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# Demographic and competition studies on *Brachionus ibericus* and *Proales similis* in relation to salinity and algal (*Nannochloropsis oculata*) density

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**Abstract** We isolated the rotifers *Brachionus ibericus* and *Proales similis* from the sediment of shrimp tanks and studied their individual demographic characters and competition between them at two food levels ($0.25 \times 10^6$, $1.00 \times 10^6$ cells ml$^{-1}$ of *Nannochloropsis oculata* at 25 °C) and salinities ranging from 10 to 30‰. Our hypothesis was that growth rates would be higher with increasing food levels and salinities. Observations were taken twice a day for life table studies and daily once for population growth experiments. Using survivorship and fecundity data, we derived various life history variables. Although the average life span (7.6 ± 0.4 days) and gross reproductive rate (33.8 ± 2.9 neonate female$^{-1}$ day$^{-1}$) of *B. ibericus* were higher than those of *P. similis* (average life span 5.4 ± 0.6 days and gross reproductive rate 13.0 ± 0.6 neonate female$^{-1}$ day$^{-1}$), the population growth experiments showed that *P. similis* had higher *r* values (0.32 ± 0.005 day$^{-1}$) than *B. ibericus* (0.23 ± 0.002 day$^{-1}$) at $1.0 \times 10^6$ cells ml$^{-1}$ of *N. oculata*. The rotifer *P. similis* was more adversely affected due to the presence of *B. ibericus* than vice versa. The data are important for developing techniques for a large-scale culture of these rotifers as food in aquaculture.

**Keywords** *Proales similis* · Marine algae · Growth rates · Live food · Aquaculture

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## Introduction

Over exploitation of resources and economic development will result in an increase in the demand for fish to meet the nutritional requirements of the constantly growing human population (Merino et al. 2012). Aquaculture plays an important role in this regard, meeting the demand for high quality animal protein (Gjedrem et al. 2012). Despite the technological progress and development, success in aquaculture practices suffers the bottleneck of larval survival of edible fish and crustacean species (Takeuchi 2014). One of the limiting factors in aquaculture success is obtaining high densities of live food that is adequate for the gape size and meets the nutritional requirements of fish and crustaceans (Hagiwara et al. 2007).

Rotifers from the *Brachionus plicatilis* species complex (different morphotypes; SS (super small) to L (large)) are commonly cultured as food for fish larvae (Segers 1995; Jamali et al. 2015). It is advantageous to use these species, since they have high growth rates, a short generation time, and are easy to culture in the laboratory reaching densities as high as 30,000 individual (ind.) ml$^{-1}$ in mass cultures (Lubzens et al. 1995; Yoshimatsu and Hossain 2014). They are considered as the best live food for feeding fish larvae in early stages (Jobling 2015).

Some aspects of the basic ecology of rotifers have direct application in aquaculture. For example, the peak population densities and intrinsic rate of increase are used to program the mass production of rotifers in aquaculture farms (Lubzens et al. 2001). However, much of the work concerning the population growth and other demographic aspects is concentrated on a few species of *Brachionus* (Yoshinaga et al. 2000; García-García et al. 2007; Wang et al. 2014). Most brachionids measure > 100 μm, while the preferred body size of the first prey items for some commercially important marine fish is often less (Cahu and Zambonino-Infante 2001). Rao (2003) also states that most fish larvae feed on prey a quarter the size of their gape. Thus, species of *Brachionus* can be too large as a first diet, especially for small (< 5 mm at birth, total length) marine fish larvae. Therefore, the search for an alternative prey species with high growth rates is important, and in this regard, one of the important species is the small euryhaline rotifer *Proales similis* (body length ca. 80 μm) (Wullur et al. 2011).

Demographic variables of rotifers such as survivorship, intrinsic rate of increase, and generation time are sensitive to changes in both biotic (e.g., food density) and abiotic (e.g., salinity) factors, as well as ecological interactions such as competition and predation (Wallace et al. 2015a). Little information is available on *P. similis* as compared to *B. ibericus* and other members of the *B. plicatilis* complex. Hagiwara et al. (2014) have shown that a strain of *P. similis* from Japan at 25‰ salinity can reach from 25 to 2400 ind. ml$^{-1}$ in 11 days and has a growth rate around of 0.40 day$^{-1}$. Although *P. similis* frequently coexists with *B. ibericus*, not much is known about the effect of food density, salinity on the growth and competition between these two taxa. Population dynamics and life table experiments provide valuable data in this regard, since they provide complementary information on the individual as well as the population. However, these two methods are simultaneously applied rarely in studies on brackish rotifers (Gabaldón et al. 2015).

The recently described *B. ibericus* has a much wider distribution than previously thought. It is now found in Europe as well as South and Central America (Segers 2008; Chaparro et al. 2011). *P. similis*, a common tropical species, has been previously recorded from inland saline waters in the Chihuahuan Desert of Mexico (Walsh et al. 2008). We found the rotifers *P. similis* and *B. ibericus* together in a shrimp farm in the State of Sinaloa, Mexico. Both these rotifer species tolerate high salinities (up to 35‰) and grow on green algae (Ciros-Pérez al. 2001; Wullur et al. 2009). Since both rotifer species are sought after in Mexico as a first food for marine fish larvae, there is a need to compare the population level responses of these rotifer species at different concentrations of alga at an optimal salinity. We hypothesized that the population growth rates would be higher with increasing food levels and salinity. Since both taxa frequently coexist in shrimp tanks in Sinaloa, we hypothesized that they would not adversely affect each other in competition experiments. We evaluated the effect of two food concentrations ($0.25 \times 10^6$ and $1.00 \times 10^6$ cells ml$^{-1}$ of *N. oculata*) on the population growth and life table of the rotifers *B. ibericus* and *P. similis* at 10, 20, and permille of salinities. We also studied the effect of competition between the species in relation to different salinity levels.

## Materials and methods

Rotifers are frequently isolated and hatched from sediment samples (García-Roger et al. 2006); in this study too, the rotifers *Brachionus ibericus* and *Proales similis* were isolated from resting eggs in the sediment of a shrimp farm located south of Mazatlán, Sinaloa, Mexico. Both species were identified using taxonomic keys (Koste 1978; Ciros-Pérez et al. 2001). Clonal cultures of both species were established separately using single parthenogenetic females, which were maintained at a salinity of 20‰ and at 25 ± 1 °C for 4 months prior to the experiments. We cultured the microalga *Nannochloropsis oculata* in 2-l transparent bottles using the f2 medium (Guillard 1975) at 30‰ salinity and at 22 ± 1 °C. Chosen salinity levels were prepared using commercial sea salts (Instant Ocean, Aquarium Systems). During the exponential phase, we harvested the alga and centrifuged it at 3000 rpm for 5 min and later re-suspended it in the saline solution. The density of *N. oculata* was estimated using a Neubauer hemocytometer. The rotifers were maintained in 500-ml beakers using saline water at 20‰, *N. oculata* (ca. $1.0 \times 10^6$ cells ml$^{-1}$) as food, in diffuse fluorescent illumination (16L:8D) at 23 ± 2 °C. The pH of the medium was 7.4 ± 0.2, and the oxygen level was 6.2 ± 0.3 mg l$^{-1}$. The nitrate-nitrogen and soluble phosphorus levels were 0.4 ± 0.05 and 1.8 ± 0.1 mg l$^{-1}$. The water in these rotifer cultures was changed completely every alternate day, but the food was added daily.

We tested the survivorship of both *B. ibericus* and *P. similis* at several different concentrations of salinity (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, and 35‰) over a 24-h period. Twenty individuals of each taxon were introduced into a beaker with 20 ml of medium, and three replicates per concentration were set up. After 24 h, we observed the survivorship. Since the mortality was minimum at 20‰ for both taxa, we conducted all further experiments at this salinity. Only in the competition experiments, to test for the impact of salinity, we used three salinity concentrations, viz., 10, 20, and 30‰.

### Population growth

Experiments were initiated using cohorts of a mixed population of females of either rotifer species distributed randomly (under a stereomicroscope at ×20) using a finely drawn Pasteur pipette. The experiments were conducted in 25-ml vials, containing 20 ml medium at 25 °C and salinity of 20‰ with concentrations of at either $0.25 \times 10^6$ or $1.00 \times 10^6$

cells ml$^{-1}$ of *N. oculata*. For each treatment, we set up four replicates. In all, we used 16 vials for both rotifer species (2 species × 2 concentrations × 4 replicates).

All individuals were counted daily under a stereomicroscope and were then transferred to new vials containing fresh medium at appropriate food densities. From a stock concentration of *N. oculata*, we prepared the appropriate medium for each treatment so that every day, the algal concentration was maintained at the desired cell concentration in each treatment.

Although the population of *P. similis* decreased earlier, the experiments were terminated when the growth of *B. ibericus* began to decline (18 days). Based on the data collected, we derived the rate of population increase using the exponential growth equation: $r = (\ln N_t - \ln N_0)/t$, where $N_0$ and $N_t$ are the initial and final population densities, and $t$ is the time in days (Krebs 1985).

## Life table

Demographic tests of *B. ibericus* and *P. similis* were conducted separately for each species. Cohorts of 10 newborn females (< 6 h old) of each rotifer species were distributed randomly (under a stereomicroscope at ×20 using a finely drawn Pasteur pipette) into 25-ml vials, containing 20 ml medium at 25 °C and salinity of 20‰, with two chosen algal concentrations ($0.25 \times 10^6$ and $1.00 \times 10^6$ cells ml$^{-1}$). For each treatment, we set up four replicates (16 vials for the two rotifer species (= 2 species × 2 concentrations × 4 replicates)).

The neonates and dead adults of the original cohort when present were counted after every 12 h and removed. The surviving adults of the cohort were then transferred to a fresh medium with the desired concentration of the alga. We terminated the experiment when the last adult of each cohort died. From the data of survival ($l_x$) and fecundity ($m_x$), we calculated demography variables using standard formulae (Krebs 1985):

1. Gross reproductive rate

$$= \sum_0^\infty m_x,$$

2. Net reproductive rate

$$= \sum_0^\infty l_x \times m_x,$$

3. Average life span

$$e_x = \frac{T_x}{n_x},$$

4. Rate of population increase

$$\sum_{x=w}^{n} e^{-rx} \times l_x \times m_x = 1,$$

where $l_x$ is the survivorship, $m_x$ is the age-specific fecundity, $R_o$ is the net reproductive rate, and $r$ is the population growth rate.

### Competitive interactions

These experiments were conducted in 27 transparent 25-ml capacity vials, each containing 20 ml of the test medium, and were initiated at a density of 1 ind. ml$^{-1}$ of each rotifer species alone or in combination. Experiments were conducted at three salinities 10, 20, and 30‰ at 25 °C and at one food density ($1.00 \times 10^6$ cells ml$^{-1}$of *N. oculata*) for both species. For each treatment, we used three replicates. Daily, we estimated the density of live rotifers in each test vials, initially in total, but later in two to three aliquots of one milliliter each. After the count, we transferred all living rotifers to fresh vials containing algal food and appropriate levels of salinity. The experiments were terminated after 18 days when most test populations began to decline. The rate of population increase of rotifers cultured at different salinities and under competition stress was calculated using the exponential growth equation.

### Morphometry

The rotifers *B. ibericus* and *P. similis* were grown in three salinities (10, 20, and 30‰) at 25 °C temperature and fed *N. oculata* ($1.00 \times 10^6$ cells ml$^{-1}$). The experiment was conducted in nine transparent vials containing 20 ml medium. We introduced ten neonate females into each vial. Eight days later, we mixed replicates of each treatment and rotifers were fixed in formalin (4% final concentration). For each treatment, we randomly separated 20 adult females of *B. ibericus* and *P. similis* and measured the body size (length and width) using an optical microscope Nikon Eclipse E600 and calibrated camera lucida Nikon Y-IDT JAPAN.

### Statistical analyses

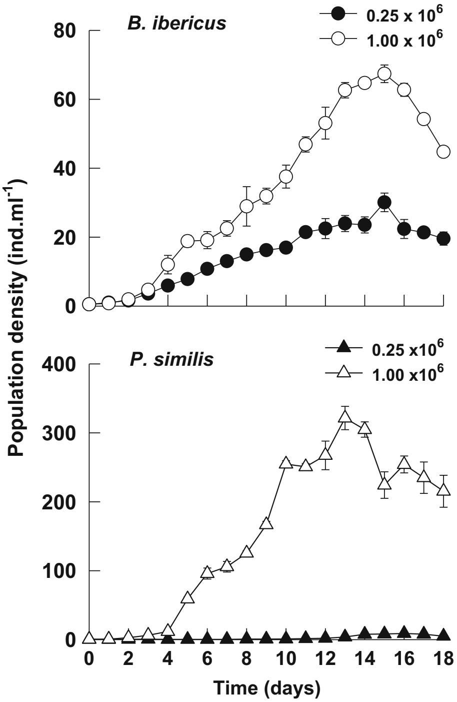
Differences among treatments for life table and population growth in relation to food density were statistically evaluated using ANOVA and Kruskal-Wallis tests and post hoc (multiple comparisons of means) Student-Newman-Keuls test. With respect to experiments on the effect of salinity and competition, the differences among treatments for peak population density and growth rates were evaluated using two-way ANOVA (Sokal and Rohlf 2011).

## Results

### Population growth

The population growth curves of *Brachionus ibericus* and *Proales similis* showed a clear relation with food concentrations (Fig. 1). At $0.25 \times 10^6$ cells ml$^{-1}$, *N. oculata* of both species had slower growth compared to that at $1.00 \times 10^6$ cells ml$^{-1}$. At both food densities, *B. ibericus* began increasing after 3 days and reached peak densities after 14 days. In the case of *P. similis*, however, a numerical difference was also observed with respect to the food concentrations. The peak population density of *P. similis* in the treatment with the lowest concentration of microalgae was equivalent to ~30% of the peak value at $1.00 \times 10^6$ cells ml$^{-1}$ (330 ind. ml$^{-1}$) (Fig. 2a). In spite of the high densities reached, we found that algal food was available even after 24 h. More food remained unutilized in *Proales* treatments as compared to *Brachionus* treatments.

**Fig. 1** Population growth curves of *Brachionus ibericus* and *Proales similis* cultured under different algal (*Nannochloropsis oculata*) concentrations (cells ml$^{-1}$). Shown are the mean ± standard error based on four replicates



In tests at a food concentration of $1.0 \times 10^6$cells ml$^{-1}$, the density of *B. ibericus* was approximately twice than that when they were fed $0.25 \times 10^6$ cells ml$^{-1}$. Under similar conditions, the peak density reached by *P. similis* was more than ten times higher than that obtained at the lower food level. The peak population density was significantly higher at the higher food level (Kruskal-Wallis test, $p < 0.029$). For both species, the rate of population increase ($r$) was significantly affected by food density ($p < 0.001$, ANOVA,

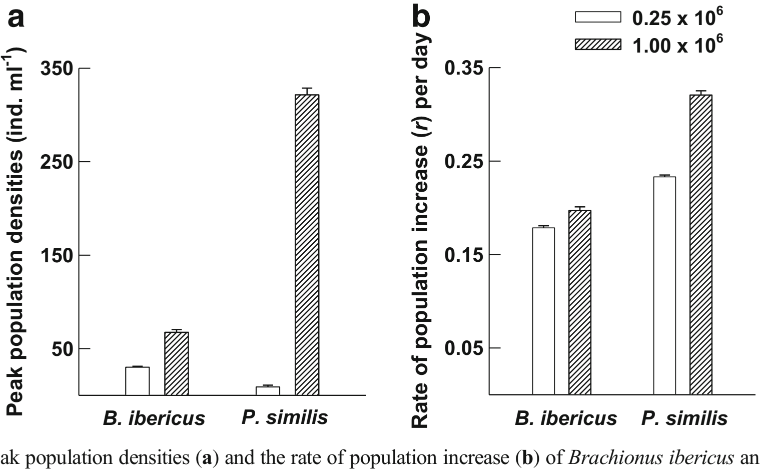


**Fig. 2** Peak population densities (**a**) and the rate of population increase (**b**) of *Brachionus ibericus* and *Proales similis* cultured under different algal (*Nannochloropsis oculata*) concentrations (cells ml$^{-1}$). Shown are the mean ± standard error based on four replicates

Table 1). It ranged between 0.18–0.23 day$^{-1}$ for *B. ibericus* and 0.19–0.32 day$^{-1}$ for *P. similis* (Fig. 2b).

## Life table

*B. ibericus* had a longer survivorship, which was similar regardless of the food concentration, as compared to *P. similis* which had survived twice as long at $1.0 \times 10^6$ cells ml$^{-1}$ than at $0.25 \times 10^6$ cells ml$^{-1}$. The fecundity of *B. ibericus* was higher at lower food concentration as compared to that at a higher level. At both food concentrations, peak offspring production was observed at day three after which it began to decline. *P. similis*, however, reached peak fecundity later (day 9) at $1.0 \times 10^6$ cells ml$^{-1}$ but on day three at $0.25 \times 10^6$ cells ml$^{-1}$ (Fig. 3).

The demographic variables of *P. similis* were significantly lower at $0.25 \times 10^6$ cells ml$^{-1}$ than those at the higher food level ($p < 0.05$, one-way ANOVA). On the other hand, this did not occur for *B. ibericus* where none of the variables were significantly different in relation to food density ($p > 0.05$, one-way ANOVA). Average life span and life expectancy at birth of *P. similis* were significantly lower than those of *B. ibericus*. While the brachionid had high gross and net reproductive rates, *P. similis* could reproduce only at the higher food level. The generation time of *P. similis* was shorter than that of *B. ibericus* and more so at the lower food level. *B. ibericus* had high growth rates (> 0.75 day$^{-1}$) at both food levels but *P. similis* had negative growth rates at the lower food level tested (Fig. 4).

## Competitive interactions

The population growth of *B. ibericus* was unaffected, regardless of salinity or competitive pressure. In contrast, the growth of *P. similis* was dependent on these two factors (Fig. 5). When *P. similis* grown alone in salinities of 10 and 20‰, it was more abundant (> 375 ind. ml$^{-1}$) than at the highests alinity tested where it reached a density of only 130 ind. ml$^{-1}$. Nevertheless, when *P. similis* was grown together with *B. ibericus*, the population density declined more so at the salinity of 30‰.

The peak population densities for *P. similis* varied from 40 to 410 ind. ml$^{-1}$, depending on the salinity level and the presence of the competitor. Under comparable conditions, the peak densities of *B. ibericus* ranged between 60 and 70 ind. ml$^{-1}$ (Fig. 6a). In general, in all tests, the presence of *B. ibericus* caused a considerable reduction in the abundance of *P. similis*; however, *Proales* had little effect on *B. ibericus* as compared to the controls.

Regardless of salinity and competition, the rates of population increase were similar across all treatments of *B. ibericus*, the highest value was recorded at a salinity of 20‰ but

**Table 1** Statistical evaluation using post hoc Student-Newman-Keuls tests on the rate of population increase of *B. ibericus* and *P. similis* in relation to different *N. oculata* concentrations

| Rotifer | Source of variation | *DF* | *SS* | *MS* | *F* |
|---|---|---|---|---|---|
| *B. ibericus* | Between groups | 1 | 0.00594 | 0.00594 | 561.08*** |
| | Error | 6 | 0.00006 | 0.00001 | |
| *P. similis* | Between groups | 1 | 0.03050 | 0.03050 | 869.67*** |
| | Error | 6 | 0.00021 | 0.00003 | |

*DF* degrees of freedom, *SS* sum of squares, *MS* mean square, *F F* ratio

***$p < 0.001$

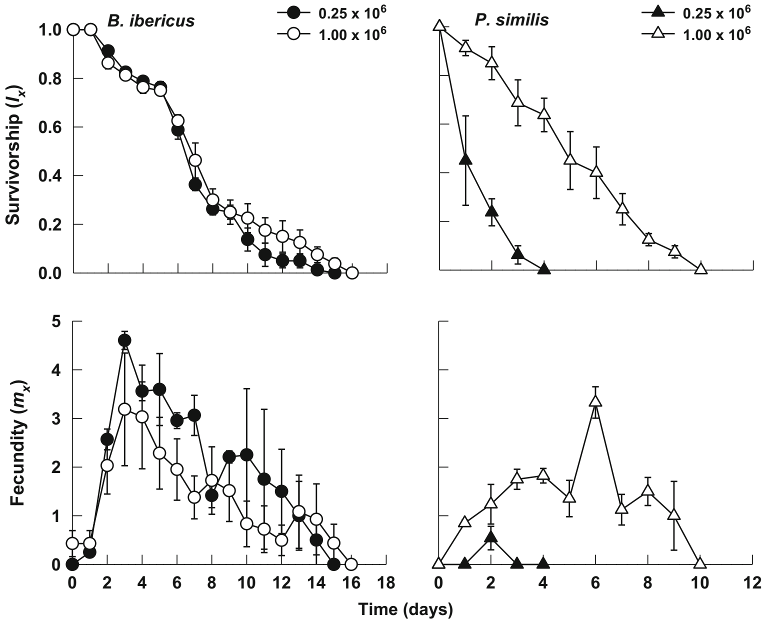


**Fig. 3** Data on fecundity and survivorship of *Brachionus ibericus* and *Proales similis* cultured under different algal (*Nannochloropsis oculata*) concentrations (cells ml$^{-1}$). Shown are the mean ± standard error based on four replicates

in the presence of *P. similis* ($0.25 \pm 0.02$ day$^{-1}$). On the other hand, the rotifer *P. similis* presented a clear trend with respect to salinity and competition (Fig. 6b). When grown alone at salinities of 10 and 20‰, it had a higher increase ($0.32 \pm 0.03$ day$^{-1}$) than when cultured together with *B. ibericus* and/or a salinity of 30‰ ($0.10 \pm 0.04$ day$^{-1}$). Statistically, the salinity had a significant influence on the rate of the population of both species ($p < 0.001$, two-way ANOVA), while competition only caused a negative effect on the growth of *P. similis* (Table 2).

## Morphometry

The morphometric measurements for the body size of *B. ibericus* and the body size of *P. similis* in relation to levels of salinity are shown in Tables 3 and 4 (Plate 1). In general, the length and width of *B. ibericus* ranged from 193 to 201 and 151 to 157 μm, respectively (Table 3). The length (Kruskal-Wallis test) and width (one-way ANOVA) were significantly higher ($p < 0.05$) at a salinity of 20‰ than at 10 or 30‰. The length (72–84 μm) and width (56–58 μm) of *P. similis* were also significantly higher ($p < 0.05$) at different salinities (Table 5).

# Discussion

Demographic studies on prey species are important for aquaculture, since they generate valuable information on optimal conditions for the production of live food (rotifers, cladocerans, and copepods). Data on variables such as growth rates under different

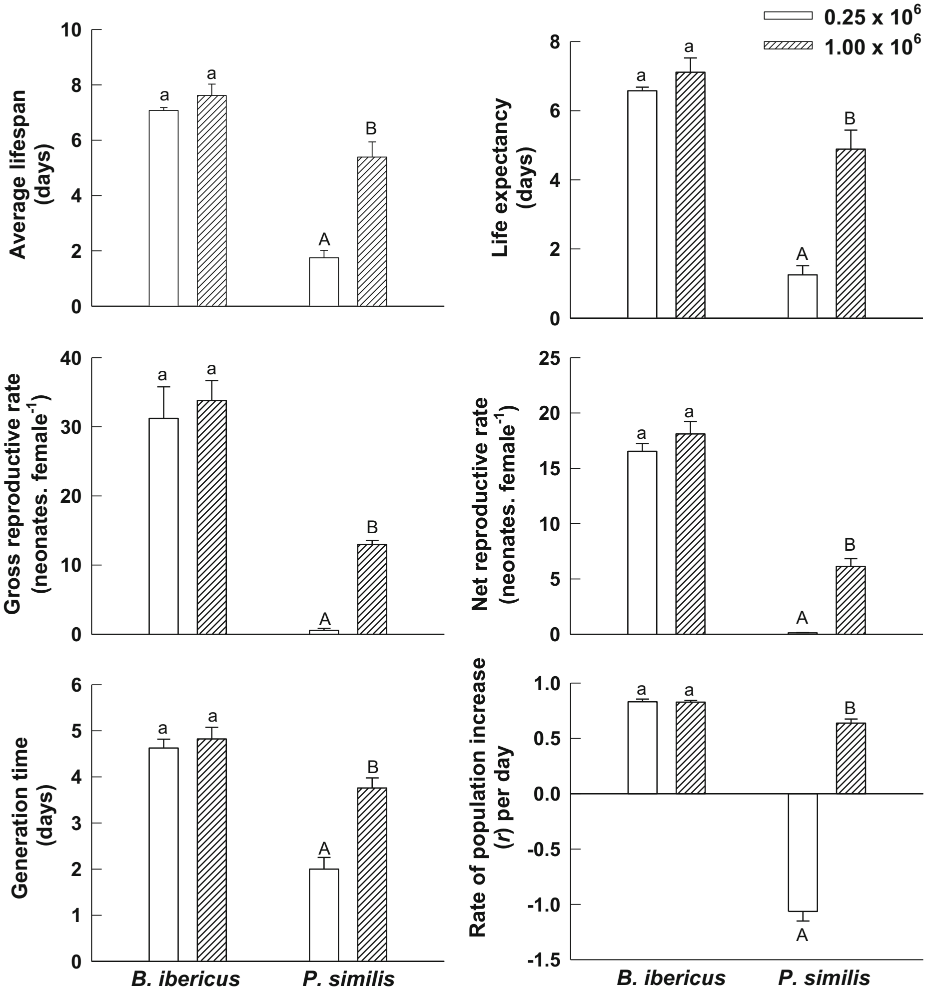


**Fig. 4** Data on the life table of *Brachionus ibericus* and *Proales similis* cultured under different algal (*Nannochloropsis oculata*) concentrations (cells ml$^{-1}$). Shown are the mean ± standard error based on four replicates

conditions of temperature, salinity, or competition with other species are helpful to optimize costs of operation in mass culture (Dhont et al. 2013). For instance, in this study, we found that in the population growth experiments, the growth rate of both *B. ibericus* and *P. similis* were significantly higher under conditions of greater food availability. On the other hand, the demography studies showed that *B. ibericus* grows equally well at low and high food levels; probably due to its low threshold food requirements which varies depending on the taxa (Nandini et al. 2007). This information will help economize on the use of algal biomass for large-scale cultures and forms the basis future culture experiments to standardize conditions for large-scale production of these rotifers for aquaculture.

Both *B. ibericus* and *P. similis* have ideal characteristics for use in the marine larviculture. They are small, appropriately sized for the gape of marine fish larvae and

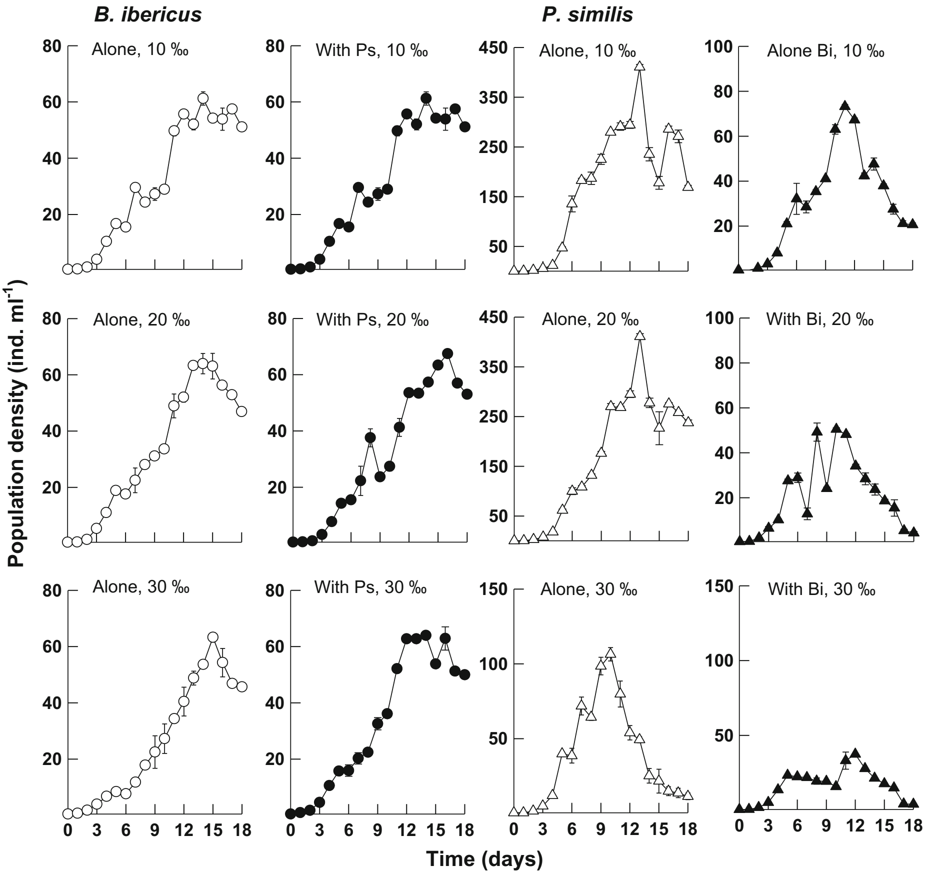


**Fig. 5** Population growth curves of *Brachionus ibericus* and *Proales similis*, grown alone or in the presence of competition and under at different levels (10, 20, and 30‰) of salinity. Shown are the mean ± standard error based on three replicates. Note differences in the scale of the *y*-axis scaling

are euryhaline (Wullur et al. 2011; Kostopoulou et al. 2015). It has been shown that fish larvae do not consume prey the exact size of the gape but about select prey which is about 25% of their gape size (Rao 2003). *B. ibericus* and other taxa of the *Brachionus plicatilis* complex are perhaps more important in the first feeding of several marine fish larvae which have a gape size of 150–400 μm at hatching such as turbot, *Scophthalmus maximus*, and various other taxa (Cunha and Planas 1999). However, other commercially important fish species such as the Mexican whitefish *Chirostoma estor estor* and the Spotted Rose Snapper, *Lutjanus guttatus* larvae have smaller gape size (90–120 μm) (Martínez-Palacios et al. 2004; García-Ortega 2009; Puello-Cruz et al. 2015) and for such taxa, *P. similis* is more appropriate than typical live food based on species of the *B. plicatilis* complex.

The nutritional quality of the prey is also an important factor determining its consumption by the predator. In a recent study, Hagiwara et al. (2014) observed that during the first days after hatcher of the seven-band grouper larvae (*Epinephelus septemfasciatus*) has a greater selectivity for *P. similis* compared to *B. rotundiformis*.

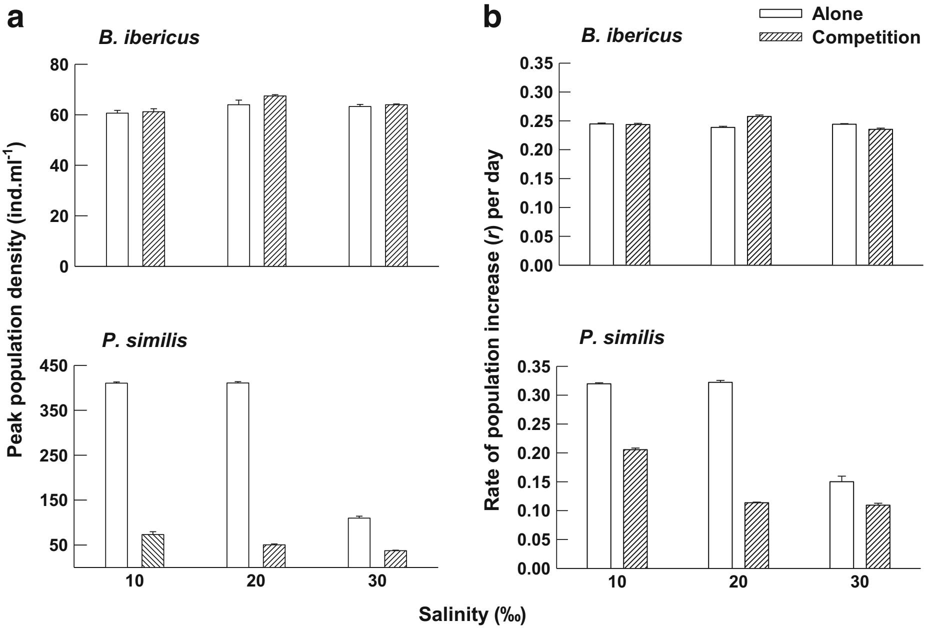


**Fig. 6** Peak population densities (**a**) and the rate of population increase (**b**) of *Brachionus ibericus* and *Proales similis*, grown alone or in the presence of competition and under at different levels (10, 20, and 30‰) of salinity. Shown are the mean ± standard error based on three replicates

The same study found that *P. similis* has a higher percentage of essential fatty acids (EFA) than *B. rotundiformis* when fed with the marine microalgae *N. oculata* and "super fresh" *Chlorella vulgaris* V-12®. Puello-Cruz et al. (2015) also show that the growth of the red snapper larvae is better when fed on the copepod *Pseudodiaptomus euryhalinus* than on the rotifer *B. plicatilis* complex. In addition to body size, nutritional quality also plays an important role in the prey utilization by fish larvae.

**Table 2** Results of analysis of variance (ANOVA) performed on the rate of population increase per day of *B. ibericus* and *P. similis* in relation to salinity and competitive interaction

| Source of variation | *DF* | *SS* | *MS* | *F* |
|---|---|---|---|---|
| Rate of population increase | | | | |
| *B. ibericus* | | | | |
| Salinity (A) | 2 | 0.00040 | 0.00020 | 23.48*** |
| Competition (B) | 2 | 0.00006 | 0.00003 | 3.95$^{ns}$ |
| Interaction of A × B | 4 | 0.00065 | 0.00016 | 19.16*** |
| Error | 9 | 0.00007 | 0.00000 | |
| *P. similis* | | | | |
| Salinity (A) | 2 | 0.0553 | 0.0276 | 414.78*** |
| Competition (B) | 1 | 0.0668 | 0.0668 | 1002.97*** |
| Interaction of A × B | 2 | 0.0221 | 0.0111 | 165.92*** |
| Error | 12 | 0.0008 | 0.0000 | |

*DF* degrees of freedom, *SS* sum of squares, *MS* mean square, *F F* ratio

***$p < 0.001$; ns: non-significant ($p > 0.05$)

**Table 3** Body size of *B. ibericus* in relation to salinity. All measurements are in micrometers (μm). Shown are the mean ± standard error based on 20 adult females

| Body size | Salinity | | |
|---|---|---|---|
| | 10‰ | 20‰ | 30‰ |
| Length | 193.82 ± 1.02 | 201.92 ± 1.94 | 198.26 ± 1.59 |
| Width | 154.29 ± 0.70 | 157.88 ± 0.85 | 153.46 ± 1.21 |

Little information apart from the seminal works of Wullur et al. (2009) is available on the food threshold and the effect of salinity on *P. similis*; the majority of the work is focused on members of the *B. plicatilis* complex which are traditionally used as the first food of fish (Romero-Romero and Yúfera 2012). We found that both food density and salinity had a large effect on the somatic and population growth of *P. similis*. Wullur et al. (2009) report that *P. similis* can grow well at salinities as low as 2‰, although this species is also found in hypersaline conditions at salinities as high as 98‰. In our study, we found that *P. similis* grew best, in terms of population growth rates and body size up to 20‰, and with a lower although positive growth rate at 30‰. Although *P. similis* is a cosmopolitan species as is *B. plicatilis* s.l. (Segers 2007), there are few studies on their population dynamics, and most studies are based on taxonomy and genetics (Brain and Koste 1993; Sørensen and Giribet 2006).

Genetic variability between different strains is reflected in the population parameters. We compared our results on the life table and population growth of *P. similis* isolated from white shrimp (*Litopenaeus vannamei*) ponds with a strain of the same species isolated in an estuary of Japan. Despite the difference between the food density and the salinities used in the present study, our results are similar to those reported in Wullur et al. (2009). We observed a negative effect on the life history variables of *P. similis* when fed a low food density. Previous studies show that *B. plicatilis* grows better with increasing food availability (Yin and Zhao 2008). The strain of *B. ibericus* we have could grow well at the lower food concentration tested which is well above the threshold food level reported for rotifers (Nandini et al. 2007).

The strain of *B. ibericus* and *P. similis* used in this study are euryhaline tolerating a wide range of salinities and thus suitable for the culture of several fish species. Similar trends have been reported for *B. plicatilis* and *B. rotundiformis* (Fielder et al. 2000). A competition between different rotifer species depends on both abiotic and biotic factors. For example, Gabaldón (2015) and Gabaldón et al. (2015) report that a competition between *B. plicatilis* and *B. manjavacas* depends on the salinity level. In other freshwater rotifers, it is known that under greater food availability, larger species tend to dominate (Sarma et al. 1996). Our

**Table 4** Body size of *P. similis* in relation to salinity. All measurements are in micrometers (μm). Shown are the mean ± standard error based on 20 adult females

| Body size (μm) | Salinity | | |
|---|---|---|---|
| | 10‰ | 20‰ | 30‰ |
| Body length | 82.26 ± 1.28 | 78.2 ± 1.26 | 72.3 ± 11.09 |
| Body width | 57.73 ± 0.81 | 56.76 ± 1.09 | 56.73 ± 0.83 |

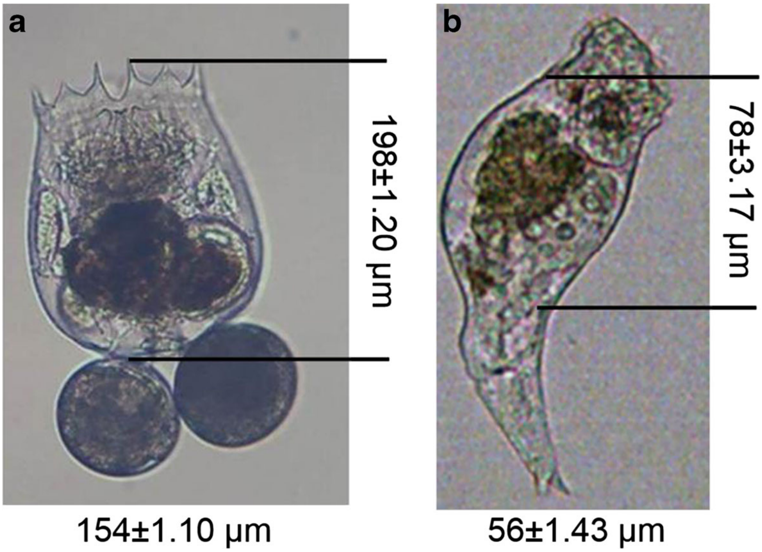


**Plate 1** **a**) *Brachionus ibericus* and **b**) *Proales similis*. Shown are the body size measurements, mean ± standard error

experiments showed that *B. ibericus* was unaffected by the presence of *P. similis* at the salinities tested; however, the growth of *P. similis* was adversely affected due to the presence of *B. ibericus*. This was most probably due to the small body size and soft body of *P. similis* which made it susceptible to the active movements of the larger *B. ibericus*. Such trends have been shown in previous studies on interactions between small and large rotifers. The rate of population increase and the peak population densities of *P. similis*, when grown individually, were higher than those of *B. ibericus*. Similar to the trends reported in a study of competition between *B. rotundiformis* and *H. jenkinae* (Sarma et al. 2002), we also observed that *B. ibericus* and *P. similis* could coexist in the same experimental salinities, but *B. ibericus* was the superior competitor.

**Table 5** Results of the analysis of variance (ANOVA) performed on the body size *B. ibericus* and *P. similis* in relation to salinity

| Source of variation | *DF* | *SS* | *MS* | *F* |
|---|---|---|---|---|
| Morphometry | | | | |
| *B. ibericus* | | | | |
| Salinity (A) | 2 | 1127.219 | 563.609 | 14.811*** |
| Characters (B) | 1 | 56,834.443 | 56,834.443 | 1493.568*** |
| Interaction of A × B | 2 | 51.282 | 25.641 | 0.674$^{ns}$ |
| Error | 114 | 4338.018 | 38.053 | |
| *P. similis* | | | | |
| Salinity (A) | 2 | 1262.011 | 631.006 | 20.539*** |
| Characters (B) | 1 | 19,635.556 | 19,635.556 | 639.132*** |
| Interaction of A × B | 2 | 904.344 | 452.172 | 14.718*** |
| Error | 174 | 5345.667 | 30.722 | |

*DF* degrees of freedom, *SS* sum of squares, *MS* mean square, *F* *F* ratio

***$p < 0.001$; ns: non-significant ($p > 0.05$)

## Conclusions

Our study highlights the importance of further studies on *B. ibericus* and *P. similis*. It is clear that both the species are euryhaline and have high growth rates. Optimal growth in large-scale cultures can be achieved by allowing them to grow individually and not in competition. Although there are some studies on the feeding behavior of fish larvae using these rotifer species, more studies are needed on the survivorship, growth, and feeding behavior of commercially important local fish species. This includes locally important taxa such as the Pike silverside (*Chirostoma estor estor*) and the Spotted Rose Snapper (*Lutjanus guttatus*) fed these two rotifer species to ensure success in larviculture.

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