Euryhaline rotifer *Proales similis* as initial live food for rearing fish with small mouth

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Abstract

The SS-type rotifer *Brachionus rotundiformis* is a common initial food for rearing fish larvae with small mouth. However, there are commercially important fish species whose mouth sizes are too small to feed on SS-type rotifers. In 2004, we isolated a small (body length= 82.7 ±10.9 µm; body width 40.5±6.4 µm), flexible, and iloricate rotifer, *Proales similis* from an estuary in Okinawa, Japan. Under laboratory conditions (25^{o}C, 2-25 ppt) *P. similis* produced its first offspring on 2.5 to 2.8 days after hatching, and produced 4.3 to 7.8 offspring within 4.0 to 4.7 days life span. Batch cultured *P. similis* fed *Nannochloropsis oculata* suspension at 28.8 µg dry weight ml^{-1} and cultured at 25^{o}C, 25 ppt filtered seawater, increased exponentially from 25 to 2400 ind ml^{-1} after 11 days of culture with an overall intrinsic rate of natural increase (*r*) of 0.42 day^{-1}. Growth rate of *P. similis* was not significantly different when fed fresh *N. oculata* and super fresh *Chlorella vulgaris*-V12®. Total lipid per wet weight of *P. similis* fed by *N. oculata* and *C. vulgaris* were 2.4 and 2.6%, respectively. The compositions of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (ARA) of *P. similis* fed *N. oculata* were 23.2, 0.0 and 5.3%, respectively, while these were 11.0, 17.5 and 0.5% respectively, when fed *C. vulgaris*. The use of *P. similis* to feed small mouth fish including seven-band grouper *Epinephelus septemfasciatus*, rusty angelfish *Centropyge ferrugata*, and humphead wrasse *Cheilinus undulatus* showed that it is an excellent starter food for these species because of their high selectivity index and improved survival. In addition, *P. similis* was ingested by Japanese eel *Anguilla japonica* larvae with complicated digestive system. The use of *P. similis* as starter feed for small mouth fish larvae is highly recommended.

Keywords

euryhaline rotifer, larval rearing, live food, small-mouth fish, *Proales*

1. Introduction

In marine fish larvae culture, the rotifers provided as starter food during the first days of exogenous feeding, depending on the mouth size of the larvae (Lubzens, 1987; Hagiwara et al., 2001). Rotifers are excellent first live food due to their small size (Tanaka et al., 2005; Akazawa et al., 2008), ability to be cultured at high density (Yoshimura et al., 1997; Hagiwara et al., 1997, 2001) and the capacity to be nutritionally manipulated (Hayashi et al., 2001; Hagiwara et al., 2001). Based on lorica size, culturists divided rotifers into L (large; 130-340 µm), S (small; 100-1200 µm) and SS (super small; 90-110 µm) type (Hagiwara et al., 1995; 2001). The SS-type is also classified as *Brachionus rotundiformis* (Segers, 1998; Kotani et al., 2005; Fontaneto et al., 2007). Due to its smaller size, *B. rotundiformis* is commonly used as starter food for fish species with small mouth gape. However, feeding mix stages of *B. rotundiformis* is infective or unsuitable for larvae of several marine fishes with even smaller mouth, including some species of groupers (Kohno et al., 1997; Okumura, 1997; Glamuzina et al., 1998, 2000), angelfishes (Olivotto et al., 2006) and wrasse (Sugama et al., 2004). Larvae of angelfishes of the family Pomachantidae for example is reported to have a gape size of approximately 160 µm (Olivotto et al., 2006; Leu et al., 2009), while larvae of Napoleon wrasse *Cheilinus undulatus* have a mouth size of 133 µm (Slamet and Hutapea, 2004). These two commercially valuable fish species require even smaller live food in the range of 40-80 µm at the initial feeding stages (Slamet and Hutapea, 2004; Olivotto et al., 2006). Despite much success achieved in the maturation and spawning of rusty angelfish and Napoleon wrasse, larval rearing has achieved little success due to the lack of starter food suitable for their larvae. If suitable size of prey is assumed from 20 to 70% of the mouth size (Cunha and Planas, 1999; Yúfera and Darias, 2007), larvae of rusty angelfish may require starter food with size from 32 to 112 μm, while larvae of Napoleon wrasse may require 26 to 93 μm food items.

Aside from small mouth gape, some fish species have complicated digestive system that requires smooth and easily digested food item. An example is the Japanese eel *Anguilla japonica*. Although eel larvae have large mouth size at initial feeding their oesophagus is narrow and without mucus cells (Yoshimatsu and Matsuda, 2008; Yoshimatsu et al., 2008), thus could not ingest rotifers with lorica or copepods with exoskeleton. At present, larvae culture of Japanese eel uses a slurry diet made of dried shark egg, particularly the egg of spiny dogfish *Squalus acanthias* (Tanaka et al., 2001, 2003; Kagawa et al., 2005). However, the use of shark egg raised concerns because of serious depletion of shark population, and the species presently is considered as endangered (Baum et al., 2003). Finding alternative dietary source for eel larvae is necessary for its sustainable aquaculture.

In July 2004, we isolated a rotifer species from an estuary in Okinawa, Japan, and tentatively identified it as *Proales similis*. The identity was confirmed by Professor Russel Shiel of Albury, NSW, Australia. *P. similis* belongs to class Monogonta, family Proalidae and genus *Proales* (Koste and Shiel, 1990; De Smet, 1996). It was firstly reported by De Beauchamp in 1907 (De Smet, 1996), and later on, reported to be found in wide range of water bodies, from freshwater (Manuel et al., 1992; Turner, 1996; Ricci and Balsamo, 2000), estuarine and brackishwater (De Smet, 1996) to hypersaline water (De Smet, 1996; Moscatello and Belmonte, 2004; Walsh et al., 2008). Its body is soft and flexible without lorica (iloricate) unlike other rotifer species (De Smet, 1996). Among the species in genus *Proales*, only *Proales sordid* (Jennings and Lynch, 1928a, 1928b) and *Proales decipiens* (Noyes, 1922) have been successfully cultured. Recognizing the demand of fish larvae on small, smooth and flexible starter food and the potential of *P. similis* to meet this demand, we conducted series of experiments in order to determine the life history, mass production, and nutritional value of *P. similis*. After establishing its culture, we tested its suitability as starter food for various fish species under laboratory conditions. For the first time, we successfully mass cultured *P. similis* at high density in the laboratory (Wullur, 2009). Our feeding experiments also proved that *P. similis* is a suitable first food for fish species with very small mouth and complicated digestive system.

2. Life history, culture, and nutritional value of *Proales similis*

The *P. similis* (Figure 1) that we explored was collected using a 45 µm mesh plankton net from an estuary in Ishigaki Island, Okinawa, Japan on July 2004. The water temperature and salinity during the collection were 27^{0}C and 2 ppt, respectively. A clonal culture of *P. similis* was subsequently acclimatized to higher salinity under laboratory conditions, fed *Nannochloropsis oculata*. The total length, body length, and body width of *P. similis* ranged from 50 to 150 µm (mean ± SD; 109 ± 15 µm), 40 to 110 µm (mean ± SD; 83 ± 11 µm), and 10 to 50 µm (mean ± SD; 40 ± 6µm), respectively. Its body length is 38% smaller than the lorica length of *B. rotundiformis* (which ranged from 70-170 µm), and its body width is 60% narrower than the lorica width of *B. rotundiformis* which ranged from 50-150 µm (Wullur et al., 2009).

Temperature and salinity are two important factors that influence the population growth of rotifers. Life history parameters of *P. similis* under a wide range of temperature and salinity measures were undertaken. Temperature showed a strong influence on the population growth of *P. similis* under batch culture method (Wullur et al., 2009). Maximum density (1,400 ind ml^{-1}) was obtained at 30 to 35^{0}C. This indicates its usefulness in feeding subtropical and tropical fish species. Results also showed that *P. similis* is an euryhaline species because it can propagate in a wide range of salinity (2-25 ppt), although it can reproduce faster at 2 ppt (Wullur et al., 2009). This salinity corresponds to the salinity to where *P. similis* was sampled. However, Brain and Koste (1993) found *P. similis* in hypersaline water (48-98 ppt). The capability of *P. similis* to tolerate a wide range of salinity is similar to that of the euryhaline rotifer *Brachionus plicatilis* sp. complex, which is reported to thrive from 1 to 60 ppt (Hoff and Snell, 1987).

We also conducted a series of life table experiments of individual cultured *P. similis*, in order to determine its lifespan, generation time, reproductive period, and fecundity under different temperatures (15, 20, 25, 30 and 35^{0}C) and salinities (2, 15, and 25ppt; Wullur et al., 2009). During the experiment, the animals were fed with $2.5x10^6$ *N. oculata* and were kept in darkness. The animals were inspected daily until they die. Life span ranged from 4.0 to 4.7 days, generation time from 2.4 - 2.8 days, reproductive period from 2.9 – 3.4 days, and fecundity 4.3 – 7.8 (Wullur et al., 2009). Based from the above results, we also conducted a mass culture (2-l) experiment in order to determine the population growth rate of *P. similis* in bigger scale. The experiment was conducted at 25^{o}C, 25 ppt and fed with *N. oculata* at 28.8 µg dry weight ml^{-1}. Results showed that *P. similis* grew from 25 ind ml^{-1} on day 0 to 2400 ind ml^{-1} on day 11, with a lag growth from day 1 to day 4, exponential growth from day 5 to day 8, and stationary phase from day 9 onwards. The mean *r*-value we obtained from 3 runs was 0.42 day^{-1} (Wullur et al., 2009).

P. similis could be nutritionally enriched by feeding *N. oculata* and Super Fresh *Chlorella*® (Wullur et al., 2011). The highly unsaturated fatty acid (HUFA) of *P. similis* is comparable to that of *B. rotundiformis* when cultured, fed or treated with the same microalgae at the same concentration (Table 1). The DHA of the *P. similis* fed with Super Fresh *Chlorella*®

was 2.6 times higher than that of *B. rotundiformis* fed with the same food. The ratios of DHA/EPA in *P. similis* and *B. rotundiformis* fed Super Fresh *Chlorella* were 1.59 and 1.08, respectively. These levels of DHAs, EPAs and of the DHA/EPA ratio were in the range of the suggested levels for marine fish larvae (Tucker, 1998; Sargent et al., 1999).

3. The suitability of *P. similis* as initial food for:

3.1. Seven-band grouper *Epinephelus septemfasciatus*

The mouth of the seven-band grouper *E. septemfasciatus* opens at 3 day after hatching (DAH), and the mouth size at first day of feeding (4 DAH) is 180 ± 20 µm (Wullur et al., 2011). On 4 DAH, the larvae showed higher selectivity on *P. similis* than *B. rotundiformis*, with selectivity index of 0.7 and 0.3, respectively. The preference became neutral on 5 DAH, and the larvae switched their preference to larger prey (*B. rotundiformis*) on 6 DAH and thereafter. Therefore, a combination of *P. similis* and *B. rotundiformis* is recommended in larval rearing of grouper, *E. septemfasciatus* (Wullur et al., 2011). The consistent better growth and survival of grouper larvae fed with the combination of two rotifer species indicated that they effectively utilized *P. similis* during the first few days of feeding, in addition to *B. rotundiformis* as an energy resource for growth and survival. Feeding *P. similis* to other grouper species with similar characteristics to *E. septemfasciatus* is therefore recommended.

3.2. Rusty angelfish *Centropyge ferrugata*

Angelfishes (family Pomacanthidae) are among the top ten families in international trade of marine aquarium species (Baensch and Tamaru, 2009). Within family Pomacanthidae, the genus *Centropyge* is among the most popular, highly prized and heavily traded (Olivotto et al., 2006; Baensch and Tamaru, 2009). Despite much success in captive maturation and spawning of angelfishes have been achieved in last three decades (Suzuki et al., 1979; Arai, 1994; Olivotto et al., 2006; Leu et al., 2009), massive mortality related to poor initial feeding of the larvae still remain a bottleneck for successful captive production of this species (Olivotto et al., 2006; Leu et al., 2009).

Wullur (2009) conducted two feeding trials on angelfish *C. ferrugata* to determine the acceptability of *P. similis* as well as other zooplankton, including *Keratella* sp. cf. *sinensis*, *Paracyclopina nana*, and SS-type rotifer *B. rotundiformis.* Larvae were stocked in a 2.5 l natural seawater (32ppt) at 25^{0}C. All test zooplankton were supplied to the larvae at 20 ind ml^{-1} starting from 3 DAH. Results showed that the feeding incidence (measured by the quantity of zooplankton found in the gut of the larvae) of the larvae fed *P. similis* was higher than those fed with other zooplankton species (Figure 2). Furthermore, survival on day 6 was higher in the larvae fed *P. similis* (18.5 to 38.0%) than those in other treatments (1 to 11.5%; Figure 3). Results of this study proved that *P. similis* is a good candidate as first food for angelfishes.

3.3. Humphead wrasse *Cheilinus undulates*

The total length of humphead wrasse *C. undulatus* after 6h of hatching was approximately 2.4 mm, then the mouth opens and the eye pigmentation were observed at 2 DAH (Hirai et al., 2013). The mouth diameter and mouth width was 154 µm and 133 µm, respectively. Due to their small mouth gape, we conducted a preliminary experiments exploring the use of particulate diets such as powdered milk and boiled chicken yolk which are small and contain high protein (Hirai et al., 2012). *C. undulatus* larvae ingest *P. similis*, boiled chicken egg yolk and powdered milk on 2 DAH, and increased ingestion of *P. similis* was observed on 3 DAH. On both days, *C. undulatus* did not ingest *B. rotundiformis*. However, on 7 DAH, the number of

B. rotundiformis in the gut of *C. undulatus* was greater than the number of *P. similis*. During this experiment, we produced 537 juveniles at 50 DAH (survival rate = 10.7%), indicating the success of *C. undulatus* seed production with the use of *P. similis* as initial food (Hirai et al., 2013).

3.4. Japanese eel *Anguilla japonica*

The mouth size of the Japanese eel *A. japonica* larvae is large (521±28 µm), but they have difficulty ingesting large and solid food items because their esophagus is characteristically narrow and devoid of mucus cells (Yoshimatsu et al., 2008). The lack of mucus cells in the esophagus may limit the larvae to ingest only soft, small, and smooth food materials. At present, the primary food of *A. japonica* larvae in captivity is a slurry diet, made of shark egg powder (Tanaka et al., 2001, 2003; Kagawa et al., 2005). However, the use of this food is not sustainable because of serious depletion of shark population (Baum et al., 2003).

We conducted a series of experiment to determine if *A. japonica* larvae could survive when fed *P. similis*, both as living and non-living diet. A slurry diet made of shark egg powder was fed to the control group. *P. similis* paste was made by concentrating the rotifer culture at exponential growth stage and the concentrated rotifers were stored in a refrigerator (4^0C) until use, while live *P. similis* diet was taken directly from the culture tanks during feeding time. Feeding started on 7 DAH and terminated on 13 DAH where survival rate and total length of survivors were determined. Results showed that survival was significantly higher in the slurry diet fed group (62.8%) than those fed non-living *P. similis* (37.2%) and living *P. similis* (0.8%). The results indicated that *A. japonica* larvae ingest only non-living diet (Wullur, 2009). In successive experiments, in addition to *P. similis*, we tested the acceptability of other minute zooplankton species including, *Synchaeta* sp. cf. *cecilia*, *B. rotundiformis*, *Keratella* sp. cf. *sinensis*, *B. angularis* and nauplii of copepod *Paracyclopina nana* as initial food for *A. japonica*. Mass cultured zooplanktons were harvested, concentrated, and paste as described above, and fed to *A. japonica*. Feeding incidence (percentage of larvae with food in the gut) of the larvae fed slurry diet (control) was 26.7 to 100.0%, and *P. similis* paste was 20.0 to 46.7% (Wullur et al., 2013). The feeding incidence of larvae fed *P. similis* was significantly higher than those of other zooplanktons (0 to 6.7%). The ingested slurry diet (20.3 to 68.9%) and *P. similis* (1.8 to 37.2%) appeared in larval foregut and mid-hindgut, while the ingested *B. rotundiformis*, *Keratella* sp., and *B. angularis* remained in the foregut. Although feeding incidence of group fed *P. similis* paste was lower than the slurry diet, the use of *P. similis* paste is a good potential as food for eel larvae because the uneaten slurry diet needs to be flushed out of the rearing tank every after feeding time to avoid deterioration of the culture water.

4. Conclusion

P. similis is so far among the smallest rotifer species successfully mass cultured in the laboratory and successfully used in the larval rearing of marine fish with very small mouth gape. Since it is iloricate, it is also better ingested and digested by fish larvae with complicated digestive system. Its culture is the same as the widely used *Brachionus* species (*B. plicatilis* and *B. rotundiformis*) with the use of either *N. oculata* or *C. vulgaris*. *P. similis* is euryhaline and eurythermic, thus it can used for freshwater and marine species as well as in subtropical and tropical fish species. Based on the above feeding experiments, *P. similis* proved to be an excellent first food for fish larvae with very small mouth gape such as groupers, wrasse, and angelfishes, and with complicated digestive system such as Japanese eel. Although small live food organisms such as ciliates, bivalve larvae, sea urchin eggs, oyster trocophores, and copepods, were accepted by fish larvae with small mouth, these live feed are either low in nutritional value or difficult to culture or obtain at high density. The use of euryhaline rotifer, *P. similis* is highly recommended for testing to other fish larvae with similar characteristics as the above tested fish species.

Acknowledgement

This research was supported by the JSPS Kakenhi (Grant-in-Aid for Scientific Research B), Grant Number 24380108 to A.H.

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Table legends

Table 1. Total highly unsaturated fatty acid (HUFA) of *P. similis* fed *N. oculata* and super fresh

Table 1

HUFA	*P. similis* fed *N. oculata*	*P. similis* fed super fresh *C. vulgaris*®	*B. rotundiformis* fed super fresh *C. vulgaris*®
C20: 4n-6	5.3	0.5	0.8
C20: 5n-3	23.2	11.0	6.1
C22: 6n-3	0	17.5	6.6
DHA/EPA	0	1.59	1.08

Figure legends

Figure 1. The *Proales similis* isolated in Ishigaki Island, Okinawa, Japan.

Figure 2. Number of zooplankton in the gut of *C. ferrugata* larvae. I, first run; II, second run. *Proales similis* (□), *Brachionus rotundiformis* (■), *Keratella* sp. cf. *sinensis* (▒) and *Paracyclopina nana* nauplii (▚).

Figure 3. Survival of *C. ferrugata* larvae in the first (■) and second run (▒).

Figure 1

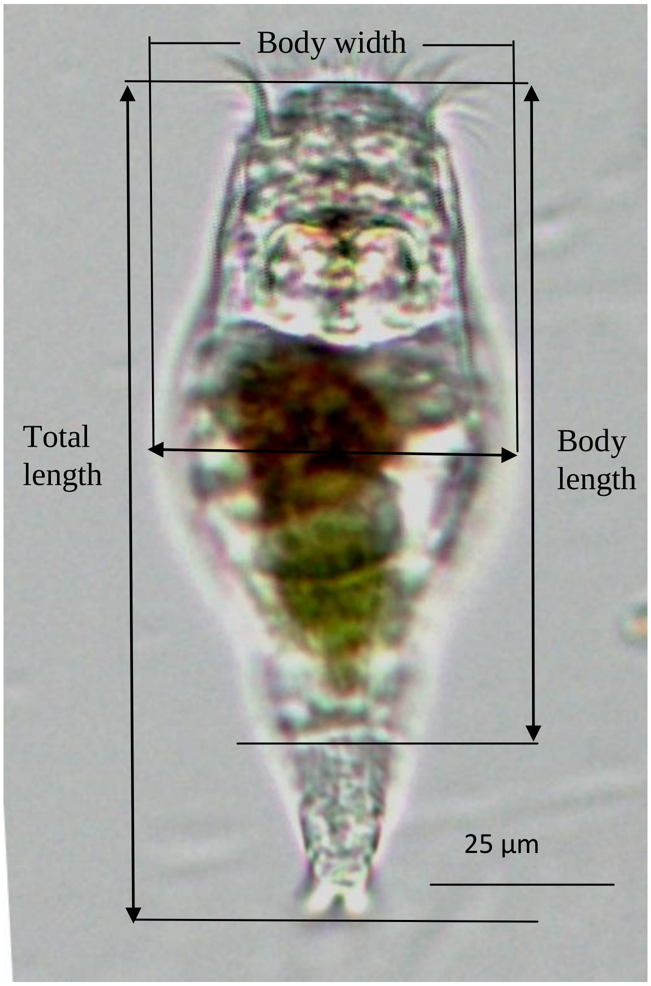


Figure 2

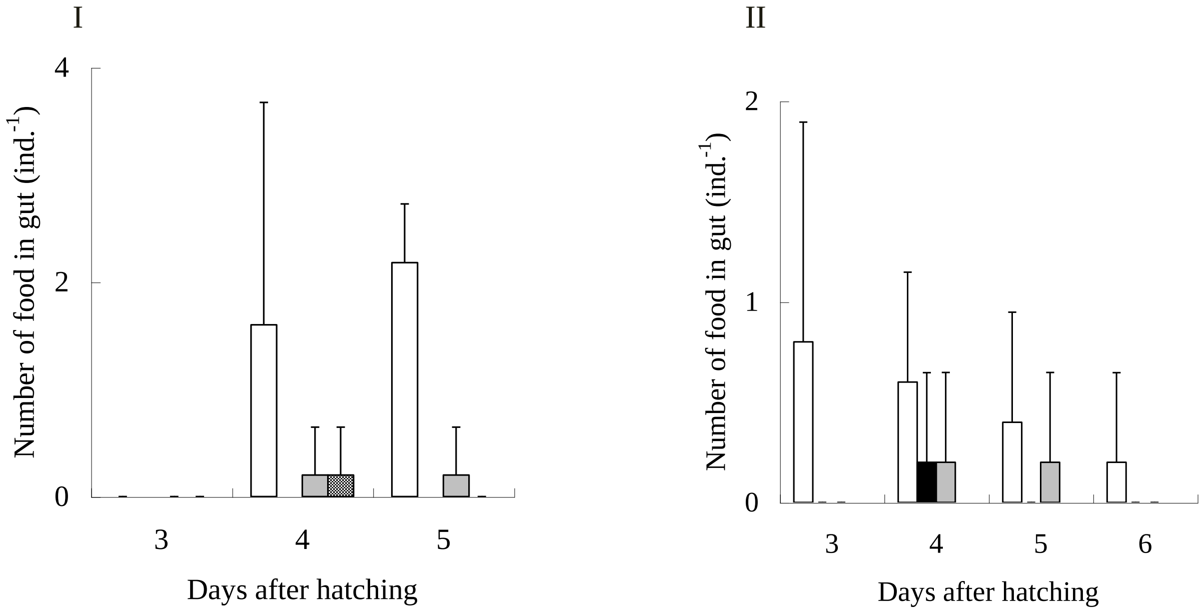
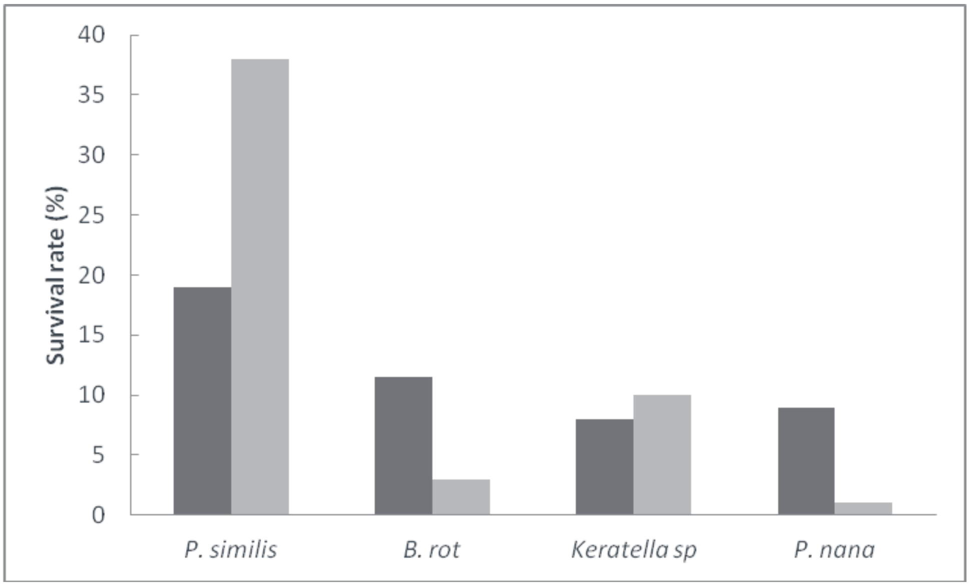

I
4
2
0
Number of food in gut (ind.$^{-1}$)
3
4
5
Days after hatching
II
2
1
0
Number of food in gut (ind.$^{-1}$)
3
4
5
6
Days after hatching

Figure 3


Survival rate (%)
40
35
30
25
20
15
10
5
0
P. similis
B. rot
Keratella sp
P. nana