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Effects of algal concentration and initial density on the population growth of *Diaphanosoma celebensis* Stingelin (Crustacea, Cladocera)*

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Abstract The effects of algal concentration and initial density on the population growth of the estuarine cladocera, *Diaphanosoma celebensis* Stingelin, were evaluated in an indoor experiment. A 2 × 4 layout that included two algal concentrations (*Chlorella pyrenoidosa*, 1×10^6 and 3×10^6 cell/mL) and four inoculation densities (100, 200, 300 and 400 ind./L) were established. *Diaphanosoma celebensis* were reared in 150 mL flasks containing 50 mL of algal medium at 22°C, under salinity of 10 and a photoperiod of 12 h L: 12 h D. The lag phase required to initiate continuous population growth following inoculation was shorter for *D. celebensis* fed 1×10^6 cell/mL and inoculated at 300 or 400 ind./L than that for *D. celebensis* fed 3×10^6 cell/mL and inoculated at 100 or 200 ind./L. However, *D. celebensis* fed 3×10^6 cell/mL and inoculated at 100 or 200 ind./L exhibited longer periods of positive population growth. The maximum population densities were 5 875 ± 324, 6 690 ± 691, 7 735 ± 1,121 and 6 365 ± 691 ind./L for *D. celebensis* fed 1×10^6 cell/mL and inoculated at 100, 200, 300 and 400 ind./L, respectively, and 15 070 ± 379, 12 215 ± 648, 11 960 ± 2,551 and 16 130 ± 880 ind./L for *D. celebensis* fed 3×10^6 cell/mL and inoculated at 100, 200, 300 and 400 ind./L, respectively. The average daily increasing rates of population were 0.076 ± 0.001, 0.065 ± 0.002, 0.055 ± 0.002 and 0.048 ± 0.003 for *D. celebensis* fed 1×10^6 cell/mL and inoculated at 100, 200, 300 and 400 ind./L, respectively, and 0.098 ± 0.001, 0.078 ± 0.002, 0.072 ± 0.003 and 0.067 ± 0.003 for *D. celebensis* fed 3×10^6 cell/mL and inoculated at 100, 200, 300 and 400 ind./L, respectively. The maximum population density and average daily increasing rate of population increased as the algal concentration increased, whereas an increase in the inoculation density led to a linear decrease in the daily increasing rate of population under both algal concentrations. The results of the present experiment indicate that the algal concentration and inoculation density significantly affect population growth of *D. celebensis*. Furthermore, the results suggest that the optimal algal concentration and inoculation density for the mass culture of *D. celebensis* should be 3×10^6 cell/mL and 100 ind./L.

Keyword: *Diaphanosoma celebensis*; population growth; algal concentration; inoculation density

1 INTRODUCTION

Cladocera, which are a major component of freshwater zooplankton, are an excellent natural food source for aquatic animals such as finfish and shellfish, and are widely used as live food in freshwater fish hatcheries. However, there are few species of cladoceran in ocean and inland saline lakes. Indeed, only eight species of cladocerans, *Penilia avirostris*, *Podon intermedius*, *P. leuckarti*, *P. schmackeri*, *P. polyphemoides*, *Evadne nordmanni*, *E. spinifera* and *E. tergestina*, are considered to be true marine cladocera (Zheng et al., 1987). In addition, Hammer (1986) identified five species of cladoceran that occurred in hypersaline waters (salinity > 30), *Daphnia similis*, *Daphniopsis pusilla*, *Moina hutchinsoni*, *M. microcephala*, and *M. mongolica*, as well as nine species that were tolerant of mesosaline waters (salinity 20 to 30), *Daphnia atkinsoni*, *D. magna*, *D. dolichocephala*, *Daphniopsis australis*, *Macrothrix hirsuticornis*, *Moina brachiata*, *M. baylyi*, *M. macrocopa* and *Scaphaloberis mucronata*. It is

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still difficult to culture marine cladocera under artificially controlled conditions (Fukusho, 1991). Accordingly, it is essential to identify species of cladoceran which are nutritious, have a suitable body size and rapid population growth, are euryhaline and tolerant of artificial dense conditions as candidates for mass culture in seawater or brackish water to overcome the deficiency of live food that are required following the first feeding diet (rotifer) in marine larviculture (He et al., 2001). Species of cladoceran that have the potential for cultivation under dense conditions in sea water include *Moina mongolica* (He et al., 1988, 2001), *M. salina* (Gordo et al., 1994) and *Diaphanosoma celebensis* (Segawa et al., 1988).

Diaphanosoma celebensis is an estuarine cladocera distributed in tropical regions of Asia (Korovchinsky, 1989, 1993). This species can survive and reproduce in salinities ranging from 0 to 30 (Achuthankutty et al., 2000). *D. celebensis* also undergoes rapid population growth via parthenogenetic reproduction (Shrivastava et al., 1999; Achuthankutty et al., 2000). In the past two decades, many studies have been conducted to evaluate the population growth and culture methods (Segawa et al., 1988; Jung et al., 1999), suitability as live food for marine larviculture (de la Pena, 2001), life span and parthenogenetic reproduction (Shrivastava et al., 1999; Achuthankutty et al., 2000; PrabhuKonkar et al., 2004), and toxicology (Helen et al., 2007) of *D. celebensis*. Nevertheless, the relationship between the population growth of *D. celebensis* and various environmental conditions (temperature, salinity and food conditions) and initial population density is still not well understood. Algal conditions (species and concentration) and initial density are important factors in the mass culture of zooplankton (Vijverberg, 1989). The quality and quantity of algae are involved in regulation of the population growth of cladocera (Alva-Martínez et al., 2001; Ovie et al., 2002; Nandini et al., 2003), and initial density affects the interactions among coexisting zooplankton species (Sarma et al., 1999; Hurtado-Bocanegra et al., 2002). Therefore, this study was conducted to evaluate the population growth of *D. celebensis* in response to different algal concentrations and inoculation densities.

2 MATERIALS AND METHODS

D. celebensis were obtained from the Faculty of Fisheries, Nagasaki University, Japan, and maintained through parthenogenetic reproduction in the Laboratory of Aquatic Ecology and Aquaculture (AEA), with *Chlorella pyrenoidosa* being provided as food. *C. pyrenoidosa* was cultured in 5 L flasks using F/2 medium (salinity 10). The concentration of *C. pyrenoidosa* was determined using a previously established regression equation that described the relationship between algal concentration and absorbance: $y = 4E\text{-}08x+0.0024$ (n=6, R^2=0.998), where x is the algal concentration (cell/mL) and y is the absorbance at 800 nm. Algal absorbance was measured with a 752 spectrometer (Jing-ke Instrument Co., Shanghai) using the method described by Wang et al. (2004).

A factorial layout including two algal concentrations (1×10^6 and 3×10^6 cell/mL) and four inoculation densities (100, 200, 300 and 400 ind./L) was designed. At the beginning of the experiment, a previously established cohort of *D. celebensis* was sorted into 32 flasks (volume 150 mL) each contained 50 mL of algal medium. Each treatment had four replicates. The flasks were randomly placed in two Zhujiang LRH-250-G incubators (Shaoguan, Guangdong, China), in which the temperature was maintained at 22 ± 1°C and the photoperiod was maintained at 12 h L: 12 h D (0 lx during the darkness period, and 2 000 to 3 000 lx during the light period). During the experiment, the number of *D. celebensis* alive in each flask was determined once every two days, after that the algal medium was renewed. The experiment was ceased when the population density of *D. celebensis* no longer increased continuously in most of the flasks.

The daily increasing rate of population (r) was calculated using the equation: $r = (\ln N_t - \ln N_0) / t$, where N_0 (ind.) is number of *D. celebensis* at the start of a time period and N_t (ind.) at the end, and t (days) is duration of the period. The average increasing rate of population (r_A) was calculated as: $r_A = \sum r_i / N$, where r_i is r during each time period, and N is the number of periods. Differences in T (the time during which the *D. celebensis* reached the maximum population density), D_M (the maximum population density) and r_A among the treatments were analyzed using ANOVA for the factorial layout, and multiple comparisons between treatments were conducted using Duncan's test. The relationship between r_A and the inoculation density was examined by multiple regression. Differences were considered to be significant at $P < 0.05$.

3 RESULTS

The population density of *D. celebensis* in each of the treatment groups increased slowly during days

1 to 5, after which it climbed rapidly (Fig.1). The maximum population densities were 5 875 ± 324, 6 690 ± 691, 7 735 ± 1, 121 and 6 365 ± 691 ind./L for *D. celebensis* fed 1×10^6 cell/mL and inoculated at densities of 100, 200, 300 and 400 ind./L, respectively. The maximum population densities for *D. celebensis* fed 3×10^6 cell/mL and inoculated at densities of 100, 200, 300 and 400 ind./L (Fig.2) were 15 070 ± 379, 12 215 ± 648, 11 960 ± 2 551 and 16 130 ± 880 ind./L, respectively. The maximum population density occurred earlier when *D. celebensis* were fed 1×10^6 cell/mL than that when fed 3×10^6 cell/mL ($P < 0.05$). When inoculated at the same density, *D. celebensis* fed 3×10^6 cell/mL had higher D_M than those fed 1×10^6 cell/mL ($P < 0.05$). No significant difference in D_M was observed among *D. celebensis* fed the same concentration of algae but inoculated at different densities ($P > 0.05$).

The daily increasing rate of population was negative in all treatments during days 1 to 3, and ranged from - 0.09 to 0.24 throughout the experiment (Fig.3). The duration for positive population growth was longer in the *D. celebensis* fed 3×10^6 cell/mL and inoculated at densities of 100 or 200 ind./L (21 days positive increase) compared with the *D. celebensis* fed 1×10^6 cell/mL and inoculated at densities of 300 or 400 ind./L (19 days positive increase). During the experiment, the r_A values were

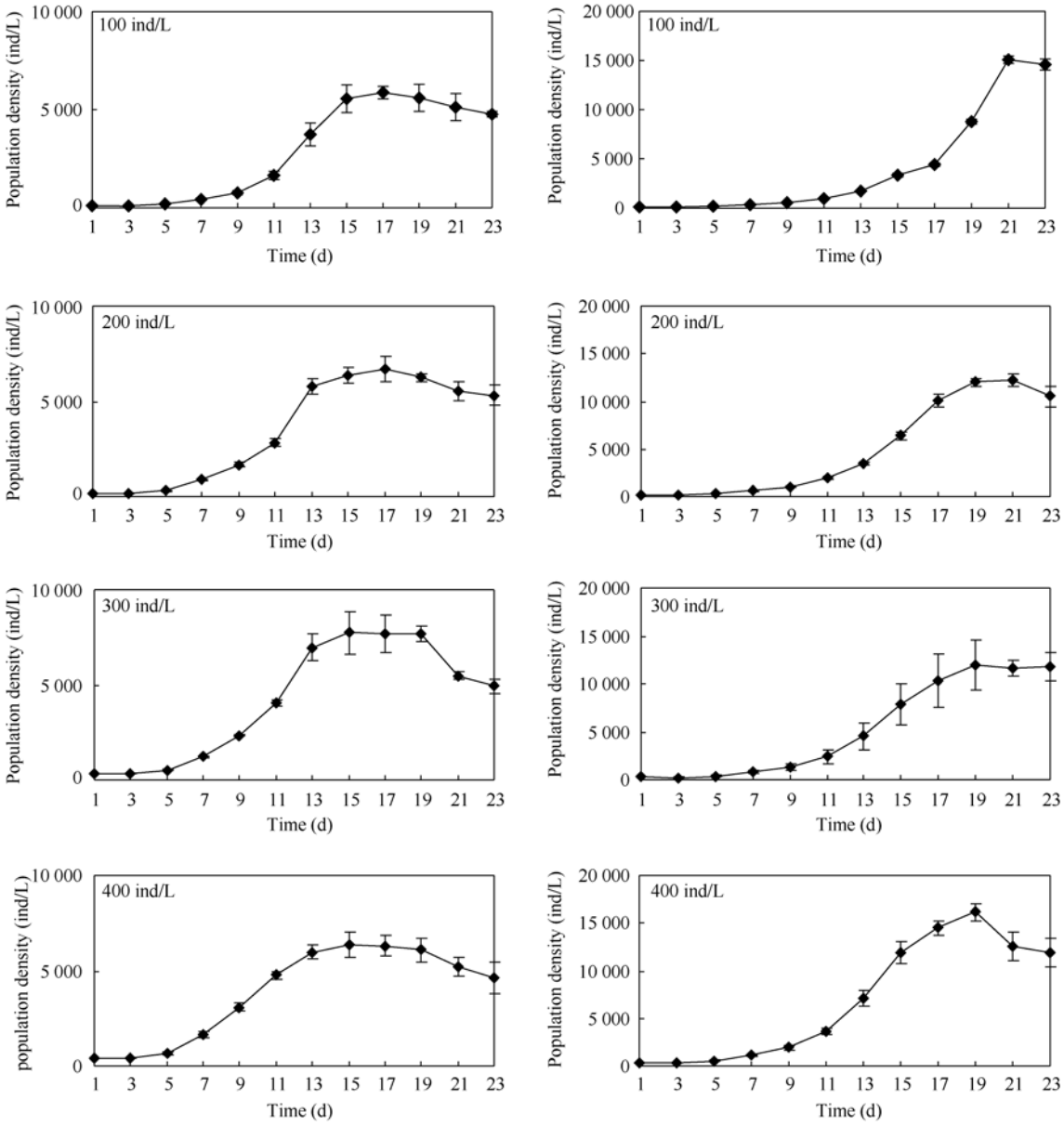

Fig.1 Population density of *Diaphanosoma celebensis* fed at two algal concentrations and inoculated at four densities

Data are expressed as the means ± SE ($n = 4$). The left panel: algal concentration 1×10^6 cell/mL; the right panel: algal concentration 3×10^6 cell/mL

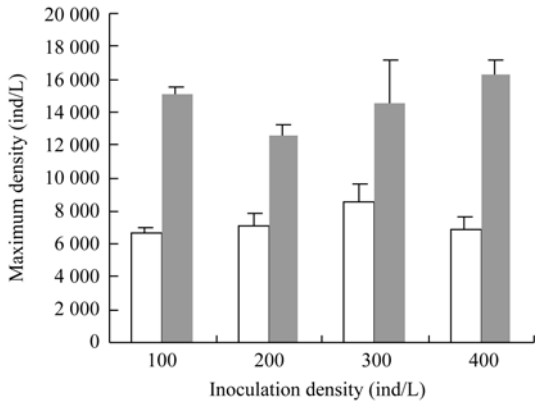

Fig.2 Maximum population density of *Diaphanosoma* celebensis fed at two algal concentrations and inoculated at four densities

Data are expressed as the means ± SE (n = 4). □ algal concentration 1×10^6 cell/mL; ■ algal concentration 3×10^6 cell/mL

0.076 ± 0.001, 0.065 ± 0.002, 0.055 ± 0.002 and 0.048 ± 0.003 for *D. celebensis* fed 1×10^6 cell/mL and inoculated at densities of 100, 200, 300 or 400 ind./L, respectively (Fig.4). The r_A values were 0.098 ± 0.001, 0.078 ± 0.002, 0.072 ± 0.003 and 0.067 ± 0.003 for *D. celebensis* fed 3×10^6 cell/mL and inoculated at densities of 100, 200, 300 and 400 ind./L, respectively (Fig.4). The average daily increasing rate of population was higher in the *D. celebensis* fed 3×10^6 cell/mL than the *D. celebensis* fed 1×10^6 cell/mL ($P < 0.05$), and these rates decreased linearly as the inoculation density increased with regardless of algal concentrations ($P < 0.05$).

4 DISCUSSION AND CONCLUSION

Shrivastava et al. (1999) reported that algal species and concentration had a significant effect on the survival time and fecundity of individually cultured *D. celebensis*. In addition, Nandini et al. (2003) reported that the population growth of seven species of cladoceran (*Alona rectangula*, *Ceriodaphnia dubia*, *Daphnia laevis*, *Diaphanosoma brachyurum*, *Moina macrocopa*, *Scapholeberis kingi* and *Simocephalus vetulus*) decreased in response to a reduction in the concentration of *Chlorella vulgaris*. However, an excessive algal concentration was reported to have a negative effect on the population growth of *Moina micrura* (Ovie et al., 2002). Feeding *Moina macrocopa* and *Ceriodaphnia dubia* high concentrations of undesired algae has been found to induce declining population density or population crash (Alva-Martínez et al., 2001). In the present study, D_M and r_A were higher in the *D. celebensis* fed 3×10^6 cell/mL than the *D. celebensis* fed 1×10^6 cell/m L, suggesting that low algal concentration limited population growth. Although the *D. celebensis* fed 3×10^6 cell/mL exhibited a longer period of positive population growth and higher D_M than that of the *D. celebensis* inoculated at the same density but fed 1×10^6 cell/mL, D_M occurred earlier in the *D. celebensis* fed 1×10^6 cell/mL than the *D. celebensis* fed 3×10^6 cell/mL. These facts could be attributed to a mechanism that the minimum algal concentration for population growth of *D. celebensis* increased with the increase of population density of the cladocera. Similarly, the minimum concentration of food required for population growth of *M. mongolica* increased as the population density increased (Li et al., 2005). Individuals carrying ephippium were observed earlier in the *D. celebensis* fed 1×10^6 cell/mL and inoculated at 300 or 400 ind./L than the *D. celebensis* fed at the same algal concentration but inoculated at 100 or 200 ind./L, indicating that low algal concentration and high inoculation density led to an earlier occurrence of food limitation and a decrease in population density. Results of the present study reveal that *C. pyrenoidosa* concentration required to initiate the mass culture of *D. celebensis* should be 3×10^6 cell/mL, and this concentration should be further elevated simultaneously with the increase in the population density of *D. celebensis*.

High population densities have been found to induce negative effects on the Daphnia population via release of chemical metabolites (Burns, 2000), while low inoculation densities have been found to lead failure of initiation of *M. mongolica* mass culture (Li et al., 2005). In the present study, *D. celebensis* inoculated at 300 or 400 ind./L exhibited earlier exponential growth than those fed the same algal concentration but inoculated at 100 or 200 ind./L, although stable population growth was achieved in *D. celebensis* inoculated at densities of 100 to 400 ind./L. These facts were likely due to the negative effect of inoculation damage on survival and population growth was relatively low in the *D. celebensis* inoculated at high densities. In the present experiment, the average daily increasing rate of population of *D. celebensis* fed either 1×10^6 cell/mL or 3×10^6 cell/mL decreased as the inoculation density increased. This trend is similar to that observed in *M. mongolica* (Li et al., 2005). Results of the present study demonstrate that the relationship between population growth and the inoculation density of *D. celebensis* is independent on the algal concentration. Furthermore, results of this study indicate that an inoculation density of 100 ind./L is

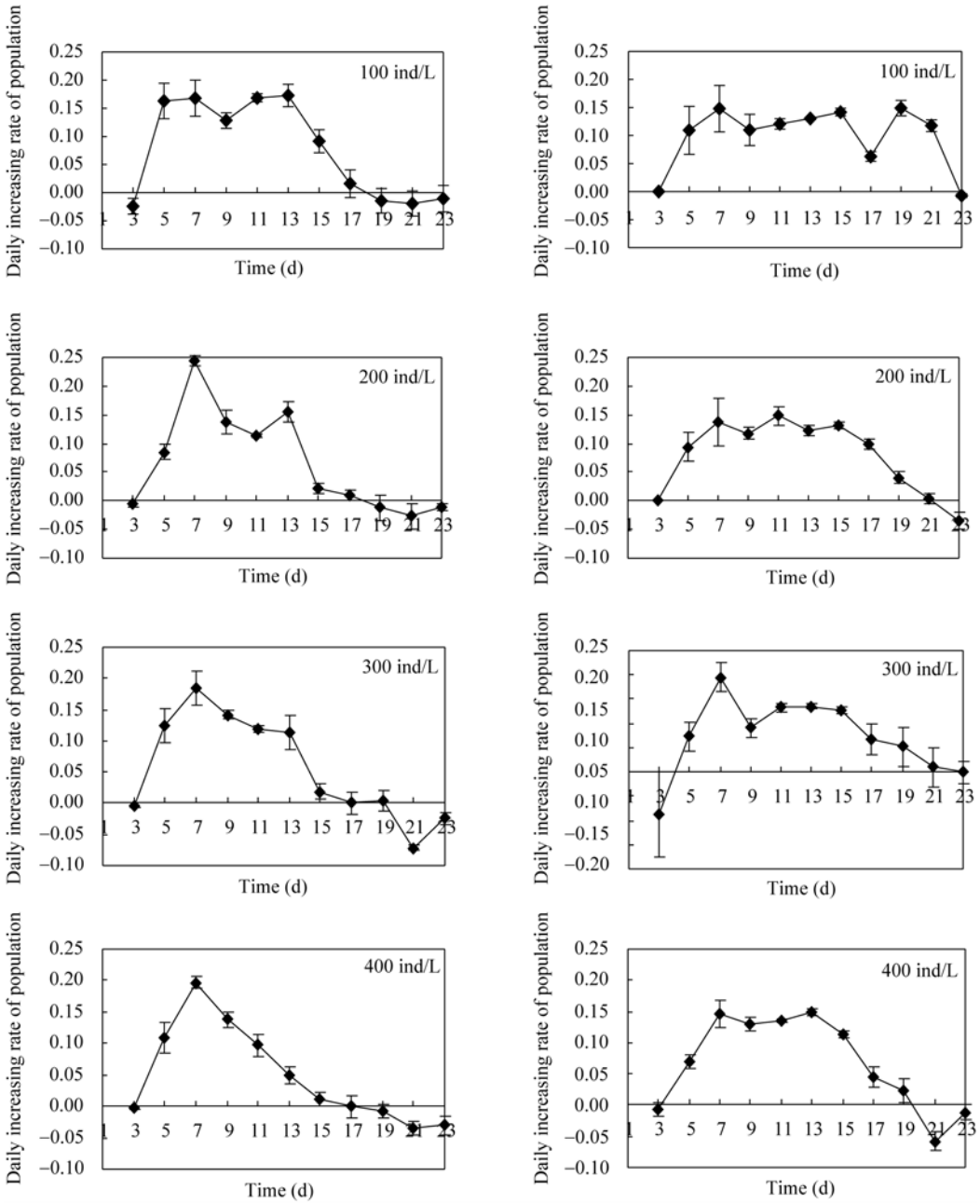

Fig.3 Daily increasing rate of population of *Diaphanosoma celebensis* fed two algal concentrations and inoculated at four densities

Data are expressed as the means ± SE (n = 4). The left panel: algal concentration 1 × 10^6 cell/mL; the right panel: algal concentration 3 × 10^6 cell/mL.

sufficient to initiate the mass culture of *D. celebensis*. In comparison, the minimum inoculation density required to initiate the mass culture of *M. mongolica* has been shown to be 150 ind./L (Li et al., 2005).

Species of cladoceran with small body size generally achieve higher population densities than large cladocer (Nandini et al., 2003). Korovchinsky (1989) reported that body length of adult *D. celebensis* ranged from 0.63 to 0.91 mm, which is smaller than that of *M. mongolica* (adult body length 1.00 to 1.40 mm, He et al., 2001). The maximum population density (16 130 ind./L) of *D. celebensis* observed in the present study was higher than that of *M. mongolica* (10 520 ind./L, Xu et al., 2003). The continuous spectrum in body size of *D. celebensis* and *M. mongolica* and their high population density achieved under artificial conditions suggest that it's potential to use *D. celebensis* and *M. Mongolica* as live foods following rotifers (the first feeding diet) in marine fish hatchery.

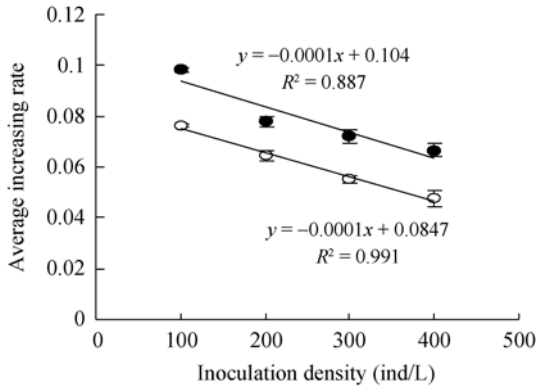

Fig.4 Average increasing rate of population of *Diaphanosoma celebensis* fed at two algal concentrations and inoculated at four densities

Data are expressed as the means ± SE (n = 4) ○: algal concentration 1×10^6 cell/ml; ●: algal concentration 3×10^6 cell/ml.

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