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Zooplankton cultivation and prawn seed-production in an artificial ecosystem

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ABSTRACT: The cultivation experiments were conducted in two parts: (1) zooplankton cultivation in a heterogeneous system as fundamental research (Experiment I); (2) prawn seed-production in a homogeneous system as applied research (Experiment II). In Experiment I, several types of apparatus were designed in order to study a feedback system for cultivation of zooplankton *(Brachionus plicatilis* and *Tigriopus japonicus).* A 550-l round transparent tank was used for reserve culture, A 150-l zigzag stream unit was made of vinyl plates, and connected with the reserve culture tank. In both cases, the water was re-circulated 20 times a day by an airlift pump. A tower-decomposer tank and two blowing cultivators were specially designed for promoting energy-flow. During a 140-day experiment, total wet weight of feed and harvest were 4817 g of yeast and 4308 g of zooplankton, respectively. The rate of food conversion was 89.4 % in terms of wet weight and 33.6 % in dry weight. In Experiment II, a 2500 m^3-hatchery tank with movable aerators and airlift circulation systems was constructed to maintain an artificial ecosystem for rearing of the prawn *Penaeus japonicus.* Feeding rate in the 2500 m^3-tank was 10.8 % lower than that in the 200 m^3-tank, but growth rate in the former was 10.6 % higher than in the latter.

INTRODUCTION

Water pollution due to commercial fish cultivation has recently become a serious problem in south-western Japan. In the Seto Inland Sea, the standing crop of macro-algae has decreased 20 % during the last 20 years. Likewise, the ecosystem has been deformed by industrial waste affluents and abundant biodeposition of fish excretion and excess food.

A similar conditions was noted when culturing aquatic animals in a tank. Faeces of the animals and residues of their diet sink down and accumulate on the bottom of the tank. Such biodeposits, when decomposed, may cause disturbances of the normal energy flow in the water. If algae are cultured in the same tank, they utilize the decomposed materials and thus keep the culture water in a homeostatic state.

In 1964, Hirata tested a system of triple player for seed production in puffer fish. The excess nutrients due to faeces and remaining residue were removed by algae which were illuminated by fluorescent lamps in an artificial stream (see also Kinne, 1976). The copepods fed on the micro-algae but they were, in turn, consumed by

the fish larvae. However, the stream was too small, and homeostasis of the ecosystem was not maintained for a long period.

In this paper, it is suggested that the culture system of aquatic animals should preferably be maintained as a balanced ecosystem consisting, as in nature, of producers, consumers, and decomposers. The experiments were conducted in two parts: (1) zooplankton culture in a heterogeneous system as a fundamental research project, and (2) prawn seed-production in a homogeneous system as an applied research project.

MATERIALS AND METHODS

Producers, consumers and decomposers

Enteromorpha intestinalis, Chaetoceros rigidus, Chlorella saccharophila var. *saccharophila* and *Nitzschia* spp. were cultured in the producer tanks prepared in Experiment I. *Brachionus plicatilis, Tigriopus japonicus* and *Penaeus japonicus* were

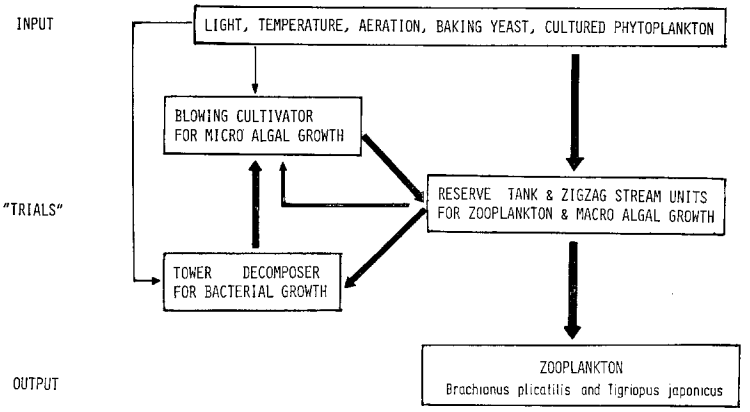

Fig. 1: Flow chart of homeostasis in zooplankton-culture ecosystem

grown as consumers in Experiments I and II, respectively. Several types of bacterial organisms were grown naturally in the waters and served as decomposers in all the experiments. Quantitative analysis of bacterial types was only done in Experiment I.

Apparatus for zooplankton cultivation

A flow chart of the heterogeneous system for zooplankton culture in Experiment I is presented in Figure 1. White beam fluorescent lamps (40 w x 8) were switched on for 15 h during day time and switched off 9 h at night from 20.30 to 5.30 (Hirata, 1975 a). Air (about 2.5 l min^{-1}) was supplied continuously for an air-lift pump; 30 g of baking yeast and 1 or 2 g of cultured diatoms were fed to the zooplankton every day, in the morning and evening.

Two 500 l transparent plastic tanks were employed for zooplankton culture; Tank *a* was employed for the closed recirculation system, Tank *b* served as the reserve tank. The water was recirculated in each tank by an air-lift pump as illustrated in Figure 2. Polyethylene nets (80 meshes) were set on the surface of the stream (Tank *a*) in order to accelerate the growth of *Enteromorpha intestinalis*. The average rate of water circulation was about 20 times day^{-1}. The current speed in the stream was about 1.3 m min^{-1}. *E. intestinalis* were harvested from the algae nets after attaining critical density.

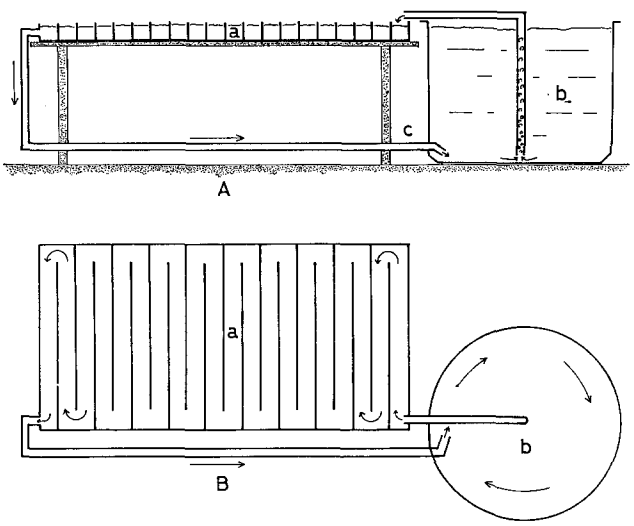

Fig. 2

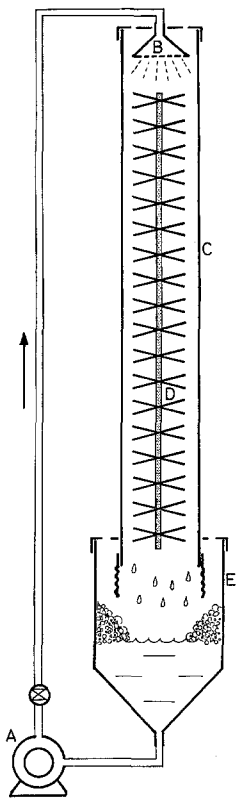

Fig. 3

Fig. 2: Schematic diagram of main tank and zigzag stream for cultivation of zooplankton *(Tigriopus & Brachionus)* and macro alga *(Enteromorpha)*. *A* side view; B top view; a 150 l zigzag stream unit; b 550 l main cultivation tank; c recirculation pipe

Fig. 3: Schematic diagram of tower decomposer. A circulation pump; B shower nozzle; C vinyl tower; D polyethylene brush; E reservoir

The faeces and diet residue deposited on the bottom of the stream were removed by a siphon and transferred into a tower decomposer tank which contained a PVC honeycomb settler as shown in Figure 3. The decomposing method used was a modification of the technique reported by Fujiwara et al. (1976). In this method, organic matters, such as faeces, was transformed into inorganic nutrients within 24 h by 2 or 3 bacteria species.

A blowing cultivator (Fig. 4) was used for the cultivation of micro algae which thrived on the excess nutrients in the zooplankton culture tank. Two 60-l tanks made of transparent plastic plates were put in a water bath with a thermo-regulator (22.0 ° ± 2.0 C °). The medium in the cultivator was aerated and illuminated by white-beam fluorescent lamps (20 w x 16) from both sides. About 30 l of waste

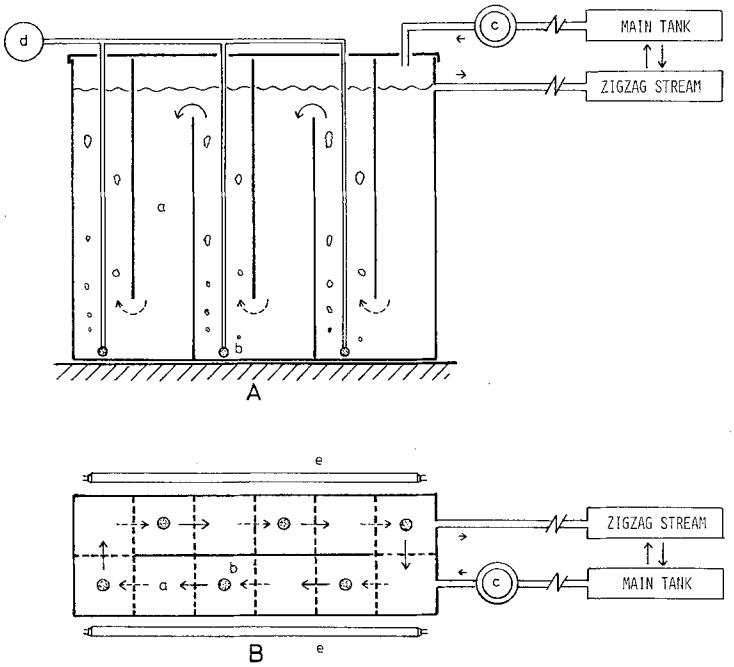

Fig. 4: Schematic diagram of blowing cultivator. A side view; B top view; a blowing cultivator; b air outlet; c recirculation pump; d air entry; e fluorescent lamps

water obtained from the by-products of animal harvesting and 0.5 l of excess nutrient from the decomposer tank were pumped into this culture enclosure immediately after the light was turned on every morning (5.30). At the same time, the micro-algae cultured in the blowing cultivator were drained into the zigzag stream unit. An automatic time switch and a diaflum pump were used in all processes mentioned above.

Prawn hatchery

For prawn hatching and rearing, a 2,500-m³ hatchery with attached, movable aerators and an airlift circulator was designed. In order to provide satisfactory aeration, a deep hatching tank was built (4 m depth). Instead of being removed, the biodeposits were recycled by the oxidation processes occurring in the tank. As shown in Figure 5, the moving pipe of the aerator supplied a strong air flow directly

at the bottom thus preventing the sedimention of particles. Oxidation was ensured by a relatively high dissolved oxygen content of 4 to 7 ppm, obtained from an air supply of 0.5 to 1.0 % of the water volume min^{-1}. The culture water in the hatchery was recirculated horizontally by 20 air-lift pipes attached to the inside wall of the tank. The diameter of each air-lift pipe was 20 cm. An example of the daily feeding schedule is presented in Table 1.

Table 1

Example of daily feeding schedule.
Temperature: 20°–25° C; salinity: 30–35 ‰; pH: 7.8–8.4; dissolved oxygen: 5–7 ppm

Nauplius	N1–4	0–1	No feeding
	N5–6	1–2	1 g S. C.*
Zoea	Z1	3–4	1 g S. C. + 0.1 g baking yeast*
	Z2	5–6	1 g S. C. + 0.5 g baking yeast
	Z3	7–8	1 g S. C. + 500 marine rotifer**
Mysis	M1	9–10	1 g S. C. + 50 brine shrimp**
	M2	11–12	1 g S. C. + 100 brine shrimp
	M3	13–14	1 g S. C. + 150 brine shrimp
Postlarvae	P1	15	150 % larval B. W. of neck clam^{+} + 100 brine shrimp
	P2	16	150 % larval B. W. of neck clam + 50 brine shrimp
	P3–5	17–19	100 % larval B. W. of neck clam and frozen shrimp^{++}
	P6–10	20–24	80 % larval B. W. of neck clam and frozen shrimp
	P11–20	25–34	80 % larval B. W. of neck clam and frozen shrimp
	P21–	35–	60 % larval B. W. of neck clam and frozen shrimp

* S. C. (soy cake) and baking yeast per 10,000 larvae
** Marine rotifer and brine shrimp per larva
$^{+}$ Neck clam meat minced
$^{++}$ Weight-ratio of neck clam and frozen shrimp is 2:1.

RESULTS

Zooplankton culture in a feedback system

As shown in Table 2, the total wet weight of feed and harvest were 4817 g of yeast and 4308 g of zooplankton, respectively. The moisture content, observed at random after the experiment was 69.4 % for baking yeast and 88.5 % for the harvested zooplankton. Therefore, average food conversion was calculated to be 89.4 % in terms of wet weight and 33.4 % in dry weight. The conversion rates increased gradually during cultivation. For example, wet-weight conversion ranged from 49.5 % to 99.7 % during the first half of the experiment, while the rates increased up to 160.6 % between the 91st and 100th day.

In general, higher conversion rates were obtained when the *Chlorella saccharophila* var. *saccharophila* growth on excess nutrients was intense. The total amount of *Chlorella* regenerated was 1314 g, and this was estimated to account for 21.4 % of all the food in this cultivation system.

Growth of *Enteromorpha intestinalis* also exerted a great influence on the propagation of zooplankton, especially on the survival of *Tigriopus japonicus*. It was interesting to note the proportion of zooplankton distribution in both zigzag stream tank and round reserve tank. Average densities of *Brachionus plicatilis* were 80 individuals ml^{-1} in the stream and 60 individuals ml^{-1} in the tank. Comparing

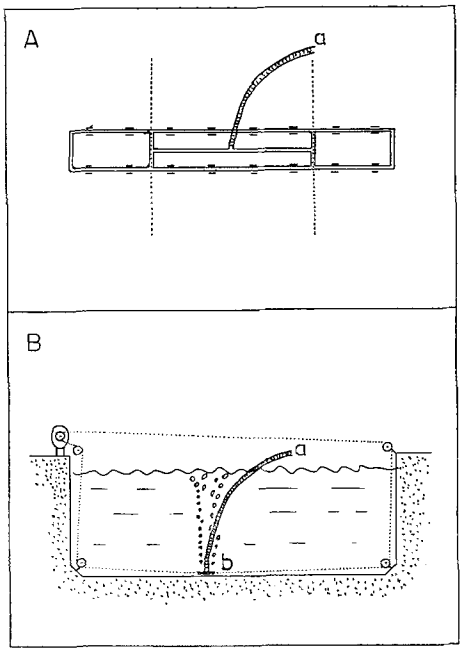

Fig. 5: Schematic views of movable aerator. A top view; B side view; a aeration tube; b air outlet

the population densities between zigzag stream and reserve tank, the *B. plicatilis* abundance was calculated to be 6:4, that is about 60 % in the stream and 40 % in the reserve tank. In contrast, the abundance of *T. japonicus* was 9:1 (90 % in the stream and 10 % in the reserve tank). Therefore, there is not much difference in the abundance of *B. plicatilis* in the stream or reserve tank, but the *T. japonicus* abundance in the stream was 9 times higher than in the reserve tank. This tendency was pronounced when *E. intestinalis* grew well near the water surface of the zigzag stream.

Standing crops of zooplankton are presented in Figures 6 and 7. The total body volume of *Brachionus plicatilis* and *Tigriopus japonicus* in both stream and reserve tank increased rapidly during the first 30 days of the experiment. Thereafter, the population density of *B. plicatilis* increased gradually from a volume of 189 to 254 ml, but became stable during the last 70 days at about 240 ml body volume, because of optimum feeding and harvesting rates. The population density of *T. japonicus* showed little variation over a range of 22 to 57 ml of body volume

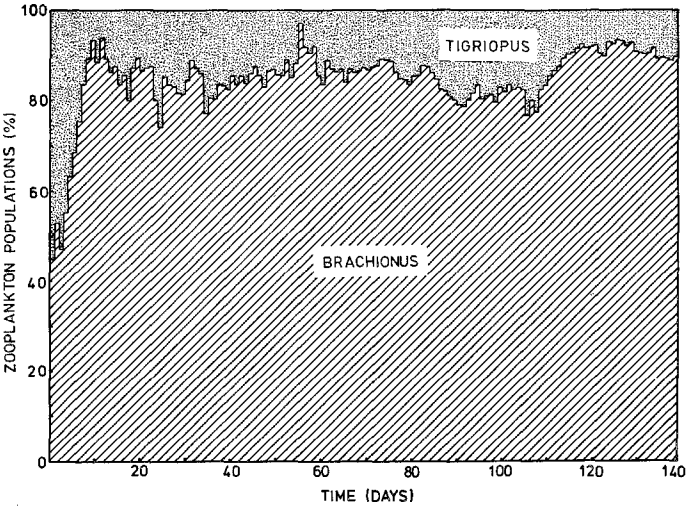

Fig. 6: Homeostasis of *Brachionus* and *Tigriopus* populations during experiment

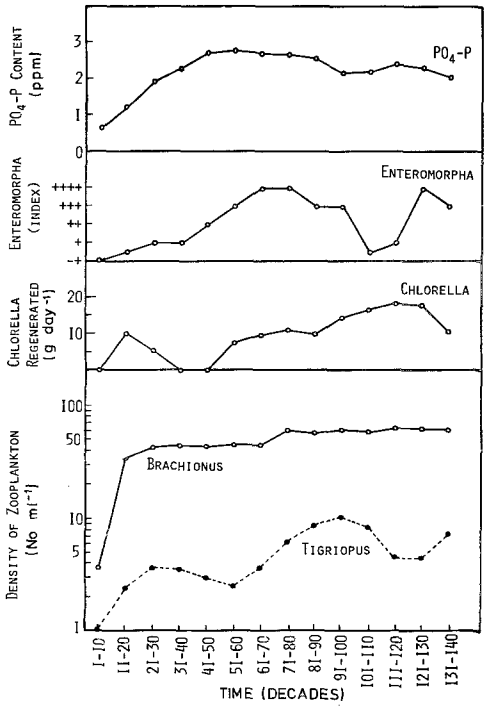

Fig. 7: Homeostatic states of zooplankton, algae and PO_4-P

throughout the experiment as shown in Table 3 and Figure 7. It seems that the population densities of *B. plicatilis* and *T. japonicus* were related to the growth of *Chlorello saccharophila* var. *saccharophila* and *Entheromorpha intestinalis* grown in stream and tank, respectively. The population of *T. japonicus* was affected indirectly by the growth of *E. intestinalis* with a delay of 20 or 30 days.

Inorganic phosphate (PO_4-P) contents of the culture water, measured each day (10-day period averages) are shown in Figure 7. The PO_4-P value at the beginning of the experiment was 0.62 ppm; it increased to 2.77 ppm in the 5th decade. Then it decreased again gradually to 2.03 ppm in the final decade of the experiment, depending on the growth of *Chlorella saccharophila* var. *saccharophila* and *Enteromorpha intestinalis* in the waters.

The experiment was initiated with the same body volume at a level of 50 % each, of *Brachionus plicatilis* and *Tigriopus japonicus*. However, *B. plicatilis* became the dominant species about 10 days later. Thereafter, the relative species abundance remained almost constant at about 80 % *B. plicatilis* and 20 % *T. japonica*.

Prawn rearing experiment

Results of prawn rearing experiments are summarized in Tables 4 and 5, comparing a 2500-m^3 hatching tank with a movable aerator to the routine method employed with 200-m^3 tanks.

The average densities of diatoms such as *Chaetoceros rigidus*, *Nitzschia* sp. in the 2500-m^3 and 200-m^3 tanks were 89.4 $\pm$ 8.0 and 73.9 $\pm$ 4.9 cells ml^{-1}, respectively. The density was calculated to be 100 in 200-m^3 tank and 212.0 in 2500-m^3 tank. Therefore on the average, diatom growth in the larger tank was 121 % higher than that in the smaller one.

Artemia salina nauplii were fed to the prawn larvae after these attained the mysis stage. Feeding rates were 215 nauplii $larvae^{-1}$ day^{-1} in the 2500-m^3 tank. The feeding index of *Artemia nauplii* in the 200-m^3 tank was 100.0; in the 2500-m^3 tank, 89.2. Hence, the feeding rate in the large tank was 10.8 % lower than that in the small one.

On the other hand average growth of postlarvae in a 2500-m^3 hatchery was 9.4 mg, and greater than that in 200-m^3 tanks (8.5 mg). The index difference between large and small tanks were 110.6 to 100.0.

Survival rates in the larger tank were 85.3 % and 78.9 % in the small tank. However, standard deviations of the mean in the large and small tanks were 13.0 and 28.1, respectively. This means that survival rates in the 200-m^3 tank fluctuated very much, and that in the large tank it was more stable.

A big difference in postlarval density was found in the two types of tanks. Average densities in the 200-m^3 tank were 12.5 x 10^3 m^{-3}; in the 2500-m^3 tank 8.8 x 10^3 m^{-3} (due to the small number of gravid females provided at the beginning of the rearing experiment). On the average, in all experiments, 0.4 female m^{-3} were used in the larger tank and 1.0 female m^{-3} in the small tanks case.

Table 2

Results of zooplankton cultivation in feedback system

Date	Period (day)	Food supplied (g)	Animal harvested (g)	Food conversion (%)	*Chlorella* regenerated (g)	PO_4-P (ppm) (mean)	NO_2-N (ppm) (mean)	Density of zooplankton *Brachionus* (No ml^{-1}) (mean)	*Tigriopus* (No ml^{-1}) (mean)	Water temp. (° C) (mean)	pH (mean)
March, 12–21	1–10	709	0	0	0	0.62	0.31	3.7	1.1	19.8	7.76
March, 22–31	11–20	416	206	49,5	101	1.20	0.21	34.8	2.4	20.0	7.57
April, 1–10	21–30	305	239	78.4	52	1.92	0.16	43.9	3.7	19.0	7.55
April, 11–20	31–40	299	250	83.6	0	2.23	0.12	45.1	3.6	21.6	7.64
April, 21–30	41–50	302	229	75.8	0	2.72	0.16	44.9	3.0	23.1	7.79
May, 1–10	51–60	312	239	76.6	76	2.77	0.39	45.6	2.6	22.3	7.81
May, 11–20	61–70	326	257	78.8	96	2.68	0.48	45.4	3.8	23.3	–
May, 21–30	71–80	324	323	99.7	110	2.66	0.27	60.8	6.2	25.3	–
May, 31–June, 9	81–90	306	381	124.5	100	2.56	0.36	58.4	8.6	25.0	7.52
June, 10–19	91–100	307	493	160.6	145	2.14	0.35	61.2	10.4	25.4	7.53
June, 20–29	101–110	306	481	157.2	167	2.16	0.39	59.5	8.4	25.8	7.58
June, 30–July, 9	111–120	300	479	159.7	184	2.39	–	64.3	4.7	26.5	7.68
July, 10–19	121–130	304	385	126.6	178	2.28	–	63.6	4.6	27.7	7.70
July, 20–29	131–140	301	346	115.0	105	2.03	–	62.0	6.5	27.3	7.76
Total (wet)		4817	4308	89.4	1314						
Dry conversion		1389.3	495.4	33.6	281.2						

Table 3

Habitat of *Brachionus* and *Tigriopus* in artificial ecosystem

Period (day)	*Brachionus plicatilis* Zigzag stream (1) ($\times 10^{-3}$ ml l^{-1})	*Brachionus plicatilis* Reserve tank (2) ($\times 10^{-3}$ ml l^{-1})	(1)/(2)	*Tigriopus japonicus* Zigzag stream (3) ($\times 10^{-3}$ ml l^{-1})	*Tigriopus japonicus* Reserve tank (4) ($\times 10^{-3}$ ml l^{-1})	(3)/(4)	*Enteromorpha* (index)
1– 10	33	33	1.0	9	7	1.3	—
11– 20	200	206	1.0	115	14	8.2	—+
21– 30	285	238	1.2	229	13	17.6	+
31– 40	312	241	1.3	204	18	11.3	+
41– 50	288	239	1.2	148	21	7.1	++
51– 60	235	257	0.9	125	15	8.3	+++
61– 70	287	247	1.2	187	10	18.7	++++
71– 80	569	283	2.0	252	8	31.5	++++
81– 90	456	289	1.6	290	13	22.3	+++
91–100	481	308	1.6	361	27	13.4	+++
101–110	305	332	0.9	258	40	6.5	—+
111–120	432	339	1.3	146	20	7.3	+
121–130	605	301	2.0	121	13	9.3	++++
131–140	717	266	2.7	169	13	13.0	+++
Mean	372	256	1.4	187	17	12.6	

Table 4

Effects of movable aerator in 2500-m³ hatchery on the reproduction of diatoms, rates of feeding and growth of larvae

Culture enclosure	Density of diatoms ($\times 10^3$ cells ml^{-1})			Rates of feeding (*Artemia* $Larva^{-1}$ Day^{-1})			Growth of postlarvae (mg)			Density of postlarvae ($\times 10^3$ Larvae m^{-3})			Survivals of larvae (% of Z–1/P–15)		
	mean	± S.D.	index	mean	± S.D.	index	mean	± S.D.	index	mean	± S.D.	index	mean	± S.D.	index
2500-m³ hatchery	89.4	8.0	121.0	21.5	20.4	89.2	9.4	0.9	110.6	8.8	3.7	70.4	85.3	13.0	108.1
200-m³ hatchery	73.9	4.9	100.0	24.1	22.4	100.0	8.5	0.6	100.0	12.5	5.0	100.0	78.9	28.1	100.0

The numbers of postlarvae produced in both types of hatching tanks are presented in Table 5. The total number of seeds (P-15) produced in 2500-m³ and 200-m³ tanks were 66000 x 10^3 larvae and 7500 x 10^3 larvae, respectively. Average seed-production at a given time was 22,000 x 10^3 larvae in the large and 2500 x 10^3 larvae in the small tank.

Table 5

Results of prawn seed-production in 2500-m³ and 200-m³ hatching tanks

Experiment	Period	Water temperature (° C)	2500-m³ hatching tank No. of larvae per tank (×10^3) Z–1	P–15	survival rate (%)	200-m³ hatching tank No. of larvae per tank (×10^3) Z–1	P–15	survival rate (%)
Exp. 11–1	April–June	20–23	16,000	14,000	87.5	3,340	3,000	89.8
Exp. 11–2	June–July	24–26	33,000	32,000	97.0	3,160	3,160	100.0
Exp. 11–3	July–August	27–29.5	28,000	20,000	71.4	2,850	1,340	47.0
Mean		24.9	25,670	22,000	85.7	3,120	2,500	80.1

Dissolved oxygen concentrations ranged from 7 to 10 ppm in the 2500-m³ tank and 3 to 6 ppm in the 200-m³ tank. Hence, the oxygen content in the large tank was about twice as high as that in the small tank because of the aeration provided by the movable aerator in the 2500-m³ tank. The amount of air supplied equaled about 0.5 to 1.0 % of the water volume min^{-1}. Minimum and maximum water temperatures were 20 ° C and 29.5 ° C, respectively, depending on the season (spring to summer). The pH values varied from 8.0 to 8.4. There was no significant difference in pH between the 2500-m³ and 200-m³ tanks because the culture water was renewed when the pH decreased below 8.0.

No sedimentation was found on the bottom of the 2500-m³ tank (diving observation). Organic aggregations remained suspended all the time due to the movable aerator, and were oxidized by the strong aeration provided (movable aerator, air-lift). This contrasts with the 200-m³ tank where biodepositions accumulated in the corners or on the floor, even during mixing with an agitator (Akazawa, 1973).

DISCUSSION

In general, the food conversion of *Brachionus plicatilis*, fed baking yeast, was estimated to be about 20 to 30 % in terms of wet weight. The corresponding conversion was 89.7 % in wet weight and 33.4 % in dry weight. These values are about 3 times higher than those obtained previously (Hirata & Mori, 1967; Hirata, 1974). Two possible reasons for this should be considered. The first reason for the high conversion rate obtained might be due to biodeposition feedback. That is, *Chlorella saccharophila* var. *saccharophila* developed due to excess nutrients originating from faeces and food remains. The amounts of *Chlorella* produced and the feed supplied were 1314.0 g and 4817.0 g, respectively. Therefore, the feedback rate was 21.5 % during the 140-day experiment. The second reason is probably due to water-quality

homeostasis regulated by the propagation of *Enteromorpha intestinalis* in the zigzag stream and of *C. saccharophila* in the blowing cultivator.

Enteromorpha intestinalis also exerted great influence on the development of *Tigriopus japonicus* nauplii. The density of the *T. japonicus* nauplii increased initially, but decreased again about 30 or 40 days after the *E. intestinalis* bloom. It may be assumed that *E. intestinalis* acted in three ways: (1) supplying shelter for *T. japonicus:* (2) using up nutrients and producing oxygen (3) serving as food (zoospores) for *T. japonicus.*

In Experiment II, the diatom density in the 2500-m^3 hatchery was higher than that in the 200-m^3 tanks. This may have resulted from the use of the movable aerator for bottom cleaning and oxygen supply (Hirata, 1975 b). The aerator prevented sedimentation of organic material on the bottom of the tank, and supplied oxygen to the suspended particles. The growth of diatoms influenced the feeding, growth and survival of the larvae. Lower feeding rates with higher larvae growth were obtained in the 2500-m^3 tank. Hence, the energy-flow could be promoted by the movable aerator in the 2500-m^3 hatchery. The experiment was repeated and practically confirmed at the Shibushi Station of the Seto Inland Sea Farming Fisheries Association in 1976.

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