



Short communication

Studies on kuruma shrimp culture in recirculating aquaculture system with artificial ecosystem



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ABSTRACT

The Closed Ecological Recirculating Aquaculture System (CERAS) simulates the food chain of hydrosphere and also includes materials cyclical type of food production systems. In the present study, the nourishment salt form kuruma shrimp aquacultural waste (waste water, foam liquid) collected from the CERAS was used as a medium to raise *Chaetoceros gracilis* and *Tetraselmis tetrathele*. F medium was used as a control. The results show that although the growth speed of *C. gracilis* which cultured in aquacultural waste + Manganese medium was 1 day slower compare with the F medium, there were no significant difference in the lipid composition and the fatty acid, amino acid compositions profile of *C. gracilis* and *T. tetrathele* between two groups. Fed shrimp larvae on the algae collected from the two groups for 3 weeks until post-larva stage and collect the shrimp larvae as analytical samples, and then they were divided into two dietary groups and the larvae fed on algae which cultured in F medium was used as a control. There were no significant differences in the larval quality and fatty acid, amino acid compositions profile of shrimp larvae between the two groups.

1. Introduction

Closed Recirculating Aquaculture System (CRAS) is land based system in which species of fisheries are grown at high density under controlled environmental conditions. Without exchange of the breeding water and drainage, CRAS is capable of reducing environmental load by highly purify breeding water (Endo, 2008).

Closed Ecological Recirculating Aquaculture System (CERAS) is the system that consists of CRAS and aquatic artificial ecosystem as a sea-food production system with material recycling for preservation of natural environment (Takeuchi et al., 1997). It was proved that using the nourishment salt of fish culture drainage and the sediment as a medium to raise phytoplankton was possible, and the phytoplankton can be used as bait in fish breeding (Endo and Takeuchi, 2004).

In this study, the rearing experiments of adult kuruma shrimp were conducted in CRAS. The nourishment salt form kuruma shrimp aquacultural waste (waste water, foam liquid) collected from the CRAS was used as a medium to raise *Chaetoceros gracilis* and *Tetraselmis tetrathele*, which are the most ideal food for shrimp larva in early developmental stages (Okauchi and Hirano, 1986). Furthermore, *C. gracilis* and *T. Tetrathele* are totally different in size, which can be used in different shrimp larva developmental stages. Fed shrimp larvae on the algae

collected from the two groups for about 3 weeks until post-larva stage and collect the shrimp larvae as analytical samples, and then they were divided into two dietary groups and the larvae fed on algae which cultured in F medium was used as a control. The larval quality and fatty acid, amino acid compositions profile of shrimp larvae were also analyzed.

2. Materials and methods

2.1. Adult shrimp culture

In the culturing of adult shrimp was conducted using 6 fully mature 1 year old shrimps (length: 14.6 ± 0.6 cm, weight: 25.1 ± 0.8 g) in CRAS (total water volume: approx. 200 L) and bottom of the rearing tank was coated with quartz sand. The culturing period was 80 days. The culture condition of water temperature set in 24 °C, salinity 32 ppt, pH 7.9–8.2, oxygen saturation concentration > 90%, average photosynthetic photon flux density (PPFD) was $100 \mu\text{mol}/\text{m}^2/\text{s}$ with a photoperiod of 14L and 10D. Adult shrimp was fed on commercial diet.

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2.2. Production of shrimp larva by environmental control

During the culturing, we remove the water supply sound and the CRAS devices noise to guide naturally copulation. Then shade the light by a black curtain around the culturing water tank, maintain a light condition from the top of the tank by controlling automatically illumination with a photoperiod of 14L and 10D. Culture rate were set to 2 males for 4 females.

After mating, only discriminated the female shrimp that had copulatory stopper and developed ovary, then accommodated it to an egg collection water tank (water temperature: 26 °C) to let it spawn.

2.3. Algae culture

The axenic stock cultures of *C. gracilis* and *T. tetraathele* were obtained from the Fisheries Research Agency (FRA), Aquaculture Technology Division. Nutrient solution was 1 L of F medium. After high pressure steam sterilization (121 °C, 30 min), polytetrafluoroethylene (PTFE) membrane was attached on the air intake filter unit (Millex FH50, Japan Millipore Corp.), a stopper (c-30 and Shin-Etsu Polymer Corp.) was installed at the exhaust, aerate adjusted with free air at a rate of 3 L/min. Constant illumination was provided with fluorescent lights (F140SS-W/37, Toshiba Corp. Ltd.), average photosynthetic photon flux density (PPFD) was 243.7 $\mu\text{mol}/\text{m}^2/\text{s}$ with a photoperiod of 12L and 12D, and the light irradiated below the culture (Aidar et al., 1994). Cultivation temperature was 27.5 ± 0.2 °C, Salinity 20 ± 1.5 ppt, pH 7.2–7.4. To determine the algal biomass, a 1 mL aliquot of the algal suspension was filtered through hematimeter every day. Each culture was 3 repetitions. When the culture was over, *C. gracilis* and *T. tetraathele* were collected by centrifuge (8300 rpm, 10 min, SPX-201, Tomy Seiko Corp.), and the obtained algae concentrate were then freeze-dried for 6 h by lyophilizer (FDU-506, Tokyo Rikakikai Co., Ltd).

2.4. Identify shortage element by element analysis

Algae sample was preprocessed by microwave digestion device (Model 7295, O. I. Analytical). Added 3 mL concentrated sulfur acid (97%) for 0.15 g dry sample and microwaved at 175 °C for 10 min, then added 3 mL of 15% hydrogen peroxide, went for 125 °C in 5 min microwave heat treatment, then diluting in measuring cylinder to 100 mL for elemental analysis. Nitrogen content of algae was measured by total organic carbon analyzer (TOC-V_{CSH}, Shimadzu Corp.). The other elements (phosphorus, potassium, calcium, magnesium, copper, iron, cobalt, zinc, manganese, molybdenum) were measured by inductively coupled plasma atomic emission spectrometry, ICP-AES (SPS7800, Seiko Instruments Inc.). The analysis elements decision was referred to Dou et al. (2013), and shown in Table 1. Then analyzed the two kinds of aquacultural waste (waste water, foam liquid) collected from the foam

Table 1

Nitrogen and mineral compositions (dry matter basis) of *Chaetoceros gracilis* and *Tetraselmis tetraathele*.

		<i>C. gracilis</i>	<i>T. tetraathele</i>
N	(mg/g)	29.92 \pm 0.26	21.26 \pm 0.12
P	(mg/g)	1.51 \pm 1.11	0.77 \pm 1.09
K	(mg/g)	21.33 \pm 0.19	9.83 \pm 0.29
Mg	(mg/g)	14.31 \pm 0.4	5.65 \pm 2.49
Ca	(mg/g)	3.37 \pm 0.25	34.58 \pm 0.08
Fe	($\mu\text{g}/\text{g}$)	1117.87 \pm 38.01	461.15 \pm 17.62
Mn	($\mu\text{g}/\text{g}$)	134.94 \pm 3.01	92.08 \pm 7.08
Co	($\mu\text{g}/\text{g}$)	4.77 \pm 1.4	2.17 \pm 1.37
Cu	($\mu\text{g}/\text{g}$)	5.36 \pm 1.6	6.52 \pm 7.57
Zn	($\mu\text{g}/\text{g}$)	19.89 \pm 2.53	14.08 \pm 0.17
Mo	($\mu\text{g}/\text{g}$)	51.16 \pm 1.85	0.57 \pm 0.18

Mean \pm S.D. (n = 3).

Table 2

Nitrogen and mineral compositions of aquacultural wastes.

		Waste water	Foam liquid
N	(mg/L)	158.85	58.56
P	(mg/L)	5.34	3.15
Mg	(mg/L)	397.01	147.24
K	(mg/L)	78.66	57.79
Ca	(mg/L)	273.08	69.00
Fe	(mg/L)	1.36	5.16
Mn	($\mu\text{g}/\text{L}$)	n.d.	n.d.
Co	($\mu\text{g}/\text{L}$)	22.91	13.29
Cu	($\mu\text{g}/\text{L}$)	21.20	31.31
Zn	($\mu\text{g}/\text{L}$)	40.23	100.13
Mo	(mg/L)	1.75	n.d.

n.d.: not detected.

separation unit and water tank after 80-day culture of adult shrimp in the same way, and shown in Table 2.

After the elemental analysis, assumed that 1 L culture medium can cultivate 1 g algae at most, each element contents of the two kinds of aquacultural waste were divided by the nitrogen content of waste water, and divided by the date of algae element contents divided by algae nitrogen content. The lack element can be identified when the result that do not reach 100%.

2.5. Culture algae by medium made from aquacultural waste

With the result of identifying of lack element, it can be concluded that Mn need to be added in process of making culture media with aquacultural waste. The medium were made as 1) aquacultural waste (including waste water and foam liquid), 2) aquacultural waste + Mn, 3) F medium (as standard, with adjustment of nitrogen content as same as algae). The pH was adjusted by strong basic mixture (NaOH, KOH, Ca (OH)₂, Mg(OH)₂) to 7.5–7.9. After high pressure steam sterilization (121 °C, 30 min), inoculated with algal suspension cultured by F medium in rate of 10 mL/L.

2.6. Nourishment analysis of algae

After largescale culture of *C. gracilis* and *T. tetraathele* with F medium and aquacultural waste + Mn medium (aquacultural waste medium) as same way in 2.3,

Samples of the algae for biochemical analysis were collected by centrifuge and saved in a – 80 °C fridge on the first day that the shrimp larvae were fed. It was assumed that there was little or no change in algal biochemical composition of cells over a 24-h period (Thompson and Harrison, 1992). The other half of the algae were collected by centrifuge, and resuspended with 20 ppt artificial seawater and saved in a 4 °C fridge, and Crude lipids, Fatty acid and Amino acid composition was analyzed.

2.7. Examine about the nutritional values of the algae cultured by aquacultural waste for kuruma shrimp larva

The nauplius used in this experiment were hatched from eggs obtained from a single female. Some hours after hatching, vigorous nauplii gather by light were collected using a pipette. Rearing of shrimp larvae were tested by feeding them on living *C. gracilis* and *T. tetraathele*, through the early developmental stages, from nauplius to post-larva. After spawning, the nauplii were collected and stocked in 2 L glass flask, at an initial density of 100 indiv./L, with three replicates. The larval rearing experiment was carried out in a temperature and light controlled room at 26 ± 1 °C and 80 $\mu\text{mol}/\text{m}^2/\text{s}$ (14L:10D), and sufficient air was supplied continuously, salinity 32 ± 2.5 ppt, pH 7.2–8.0. To maintain microalgae concentrations and eliminate faecal residues or food remains, a 20% water exchange was done daily

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