Low Water Pollution Rearing by means of Polyculture of Larvae of Kuruma Prawn *Penaeus japonicus* with a Sea Lettuce *Ulva pertusa*

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In recent years, seed production of finfish and crustaceans has been widely conducted. In this type of seed production, larval rearing is usually performed under running water at a turnover rate of a few times a day, or exchanged periodically with new water to maintain good water quality.^{1–3} In this manner, pollution of the rearing water by excreted matter from the larvae is unavoidable if water quality is not managed. Generally, in the natural ecosystem, pollutional excreted matters such as feces and urine of animals are absorbed directly or indirectly by photosynthetic organisms in the material cycle to maintain a clean environment.⁴ Prevention of the rearing water pollution of kuruma prawn *Penaeus japonicus* larvae by a sea lettuce *Ulva pertusa*, and its influence on the growth of the larvae, were tested in this experiment.

The sea lettuce *U. pertusa* grows throughout the year in Ohmura Bay according to Migita.⁵ The thalli of *U. pertusa* were preliminarily cultured in 30 l polycarbonate tanks containing sea water enriched with PES medium.⁶ The tanks were located by a window in a laboratory. Aeration was provided at 1 l/min. The thalli were cut into smaller sizes and cultured in the new medium when they grew. When the prawn larvae rearing was initiated, circular disks of 1.8 cm in diameter of *U. pertusa* were used. One glass aquarium of 60 l volume for larval rearing contained 250 disks.

Three treatments were prepared for larval rearing of the kuruma prawn, that is, 1) larvae with *U. pertusa* thalli disks, 2) larvae with vinyl membrane disks, and 3) larvae alone as control. In the second treatment, 250 circular vinyl membrane disks of 1.8 cm in diameter were supplied in one aquarium. These disks were used as an attaching substratum of bacteria, though the quality was not good enough for suspension. Glass aquariums of 60 l in volume containing 45 liters of natural sea water (34 ppt salinity) were used for the prawn larvae rearing. Three glass aquariums were prepared for each treatment. Fifth nauplius stage larvae, which were hatched in our laboratory, were transferred into each aquarium at a density of 67 indiv./l. These aquariums were set outdoors (28–30°C), and were shaded from direct sunlight. Aeration was provided at 3 l/

min through a bubbling stone to supply oxygen and to suspend the *U. pertusa* thalli disks. *U. pertusa* thalli disks suspended and scraped attaching substances off the bottoms and sides of the aquarium, however, vinyl membrane disks did not suspend smoothly. Subsequently, the bottoms and sides of the aquariums which contained vinyl membrane disks were cleaned with a glass wiper every morning.

Chaetoceros caritrance, which was cultured outdoors in normal sea water enriched with PES medium, was used as food and maintained at 10×10^4 cells/ml during the zoeal larval stage. *C. caritrance* was supplied with culture medium. The same volume of rearing water was discarded at feeding. When above 60% of the larvae metamorphosed into the mysis stage, feeding of newly hatched *Artemia* nauplii was started. The nauplii were supplied so as to be entirely consumed by the next morning. After the mysis stage, no rearing water was exchanged in all aquariums. Larval rearing was continued for 20 days until the 10th day of the post larva stage.

Dissolved inorganic nitrogen concentration, namely, $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$, and $\text{NO}_3\text{-N}$, was determined by the Strickland and Parsons method⁷) as an indicator of water pollution. These concentrations were analyzed on the initial and final day of the experimental period. At the end of the larval rearing, the number of larvae was counted, and the body length of 20 larvae was measured for each aquarium. The diameter of *U. pertusa* thalli disks was also measured using 20 samples from each aquarium. In addition, the weight of suspended substances contained in the rearing water and weight of deposited substances on the bottom of the aquariums were measured in each aquarium. Suspended and deposited substances were collected by centrifugation to determine the dry weight after removing salt by washing with distilled water. Bottom sediments were collected by siphoning and concentrated by centrifugation. The dry weight was obtained using the same method of suspended substances. Suspended bacterial density in the rearing water was also determined on the initial, middle, and final day of the culture period. Bacterial density was observed by colony formation on modified Zobel 2216E medium.⁸) During this culture period, light intensity

Table 1. Conditions of larval growth and the environment in kuruma prawn *Penaeus japonicus* rearing (mean and standard deviation)

	Treatments		
	<i>Ulva</i>	Vinyl membrane	Control
Survival rate (%)	86.1* $\pm$ 2.5 (n=3)	71.3 $\pm$ 5.0 (n=3)	71.3 $\pm$ 3.4 (n=3)
Body length (mm)	11.8* $\pm$ 0.9 (n=60)	10.9 $\pm$ 1.0 (n=60)	11.1 $\pm$ 1.1 (n=60)
IN increase (μ g-at/l)	10* $\pm$ 9 (n=3)	133 $\pm$ 37 (n=3)	96 $\pm$ 19 (n=3)
Suspended substances (dry-g/aquarium)	0.02* $\pm$ 0.01 (n=3)	0.43 $\pm$ 0.06 (n=3)	0.37 $\pm$ 0.08 (n=3)
Deposited substances (dry-g/aquarium)	0.01* $\pm$ 0.01 (n=3)	0.39 $\pm$ 0.11 (n=3)	0.42 $\pm$ 0.09 (n=3)
Bacterial density (CFU/ml)	1.6 $\times$ 10 ⁴ * $\pm$ 0.2 $\times$ 10 ⁴ (n=9)	2.6 $\times$ 10 ⁴ $\pm$ 0.3 $\times$ 10 ⁴ (n=9)	2.4 $\times$ 10 ⁴ $\pm$ 0.3 $\times$ 10 ⁴ (n=9)
<i>Ulva</i> growth (times by area)	16.4 $\pm$ 0.8 (n=60)	—	—
pH	8.6* $\pm$ 0.1 (n=20)	8.2 $\pm$ 0.1 (n=20)	8.2 $\pm$ 0.1 (n=20)
Light intensity (mol/m ² /day)		15.3 $\pm$ 3.3 (n=20)	

IN: Inorganic nitrogen concentration, CFU: Colony formation unit.

*: Significant difference among treatments ($P < 0.01$).

was recorded by a data logger (LI-Cor Inc. LI-1000).

The growth conditions of the prawn larvae, water quality, particulate substances contained in the rearing tank, *U. pertusa* growth, and other environmental parameters are shown in Table 1. Higher survival and better growth of prawn larvae were obtained in the treatment with *U. pertusa*. However, inorganic nitrogen concentration was maintained at almost the same concentration as the initial one in the treatment with *U. pertusa*, whereas the concentration increased rapidly in other treatments. Suspended and deposited substances, which are also indicators of water pollution, remained scarce also in the treatment with *U. pertusa*. The bacterial density in the rearing water showed the same numerical values, however, the density in the treatment with *U. pertusa* was significantly lower. pH also showed a different value in the treatment with *U. pertusa* that was significantly higher because of good growth of 16.4 times by area throughout the rearing period. Light intensity was 15.3 $\pm$ 3.3 mol/m²/s.

It was reported that *Pseudomonas* was the dominant species among the bacteria, which attached on the *U. pertusa* thalli.⁹⁾ *Pseudomonas* has a capacity to decompose organic matter,¹⁰⁾ and is also an effective food for kuruma prawn larva.¹¹⁾ It can be considered that the feces and urine of the kuruma prawn larvae traveled more smoothly directly to *U. pertusa* as nutrients and also indirectly to *U. pertusa* and the kuruma prawn larvae by the bacterial growth, in the treatment with *U. pertusa*. It could be concluded that the small-scale ecosystem functioned more effectively by the addition of *U. pertusa* and the attaching microorgan-

isms.

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