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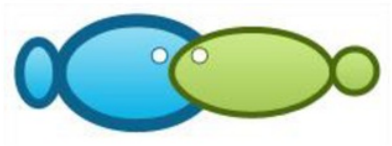

Assessment of different minute zooplankton in the larval rearing of rusty angelfish *Centropyge ferrugata*

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Abstract. Angelfishes of the genus *Centropyge* are commercially valuable aquarium fish. Although success in domesticating the broodstock of angelfishes is already reported, larval rearing remains a failure because their larvae require very small live food. Recognizing this demand, we assess the acceptability of four minute zooplankton including rotifers *Proales similis*, *Keratella sinensis*, *Brachionus rotundiformis* (SS-type), and nauplii of copepod *Paracyclopina nana* as feed for rusty angelfish *Centropyge ferrugata* larvae. The average size of the four zooplankton we used ranged from 80 to 160 μm. Results showed that feeding incidence on *P. similis* on the first day of feeding (3 days after hatching, 3 DAH) was 40%, and as high as 60% on day 6 after hatching (6 DAH), while it was 0 to 20% on other zooplankton tested. Larvae in the control group (without feeding) did not survive on 5 DAH. Standard length of the larvae in the two trials was not significantly different among treatments, but survival was significantly higher in *P. similis* (19 to 38%) than other zooplanktons (0 to10%). Results of this study indicate the usefulness of *P. similis* as starter food for *C. ferrugata* larvae.
Key Words: *Proales similis*, *Brachionus rotundiformis*, rotifer, live food, copepod.

Introduction. Angelfishes of the genus *Centropyge* are among the highly prized and often sought coral reef fish because of their prominent coloration (Olivotto et al 2006). At present, the main source of angelfishes for aquarium rearing is from the wild (Wood 2001), especially the coral reef ecosystem where these fishes reside. However, collection from the wild is often unsustainable and stresses the coral reef ecosystems (Rubec 1988; Jones & Steven 1997; Jones et al 1999). Furthermore, the declining condition of coral reefs has caused increasing regulation on any activities that impact the reef environment including the restriction or even banning of ornamental fish collection activities which includes angelfishes (Rubec 1988; Wood 2001). These problems have created compelling need to develop an alternative mariculture technology to wild collection in order to meet the market demand and possibly restore degraded wild population (Laidley et al 2008). Despite much success in captive maturation and spawning of angelfishes in the last three decades (Suzuki et al 1979; Arai 1994; Hioki et al 1990; Olivotto et al 2006; Leu et al 2009), massive mortality related to poor initial feeding of the larvae remain a bottleneck for their successful captive production (Olivotto et al 2006; Leu et al 2009).

Larvae of angelfishes require small food due to their small mouth size (Olivotto et al 2006; Laidley et al 2008; Leu et al 2009). SS-type rotifer *Brachionus rotundiformis* (lorica length 90 to 240 μm) which is a common starter food for rearing marine fish with small mouth size (Lubzens et al 1989; Hagiwara et al 2001; Ogello et al 2018) is ineffective in rearing angelfish larvae (Olivotto et al 2006; Laidley et al 2008; Leu et al 2009). Efforts had been geared to identify appropriate starter food organisms for angelfish larvae including the use of copepod nauplii (mean body length <100 μm, Olivotto et al 2006; Laidley et al 2008), dinoflagellate (*Gonyaulax* sp., 65 μm in length and 38 μm in width, Leu et al 2009), sea urchin eggs, ciliates or oyster trochopores (mean diameter

<70 μm, Laidley et al 2008). The use of copepods showed promising results on growth and survival of angelfish larvae (Otto et al 2006; Laidley et al 2008), but since most the tested copepod species are difficult to culture at high density (Schipp et al 1999; McKinnon et al 2003; Shields et al 2005), their use as starter food is still limited to experimental scale only. The use of other food organisms including dinoflagellates, sea urchin, ciliates or oyster trochopores were reported to have modest success (Laidley et al 2008) or no beneficial effect on the larval growth and survival (Leu et al 2009). Although their minute size could fit the mouth of the larvae, they are nutritionally inferior and could not meet the requirement of the larvae for growth and survival. Moreover, nutritional enrichment of these food organisms is difficult because at the stage where their size fits the mouth of the angelfish larvae, the organisms are not yet on their feeding stage (Hoff & Snell 1987).

Recognizing the demand of smaller starter food, we conducted two feeding trials on rusty angelfish (*Centropyge ferrugata*) using four-minute zooplankton including rotifers *Proales similis*, *Keratella sinensis*, SS-type *Brachionus rotundiformis,* and nauplii of a copepod *Paracyclopina nana*. These zooplanktons were selected mainly because of their small size and easiness of culture.

Material and Method

Zooplankton food. Zooplanktons used in the present study were collected from various habitats around the world. The rotifer *P. similis* was collected in Ishigaki island, Okinawa, Japan (Wullur et al 2009, 2011, 2013), *B. rotundiformis* in Bali island, Indonesia (Hagiwara et al 1995; Wullur et al 2013), *K. sinensis* in South-Korea (J.C. Park, Kangnung National University, South-Korea; Wullur et al 2013) and copepod *P. nana* in Hwajinpo salt lake, Gangwondo, South-Korea (Lee et al 2006; Wullur et al 2013).

All zooplanktons were cultured in 2-5 L culture containers using diluted seawater (25 ppt) at 25°C, adapting the semi-continuous culture method and feed with super fresh *Chlorella vulgaris* V-12® (Chlorella Industry Co. Ltd., Fukuoka, Japan). Our previous study showed that by feeding super fresh *C. vulgaris* V-12 to *P. similis*, nutritional value greatly increased (Wullur et al 2011), and can be used to feed directly to the fish larvae. The population growth of the cultured zooplanktons was monitored daily by taking aliquot samples from each culture tank and counting under a stereomicroscope. The density was maintained at 100 to 300 ind/mL for *P. similis*, *B. rotundiformis* and *K. sinensis*, and at 5 to 10 ind/mL for *P. nana* by harvesting certain culture volume of the culture and replenishing with diluted seawater depending on their density. The zooplanktons were harvested using plankton net (mesh size = 10 to 45 μm mesh size depending on body size of the zooplankton). Nauplii of *P. nana* were obtained by separating them from the mother and copepodids using a 100 μm net.

Feeding experiment. Feeding experiment was conducted in an aquarium filled with 2.5 L filtered natural seawater (32 ppt) at a temperature-controlled (25°C) and well lighted (500-600 lux) room. The room was lighted all day (24L:0D) and the aquarium was provided with slow (5 mL/min) aeration. Two feeding trials were conducted.

In both trials, fertilized eggs of naturally spawned *C. ferrugata* were obtained from a private hatchery in Kagawa, Japan. In the first trial, newly hatched larvae were stocked at 200 larvae/aquarium and the water temperature was maintained at 25°C, while in the second trial, the larvae were stocked at 50 larvae/aquarium and the temperature was maintained at 28°C.

In our preliminary experiments, we knew that first feeding of C. *ferrugata* occurred on day 3 after hatching (3 DAH). Thus, during the experiment, zooplankton foods were added in each aquarium on 3 DAH. *P. similis, K. sinensis*, and *B. rotundiformis* were added in each aquarium at 20 ind/mL, while *P. nana* at 10 ind/mL. We prepared another aquarium stocked with 50 larvae, but no food was added, as our control. Super fresh *C. vulgaris* V-12® was added into the culture water as background algae.

Starting from 3 DAH, five larvae were sampled from each aquarium, anesthetized with 0.01% MS 222 (Tricaine; Sigma Chemical Co., St Louis, MO, USA), fixed with 5% formaldehyde, and measured under a digital microscope (VH-6300, Keyence Corp., Japan). Feeding incidence (percent of larvae with rotifers inside the gut) and feeding amount (number of rotifers in larval gut) were investigated by dissecting the larval gut under a stereomicroscope. Feeding amount was determined by counting the trophi of digested rotifers as well as number of undigested body of rotifers or copepod (Akazawa et al 2008). Percent survival of the larvae in all treatments was estimated by counting the number of surviving larvae at the end of the experiment.

Results. The body length and width of the four zooplankton used in the present study are shown in Table 1. Among the zooplankton, mean body size of *P. similis* (90.9±10.9 μm in length and 44.4±6.8 μm in width) was the smallest, while *B. rotundiformis* was the largest (137.5±15.7 μm in length and 106.9±15.6 μm in width). Mouth size of *C. ferrugata* larvae on 3 DAH (when the mouth opens) was 160±16 μm.

Table 1

Body length and width (mean±SD) of the zooplankton used in present study (each n=50)

Zooplankton species	*Body dimension (μm)*	
	Length	*Width*
Proales similis	90.9±10.9	44.4±6.8
Keratella sinensis	107.3±10.2	62.3±7.5
Brachionus rotundiformis	137.5±15.7	106.9±15.6
Paracyclopina nana	113.5±46.9	61.8±11.8

Feeding incidence of the larvae in the first trial occurred from 4 DAH, with 60% feeding incidence (food intake, 1.6 ind/larvae) on *P. similis* while it was 0 to 20% (food intake, 0 to 0.2 ind/larvae) for those fed with other zooplankton. The larvae kept ingesting *P. similis* (feeding incidence 40%, feeding amount 2.2 ind/larvae) and *K. sinensis* (feeding incidence 20%, feeding amount 0 to 0.2 ind/larvae) on the next day (5 DAH) while no ingestion by the larvae was observed for *B. rotundiformis* and *P. nana* (Figure 1).

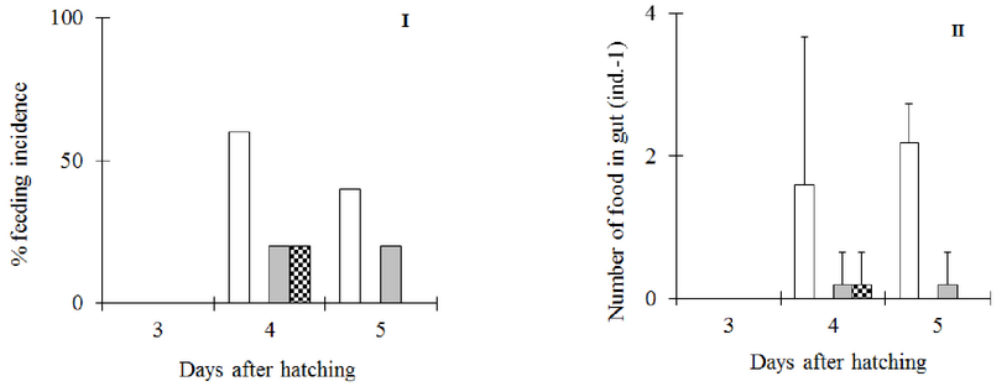

Figure 1. Feeding incidence (mean%±SD, n=3) (I) and feeding amount (mean±SD, n=3) (II) of *Centropyge ferrugata* larvae in first trial fed with *Proales similis* (□), *Brachionus rotundiformis* (■), *Keratella sinensis* (▢) and nauplii or the copepod *Paracyclopina nana* (▩).

In the second trial, we observed the first feeding on 3 DAH (feeding incidence = 40%, feeding amount = 0.8 ind/mL) in *P. similis*-fed treatment and none in other treatments. From 4 DAH to 6 DAH, 20 to 60% feeding incidence was observed on *P. similis* while it was 0 to 20% on other zooplankton (Figure 2).

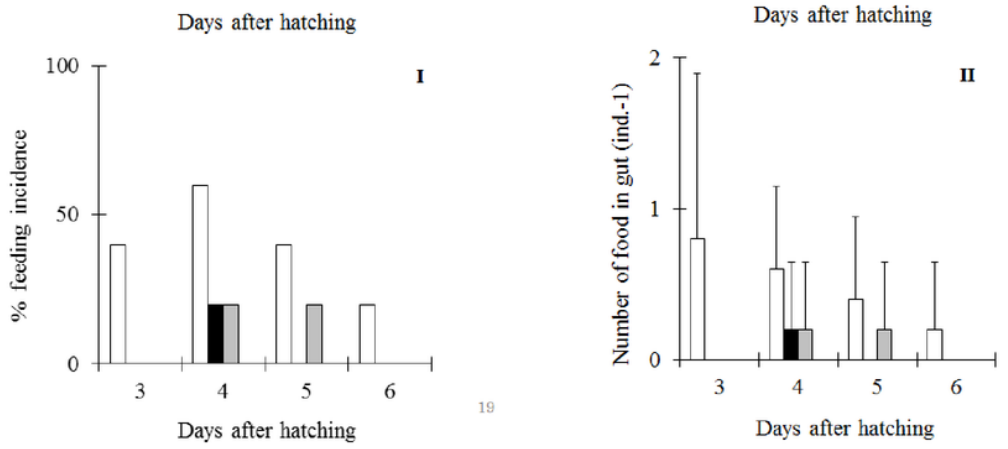

Figure 2. Feeding incidence (mean%±SD, n=3) (I) and feeding amount (mean±SD, n=3) (II) of *Centropyge ferrugata* larvae in second trial fed with *Proales similis* (□), *Brachionus rotundiformis* (■), *Keratella sinensis* (▢) and nauplii or the copepod *Paracyclopina nana* (▣).

Standard length of larvae fed with *P. similis* (2.2 ±0.0 μm), *K. sinensis* (2.1±0.1 μm), *B. rotundiformis* (2.0±0.2 μm), *P. nana* (2.1±0.1 μm) was not significantly different among treatments, but survival was significantly higher on *P. similis* (18.5%) than other zooplankton (6 to 11.5%) (Tukey-Kramer test, $p<0.05$) (Figure 3-I). Larvae in the control group (no feeding) did not survive on 5 DAH.

No significant difference was observed in the standard length of larvae in all treatments. The standard length of larvae fed with *P. similis* was 2.2±0.1 μm, 2.1±0.1 μm for those fed with *K. sinensis* and 2.0±0.1 μm with *B. rotundiformis*. Survival was significantly higher on *P. similis* (38%) than other zooplankton (0 to10%) (Tukey-Kramer test, $p<0.05$) (Figure 3-II). No larvae survived in the control group (no feeding) on 6 DAH.

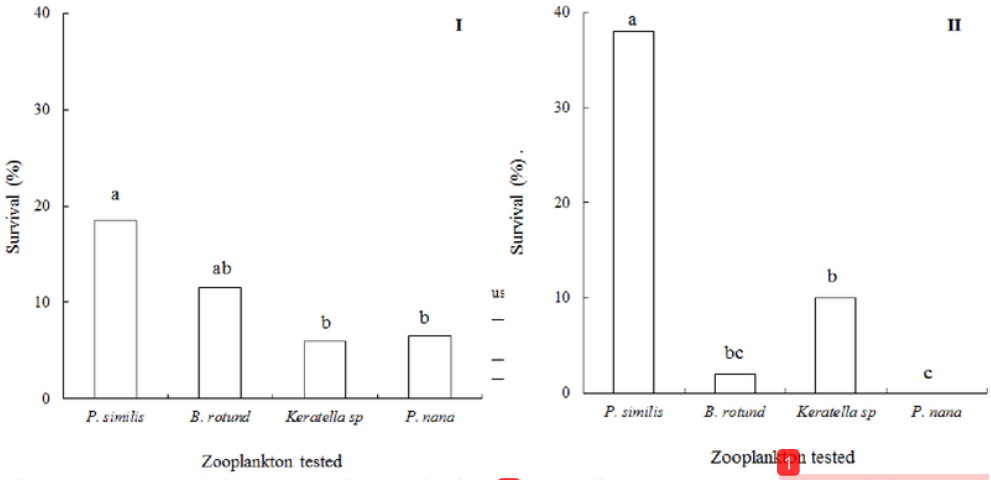

Figure 3. Survival (mean%±SD, n=1) of rusty angelfish *Centropyge ferrugata* larvae in the first (I) and second (II) trial fed with *Proales similis*, *Brachionus rotundiformis*, *Keratella sinensis* and nauplii of copepod *Paracyclopina nana*. Different alphabetical letters indicates significant differences, Tukey Kramer test, $p<0.05$, a>b>c.

Discussion. The first characteristic to consider in choosing food organism for small-mouth fish larvae is the body size of their prey (Shirota 1970; Fernandez-Diaz et al 1994; Cunha & Planas 1999; Olivotto et al 2006; Yufera & Darias 2007). The mouth size of *C. ferrugata* larvae in the present study was 160±16 μm at first opening. It has been known that most fish larvae could prey on food 20 to 70% smaller than their mouth size (Shirota 1970; Fernandez-Diaz et al 1994; Cunha & Planas 1999; Yufera & Darias 2007). Considering that *C. ferrugata* used in this study has a mouth size of 160±16 μm, this suggests that they require food with size from 32 to 112 μm. *P. similis* (mean±SD; 90.9±10.9 μm in length and 44.4±6.8 μm in width) and *K. sinensis* (107.3±10.2 μm in length and 62.3±11.5 μm in width) are within the required size, but those of *B. rotundiformis* (137.5±15.7 μm in length and 106.9±15.6 μm in width) and nauplii of *P. nana* (113.5±46.9 μm and 61.8±11.8 μm) slightly exceed the requirement.

At first feeding (3 DAH) we found that *C. ferrugata* larvae could ingest only *P. similis* (Figure 1) and occurred only when the larvae were reared at higher temperature (28°C, first run), and not at 25°C. We hypothesized that in addition to the size of food, environmental factors such as temperature also affect the initial feeding of *C. ferrugata* larvae. Furthermore, larvae on 4, 5 and 6 DAH showed 20 to 60% feeding incidence on *P. similis*, while it was less than 20% for those larvae in other treatments indicating the potential of *P. similis* to be used as starter food for angelfish larvae. Less feeding incidence by the larvae in the treatments of *Keratella* sp., *B. rotundiformis* and copepod *P. nana* nauplii could be due to the less proportion of food with suitable size for larvae available in the water column affecting the encounter rate with the larvae and the prey.

Past feeding trials on angelfish larvae used bigger tanks of 150 L or more (Olivotto et al 2006; Leu et al 2009), while we used small (2.5 L) aquaria and with high stocking density (50 to 200 larvae), and also high food density (10 to 20 ind/mL). With these rearing conditions, water quality is difficult to maintain for an extended time which was the reason why we conducted the experiment for a short period by counting the survival on 5 or 6 DAH where the larvae stocked without feeding in two trials showed zero survival. On these days, growth of the larvae in the two trials was not significantly different among treatments but survival was significantly higher on larvae fed *P. similis* (18.5 to 38%) than those fed with other zooplankton (0 to 11.5%). As survival during the early larval stage depends on various factors including feeding, environmental requirements or age at which the survival were recorded (Yufera & Darias 2007), it is suggested that angelfish larvae to be reared at suitable environmental condition using *P. similis* as food source so that survival of the larvae could be enhanced and prolonged. Higher survival of angelfish larvae in the treatment of *P. similis* correlated to the feeding of the larvae in this treatment during the experiment.

The result of the present study could serve as a starting point for successful larval rearing of angelfish and other fish larvae with small mouth size including humphead wrasse (*Cheilinus undulatus*) by providing *P. similis* as starter food. Besides their small size, more advantages of *P. similis* is that it can be mass propagated and nutritionally enriched (Wullur et al 2009, 2011; Hirai et al 2012; Hagiwara et al 2014). These features are in contrast with those food organisms that have been previously evaluated for their potentials as starter food for angelfish larvae including copepod naupliar (Olivotto et al 2006; Laidley et al 2008), dinoflagellates (Leu et al 2009), sea urchin eggs, ciliates or oyster trochophores (Laidley et al 2008), which are difficult to mass culture (Schipp et al 1999; McKinnon et al 2003; Shields et al 2005) or nutritionally enrich (Hoff & Snell 1987).

Conclusions. Our results demonstrate the usefulness of *P. similis* as a starter food for *C. ferrugata* larvae during the onset of first feeding. The larvae effectively consumed *P. similis* and were able to use nutritional benefits of the rotifer to support their survival. The present results can be a starting point for successful larval rearing of marine fish species with small mouth size at first feeding by providing *P. similis* as starter food.

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