert, 2011; Dodson and Frey, 2001). Consequently, even when identical diets are provided, differences in water chemistry may result in considerable variation in population growth, survival, reproductive performance, and culture stability. Freshwater used for aquaculture differs substantially in its physicochemical properties depending on its origin. Borewell water generally contains relatively high concentrations of dissolved minerals and hardness-producing ions such as calcium and magnesium, whereas rainwater is comparatively soft and contains lower concentrations of dissolved salts. In contrast, reverse osmosis (RO) water is characterized by extremely low concentrations of dissolved minerals because most ions are removed during the purification process. These differences influence important water-quality parameters including electrical conductivity, total dissolved solids, alkalinity, hardness, and ionic composition, all of which regulate the physiological performance of freshwater zooplankton (Wetzel, 2001; Lampert, 2011). Appropriate concentrations of calcium and magnesium are essential for exoskeleton formation, successful moulting, muscle function, and reproduction, whereas excessive mineral concentrations or severe mineral deficiency may impair normal physiological processes and reduce reproductive success (Jeziorski et al., 2008). Likewise, dissolved oxygen, pH, alkalinity, and electrical conductivity influence metabolic activity, filtration efficiency, nutrient uptake, and the maintenance of ionic balance, ultimately determining the growth and stability of cladoceran populations (Rajagopal et al., 2010; Otero et al., 2013). Several studies have investigated the effects of feed type, algal species, probiotic supplementation, temperature, photoperiod, salinity, stocking density, and nutrient enrichment on the culture performance of cladocerans

(Ricci et al., 2003; Samat et al., 2020b). These investigations have substantially improved current knowledge regarding nutritional requirements and culture management. Nevertheless, comparatively little attention has been given to evaluating the influence of commonly available freshwater sources that differ naturally in mineral composition and physicochemical characteristics under standardized feeding conditions. In practical hatchery operations and laboratory cultures, Borewell water, rainwater, and RO water are frequently used based on their local availability rather than on scientific evidence supporting their suitability for cladoceran production. Consequently, the selection of an appropriate water source often remains empirical, resulting in inconsistent culture performance and reduced biomass production. The present study addresses this knowledge gap by evaluating the culture performance of two economically important freshwater cladocerans, *Daphnia pulex* and *M. micrura*, in three commonly available water sources differing in physicochemical characteristics while maintaining identical feeding conditions using baker's yeast as the sole diet. We hypothesized that differences in the mineral composition and water quality of Borewell water, rainwater, and reverse osmosis water would significantly influence the population density, reproductive performance, and survival of both cladoceran species. Therefore, the objectives of this study were to compare the population density, intrinsic growth rate, and survival of *D. pulex* and *M. micrura* cultured in these three water media and to identify the most suitable water source for efficient and sustainable cladoceran production. The findings of this study are expected to contribute to the development of standardized, cost-effective, and reliable live-feed culture protocols that can enhance freshwater hatchery production and support sustainable aquaculture practices.

2. Materials and Methods

2.1 Experimental organisms

Laboratory cultures of *D. pulex* and *M. micrura* were established from stock cultures maintained in the PG and Research Department of Zoology, VHNSN College, Virudhunagar. Healthy and actively reproducing parthenogenetic individuals were selected for the experiment to ensure uniformity among treatments. Prior to the experiment, both species were acclimatized to laboratory conditions for seven days. Representative photomicrographs of the experimental cladoceran species are shown in Figure 1.

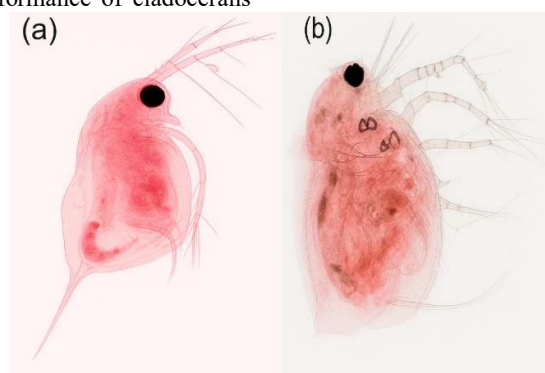


Figure 1. (a) *Daphnia pulex* and (b) *Moina micrura*.

2.2 Physicochemical characteristics of water

The physicochemical characteristics of the three experimental water sources, namely Borewell water (T₁), rainwater (T₂), and reverse osmosis (RO) water (T₃), were analysed before the commencement of the culture experiment following the procedures described by Madasamy and Jaiswal (2021). The parameters analysed included pH, water temperature, dissolved oxygen, electrical conductivity, total dissolved solids, total hardness, carbonate, bicarbonate, chloride, sulphate, calcium, magnesium, sodium, potassium, residual sodium carbonate, sodium adsorption ratio, and magnesium-to-calcium ratio. The physicochemical parameters were analysed at the Department of Agriculture and Farmers Welfare, Virudhunagar. Water temperature was measured using a laboratory thermometer, whereas dissolved oxygen was determined by the Winkler method using starch as the indicator (Ekramuddin, 2024).

2.3 Experimental design

The experiment was conducted under laboratory conditions using a completely randomized design. Each cladoceran species (*D. pulex* and *M. micrura*) was cultured separately in the three different water media (Borewell water, rainwater, and RO water), resulting in six treatment combinations. Cultures were maintained in 25-L plastic containers with a working volume of 20 L. Each treatment was performed in triplicate. The initial stocking density was 20 individuals L⁻¹, corresponding to 400 individuals per culture container. The experimental period lasted 10 days, during which no water exchange or aeration was provided. Cultures were maintained under the natural photoperiod at ambient laboratory temperature throughout the study.

2.4 Feeding protocol

Both *D. pulex* and *M. micrura* were fed exclusively with baker's yeast (*Saccharomyces cerevisiae*) throughout the experimental period. A fresh yeast stock solution was prepared daily by dissolving 0.5 g of baker's yeast in 1 L of distilled water. After thorough mixing, 250 mL of the yeast suspension was added once daily to each 20 L culture container. The feeding protocol was adopted from Hena et al. (2025) with slight modification to suit the experimental conditions. An identical feeding regime was maintained for all treatments to ensure that any differences in population growth, survival, and reproductive performance were attributable to the physicochemical characteristics of the culture media rather than differences in feed availability.

2.5 Population assessment

Population density was determined daily for each treatment using a Sedgwick–Rafter counting chamber (50 × 20 × 1 mm; capacity 1 mL). Before sampling, the culture medium was gently mixed to ensure a homogeneous distribution of cladocerans. A 100 mL sample was collected from each culture container, thoroughly mixed, and a 1 mL aliquot was immediately transferred to the counting chamber. Cladocerans were

counted under a binocular microscope at 10× magnification. Population density was expressed as individuals per millilitre (ind. mL⁻¹). The mean population density for each treatment was calculated from three independent replicates. The intrinsic population growth rate (*r*) was calculated following Krebs (1985) using the equation:

$$r = \frac{(\ln N_t - \ln N_0)}{t}$$

Where *N*₀ is the initial population density (ind. mL⁻¹), *N*_t is the maximum population density (ind. mL⁻¹) attained during the exponential growth phase, and *t* is the time interval (days) between the initial population and the day on which the maximum population density was recorded. In the present study, *t* = 6 days for *D. pulex* and *t* = 7 days for *M. micrura*.

2.6 Statistical analysis

All experiments were conducted in triplicate, and the results are presented as mean ± standard deviation (SD). Prior to statistical analysis, the assumptions of normality and homogeneity of variances were evaluated using the Shapiro Wilk and Levene's tests, respectively. Differences in population density and intrinsic population growth rate among the three water media were analysed separately for *D. pulex* and *M. micrura* using one-way analysis of variance (ANOVA). When significant differences were observed, treatment means were separated using Tukey's Honestly Significant Difference multiple comparison test. To determine the effects of water medium, culture period (days), and their interaction on population density, a two-way analysis of variance (ANOVA) was performed separately for each cladoceran species. All statistical analyses were carried out using IBM SPSS Statistics version 21 and differences were considered statistically significant at *P* < 0.05.

3. Results

3.1 Physicochemical characteristics of the culture media

The physicochemical characteristics of Borewell water, rainwater and reverse osmosis (RO) water used for culturing *D. pulex* and *M. micrura* are presented in Table 1. The pH of the three water sources ranged from 6.5 to 7.8, with Borewell water exhibiting slightly alkaline conditions, whereas rainwater and RO water remained close to neutral. Electrical conductivity was highest in Borewell water (7.3 μS m⁻¹), followed by RO water (6.0 μS m⁻¹) and rainwater (4.0 μS m⁻¹), indicating a greater concentration of dissolved ions in Borewell water. Among the major ions, Borewell water contained the highest concentrations of bicarbonate, chloride (45.4 meq L⁻¹), sulphate (14.0 meq L⁻¹), calcium (8.7 meq L⁻¹), magnesium (18.7 meq L⁻¹) and sodium (44.8 meq L⁻¹), whereas considerably lower concentrations were recorded in rainwater and RO water. Total dissolved solids and total hardness followed a similar trend, with Borewell water exhibiting the highest values. Overall, rainwater and RO water were characterized by lower mineral content and electrical conductivity than Borewell water, providing more favourable physicochemical conditions for the culture of *D. pulex* and *M. micrura*.

Table 1. Physicochemical characteristics of borewell water (T₁), rainwater (T₂), and reverse osmosis (RO) water (T₃) used for culturing *D. pulex* and *M. micrura*.

Parameter	Borewell water (T ₁)	Rainwater (T ₂)	RO water (T ₃)
pH	7.8	6.5	6.9
Electrical conductivity (dS m ⁻¹)	7.30	0.14	0.16
Carbonate (meq L ⁻¹)	0.8	0.5	0.5
Bicarbonate (meq L ⁻¹)	12.8	1.1	1.1
Chloride (meq L ⁻¹)	45.4	0.2	0.3
Sulphate (meq L ⁻¹)	14.0	0.1	0.1
Calcium (meq L ⁻¹)	8.7	0.8	0.6
Magnesium (meq L ⁻¹)	18.7	0.2	0.4
Sodium (meq L ⁻¹)	44.8	0.1	0.1
Potassium (meq L ⁻¹)	0.2	0.1	0.1
Residual sodium carbonate	0.0	0.6	0.6
Sodium adsorption ratio	12.10	0.14	0.14
Magnesium : Calcium ratio	2.15	0.25	0.67

3.2 Population density of *D. pulex* and *M. micrura* cultured in different water source

Water source significantly influenced the population density of *D. pulex* and *M. micrura* cultured with baker's yeast as the sole feed. Among the three water sources evaluated, rainwater consistently supported the highest population densities, followed by RO water, whereas Borewell water produced the lowest populations throughout the culture period (Figures 2 and 3). In both species, population density increased rapidly during the initial culture phase, reached a maximum between Days 6 and 7, and subsequently declined during the remaining culture period. This decline was most likely associated

with increased stocking density, depletion of available food, accumulation of metabolic wastes and deterioration of water quality. For *D. pulex*, the highest population density (107.0 ± 6.48 ind. mL⁻¹) was recorded in rainwater on Day 6, followed by RO water (83.0 ± 2.40 ind. mL⁻¹), whereas Borewell water supported the lowest peak population (51.0 ± 0.89 ind. mL⁻¹). Likewise, *M. micrura* attained the highest population density (119.0 ± 2.33 ind. mL⁻¹) in rainwater on Day 7, followed by RO water (102.0 ± 2.90 ind. mL⁻¹) and Borewell water (44.0 ± 1.20 ind. mL⁻¹). These findings indicate that rainwater provided more favourable physicochemical conditions for cladoceran growth than bore well or RO water.

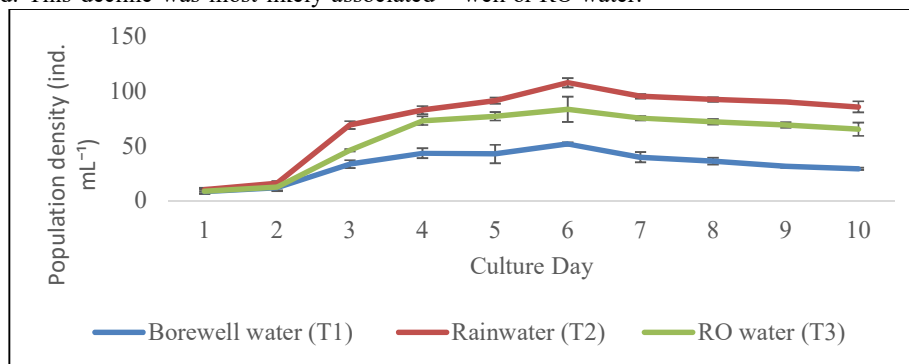


Figure 2. Population density (ind. mL⁻¹) of *Daphnia pulex* cultured in borewell water (T₁), rainwater (T₂), and reverse osmosis (RO) water (T₃) over a 10-day culture period. Values are presented as mean $\pm$ SD of three independent replicates (n = 3).

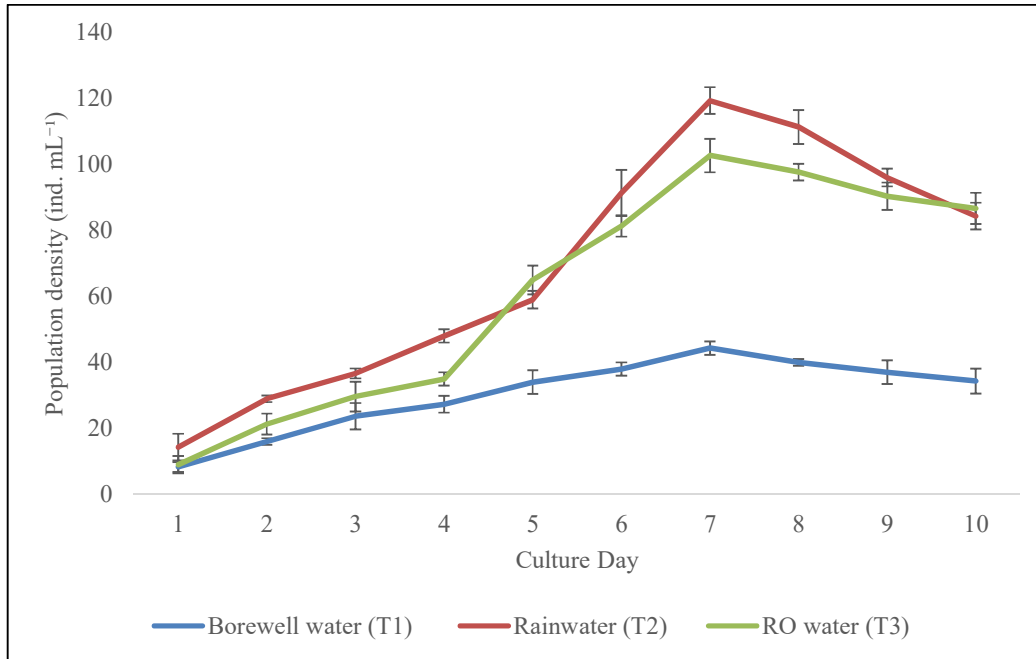


Figure 3. Population density (ind. mL⁻¹) of *Moina micrura* cultured in borewell water (T₁), rainwater (T₂), and reverse osmosis (RO) water (T₃) over a 10-day culture period. Values are presented as mean $\pm$ SD of three independent replicates (n = 3).

3.3 Intrinsic population growth rate of *D. pulex* and *M. micrura* cultured in different water sources

The intrinsic population growth rate of *Daphnia pulex* and *Moina micrura* differed significantly among the three water sources (Figure 3). In *D. pulex*, the highest intrinsic population growth rate was recorded in rainwater (0.480 ± 0.055 day⁻¹), followed by RO water (0.455 ± 0.032 day⁻¹), whereas borewell water exhibited the lowest growth rate (0.367 ± 0.031 day⁻¹). One-way ANOVA revealed a significant effect of water source on growth rate ($F_{2, 6} = 6.339$, $P = 0.033$). Tukey's HSD test indicated that rainwater supported a significantly higher

growth rate than borewell water ($P < 0.05$), while RO water did not differ significantly from either treatment. Similarly, *M. micrura* exhibited significant differences in intrinsic population growth rate among the water sources ($F_{2, 6} = 7.705$, $P = 0.022$). The highest growth rate was recorded in RO water (0.410 ± 0.041 day⁻¹), followed by rainwater (0.358 ± 0.045 day⁻¹), whereas borewell water produced the lowest growth rate (0.280 ± 0.037 day⁻¹). Tukey's HSD test showed that RO water supported a significantly higher growth rate than borewell water ($P < 0.05$), while rainwater did not differ significantly from either RO water or borewell water.

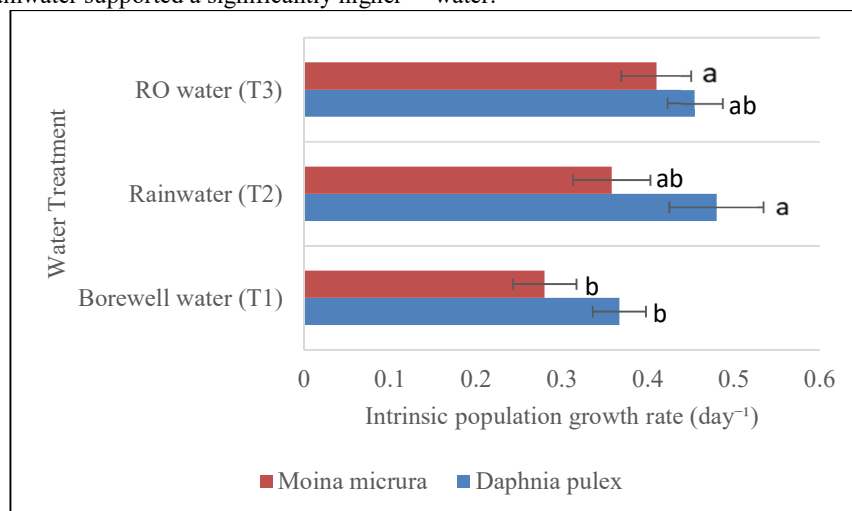


Figure 4. Intrinsic population growth rate (day⁻¹) of *Daphnia pulex* and *Moina micrura* cultured in borewell water (T₁), rainwater (T₂), and reverse osmosis (RO) water (T₃). Values are presented as mean $\pm$ SD (n = 3). Different lowercase letters indicate significant differences among water sources within each species according to Tukey's HSD test ($P < 0.05$).

3.4 Effect of water source on cladoceran population density

One-way ANOVA demonstrated that water source significantly influenced the mean population density of both *Daphnia pulex* and *Moina micrura*. In *D. pulex*, population density differed significantly among the three water sources ($F_{2, 27} = 6.365$, $P = 0.005$). Tukey's HSD multiple comparison test showed that both rainwater and RO water supported significantly higher population densities than borewell water ($P < 0.05$), whereas no significant difference was observed between rainwater and RO water ($P > 0.05$). Similarly, *M. micrura* exhibited significant differences in mean population density among the three water sources ($F_{2, 27} = 4.740$, $P = 0.017$). Tukey's HSD test likewise indicated significantly greater population densities in rainwater and RO water than in borewell water ($P < 0.05$), while the difference between rainwater and RO water was not statistically significant ($P > 0.05$).

3.5 Effect of culture day and water source on cladoceran population (Two-way ANOVA)

Two-way ANOVA revealed significant effects of culture day, water source, and their interaction on the population density of both *Daphnia pulex* and *Moina micrura*. For *D. pulex*, Levene's test indicated significant heterogeneity of error variances among treatment combinations ($F_{29,60} = 2.790$, $P < 0.001$), whereas the assumption of homogeneity of variances was satisfied for *M. micrura* ($F_{29,60} = 1.277$, $P = 0.209$). Culture day significantly affected population density in both *D. pulex* ($F_{9,60} = 341.249$, $P < 0.001$, partial $\eta^2 = 0.981$) and *M. micrura* ($F_{9,60} = 553.688$, $P < 0.001$, partial $\eta^2 = 0.988$), indicating very large effects of culture duration. Water source also significantly affected population density in *D. pulex* ($F_{2,60} = 827.255$, $P < 0.001$, partial $\eta^2 = 0.965$) and *M. micrura* ($F_{2,60} = 1046.261$, $P < 0.001$, partial $\eta^2 = 0.972$). Significant culture day $\times$ water source interactions were observed for both *D. pulex* ($F_{18,60} = 24.361$, $P < 0.001$, partial $\eta^2 = 0.880$) and *M. micrura* ($F_{18,60} = 53.997$, $P < 0.001$, partial $\eta^2 = 0.942$), indicating that the effect of water source varied across the culture period. The models showed very high explanatory capacity for *D. pulex* ($R^2 = 0.989$; adjusted $R^2 = 0.983$) and *M. micrura* ($R^2 = 0.993$; adjusted $R^2 = 0.989$). For both species, population density was highest in rainwater, followed by RO water and borewell water, with Bonferroni-adjusted comparisons confirming significant differences among all water sources ($P < 0.001$). Population density increased during the initial culture period, reached its highest overall mean on Day 6 in *D. pulex* and Day 7 in *M. micrura*, and subsequently declined through the end of the culture period. The significant interactions further indicated that the magnitude and temporal pattern of population change differed among water sources.

4. Discussion

The present study demonstrated that water source significantly influenced the culture performance of *D. pulex* and *M. micrura*. Among the three water sources evaluated, rainwater consistently supported the highest population density of both species, followed by reverse osmosis (RO) water, whereas Borewell water produced

the lowest population densities. These differences were closely associated with variations in the physicochemical characteristics of the culture media. Borewell water contained higher electrical conductivity, hardness and dissolved mineral concentrations than rainwater and RO water. Elevated concentrations of bicarbonate, chloride, sulphate, calcium, magnesium and sodium indicated a greater ionic load, which may impose osmotic stress on freshwater cladocerans and consequently reduce reproductive performance. In contrast, rainwater exhibited lower conductivity and lower dissolved mineral content while maintaining a near-neutral pH, providing more favourable conditions for growth and reproduction. Although RO water was produced from Borewell water, the reverse osmosis process substantially reduced dissolved salts and hardness, thereby improving its suitability for cladoceran culture. These observations agree with the findings of Mona *et al.* (2014), who reported that increasing salinity significantly reduced survival, reproduction and population growth of *Daphnia longispina*. The authors concluded that although freshwater cladocerans tolerate moderate ionic concentrations, excessive salinity adversely affects physiological performance by increasing the energetic cost of osmoregulation. Similar mechanisms probably contributed to the lower population densities observed in Borewell water in the present study. Both *D. pulex* and *M. micrura* exhibited a characteristic sigmoid population growth pattern. Population density increased rapidly during the first week of culture, reached its maximum on Day 6 in *D. pulex* and Day 7 in *M. micrura*, and subsequently declined. The decline observed after the exponential growth phase is likely associated with increased population density, depletion of available food, accumulation of metabolic wastes and progressive deterioration of water quality, which collectively limit reproduction and survival under batch culture conditions. The superior population growth observed in rainwater is consistent with previous investigations on freshwater cladocerans. Alva-Martínez *et al.* (2007) reported that *Moina macrocopa* attained maximum population density within approximately 10 days under favourable freshwater conditions and adequate algal nutrition. Similarly, Gama-Flores *et al.* (2010) demonstrated that *D. pulex* achieved maximum population growth under optimal environmental conditions with sufficient food availability. More recently, Samara *et al.* (2025) reported that *Moina macrocopa* cultured in diluted biofloc wastewater exhibited significantly greater population density than cultures maintained in highly concentrated wastewater, indicating that excessive nutrient and dissolved solid concentrations negatively affect cladoceran production. These studies collectively support the present findings that water quality is a major determinant of cladoceran productivity. The intrinsic population growth rate further confirmed the influence of water source on cladoceran culture performance. *Daphnia pulex* exhibited the highest intrinsic growth rate in rainwater, whereas *Moina micrura* attained the highest growth rate in RO water. In both species, borewell water consistently produced the lowest growth rates, indicating that elevated dissolved mineral concentrations adversely affected population development. The higher growth rates observed in rainwater and RO water suggest that reduced ionic concentration and improved

water quality promote more favourable physiological conditions for reproduction during the exponential growth phase. Similar relationships between water quality and cladoceran performance have been reported by Samat et al. (2020), Hena et al. (2025) and Contreras-Tapia et al. (2026), who demonstrated that favourable environmental conditions and appropriate culture management significantly enhance biomass production and reproductive performance. Statistical analyses further supported these observations. One-way ANOVA demonstrated that water source significantly influenced both the population density and intrinsic population growth rate of the two cladoceran species. In addition, two-way ANOVA showed that culture day, water source and their interaction significantly affected population density, indicating that cladoceran production depends on both water quality and culture duration. Previous investigations have also demonstrated that feed quality strongly influences cladoceran production. Latib et al. (2020) reported greater population density of *M. micrura* when cultured with *Chlorella vulgaris* than with baker's yeast alone. Likewise, Ritu et al. (2023) observed enhanced population density and reproductive performance when microalgae were combined with baker's yeast. Peña-Aguado et al. (2005) further demonstrated that mixed microalgal diets supported higher population densities than single diets or yeast alone. Similar improvements in cladoceran productivity using algal diets have been reported by Munirasu et al. (2016), Kaeoprakan et al. (2025), Turcihan et al. (2022) and Castro-Mejía Jorge et al. (2016). Although baker's yeast was used as the sole feed in the present study, the favourable response obtained in rainwater indicates that optimisation of water quality alone can substantially improve cladoceran production. Combining suitable water quality with nutritionally superior algal diets may therefore further enhance biomass production for aquaculture. Overall, the present study demonstrates that rainwater and RO water are both suitable culture media for the production of *D. pulex* and *M. micrura*. Rainwater supported the highest population density of both species, whereas RO water also provided favourable conditions for population growth and yielded the highest intrinsic growth rate in *M. micrura*. In contrast, untreated borewell water, owing to its higher dissolved mineral content and electrical conductivity, was less suitable for cladoceran culture. These findings provide useful baseline information for developing economical and efficient live-feed production systems for freshwater aquaculture.

5. Conclusion

The present study demonstrated that water source is a critical factor influencing the culture performance of *Daphnia pulex* and *Moina micrura*. Among the three water sources evaluated, rainwater consistently supported the highest population density and exhibited the most favourable physicochemical characteristics for cladoceran culture. RO water also supported satisfactory growth and may serve as a practical alternative where rainwater is unavailable. In contrast, borewell water, characterized by higher electrical conductivity, hardness and dissolved mineral concentrations, resulted in

significantly lower population density and intrinsic population growth rate in both species. Both cladocerans exhibited a typical sigmoid population growth pattern, reaching peak abundance on Day 6 in *D. pulex* and Day 7 in *M. micrura*. Statistical analyses confirmed that water source, culture duration and their interaction significantly influenced population density. These findings highlight the importance of selecting an appropriate culture medium to maximize cladoceran production. Overall, rainwater is recommended as the most suitable water source for the mass culture of *D. pulex* and *M. micrura* for freshwater aquaculture. Future studies should investigate the combined effects of water quality, microalgal diets and culture management practices to further optimize cladoceran biomass production for live-feed applications.

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Author Contributions

S. Robinson Davidraj: Performed the field and laboratory experiments, analysed the data, and prepared the original draft of the manuscript.

M. Kannan: Conceived the study, designed the research, supervised the work, and critically reviewed the manuscript.

N. Alagumanikumar: Provided expertise in laboratory analyses and contributed to data interpretation.

M. Vigneeswaran: Reviewed and edited the manuscript.

P. Lokesh: Assisted in preparing the initial manuscript framework and organization.

S. Palpperumal: Performed the statistical analyses and assisted in data interpretation.

K. Madhavan: Interpreted the results and contributed to manuscript revision.

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Ethical Approval: This study involved laboratory culture of freshwater cladocerans (*Daphnia pulex* and *Moina micrura*). No specific ethical approval was required for this work.

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