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BODY SIZE, POPULATION GROWTH AND LARVAL INGESTION OF A MINUTE EURYHALINE ROTIFER *COLURELLA* SP ISOLATED FROM AN INDONESIAN ESTUARY

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Introduction

Current rearing procedures for marine finfish larvae involve feeding the rotifer *Brachionus rotundiformis* during the initial development stages. However, *B. rotundiformis* is regarded as too large an initial feed type for larvae of several tropical marine fishes including groupers (Okumura, 1997; Knuckey et al., 2004), Napoleon wrasse *Cheilinus undulatus* (Slamet and Hutapea, 2004) and rabbitfish *Siganus* spp. (Juario et al., 1985). For grouper larvae, even when *B. rotundiformis* is used as the initial feed, massive mortality related to poor feeding is commonly observed. The small larval mouth size is widely suspected as a cause of this mortality (Okumura, 1997; Wullur et al., 2009; 2011; Hagiwara et al., 2014). This study investigated the potential use of a minute rotifer *Colurella* sp., isolated from an estuary in North Sulawesi, Indonesia, as a starter food for small-mouthed marine fish larvae.

Materials and methods

A species of minute rotifer, *Colurella* sp., was isolated from an estuary in Mangket, North Minahasa, North Sulawesi, Indonesia, in July 2013 by using a 45-µm mesh plankton net. Salinities at the time of collections were 2-5ppt and the rotifers were gradually acclimatized to higher salinity (20ppt) in the laboratory. Clonal cultures of the rotifers were maintained by feeding the rotifers with the microalga *Nannochloropsis oculata*.

To measure body size of the rotifer, five adult females were cultured in conditions similar to the stock culture, i.e salinity 20ppt, temperature 25°C and *N. oculata* density 1×10^{7}cells.ml^{-1}. About 60 adult females of the rotifer were obtained from the culture and were measured for their lorica length and width under a digital microscope at 400–1000× magnification, after they were immobi-

lized with 5% formalin. As a comparison, the local strain of rotifer *B. rotundiformis* cultured in similar conditions was measured for lorica length and width.

Population growth of *Colurella* sp. was evaluated under four different densities of the microalga *N. oculata*: 3×10^6, 6×10^6, 9×10^6, and 12×10^6cells.ml^{-1} in triplicates. The rotifer was cultured in 24-well polystyrene plates (Iwaki, Japan) containing 1 ml seawater at salinity 20ppt and the plates were placed in a controlled temperature room at 25°C. Five adult rotifers were transferred to each well and the initial day was designated day 0. Observation was made daily by counting the number of rotifers while transferring the rotifer individuals to new wells containing fresh culture medium.

A feeding experiment to investigate whether fish larvae ingested the rotifer was conducted using humpback grouper (*Cromileptes altivelis*) larvae fed *Colurella* sp. The grouper larvae were stocked at a density of about 1 ind.l^{-1} in 200-l fibreglass tanks. Mouth opening of the larvae occurred at 3 days post hatching (dph), at which time rotifers were added to the rearing tanks at a density of 10ind.ml^{-1}. The distribution of rotifers inside larval rearing tanks was observed for 12 hours from 0700 to 1900. Ingestion by the larvae of the rotifer was observed by selecting 5 larvae from the rearing tanks and viewing the gut contents of the larvae using a microscope.

Results and discussion

The body length of the minute rotifer *Colurella* sp. ranged from 82.8 to 103.2µm with a mean ± standard deviation of 96.0±3.81µm. The mean body length of *Colurella* sp. was significantly less than the mean body length of rotifer *B. rotundiformis* (175.3±9.2µm) (t-test, $p<0.05$). *Colurella* sp. body width (range 46.8-61.7µm, mean ± standard deviation 53.6±3.1µm) was significantly narrower than the mean body width of *B. rotundiformis* (123.5±7.7µm; t-test, $p<0.05$).

The body length and width of rotifer *Colurella* sp. cultured in these experiments was similar to that of the rotifer *C. dicentra* (length 93µm, width 49µm; Suchar and Chigbu, 2006), but slightly larger than the rotifer *Proales similis* (length 83±11µm, width 40±11µm) (Wullur et. al. 2009; 2011; Hagiwara et al., 2014). *Proales similis* has been successfully trialled as a live feed for initial feeding of small-mouthed fish larvae (Wullur et al., 2011; Hagiwara et al., 2014).

Rotifer *Colurella* sp showed different responses to growth in different densities of the microalga *N. oculata*. *Colurella* sp. cultured in *N. oculata* at 6×10^6cells.ml^{-1} attained maximum density (on day 14) faster than the other three microalgal densities used (Table I). This microalgal density also provided the highest rotifer density of the four treatments (Table I). These results suggest that 6×10^6 cells.ml^{-1} is the optimal microalgal density for *Colurella* sp. reared on

N. oculata, and that higher microalgal densities do not sustain higher densities of rotifers.

Table 1. Peak densities of rotifer *Colurella* sp. cultured using different densities of the microalga *N. oculata*, and the day of culture on which that peak density occurred.

***N. oculata* density (cells.ml^{-1})**	**Peak rotifer density**	
	Days of culture	**Rotifer density (ind.ml^{-1}) mean ± standard deviation**
3×10^6	Day 26	646.2 ± 85.4
6×10^6	Day 14	774.2 ± 167.0
9×10^6	Day 18	655.6 ± 139.4
12×10^6	Day 34	560.5 ± 58.0

After stocking in 200-l larval rearing tanks, *Colurella* sp. were distributed mainly towards the bottom of the tank (44%), with 28% of rotifers towards the top of the water column and 28% in the middle of the tank. Humpback grouper larvae ingested rotifer *Colurella* sp. from 4dph. Feeding incidence of the grouper larvae was 60% and the number of rotifers in the gut of the sampled larvae range from 2 to 8 individual rotifers.

In summary, the results of the present study shows that rotifer *Colurella* sp. has good potential to serve as an initial live feed for marine finfish larvae. It has a smaller body size than *B. rotundiformis* (96µm in length and 54µm in width) and has the capability to be cultured at high density (> 500ind.ml^{-1}). The ability of humpback grouper larvae to ingest the rotifer makes it potentially useful for successful rearing of small-mouthed fish larvae.

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