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The Culture of *Biddulphia longicuris* and Its Use as a Food for *Penaeus monodon* Larvae

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Abstract - *Peneaus monodon* larvae reared to Postlarva-1 stage were fed with the diatom *Biddulphia longicuris*. A density of 5,000-10,000 cells·ml^{-1} of *B. longicuris* was maintained in larval rearing tanks. The mean survival rate from Zoea-1 to Mysis-1 larval stage was 88.7%, and Mysis-1 to Postlarva-1 stage was 91.6%. The Zoea larva metamorphosed to the Mysis stage within four days at a temperature of 29-31°C. The Zoea larvae were fed only the *B. longicuris*; a microencapsulated feed was given at the Mysis stage along with the same diatom.

Several types of phytoplankton are used as live food items in shrimp hatcheries all over the world. The most common species used in larviculture of penaeid shrimps are *Skeletonema costatum, Chaetoceros calcitrans, C. gracilis* and *Tetraselmis* spp. The nutritional values of these algae vary and the crude protein level of the above mentioned organisms ranges from 29.0 to 60.6% on a dry-weight basis (Rao 1983). In addition, brine shrimp (*Artemia*) instars are used as a live food organism for Mysis and Postlarval stages.

Our study was conducted to determine the suitability of *Biddulphia longicuris* as a food organism for *Penaeus monodon* larvae, since this diatom is commonly found in coastal waters of Sri Lanka.

B. longicuris were collected from the sea by means of a plankton net. Particular species were isolated by the capillary pipette isolation method. Starter cultures were developed in an indoor plankton room using Guillard and Ryther's F medium. Mass cultures were developed in 10-tonne outdoor plankton tanks. The fertilizer mixture given in Table 1 was used in the development of large-scale unialgal cultures.

Table 1. Fertilizer mixture for mass production of *B. longicuris* in outdoor tanks.

Component	Concentration (ppm)
Ammonium sulfate	20.0
Dicalcium phosphate	10.0
E.D.T.A. disodium salt	1.0
Ferric chloride	0.1
Potassium nitrate	10.0
Sodium silicate	2.0
Urea	10.0

P. monodon larvae were reared up to postlarva-1 (PL-1) stage in 2.5-3.0 t cement tanks. Two runs with two replicates each were done. Nauplii were obtained from spawners collected from the sea. Nauplii of the same spawner were used in each replicate and they were stocked in filtered seawater at a rate of 50·l^{-1}.

Vigorous aeration was provided throughout the culture period. On the first day, the phytoplankton density of larval rearing tanks was about 1,000 cells·ml^{-1}. From the second day and onwards it was maintained at 5,000-10,000 cells·ml^{-1}. Additional *B. longicuris* were introduced daily when cell density dropped below 5,000 cells·ml^{-1}. In addition to diatoms, Mysis larvae were fed with a commercially available microencapsulated larval diet (MED) at a rate of 1.0 g per tonne per day. Cell density of *B. longicuris*, larval density, salinity, temperature, pH and dissolved oxygen level of the rearing water were measured daily. Everyday, a sample of larvae in each batch was examined through the microscope in order to determine the larval stage, health condition and feed consumption rate of the shrimp.

No antibiotics, fungicides, drugs, UV light or chemicals were used. Seawater was filtered by means of a sand filter bed. Rearing water was renewed 30-50% daily when the larvae reached the Mysis stage. Numbers of larvae were computed daily by random sampling.

The particular strain of *B. longicuris* could be seen either as single cells or as strands with two to three cells. Each cell was 10-90 μm in length and 5-50 μm in width. The biomass of the diatoms was 87.5% water and 12.5% organic matter (Table 2). *B. longicuris* mass cultures reached

Table 2. Chemical and nutritional composition of *B. longicuris*.

a. Water	87.5%
Anhydrous component	12.5%
b. Nutritional composition of anhydrous component	
Crude protein	36.3%
Crude fat	9.1%
Ash	41.6%

the stationary phase within 24 hours under sunlight. Salinity and temperature of the mass culture were 28-30 ppt and 27-31°C, respectively. Maximum cell density of the outdoor diatom cultures was 70,000 cells·ml^{-1} and the plankton culture remained in this phase for 60-72 hours. During the stationary phase, the diatom culture was brown to dirty green.

The Nauplii metamorphosed to Zoea on the second day of stocking. Mean survival rate from Nauplius to Zoea-1 (Z-1) was 92.2% with a maximum of 96% and a minimum of 88%. Survival rate from Zoea-1 to Mysis-1 (M-1) was 82.2-95.5%, mean 88.7% (Table 3).

Table 3. Survival rates of *P. monodon* larvae fed with *B. longicuris*.

Batch No.	Initial no. of larvae* Nauplius	Survival rates(%) N - Z.1	Z.1 - M.1	M.1 - PL.1
E-1	150,000	88.0	95.5	97.6
E-2	150,000	95.3	92.3	90.9
E-3	125,000	96.0	83.3	82.0
E-4	125,000	89.6	82.2	95.7
Mean value	137,500	92.2	88.7	91.6

*Computed to nearest thousand.

Feeding rate and the growth of Zoea and Mysis larvae seemed to be very good. Long fecal threads were observed during Z-2 and Z-3 larval stages.

According to our analysis, *B. longicuris* contains 36.3% protein on a dry-weight basis (Table 2). Nutritionally this diatom can be accepted as a suitable diet for penaeid shrimp larvae, although it falls near the lower end of the range for diatoms previously used in hatcheries (29-62%) (Rao 1983).

Yang (1975) observed that early Zoea larvae of penaeid shrimps can only ingest particles within the range 3-5 μm. However, the late Zoea larva can ingest feed particles of 200 μm (Tseng 1987). It was found in our study, though the *B. longicuris* cells were around 10-90 μm long, they were accepted and ingested by all the stages of Zoea larvae.

Mean survival rate from Zoea-1 to Mysis-1 stage of 88.7% appeared to be very good because the survival of Zoea larvae is comparatively lower than other larval stages. Sunaz (1980) observed

that survival rates of Zoea larvae of *P. monodon* fed with different diatoms were 8.95-62.9% (Table 5) and Tseng (1987) recorded it as 30-50%.

Zoea larvae fed with *B. longicuris* had a good growth rate and they metamorphosed to Mysis stage within 4 days (at a temperature of 29-31°C and salinity of 28-31 ppt). This indicates that food ingested by shrimp larvae consisted of adequate amounts of nutrients required for their growth.

The phytoplankton density of larval rearing water is another interesting point to be discussed. Usually in the larviculture of *P. monodon*, the recommended plankton density varies with the type of plankton, larval stage and culture system. With reference to algae such as *Chaetoceros* spp., *Skeletonema* spp., *Tetraselmis* spp. and mixed diatoms, the recommended cell densities in larval rearing tanks are 30,000-100,000 cells·ml^{-1} (New 1979), 50,000-100,000 cells·ml^{-1} (Sunaz 1980), 5,000-20,000 cells·ml^{-1} (Rao 1983), 30,000-

Table 4. Growth and survival of *P. monodon* larvae (E = batch no.).

Day of culture	Larval stage	Survival rate on initial stocking nos. (%)				
		E-1	E-2	E-3	E-4	Mean
01	Nauplius	100.0	100.0	100.0	100.0	100.00
02	Zoea-1	88.0	95.3	96.0	89.6	92.2
03	Zoea-2	86.0	87.0	85.0	78.9	84.2
04	Zoea-2	84.9	85.9	83.6	76.8	82.8
05	Zoea-3	84.5	82.0	80.0	73.5	80.0
06	Mysis-1	84.0	88.0	80.0	73.6	81.5
07	Mysis-2	84.0	80.0	80.0	70.0	78.5
08	Mysis-2	86.2	79.5	80.5	65.8	78.0
09	Mysis-3	83.7	80.0	70.2	73.3	76.8
10	Postlarva-1	82.0	80.0	65.6	70.4	74.5

Table 5. Survival and growth of *P. monodon* Zoea larvae fed different types of diatoms (from Sunaz 1980).

Treatments	Survival rate (%)	Zoeal period (days)
Mixed diatoms	60.43	5
S. costatum	8.95	6
C. gracilis	62.90	5
C. calcitrans	43.28	6

40,000 cells·ml^{-1} (Frippak hatchery practices, 1987) and 40,000-60,000 cells·ml^{-1} (Gold Coin hatchery feeds, 1991). The plankton density in our study was maintained at 5,000-10,000 cells·ml^{-1}, very low compared to the above mentioned recommendations. However, this did not affect the growth and survival of Zoea larvae.

The mean survival rate from Mysis-1 to Postlarva-1 stage was 91.7%. In addition to *B. longicuris*, Mysis larvae were fed with a microencapsulated diet which served as a supplementary feed at this stage. Therefore, a conclusion could not be reached on the effect of *B. longicuris* on the growth and survival of Mysis larvae. However, microscopical observations proved that *B. longicuris* cells were ingested by Mysis larvae.

According to the results and observations of this study, *B. longicuris* can be considered as a suitable diet for larviculture of *P. monodon*. In addition, high survival and growth rates can be obtained by using this diatom as a food organism for Zoea larvae.

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