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Citation	Aquaculture, 293(1-2), pp.62-67; 2009
Issue Date	2009-08-01
URL	http://hdl.handle.net/10069/22002
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The minute monogonont rotifer *Proales similis* de Beauchamp: culture and feeding to small mouth marine fish larvae

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Abstract

The body length and width of *Proales similis* (mean±SD; 83±11 µm and 40±6 µm, respectively) is 38.1% smaller and 60.3% narrower than that of *Brachionus rotundiformis*. Due to its small size, *P. similis* has potential for rearing marine fish larvae which require first food smaller than *B. rotundiformis*. We examined the feasibility of using *P. similis* as live food by analyzing its life history, population growth and feeding incidence by fish larvae. *P. similis* produced first offspring on 2.5-2.8 days after hatch. Females produced 4.3-7.8 offspring during their 2.9-3.4 day reproductive period. *P. similis* grew well at temperatures 25 to 35°C (density = 250 to 1030 ind./ml; $r = 0.68$ to 0.81 day^{-1}) and at salinities 2 to 15 ppt (density = 360 to 500 ind./ml; $r = 0.73$ to 0.78 day^{-1}). Population density of *P. similis* was higher than *B. rotundiformis* after 8 days of culture period with either *N. oculata* and *C. vulgaris* as food. In mass culture, population density of *P. similis* increased from 25 to 2,400 ind./ml ($r = 0.42 \text{ day}^{-1}$) after 11 days. Results from feeding experiments confirm that *P. similis* is consumed by seven-band grouper (*Epinephelus septemfasciatus*) larvae. The larvae demonstrated the highest feeding rate at 20 ind./ml of *P. similis*.

Keywords: Rotifera, *Proales similis*, *Brachionus rotundiformis*, culture, live food, larviculture, *Epinephelus septemfasciatus*.

1. Introduction

Marine fish hatcheries culture rotifers of the *Brachionus plicatilis* species complex as the initial live food for several marine fish larvae. The *B. plicatilis* species complex were traditionally classified morphologically as SS (super-small), S (small) and L (large) type, with lorica length around 90-150, 100-210 and 130-340 μm , respectively (Hagiwara et al., 2001). We now recognize these morphotypes as different species, with the SS type being classified as *B. rotundiformis* (Segers, 1995). Due to its small lorica size, *B. rotundiformis* (Fig. 1) is mostly used as a starter food for feeding fish larvae with small mouths, but it is not satisfactory as starter food for those fish with even smaller mouths, like groupers in the genus *Epinephelus* (Okumura, 1997), Napoleon fish (*Cheilinus undulatus*) or Angelfishes (family Pomachantidae). Larvae of the two latter fish, require specifically small live food of about 40-80 μm at initial feeding stages (Slamet and Hutapea., 2004; Olivotto, et al., 2006). Recognizing this demand for smaller sized live food, we have developed *P. similis* as a starter live food organism for rearing small mouth fish larvae. For that purpose, we explored the culture of *P. similis* under various environmental conditions to determine optimal conditions for the culture of this species. Population growth and mean body size was compared to *B. rotundiformis* cultured in similar conditions. We also investigated the potential of *P. similis* as live food for fish larviculture by observing whether seven-band grouper larvae can ingest this rotifer.

2. Materials and methods

P. similis (Fig. 1) was collected using a 45 µm mesh plankton net from an estuary (24°N:124°E) on Ishigaki Island, Okinawa, Japan on July 2004. The water temperature and salinity at the time of collection were 27°C and 2 ppt, respectively. *P. similis* was acclimatized to higher salinity and a clonal culture of *P. similis* was established in the laboratory in 2 and 22 ppt diluted seawater at 25°C. *Nannochloropsis oculata* grown in KW-21 medium (Daiichi Seimou Co.Ltd, Japan) was provided as food. The *B. rotundiformis* (Indonesian strain) (Hagiwara et al., 1995; Rumengan et al., 1998) was used for comparison to *P. similis*. Both species are common in tropical and subtropical region.

2.1. Life history parameters

We investigated life history parameters of *P. similis* at salinities of 2, 15, and 25 ppt, at 25°C in darkness. *N. oculata* was provided at a density of 2.5×10^6 cells/ml. Prior to the experiment, *P. similis* was cultured in the salinity treatments for at least one week to permit acclimation. From these cultures, a number of adults identified by their body size were selected and were allowed to produce amictic eggs. Fifty neonates (< 4h old) were selected and cultured individually in 1 ml fresh medium and used as test animals. Observations were conducted every 12 h to distinguish between an adult rotifer and newly formed neonates and the data pooled daily. The number of offspring produced by each female was counted and only the maternal female was transferred to fresh medium until the

all test animals died. Lifespan, reproductive period, generation time, fecundity and intrinsic rate of population increase (r) were determined. Intrinsic rate of natural increase r was calculated by using the following equation (Lotka, 1913);

$$\sum_{x=0}^n l_x m_x \exp(-rx) = 1$$

Other demographic parameters were obtained by the following:

Net reproduction rate:
$$Ro = \sum_{x=0}^n l_x m_x$$

Generation time:
$$T = 1/ Ro \sum_{x=0}^n l_x m_x x$$

where, x = age (0, 1, ...n days), l_x = age-specific survival from birth to age x , m_x = age-specific fecundity, Ro = net reproductive rate and T = generation time. A one-way ANOVA and Tukey-Kramer's test was performed to compare live history parameters among the salinity levels.

2.2. Population growth and body size

Three different experiments were performed to examine the effect of temperature, salinity and food density on population growth of *P. similis*. A first experiment to test the temperature effect was performed by combining 15, 20, 25, 30 and 35⁰C with 2 and 25 ppt salinity. A second experiment to test the effect of salinity was performed using 2, 15, 20, 25 and 30 ppt at 25⁰C. A third experiment focused on the food density effect was

performed at 25⁰C, by combining 2 and 25 ppt salinity with two types of food (the microalgae *N. oculata* and *Chlorella vulgaris*; Super Chlorella V-12®, Chlorella Industry Co.) and four food levels (1.2, 5.8, 17.3 and 28.8 µg dry weight/ml), altogether summing up to 16 experimental conditions. The two microalgae used were obtained as concentrated liquid solution and a dry weight of 28.8 µg/ml corresponds to 12.5x10⁶ and 4.3x10⁶ cells/ml for *N. oculata* and *C. vulgaris*, respectively. Experiments were conducted using 24-well polystyrene plates (Iwaki, Japan) containing 3 ml culture medium in darkness. For each treatment, there were three to six replicates.

To begin each experiment, three adult rotifer were transferred into their treatment and the initial day was called day 0. Observations were made once every two days, when the number of living individuals was counted and transferred to new wells containing fresh culture medium and food suspension. Population growth of *P. similis* was compared to *B. rotundiformis* cultured in similar conditions. The population growth rates (*r*) were calculated using the following equation (Krebs, 1985);

$$r = (\ln N_t - \ln N_0)/t$$

Where, ln= natural logarithm, N_t= population after time t (days) and N₀= initial population. Population density of the rotifers on day 8 in the experiments of temperature and food density were compared using a two-way ANOVA, while the experiment on salinity was compared using one-way ANOVA. A Tukey-Kramer test was calculated to determine significantly different treatments in each experiment. Student *t*-test was used for

comparing the population density of the rotifers in each food density.

A total of 480 individuals of *P. similis* and *B. rotundiformis* obtained on day 8 from each food treatment in the experiment of food density (25 ppt; 25⁰C) were measured for their total length, body length and width (Fig.1) at 450x of magnification, with a digital microscope (Keyence VH-8000, Keyence Corp.). Prior to measurement, the rotifers were anesthetized with 0.002% MS-222 (Tricaine; Sigma Chemical Co., USA) to prevent shrinkage. A Student *t*-test was used to compare body size of the two species of rotifers.

2.3. Mass culture and salinity change

Mass culture of *P. similis* was conducted in three 2-l polyethylene containers containing 2 l diluted seawater (25 ppt salinity) maintained at 25⁰C. *N. oculata* was used as food at 1.25×10^7 cells/ml (equal to 28.8 μ g dry weight/ml). The density was controlled daily by counting the number of microalgae in a haemocytometer and adjusting the density. Constant aeration at 100 ml/min controlled by a flow meter (Kofloc RK-1350V) was provided to each culture. The experiment was initiated by stocking *P. similis* at 25 individuals/ml and the culture was observed until exponential growth stopped. Daily sampling consisted of taking a 1 ml sample from each container and the number of rotifers was counted to determine density. Population growth rate was calculated using the same equation as described previously.

To test *P. similis* survival after changing salinity from 25 to 35 ppt, the following

experiment was conducted. A total of 21 wells of a 24-well polystyrene plate were filled with 1 ml seawater (35 ppt) containing *C. vulgaris* at density 0.5×10^6 cells/ml at 25°C. Ten individual of *P. similis* cultured at salinity 25 ppt were individually transferred to the 35 ppt medium by a micropipette. Observation was made by counting the total number of live rotifers in each culture medium at 0, 4, 8, 12, 24, 48 and 72 hours (in three replicates) after the transfer.

2.4. Feeding experiment on fish larvae

To investigate whether fish larvae can consume *P. similis*, seven-band grouper *E. septemfasciatus* larvae were tested due to their small mouth size (180 ± 20 μm). The experiment was conducted in fifteen 30-l polycarbonate tanks in a controlled-room temperature at 25°C. Photoperiod during the experiment was 12L:12D. The rearing tanks were filled with artificial seawater with salinity at 34 ± 1 ppt (Marine artHi, Tomita Pharmaceutical Co. Ltd., Tokushima, Japan). The bottom of the rearing tank was filled with ceramic sand (MS-10, Micros Ceramic, Norra Co. Ltd, Kyoto, Japan) to maintain water quality. Gentle aeration was provided by an air stone placed on the bottom center of the rearing tanks. Fertilized eggs of seven-band grouper larvae obtained from Nagasaki Prefectural Fisheries Experimental Station were stocked in the rearing tanks at a density of 10 eggs/l. The eggs hatched on the following day. An oil film (Yamaoka et al., 2000) was introduced on the surface of each rearing tank at a concentration of 0.2 ml/m^2 , to prevent

surface entrapment of the larvae. First feeding occurred on day 4 after hatching (DAH), at which time five dietary treatments of *P. similis* (10, 20 and 30 ind./ml), *B. rotundiformis* (10 ind./ml) and a mixture of the rotifer species (consisting of 5 ind./ml of each *P. similis* and *B. rotundiformis*) were offered to the larvae in three replicates. Prior adding to the larval rearing tanks, the rotifers were cultured (25 ppt; 25⁰C) with concentrated liquid *C. vulgaris* (Super fresh Chlorella V-12®, Chlorella Industry Company), for nutritional enrichment. The concentrated liquid *C. vulgaris* was also introduced to the larval rearing tanks at density of 0.5×10^6 cells/ml. The fish larvae were fed for 24 hours and all live larvae were counted 48 hours later to determine the survival. Percent survival was calculated from the numbers of eggs stocked. In order to examine feeding of the larvae, about 10 larvae from each tank were anesthetized with 0.01% MS-222 and fixed with 5% formalin solution. Number of larvae that fed on rotifers was investigated by examining the presence of trophi or undigested rotifers inside the gut of larvae. A one-way ANOVA and Tukey-Kramer's test was used to compare feeding incidence of the fish larvae.

3. Results

3.1. Life history parameters

Lifespan, reproductive period and generation time of *P. similis* ranged from 4.0 - 4.7, 2.4 - 2.8 and 2.4 - 2.8 days, respectively. The fecundity was significantly higher at 2 ppt than at higher salinities (Tukey-Kramer test, $p < 0.05$). The intrinsic rate of population

increase (r) ranged from 0.63 to 0.93 day⁻¹ at salinities of 2 to 25 ppt, respectively (Table 1)

3.2. Population growth and body size

Maximum population densities of *P. similis* during 8 day culture period were compared among temperature, salinity (Fig. 2) and food density treatments (Fig. 3). Rotifer density reached to maximum on day 8 except at 15 and 20°C. A two-way ANOVA showed that the effect of temperature was significant on population density of *P. similis* cultured at salinity 2 and 25 ppt ($p < 0.05$). Tukey-Kramer tests for the two salinity groups (2 and 25 ppt) showed that rotifer density at temperatures 25 and 35°C (250 to 1030 ind./ml with $r = 0.68$ - 0.81 day⁻¹) was significantly higher than that at $< 20^\circ\text{C}$ (density < 2 ind./ml) (Fig. 2-I). The effect of salinity was also significant on population density of *P. similis* (ANOVA, $p < 0.05$). Significantly higher rotifer density was observed at salinity 2 and 15 ppt (500 and 360 ind./ml with $r = 0.78$ and 0.73 day⁻¹, respectively) than those at higher salinities (< 120 ind./ml) (Tukey-Kramer, $p < 0.05$) (Fig. 2-II). The effect of the experimental environment 25°C and 25 ppt was tested twice, one in each of the experiments reported above. Maximum population densities (mean \pm SD, $n=3-6$) were quite different (250 \pm 116 ind./ml with $r = 0.68$ day⁻¹, 25°C in Fig. 2-I to 110 \pm 118 ind./ml with $r = 0.55$ day⁻¹ for salinity 25 ppt, in Fig. 2-II). However, they were not significantly different (t -test, $p < 0.05$). Two-way ANOVA showed that the density of *N. oculata* significantly affected population density of *P. similis* cultured at 2 and 25 ppt ($p < 0.05$). Tukey-Kramer

test on the two salinity groups (2 and 25 ppt) revealed that the population densities of *P. similis* at *N. oculata* densities of 17.3 and 28.8 µg dry weight/ml (2,270 to 2,450 ind./ml) were significantly higher than those at 1.2 and 5.8 µg dry weight/ml (250 to 520 ind./ml) at 2 ppt, but not significant at salinity 25 ppt. *P. similis* cultured at salinity 25 ppt showed significantly high population density than *B. rotundiformis* at most *N. oculata* densities (*t*-test, $p < 0.05$). The *B. rotundiformis* showed a significantly different population density with increasing *N. oculata* density (Tukey-Kramer, $p < 0.05$) (Fig. 3-I). Similar effects on population density of the two rotifers as in the *N. oculata* were also observed on the densities of *C. vulgaris* (Fig. 3-II).

Total length, body length and width of *P. similis* did not vary among food treatments. The total length distributed between 50-150 µm (mean±SD; 109±15 µm) while the body length distributed between 40-110 µm (83±11 µm). The body length was significantly smaller (38.1% smaller) than lorica length of *B. rotundiformis* which ranged from 70-170 µm (134±14 µm) (*t*-test, $p < 0.05$). Body width of *P. similis* ranged from 10-50 µm (40±6 µm) and was significantly narrower (60.3%) than lorica width of *B. rotundiformis*, which ranged from 50-150 µm (102 ±12 µm) (*t*-test, $p < 0.05$) (Fig. 4).

3.3. Mass culture and salinity change

Exponential growth of *P. similis* mass-cultured in 2-l containers by feeding *N. oculata* at 28.8 µg dry weight/ml is shown in Fig. 5. *P. similis* grew from 25 ind./ml on

day 0 to 2,400 ind./ml on day 11, with a mean r -value of 0.42 day^{-1} .

Transferring *P. similis* from salinity 25 to 35 ppt for 0, 4, 8, 12, 24, 48 and 72 hours did not significantly influence the total number of rotifers. Since rotifers grew during observation, percentages of the initial number of rotifers after salinity change were 100%, $103.3\% \pm 5.8$, $107.7\% \pm 5.8$, $103.3\% \pm 5.8$, $113.3\% \pm 11.5$, $100\% \pm 10.0$ and $103\% \pm 20.8$, respectively.

3.4. Feeding experiment on fish larvae

Percent survival of seven-band grouper larvae after 24 hours of feeding experiment were 2.4, 6.7, 4.5, 1.0 and 3.2 in dietary treatments of *P. similis* at 10, 20 30 ind./ml, *B. rotundiformis* at 10 ind./ml and mixed rotifer species at 10 ind./ml, respectively, and was not significantly different among treatments. Based on gut content analysis, higher feeding incidence and lower variation among rearing tanks at the onset of first feeding (4 DAH) of seven-band grouper larvae was observed in the treatments fed *P. similis* 20 ind./ml than those at 10 and 30 ind./ml (Fig. 6). In this treatment, the percentage of feeding larvae reached 95% while it was only 55% and 75% in the treatments fed *B. rotundiformis* alone or a mixture of the two rotifer species, respectively.

4. Discussion

Determining optimal culture conditions and limits for population growth of live

food for fish larviculture is required prior to its mass production. Another important factor is to determine whether fish larvae ingest the organism. For mass culture, salinity, temperature and food levels are considered as important. Results in the present study show that salinity ranging between 2 and 30 ppt has no significant effect on lifespan and reproductive period of *P. similis*, but low salinity (2 ppt) significantly raised fecundity of *P. similis*, which contributed to a high intrinsic rate of population increase. This salinity corresponds to the salinity at the sampling location of *P. similis* in nature. Population density of *P. similis* cultured at 2 ppt in batch culture was not significantly different from that at 15 ppt (Fig. 2-II), suggesting that the salinity range from 2 to 15 ppt is optimal for *P. similis* growth. Decreasing population density of *P. similis* at higher salinity may be due to the increasing energy demands for osmoregulation (Miracle and Serra, 1989) and decreased filtration rate (Hirayama and Ogawa, 1972) as reported in *Brachionus*. Our study also demonstrated that *P. similis* is capable of growing at a wide salinity range, indicating that *P. similis* is a euryhaline rotifer. Brain and Koste (1993) and Moscatello and Belmonte (2004) reported that the rotifer is found inhabiting hypersaline water of 48-98 ppt. Capability of *P. similis* to tolerate a wide range of salinity is probably similar to the euryhaline rotifer *B. plicatilis* sp. complex, which can tolerate salinity from 1 to 60 ppt as reported in Hoff and Snell (1987).

Temperature also showed strong influence on the population growth of *P. similis*. It has been shown that increasing temperature accelerates rotifer reproduction (Miracle and

Serra, 1989), as a result of accelerating metabolism and development (Hirayama and Rumengan, 1993). High population growth of *P. similis* at high temperature indicates the possibility of the species to be used as first food for culturing tropical fish larvae.

N. oculata and *C. vulgaris* are the most widely used microalgal species for feeding rotifers in hatcheries and laboratories (Hoff and Snell, 1987). We successfully used these microalgae to culture *P. similis*. It has been reported that some species of rotifers including *Platyias quadricornis* (Enriquez-Garcia et al., 2003), *Synchaeta cecilia* (Egloff, 1988) and *Synchaeta cecilia valentina* (Oltra et al., 2000) are difficult to grow using *N. oculata* or *C. vulgaris*. Our result further shows that the effect of the two microalgae densities on population growth of *P. similis* was stronger at salinity 2 ppt than at 25 ppt. It is suggested that lower filtration rate at higher salinity (Hirayama et al., 1972) and low initial density of the rotifer (1 ind./ml) in this study are probably the explanation why *P. similis* cultured at 25 ppt need lower food density for population growth. *P. similis* cultured at 25 ppt reached higher population density than *B. rotundiformis* cultured at the same food density for 8 days, indicating faster population growth rates for *P. similis*. Results in mass culture confirmed the high population growth rate of *P. similis*. From an initial density of 25 ind./ml, *P. similis* could reach population density of 2,400 ind./ml ($r=0.42 \text{ day}^{-1}$) on day 11. Further, results of our study shows that there was no significant effect on the total number of *P. similis*, 72 hours after they were transferred from 25 to 35 ppt salinity.

P. similis is among the smallest euryhaline rotifers that have been successfully mass cultured in the laboratory (Chigbu and Suchar, 2006). Its mean body length and width were 83 ± 11 μm and 40 ± 6 μm respectively, which is 38.1% smaller and 60.3% narrower than lorica size of *B. rotundiformis*. It has been suggested that the availability of small live food organisms is important for successful rearing of fish larvae with small mouths (Eda, et al., 1990; Polo et al., 1992; Doi et al., 1997; Okumura, 1997; Tanaka et al., 2005; Olivotto et al., 2006; Akazawa et al., 2008; Soyano et al., 2008). Small live food organisms that have been offered to fish larvae include ciliate, bivalve larvae, sea urchin eggs, barnacle nauplii, oyster trochophores or copepods, but the results are still unsatisfactory due to the low nutritional value or difficulties of culturing the organisms at high density (Rimmer, 2000). As rotifers can be cultured at high density and their nutritional content can be manipulated to match fish larvae requirements, efforts to reduce the lorica size of rotifer *B. plicatilis* have also been conducted (Gallardo et al., 1997; Hagiwara et al., 2007). However, since lorica size is genetically constrained (Snell and Carrillo, 1984; Fu et al., 1991a,b), collecting rotifer strains with small body size from nature is also important strategy (Ito et al., 1981; Yufera, 1982; Fukusho and Okauchi, 1983; Serra and Miracle, 1983). Recently, Chigbu and Suchar (2006) reported their success in culturing *Colurella dicentra*, a small rotifer with lorica length of 93 μm and width of 49 μm . But the effectiveness of this species as first food for fish larvae has not yet been examined. In this study, we demonstrated that seven-band grouper larvae feed

actively on *P. similis* at the onset of first feeding. Grouper larvae fed *P. similis* at 20 ind./ml had a 95% feeding incidence, while it was only 55% for the larvae fed *B. rotundiformis*. This suggests that there is an opportunity to improve the initial survival of groupers and other small mouth fish larvae by providing *P. similis*.

The results in this study demonstrated that *P. similis* possesses characteristics that are useful for aquaculture. First, among rotifer species already used in most hatcheries, *P. similis* has the smallest body size. This species also lacks a lorica, which may result in better ingestion and digestion. Second, *P. similis* can be mass cultured using the same techniques as *B. plicatilis*, by feeding *N. oculata* or *C. vulgaris*. Third, *P. similis* is a promising live food for fish larviculture, particularly for species that require an initial food smaller than *B. rotundiformis*. Future study should include evaluation of culture stability and investigation of the nutritional content of *P. similis*, as well as its use for rearing trials of fish larvae to examine survival and growth.

Acknowledgements

The rotifer *Proales similis* was collected during the 189th cruise of Kakuyo-maru, Faculty of Fisheries, Nagasaki University in July, 2004. The authors express thanks to Terry Snell for reviewing the manuscript and Russ Shiel for identification of rotifer species. This research was supported in part by the Ministry of Education, Science, Sports and Culture, Grant-in-Aid for Scientific Research (B), 2006-2008, No. 18380118, and the

Nagasaki prefecture Collaboration of Regional Entities for the Advancement of Technological Excellence, Japan Science and Technology.

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Figure legends

Fig. 1. Body dimensions of *Proales similis* and *Brachionus rotundiformis*. Bar 50 μm .

Fig. 2. Maximum population density (mean \pm SD) of *P. similis* cultured at five temperatures at 15, 20, 25, 30 and 35 $^{\circ}\text{C}$ (at 2 \square and 25 ppt \blacksquare) (I), and five salinities at 2, 15, 20, 25 and 30 ppt (at 25 $^{\circ}\text{C}$) (II). Capital letter indicates significant difference in temperature treatment among *P. similis* at 2 ppt, while lowercase indicates significant differences among temperature treatment at 25 ppt and among salinity treatment (A>B>C, a>b>c, Tukey-Kramer test, $p<0.05$, n=3-6).

Fig. 3. Maximum population density (mean \pm SD) of *P. similis* and *B. rotundiformis* cultured under four densities of *N. oculata* (I) and *C. vulgaris* (II). Culture salinity of *P. similis* was at 2 (\square) and 25 (\blacksquare) ppt, and that of *B. rotundiformis* at 25 ppt (\blacksquare). Capital letter indicates significant difference in food treatment among *P. similis* at 2 ppt, while lowercase indicates significant differences among *B. rotundiformis* at 25 ppt (A>B, a>b>c, Tukey-Kramer test, $p<0.05$, n=3). There were no significant differences among *P. similis* at 25 ppt. Asterisk indicates significant difference in population density at each food density treatments between *P. similis* and *B. rotundiformis* cultured at 25 ppt (t -test, $p<0.05$, n=3)

Fig. 4. Histogram of body or lorica length and width of *P. similis* and *B. rotundiformis* (each n=480). Measured rotifers were taken from culture at 25⁰C and 25 ppt fed by *N. oculata* or *C. vulgaris*.

Fig. 5. Population density (mean±SD, n=3) of *P. similis* mass-cultured at 25⁰C and in 25 ppt diluted seawater, fed daily by 28.8 µg dry weight/ml *N. oculata*.

Fig. 6. Feeding incidence at the onset of first feeding (mean %±SD) of seven-band grouper larvae (10 larvae in three replicates), fed with *P. similis* at 10 ind./ml (10-PS), 20 ind./ml (20-PS), 30 ind./ml (30-PS), *B. rotundiformis* at 10 ind./ml (10-SS) and mixed rotifers at 10 ind./ml consisted of 5 ind./ml of *P. similis* and 5 ind./ml of *B. rotundiformis* (10-Mix). Letters indicate significant differences in each treatment at the same age group (a>b, Tukey-Kramer test, $p<0.05$, n=3).

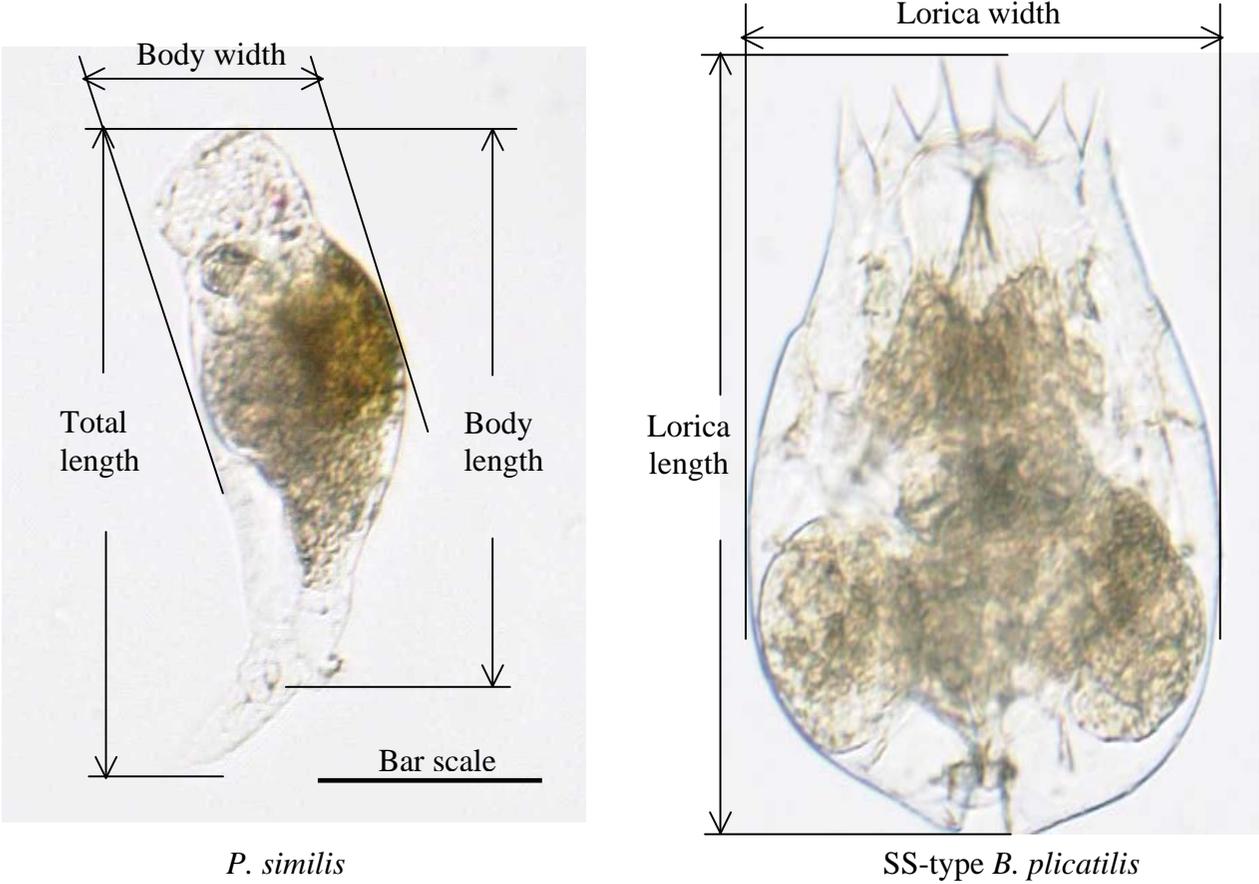


Fig. 1

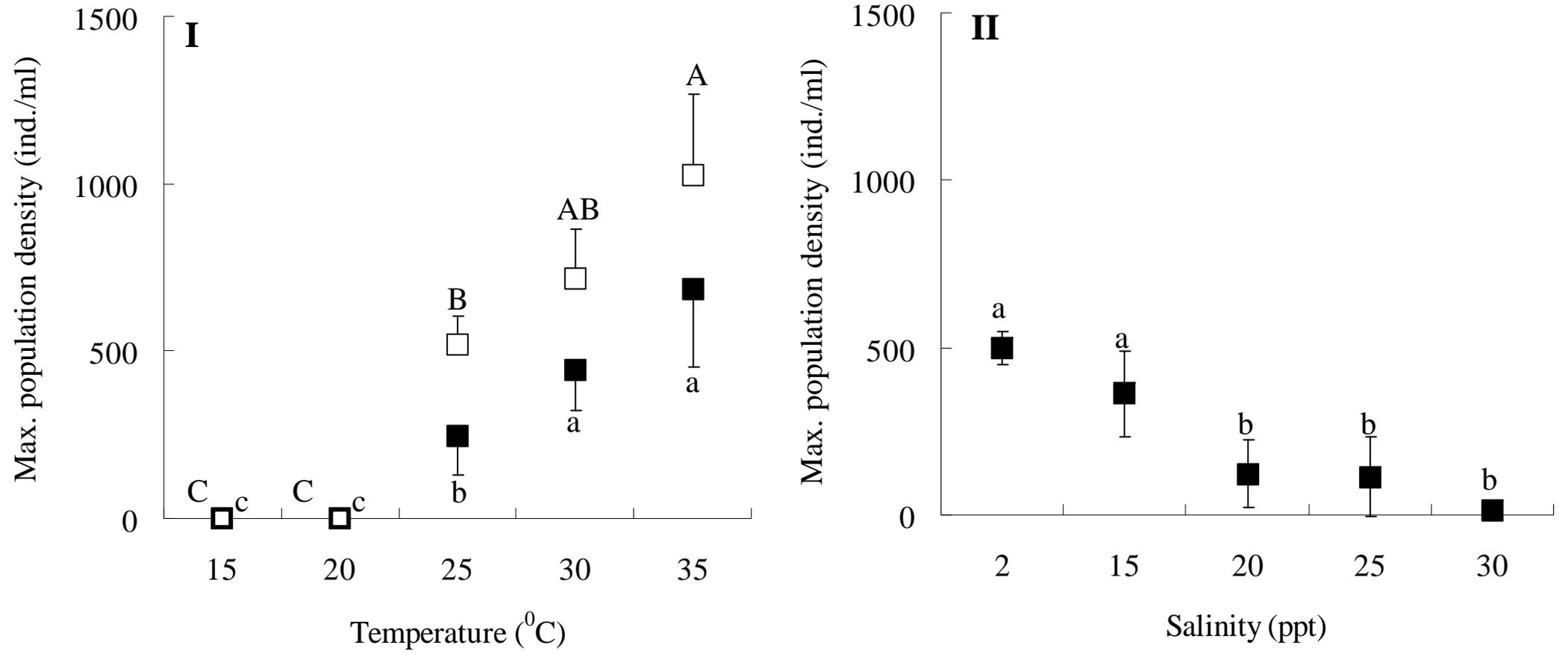


Fig. 2

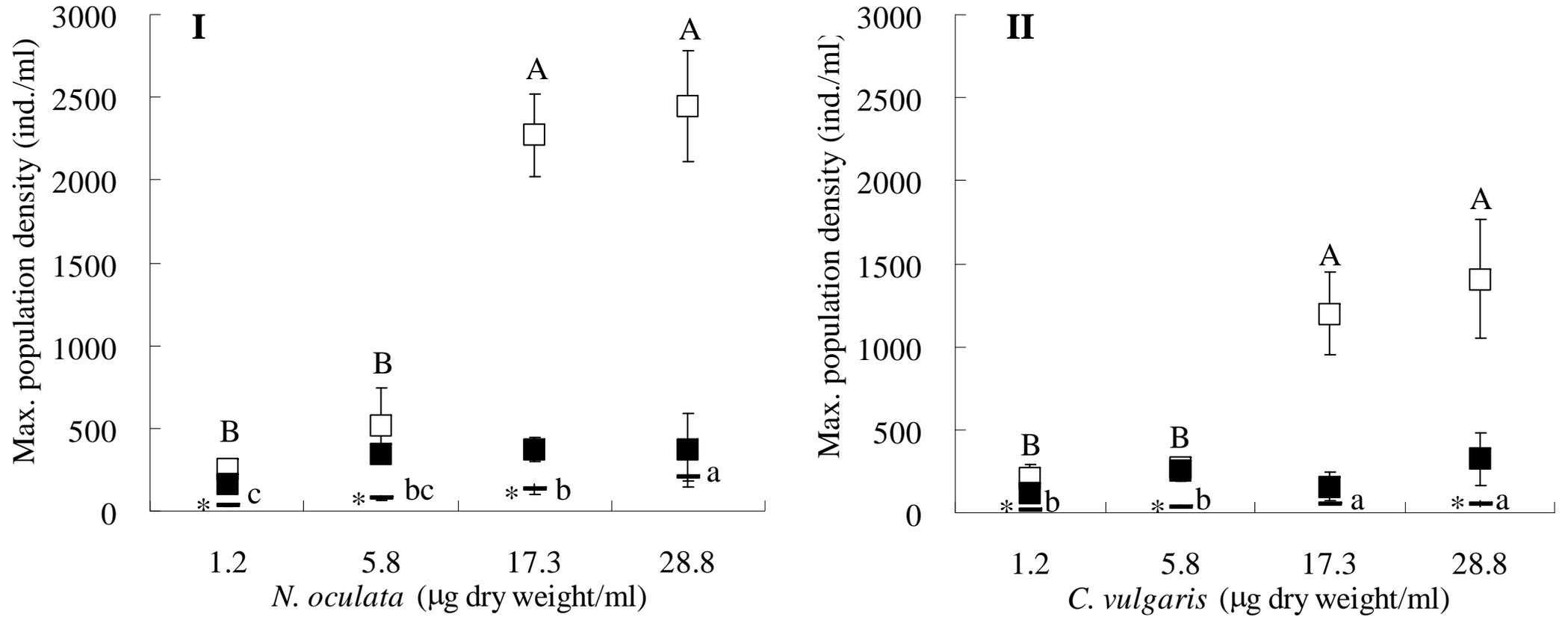


Fig. 3

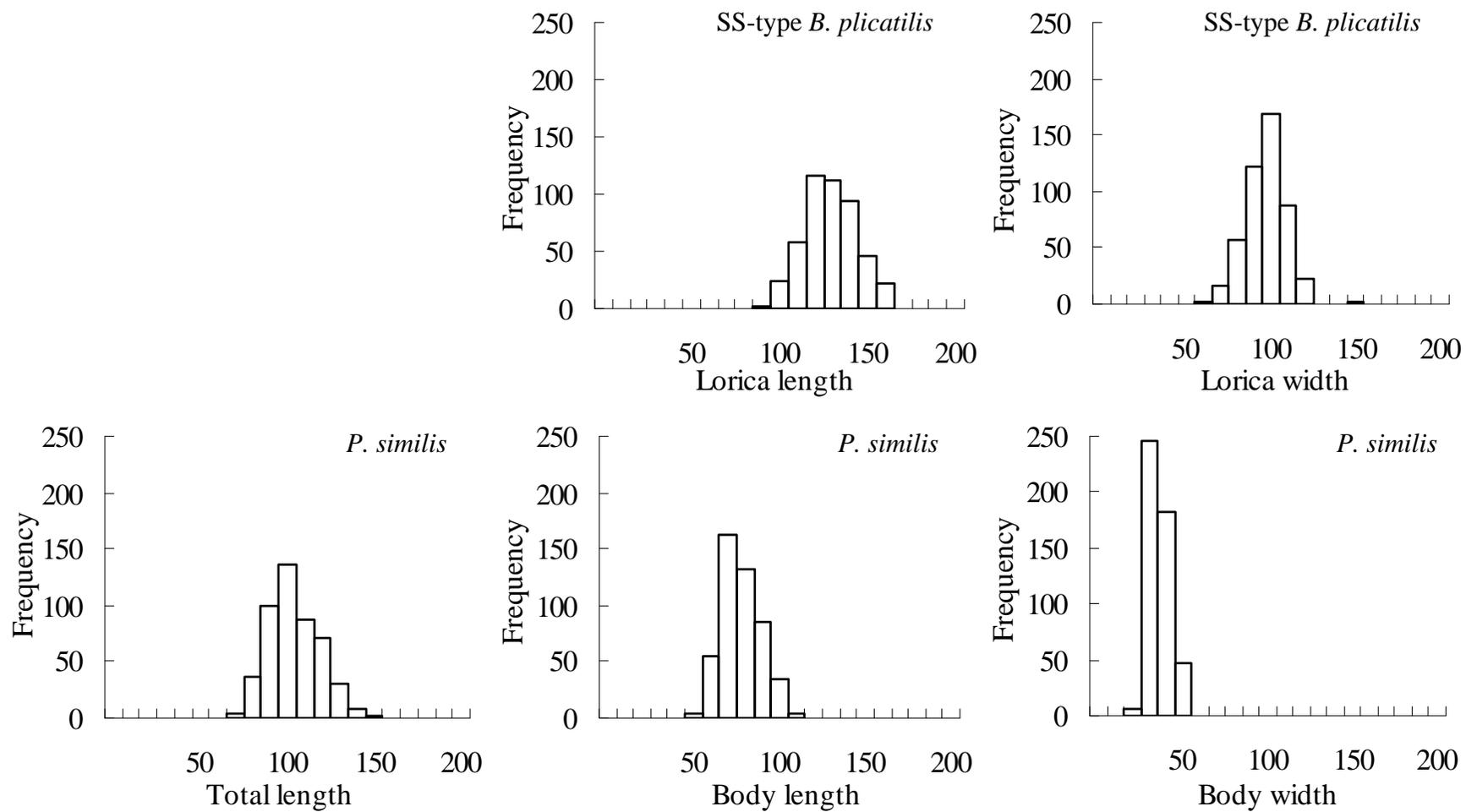


Fig. 4

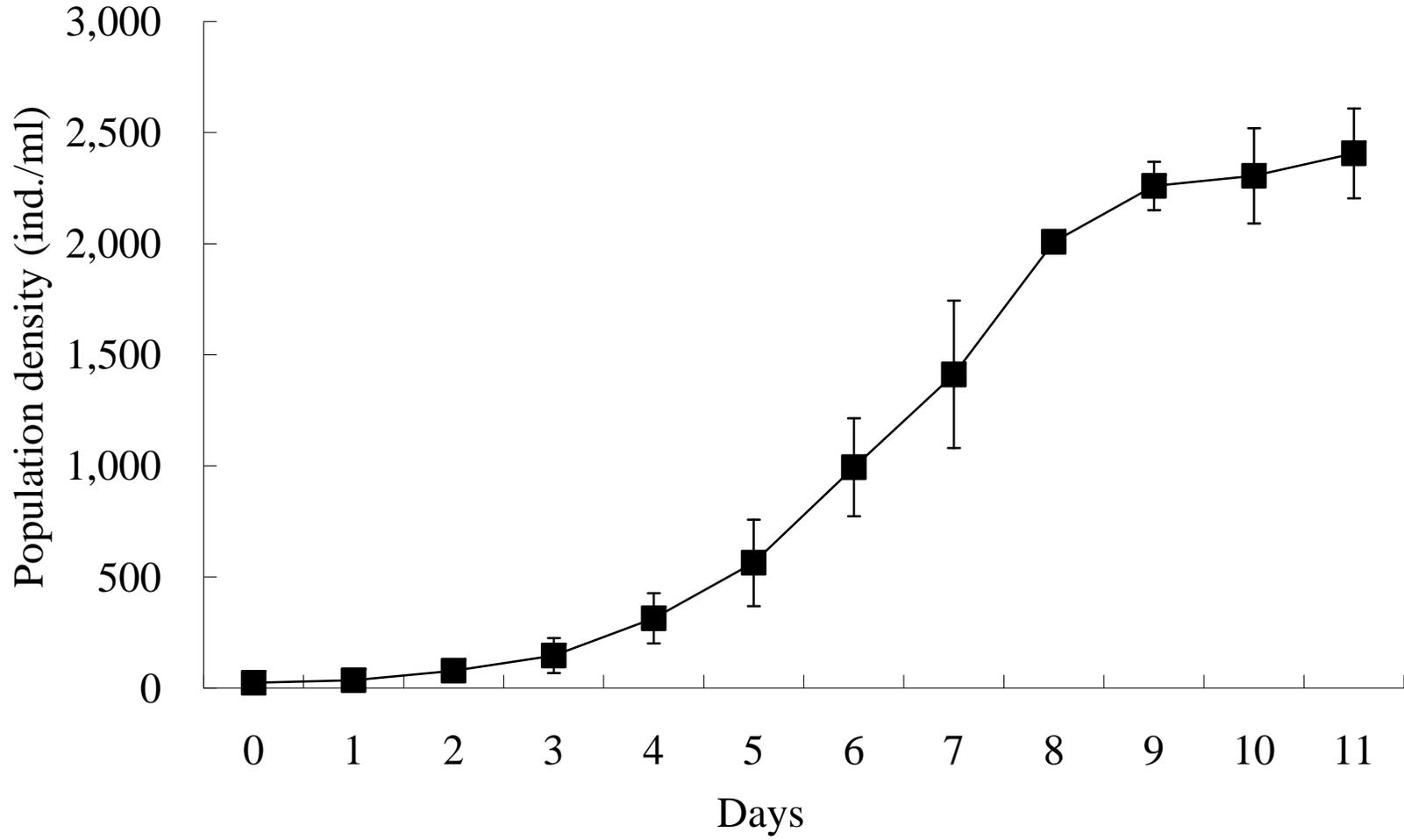


Fig. 5

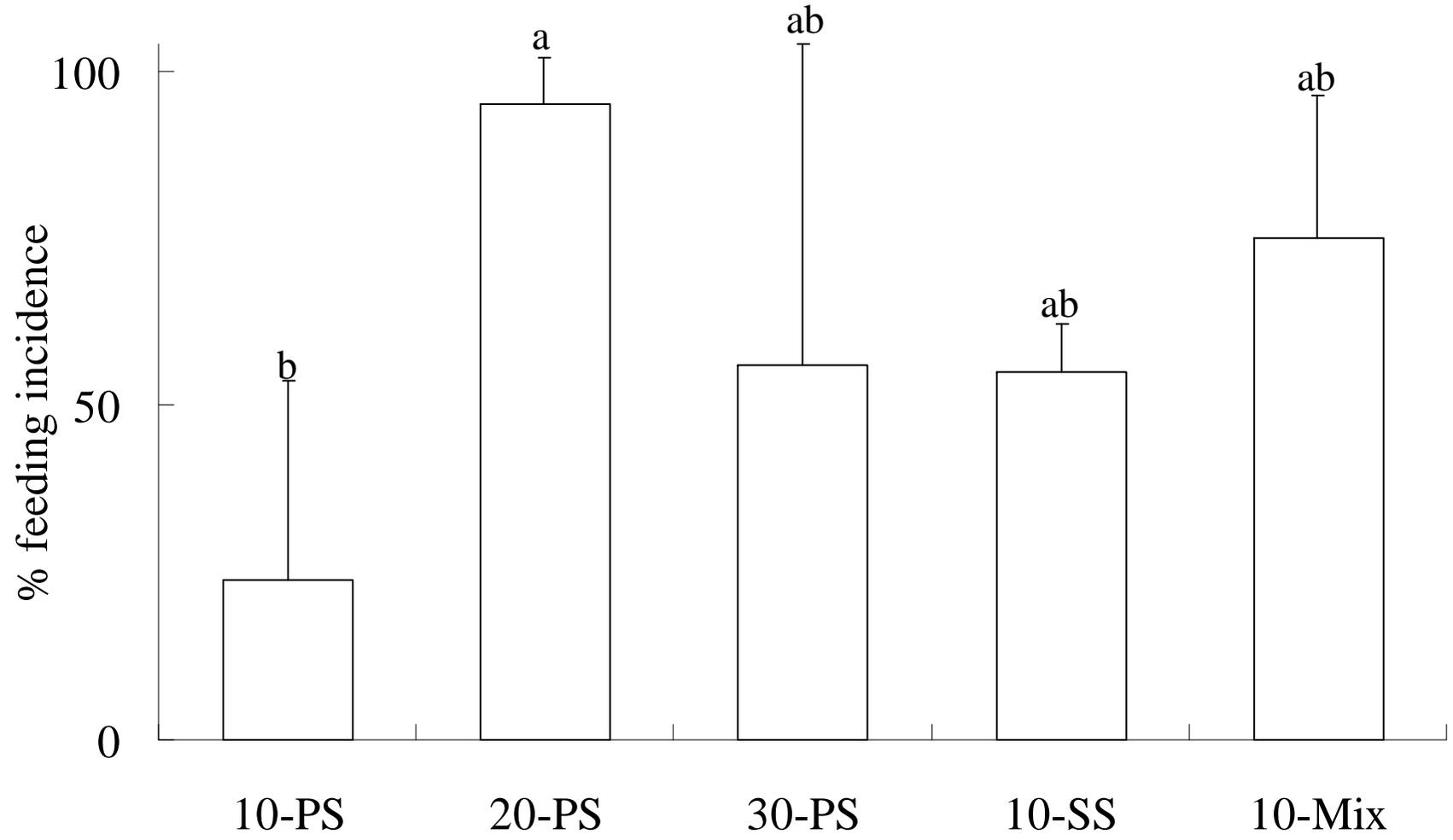


Fig. 6

Table 1. Life history parameters of *P. similis* cultured under different levels of salinity. Data of life span, reproductive period and fecundity indicate mean \pm SD of 50 individuals.

Life history Parameters	Salinity (ppt)		
	2	15	25
Life span (days)	4.6 \pm 1.7	4.0 \pm 1.4	4.7 \pm 2.0
Generation time (days)	2.5	2.4	2.8
Reproductive period (days)	2.9 \pm 1.2	3.4 \pm 1.1	3.2 \pm 1.1
Fecundity (female ⁻¹)	7.8 \pm 3.9 ^a	4.3 \pm 1.4 ^b	4.3 \pm 2.6 ^b
r (day ⁻¹)	0.93	0.70	0.63

a>b, Tukey-Kramer *post hoc* test, $p<0.05$