Borneo Journal of Resource Science and Technology (2025), 15(2): 102-109
DOI: https:doi.org/10.33736/bjrst.9334.2025

Influence of Temperature and Oxygen Injection on Population Growth of Marine Rotifers, *Brachionus plicatilis*

RIZAL ISMAIL[1], NORFAZREENA MOHD FAUDZI[1], CHING FUI FUI[1], KENNETH TEO[2], NAJAMUDDIN ABDUL BASRI[1], VUTTHICHAI ONIAM[3] & SITTI RAEHANAH MUHAMAD SHALEH[*1]

[1]Higher Institution Centre of Excellence (HICoE), Borneo Marine Research Institute, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia; [2]Modelling, Simulation and Computing Laboratory, Level 3, Block C, School of Engineering and Information Technology, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia; [3]Klongwan Fisheries Research Station, Academic Supporting Division, Faculty of Fisheries, Kasetsart University, Mueang, Prachuap Khiri Khan, 7700 Thailand
[*]Corresponding author: sittirae@ums.edu.my
Received: 24 March 2025 Accepted: 18 August 2025 Published: 31 December 2025

ABSTRACT

Rotifers (*Brachionus plicatilis,* Brachionidae) are critical live feed for marine, crustaceans, and ornamental fish during their early developmental stages. This study investigated the effects of temperature and oxygen injection on rotifer population growth. A five-day experiment was conducted with nine treatments combining temperatures (26 °C, 28 °C, and 30 °C) and oxygen injection frequencies (0, 2, 4, and 6 times/day). Each treatment was triplicated, with 27 experimental jars containing 4 L of water, a filter, and aeration at 200 mL/min. Commercial *Nannochloropsis* sp. was used as feed through automated feeders. The results show significant effects of temperature and oxygen injection on population density, growth rate, and egg production ($p<0.01$). Statistical analysis was conducted using two-way ANOVA to assess the significance of main and interaction effects. The highest population density (308 individuals/mL) and growth rate (1.14 day^{-1}) were observed at 30 °C with 6 oxygen injections/day, followed by 256 individuals/mL with 4 injections/day. The lowest density (32 individuals/mL) and growth rate (0.62 day^{-1}) were recorded at 26 °C with 4 injections/day. Egg production was highest (27.09 ± 2.49%) at 30 °C with 6 injections/day. These findings indicate the critical role of temperature and oxygen injection frequency for optimizing rotifer production for aquaculture.

Keywords: Aquaculture, hatchery, live feed, mariculture, population growth, rotifer

INTRODUCTION

Due to its favorable nutritional profile, appropriate body size, relatively slow swimming behavior, and exceptional tolerance to a wide range of salinities, the marine rotifer *Brachionus plicatilis* is a zooplankton species that has been extensively studied. It is also vital to aquatic food webs and is heavily used extensively in aquaculture. *Brachionus plicatilis* is a perfect live feed for the early stages of growth of many marine fish and shellfish because of its capacity to stay suspended in the water column (Edward *et al.,*2020). According to Contente *et al.* (2024), the species is highly prized for its quick reproduction rate, ease of enrichment with essential fatty acids, and potential as a vector for delivering probiotics or antibiotics to target aquaculture species. *Brachionus. plicatilis* is currently used to feed over 60 marine finfish and 18 species crustaceans (Lubzens & Zmora, 2003; Yona, 2018).

Rotifers' development, reproduction, and population dynamics are greatly impacted by environmental factors as temperature and dissolved oxygen (DO). While temperature is essential for metabolic activity, reproduction, and survival (Nhinh *et al.,*2020), prior research has shown that DO influences rotifer feeding behaviour and growth performance (Koiso & Hino, 2006). Up to an ideal thermal threshold, higher temperatures often speed up metabolic rates, which encourages population expansion (Nhinh *et al.,*2020; Yoo *et al.,*2023). However, high temperatures can also cause DO levels to drop, which could reduce the productivity and health of rotifers (Chaturvedi & Misra, 2020).

Although much research has been done on the effects of temperature and DO separately on rotifer cultures, little is known about how these two variables interact, particularly how changes in the frequency of oxygen injections under various temperature regimes affect *B. plicatilis* growth and reproductive success. Given the rising need for scalable, highly efficient live feed production systems to service the burgeoning aquaculture industry, this constitutes a substantial knowledge gap.

By examining the combined impacts of temperature and oxygen enrichment frequency on the population expansion of *B. plicatilis*, this study seeks to close this gap. This study aims to determine the ideal environmental conditions for rotifer productivity by assessing these interdependent impacts. In addition to advancing our ecological knowledge of rotifer physiology, the results will offer valuable suggestions for enhancing the effectiveness of rotifer culture systems in commercial aquaculture environments. This work examines the combined impacts of temperature and DO regulation, providing fresh perspectives for improving rotifer cultivation in contrast to earlier research that mainly concentrated on single-factor influences.

MATERIALS AND METHODS

Rotifer Stock Culture

Rotifers, *Brachionus plicatilis*, L-strain had an average lorica length of 195.5 µm. They were sourced from the University Malaysia Sabah Crustacean Hatchery. They were cultured in a 100-liter conical tank preserved at a temperature of 28 °C. The initial stocking density was 10 rotifers/mL, and cultivation continued until the population density reached 100–150 rotifers/mL within 3 days. The culture salinity was set at 30‰. Concentrated microalgae (*Nannochloropsis* sp., Nano3600) were used as feed, supplied twice daily at 10.0×10^6 cells/mL. The rotifers were grown in batch culture, with water parameters such as temperature and dissolved oxygen (DO) monitored daily throughout the cultivation of the rotifer stock.

Experimental Design

Nine temperature and pure oxygen injection frequency combinations were evaluated using a factorial design (Table 1). Each experimental jar (4 L plastic jar) was filled with 3 L of seawater at 30‰ salinity and initially stocked with rotifers at a density of 10 rotifers/mL. The temperatures tested were 26 °C, 28 °C, and 30 °C. The oxygen injection frequencies were 0, 2, 4, and 6 times per day. The experimental jars were equipped with filters and aeration systems, with aeration regulated to 200 mL/min and maintained throughout the study. A water bath with submersible heaters was used to maintain the desired temperature levels. The experiment followed a batch culture method over five days. Daily measurements of water temperature (°C), dissolved oxygen (mg/L), and salinity (‰) were taken and recorded using a BLE-9100 Dissolved Oxygen Analyzer. The BLE-9100 Dissolved Oxygen Analyzer was calibrated prior to the start of the experiment following the manufacturer's recommended procedure, using a two-point calibration with air-saturated water (100% saturation) and sodium sulfite solution (0% saturation) as references. Calibration was conducted weekly to ensure accuracy in measurement throughout the experiment. The filters were cleaned every two days to remove debris and waste to maintain water quality.

Table 1. The combination of temperature (°C) and pure oxygen injection frequencies (POI) of each treatment

°C / Day^{-1}	26	28	30
0	(No injection, 26 °C)	(No injection, 28 °C)	(No injection, 30 °C)
2	(2, 26 °C)	(2, 28 °C)	(2, 30 °C)
4	(4, 26 °C)	(4, 28 °C)	(4, 30 °C)
6	(6, 26 °C)	(6, 28 °C)	(6, 30 °C)

Rotifer Diet Preparation

The concentrated microalgae paste was first diluted with filtered seawater and stored in one-liter plastic jars. To prevent the algae from settling at the bottom of the containers, the contents were mixed using a SOBO Aquarium Wave Maker, which was programmed to activate automatically at each feeding time. The jars were then stored in a styrofoam box containing ice packs to maintain a low temperature, preserving the quality of the microalgae throughout the experiment. The feeding process was fully automated and was carried out twice daily. In total, 100 mL of the diluted microalgae paste was dispensed into each experimental jar during each feeding. This feeding rate corresponded to an estimated density of 200,000 cells/rotifer.

Rotifer Counting

Every day at 0800 hours, 1 mL of rotifer culture was sampled from each experimental jar for counting. Acidic Lugol's solution was used to immobilise the rotifers before counting with a Sedgwick-Rafter counter. Empty loricae from dead rotifers were not included in the count. Every sample was counted three times. The mean value was recorded. The population growth rate (GR) of *B. plicatilis* was calculated using the formula: $G = {}^{1}/_{T} \ln(N_T - N_0)$. T is the period of culture days, N_0 is the initial number of rotifers, N_T is the total number after T days of culture. The egg percentage of rotifer (EP) was estimated using the formula: $EP = Nt \,/\, N \, x \, 100$. EP is the egg percentage of rotifer (%), N is the total number of *B. plicatilis,* Nt is the number of egg-bearing rotifer (Radhakrishnan *et al.,*2017).

Statistical Analysis

The Shapiro-Wilk test was used to determine whether the data were normal, and Levene's test was used to see whether the variances were homogeneous. The significance level was set at $p<0.05$. To assess both individual and interaction effects of the treatments, a two-way Analysis of Variance (ANOVA) was used to examine how temperature and oxygen injection frequency affected rotifer development characteristics. Post hoc comparisons of treatment means were performed using Duncan's multiple range test to find significant changes between groups. The Statistical Package for Social Sciences (SPSS), Version 29.0.2.0, was used for all statistical analyses.

RESULTS

Population Growth

Based on the population growth curve (Figure 1), the population growth of *Brachionus plicatilis* varied significantly based on temperature and the frequency of pure oxygen injection. Across all treatments, population growth generally increased over time. However, higher temperatures (30 °C) and more frequent oxygen injections (6 times/day) led to the highest densities. Conversely, lower temperatures (26 °C) and the absence of oxygen injection resulted in the slowest growth.

At 26 °C, population growth remained low regardless of oxygen injection frequency. The treatment without oxygen injection showed the slowest and most stagnant growth. In contrast, even the highest oxygen injection frequency (six times/day) did not lead to substantial population increases compared to higher temperature treatments. Rotifers at 28 °C exhibited moderate growth, with the 6 times/day oxygen injection treatment indicating a noticeable increase from Day 2 before stabilising around Day 4. The 4 times/day treatment followed a similar trend but at a slightly lower rate, while the treatment without oxygen injection resulted in minimal growth.

The most rapid and highest population growth was observed at 30 °C, where rotifers with six oxygen injections per day reached the greatest density by Day 5, with a steep growth increase starting from Day 2. The 4 times/day treatment was followed closely, exhibiting steady but slightly lower growth, while the 2 times/day treatment showed a slower increase. At 30 °C, the absence of oxygen injection still led to some growth, but at a significantly lower rate compared to treatments with oxygen supplementation.

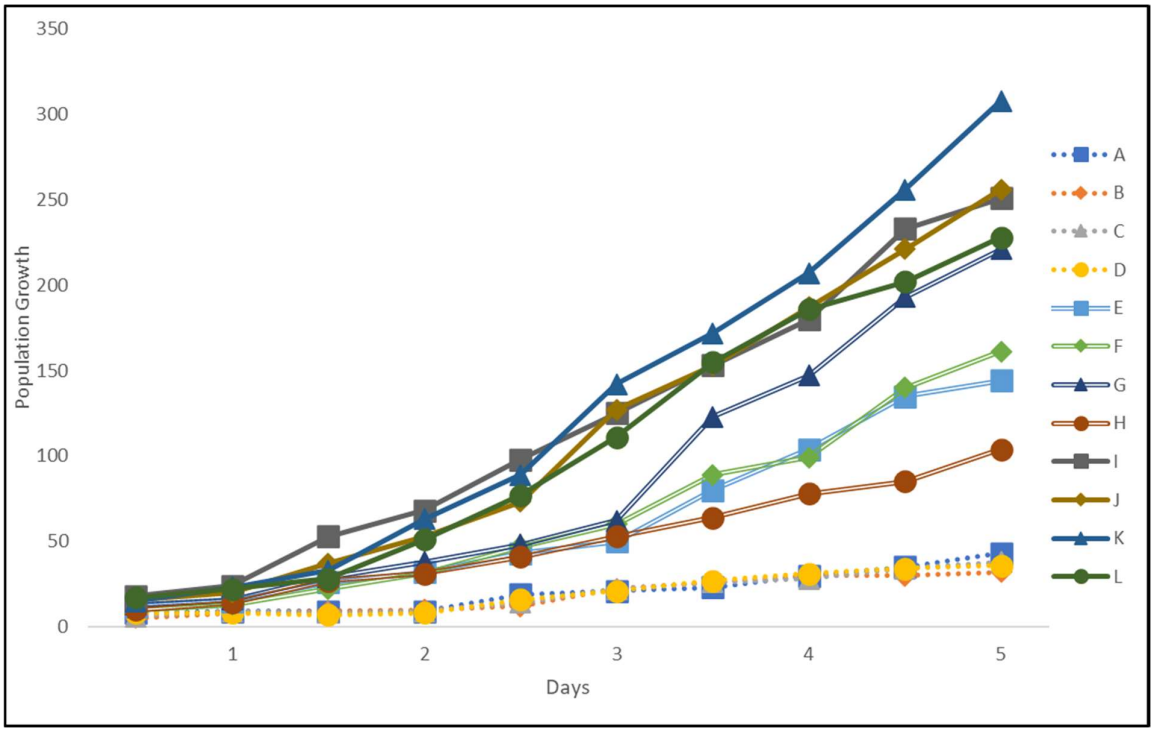

Figure 1. Growth curves of *Brachionus plicatilis* cultured under different combinations of temperature and oxygen injection frequencies: A (26 °C, 2 times/day), B (26 °C, 4 times/day), C (26 °C, 6 times/day), D (26 °C, no injection), E (28 °C, 2 times/day), F (28 °C, 4 times/day), G (28 °C, 6 times/day), H (28 °C, no injection), I (30 °C, 2 times/day), J (30 °C, 4 times/day), K (30 °C, 6 times/day), and L (30 °C, no injection).

Highest Population Density (HPD)

The highest population density (HPD) was observed on the fifth day (Table 2), revealing significant differences among all treatments. The HPD increased with higher temperatures and more frequent oxygen injections. At 30 °C, the HPD rose from 228 ± 26.69 rotifers/mL with no oxygen injection to 308 ± 17.14 rotifers/mL with six oxygen injections daily. In contrast, at 26 °C, the HPD remained consistently low across all POI levels, reaching a maximum of only 43±4.73 rotifers/mL at two oxygen injections per day. The effect of oxygen injection on HPD depends on temperature. Oxygen injection has minimal impact at lower temperatures (26 °C), but at higher temperatures (28 °C and 30 °C), it significantly enhances HPD.

Growth Rate (GR)

Growth rates followed a similar pattern, increasing with higher temperatures and more frequent oxygen injections. At 30 °C, the growth rate improved from 1.08±0.024 day^{-1} with no oxygen injection to 1.14±0.012 day^{-1} at six oxygen injections daily. Conversely, at 26 °C, the growth rate remained consistently low, ranging from 0.62±0.018 day^{-1} to 0.70±0.295 day^{-1}, regardless of the oxygen injection frequency (Table 2). Oxygen injection has a stronger effect on GR at higher temperatures (28 °C and 30 °C) than lower temperatures (26 °C), where it has a negligible effect.

Egg Percentage (EP)

Egg percentage (EP) followed a similar trend, with temperature and oxygen injection frequency (POI) significantly influencing EP ($p<0.01$) (Table 2). At 26 °C, egg percentage (EP) remained relatively low, ranging from 9.57% to 13.15%, and showed no correlation with oxygen injection. As the temperature increased to 28 °C, EP values improved overall (20.21% to 23.55%). However, no consistent trend was observed in response to oxygen injection. In contrast, at 30 °C, EP increased significantly with higher oxygen injection frequencies, reaching its peak 6 times per day (60.50±2.20%). Oxygen injection has a minimal on EP at lower temperatures (26 °C and 28 °C). However, at 30 °C, it significantly enhances EP, with the greatest effect at 6 times/day.

Table 2. Growth rate (GR, day^{-1}), highest population density (HPD, rotifer/ml) and egg percentage (EP, %) of *B. plicatilis* culture at different temperature (T, °C) and pure oxygen injection frequency (POI, times/day).

Parameters	T(°C)	POI (time/day)			
		0	2	4	6
GR (day^{-1})	26	0.70±0.295^{cd}	0.62±0.018^{cd}	0.67±0.014^{cd}	0.65±0.031^{cd}
	28	0.91±0.028^{c}	0.98±0.006^{bc}	1.00±0.005^{bc}	1.07±0.019^{e}
	30	1.08±0.024^{a}	1.10±0.020^{a}	1.10±0.017^{a}	1.14±0.012^{b}
HPD (rotifer/ml)	26	36±4.04^{f}	43±4.73^{f}	32±2.00^{f}	38±2.00^{f}
	28	105±3.31^{d}	144±4.04^{e}	161±4.16^{c}	221±19.13^{cd}
	30	228±26.69^{a}	251±24.56^{a}	256±20.60^{a}	308±17.14^{b}
EP (%)	26	12.98±2.44^{a}	13.15±4.63^{a}	10.37±4.68^{a}	9.57±3.82^{a}
	28	23.03±3.43^{b}	20.21±2.35^{b}	23.55±2.55^{b}	21.49±2.33^{b}
	30	29.33±4.04^{c}	42.00±2.64^{cd}	52.13±3.03^{cd}	60.50±2.20^{f}

Notes: Different letter superscripts indicate significance differences (Two-way ANOVA; $p<0.05$). Growth rate (GR, day^{-1}), highest population density (HPD, rotifer/ml) and egg percentage (EP, %) of *B. plicatilis* culture at different temperature (T, °C) and pure oxygen injection frequency (POI, times/day).

Interaction

Based on Table 3, the two-way ANOVA analysis revealed that temperature (T) and pure oxygen injection frequency (POI) had statistically significant effects on all measured parameters, including growth rate (GR), highest population density (HPD), and egg percentage (EP) of the rotifer *B. plicatilis*. For each parameter, the main effects of temperature and POI and their interaction were highly significant with p-values of 0.001. Temperature significantly influenced the GR of rotifers ($p = 0.001$), while POI also showed a significant effect on GR ($p = 0.001$). The interaction between temperature and POI showed a significant combined effect on GR ($p = 0.001$). This indicates that the response of the growth rate to one factor relies on the level of the other factor.

The HPD and EP showed a similar pattern, with temperature, POI, and their interactions being statistically significant ($p = 0.001$ in all cases). Given that the combined impacts of temperature and POI frequency are crucial in determining the development dynamics and reproduction of rotifers, these results imply that careful adjustment of these variables can lead to optimal rotifer output.

Table 3. Summary of two-way ANOVA (p-value) results analyzing the effects of temperature (T, °C), Pure oxygen injection interval (POI, time/day), and their interaction on growth rate (GR), highest population density (HPD), and egg percentage (EP)

Parameters	T(°C)	POI (time/day)	Interaction
GR	0.001	0.001	0.001
HPD	0.001	0.001	0.001
EP	0.001	0.001	0.001

DISCUSSION

This study shows *Brachionus plicatilis* reached its maximum population density (HPD) at 30 °C while receiving six oxygen injections daily. This result emphasizes how crucial it is to provide the perfect habitat for rotifer formation by maximising the temperature and oxygen supply. The lowest HPD, on the other hand, was recorded at 26 °C, underscoring the way that suboptimal temperatures hinder the dynamics of rotifer populations. These findings are in line with earlier research that highlighted how important temperature is in affecting aquatic species' development, metabolism, and reproduction (Nhinh *et al.,* 2020). For instance, *B. calyciflorus* reached its maximal population growth at 27 °C, according to Nhinh *et al*. (2020), with growth being considerably slower at lower temperatures. In a similar vein, Yona (2018) discovered that although greater temperatures limited the lifetime of rotifers, they

also increased the reproduction rate. This shows an increase in population growth overall.

Temperature has a significant impact on reproductive output in addition to lifespan. In contrast to previous temperature treatments, rotifers cultivated at 30 °C in this study produced more eggs, which directly increased population expansion. Similarly, Yona (2018) found that although overall lifespans were shorter, reproductive periods varied with temperature, with higher reproductive rates happening at warmer temperatures. These results are consistent with the idea that higher temperatures raise metabolic rates, which speed up rotifer reproduction and senescence.

Another important element affecting rotifer growth and production was dissolved oxygen (DO). Maintaining aerobic respiration, metabolic activity, and effective food utilisation in rotifer cultures requires adequate DO levels (Ramaekers *et al.,*2022; Yang *et al.,*2024). Oxygen saturation must be kept within ideal levels to avoid hypoxic situations, particularly in dense cultures with high oxygen demand (Gerasimova & Sadchikov, 2022). However, as prior studies have demonstrated, overaeration can cause physical harm, foaming, and mechanical stress to fragile rotifers, especially those carrying eggs (Yoshimura *et al.,*1996). In order to overcome this, the current work used pure oxygen injection to effectively raise DO levels while reducing mechanical disruptions. This strategy worked because higher HPD and better reproductive outcomes were positively connected with more frequent oxygen injections.

Our findings are in line with those by Koiso and Hino (2006) and Yoo *et al.* (2023), who reported that a sudden reduction in DO can result in a significant decrease in rotifer feeding behaviour and reproductive success. Our results demonstrate that oxygen enrichment is particularly effective when combined with increased temperatures. The interaction between temperature and oxygen injection frequency was significant for all key variables HPD, GR, and EP ($p = 0.001$). Notably, oxygen injection had minimal impact at lower temperatures but became increasingly critical at higher temperatures, with greater metabolic demands. These results indicate that temperature directly influences the efficiency of oxygen utilisation by rotifers, suggesting that can more effectively convert available oxygen into reproductive output when cultured under thermally optimal conditions.

While the DO levels achieved in this study exceeded the general optimal recommendations (>6.0 mg/L; Yang *et al.,* 2024), not all treatments yielded proportional increases in GR or HPD. This indicates a potential threshold beyond which further oxygen enrichment does not confer additional reproductive or growth benefits. The interaction likely stems from a synergistic relationship wherein higher metabolic rates at elevated temperatures increase oxygen demand, and oxygen enrichment fulfils this requirement, leading to improved culture outcomes. However, beyond this synergistic range, further oxygenation may yield diminishing returns.

These effects can be explained by known physiological mechanisms in rotifers. Increased temperatures increase enzymatic activity, protein synthesis, and reproductive cycles. Simultaneously, sufficient DO ensures that aerobic respiration remains efficient, offering the necessary energy for heightened reproductive activity. Conversely, insufficient oxygen availability at high temperatures may lead to metabolic stress, reducing fecundity and potentially increasing mortality rates.

The results obtained have practical implications for large-scale aquaculture operations, particularly in the production of live feeds for marine hatcheries. The identification of 30 °C with six oxygen injections per day as an optimal condition offers aquaculture practitioners a reliable guideline for maximising rotifer output. However, the scalability of pure oxygen injection should be considered, as it may incur additional operational costs. Hatcheries aiming for cost-effective production must balance the benefits of higher rotifer yields against the expenses associated with oxygen supplementation. Moreover, integrating efficient oxygen delivery systems, such as fine diffusers or membrane oxygenation, could mitigate costs while improving culture performance.

While this study offers valuable insights into the effects of temperature and oxygen enrichment on *B. plicatilis* culture, further research is crucial to build on these findings. Future studies should focus on long-term culture

experiments to evaluate the cumulative effects of sustained high-temperature and oxygen enrichment on rotifer health, reproductive stability, and potential genetic adaptations over multiple generations. Additionally, investigating the effects of combining optimal physical conditions with targeted nutritional enrichment, such as the incorporation of essential fatty acids or probiotics, could provide further improvements in rotifer quality for aquaculture feed applications. Investigations into the selection or development of rotifer strains that show enhanced tolerance to high-density and high-productivity culture systems would also be beneficial for improving culture efficiency. Furthermore, it is essential to conduct economic feasibility assessments to determine the practicality and cost-effectiveness of implementing oxygen injection systems in commercial aquaculture hatcheries. Finally, future research should further explore the interactive effects of other abiotic factors, including salinity fluctuations, pH variation, and nutrient availability, to develop more comprehensive and robust best practices for rotifer culture management in diverse aquaculture settings.

CONCLUSION

This study demonstrated that temperature and pure oxygen injection (POI) significantly influenced rotifer production. Culturing rotifers at optimal temperatures, combined with POI, effectively enhanced population growth. However, further research is required to assess the nutritional quality of rotifers produced under these conditions.

ACKNOWLEDGEMENTS

This study was funded by the UMS Flagship grant (DFM 2101). The author would like to express gratitude to all staff and research assistants at the Borneo Marine Institute, University Malaysia Sabah, Kota Kinabalu, Malaysia, for their technical support throughout the study.

REFERENCES

Chaturvedi, V. & Misra, S. (2020). Reductive metabolites of curcumin and their therapeutic effects. *Heliyon*, 6(5): 54-69. DOI: https://doi.org/10.1016/j.heliyon.2020.e05469

Contente, D. M. L., Díaz-Formoso, L., Feito, J. A., Hernández, P. E., Muñoz-Atienza, E., Borrero, J., Poeta, P. & Cintas, L. M. (2024). Genomic and Functional Evaluation of Two *Lacticaseibacillus paracasei* and Two *Lactiplantibacillus plantarum* Strains, Isolated from a Rearing Tank of Rotifers (*Brachionus plicatilis*), as Probiotics for Aquaculture.*Genes*,15(1): 64. .DOI:https://doi.org/10.3390/genes15010064

Edward, L., Laxmilatha, P., Sreeramulu, K., Ranjith, L. & Megarajan, S. (2020). Influence of certain environmental parameters on mass production of rotifers: A review. *Journal of the Marine Biological Association of India*, 62(1): 49–53. DOI: https://doi.org/10.6024/jmbai.2020.62.1.2176-08

Gerasimova, T. N. & Sadchikov, A. P. (2022). The role of rotifer in water quality improvement in a fishpond. *Vestnik MGTU*, 25(2): 120–124. DOI: https://doi.org/10.21443/1560-9278-2022-25-2-120-124

Koiso, M. & Hino, A. (2006). Effect of sudden drop of dissolved oxygen on population growth and feeding in the rotifer *Brachionus plicatilis*. *Aquaculture Science*, 54(1), 37–45. DOI:https://doi.org/10.11233/aquaculturesci1953.54.37

Lubzens, E. & Zmora, O. (2003). Production and nutritional value of rotifers. In Støttrup, J. G. & McEvoy, L. A. (eds.) *Live feeds in marine aquaculture*. Oxford, UK, Blackwell Publishing. pp. 17–64. https://doi.org/10.1002/9780470995143.ch2

Nhinh, D. T., Hong, P. T. L., Hoai, T. D. & Van Van, K. (2020). Effects of temperature on population growth and resting egg production of freshwater rotifer (*Brachionus calyciflorus*). *Vietnam Journal of Agricultural Sciences*, 3(4): 798–805. DOI: https://doi.org/10.31817/vjas.2020.3.4.07

Radhakrishnan, K., Aanand, S., Rameshkumar, S. & Divya, F. (2017). Effect of feeding rate and feeding frequency in mass culture of *Brachionus plicatilis* in a semi-continuous method with a yeast-based diet. *Journal of Fisheries and Life Sciences*, 2(1): 40–44.

Ramaekers, L., Pinceel, T., Brendonck, L. & Vanschoenwinkel, B. (2022). Direct effects of elevated dissolved CO2 can alter the life history of freshwater zooplankton. *Scientific Reports*, 12(1): 61-34. DOI: https://doi.org/10.1038/s41598-022-10157-2

Yang, D., Li, W. & Ma, X. (2024). Suitable acidified eggshell powder food promotes *Brachionus calyciflorus* growth and reproduction: From antioxidant capacity insight. *Aquaculture Nutrition*, 20(24): 1–10. DOI: https://doi.org/10.1155/2024/7875547

Yona, G. K. (2018). Effect of culture temperature on rotifers (*Brachionus plicatilis* sensu strictu) size and reproduction activities. *Tanzania Journal of Agricultural Sciences*, 17(1): 10–17.

Yoo, H. K., Kim, S. S., Lee, K. W., Lee, S. Y., Jung, M. M. & Woo, S. J. (2023). Determination of the optimal conditions for the mass culture of large-type rotifers (*Brachionus plicatilis*) at low temperatures. *Water*, 15(18): 3310. DOI: https://doi.org/10.3390/w15183310

Yoshimura, K., Ohmori, Y., Yoshimatsu, T., Tanaka, K. & Ishizaki, A. (1996). On the aeration method in high-density culture of rotifer *Brachionus rotundiformis. Nippon Suisan Gakkaishi*, 62(6): 897–902.