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Effect of Different Salinity Levels on Population Dynamics and Growth of the Cyclopoid Copepod *Oithona nana*

Fawzy I. Magouz [1], Mohamed A. Essa [2], Mustafa Matter [2], Abdallah Tageldein Mansour [3,4,*], Ahmed Gaber [5] and Mohamed Ashour [2]

1 Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh 33516, Egypt; Fozymagouz22@yahoo.com
2 National Institute of Oceanography and Fisheries (NIOF), Cairo 11516, Egypt; messa51@yahoo.com (M.A.E.); mhsy4@yahoo.com (M.M.); microalgae_egypt@yahoo.com (M.A.)
3 Animal and Fish Production Department, College of Agricultural and Food Sciences, King Faisal University, P.O. Box 420, Al-Ahsa 31982, Saudi Arabia
4 Fish and Animal Production Department, Faculty of Agriculture (Saba Basha), Alexandria University, Alexandria 21531, Egypt
5 Department of Biology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia; a.gaber@tu.edu.sa
* Correspondence: amansour@kfu.edu.sa; Tel.:+966-56-508-9202

Abstract: Copepods are one of the most abundant and diverse live food sources for mesopelagic and bathypelagic fishes and crustaceans. They could contribute to the overlap of the transition period from live feed to an artificial weaning diet in marine larvae production. However, the culture conditions still need optimization to provide sufficient production to cover the increasing demand for marine hatcheries. Therefore, the present study investigated the effects of different salinity levels (5, 10, 15, 20, 25, and 30 ppt) on the population growth, growth rate, and population composition (males, females, copepodite, and nauplii ratio) of the marine copepod, *Oithona nana*. The experiment continued for 15 days, under laboratory-controlled conditions of temperature (27 ± 1 °C), pH (7.7 ± 0.15), and continuous gentle aeration in 30 L glass aquaria. The copepod culture aquaria were supplemented with a mixture of soybean and yeast (0.5 g 10^{-6} individual^{-1} 24-h^{-1}) as a feed source. The highest significant population growth and population growth rate of *O. nana* were achieved with a salinity level of 20 ppt. Regarding population composition, *O. nana* cultured at the salinity level of 20 ppt recorded the highest significant percentages of copepodite and nauplii. The results concluded that copepod, *O. nana*, is capable of withstanding abrupt changes in the salinity, but there are limits to their tolerance, with an optimal salinity level of 20 ppt. This salinity level achieved the highest population growth and the highest percentages of copepodite and nauplii of marine Copepoda, *O. nana*.

Keywords: aquaculture; live feeds; salinity; copepodite; growth; population composition

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1. Introduction

Aquaculture is one of the fastest-growing global industries that supply animal protein to face the demand of an increasing global population [1]. To realize successful and sustainable marine aquaculture applications, continuous isolation and screening are required to discover native aquatic organisms [2]. Copepods are the most important group that links the primary producers with the secondary consumers in aquatic ecosystems. In marine hatcheries, the production of the appropriate live feed is considered one of the most important bottleneck factors that limit the success of marine larvae production [3–7]. Besides rotifer and Artemia, which were extensively utilized as prey for shrimp and fish larvae [8–17], copepods are the most suitable live prey [3–6]. In the wild, among zooplankton, copepods are the primary prey of mesopelagic and bathypelagic fish [18,19]. Copepods are

remarkable because they are the most abundant and diverse under different environmental conditions [10].

The copepod family Oithonidae comprises an important part of the copepod biomass in temperate areas, displaying high population densities [20]. The Oithonidae family are the most efficient free-living cyclopoids in the marine epipelagic ecosystem. They are small (usually less than 1 mm in body length) and exist in the ocean at relatively high densities [21]. *Oithona nana* is the most distributed *Oithonidae* species that comprises more than 90% and 67% of the abundance and the biomass, respectively, of the zooplankton in many coastal areas [22]. Although the ecological importance of this *Oithonidae* species is high, the literature is sparse about its reproduction and ecosystem conditions [20,23,24].

Delicate determination of the population dynamics of copepods in the mass production system is very important in predicting the quantity of production and providing the hatchery's requirement of live food, which can be achieved successfully in a laboratory environment under controlled ranges similar to environmental conditions [25]. It is very important to identify the optimum environmental conditions for a potential aquaculture species to establish a population of that organism, which will be positively reflected in the revenue of production [8,26,27]. Such information is important for the successful culturing of copepods to be utilized as live feed in the aquaculture industry [28].

Among all the environmental parameters, temperature and salinity have been reported as the major variables affecting copepod species. The salinity significantly affects the population dynamics of the copepod *O. rigida* [29]. Many authors have reported that the variation in salinity strongly influences population growth, growth rate, composition, survival, and hatching success of several species of marine copepods [25,30–39]. Therefore, studying the effects of salinity levels on marine copepod production is still required to find out the ideal concentration for marine aquaculture. Milione et al. [36] reported that the highest population growth of calanoid copepod *A. sinjiensis*, cultured at different salinity levels ranging from 10 to 50 g L^{-1}, was reported at 30 g L^{-1}. Pan et al. [39] studied the effects of different salinity levels (0–35 ppt) on the population growth and composition of different developmental stages of the copepod *Apocyclops royi* and reported that salinity levels had varying effects on *A. royi* regarding its composition at different developmental stages. The current study aims to evaluate the population dynamics and growth of the cyclopoid copepod *O. nana* cultured under different salinity levels.

2. Materials and Methods

2.1. Isolation and Stock Culture of Copepods

The studied copepod, *O. nana*, was isolated during the 2017 spring season from earthen ponds at El-Max Research Station, Alexandria Branch of the National Institute of Oceanography and Fisheries, (NIOF), Egypt. During the collection period, the water quality factors of salinity (31 ± 1 ppt), temperature (23 ± 2 °C), and pH (7.37 ± 0.10) of earthen ponds were recorded at noon. Copepod samples were collected using the protocol described by Abo-Taleb et al. [9]. Morphological identifications of isolated adult individuals were firstly examined under a binocular stereomicroscope (Optika Microscopes, B190/B-290, Italy) and then taxonomically characterized by the Hydrobiology Lab., Marine Environment Division, NIOF, Egypt. The adult individuals of *O. nana* were cultured in stock tanks under laboratory-controlled conditions (27 ± 1 °C, 30 ± 1 ppt, pH 7.7 ± 0.15, and continuous gentle aeration) and enriched with a native identified marine microalga, *Nannochloropsis oceanica*, NIOF15/001 at a concentration of 2 × 10^6 cells^{-1} ml^{-1} day^{-1} [40], which was diluted from the algal culture medium (5 × 10^6 cells^{-1} ml^{-1} day^{-1}) [13].

2.2. Experimental Design

Before the start of the experiment, the adult individuals of *O. nana* were collected from the stock tanks and transferred to the new culture water for 24 h as a gut-evacuation period [39,41]. The adult individuals of copepod *O. nana* (average size: 625 µm) were cultured in an experimental glass tank with 30 L of 1 µm bag-filtered, chlorine-disinfected

seawater (30 ppt). According to references [26,38], the standard culture densities for copepods species ranged from 0.5 to 1.0 mL. Therefore, the initial stock density of *O. nana* in the current study was selected according to the previous reference with approximately 1 individual ml^{-1} (about 1000 ind. L^{-1}).

In the current study, the effects of six salinity levels (5, 10, 15, 20, 25, and 30 ppt, with three replicates for each level) on population dynamics, including growth, growth rate, and population composition (male, female, copepodite, and nauplii) of marine isolated copepods, *O. nana*, were determined. To prepare the salinity levels, saline water (30 ± 1 ppt) was diluted with distilled water to obtain the needed level. During the experiment, all treatments were fed a mixture of commercial-grade baker's yeast, *Saccharomyces cerevisiae* (supplied by Starch, Yeast and Detergents Company, Alexandria, Egypt), and a very finely ground commercial grade of soybean (supplied by Fish Feed Factory, located in Alexandria, Egypt), with a concentration of 0.5 g 10^{-6} individual 24-h^{-1} of each feed source.

The needed concentration of feed was estimated depending on the previously counted copepods' individual mL^{-1}, which was measured every three days (day-0, day-3, day-6, day-9, day-12, and day-15) [42,43]. The experiment was continued for 15 days (from day-0 to day-15), under laboratory-controlled conditions of temperature (27 ± 1 °C, using a digital thermometer) and pH (7.7 ± 0.15). The replicates were conducted without water replacement and were supplied with gentile aeration to keep the dissolved oxygen (DO) over 4 mg/l (measured using Oxymeter, HI-9142, HANNA Instruments, Woonsocket, RI, USA). In all salinity levels, the ammonia (NH_3) concentration (measured using digital multi-meter, Crison Model MM41, Spain) was < 0.45 ± 0.05 mg L^{-1}.

2.3. Tested Parameters

2.3.1. Population dynamics

Every three days, from each replicate, a constant volume (25 ml) of culture water was taken to calculate the population growth of copepods (ind. ml^{-1}). The population growth rate (*r*) was calculated depending on the population growth, according to the equation of Yin et al. [44], as the following:

$$R = (\ln N_t - \ln N_0)/t \quad (1)$$

where N_0 and N_t are the initial and final population densities, respectively, and t is the incubation time in days.

2.3.2. Population Composition

To determine the percentage of different developmental stages (male, female, nauplii, and copepodite) of population composition, approximately one hundred individuals were randomly harvested from each replicate (using a plankton net of a 38 µm mesh), and fixed with a 4% formalin solution, and investigated on slides under a microscope (Optika Microscopes, B190/B-290, Ponteranica, Italy).

2.4. Data Analysis

The statistical analyses were determined using SPSS Ver. 16. The data are presented as the mean ± standard error (n = 3). To compare differences among individual means at a significance level of $p \leq 0.05$, all variables were calculated using one-way analysis of variance (ANOVA) followed by Duncan's multiple-range tests.

3. Results

Data presented in Figure 1 show that the population growth of marine isolated copepoda, *O. nana*, was significantly affected by different salinity levels (5, 10, 15, 20, 25, and 30 ppt). Among all salinity levels, the population growth of *O. nana* cultured at a level of 20 ppt exhibited the highest significant ($p \leq 0.05$) population growth in all investigated days, as follows: day-3 (1300 ind. L^{-1}), day-6 (1800 ind. L^{-1}), day-9 (2800 ind. L^{-1}), day-12 (3533 ind. L^{-1}), and day-15 (4733 ind. L^{-1}).

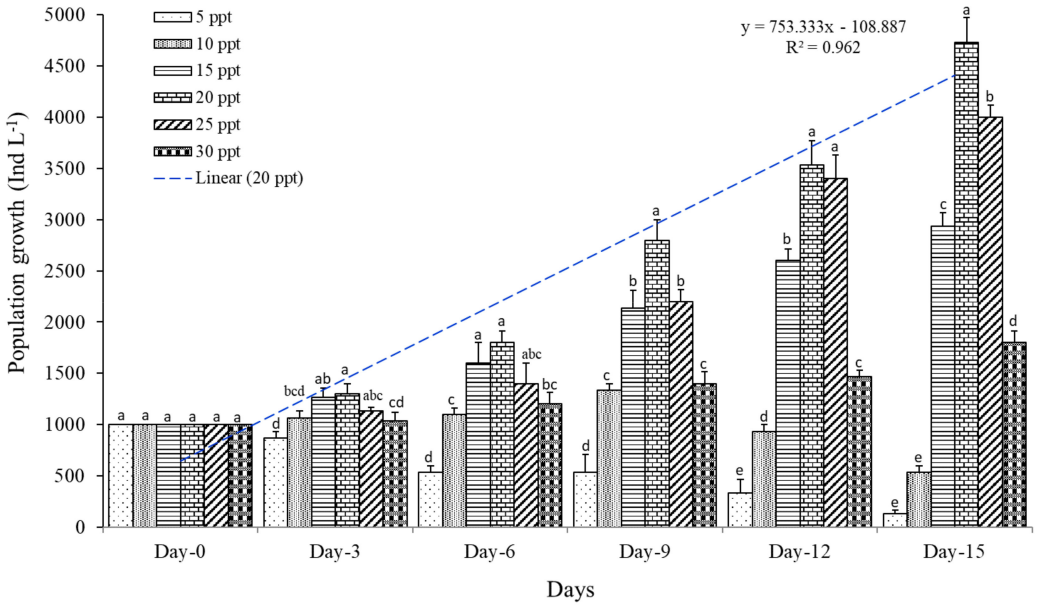

Figure 1. Effect of different salinity levels on the population growth (individual l^{-1}) of Copepoda, *Oithona nana*. Data are presented as the mean ± standard errors. The letters (a, b, and c) above each bar indicate the significant differences ($p \leq 0.05$) between different diets in the same day. The blue dash line is the linear regression of population growth of *O. nana* reared at 20 ppt salinity level over time.

As presented in Figure 1, at the salinity level of 20 ppt, the increase in population growth followed a linear trend with R^2 = 0.96 and y = 753.333x − 108.887. Between measurements, the salinity level of 25 ppt exhibited the second-highest population growth of *O. nana*, while the lowest population growth was reported at 5 ppt. At the end of the experiment, day-15, the highest significant increase in population growth was obtained by *O. nana* cultured at the salinity level of 20 ppt (4733 ind. L^{-1}), followed by 25 ppt (4000 ind. L^{-1}), 15 ppt (2933 ind. L^{-1}), 30 ppt (1800 ind. L^{-1}), and 10 ppt (533 ind. L^{-1}). Meanwhile, the copepod population maintained in the salinity levels of 5 and 10 ppt significantly decreased compared to other treatments from the initial stocking density. In addition, the effect of salinity on population growth after 15 days revealed a polynomial second-order regression with a strong correlation (R^2 = 0.80) and the regression equation as follows: $y = -18.238x^2 + 755.67x - 3953.3$ (Figure 2).

As shown in Figure 3, among all salinity levels at day-15, the highest population growth rate (0.518) of *O. nana* was reported with the salinity level of 20 ppt and the increase was time-dependent (with R^2 = 0.993 and y = 0.107x-0.114), followed by 25 ppt (0.462), 15 ppt (0.358), 30 ppt (0.195), 10 ppt (−0.215), and the lowest was at 5 ppt (−0.691) (Figure 3). The effect of salinity was a second-order polynomial pattern with R^2 = 0.98 and the regression equation is $y = -0.0044x^2 + 0.1935x - 1.5955$ (Figure 4).

The percentages of the population composition of *O. nana* cultured on different salinity levels (Figure 5) revealed significant differences between the developmental stages (male, female, nauplii, and copepodite). Copepoda *O. nana* cultured on salinity levels of 20, 25, and 30 ppt exhibited the highest significant percentages of nauplii (18.67%, 13.59%, and 11.33%, respectively) and copepodite (16.65%, 14.33%, and 10.33%, respectively), while the salinity levels of 15, 10, and 5 ppt exhibited the lowest significant percentages of nauplii (3.33%, 2.00%, and 1.42%, respectively) and copepodite (2.65%, 2.34%, 2.03%, respectively), as presented in Figure 5.

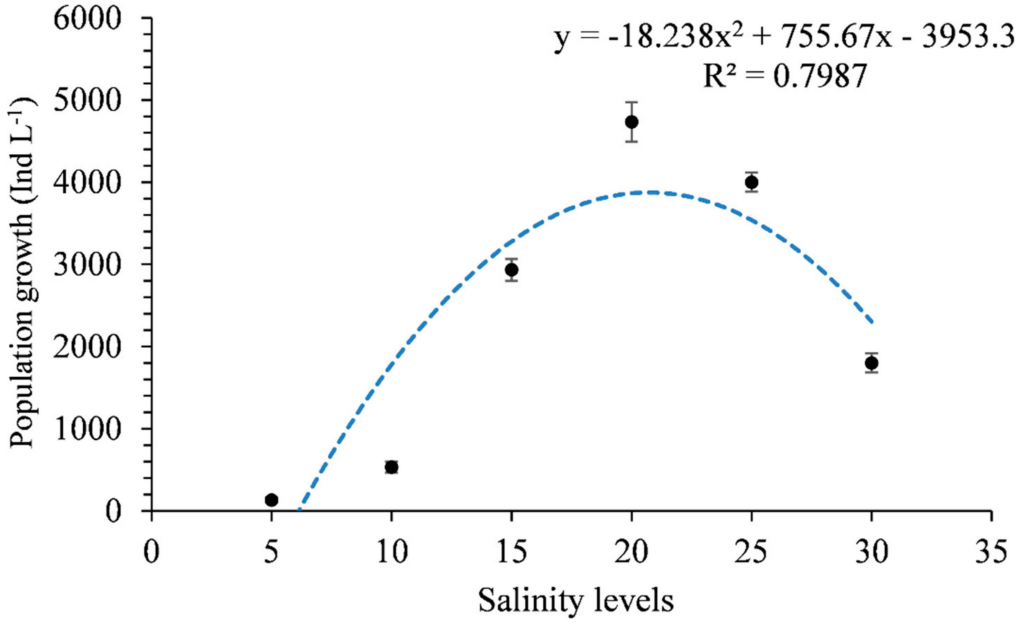

Figure 2. Polynomial second-order regression of Copepoda, *Oithona nana*, population growth reared at different salinity levels after the 15 days of the experiment.

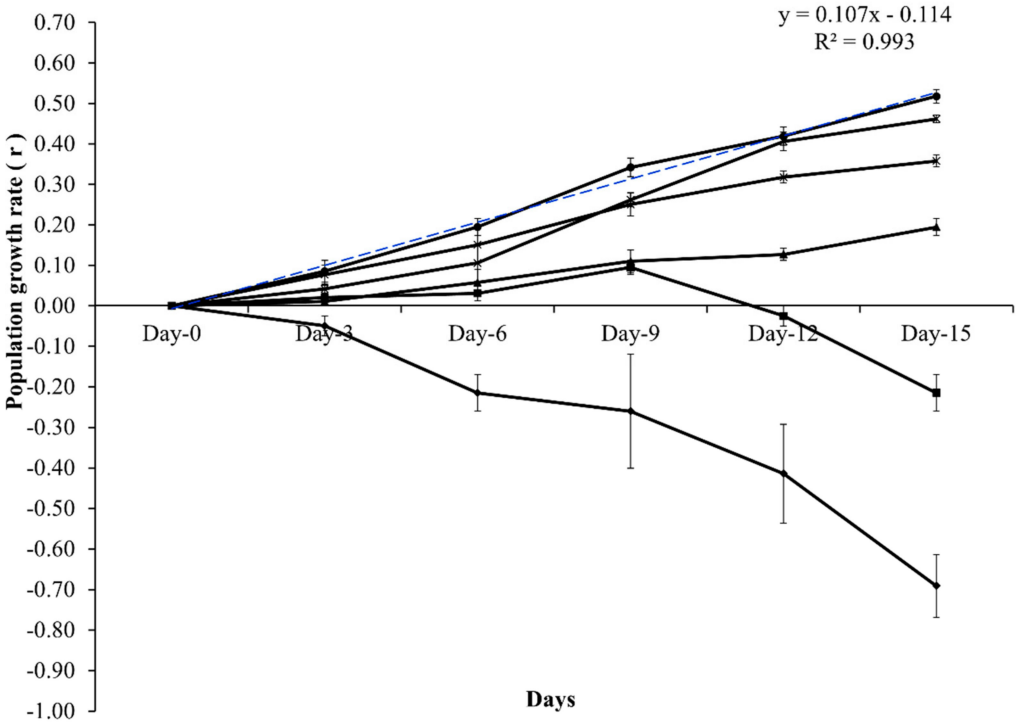

Figure 3. Effect of different salinity levels on the population growth rate (*r*) of Copepoda, *Oithona nana*.

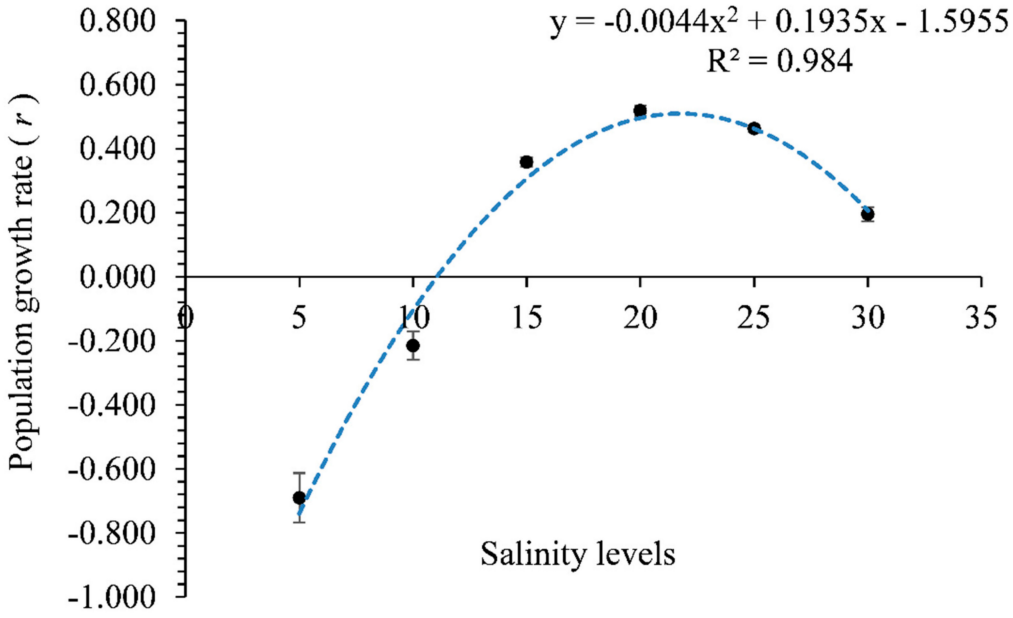

Figure 4. Polynomial second-order regression of Copepoda, *Oithona nana*, population growth rate (*r*) reared at different salinity levels after the 15 days of the experiment.

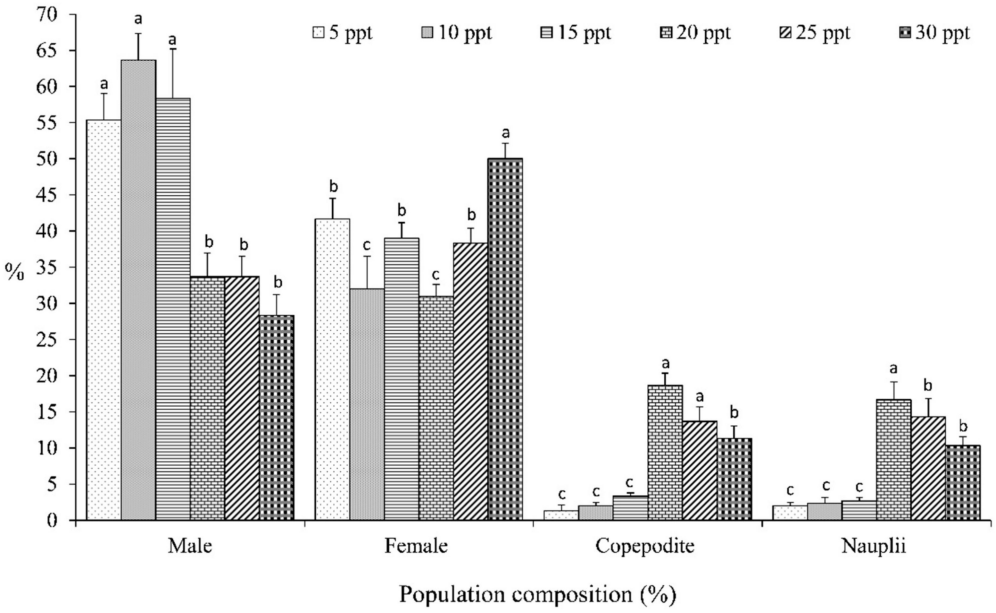

Figure 5. Effect of different salinity levels on the percentage of developmental stages of Copepoda, *Oithona nana*. Data are presented as the mean ± standard errors. The letters (a, ab, b, and c) above each bar indicate the significant differences ($p \leq 0.05$) between the developmental stages (adult males, adult females, nauplii, and copepodites) at different salinities.

4. Discussion

Copepoda *Oithona* sp. is an egg-bearing species with a global distribution [40], and is the prevailing copepod in many tropical, subtropical, temperate coastal marine, and estuarine environments [11,45,46]. *Oithona nana* is considered one of the most appropriate species for mass production in marine hatcheries [47,48]. The low yields, long generation time, and seasonal variations in production under certain conditions are the major obstacles limiting the success of the farming of aquatic organisms [3,11,49,50].

Studying the effects of the salinity on the different growth population parameters, such as population growth and growth rates, of copepod species might clarify the pop-

ulation dynamics in extensive aquaculture ponds or their wild habitats. In addition, a comprehension of ideal salinity could improve copepod productivity in aquaculture environments [39]. In the current study, different salinity levels (5, 10, 15, 20, 25, and 30 ppt) were used to determine the optimal salinity level for the population growth, population growth rate, and population composition of the copepod, *O. nana*. The results disclosed that the salinity levels significantly affected the population growth, population growth rate, and population composition of *O. nana* (Figures 1–3).

In the current study, after a 15-day culture period, *O. nana* cultured with a salinity level of 20 ppt showed the highest significant population growth (4733 ind. L^{-1}, $R^2 = 0.962$) and growth rate (0.518, $R^2 = 993$), followed by 25 ppt (4000 ind. L^{-1}, 0.462, respectively). In contrast, the lowest significant population growth and growth rate were revealed in both the low- and high-salinity levels of 5, 10, 15, and 30 ppt. Our results are in line with the results of Pan Allam et al. [51], who reported a significantly lower population growth of Cyclopoida copepod, *A. royi*, was obtained in both the low- and high-salinity levels of 5, 30, and 35 ppt, after a 14-day culture period. Moreover, Santhanam et al. [29] cited that the ideal population of copepod, *O. rigida*, was maintained between 28 and 34 ppt, decreasing the salinity to less than 20 ppt significantly reduced the population, and the *O. rigida* population disappeared in the culture tanks when the salinity was decreased to less than 10 ppt.

In estuary habitats, salinity variation is the pivotal factor that affects the distribution of copepod populations. The differences in salinity tolerance demonstrate the distribution of prevalent copepod species in salinity gradients [52]. In defining the feasibility of copepod species to be utilized as live prey in aquaculture, it is necessary to understand the effects different salinities have on production parameters. Many copepod species can survive in different conditions while having very different productivity [3]. Having wide salinity tolerance is a very useful feature for the live prey in aquaculture and allows copepods to be fed to cultured larvae at different salinity levels [38,53].

The findings of the current study indicate that the highest developmental-stage percentages of nauplii and copepodites of copepod, *O. nana*, were found when cultured at the salinity level of 20 ppt followed by 25 ppt, while salinity levels lower than 20 ppt (5, 10, and 15 ppt) showed very low developmental-stage percentages. Our results agree with Santhanam et al. [29], who reported that salinity levels strongly influence the population and developmental stages of copepod, *O. rigida*, with consideration of lower salinity resulting in longer development times. The authors of [38] report on the salinity affecting the time to maturation, percentage of females, and total nauplii produced by copepod, *Pseudodiaptomus pelagicus*.

Our results conclude that the adult copepods, *O. nana*, were unable to tolerate rapid changes in the salinity from 30 ppt to lower than 15 ppt. These findings show that *O. nana* is able to resist sudden changes in the salinity levels but displays a limit to optimal tolerance of 20 ppt. These findings could be attributed to the physiological stress, the additional osmoregulation, and respiration demands at these salinities. Our findings agree with [38], who reported that copepod *P. pelagicus* adults can withstand abrupt changes in salinity levels from 35 to 15 g L^{-1} and up to 48 g L^{-1}. In the current study, the total nauplii produced at the salinity levels of 20 and 25 ppt were more than double the number produced at 30, 15, 10, and 5 ppt, although there was a high variation [38]. Chen et al. [34] cited that adult copepods, *P. annandalei*, were not capable of producing nauplii at 35 ppt. Payne et al. [54] reported that cultured *G. imparipes* were not able to produce nauplii at four salinity levels.

5. Conclusions

Studying the effects of different salinity levels on the different growth population parameters of the copepod species is useful in clarifying its population dynamics in marine aquaculture, as well as defining its feasibility to be utilized as live prey in marine hatcheries. The salinity levels of the culture water significantly influence the population growth, population growth rate, and the percentages of developmental stages of cultured *O. nana*.

The findings of our study could be used to develop and improve the culture conditions of copepod, *O. nana*. The current study concluded that copepod, *O. nana*, can withstand abrupt changes in the salinity, but exhibits limits to its tolerance, with an optimal salinity level of 20 ppt.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the authors upon request.

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Conflicts of Interest: The authors declare no conflict of interest.

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