Effects of Food Concentration and Photoperiod on Egg Production, Female Life Expectancy and Population Dynamics of the Paracalanid Copepod, *Bestiolina amoyensis*

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The paracalanid copepod, *Bestiolina amoyensis*, is a widely distributed species occurring in subtropical inshore waters across the Pacific Ocean. Its small size, herbivorous feeding habit, and high adaptability make the species one of the most promising candidates as potential live feed for hatchery larval rearing. This study investigated effects of different feeding density of microalgae *Isochrysis* spp. (1×10^5, 2×10^5, 3×10^5, 4×10^5, and 5×10^5 cells ml^{-1}) and photoperiod (8L:16D, 12L:12D, and 16L:8D) on productivity-related parameters, including egg production, female life expectancy and population dynamics of *B. amoyensis*. Results showed that total egg output over female lifespan, final population size and intrinsic rate of population of 12L:12D photoperiod treatments were always the highest among three photoperiod conditions, especially at the food concentration of 4×10^5 cells ml^{-1}, indicating *B. amoyensis* had high reproductive performance and the population was in a more stable status. The number of nauplii from 4×10^5 cells ml^{-1} algal concentration treatment accounted for 75% of the population, and the ratio of females to males approaching 1:1 when photoperiod was 12L:12D; female life expectancy was 10.5 ± 0.6 days. In conclusion, our results showed that *Isochrysis* spp. is a suitable feed for *B. amoyensis* with an optimal concentration at 4×10^5 cells mL^{-1}; the optimal photoperiod for *B. amoyensis* rearing is 12L:12D. The relatively long reproductive lifespan and high intrinsic population increase rate make *B. amoyensis* a good candidate to develop culture techniques for hatchery larval rearing.

Keywords: copepod, sex ratio, productivity, live prey, algal feeding density

INTRODUCTION

Providing palatable nutritional food of appropriate size to marine larvae at different development stages is one of the most challenging tasks in marine hatcheries. For some larvae with small mouth gapes, the traditional live prey of rotifers (mean length 135 μm of SS-type rotifer *Brachionus rotundiformis*) and *Artemia* nauplii (typically between 400–500 μm) could be too large to be

ingested by them (Fielder et al., 2000; Assavaaree et al., 2003; Gopakumar and Santhosi, 2009; Wullur et al., 2009; Conceição et al., 2010; Lindley et al., 2011; Hagiwara et al., 2014), and lacking of appropriate live prey to feed such larvae has been a crucial technical bottleneck. Copepods are the natural food for most marine larvae and nauplii of many copepods are less than 100 μm in size, which can meet the first feeding requirement of even small mouth- gaped fish larvae (McKinnon et al., 2003; Camus and Zeng, 2010; Bradley et al., 2013; Hill et al., 2020). Moreover, copepods typically develop through six naupliar and six copepodite stages with each stage of different size, making them suitable prey for fish larvae with different sizes of mouth gapes, as well as at different developmental stages (Schipp et al., 1999).

Several paracalanid species, including *Parvocalanus crassirostris* (Alajmi and Zeng, 2015) and *Bestiolina similis* (McKinnon et al., 2003; Camus et al., 2009; VanderLugt et al., 2009; Camus and Zeng, 2010), have been recommended as good candidates as larvae prey for fish larvae with small mouth gape. *B. amoyensis,* a species belonging to the same genus of *B. similis,* has been reported to co-exists with *B. similis* in the tropical coastal and estuary waters (McKinnon et al., 2003). The species distributes widely in Pacific Ocean, especially in western Pacific Ocean, and it is a dominant species during autumn (August – November) in subtropical Xiamen Harbor and Dongshan Bay of China (Yang, 2007; Zheng, 2009). However, no attempts have been made to culture the species as the live prey for feeding fish larvae previously.

Comparing to the traditional live prey, the main problem of copepods as live feed for larval culture is their difficulty for intensive culture and/or low culture productivity. Therefore, optimizing the culture conditions has been the focus of previous research. Many factors could affect copepod culture reproductivity and past research has been focused on temperature, salinity, and both quality and quantity of their food (e.g., Holste and Peck, 2006; Camus et al., 2009; Camus and Zeng, 2010; Nogueira et al., 2017; Nguyen et al., 2020; Choi et al., 2021; Dayras et al., 2021), while the effects of photoperiod is less studied despite photoperiod has been shown to significantly affect culture productivity of two *Acartia* species, i.e., *Acartia tonsa* (Peck and Holste, 2006) and *Acartia sinjiensis* (Camus and Zeng, 2008). Photoperiod is well known as one of the most important environmental factors regulating reproductive activity of aquatic animals, including copepods, and it has been reported to influence number of eggs produced by females, as well as the type (i.e., subitaneous vs. diapause eggs) of eggs produced (Marcus, 1982; Peck and Holste, 2006; Camus, 2012; Fereidouni et al., 2015). Furthermore, the physiological "clock" of aquatic animals allows them to response to changes in light condition, which could affect their feeding and reproductive activity (Miliou, 1992). For example, marine zooplankton, especially copepods, are known to undertake diurnal vertical migration (Haney, 1988) with a typical feeding pattern showing higher ingestion rate during night (Fuller, 1937; Petipa, 1958; Dagg and Grill, 1980; Stearns, 1986; Saito and Taguchi, 1996; Calbet et al., 1999), and some calaniod copepods, such as *P. crassirostris* (Sun, 2008) and Calanus (Harding et al., 1951; Zhang, 2003),

spawn occurs more frequently at night. Hence, the goal of the current study was to evaluate the effects of photoperiod and microalgae feeding concentration and their interaction effects on culture productivity of *B. amoyensis*, seeking to answer the questions such as is there an optimum photoperiod regime for the cultivation of *B. amoyensis*? Whether there exist interactions between algae feeding concentration and photoperiod? For instance, whether longer darkness hours leading to enhanced egg production may require more abundant food supply to sustain higher productivity?

MATERIALS AND METHODS

Microalgal Culture

The microalgae *Isochrysis* spp. was cultivated in a temperature-controlled room. Several 70 L cylindrical acrylic cylinders filled with 0.01 μm filtered, UV irradiated, and Chlorine treated seawater (salinity 28 psu) were used for the algae culture. All cultures were maintained at $25 \pm 1°C$ with vigorous aeration and f/2 medium was used for the cultures (Guillard and Ryther, 1962). The photoperiod was set as constant light (24 h light) with a light intensity of approximately 4000–5000 Lux. The algal cultures in its exponential growth phase were used for feeding copepods.

Sampling and Isolation of Copepods

Bestiolina amoyensis were initially obtained from a plankton tow performed in coastal water of Jimei District, Xiamen City, Fujian Province, China, on May 20th, 2019. The plankton sample was immediately transported back to a laboratory at Jimei University for isolation of *B. amoyensis*.

To isolate *B. amoyensis*, healthy copepods were firstly attracted to a light source, they were then transferred by a pipette to several petri dishes, where they were sorted according to their morphological characteristics and movement mode. The *B. amoyensis* identified (Li and Huang, 1984; Lian et al., 2018) were gently pipetted individually to wells of a 12-well plate filled with autoclaved seawater, wells were then carefully checked to make sure there was no contamination of other animals. Female *B. amoyensis*, identified by their swollen genital segments, were further sorted and individually cultured in 12-well cell culture plates. The culture volume of each well was 5 ml with *Isochrysis* spp. added at 1×10^5 cells ml^{-1}. Plates were incubated in a unit with light illustrated from down under. The light intensity was 500–800 Lux and photoperiod was set as 12 h L:12 h D.

Bestiolina amoyensis Stock Culture

Following separation, *B. amoyensis* cultures were gradually scaled up and eventually kept in several 300 L tanks filled with 0.01 μm filtered seawater and with gentle aeration. The salinity was 28 ± 1 psu and temperature was maintained at $26 \pm 1°C$. Light intensity was ~ 500 Lux with a light: dark cycle of 12 h:12 h. Approximately 30% of the culture water was exchanged daily by gentle siphoning with a 5 cm diameter hollow cylindrical isolation device fitted to the front of the siphon tube, the bottom of which was covered with 48 μm mesh to prevent removal of copepods and reduce damage. *B. amoyensis* was

fed daily with microalgae *Isochrysis* spp. at a concentration of 1×10^5 cells ml^{-1}. Culture vessels were totally drained every 15 days for cleaning when adult copepods were caught with 500 μm special nets and juveniles with 75 μm nets, and the juveniles were transferred to culture carboys cleaned and sterilized with chlorine.

Experimental Design and Setup

A series of experiments were carried out to assess the influence of food concentration and photoperiod on the following parameters related to *B. amoyensis* culture productivity: (1) egg production, (2) adult female life expectancy, and (3) population growth over a 12 days culture period.

Production of Adult Copepods for the Experiment

For all experiments, mature *B. amoyensis* were retrieved with a special 500 μm sieve from stock culture and immediately transferred to a 2.5 L container with the bottom covered by a 75 μm mesh, the container was suspended in a 5 L transparent vessel containing 4 L of 0.01 μm filtered seawater. This design facilitated the separation of adult copepods and eggs produced by the females. After leaving the copepods to spawn for 6 h, each 2.5 L container containing the adult copepods was transferred to a new 5 L container, but the eggs produced by them were kept in the original 5 L container for incubation until hatching. The newly hatched nauplii were cultured for 3 days in the 5 L container before being transferred to an 18 L container for further cultivation until reaching the copepodite V stage (C_V). The culture conditions and feeding were initially the same as those described for stock culture (i. e. 26 ± 1°C; 28 ± 1 psu) and the concentration of feeding algae was 1×10^5 cells mL^{-1}. The last stage copepodites were then transferred to 30 ml Petri dishes and were monitored for molting daily with a microscope until they all developed to adults and ready for the following experiments. Adult *B. amoyensis* were transferred to a 1 L container containing filtered seawater and acclimated for 24 h to eliminate potential residual effects under the previous culture condition. The mixed population of both males and females during acclimation ensured that copepods used for the experiment were fertilized before being introduced into the Petri dishes for experiment.

Egg Production and Adult Female Life Expectancy Experiment

Following the acclimation, pairs of adult *B. amoyensis* that were actively swimming and with intact appendages were selected from the 1L beaker and carefully transferred to each of a 30 mL Petri dishes containing 20 mL seawater to monitor daily egg production. In the case that a male died during the experiment, a new male was introduced (Hall and Burns, 2001). If a female had not produced any eggs by the 3rd days of the experiment, a new female was introduced. Cultures were maintained in a constant temperature incubator and light intensity was set as ∼ 500 Lux.

This experiment used 5 × 3 factorial design with five food concentrations (1×10^5, 2×10^5, 3×10^5, 4×10^5 and 5×10^5 cells ml^{-1}) and 3 photoperiod conditions (8 h:16 h, 12 h:12 h, and 16 h:8 h), hence a total of 15 treatments. With 12 replicate pairs of adult copepods per treatment, a total of

180 Petri dishes were set up. The copepods were starved for 24 h prior to the formal commencement of the trial. Microalgae in exponential growth phase were use in present experiment and five samples were measured daily using an automated cell counter (Count-star IA1000, Shanghai Ruiyu Biotech Co., Ltd.) to maintain the designated concentrations of the experiment. Constant temperature (26°C) incubators were used to set up three photoperiod conditions and the light intensity was ∼ 500 Lux. During the experiment, each pair of copepods were removed from the Petri dish and transferred to new one with fresh seawater and microalgae supplied at the designated concentration every 24 h. The egg production was monitored and recorded using a Leica DMi8 microscope. Any deaths over the 24 h period was also recorded. Experiment was continued until all copepods in a treatment had died, and the mean life expectancy of adult *B. amoyensis* in the treatment was obtained by averaging individual lifespans all 12 replicates of the treatment. The daily egg production and the total egg production over female lifespan for each treatment was calculated by averaging the data obtained from 12 replicates (Camus and Zeng, 2010).

Population Growth Experiment

The same 5 × 3 factorial design of 5 food concentrations and 3 photoperiod conditions was used for the population growth experiment, which lasted for 12 days. There were three replicates per treatment. Adult copepods were produced as described in the section "Production of Adult Copepods for the Experiment." After acclimation, 12 *B. amoyensis* adults (4 males and 8 females) were introduced into each of a series of 45 × 1 L beakers (each 1 L beaker as a replicate) to start the experiment. Cultures were maintained in an incubator with constant temperature (∼ 26°C), salinity 28 psu, and a light intensity of ∼ 500 Lux over 12 days. Approximately 30% of the culture water was exchanged daily by gently siphoning using the cylindrical isolation device described above.

After 12 days of culture, the contents of each beaker were emptied onto a 48 μm sieve, all eggs, nauplii, copepodites and adults retained on the sieve were subsequently rinsed into Petri dishes, and 4–5% formalin was added to fix them for later counting. Eggs, nauplii, copepodites and adults of *B. amoyensis* were counted in each replicate and the final population size averaged from three replicates. Intrinsic rate (r) of population growth was then calculated for each treatment using the following formulation:

$$r = \frac{\ln \frac{N_0}{N_1}}{t}$$

where N_0 = population number at the beginning of the experiment, N_1 = population number at the end of the experiment while t (days) is the duration of the experiment (Fenchel, 1974). Meanwhile, adults were sexed to yield the sex ratio of the population.

Data Analysis

Data are presented as mean ± standard error (SE). A statistical probability of $p < 0.05$ was accepted as significant in all tests. The effects of food concentration and photoperiod and

their interaction on egg production, female life expectancy and population dynamics were analyzed using two-way ANOVA. All data were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) prior to analysis of ANOVA. When significant interaction between the two factors of food concentration and photoperiod was found, the Tukey's multiple comparisons test was performed to determine specific significant differences among treatments. If the assumption of normality was not met, the correlation between two variables was tested by the nonparametric Scheirer-Ray-Hare test (daily egg production data).

RESULTS

Daily Egg Production

Figure 1 shows average daily egg production of *B. amoyensis* when cultured under various combinations of microalgae concentrations and photoperiods. Statistical analysis showed that there was an interaction effect between food concentration and photoperiod on daily egg production ($p < 0.001$). However, there was no significant effects among different photoperiods at all 5 diet concentrations ($p > 0.05$, **Figure 1**). On the other hand, the average daily egg production of the diet concentration treatment of 1×10^5 and 2×10^5 cells ml^{-1} were significantly lower than that of 3×10^5, 4×10^5, and 5×10^5 cells ml^{-1} treatment ($p < 0.01$) under all 3 photoperiod regimes (**Figure 1**).

In term of total egg production over female lifespan, both food concentration ($p < 0.001$) and photoperiod ($p < 0.001$) had a main effect, and there was also a significant interaction effect between food concentration and photoperiod on the total egg production ($p < 0.05$, two-way ANOVA). Under all 3 photoperiods tested, the total egg production over female lifespan increased continuously with increasing food concentration, and reached a peak at 4×10^5 cells ml^{-1} ($p < 0.05$), however, the trend reversed as microalgae concentration further increased to 5×10^5 cells ml^{-1} ($p < 0.05$, **Figure 2**). Indeed, copepod from the 4×10^5 cells ml^{-1} treatment produced the highest number of eggs under all 3 photoperiod conditions, which was 2.3 times higher than the lowest value of the 1×10^5 cells ml^{-1} treatment (**Figure 2**). Similarly, photoperiod also had a significant effect on the total egg production of female at various food concentrations ($p < 0.001$): At microalgae concentration of 3×10^5, 4×10^5, and 5×10^5 cells ml^{-1}, the highest egg production was all found under the photoperiod of 12 h L:12hD, followed by the photoperiod of 8 h L: 16 h D, while the 16 h L:8 h D photoperiod always had the lowest egg production (**Figure 2**). Overall, the highest total egg production over female lifespan was found under the combination of 4×10^5 cells ml^{-1} microalgae and 12 h L:12 h D photoperiod, which reached 139.7 ± 4.4 eggs female^{-1} (**Figure 2**).

Adult Female Life Expectancy

Both food concentration and photoperiod significantly impacted female lifespan of *B. amoyensis*, and there was a significant interaction between the two factors ($p < 0.01$, **Figure 3**). The female life expectancy of 12L:12D photoperiod was not

significantly different from that of other 2 photoperiod groups ($p > 0.05$) in 1×10^5 cells mL^{-1} group, while that of 16L:8D photoperiod group (11 ± 0.8 d) was significantly higher than that of the 8L:16D photoperiod group (8 ± 1.4 d) ($p < 0.01$). In 3×10^5 cells mL^{-1} group, the female life expectancy of the 12L:12D photoperiod group (10 ± 2.1 d) was significantly higher than that of the other 2 photoperiod groups ($p < 0.05$), and the difference between 8L:16D photoperiod and 16L:8D photoperiod groups was not significant ($p > 0.05$). When food concentrations were 2×10^5 cells mL^{-1}, 4×10^5 cells mL^{-1} and 5×10^5 cells mL^{-1}, the female life expectancy from 3 photoperiod groups did not differ significantly ($p > 0.05$, **Figure 3**).

There was no statistical difference among different food concentrations under 8L: 16D and 12L: 12D photoperiods for female life expectancy ($p > 0.05$, **Figure 3**). Under the photoperiod regime of 16L:8D, the female lifespan of the 1×10^5 cells mL^{-1} diet treatment was not significantly different from that of the 2×10^5 cells mL^{-1}, and 4×10^5 cells mL^{-1} diet treatments ($p > 0.05$), but significantly higher than those of the 3×10^5 cells mL^{-1}, and 5×10^5 cells mL^{-1} food concentrations ($p < 0.01$, **Figure 3**). At the same time, the female life expectancy of the 2×10^5 cells mL^{-1} treatment was significantly higher than that of the 5×10^5 cells mL^{-1} treatment ($p < 0.05$) although both of them were not significantly different from that of the 3×10^5 cells mL^{-1}, and 4×10^5 cells mL^{-1} treatments ($p > 0.05$, **Figure 3**).

Population Growth and Composition

The average final population sizes of *B. amoyensis* after 12 days of culture under different combinations of food concentrations and photoperiods are presented in two categories: "All Stages Included", which included eggs, and "All Post-Egg-Stages", which excluded eggs (**Table 1**). For "All Stages Included" final population, statistical analysis showed that the final population sizes were significantly affected by both food concentration ($p < 0.01$) and photoperiod ($p < 0.01$), and there was an interaction effect between food concentration and photoperiod ($p < 0.001$).

Of the 3 photoperiods tested, at a same algal concentration, the 12 h L:12 h D photoperiod always produced the largest and significantly bigger final population than those of the other two photoperiods ($p < 0.01$, **Figure 4**). Meanwhile, under both 8 h L:16 h D and 12 h L:12 h D photoperiods, population size increased with increasing food concentration, and reached a peak at 4×10^5 cells ml^{-1} (4386.7 ± 112 and 7979.7 ± 172.2 for photoperiod 8 h L:16 h D and 12 h L:12 h D, respectively) before dropped when food concentration further increased to 5×10^5 cells ml^{-1} (**Figure 4**). At photoperiod regime of 16 h L:8 h D, population size similarly increased significantly with increasing algal concentration but the maximum population size was observed at microalgae concentration of 3×10^5 cells ml^{-1} as population decreased significantly as algal concentration further increased to 4×10^5 and 5×10^5 cells ml^{-1}, respectively (**Figure 4**). It is worth noting that levels when compared to the other two photoperiods, at all food concentrations the final population of the photoperiod 12 h L:12 h D remained at relatively high levels (**Figure 4**).
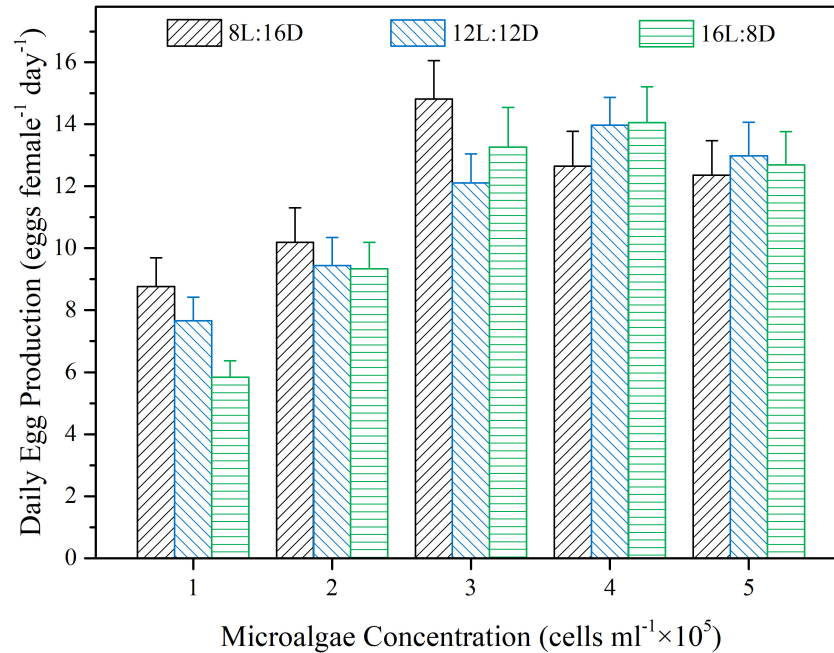
FIGURE 1 | Effects of different concentrations of the microalgal diet and photoperiods on average daily egg production of *Bestiolina amoyensis* (*N* = 12). Data is presented as mean ± standard error (SE).
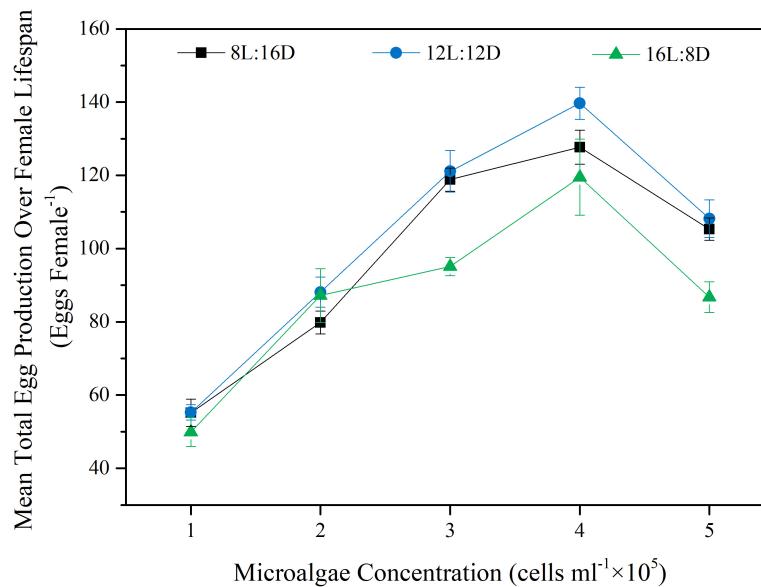

FIGURE 2 | Effects of different concentrations of the microalgal diet and photoperiods on total egg production over female lifespan of *B. amoyensis* (*N* = 12). Data is presented as mean ± SE.

Figure 4 shows final population composition of *B. amoyensis* (eggs, nauplii, copepodites, female and male adults) at the end of the experiment. The maximum amount of nauplii and copepodite was reached under photoperiod of 8L:16D in all diet treatments (**Figure 4**, **Table 1**). Under photoperiod 12 h L:12 h D and 16 h L:8 h D, nauplii stage accounted for more than 50% of

the population size. In particular, under the culture condition of photoperiod 12 h L:12 h D and 4 × 10^5 cells ml^{-1} algal feeding concentration, nauplii accounted for 75% of the total population (**Figure 4**).

For the female to male sex ratio, under photoperiod 8 h L:16 h D, it ranged from 1.5:1 of 3 × 10^5 cells ml^{-1} food concentration
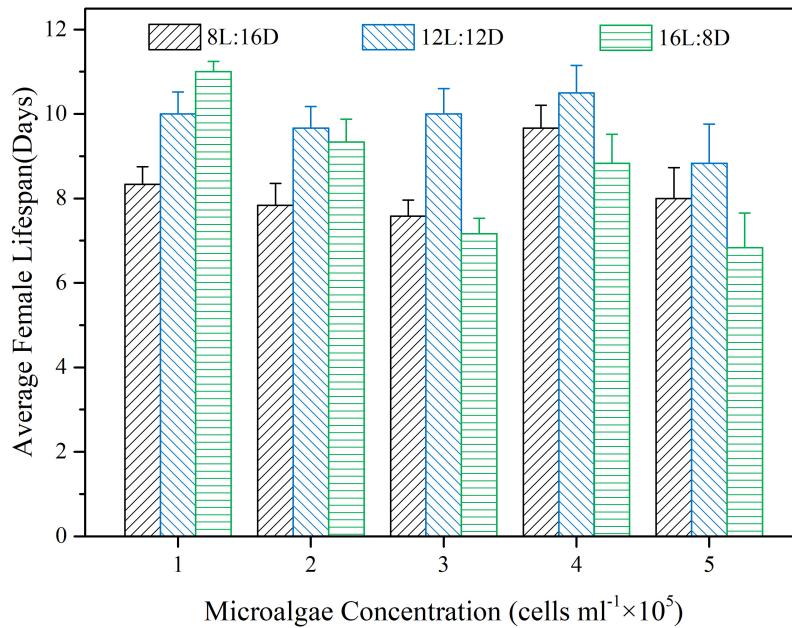
FIGURE 3 | Effects of different concentrations of the microalgal diet and photoperiods on female lifespan of *B. amoyensis* (N = 12). Data is presented as mean ± SE.

TABLE 1 | Final population size and intrinsic rate (r) of *B. amoyensis* cultured over a 12 days period fed on different microalgal diets under three photoperiod regimes.

Photoperiod regime	Microalgal concentration (cells mL^{-1})	Total number of final population – All stages included	r – All stages included	Total number of final population – All Post-Egg-Stages	r – All Post-Egg-Stages	Sex ratio Female:Male
8L:16D	1 × 10^5	1684.3 ± 54.9^c	0.40 ± 0.02^d	1474.3 ± 49.1^c	0.39 ± 0.03^d	(2.23 ± 0.03):1
	2 × 10^5	2275.0 ± 41.1^c	0.43 ± 0.02^c	2061 ± 32.8^{bc}	0.42 ± 0.03^c	(1.88 ± 0.08):1
	3 × 10^5	3334.7 ± 132.6^b	0.46 ± 0.03^b	2861.3 ± 145.3^{ab}	0.44 ± 0.02^b	(1.53 ± 0.02):1
	4 × 10^5	4386.7 ± 112^a	0.48 ± 0.01^a	3746.3 ± 137.3^a	0.47 ± 0.02^a	(1.70 ± 0.01):1
	5 × 10^5	3345.3 ± 113.5^{ab}	0.46 ± 0.02^b	2827.3 ± 114.3^{ab}	0.44 ± 0.03^b	(1.77 ± 0.07):1
12L:12D	1 × 10^5	6130.7 ± 100.5^B	0.51 ± 0.03^B	5110.0 ± 107.3^B	0.50 ± 0.03^B	(1.76 ± 0.07):1
	2 × 10^5	6329.7 ± 189.8^B	0.51 ± 0.02^{BC}	5665.0 ± 212.5^B	0.51 ± 0.02^{AB}	(1.58 ± 0.02):1
	3 × 10^5	7597.0 ± 283.8^A	0.52 ± 0.02^{AC}	5877.2 ± 240^{AB}	0.50 ± 0.02^{AB}	(1.62 ± 0.04):1
	4 × 10^5	7979.7 ± 172.2^A	0.53 ± 0.02^A	6839.7 ± 160.5^A	0.52 ± 0.02^A	(1.06 ± 0.01):1
	5 × 10^5	6085.7 ± 118^B	0.51 ± 0.03^B	4708.3 ± 119.4^{AB}	0.49 ± 0.04^B	(1.83 ± 0.02):1
16L:8D	1 × 10^5	2866.0 ± 17.5^J	0.44 ± 0.02^K	2170.3 ± 9.2^J	0.42 ± 0.03^J	(2.0 ± 0.06): 1
	2 × 10^5	3269.7 ± 39.2^J	0.45 ± 0.02^{JK}	2704.7 ± 32.7^{IJ}	0.44 ± 0.03^{IJ}	(1.54 ± 0.02):1
	3 × 10^5	6224.4 ± 69.3^H	0.51 ± 0.02^H	5247.7 ± 89.9^H	0.49 ± 0.03^H	(1.51 ± 0.07):1
	4 × 10^5	4815.3 ± 11.7^I	0.49 ± 0.03^I	3250.3 ± 6.7^I	0.45 ± 0.04^I	(1.09 ± 0.05):1
	5 × 10^5	3435.0 ± 9.5^J	0.46 ± 0.04^J	2388.6 ± 26^{IJ}	0.43 ± 0.05^J	(1.50 ± 0.02):1

Data are presented as Mean ± SE; different superscript letters in a same column indicate significant differences (p < 0.05) among different diet concentrations under the same photoperiod regime.

to 2.2:1 of 1 × 10^5 cells ml^{-1} food concentration (**Table 1**). Under both photoperiods 12 h L:12 h D and 16 h L:8 h D, the lowest female to male sex ratio was both 1.1:1 and from the 4 × 10^5 cells ml^{-1} food concentration treatment. For other food concentration treatments, the sex ratio ranged from 1.6:1 to 1.8 and 1.5:1 to 2.0:1 under photoperiod 12 h L:12 h D and 16 h L:8 h D, respectively (**Table 1**).

It is worth noting that both food concentration and photoperiod had a significantly effect on the intrinsic population

increase rates(r) of *B. amoyensis* (p < 0.001). The intrinsic rates under photoperiod 12 h L:12 h D was significantly higher than that of other 2 photoperiods (p < 0.01) at all 5 food concentrations. The intrinsic rates of 8L:16D photoperiod treatment was significantly lower than that of other 2 photoperiods in 1 × 10^5 cells ml^{-1} to 3 × 10^5 cells ml^{-1} food concentrations (p < 0.001), while at 4 × 10^5 cells ml^{-1} and 5 × 10^5 cells ml^{-1} food concentrations, the intrinsic population increase rates(r) of 8L:16D photoperiod group were
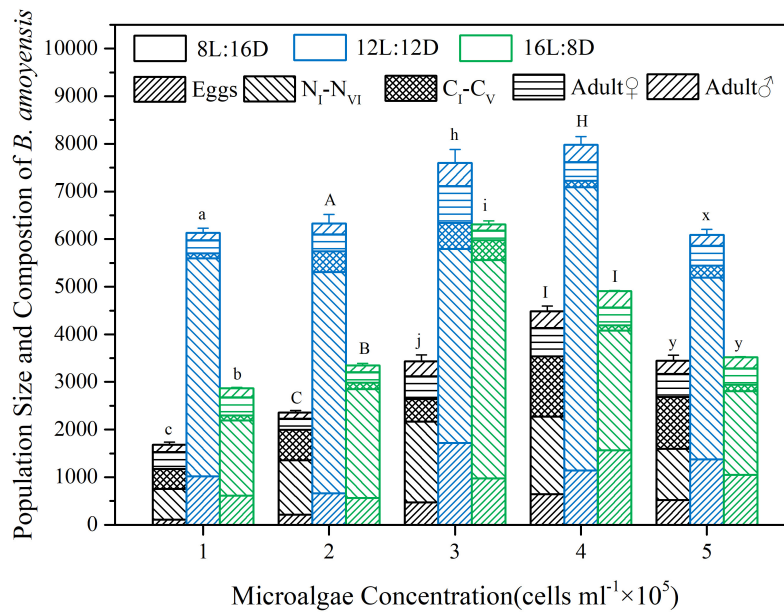
FIGURE 4 | Effects of different concentrations of the microalgal diet and photoperiod regimes on population size and population structure of *B. amoyensis* over 12 days of cultivation (*N* = 3). Data is presented as mean ± SE. Different letters on the top of bars indicate significant differences of different photoperiod treatments at the same microalgae feeding concentration (*p* < 0.05).

not significantly different from that of 16L:8D photoperiod (**Table 1**). When all stages were included, the intrinsic rates of 12 h L:12 h D photoperiod ranged from 0.51 to 0.53, the highest *r* value was reached at food concentration of 4×10^5 cells ml^{-1}, which was significantly higher than other food concentration treatments except the 3×10^5 cells ml^{-1} treatment. On the other hand, the intrinsic rates of under photoperiods 8 h L:16 h D and 16 h L:8 h D were ≤0.48 (4×10^5 cells ml^{-1}) and 0.51 (3×10^5 cells ml^{-1}), respectively (**Table 1**). Similar trend was shown when eggs were excluded with the intrinsic rates ranged from 0.39 to 0.47, 0.49 to 0.52, and 0.42 to 0.49 for photoperiod of 8 h L:16 h D, 12 h L:12 h D, and 16 h L:8 h D, respectively (**Table 1**).

DISCUSSION

Reproductive ability of copepods is affected by the nutritional condition of mature females (Castro-Longoria, 2003), and food quantity is an important factor that can significantly affect female condition. For example, the egg production has been reported to typically increase with the food concentration, reach a maximum level at a certain concentration (Runge, 1984; Hirche et al., 1997; Camus, 2012), but tend to decrease when the food concentration exceeds a threshold level (Zhao et al., 2014). Therefore, determining the optimal microalgae feeding quantity is crucial for optimizing the culture protocol of *B. amoyensis*.

Most of paracalanid copepods are herbivorous (Paffenhöfer, 1984) although a few of them have been reported as could be carnivorous (Suzuki et al., 1999). Similar to its congener *B. similis* (Vineetha et al., 2018; Siddique et al., 2021), we found *B. amoyensis* as herbivorous, feeding mainly on

phytoplankton. In the present study, the microalgae *Isochrysis* spp., one of the most commonly used marine unicellular algae for feeding calanoid copepods (Payne and Rippingale, 2000; Vengadeshperumal et al., 2010; Santhanam et al., 2013; El-Tohamy et al., 2021), was confirmed could fulfill the growth and reproduction needs of *B. amoyensis*. *Isochrysis* spp. is known for their high nutritional value, such as rich in polyunsaturated fatty acids (PUFAs), e.g., DHA and EPA (Sukenik and Wahnon, 1991; Gouveia et al., 2008) and grows well in mass cultures, either indoors or outdoors (Kaplan et al., 1986; Fidalgo et al., 1998). When at exponential phase of growth, the flagellated microalgae are not easily accumulated at the bottom like some other microalgae species, such a feature benefited the present experiments: the concentrations of *Isochrysis* spp. largely remained at designated levels during the 24 h period between daily water changes as confirmed by intermittent sampling of culture waters from replicates to estimate the algal concentrations from different treatments (see **Supplementary Table 1**).

Copepods productivity has commonly been indicated by daily egg production of females (Klepper et al., 1998). However, Camus and Zeng (2010) have adopted total egg production over female lifespan to estimate female productivity, and noted that this method is more accurate since it takes female life expectancy and reproductivity changes during lifespan into account. In this study, we have measured both daily egg production and egg production over female lifespan to provide a comprehensive information on egg production of *B. amoyensis* under different culture conditions. Our results showed that under all 3 photoperiods used, average daily egg production of *B. amoyensis* was significantly higher when they were fed *Isochrysis* spp. at a concentration ≥3×10^5 cells ml^{-1} when

compared to that at $\leq 2 \times 10^5$ cells ml^{-1}. In the case of total egg production over female lifespan, under all photoperiods used, the lowest total egg production was found in the lowest algal concentration of 1×10^5 cells mL^{-1}, and total egg production increased by the algal concentration, reaching a maximum level at 4×10^5 cells mL^{-1} before decreased with further increase of food concentration. Similar results were also reported for other copepod species, such as *Calanus pacificus* (Runge, 1984), *Calanus finmarchicus* (Hirche et al., 1997), and *Tisbe furcate* (Zhao et al., 2014). Such a result likely can be explained by that when food concentration is low, copepods need to move actively to catch more algae and with increase movement frequency of feeding appendage to enhance feeding (Paffenhöfer and Lewis, 1990), which increases energy expenditure. As the result, less energy can be channeled for egg production. Indeed, it has been observed that in some calanoids, clearance rate, i.e., the water volume sweeps clear by a copepod per unit time, increased with decreasing food concentration (Corner et al., 1972). Clearly, clearance rate increase is associated with higher energy expenditure. With higher food concentrations, copepods could obtain enough food with a lower clearance rate, which reduces energy consumption while still meeting the energy demands for both maintenance and reproduction (Zhao et al., 2014). However, when the food concentration is too high, it was found that microalgae could adhere to the feeding appendages and body surface of copepods, which could impair their feeding and motility, leading to lower food ingestion, and subsequent lower egg output and shorter female life expectancy. While Garrido et al. (2013) found that ingestion rate of calanoid copepod *Centropages chierchiae* increased linearly with the range of food concentrations they used (6.4–393.8 μC L^{-1}), but out results suggest that the ingestion rate of *B. amoyensis* does not increase infinitely with food concentration and a threshold exists, beyond which the clearance rate would not further increase, or even decrease.

Female life expectancy is one of the factors that could affect how much a female contributes to the total pool of eggs produced in the culture, and hence culture population growth. When food concentrations were $\geq 3 \times 10^5$ cells mL^{-1}, adult females cultured under 16 h L:8 h D photoperiod had a shorter life expectancy than other two photoperiods. The prolonged light phase likely kept *B. amoyensis* active for longer, and the higher metabolic rate may result in shorter female lifespan. Similarly, *A. sinjiensis* cultured under constant light had a relative shorter life expectancy than other photoperiods (Camus and Zeng, 2008).

The results of population growth experiment showed that feeding on *Isochrysis* spp. alone, *B. amoyensis* could significantly expanded its population at all concentrations tested, ranging from 1×10^5 to 5×10^5 cells ml^{-1} and under different photoperiods. Interestingly, at all 5 algal feeding concentrations, *B. amoyensis* cultured under 12 h L:12 h D photoperiod consistently had the maximum and significantly bigger population sizes at the end of the experiment than other two photoperiods, indicating the important of photoperiod in regulating population growth. A possible explanation of such

a result is that 12 h L:12 h D mimics typical photoperiod of Xiamen region during autumn when *B. amoyensis* reportedly is a dominant copepod species in Xiamen Harbor of China (Yang, 2007) where *B. amoyensis* used in this study were collected from. Indeed, the photoperiod of Xiamen region in spring and autumn ranges from 12 h L:12 h D, hence *B. amoyensis* may have adapted to such a photoperiod condition for maximum production. Indeed, under 12 h L:12 h D photoperiod, female *B. amoyensis* typically had the longest life expectancy among 3 photoperiods except at 1×10^5 cells ml^{-1} algal concentration, which was also not significant different from the longest life expectancy under 16 h L:8 h D. This should also lead to higher overall egg production while suggesting that 12 h L:12 h D is in fact optimal photoperiod for *B. amoyensis*.

The relative numbers of nauplii and copepodites in a culture population typically indicate the status of the culture (VanderLugt et al., 2009). VanderLugt and Lenz (2009) pointed out that populations dominated by nauplii typically indicate high reproductive rates of females. The maximum numbers and proportion of nauplii in the final populations of the population growth experiment were also found from the 12 h L:12 h D photoperiod, and at all 5 algae concentrations exceeding 50% of the total population. In particular, the number of nauplii of the 4×10^5 cells mL^{-1} treatment achieved 75% (**Figure 3**), indicating that *B. amoyensis* had a high reproductive rate under 12 h L:12 h D photoperiod and when fed *Isochrysis* spp. at 4×10^5 cells mL^{-1}.

Sex-determining in copepods are related to the population size and sex ratio (Mauchline, 1998; Voordouw and Anholt, 2002). In planktonic calanoid copepods, sex may also be controlled by environmental factors (Mauchline, 1998). The female-biased adult sex ratios had been reported in many species, including *Acrocalanus* sp., *Parvocalanus* sp., *Paracalanus indicus*, *B. similis* (McKinnon and Duggan, 2001; McKinnon et al., 2005; Duggan et al., 2008; Gusmão and McKinnon, 2009), *Paracalanus nanus* (McKinnon and Duggan, 2001; Gusmão and McKinnon, 2009), and *Paracalanus aculeatus* (McKinnon and Duggan, 2001; McKinnon et al., 2005; Gusmão and McKinnon, 2009). The sex ratios of *Calanus* spp. vary seasonally, and the food quality and quantity could also significantly affect sex ratio. For example, more males could be observed during the phytoplankton boom season comparing to the pre-phytoplankton boom season (Irigoien et al., 2000). Similarly, there were more male *B. amoyensis* found in the populations of 4×10^5 cells mL^{-1} treatments under 12 h L:12 h D photoperiod with the sex ratio approaching 1:1, indicating the culture population was at a stable status (Fisher, 1930) under the culture condition.

CONCLUSION

The results of this study showed that *Isochrysis* spp. is a suitable diet for *B. amoyensis* culture as the population expanded significantly when it is fed to the copepod at concentrations ranging from 1×10^5 to 5×10^5 cells ml^{-1}, and under photoperiods of 8 h L:16 h D, 12 h L:12 h D and 16 h L:8 h D. However, summarizing the outcomes of a range productivity

related parameters measured in *B. amoyensis*, including egg production, female life expectancy, and population growth and composition, the optimum *Isochrysis* spp. feeding concentration and photoperiod for the culture productivity of the species was determined to be 4×10^5 cells mL^{-1} and 12 h L:12 h D, respectively.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

SW and LW conceived the original idea of this study and designed the experiments. LW conducted the experiments and collected the data. LW, SW, and CsZ performed the data analyses. LW wrote the draft of the article and revised it with SW and CsZ. YW and CxZ attended the field works and performed part of data collection. SW supervised the research. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.788744/full#supplementary-material

Supplementary Table 1 | Concentrations of *Isochrysis* spp. from five different treatment groups after 24 h of incubation with the experimental *Bestiolina amoyensis*.

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