Mem. Fac. Fish., Kagoshima Univ.
Vol. 34, No. 1, pp. 27～36 (1985)

Evaluation of the Use of *Brachionus plicatilis* and *Artemia* nauplii for Rearing Prawn *Penaeus japonicus* Larvae on a Laboratory Scale

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Abstract

In order to know the energy requirement of prawn (*Penaeus japonicus*) larvae under feeding conditions of *Brachionus plicatilis* and *Artemia* sp. nauplii, daily consumption rate of those food organisms by prawn larvae at different developmental stages was observed. Prawn larvae were reared individually from 2nd zoea to 2nd post larva stage at food density of 10 ind·ml^{-1} for rotifers and 5 ind·ml^{-1} for *Artemia*. The consumption rate of rotifers per larva increased to 308 ind·larva^{-1}·day^{-1} until 3rd zoea stage. After 1st mysis stage, the consumption rate decreased. The consumption rate of *Artemia* was almost constant at 50 to 80 ind·larva^{-1}·day^{-1} until 3rd mysis stage. In 1st post larva stage, *Artemia* consumption increased about 1.6 times as that of 3rd mysis stage. Energy intake rate per larva was 0.60 cal·larva^{-1}·day^{-1} at 3rd zoea stage in the feeding conditions of both rotifers and *Artemia*. After 1st mysis stage, the intake rate of rotifer decreased gradually. However, the intake rate of *Artemia* increased to 1.26 cal·larva^{-1}·day^{-1} in 2nd post larva stage and about 3 times as that of rotifer feeding.

It is important to find out the daily amount of food consumption in order to supply the suitable amount of food, and also to carry out the mass production of live foods[1]. About ten years earlier, HUDINAGA and KITTAKA[2] expressed the need to "discover the most suitable and economical food that can be supplied to the several stage of the developing larvae".

The present work aims 1) to determine the consumption rates of *Penaeus japonicus* larvae feeding on freshly hatched *Artemia* sp. nauplii and the rotifer *Brachionus plicatilis* fed on *Chlorella saccharophila*, and 2) to evaluate their use for the larval rearing of this prawn. For the last purpose *Artemia* sp. nauplii were supplied from Z_2, N_1 or during Z_3—M_1 substage (after *Brachionus* was supplied during Z_2), and survival rates and growth index were recorded daily. In another treatment *Brachionus* was supplied from Z_2 to P_3. At termination of the experiment, total body length and dry body weight of the larvae from each treatment was measured to determine any significant difference among treatments due to feeding

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strategy. Any benefit from the introduction of *Artemia* nauplii or *Brachionus* from the Z_2 substage is discussed.

MATERIALS AND METHODS

Wild gravid females were obtained from the Matsumoto Fish Market in Nobeoka city, Miyazaki Prefecture. The spawners were placed separately in 500L concrete tanks previously cleaned, dried and filled with fresh natural seawater. After hatching and when nauplii metamorphosed to nauplius 6 substage (N_6), they were transferred to the laboratory. Before metamorphosing of the N_6 to Z_1, diatom *Chaetoceros calcitrans* was supplied at a density of $10\sim15\times10^4$ cells$\cdot ml^{-1}$. When larvae became zoea food was available for the weak larvae and survival was improved[3].

Chaetoceros calcitrans culture, *Brachionus plicatilis* fed on *Chlorella saccharophila* and *Artemia* (Tsientsin strain) hatching procedures were carried out as described by NAVRIKOS[4]. Larval rearing experiments were conducted in 21 polyvinyl cylindrical vessels, each with 1L in suspension, immersed in a waterbath at 27～28℃. The experimental vessels were placed in a completely randomized design[5]. Salinity of the 5μ filtered natural seawater was 35 ppt. Vessels, airstones and airtubes were sterilized with boiling water. 30 larvae were initially stocked in each container and gentle aeration was provided in the medium by air bubbling from the bottom of the cultures to assure homogeneous distribution of prey and predator organisms and dissolved oxygen near saturation levels. Larval counts were taken every 24h to calculate cumulative survival rates at each substage. Growth was quantified by the growth index of the larvae[6]. Water and food were changed daily and larvae were transferred by a wide-bore pipette into clean containers with fresh seawater and food. No precautions were taken to control bacterial infection.

Two predation experiments were conducted to determine the consumption rates of *Penaeus japonicus* larvae feeding on freshly hatched *Artemia* nauplii and *Brachionus plicatilis*, following the method of EMMERSON[7-8]. Preliminary studies showed that the optimal feeding density was 5 nauplii$\cdot ml^{-1}$. for *Artemia* feeding and 10 rotifers$\cdot ml^{-1}$ for *Brachionus* feeding. For each experiment, tests and control (holding no larvae) were duplicated. Consumption rates were calculated according to the following formula:

$$I=\frac{V}{t\cdot n}(C_o-C_t)-A \qquad A=\bar{C}_o-\bar{C}_t$$

Where, I: ingestion rates, t: time expressed in days, n: number of larvae, calculated from the mean of larvae present at time 0h and 24h, V: volume of the culture medium, expressed in ml, C_o, C_t: concentration of prey at time 0h and 24h, respectively, $\bar{C}_o$, $\bar{C}_t$: mean concentration of prey in the two replicates of the control at time 0h and 24h, respectively, A: correction term for changes in the control with final concentration C_t after time t (24h). Daily initial and final concentrations of prey, in the test and control vessels, were calculated from the mean value of ten counts taken from a corresponding number of 1ml samples fixed with LUGOL's iodine solution.

At the third experiment, five feeding regimes were applied to compare their influence on

the survival and development of the larvae. At termination of the experiment aside from the survival and growth rates, calculated at each substage, 10 larvae per treatment were taken to measure total body length and dry body weight. Total body length was measured with a calibrated eyepiece micrometer. Dry body weight was measured on a Sartorius 2474 balance, accurate to 0.01 mg, after the samples of 10 larvae were oven dried at 60℃ for 48 hours. The wight obtained was divided by the number of animals in the sample to yield the dry weight per postlarva. Survival rates, growth index and body length data were statistically analyzed by ANOVA and DUNCAN'S Multiple Range Test[9]. All treatments of this experiment consisted of three replicates and temperature of the waterbath was maintained at 28.2±0.9℃.

RESULTS

Fig. 1 and Table 1 show the consumption rates of *Artemia* sp. nauplii by *P. japonicus* larvae from Z_3 to P_2 at a density of approximately 5 nauplii·ml^{-1}. Larvae consumed a significant amount of brine shrimp nauplii (a mean of 60 naup. ·larva^{-1}·day^{-1}) during Z_3 to M_1 substage, followed by a two fold decline during M_1 to M_2 substage. However, during M_2 $-M_3$, M_3-P_1 and P_1-P_2 substages a continuous increase of prey consumption was occurred by the larvae. The highest ingestion of prey obtained for *P. japonicus* larvae was 124 naup. ·larva^{-1}·day^{-1} during P_1-P_2 substage. During that period the final density of

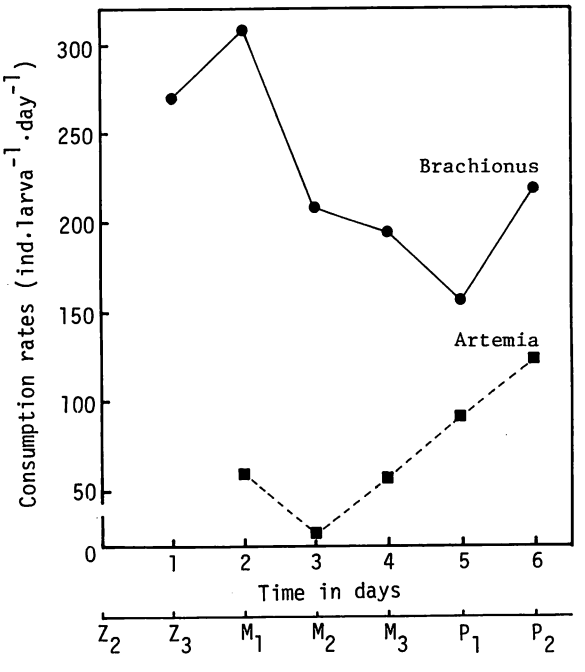

Fig. 1. Consumption rates of *P. japonicus* larvae feeding on *Artemia* sp. nauplii (□) or *Brachionus plicatilis* (○).

Table 1. Consumption rates of *Artemia* nauplii and *Brachionus plicatilis* by *P. japonicus* larvae, expressed in *Artemia* · larba^{-1} · day^{-1} and *Brachionus* · larva^{-1} day^{-1} respectively. Control corrected values were calculated from any difference between Oh and 24h counts in the control vessels holding no larvae. A and B represent the two replicates and $\bar{x}$ A+B the average of the two replicates.

Stage	*Artemia*/ml at time Oh	A Rate	Control Corrected	B Rate	Control Corrected	$\bar{x}$A+B Rate	Control Corrected
Z_3~M_1	5.5	67	53.3	80	66.7	73.5	60
M_1~M_2	5.2	32	26.8	28	27.1	30	26.9
M_2~M_3	5.6	63	63	52	52	57.5	57.5
M_3~P_1	5.4	96	102	75	82.5	85.5	92.2
P_1~P_2	5.3	150	141.7	123.5	105.9	137	123.8
Larval Stage	*Brachionus*/ml at time Oh	A Rate	Control Corrected	B Rate	Control Corrected	$\bar{x}$A+B Rate	Control Corrected
Z_2~Z_3	9.7	230	253.3	263.3	290	246.7	270
Z_3~M_1	10.6	280	315	270	301.7	275	308.3
M_1~M_2	10.6	133	206.7	152	210.2	142.5	208.4
M_2~M_3	11.2	173	185	193	205.2	183	195
M_3~P_1	10.6	177	168.3	155	146.6	166	157.4
P_1~P_2	11.5	230	210	250	228.6	240	219.3

Artemia nauplii was very low in the cultures, which might be a limiting factor for further ingestion by the postlarvae.

Consumption rates of *P. japonicus* larvae feeding on *Brachionus plicatilis*, at approximately 10 rotifers·ml^{-1}, are shown in Table 1 and Fig. 1. In contrary with *Artemia* feeding, *P. japonicus* larvae consumed the highest amounts during zoea stages. During Z_2—Z_3 substage the amount of 270 rotifers·larva^{-1}·day^{-1} were ingested and attained a peak of 308 rotifers·larva^{-1}·day^{-1} during Z_3—M_1 substage. The highest ingestion of *Brachionus* by the prawn larvae was obtained during the same period (315 rotifers·larva^{-1}·day^{-1}). Thereafter, a continuous drop of *Brachionus* predation was recorded during the mysis substages attaining a level of 157 rotifers·larva^{-1}·day^{-1} at M_3—P_1 substage ; almost half the amount of that ingested during Z_3—M_1 substage. At P_1—P_2 an increase in *Brachionus* consumption was observed, but still remained at a much lower level than that obtained during Z_3—M_1.

Energy intake rate of the prawn larvae, shown in Fig. 2 and Table 2, were approximately estimated by converting the consumption of the prey organisms into ingested dry mass and by further calculation of the dry mass energy content. Dry mass and energy content of *Artemia* sp. nauplii was calculted as the mean dry mass and energy content of the nauplii hatched 24h and 48h after incubation of the cysts. Dry mass and energy content of the nauplii after 48h dropped to 9.6% and 17% respectively. By that time nauplii have already molted into instars II and III and, therefore, all the energy reserves of their yolk have been consumed[10]. Dry mass and energy content of *B. plicatilis* fed *C. saccharophila* are calculated according to YAMASAKI and HIRATA[11].

Energy intake rates obtained from *Artemia* predation were 2.6 J·larva^{-1}·day^{-1} during Z_3—

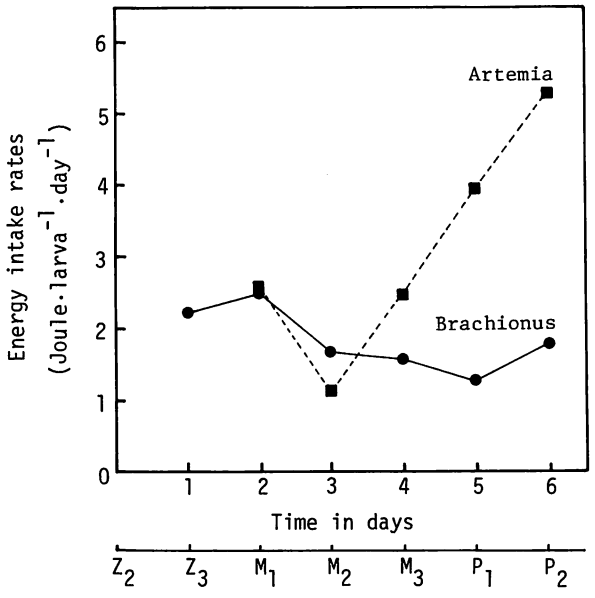

Fig. 2. Energy intake rates of *P. japonicus* larvae feeding on *Artemia* (□) or *B. plicatilis* (○).

Table 2. Daily ingested dry mass and energy intaks for the various developmental stages of *P. japonicus* larvae feeding on *B. plicatilis* and *Artemia* sp. nauplii. Dry mass (ash free) and energy values are calculated on : 0.414 μg mass per individual *Brachionus* (average of 0.37, 0.35, 0.52, 0.42 and 0.41 μg per individual *Brachionus* cultured in different densities of *Chlorella saccharophila* cultures ; YAMASAKI and HIRATA, 1985) ; 0.0047 cal/g ash free dry mass *Brachionus* (YAMASAKI and HIRATA, 1985) ; 1.89 μg per individual *Artemia* (average of 0.0056 and 0.0052 cal/g)

Stage of development	No of prey larva^{-1}·day^{-1}	Ingested dry mass μg·larva^{-1}·d^{-1}	cal·larva^{-1}·d^{-1}	J·larva^{-1}·d^{-1}
Brachionus plicatilis				
$Z_2 \sim Z_3$	270	111.78	0.53	2.22
$Z_3 \sim M_1$	308	127.51	0.60	2.51
$M_1 \sim M_2$	208	86.11	0.40	1.67
$M_2 \sim M_3$	195	80.11	0.37	1.59
$M_3 \sim P_1$	157	65.00	0.31	1.28
$P_1 \sim P_2$	219	90.67	0.43	1.78
Artemia				
$Z_3 \sim M_1$	60	113.40	0.61	2.55
$M_1 \sim M_2$	27	51.03	0.28	1.17
$M_2 \sim M_3$	58	109.62	0.59	2.47
$M_3 \sim P_1$	92	173.88	0.94	3.93
$P_1 \sim P_2$	124	234.36	1.26	5.27

M_1 substage, almost the same value as that obtained from *Brachionus* predation during the same developmental period of the larvae (2.5 J·larva^{-1}·day^{-1}). However, during *Artemia* feeding energy intake rates increased continuously from M_2-P_1 substages and reached a peak of 5.3 J·larva^{-1}·day^{-1}. During the same developmental period energy intake rates from *Brachionus* feeding decrease and attained a level of only 1.8 J·larva^{-1}·day^{-1} at P_1-P_2 substage.

Table 3 and Fig. 3 show the survival rates of each larval feeding regime, during the last experiment. Growth index, shown in Table 5 was not recorded from the first postlarval stage (P_1) since postlarval stages were described not according to morphological characteristics but counting from the day they metamorphosed to P_1 stage.

Survival rates and growth index were not significantly different among treatments ($p<0.01$) at termination of the experiment. In treatment I, where larvae were fed on *B. plicatilis* during Z_2-Z_3 substage and thereafter *Artemia* sp. nauplii, survival rates at each substage were relatively higher than in other treatments (except for the control). Larvae fed only on *Brachionus* had survial values at high levels and almost constant during Z_2-M_2 substages, but thereafter a continuous decline was observed to attain a level of 78 % at three-days-old postlarva (P_3). When larvae were fed only on *Artemia* nauplii from Z_2-P_3 substages survival was higher in comparison with treatment II but attained the same level at M_2 substage. Nevertheless, during postlarval stages survival remained constant at level of

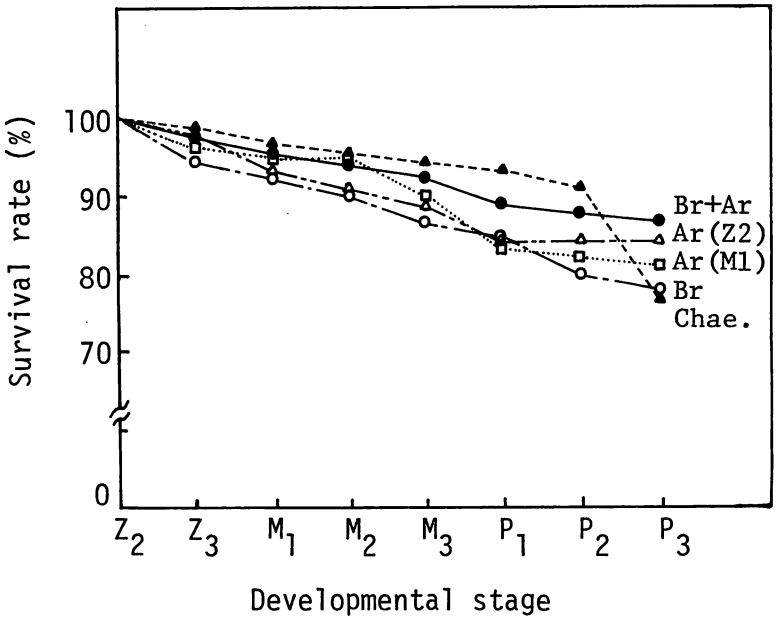

Fig. 3. Cumulative survival rates of *P. japonicus* at various feeding regimes : (●) *Brachionus* at Z_2-Z_3 and *Artemia* from Z_3-P_3, (○) *Brachionus* from Z_2-P_3, (△) *Artemia* from Z_2-P_3, (□) *Artemia* from M_1 and (△) *Chaetoceros* only from Z_2-P_3 stages (control).

Table 3. Cumulative survival rates at each substage of *P. japonicus* larvae reaerd on various feeding regimes from the second zoea substage (Z_2) to the 3-days-old postlarva (P_3). Larvae were fed on the diatom *Chaetoceros calcitrans* plus *Brachionus plicatilis* beginning at Z_2 to Z_3, and or *Artemia* sp. nauplii from Z_2 or M_1.

Larval stage	Survival (%) ± SD at each substage Treatments				
	I	II	III	IV	V
Z_3	97.8±3.1	94.5±3.1	97.8±1.6	96.7± 2.8	98.9± 1.6
M_1	95.6±3.1	92.2±6.3	93.3±2.7	95.6± 1.6	96.7± 0.0
M_2	94.4±1.6	90.0±4.7	90.0±0.1	95.6± 1.6	95.6± 1.6
M_3	92.2±4.2	86.7±2.7	88.9±1.6	90.0± 9.4	94.4± 1.6
P_1	88.9±4.2	84.4±4.2	84.5±3.1	83.3±11.9	93.3± 2.7
P_2	87.8±5.7	80.0±5.5	84.5±3.1	82.2±11.3	91.1± 5.7
P_3	86.7±5.4	77.8±6.9	84.5±3.1	81.1±10.3	76.7±14.4

Table 4. Growth index (absolute values) of *P. japonicus* larvae reared on various feeding regims from Z_2-P_3.

Treatment	Days	Larval Stage	Growth Index
I	1	Z_2~Z_3	2.9±0.05
	2	Z_3~M_1	3.3±0.12
	3	M_1~M_2	4.8±0.08
	4	M_2~M_3	6.0±0.00
II	1	Z_2~Z_3	2.8±0.14
	2	Z_3~M_1	3.4±0.12
	3	M_1~M_2	4.7±0.21
	4	M_2~M_3	6.0±0.05
III	1	Z_2~Z_3	2.8±0.08
	2	Z_3~M_1	3.4±0.22
	3	M_1~M_2	4.9±0.14
	4	M_2~M_3	6.0±0.00
IV	1	Z_2~Z_3	2.9±0.14
	2	Z_3~M_1	3.3±0.22
	3	M_1~M_2	4.6±0.42
	4	M_2~M_3	5.5±0.37
V	1	Z_2~Z_3	3.0±0.00
	2	Z_3~M_1	3.4±0.17
	3	M_1~M_2	4.5±0.21
	4	M_2~M_3	5.7±0.24

84.5%, while for *Brachionus* feeding larvae it was still declining. For those larvae fed *Artemia* from M_1 substage, survival rates were higher than in the other treatments up to M_3 substage, but dropped to 83.3% during P_1 substage ; during postlarval stages survival

Table 5. Average dry weights and body lengths of *P. japonicus* postlarvae (P_3) reared from the 2nd zoea substage on different feeding regims.

Treatments	Average dry weight at (P_3) stage ($\mu g \cdot larva^{-1}$)	Body length ($\pm$ SD) (mm)
I	294	7.8±0.5
II	312	7.6±0.2
III	325	8.1±0.4
IV	277	7.7±0.4
V	57.2	5.2±0.3

remained almost constant as in treatment I. In the control, where larvae were fed exclusively on *Chaetoceros calcitrans*, survival remained at surprisingly high level and dropped only at P_3 stage due to cannibalism.

The growth index was high in all treatments, the lower ones recorded in treatments IV and V (Table 4).

Growth, in terms of average dry body weight and total body length, was similar for all treatments except for the control, where it was significantly lower (Table 5). Dry body weight and body lenght, was similar, the highest being for those larvae fed *Artemia* nauplii from Z_2 to P_3 substages (325 $\mu g \cdot larva^{-1}$ and 8.1 mm, respectively). Nevertheless, no significant difference ($p<0.01$) was observed among the larvae of these vessels, in terms of body length (Table 5). Dry body weight of those larvae fed only on *Brachionus* was very similar with those larvae fed only on *Artemia*.

DISCUSSION

GOPALAKRISHNAN[11] found that *P. marginatus* larvae were able to consume *Artemia* sp. nauplii from the first protozoea substage. WILKENFELD et al.[12] showed that consumption of live *Artemia* nauplii started from the third protozoea substage for *P. setiferus* larvae. Predation experiments for *P. indicus* larvae showed that they were able to consume a significant amount of *Artemia* from the third protozoea substage[8]. However, YUFERA et al[13] reported that *P. kerathurus* ingested *Artemia* from the second mysis substage (M_2). The present results showed that *P. japonicus* larvae could consume a significant amount of *Artemia* from the Z_3-M_1 substage (60 naup$\cdot larva^{-1} \cdot day^{-1}$). Ingestion of the nauplii by the larvae was increasing with progressive development which has been reported by several workers[3,8,11,14,15]. The highest consumption of *Artemia* was obtained at a rate of 219 nauplii$\cdot larva^{-1} \cdot day^{-1}$ at an initial density of 5 nauplii$\cdot ml^{-1}$ during the postlarval stage (P_1-P_2). GOPALAKRISHNAN[11] reported an ingestion of 210 nauplii$\cdot larva^{-1} \cdot day^{-1}$ for *P. marginatus* postlarvae and the same initial prey density. EMMERSON[13] obtained a maximal ingestion of *Artemia* at a rate of 187 nauplii$\cdot larva^{-1} \cdot day^{-1}$ at 9 nauplii$\cdot ml^{-1}$ initial prey density during the postlarval stage. He[13] also concluded that a density of 5 nauplii$\cdot ml^{-1}$ limited ingestion by the larvae of *P. indicus*. Recently, YUFERA et al[14] reported a maximal ingestion of *Artemia*

nauplii between 77 and 100 nauplii·larva^{-1}·day^{-1} at food densities of 15−18 nauplii·ml^{-1} for *P. kerathurus* postlarvae. These differences found among the various reports are largely attributed to species characteristics, condition of the tested larvae, methodology and dry weights of the food organisms supplied to the larvae.

Results obtained from *Brachionus* feeding show that *P. japonicus* larvae were able to consume as much as 270 *Brachionus* ·larva^{-1}·day^{-1} at Z_2-Z_3 substage, with a maximal ingestion of 308 *Brachionus* ·larva^{-1}·day^{-1} during d Z_3-M_1 substage. During mysis stage a continuous decline of *Brachionus* ingestion was observed while predation of *Artemia* was increasing. This is attributed to the constantly small size of *B. plicatilis* while prawn larvae are developing and their thoracic appendages become less efficient in catching prey of small size. Similar results were found by EMMERSON[13] and YUFERA et al[14].

Recently, HIRATA et al.[16,17] have reported higher suvival rates, about 80−90%, of *P. japonicus* larvae fed frozen *Brachionus* at Z_1-M_3 substages and artificial diets at post larval stage. Especially, *Artemia* has been not supplied for the mass production of *P. japonicus* in several hatcheries in Japan. The results obtained in the present experiments might be suggested that *P. japonicus* larvae grow well by feeding the rotifer during zoea and misys stages.

Comparing the results of energy intake rates from *Brachionus* and *Artemia* feeding it is demonstrated that energy intake from *Artemia* predation is sufficient and almost the same with that from *Brachionus* predation during the late zoea and early mysis substages of the prawn larvae. EMEERSON[13] reported that energy intake from *Artemia* predation could overlap with that from filter feeding of the diatom *Thalassiosira weissflogii* by *P. indicus* larvae. Therefore, it was concluded that *Brachionus* could be dispensed with in practical mass culture of this prawn.

This conclusion is also supported by the results obtained from the last experiment of the present study, where it was shown that was no significant difference ($p<0.01$) in survival and growth rates and total body length of the larvae fed either on *Brachionus* and *Artemia*, *Brachionus* only or *Artemia* only from Z_2-P_3 substages. In parallel, the use of *Brachionus* as food for the prawn larvae is not excluded and can be mainly supplied during the late zoea and first mysis substage when *Artemia* cysts are not available in the market or when the cost of latter is high. In the present study it was also shown that there was no benefit by introducing *Artemia* from Z_2 instead of M_1 substage. Hence, *Chaetoceros calcitrans* can be fed to the larvae during the zoea stage and *Brachionus* or *Artemia* during the mysis stage. This has been the practice mass larval culturing in the prawn hatchery reducing in this way the cost by using *Brachionus* from mysis stage, since the technique of mass production of *Brachionus* has been already established in Japan[18,19]

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