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Original Article

Optimization of salinity levels with respect to survival, reproductive performance and population dynamics for the culture of cyclopoid copepod *Apocyclops* sp.

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Abstract
Apocyclops sp. is a widely used cyclopoid copepod in finfish and shellfish larviculture because of its superior nutritional value and suitable size for larval feeding. However, the optimal salinity for its mass culture remains inadequately defined. This study evaluated the effects of salinity on the survival, growth, reproduction, and population dynamics of *Apocyclops* sp. Adult and naupliar survival were first assessed after abrupt exposure to salinities ranging from 5 to 40 ppt, with survival recorded after 24 hr. Reproductive and population parameters were subsequently evaluated at 20, 25, 30, and 35 ppt to identify the most suitable culture salinity. Results showed that 25 ppt supported the highest adult (93.33±5.77%) and naupliar survival (84.17±3.55%). Reproductive performance was also greatest at 25 ppt, with the highest clutch size (15.17±1.17 eggs female^{-1}) and nauplii production (14.00±1.41 female^{-1}), while egg diameter and hatching rate remained unaffected by salinity. Maximum population density (6291.67±1120.36 individuals L^{-1} on day 5) and significantly higher ($p < 0.05$) growth rate were also recorded at 25 ppt. Stage composition analysis indicated that 25 ppt maintained a balanced distribution of developmental stages, supporting continuous recruitment. Although the proportions of nauplii and ovigerous females were highest at 30 ppt, overall culture performance was superior at 25 ppt. These findings identify 25 ppt as the optimal salinity for hatchery-scale culture of *Apocyclops* sp., providing a practical guideline for its large-scale production as a live feed in marine aquaculture.

Keywords: *Apocyclops*; copepod; growth; live food; population composition; salinity

1 | INTRODUCTION

Live feeds are a major dietary component for cultured fish larvae and are especially important in the rearing of altricial marine fish larvae (Conceição *et al.* 2010). An adequate supply of live feed is essential in hatcheries for rearing fish larvae, fry and fingerlings. Phytoplankton and

zooplankton are commonly used as starter feeds for many aquaculture species due to their rich macro- and micro-nutrient content, earning them the name "living capsules of nutrition" (Das *et al.* 2012; Radhakrishnan *et al.* 2019). Live feeds are readily ingested and highly digestible (Kinne 1997), while also exerting minimal impact on water quality (Watanabe *et al.* 1978; Radhakrishnan *et al.* 2019). Their movement in the water column stimulates the feeding response of larvae through their characteristic jerky motions (Bengtson 2007). They serve as rich sources of essential nutrients, including proteins, lipids, carbohydrates, vitamins, minerals, amino acids and fatty acids, which are critical for aquatic larvae (Barad *et al.* 2017). The timely provision of appropriately sized live feed is a critical factor in maximizing growth performance and survival rates of finfish and shellfish larvae (Das *et al.* 2012; Barad *et al.* 2017). Although formulated dry feed offers economic benefits, considerable research has focused on replacing live feed with artificial diets (Lahnsteiner *et al.* 2023). However, reliable formulations remain limited, primarily due to nutrient imbalances and poor digestibility associated with the underdeveloped digestive systems (Gavhane *et al.* 2026) of fish larvae during early developmental stages (Lahnsteiner *et al.* 2023).

A diverse range of live feed organisms are extensively employed in aquaculture, including rotifers (*Brachionus* sp.), copepods and *Artemia*. These taxa are particularly preferred due to their rapid reproductive turnover, ability to achieve exponential population growth within a short period and tolerance to variable or suboptimal environmental conditions, thereby enhancing their suitability for large-scale hatchery production systems (Neelakantan *et al.* 1988; Radhakrishnan *et al.* 2019). Although *Artemia* nauplii and rotifers are widely utilized as live feed, it has been reported that rotifers can synthesize polyunsaturated fatty acids (PUFAs), but the quantity produced is often insufficient to support high survival of fish larvae (Paulo *et al.* 2020). In addition, their size range may not be suitable for the small mouth openings of certain marine fish larvae, and their nutritional quality, is often inadequate unless properly enriched (Marjuk *et al.* 2026). Similarly, *Artemia* species are generally deficient in essential fatty acids, and their cost, physical characteristics and nutritional quality vary considerably among different sources (Ananthi *et al.* 2011).

Copepods are the most abundant planktonic crustaceans found in nearly all aquatic environments. This group includes more than 210 families, 2,400 genera and 24,000 identified species (Ajiboye *et al.* 2011). Among metazoans, planktonic copepods are considered one of the most abundant organisms on Earth. The three major copepod orders-Cyclopoida, Calanoida and Harpacticoida-have been evaluated for their suitability as feed for larval and juvenile fish (Ajiboye *et al.* 2011). In recent years, copepod culture has gained attention due to the important role of copepods as live feed in marine fish larviculture (Ballesteros-Redondo *et al.* 2023), given their high abundance and numerical dominance within the metazoan plankton across all oceanic environments (Magouz *et al.* 2021). They are widely regarded as superior to rotifers and *Artemia* in larval fish culture because of their balanced nutritional composition and appropriate digestive enzyme content. They are rich in essential fatty acids such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA), which support improved growth and survival of fish larvae (Radhakrishnan *et al.* 2019).

Apocyclops Lindberg, 1942 is one of the most important genera among cyclopoid copepods which mainly inhabit the estuarine and coastal waters throughout the world (Hołyńska *et al.* 2016). It is the most widely used cyclopoid copepod for larviculture of finfishes and shrimps due to the body length of nauplii (122.7+10.2 µm) which is similar to that of the newly hatched *Brachionus rotundiformis* (super small rotifer) and it also contains higher nutritional value in terms of DHA, EPA and vitamin B_{12} compared to rotifers and *Artemia* (Sung and Sung 2014). Furthermore, the active swimming behavior of adult *Apocyclops* sp. enhances prey visibility and stimulates the feeding responses of fish larvae, thereby improving prey capture efficiency and feeding success (Turner 2004). Despite their numerous advantages, the large scale utilization of these copepods in aquaculture remains limited due to challenges associated with mass culture, efficient harvesting and the maintenance of stable population under intensive production conditions (Støttrup 2006).

Understanding how environmental factors influence reproduction and growth is crucial for understanding copepod population dynamics (Hairston and Bohonak 1998). Knowledge of environmental parameters is crucial for understanding copepod population dynamics (Devreker *et al.* 2009). Temperature and salinity are the major environmental factors that have potential impacts on the diversity and distribution of marine organisms (Meir *et al.* 2006). Particularly, Salinity is one of the key factors influencing the distribution and abundance of zooplankton in marine ecosystems. Moreover, the change in salinity levels disrupts the physiological and biological parameters of the copepod, as a result, it affects the abundance and distribution of cyclopoid copepods in aquatic environments (Darsana *et al.* 2022).

The seasonal and temporal distribution of copepods is largely influenced by environmental parameters, including food availability, temperature and salinity (Milione and Zeng 2008). Physiological stress caused by fluctuations in salinity can result in acute zooplankton mortality, thereby diminishing population growth and disrupting the functioning of the aquatic food web (Chintada *et al.* 2023). According to Weissenberg *et al.* (2022), suboptimal

salinity negatively influences egg production in marine copepods, as salinity is a key abiotic factor regulating their oxidative status and overall physiological performance. Additionally, salinity has an impact on spawning, incubation, growth, survival rate and osmotic balance of the copepods. Therefore, studying the effects of varying salinity levels is required to understand the dynamics of marine copepod production, and it is essential to determine the optimal salinity for *Apocyclops* sp. copepods to support stable hatchery production and use as live feed (Peterson 2001; Pan *et al.* 2016).

Building upon the existing knowledge gaps, the present study evaluated the effects of different salinity levels on the survival, population growth and reproductive performance of *Apocyclops* sp. The experiment was conducted to identify the optimal salinity for the culture of this copepod species. The findings of this study are expected to provide valuable insights for establishing suitable culture conditions and supporting the reliable production of *Apocyclops* sp. as a live feed organism in marine larviculture.

2 | METHODOLOGY

2.1 Culture of microalgae (*Nanochloropsis gaditana*)

Microalgal culture of *Nanochloropsis gaditana* was carried out under regulated laboratory conditions using Guillard's F/2 medium at the Mandapam Center for Sustainable Aquaculture, Tamil Nadu, India. Initially, stock cultures were reared in 5 liter (L) Erlenmeyer flasks at 24±1°C. Then the stocks were transferred into 20 L carbouy containers at a salinity of 35 ppt with consistent aeration provided to keep growth on an even keel. Algal cells were harvested at a late exponential phase (3×10^6 – 3.66×10^6 cells/ml) to serve as copepod feed. Algal density was pinned down using a Neubauer haemocytometer under a binocular microscope (Carl Zeiss Microscopy (Zeiss Primostar, Germany).

2.2 Culture of *Apocyclops* sp.

A stock culture of the copepod *Apocyclops* sp. has been maintained in laboratory conditions at Mandapam Center for Sustainable Aquaculture, Tamil Nadu, India. Filtered seawater was chlorinated (10 ppm) and then dechlorinated after 24 hours, followed by a pass through a 0.5 µ filter bag to remove the particulate matter, and stored in 2500 L tanks for culture purposes. Adult *Apocyclops* sp. were separated and cultured in treated seawater at a salinity of 35 ppt and a temperature of 30±2°C. Initially, the culture process started off in 500 ml conical flasks before being scaled up through 5 L plastic carbouy cans and 50 L buckets.

2.3 Experimental design

To determine the viable salinity range, a preliminary pilot study was conducted, subjecting the organisms to an abrupt salinity shift from 5 to 40 ppt over a period of 24 hours (hr). From the results of pilot study, salinities below 20 and above 35 ppt were ruled out for subsequent trials, while salinities below 15 ppt and above 35 ppt showing less survival were excluded. All experimental treatments (20, 25, 30 and 35 ppt) were maintained in 1-liter containers containing 800 mL of seawater, with an initial stocking density of 1 individual mL^{-1}. Each treatment was studied in triplicate and fed with microalgae (*N. gaditana*) at a density of 30,000 – 60,000 cells mL^{-1}. The experiment was conducted for 14 days under a 12 hr light: 12 hr dark photoperiod, with 10–20% water exchange carried out once every three days. To get the target salinity levels, natural seawater (35 ppt) was either diluted with treated freshwater for lower salinities (Darsana *et al.* 2022) or usage of commercial sea salt for higher salinity levels (Cruz-Rosado *et al.* 2020). Salinity levels was regularly monitored using an Automatic compensation salinity refractometer (Atago (Master-S/Mill α), Japan) and adjusted whenever necessary to minimize fluctuations. Water quality parameters like pH, Dissolved oxygen (mg/L), Nitrite (mg/L), Nitrate (mg/L) and Ammonia (mg/L) were assessed in the interval period of 3 days (Table 1). Copepods and their larval stages were enumerated under a binocular microscope (Carl Zeiss Microscopy, Zeiss Primostar, Germany).

TABLE 1 Parameters of seawater assessed under different salinity.

Salinity (ppt)	pH	DO (mg/L)	NO_2 (mg/L)	NO_3 (mg/L)	TAN (mg/L)
20	8.11±0.18	4.73±0.39	0.43±0.15	0.10±0.08	0.13±0.06
25	8.01±0.16	4.97±0.30	0.38±0.21	0.07±0.05	0.15±0.05
30	8.14±0.16	4.44±0.38	0.51±0.18	0.12±0.04	0.15±0.09
35	8.12±0.13	4.87±0.41	0.55±0.19	0.14±0.09	0.13±0.09

Presented data are in mean ± SD. pH = Power of hydrogen, DO = dissolved oxygen, NO_2 = nitrite, NO_3 = nitrate, TAN = total ammonical nitrogen

2.4 Effect of abrupt salinity change in *Apocyclops* sp.

2.4.1 Adult survival: To assess the effect of abrupt salinity shifts on survival, a 24 hr experiment was carried out across eight distinct levels (5, 10, 15, 20, 25, 30, 35 and 40 ppt) without prior acclimatization. For each treatment, 10 adult individuals were isolated from the stock population

using a 250 µ mesh sieve and were introduced into 100 ml conical flasks containing 50 ml of seawater. Each treatment was maintained in triplicate, with the temperature held at 30±2°C. The copepods were fed with *N. gaditana* and after 24 hr, live and dead individuals were counted to determine the survival rate.

2.4.2 Naupliar survival: To evaluate naupliar survival, adult copepods were isolated and gravid females were set aside in separate containers (1 L) filled with seawater (500 ml) and microalgal diet to facilitate naupliar production. The newly hatched nauplii were collected after 30 hr (Doan *et al.* 2019) using a 50 µm mesh and stocked at a density of 200 individual L^{-1} across various test salinities (5, 10, 15, 20, 25, 30, 35, 40 ppt) without any prior acclimation. The trials were carried out until the nauplii had successfully transitioned into the copepodite stage and survival was calculated based on the number (No.) of copepodite individuals present in each trials.

2.5 Adult longevity

In total, 20 newly moulted adult copepods were randomly selected from the acclimatized culture and stocked in a 500 ml conical flask filled with 250 ml of water to assess adult longevity of *Apocyclops* sp. in 20, 25, 30 and 35 ppt salinities. The newly produced nauplii were removed from the flask, and the experiment was continued until no copepods survived. During the experiment, fresh microalgae, *N. gaditana*, were given and daily mortality was recorded. To determine adult longevity of *Apocyclops* sp., average individual lifespans across all replicates in the treatment were calculated (Wang *et al.* 2021).

2.6 Reproductive parameters

2.6.1 Clutch size per female: To evaluate clutch size, 5 egg-bearing females were picked out at random from the populations cultured in different salinity trials (20, 25, 30 and 35 ppt), following the sampling approach described by Darsana *et al.* (2022) and placed on a petri plate (with 5 ml of seawater) and treated with 4% sodium hypochlorite solution to tease the eggs away from their sacs, following the approach laid by Ohs *et al.* (2010). Once the eggs were separated from both sacs, they were counted under a binocular microscope (Carl Zeiss Microscopy, Zeiss Primostar, Germany).

2.6.2 Egg diameter: To assess the size of eggs, 5 females were taken from each treatment, and then they were placed on a cavity slide with 1 ml of seawater. The ovisac of the copepod was teased using a small needle until the eggs were detached from the ovisac. After the eggs were separated, they were measured using the micrometer scale installed in the binocular microscope (Carl Zeiss Microscopy, Zeiss Primostar, Germany).

2.6.3 Ovisac length and width: To determine the effect on the ovisac length and width, 5 female copepods were taken from the respective salinity trials and placed on the petri plate filled with 5 ml of seawater and were fixed using 4% formaldehyde. Then the length and width of the ovisac were measured using the scale incorporated in the binocular microscope (Carl Zeiss Microscopy, Zeiss Primostar, Germany).

2.6.4 Hatching rate: To determine the hatching percentage of eggs in females, 5 egg-carrying females were collected randomly from each experimental treatment and each individual was transferred separately to a 25 ml conical flask filled with 15 ml of seawater and microalgae. Then the flasks were placed at the same temperature and salinity for 30 h. Hatching rate was determined by observing the presence of nauplii in each flask using the binocular microscope (Carl Zeiss Microscopy, Zeiss Primostar, Germany).

2.6.5 Nauplii production: To determine the production of nauplii, 5 ovigerous females were collected from each experimental treatments of different salinity and were transferred to petri plate with seawater and algae and placed in the same experimental conditions. After 30 hr, all females that either released nauplii or had unhatched ovisacs were removed. The hatched nauplii were collected from each flask and counted under a microscope (Doan *et al.* 2019). Nauplii production was determined as the average number of nauplii produced by a female after 30 h of incubation in the experimental conditions. During the period of the trials, cannibalism was not observed.

2.7 Population parameters

2.7.1 Population density: The assessment of the total population of *Apocyclops* sp. was carried out by rearing later-stage copepodites in different salinities following the standard protocol described by Milione and Zeng (2008). Magouz *et al.* (2021) described the standard stocking density for copepods as 0.5-1 number/ml (No./ml) from the above reference, the acclimated individuals were stocked at a density of 1 No./ml in experimental containers. Population density was monitored at regular intervals (3 days) by collecting a fixed volume of 5 ml of culture water from each replicate. The number of individuals was counted under a binocular microscope (Carl Zeiss Microscopy, Zeiss Primostar, Germany) to determine the density in the culture.

2.7.2 Population growth rate: The specific population growth rate (*K*) of *Apocyclops* sp. was calculated using the following formula (Omori and Ikeda 1984; Hada and Uye 1991):

$$K = (\ln N_f - \ln N_o) / t$$

Here, t is the culture duration, and N_o and N_f are the initial and final copepod densities, respectively.

2.7.3 Population composition of *Apocyclops* sp.: Population composition for each salinity level was determined by randomly sampling approximately 100 individuals using a plankton net (20 µm mesh) as suggested by Magouz *et al.* (2021) and Darsana *et al.* (2022). The samples were preserved in 4% formalin and examined under a binocular microscope (Zeiss Primostar) to identify and quantify different developmental stages, including nauplii, copepodites, adults and ovigerous females.

2.8 Data analysis

Data from the different salinity treatments were subjected to one-way ANOVA after testing for homogeneity of variance. Tukey's post hoc multiple comparison test was used to identify significant differences among treatments ($p < 0.05$). The population density over time and growth rate was measured using repeated measure ANOVA. Statistical analyses were conducted using SPSS version 22.0 (SPSS Inc., Chicago, USA), and the data are presented as mean ± standard deviation (mean ± SD).

3 | RESULTS

3.1 Effect of abrupt salinity change on *Apocyclops* sp.

3.1.1 Adult survival: The adult survival of marine cyclopoid copepod *Apocyclops* sp. was significantly (ANOVA: $F_{7,16} = 21.922$, $p < 0.05$) affected by different salinity levels (5, 10, 15, 20, 25, 30, 35 and 40 ppt) as shown in Figure 1. The highest adult survival rate was recorded at 25 ppt (93.33±5.77%), followed by 30 ppt (90.00±10.00%) and 20 ppt (83.33±5.77%), which were not significantly different from each other. In contrast, the lower adult survival was observed at salinities of 5 ppt (30.00±10.00%), 10 ppt (36.67±5.77%), 15 ppt (43.33±15.26%) and 40 ppt (50.00±10.00%). These results indicate that the salinity range of 20–35 ppt supports significantly ($p < 0.05$) higher adult survival compared to salinities above or below this range.

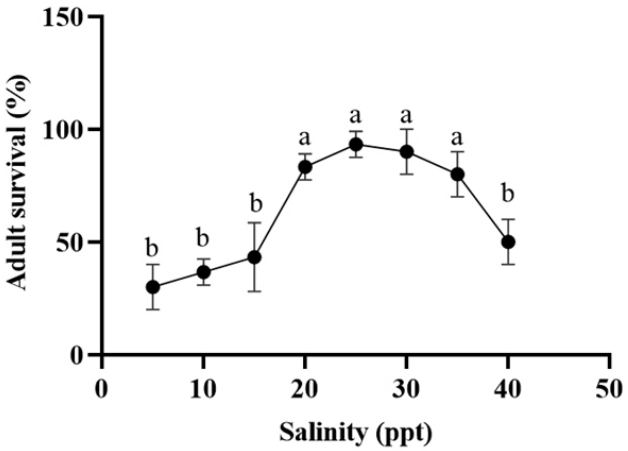

FIGURE 1 Effect of abrupt salinity on survival rate of Adult (%) of *Apocyclops* sp. after 24 hr exposure under different salinities (5–40 ppt). Presented data are in mean ± SD. Means with different superscript letters indicate that data are significantly different ($p < 0.05$).

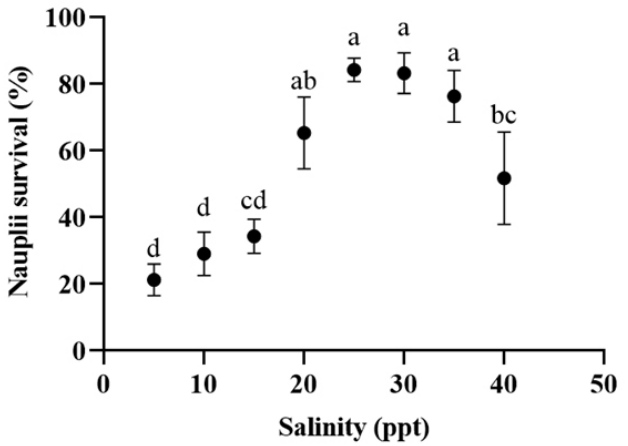

FIGURE 2 Naupliar survival (%) of *Apocyclops* sp. under different salinity treatments (5–40 ppt). Presented data are in mean ± SD. Means with different superscript letters indicate that data are significantly different ($p < 0.05$).

3.1.2 Naupliar survival: Sudden changes in different salinity levels ranging from 5 to 40 ppt had a significant effect ($F_{7,16} = 29.93$, $p < 0.05$) on the naupliar survival of *Apocyclops* sp. copepod (Figure 2). The survival rate increased gradually from 5 ppt (21.17±4.80%) to reach a peak at 25 ppt (84.17±3.55%). The highest naupliar survival was observed at 25 ppt (84.17±3.55%), which was not significantly ($p > 0.05$) different from the survival rate at 20 ppt (65.33±10.77%), 30 ppt (83.16±6.11%) and 35 ppt (76.33±7.75%) salinity. Significantly ($p < 0.05$) lower naupliar survival was recorded at 5 ppt (21.17±4.80%), 10 ppt (29.00±6.56%) and 40 ppt (51.66±13.87%). These findings suggest that naupliar survival is optimal within the salinity range of 20–35 ppt and declines at lower and higher salinities.

3.1.3 Adult longevity: Results shown that salinity had a significant ($p < 0.05$) effect on adult longevity, where the adult longevity was maximum at salinity of 25 ppt (28.33±1.26 days) and there was a slight decrease shown in the salinities such as 20 ppt (27.22±0.75 days), 30 ppt (25.50±1.50 days) and 35 ppt (24.17±1.26 days) (Figure 3).

3.2 Reproductive parameters

3.2.1 Clutch size/female: The highest clutch size was recorded at 25 ppt (15.17±1.17 eggs female^{-1}), which was significantly ($p < 0.05$) higher than other treatments (Table 2). No significant difference was observed between the treatments at salinities of 20 ppt (12.17±0.75 eggs female^{-1}), 30 ppt (11.00±1.41 eggs female^{-1}) and 35 ppt (9.50±1.05 eggs female^{-1}).

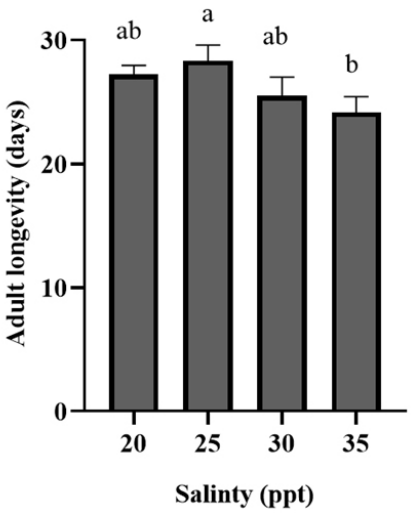

FIGURE 3 Adult longevity (days) of *Apocyclops* sp. under experimental salinity treatments. Presented data are in mean ± SD. Means with different superscript letters indicate that data are significantly different ($p < 0.05$).

3.2.2 Egg diameter: No significant difference ($p > 0.05$) in egg diameter was detected across the four salinity treatments (Table 2). The egg diameters were almost similar across treatments as 85.22±5.38 µm at 25 ppt, 82.72±9.74 µm at 35 ppt, 81.72±7.52 µm at 30 ppt and 80.34±6.27 µm at 20 ppt.

3.2.3 Ovisac width: As shown in Table 2, the widest ovaries were observed at 25 ppt (172.34±7.47 µm), which was significantly ($p < 0.05$) different from 30 ppt (147.66±11.85 µm). There was no significant ($p > 0.05$) difference in ovisac width at 20 ppt (154.27±11.99 µm) and 35 ppt (157.680±13.53 µm).

3.2.4 Ovisac length: Ovisac length did not differ significantly (p>0.05) among salinity treatments (Table 2). The longest ovisac were measured at 20 ppt (237.62±31.13 µm), followed by 25 ppt (260.55±26.47 µm), 30 ppt (229.33±33.19 µm) and 35 ppt (216.49±16.26 µm), indicating that the salinity range of 25–35 ppt does not have a significant ($p > 0.05$) effect on ovisac length.

3.2.5 Hatching rate: There was no significant ($p > 0.05$) difference in the egg hatching rate across four salinity treatments (Table 2). Egg size of *Apocyclops* sp. varied slightly across different salinity levels (20–35 ppt). Mean hatching rates were 89.06±8.29% at 20 ppt, 92.25±4.93% at 25 ppt, 87.42±13.24% at 30 ppt and 84.38±11.59% at 35 ppt.

3.2.6 Nauplii production: The average number of nauplii produced from females within 30 hr varied significantly ($p < 0.05$) among salinity treatments (Table 2). The highest nauplii production was recorded at 25 ppt (14.00±1.41), which was significantly ($p < 0.05$) different from all other treatments. At 20 ppt (10.83±1.17), nauplii production was intermediate and was not significantly ($p > 0.05$) different from 30 ppt (9.50±1.05). The lowest nauplii production per female was observed at 30 ppt (9.50±1.05) and 35 ppt (8.00±1.26). These results indicate that 25 ppt maximizes naupliar yield per female per clutch produced.

TABLE 2 Effect of different salinity on Reproductive parameters of cyclopoid copepod *Apocyclops* sp.

Salinity (ppt)	Clutch size (eggs/female)	Egg diameter (µm)	Ovisac length (µm)	Ovisac width (µm)	Hatching rate (%)	Nauplii/female /clutch
20	12.16±0.75^{b}	80.34±6.27	237.62±31.13	154.27±11.99^{b}	89.06±8.29	10.83±1.17^{b}
25	15.16±1.17^{a}	85.22±5.38	260.55±26.47	172.34±7.47^{a}	92.25±4.93	14.00±1.41^{a}
30	11.00±1.41^{bc}	81.72±7.52	229. 33±33.19	147.66±11.85^{b}	87.42±13.24	9.50±1.05^{bc}
35	9.50±1.05^{c}	82.72±9.74	216.49±16.26	157.680±13.53^{ab}	84.38±11.59	8.00±1.26^{c}

Presented data are in mean ± SD. Means with different superscript letters indicate that data are significantly different ($p < 0.05$)

3.3 Population parameters

3.3.1 Population density: A significant interaction between salinity and exposure period was observed ($F = 29.634$, $p < 0.05$). Salinity significantly ($p < 0.05$) affected the response on Days 2, 5, 8 and 11 ($p < 0.05$), but not on Day 14 ($p = 0.078$). Bonferroni-adjusted pairwise comparisons revealed distinct salinity-dependent responses across sampling days. On Day 2, 20 (2783.33±644.85 individuals L^{-1}) and 25 ppt (3373.67±232.59 individuals L^{-1}) were statistically similar, while 30 and 35 ppt differed significantly ($p < 0.05$) from the other treatments. On Day 5, 25 ppt (6291.67±1120.36 individuals L^{-1}) differed significantly ($p < 0.05$) from 20 ppt (2708.00±531.63 individuals L^{-1}), 30 ppt (999.66±166.50 individuals L^{-1}) and 35 ppt (1235.00±204.75 individuals L^{-1}), whereas the latter three were comparable. All salinity treatments (20 ppt (3041.33±181.58 individuals L^{-1}), 25 ppt (2444.33±96.42 individuals L^{-1}), 30 ppt (847.00±24.25 individuals L^{-1}), 35 ppt (2013.66±145.98 individuals L^{-1}) differed significantly from one another on Day 8. On Day 11, 25 ppt

(1833.00±167.00 individuals L^{-1}) differed significantly from the remaining treatments, while 20 ppt (749.66±83.50 individuals L^{-1}), 30 ppt (1013.66±145.98 individuals L^{-1}) and 35 ppt (791.33±110.55 individuals L^{-1}) were comparable. No significant differences among salinity treatments were observed on Day 14 (Figure 4).

3.3.2 Population growth rate: The population growth rate of Apocyclops sp. was significantly ($F_{3,8}$ = 155.686, p < 0.05) affected by salinity and culture period (Greenhouse–Geisser corrected: F = 105.550, p < 0.05). A significant interaction between salinity and culture period was also observed (Greenhouse–Geisser corrected: $F_{5.7,15.3}$ = 14.492, p < 0.05), indicating that the effect of salinity on population growth rate varied with culture period (Figure 5). The population growth rate increased during the initial culture period and subsequently declined towards the end of the experiment. The highest growth rate was recorded at 25 ppt on day 5 (K = 0.609±0.063), followed by 20 ppt on day 8 (K = 0.37±0.02), whereas the lowest growth rate was observed at 35 ppt on day 14 (K = −0.276±0.078).

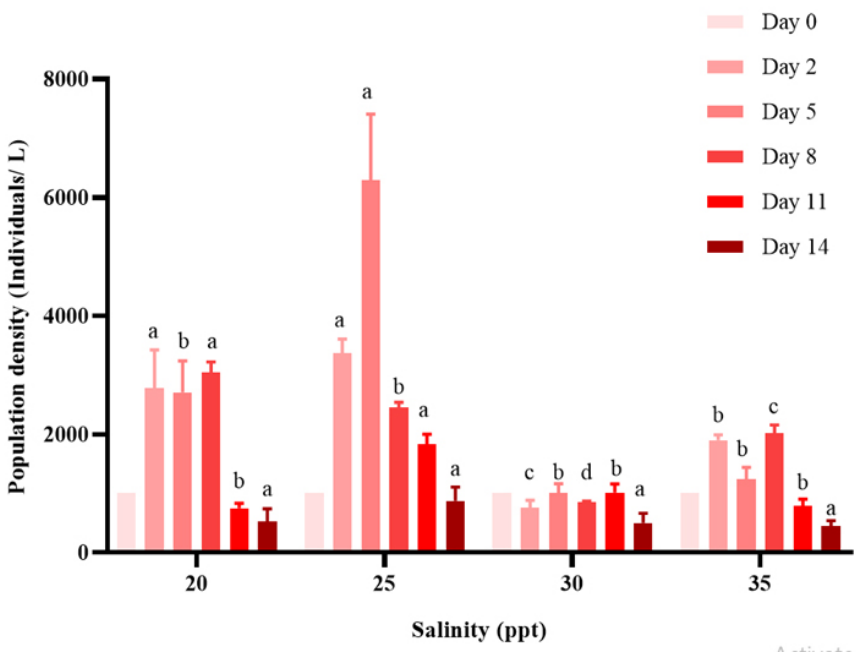

FIGURE 4 Total population density of *Apocyclops* sp. at four different salinity treatments accros 14 days interval. Presented data are in mean ± SD. Means with different superscript letters indicate that data are significantly different (p < 0.05).

3.3.3 Population composition of *Apocyclops* sp.: The stage wise percentage composition of *Apocyclops* sp. varied significantly (p < 0.05) across different salinity levels (20–35 ppt) as shown in the Figure 6. The proportion of adults was significantly ($F_{3,8}$= 5.611, p < 0.05) highest at 20 ppt (62.76±1.04%) and showed a decreasing trend with increasing salinity, reaching lowest at 35 ppt (50.63±7.37%). The highest copepodite proportion was observed at 25 ppt (37.54±1.37%) which was not significantly ($F_{3,8}$= 9.278, p > 0.05) different from 35 ppt (36.91±8.94%) and 20 ppt (28.37±1.18), while the significantly ($F_{3,8}$= 9.278, p < 0.05) lowest copepodite proportion was recorded at 30 ppt (19.91±2.48%). Naupliar composition was significantly ($F_{3,8}$ = 38.396, p < 0.05) higher at 30 ppt (17.94±2.65%) compared to 20 ppt (5.13±0.15%), 35 ppt (5.08±2.84%) and 25 ppt (2.12±0.67%). Similarly, no significant ($F_{3,8}$ = 32.978, p > 0.05) difference was observed between 30 (8.95±1.47%) and 35 (7.97±0.84 %) ppt salinity in the percentage of ovigerous females; however, both were significantly ($F_{3,8}$ = 32.978, p < 0.05) different from 20 (3.81±0.18%) and 25 (2.72±0.71%) ppt salinity.

4 | DISCUSSION

Salinity variations in estuarine and brackish-water environments play a key role in determining the distribution and abundance of copepod populations (Pan *et al.* 2016). Also it plays a crucial role in regulating copepod metabolism as it directly affects osmotic balance (Lee *et al.* 2017) and the organism's ability to maintain ionic homeostasis within their cells (Huanacuni *et al.* 2025). In the present study, it was shown that salinity becomes a critical parameter for *Apocyclops* sp. in regulating the survival, reproduction and population dynamics with 25 ppt shown as the optimal condition for overall development. Although the species exhibited broad tolerance to the salinity within the 20–35 ppt range, the results indicate that even a small variation from the optimum level has a significant influence on the biological responses at both in-

dividual and population level of this organism. Having a wider tolerance to salinity, a useful feature for the live prey to become a successful larval feed in aquaculture (Vijayaraj *et al.* 2025).

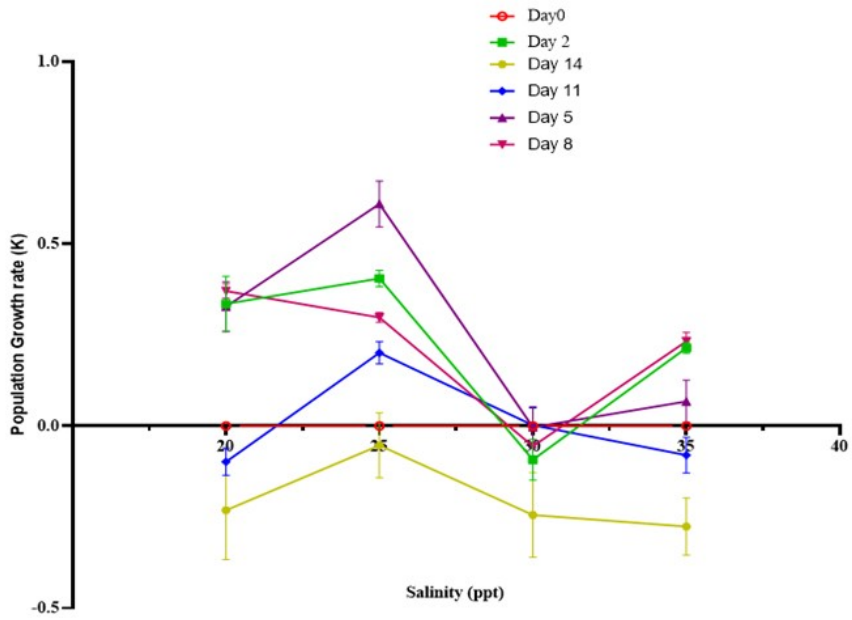

FIGURE 5 Population Growth rate (*K*) of *Apocyclops* sp. under different salinity levels. Presented data are in mean ± SD.

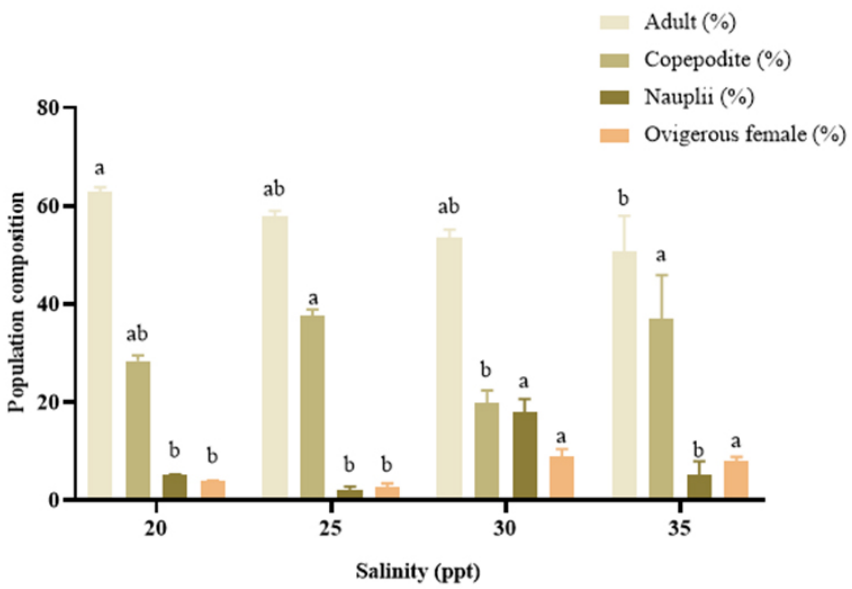

FIGURE 6 Percentage of adult, copepodities, nauplii and ovigerous female of *Apocyclops* sp. reared under different salinities. Presented data are in mean ± SD. Means with different superscript letters indicate that data are significantly different ($p < 0.05$)

4.1 Adult survival

Understanding the salinity tolerance of individual copepod species is crucial for assessing their potential use in fish larval rearing across different salinity regimes. Responses to salinity variations are highly species-specific, with different copepods exhibiting varying levels of tolerance and adaptability (Chintada *et al.* 2023). The cyclopoid copepod *Apocyclops* sp. exhibited high adult survival throughout the salinity range of 20–35 ppt, with maximum survival recorded at 25 ppt, which highlights the euryhaline nature and its adaptability to diverse aquatic environments like coastal and brackish waters (Cruz-Rosado *et al.* 2020; Yoshino *et al.* 2022). The present findings are consistent with the previous studies, where *Acartia bilobata* shows optimal survival between 25 and 30 ppt with a decline at lower (0–15 ppt) and higher salinity (35–45 ppt) levels (Chintada *et al.* 2023). A similar response was documented for *Pseudodiaptomus pelagicus* which exhibited optimal survival within moderate salinity ranges (15–35 ppt) (Ohs *et al.* 2010). Likewise, the same

pattern was observed in *Apocyclops royi*, whose survival was optimal between 10 and 32 ppt (Hansen 2023). Cruz-Rosado *et al.* (2020) reported that *Apocyclops panamensis* is commonly survive at the salinity range of 28–32 ppt. Furthermore, studies on *Dioithona oculata* showed maximum survival between 25–30 ppt with reduced survival at extreme salinities (below 20 ppt and above 35 ppt) (Darsana *et al.* 2022). Osmoregulation is one of the process which paves a way for copepod to adapt high salinity fluctuations (Svetlichny and Hubareva 2014) by concentrating free amino acids inside the cells makes them survive (Darsana *et al.* 2022). The decline in survival at extreme salinities may be associated with increased metabolic costs for maintaining the ionic balance, a pattern also observed in *Acartia tonsa*, where osmotic stress has been associated with changes in metabolic pathways (Makri *et al.* 2024; Hahn *et al.* 2026). This assessment of survival will be helpful in determining the range of salinities for utilization of copepod as live feed in larval rearing.

4.2 Naupliar survival

Nauplii are the most sensitive life stages and their survival significantly decreases under suboptimal salinities due to limited tolerance (Darsana *et al.* 2022). It is a rich source of free amino acids more than twice the amount than *Artemia* and contains higher levels of high unsaturated fatty acids (Rayner *et al.* 2017). In the present study, the naupliar survival exhibited a similar pattern, with the optimal performance at 25 ppt and significant reductions below 20 ppt, indicating the greater sensitivity of earlier life stages. This aligns with the findings for *Dioithona oculata*, where stable naupliar survival occurs within the moderate salinity ranges (25–35 ppt) (Darsana *et al.* 2022). Chinnery and Williams, 2004 reported that survival declines in *Acartia* species at salinity extremes (15–33 ppt) corresponding to habitat conditions. Consistent with the present findings, studies on *Apocyclops royi* have shown that naupliar survival decreases substantially at lower salinities may be associated with increased energetic costs associated with osmoregulation (Gréve *et al.* 2020; Hansen *et al.* 2022). Magouz *et al.* (2021) reported that cyclopoid members like *Oithona* and *Dioithona* have better survival in lower salinities (15–25 ppt). The decline in survival observed at both hypo-and hypersaline conditions may reflect that physiological stress disrupts the normal development of copepod by depleting protein reserves (Kimmel and Bradley 2001).

4.3 Adult longevity

Salinity variations play a pivotal role in regulating the energy budget of copepods, triggering the synthesis of stress related proteins, influencing reproductive success and determining spatial distribution across various environments (Lee *et al.* 2017). In our study, adult longevity was significantly influenced by salinity, with a maximum lifespan recorded at 25 ppt, which may be attributed to reduced osmotic stress and energy allocated towards somatic maintenance and survival rather than the osmoregulatory process (Rippingale and Hodgkin 1977). The decline in adult longevity in 30 ppt and 35 ppt is possibly due to increased oxidative stress. A similar trend was observed in *Tigriopus kingsejongensis*, in which exposure to both hypo- and hypersaline conditions resulted in reduced lifespan compared with individuals maintained at the control salinity of 34 ppt (Kim *et al.* 2022).

4.4 Reproductive parameters

Cyclopoid copepods, are highly sensitive to osmotic stress due to deviation from the ideal salinity range affecting the reproductive outputs (Yoshino *et al.* 2022) and its productivity is related with several factors like egg production and egg hatching success (Milione and Zeng 2008). In the present study, highest reproductive performance was recorded at 25 ppt, characterized by increased clutch size and enhanced nauplii production. The reproductive optimum observed in the present study is in agreement with previous studies reported in *Acartia bilobata*, with the highest reproductive performance observed within a salinity range of 25–30 ppt (Vu *et al.* 2024) and for *Dioithona oculata*, which exhibits optimal reproductive performance at approximately 30 ppt salinity (Darsana *et al.* 2022; Sancho 2022). This observation align with the reports on *Apocyclops dengizicus*, confirming that clutch production and performance parameters are reduced at salinities above and below 25 ppt (Farhadian *et al.* 2014). In contrast, the reduced clutch sizes observed at suboptimal salinities (15–20 ppt) may be associated with increase in energy expenditure for osmoregulation by limiting reproductive performance (Kimmel and Bradley 2001; Lee *et al.* 2016). This is similar to *Eurytemora affinis*, where salinity range of 5 to 25 ppt disrupts egg membrane stability (Devreker *et al.* 2010) and clutch is often affected because of the hyperosmotic stress which could be attributed to reduction in growth and nauplii production. Chen *et al.* (2006) reported that egg production of *Pseudodiaptomus annandalei* were reduced at high salinity of 30–35 ppt. Hatching success of copepod was influenced at high salinity levels (>15 ppt) (Devreker *et al.* 2009). Although reproductive output varied among salinity treatments, egg diameter and hatching success did not significantly within the 20–35 ppt range, suggesting that egg quality was preserved under moderate salinity conditions. Comparable observations have been documented in Calanoid copepods, whose egg size is influenced by salinity (Milione and Zeng 2008) in which egg hatching rate of *Acartia tonsa* (Danish strain) was higher at 20 ppt (Peck and Holste 2006) and A. tonsa (Baltic) shows higher at 25 ppt (Holste and Peck 2006) and in *Acartia ohtsukai*, where hatching success remains stable across moderate salinity ranges (10–30 ppt) (Choi *et al.*

2021). This evidence, together with the sustained reproductive success of *Apocyclops dengizicus* under salinity regimes between 5 and 35 ppt (Farhadian *et al.* 2014), further emphasizes the salinity tolerance characteristic of the genus *Apocyclops* at the embryonic stages. The observed stability likely reflects the protective properties of the ovisac or plasma membrane, which buffers embryos from external osmotic fluctuations (Højgaard *et al.* 2008). Morphological parameters, namely egg size and ovisac length were consistent across salinity treatments ranging from 20–35 ppt. Conversely, ovisac width showed variation across treatments and was positively associated with reproductive output, especially at 25 ppt. Similar patterns of morphological stability have been reported in copepods in which reproductive structures remain relatively constant, whereas volume–associated traits adjust to accommodate clutch size (Pan *et al.* 2017; Doan *et al.* 2019). This selective phenotypic plasticity enables copepods to ensure efficient egg packing and reproductive functionality across varying environmental conditions (Vehmaa *et al.* 2012).

4.5 Population parameters

Salinity is one of the most important factor which affect the copepod population (Devreker *et al.* 2009). Population density and structure are the major characteristics that make the copepod preferred as live feed under culture conditions. Among the salinity treatments, 25 ppt salinity consistently supported the most favorable population performance, indicating that this condition optimizes multiple biological processes simultaneously. A similar relationship between salinity and population performance has been reported in other cyclopoid copepods, like *Oithona nana*, which showed a maximum total population at salinity 20 ppt (Magouz *et al.* 2021) and *O. rigida*, which showed a maximum at a range of 28–34 ppt (Santhanam and Perumal 2012). This may be due to osmoregulatory effects of salinity stress on species-specific copepods and their life history traits (Lee *et al.* 2016).

The high population density and growth rate observed at 25 ppt can be attributed to the synchronization between important life history characteristics, such as high adult survival, maximum clutch size, stable hatching success and efficient naupliar development (Milione and Zeng 2008). Such coordination promotes consistent recruitment across developmental stages, preventing stage-specific bottlenecks and supporting sustained population expansion. In contrast, although hatching success remained stable across treatments, the reduced clutch size and delayed development observed at sub-optimal salinities, particularly at 30 ppt, resulted in lower effective recruitment and slower population growth. This aligns with the result obtained by Mau *et al.* (2023), where the population density of *Apocyclops dengizicus* shows the lowest population at 30 ppt salinity. It is evident that population growth may be associated with developmental efficiency and reproduction than by hatching success alone and also copepods cultured under unfavourable conditions often undergo a lag phase in growth, where initial reproduction or development is delayed, resulting in suppressed or even negative growth rates during early stages (Darsana *et al.* 2022).

The analysis of population composition revealed that salinity plays an important role in influencing developmental progression and stage distribution, thereby affecting the relative proportions of nauplii, copepodites, adults and ovigerous females. A significantly higher proportion of nauplii was recorded at 30 ppt, indicating that individuals are retained longer in early developmental stages, suggesting slower progression to copepodite and adult stages (Darsana *et al.* 2022). This progress reflects a developmental constraint that slows population turnover and limits population growth, even when reproductive activity is underway. Similar stage specific developmental delays have been reported in copepods exposed to suboptimal environmental conditions whereby slower development leads to low population productivity (Karlsson *et al.* 2018; Yoshino *et al.* 2022). Additionally, similar trends were shown that salinity affects developmental rates and stage composition in *Dioithona oculata* (Darsana *et al.* 2022). The relatively high proportion of adults recorded at 20 ppt may indicate prolonged adult survival (Darsana *et al.* 2022). Despite this advantage, population density remained lower than that observed at 25 ppt, suggesting that enhanced survival alone is insufficient to maximize population growth. This proves the importance of reproductive performance for population expansion. Thus 20 ppt may favour population stability, whereas 25 ppt promotes rapid and sustained population growth.

The population performance not only depends on individual characteristics but also on their effective integration across life stages (Darsana *et al.* 2022). The increased proportion of nauplii at 30 ppt, coupled with reduced clutch size and slower development, indicates that even slight deviation from the optimal salinity can disrupt synchronization of life- history traits and reduce overall productivity. The balanced stage composition observed at 25 ppt suggest that developmental progression occurred efficiently throughout the life cycle by ensuring continuous recruitment and minimizing losses between the stages. The highest proportion of ovigerous females is often influenced by salinity (30–35 ppt), where increased reproductive activity at certain salinities may not necessarily transferred into higher populations density, due to constraints in development or survival (Chen *et al.* 2006).

The temporal dynamics further highlight that population performance is strongly phase dependent. In the initial stages of culture, variations in survival and reproductive performance driven by salinity influence population growth, resulting in distinct differences among

treatments. However, as the culture progresses, density dependent factors such as resource depletion, accumulation of metabolic wastes and space limitation begin to regulate population size, leading to convergence of population responses across treatments. This shift from environmental to nutrient driven control of copepod population size represents a common phenomenon in batch cultures (Milione and Zeng 2008; Hansen 2023).

Moreover, the fact that high population density remains even at 25 ppt during the decline phase indicates greater resilience under resource limited conditions. This resilience may be attributed to more energy efficiency, constant reproductive performance and a balanced stage structures, all of which contribute toward better resistance against environmental stress (Støttrup 2006). Such characteristics are particularly advantageous in aquaculture systems, where fluctuations in food availability and culture conditions are common. The population level responses observed in the present study provide an integrated understanding of how salinity influences the overall productivity of *Apocyclops* sp., as these parameters reflect the combined effects of survival, reproduction, hatching success and developmental progression.

The relatively small number of females (n = 5 per treatment) used for reproductive assessments is a limitation of the present study, and further studies with larger sample sizes are recommended to validate these findings.

4 | CONCLUSIONS

The current study demonstrated that the salinity is a critical environmental factor governing the survival, reproduction and population performance of the marine cyclopoid copepod *Apocyclops* sp. Among the various experimental salinities (5–40 ppt for survival; 20–35 ppt for reproductive and population parameters), 25 ppt emerged as an ideal condition for all the measured biological outputs. At this salinity, *Apocyclops* sp. achieved a higher adult survival, naupliar survival, maximum clutch size, nauplii production per female and increased population parameters during the early culture phase. Egg diameter, hatching success and ovisac length remained largely stable across the 20–35 ppt range, indicating that embryonic development is well buffered against moderate salinity fluctuations. Ovisac width showed some variation, reflecting adaptive changes in reproductive organ size in response to clutch size differences. The synchronization of survival, reproduction and developmental progression at 25 ppt highlights that population productivity in *Apocyclops* sp. is an emergent outcome of the integration of multiple biological processes rather than any single parameter in isolation. These findings reinforce the importance of maintaining salinity within a narrow optimal range to maximize culture efficiency and to avoid hidden developmental bottlenecks that may not be evident from single parameter evaluations. Based on the present study, 25 ppt was identified as the most suitable salinity for maintaining and culturing *Apocyclops* sp. under the tested experimental conditions. Further validation under commercial-scale hatchery conditions, including different stocking densities and feeding regimes, is required before recommending 25 ppt as an optimal salinity for large-scale production.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

AK: Investigation, Formal analysis, Methodology, Data curation, Writing – Original draft, editing. CA: Conceptualisation, Supervision, Validation, Methodology, Review. SS: Formal analysis, Writing–Review & Editing. NM: Methodology, Review & Editing. SP: Review & editing. SVG: Formal analysis, Review & editing. KB: Review & Editing. PV: Review & editing.

AI USE DECLARATION

In the preparation of this manuscript, generative AI tools (ChatGPT developed by OpenAI) were used only to assist in language editing, grammar correction and improving clarity. The author take full responsibility for the content, accuracy and originality of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on a reasonable request from the corresponding author.

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