The use of non-*Brachionus plicatilis* species complex rotifer in larviculture

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Abstract

Due to the expanding world aquaculture production, the demand for high quality and quantity of fish larvae has also increased. Up to date, the bottleneck in larviculture is the stable and ample production of appropriate live food such as rotifers and copepods. Among rotifers, *Brachionus plicatilis* species complex, which encompasses 15 species with varied sizes ranging from 100-400 µm, is commonly used in most hatcheries. The use of *B. plicatilis* species complex (*B. plicatilis*, *B. koreanus* and *B. rotundiformis*) in larviculture is reported in several review papers. In this review, we first described rotifer species not classified under *B. plicatilis* species complex, some of which are already used in larviculture, while some have high potential for use based on their characteristics, life history, and distribution. Rotifers, *Brachionus angularis*, *Brachionus calyciflorus* and *Proales similis* are described in details in comparison with *B. plicatilis* species complex. Furthermore, we discussed some characteristics of rotifers which can affect their predation.

Keywords: Rotifera, live food, larval culture, rotifer mass culture, *Brachionus*, *Proales similis*

Introduction

Aquaculture is the world's fastest growing food producing sector, with an annual growth rate of 8.8% compared to 1.2% for capture fisheries and 2.8% for terrestrial meat production (FAO, 2016). Parallel to the growth of aquaculture is the demand of high quality and quantity of larvae needed to be stocked in either cages or fish ponds. Although aquaculture had advanced this far, larviculture for most fishes is still dependent on live food such as rotifers, copepods, cladocerans, and *Artemia*, especially during the transition from endogenous to exogenous feeding. This is due to the fact that most fish larvae cannot readily assimilate formulated diets during the first days of feeding (Conceição et al., 2010). In addition, fish larvae are believed to be predominantly visual feeders, therefore preferably selecting moving prey items (Conceição et al., 2010).

Among live feed, rotifers (genus *Brachionus*) are ideal for larviculture because of their varied body size, their nutritional quality which can be controlled with commercial enrichment products, and their established culture techniques (Lubzens, 1987; Dhert et al., 2001; Hagiwara et al., 2017). The use of rotifer, *Brachionus plicatilis* species complex (which comprises approximately 15 species; Mills et al. 2017), in larviculture is well established since its first usage as live food in the 1960s. Papakostas et al. (2006) found five species from hatcheries around the world. *Brachionus plicatilis* Muller, *Brachionus koreanus* Hwang, Dahms, Park & Lee and *Brachionus rotundiformis* Tschugunoff, corresponding to L, S, and SS morphotype, repectively (Hagiwara et al., 2007), are widely used, and their biological information is well examined. Production techniques of these species are already established and, due to their varied sizes, culturists can choose the rotifer species to use according to the mouth size of their cultured species, and are given to the fish larvae upon hatching up to 10-20 days after its mouth opening (Lubzens et al., 1987; Conceição et al., 2010). Thereafter, larvae are fed with larger live feed such as *Artemia*, copepods and cladocerans, or artificial formulated diet.

The demand for the ornamental fishes is always high (Lim et al., 2003; Whittington & Chong, 2007). Most (about 90%) of the ornamental fish in the market are freshwater species and are farm-bred, while marine species are predominantly from the wild (Whittington & Chong, 2007). Therefore, the major goal of the aquaculture industry is to reduce collection pressure on wild populations by developing captive culture techniques of marine species (Majoris et al., 2018). At present, larviculture of marine fish species is usually done by using the so-called "green water technique" and feeding with small brachionid rotifers (e.g. *B. rotundiformis* with 150-190 µm in lorica length) from hatching up to 14 days, or by raising the breeders in a fish pond where hatchlings can eat a variety of live food from the environment (Lim & Wong, 1997; Majoris et al., 2018). At commercial scale, ornamental marine fish species with a too small mouth size to ingest *B. rotundiformis* are either not cultured successfully or fed with inert food such as milk powder, egg yolk, and powder feeds (Lim et al., 2003; Hirai et al., 2012). Therefore, there is a high demand for rotifer species smaller than *B. rotundiformis* for the commercial production of ornamental fishes.

Culture of rotifers can either be done intensively or extensively. In intensive culture, rotifers are reared in a highly controlled environment, fed with condensed or concentrated food, and supplied with either aeration or pure oxygen. Under these conditions, culturists can produce as high as 2.1 billion of rotifers per day in 1 m^3 culture volume (Hagiwara et al., 2017). Although this procedure produces high quality and quantity of rotifers, it also entails skills and high costs. On the other hand, in extensive culture, rotifers are grown in a fish pond and animal manures/excreta are supplied to promote plankton productivity (Dahril, 1997; Agbakimi et al., 2017). Animal excreta enter the food web in the pond through direct consumption by phytoplankton. These wastes also serve as source of minerals and organic substrates for heterotrophic microorganisms. Phytoplankton and microorganisms are, in turn, consumed by zooplanktons (including rotifers). In this practice, however, the environmental factors that would affect the growth of rotifers, such as temperature, pH, and ammonia concentration, are difficult to control. In addition, rotifer density in the pond is relatively low, probably due to competition with other zooplankton with the same food or due to predation by other rotifers. Therefore, although extensive aquaculture has lower operating costs and easier management, this method was found to be not effective for mass production of larval fish in terms of labor cost and space.

High population growth, appropriate size, ubiquitous distribution, and ease of culture are among the most important qualities of a rotifer species to be considered as a good candidate for use in commercial hatcheries. Therefore, research efforts are being directed into finding rotifer species with these characteristics.

In this review, we described non-*B. plicatilis* species which are already used in larviculture, in comparison to *B. plicatilis* species complex. Next, we listed some of the species with high potential for usage for larviculture based on their characteristics, life history, and distribution. Third, we discussed some characteristics of rotifers which can affect their predation.

Brachionus plicatilis **sp. complex**

B. plicatilis, *B. koreanus* and *B. rotundiformis*

The euryhaline rotifer *B. plicatilis* species complex, which encompasses around 15 species with varied sizes ranging from 100-400 µm (Mills et al., 2017), is the most common species used in marine fish hatcheries worldwide. Their culture techniques and usage as live food are well known and reviewed by several authors (*e.g.* Dhert et al., 2001; Conceição et al., 2010; Sakakura, 2017; Hagiwara et al., 2017). With several modifications through the years of experimentation, a stable, reliable, economical and continuous culture system which can produce up to 2.1 billion of rotifers in 1 m^3 culture volume on daily basis have been produced (Hagiwara et al., 2017). The highest density obtained for these species complex was 160,000 ind/ml (Yoshimura et al., 2003; Yoshimatsu & Hossain, 2014).

Reproductive characteristics of *B. plicatilis* species complex in comparison to other rotifer species that are currently used in aquaculture are presented in Table 1. Because of changes in taxonomy of this group, S-morphotype species such as *Brachionus koreanus* was recognized as *B. rotundiformis* in some literatures (e.g. Yoshimura et al., 2003).

The importance of the *B. plicatilis* complex in larviculture is difficult to overestimate and reviewed by many authors (*e.g.* Lim et al., 2003; Conceição et al., 2010; Sakakura, 2017).

Non-*Brachionus plicatilis* sp. complex

Species used in larviculture

1) *Brachionus angularis*

Brachionus angularis Gosse is a common freshwater species. Its body size ranges from 85 to 140 mm; tropical species isolated from Kenya and Laos are smaller compared to those isolated from temperate countries (*e.g.* from Europe and China; Ogello et al., 2016; Ogata, 2017). *B. angularis* isolated from Laos has a round-shaped lorica, can reproduce both sexually and asexually, and lorica length of adult egg-carrying females has a size (86.0 ± 4.9 μm), smaller than that of other *B. angularis* and strains in *B. plicatilis* species complex (Ogata et al., 2011). Ogata et al. (2011) found that the optimum culture conditions for this strain include culture temperature between 24 to 27ºC and feeding with 7×10^6 cells/ml of *Chlorella vulgaris* Beyerinck. At these culture conditions, they obtained rotifer density of more 2,000 ind/ml within 10 days. During their experiments, the highest density they obtained for this species was 3,300 ind/ml.

Ogata (2017) used *B. angularis* to culture silver barb, *Hypsibarbus malcolmi* (Smith), a Laotian indigenous cyprinid. The larvae were fed with an increasing number of *B. angularis* at 5-10 ind/ml starting from hatching to day 12, and growth and survival were compared to those without feeding. After 12 days of culture, survival was 100% with food, while none survived in without food treatment. Rotifer-fed larvae also grew from 2.8 mm to 5.8 mm, proving that rotifer supported the growth of silver barb larvae. After confirming that *B. angularis* is useful for rearing *H. malcolmi* larvae, Ogata (2017) conducted another experiment to compare *H. malcolmi* fed with *B. angularis*, *Artemia*, copepods, *Moina* spp., and catfish pellets from 2 days after hatching (2DAH) to 28DAH with *H. malcolmi* fed with mixed natural zooplankton collected from an aquaculture pond. Results showed that the first group and second group have 94% and 6% survival rate on 28DAH, respectively, and there is a large variation in total length of the survivors in the second group, while the first group grew from 2.8 mm to 15.2 mm at 28DAH.

Ogata & Kurokura (2012) tested *B. angularis*, paramecium *Paramecia* sp., and *Artemia* as live food sources for Siamese fighting fish, *Betta splendens* Regan. Larviculture of *B. splendens* is presently done by feeding protozoans. Their results showed that survival (97.5–100%) was high in all fed treatments. The fastest growth rate was observed in larvae fed a combination of rotifer and *Artemia*, wherein growth increased by 282% by 18 DAH relative to 3 DAH. The next fastest growth rate was observed in rotifer-fed larvae and then in paramecia-fed larvae with 158% increase and 54.3% increase in growth, respectively.

In 2016, we had isolated *B. angularis* from a pond in Kegati, Kenya. The size of the lorica (length = 85.6 ± 3.1 µm; width = 75.4 ± 3.6 µm) is slightly smaller to that found in Laos (Ogello et al., 2016). The optimum conditions for culturing this species were at 25°C and fed 2.5×10^6cells/ml *C. vulgaris*. Under these conditions, the net reproductive rate and intrinsic rate of natural increase (*r*) were 8.43 ± 0.24 and 0.74 ± 0.02/ day, respectively. Under mass culture (300 ml total volume) and optimum culture conditions, the highest population of 255.6 ± 12.6 ind/ml was obtained (Ogello et al., 2016). We also found that addition of chicken manure at 2.0 ml/l enhances the population growth of this strain (Ogello & Hagiwara, 2015).

In China, Hu & Xi (2006, 2008) found that different strains of *B. angularis* isolated from different provinces within the country vary in size and life history parameters (generation time, *r*, and life span) and are influenced by food they consumed. Rotifers fed *Scenedesmus obliquu*s (Turpin) Kützing had higher reproduction rates than those fed *Chlorella pyrenoidosa* H. Chick. As is known in *B. plicatilis* species complex (Hagiwara et al., 1995, 2001; Mills et al., 2017), *B. angularis* strains with smaller size show higher population growth even though their net reproduction rates are similar. The *r* and net reproductive rates of these strains fed *S. obliquus* were 0.059-0.115 per hour and 13.38-16.35, respectively.

2) *Brachionus calyciflorus*

Brachionus calyciflorus Pallas is one of the widely studied freshwater rotifer with ubiquitous distribution (Rico-Martinez & Dodson, 1992).

The lorica length of *B. calyciflorus* from different geographic region in China ranges from 187 to 227 µm with an average *r* of 0.84/day at 20-30°C (Xi et al., 2005). The net reproductive rate of the three strains collected from different regions varies according to temperature, and ranging from 10-27 ind/ml (Xi et al., 2005). In Mexico, Rico-Martinez & Dodson (1992) found that the optimum culture conditions of *B. calyciflorus* isolated from a fish pond were at 30°C and fed 10^7 cells/ml *C. vulgari*s. Under these culture conditions, and at a volume of 500 ml, they were able to produce 81,080 rotifers/day. Bennett & Boraas (1988) was able to maintain *B. calyciflorus* in a turbidostat for eight months with maximum specific growth rate of 0.08/h which is equivalent to a doubling time of 8.7h.

Some studies have shown that animal and human excreta can promote *B. calyciflorus* growth. For example, Agbakimi et al. (2017) found that *B. calyciflorus* reared with cow dung and chicken droppings can reach 217 ind/ml after 5 days of culture. Dahril (1997) also showed that *B. calyciflorus* can grow up to 120 ind/ml using low concentrations of human and animal excreta including humans, chicken, duck, quail, horse, and buffalo by promoting the growth of *Chlorella,* which in turn serves as food for *B.*

calyciflorus. Under intensive culture, Park et al. (2001) conducted a batch culture (5-li vessel) experiments on *B. calyciflorus* at 28°C, feeding with freshwater *Chlorella* and supplied with pure oxygen. With these conditions, a maximum density of 19,200 ind/ml was reached, in contrast to 8,600 ind/ml obtained when usual aeration is supplied. They improved their system further by adjusting the pH of the culture water. At pH 7.0 and at 32°C with a continuous oxygen supply, a density of 33,500 ind/ml was obtained (Park et al., 2001).

There are considerable reports on the success and high growth rate of fish larvae when using *B. calyciflorus* as live food. For example, Lim & Wong (1997) showed that Dwarf gourami, *Colisa lalia* larvae (2-12 DAH), have higher growth and survival compared to those fed with egg yolk. At metamorphosis, the overall survival rate of larvae fed rotifers (65.1-74.4%) was about four times of those cultured in an open pond (17.5%). Similarly, Lim & Wong (1997) successfully cultured larvae of Brown discus, *Symphysodon aequifasciata axelrodi* L. P. Schultz using *B. calyciflorus*. Larviculture of Brown discus is usually done by rearing them together with their parents, where the larvae are eating body slime of the parents (called "parental feeding") during the first two weeks of endogenous feeding (Lim & Wong, 1997). Results of their study showed that growth and survival rate of Brown discus fed on rotifers and parental feeding were comparable. Feeding solely with rotifer is advantageous because it eliminates the risk of the larvae to be eaten by the parental fish. The use of *B. calyciflorus* is also reported on zebrafish *Danio rerio* (Aoyama et al., 2015). Zebrafish larviculture was previously done by feeding marine rotifer *B. plicatilis*, which either or both rotifer or fish experience salinity shock, resulting in mortality (Aoyama et al., 2015). Nandini & Sarma (2000) also found that mollies, *Poecilia sphenops* Valenciennes continuously fed on *B. calyciflorus* from day 5 to day 55 of culture. Harzevili et al. (2003) obtained significantly higher survival of *B. calyciflorus*-fed turbot *Lota lota* compared to *Artemia*-fed group. The survival is further enhanced in the presence of "green water" (*Chlorella* sp.). Awaiss et al. (1996) obtained 95.5% survival rate on gudgeon, *Gobio gobio* (Linnaeus) fed with *B. calyciflorus* versus

63.7% on dry diet, with the final weight 15.5 mg versus 10 mg. Awaiss et al. (1996) also successfully used *B. calyciflorus* to feed catfish *Clarias gariepinus* (Burchell) during the first week of larval feeding.

3) *Proales similis*

Proales similis de Beauchamp is one of the common rotifer in saline systems, and so far in many countries including Mexico and Japan. In 2004, our group isolated a *P. similis* in an estuary of Okinawa, Japan. This rotifer is small (body length = 82.7 ± 11 µm; body width = 40 ± 6 µm), which is 38% smaller and 60% narrower than the SS-type rotifer, *B. rotundiformis* (Wullur et al. 2009). We also found that this species is also illoricate, has high population growth rate, and has nutritional value that can be manipulated just like other rotifer species (Wullur et al., 2009; Hagiwara et al., 2014). Since we found that this species is a promising species for larviculture, we conducted experiments to mass culture and fish feeding experiments using this species.

Wullur et al. (2009) found a female *P. similis* that can produce 4.3-7.8 offspring during its 2.9-3.4 day reproductive period. *P. similis* grew well at temperatures 25 to 35°C, salinities between 2 to 15 ppt and both *N. oculata* and *C. vulgaris* as feed. Under above conditions, the *r* is ranging between 0.68 to 0.81/day, and a density of 250 to 1030 ind/ml can be obtained. In mass culture, starting from 25 ind/ml, the density can reach up to 2,400 ind/ml, with an average *r* of 0.42/day after 11 days was obtained.

We also observed that *P. similis* tends to stay at the bottom of the culture container. We hypothesized that if we increase the culture surface area of the container, then we can obtain more rotifers. Two containers, one with a total surface area of 2,240 cm^2 and the other with 507 cm^2 were tested. From an initial density of 1 ind/ml, we obtained a density of 2,840 ind/ml and 717 ind/ml on the 7th day of culture from 2,240 cm^2 and 507 cm^2 surface area, respectively (Hagiwara et al., personal communication). We are currently innovating a rotifer apartment-like culture container to provide wider spaces for *P. similis* to graze.

We also conducted an experiment to determine if bacteria coming from decomposing animal wastes could sustain *P. similis* culture as other rotifer species. Our results showed that addition of fish wastes (0.75 g/ml) is beneficial to *P. similis*. At initial stocking density of 53 ind/ml, a density as high 1,605 ± 45 ind/ml could be obtained on day 10 (Kagali et al., 2018). We hypothesized that *P. similis* uses micro-aggregates of organic materials present in the decomposing fish wastes to enhance probiotic bacterial bloom. Indeed, Le et al. (2017) showed that bacterial community is important in the proliferation of *P. similis.* The population density of *P. similis* with the addition of live mixture of bacteria was 755% higher than those fed with probionts in the presence of antibiotic (Le et al., 2017). Although *P. similis* can thrive in the presence of some species of bacteria, the presence of protozoa in the culture water is detrimental to the culture (Hagiwara et al., personal communication). Therefore, it is necessary to provide clean and protozoa-free culture water to *P. similis*.

Several experiments to determine if aeration is necessary for the proliferation of *P. similis* were also conducted. Our studies showed that culture of *P. similis* starting from a 1 ind/ml can exponentially increase and be stable for up to 13 days, with a peak density of 4,046 ± 47 ind/ml even without aeration; and a similar density with aeration (Hagiwara et al., personal communication).

Unlike the Japanese strain, the Mexican strain of *P. similis* is more resilient to high salinities. Reyes et al. (2017) found that *P. similis* isolated from a fish pond in Mexico can thrive at 5-35 ppt, with an *r* ranging from 0.46 to 0.51/day, and a duplication time ranging from 1.36 to 1.51 days. Although the maximum density at 35 ppt (1,703 ind/ml), was lower than that at 5-25 ppt (maximum values were between 2,488 to 2,560 ind/ml).

We also successfully tested the usefulness of *P. similis* to fish larvae with very small mouth gape including grouper, angelfish, and humphead wrasse as well as fish with complicated digested system such as eel (Wullur et al., 2009; Wullur et al., 2011; Hagiwara et al., 2014). So far, *P. similis* is one of the most promising smallest rotifer that can be used in culturing larvae that cannot accept SS-type rotifer.

Potential rotifer species for larviculture

In this review, we listed some of the non-*B. plicatilis* species complex with high potential for usage for larviculture based on their characteristics, life history and distribution. The summary of the life history of these rotifer species is presented in Table 2.

Chigbu & Suchar (2006) evaluated the possibility of culturing *Colurella dicentra* (Gosse) isolated from a Mississippi Gulf Coast estuary. The average lorica length of this species is 93 mm and a width of 49 mm. They conducted experiments to determine the effects of salinity (10–47 ppt) on its population growth rate, fed with *N.oculata* at a density of 100,000 cells/ml. The culture duration is 15 days. Their results showed that *C. dicentra* survived in 10–47ppt. The best salinity to cultivate this species is at 15ppt, with an *r* ranging from 0.37–0.42/day, and the highest density was 259 ± 70 ind/ml.

Another species potential for larviculture is *Keratella* sp. Lee et al. (2013) investigated the optimum salinity and temperature conditions for the mass culture of *Keratella* sp. The maximum density of 1,007 ind/ml was observed in freshwater or 0‰. Also the highest number of offspring per female (10.2) and lifespan of the female (10.7 days) were obtained at 0‰, but were not significantly different at 5‰. In their temperature experiments (16-32°C), the highest maximum density (1,766 ind/ml) was observed at 24°C. The number of offspring per female significantly increased with increasing temperature, and the highest number of offspring per female was 10.4 individual. At 24°C, the lifespan of female increased with decreasing temperature, with the longest lifespan lasting 12.8 days.

With the aim of using rotifer in larval rearing of catfish in Nigeria, Ajah (2010) conducted a mass culture experiment on local rotifer species, the *Brachionus quadridentatus* Hermann. Result of his study showed that best food for this species is *Scenedesmus quadricauda* (Turpin). Using 3m^3 concrete tank, he was able to maintain the culture for two years, with the density of 176,000 ind/l. The doubling time is at the average of 20 h.

Oltra et al. (2000) also conducted series of studies to mass culture a marine rotifer *Synchaeta cecilia valentina* Oltra & Todolí, a species ubiquitously found in Spain. Under culture conditions of 24ºC, 20-37 ppt, and fed *Tetraselmis* sp., this species can reach up to 4,800 ind/l. The fatty acid content of this species is similar to those of *B. plicatilis* when given *Tetraselmis* species (both *Tetraselmis* sp., and *Tetraselmis chuii* Butcher) as food at a concentration of 5.55 µg/ml dry weight.

Farhadian et al. (2013) studied the population growth and production of the freshwater rotifer, *Euchlanis dilatata* Ehrenberg fed different microalgal food with the addition of alfalfa (*Medicago* spp.) meal. The highest density they attained with this species is 255 ind/ml in treatment fed with *Scenedesmus quadricauda* and alfalfa meal. The mean population growth rate is also high (0.58/d) in this treatment which is not significantly different from those fed with *C. vulgaris* (0.59/d).

Factors affecting predation of rotifers

Although rotifers are superior among live food, rotifers possess defensive structures *e.g.* long spines, and have the capability to adjust their morphology and behavior to prevent predation (Gilbert, 2014; Yin et al., 2017; Zhang et al., 2017 Xue et al., 2017). For example, Yin et al. (2017) found that *B. angularis* increased lorica thickness and enhanced lorica hardness in the presence of the predator *Asplanchna brightwellii* Gosse, while *B. calyciflorus* developed longer posterolateral spines and increased in body size within the presence of the same predator. Gilbert (2014) found that *Brachionus variabilis* Hempel when cultured with *Asplanchna girodi* Guerne have larger (13%) lorica, longer (30-40%) anterior spines, and longer (150%) posterior spine. *B. calyciflorus*, which originated from different environments in China, developed stable long posterior lateral spines and smaller body size in the presence of predators including fish, copepods and *Asplanchna* (Xue et al., 2017). Rotifer *Keratella cochlearis* (Gosse) is somewhat special in which they are known to have bi-directional change in spine length, depending on the size of the predator (Zhang et al., 2017). Zhang et al. (2017) both on laboratory and field studies showed that, in the presence of larger predators, *K. cochlearis* shortened or reduced their spine length and then elongated it in the presence of small-sized predators. In the case of fish as the predator, our group found that the swimming speed of rotifer *B. plicatilis* is significantly faster (0.49 vs. 0.58 mm/sec) when cultured in a culture medium with the seven band grouper *Epinephelus semtemfasciatus* (Thunberg) as the predator. In addition, Alanis et al. (2009) found that the larvae of red-eyed tetra, *Moenkhausia sanctaefilomenae* (Steindachner) prefers to prey on *Brachionus rubens* (Ehrenberg) and *B. calyciflorus* which has shorter spines (about 10 µm) than *Brachionus havanaensis* Rousselet and *Brachionus patulus* Varga, which have longer spines.

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Table 1. Characteristics of rotifer species commonly used in larviculture

Species	Size of egg bearing females (μm)	Reproductive characteristics	Reference (Food, temperature, salinity)
Brachionus plicatilis species complex			
Brachionus plicatilis	Lorica length = 325 ± 24	*r* = 0.29 - 0.31 Highest density = 425 ind/ml	Hagiwara et al. (1993, 2007) (*Nannochloropsis oculata* and baker's yeast, 18-21 ºC, 10-15 ppt)
Brachionus koreanus	Lorica length = 192 - 213	*r* = 0.57 - 0.64 Highest density = 950 ind/ml	Hagiwara et al. (1989); Hwang et al. (2013) (*Tetraselmis tetrathele*, 25.5 - 34 ºC, 8 -32 ppt)
		Highest density = 160,000 ind/ml	Yoshimura et al. (2003) ; Yoshimatsu & Hossain (2014) (*Chlorella vulgaris*, 32 ºC, 33 - 35 ppt)
Brachionus rotundiformis	Lorica length = 187 ± 5	*r* = 0.23 - 1.57 Highest density =3,500 ind/ml	Hagiwara et al. (1995a, b) (*N. oculata*, 25 - 35 ºC, 11 - 34 ppt)
Brachionus angularis	Lorica length = 86.0 ± 4.9	Highest density =3,500 ind/ml	Ogata et al., 2011; Ogata, 2017 (*C. vulgaris*, 24 - 27 ºC)
	Lorica length = 85.6 ± 3.1	*r* = 0.41-0.74 Ro = 4.7- 6.3 Highest density = 256 ind/ml	Ogello et al., 2016 (*C. vulgaris*, 20-30 ºC)
	Body size = 2.7 - 4.8 (x10^5 μm^3)	*r* (/h) = 0.06-0.12 Ro = 13.4 - 16.4	Hu & Xi, 2006, 2008 (*Scenedesmus obliquus*, 25 ºC)

Brachionus. calyciflorus	Lorica length = 231	*r* = 1.04 Highest density = 33,500 ind/ml	Park et al., 2001; (*C. vulgaris*, 32 ºC)
		r =0.9 - 1.7 Ro = 22	Xi et al., 2005 (*S. obliquus*, 25 ºC)
Proales similis	Body length = 83 ± 11	*r* = 0.63 - 0.93 Highest density = 4,046 ind/ml	Wullur et al., 2009; (*N. oculata*, *C. vulgaris*, 25－35 ºC , 2-25 ppt)
		r =0.46 - 0.52 Highest density = 1,703-2,560 ind/ml	Reyes et al., 2017 (*N. oculata*, 25 ºC, 5-35 ppt)
		Highest density = 1,605 ind/ml	Kagali et al., 2018 (Fish waste diet, 26 ºC, 8 ppt,)

r (/day) – intrinsic rate of natural increase; Ro-net reproductive rate

Table 2. Characteristics of rotifer species with potentials for use in larviculture

Species	Size (µm)	Reproductive characteristics	Reference (Food, temperature, salinity)
Colurella dicentra	Lorica length = 93µm	*r* = 0.37-0.42 Highest density = 259 ± 70 ind/ml	Chigbu & Suchar, 2006 (*N. oculata*, 21 - 24ºC, 15 ppt)
Keratella sp.		*r* = 0.75 Ro = 10.4 Highest density = 1,766 ind/ml	Lee et al., 2013 (*Tetraselmis suecica*, 24 ºC, 0 - 34 ppt)
Brachionus quadridentatus		Doubling time = 20h Highest density = 17.6 ind/ml	Ajah, 2010 (*Eudorina elegans*, 28 ºC, 0 ppt)
Syncheta cecilia valentina		*r* = 1.0 Ro = 11.7	Oltra et al., 2000 (*Tetraselmis* sp., 20ºC, 25 ppt)
Euchlanis dilatata		*r* = 0.58-0.59 Highest density = 255 ind/ml	Farhadian et al., 2013 (*Scenedesmus quadricauda*,and alfalfa meal, 25 ºC, 0 ppt)

r – intrinsic rate of natural increase; Ro-net reproductive rate