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# Larval Growth and Survival of *Penaeus Indicus* (Decapoda: Penaeidae) On Live Feeds

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**Abstract:** This study was conducted to compare the suitability of three microalgal species (*Tetraselmis chuii*, *Skeletonema costatum* and *Rhinomonas reticulata*), both individually and in combination, as food for *Penaeus indicus* during larval culture. To determine the best feeding density, each alga was fed to protozoea 1 (PZI) larvae from 10 to 80 cells $\mu L^{-1}$.

The best algal-cell density, promoting the highest survival rate, greatest growth and fastest larval development, was obtained at 60-70 cells $\mu L^{-1}$ with a combination of *T. chuii and S. costatum*. Low cell densities (10-20 cells $\mu L^{-1}$) were not effective. The diatom *S. costatum* produced better larval survival and growth than those of the flagellates ($P<0.05$). *R. reticulata,* both individually and in combination with the other algal species, was not suitable for the culture of *P. indicus* at any of the cell densities tested. The addition of algae while feeding on *Artemia* during the mysis stages did not produce better results than feeding only on *Artemia* ($P>0.05$).

These results demonstrate that *P. indicus* PZI larvae, at 28°C and 25ppt salinity, can be reared successfully on the mixed algal feed of *T. chuii* (25 cells $\mu L^{-1}$) and *S. castatum* (35 cells $\mu L^{-1}$) together with five *Artemia* nauplii $\mu L^{-1}$ from MI onwards until metamorphosis in only 6-7 days.

Key Words: *Penaeus indicus*, shrimp, larvae, microalgae, live feed, culture.

## Canlı Yemlerle Beslenen *Penaeus indicus* (Decapoda: Penaeidae) Larvalarında Yaşama ve Büyüme

**Özet:** Bu çalışmada, üç farklı mikroalg türünün (*Tetraselmis chuii, Skeletonema costatum* ve *Rhinomonas reticulata*), bireysel yada karışık olarak verildiklerinde, hangisinin *Penaeus indicus* larvalarının beslenmesinde daha uygun olduğunu belirlemek amacıyla yapılmıştır. En uygun yemleme yoğunluğunu saptamak için, bu fitoplankton türleri, protozoea 1 (PZ1) larvalarına 10 ile 80 hücre $\mu L^{-1}$ arasında verilmiştir.

En yüksek yaşama oranı, en hızlı larval gelişim ve büyüme 60-70 hücre $\mu L^{-1}$ besleme yoğunluğunda ve *T. chuii* ile *S. costatum* kombinasyonunda elde edilmiştir. Düşük hücre yoğunluklarında (10-20 hücre $\mu L^{-1}$) larval yaşama ve büyüme, besin yetersizliği nedeniyle, çok düşük olarak gerçekleşmiştir. Diatomlardan olan *S. costatum*, test edilen diğer iki flagellat mikroalg türünden daha iyi larval yaşama oranı ve büyüme sağlamıştır ($P<0.05$). Hiçbir hücre yoğunluğunda, *R. reticulata*'nın, ne tek başına ve ne de diğer alg türleriyle birlikte beslemede, *P. indicus* larvalarının beslenmesinde uygun olmadığı bulunmuştur. Mysis dönemlerindeki beslemede, *Artemia*'ya ek olarak mikroalg vermeye devam edilmesi, aynı dönemde sadece *Artemia* ile yapılan beslemeden daha iyi sonuç vermemiştir ($P>0.05$).

Bu çalışmada, *P. indicus* larvalarının, PZ1 döneminden itibaren, *T. chuii* (25 hücre $\mu L^{-1}$) ve *S. costatum* (35 hücre $\mu L^{-1}$) karışımından ve ek olarak mysis 1'den itibaren de 5 *Artemia* $\mu L^{-1}$ bir yemleme ile 28°C ve ‰25 tuzlulukta 6-7 günde içinde postlarval döneme başarıyla ulaştırılabileceği belirlenmiştir.

**Anahtar Sözcükler :** *Penaeus indicus,* Karides, larva, mikroalg, canlı yem.

## Introduction

Penaeid larvae are generally cultured on live unicellular algae during the protozoeal stages and animal prey are added along with algal feeds during the mysis and early postlarval stages (1,2). It has been reported that several species of algae, e.g. the phytoflagellates *Tetraselmis* (3,4) and *Isochrysis* (5), and the diatoms *Skeletonema* (6,7), *Thalasiosira* (8,9) and *Chaetoceros* (5,9,10) are adequate food for penaeid larvae.

Larval cultures of *Penaeus indicus* have received considerably less attention than other commercially important penaeid species. The larvae of this species have been cultured successfully on various single algal species, such as *Thalassiosira weissflogii* (11), and on mixed algal diets, such as *Chaetoceros gracilis, Platymonas* sp., and *Isochrysis galbana*, and *Artemia* salina nauplii after the PZ3 stage (12). Aquacop (5) suggests the use of mixed algae, *Isochrysis* and *Chaetoceros,* in rearing *P. indicus* larvae. The optimal cell concentration for the growth and survival of penaeid larvae varies with the larval developmental stages and the cell size of the algal species used. Emmerson (11) obtained 96% survival at PL1 when he maintained the algal cell density of *T. weissflogii* at 7 cells $\mu L^{-1}$ between the PZ1 and PZ3 stages. Aquacop (5) recommends 100 cells $\mu L^{-1}$ of mixed algae *Chaetoceros* (20%) and *Isochrysis* (80%) between the PZ1 and PZ3 stages. Galgani and Aquacop (12) report that an algal cell density of 30-40 cells $\mu L^{-1}$ of *C. gracilis, Platymonas* sp., and *Isochrysis galbana* was sufficient to rear *P. indicus* larvae during the protozeal stages. Emmerson and Andrews (13) studied the effect of stocking density on the growth, survival and development of *P. indicus* and concluded that levels decrease with increasing larval stocking density.

Preliminary experiments with *P. indicus* larvae obtained in the School of Ocean Sciences, Menai Bridge, UK, have shown that mixed algae *(T. chuii/R. reticulata)*, which have been used successfully to rear *P. monodon* (4,14) at a cell density of 45-50 cells $\mu L^{-1}$, are not suitable for culturing *P. indicus.* Consequently, in this study, an evaluation of live diets was conducted to determine the best diet promoting high survival and rapid growth in *P. indicus*. In the first experiment, *Tetraselmis chuii, Rhinomonas reticulata* and *Skeletonema costatum* were tested individually and in combination from 10 to 50 cells $\mu L^{-1}$ from the PZ1 to the PL1 stage. In the second experiment, the best algal feeds were selected and the algal cell concentration was increased from 50 to 80 cells $\mu L^{-1}$ as the first experiment showed that the optimal algal cell concentration was in this higher range. The third experiment investigated the possibility of eliminating the algae completely during the mysis stages. A fourth experiment was conducted to investigate possible reasons why *R. reticulata* is

not a suitable live food either alone or in combination with other algae, for *P. indicus* larvae. The primary aim of this study was simply to ascertain the best of the available algal species and cell concentrations together with the optimum environmental conditions promoting maximum larval growth and survival in *P. indicus*.

## Material and Methods

### Experiment 1

*P. indicus* larvae were obtained from broodstock, originating from India, kept at the School of Ocean Sciences, Menai Bridge, UK., in two 10-ton circular tanks. Gravid females were spawned in 100-L tanks in filtered (0.2 μm) and U/V-irradiated seawater. Following the non-feeding nauplius stages, PZ1 (protozoea 1) larvae were stocked in filtered and U/V-treated seawater at 33.5 ppt salinity in 2-litre round bottom glass flasks, which were placed in a water bath at 28°C. Live monospecific algal cultures from three species, *Tetraselmis chuii* (Butcher), *Skeletonema costatum* (Greville) and *Rhinomonas* reticulata (Lucas), grown in a semi-continuous culture, as described by Walne (15), were fed to the *P. indicus* larvae singly and in combination (50% from each algal species) at cell densities of 10, 20, 30, 40 and 50 cells $\mu L^{-1}$ $day^{-1}$. The algal diets used in the experiment were; *T. chuii, S. costatum, R. reticulata, T. chuii/S. costatum, T.chuii/R.reticulata* and *R. reticulata/S.costatum.*

The algal cultures were maintained in the exponential-growth phase and Conway Medium was used as the source of nutrients (15). Every day the algal cell densities were estimated using a haemocytometer and/or a Coulter Counter (Model ZB: Coulter Electronics) both in the algal-culture medium and larval-culture flaks so that the desired experimental algal-cell densities could be maintained. Table 1 gives the description and nutritional contens of the algal species used in the experiments. Complete water exchanges were carried out every other day when 10-15 larvae were measured under a binocular microscope from the tip of the rostrum to the end of the tail (total length = TL), counted using glass pipettes and staged according to the method of Silas et al., (16). Five newly hatched (at 28°C and 34 ppt for 24 h) *Artemia* nauplii $\mu L^{-1}$ (cysts were obtained from INVE AQUACULTURE, Belgium) were fed to the larvae along with the algal feeds from PZ3/M1 onwards. Larval growth and survival were assessed from two replicates from the PZ1 to PL stages.

| Species | Cell size (μm) | Description | Protein (%) | Carbohydrate (%) | Lipid (%) | Ash (%) |
|---|---|---|---|---|---|---|
| *Tetraselmis chuii* | 10-15 | Flagellate | 48.80 | 24.70 | 4.3 | 22.2 |
| *Skeletonema costatum* | 8-10 | Diatom | 33.30 | 22.60 | 8.1 | 36.0 |
| *Rhinomonas reticulata* | 8-10 | Flagellate | 52.00 | 33.70 | 4.3 | 4.3 |

Table 1. Microalgal species and their nutritional contens (obtained from 17) used in the experiments.

### Experiment 2

The first experiment showed that the optimal cell density for *P. indicus* was higher than the range (10-50 cells $\mu L^{-1}$) tested. Hence, higher cell densities of 50, 60, 70 and 80 $\mu L^{-1}$ per day of mixed *T. chuii/S. costatum* and *S. costatum* singly were tested for growth and survival in this experiment. The cell density of *T. chuii* was kept constant at 25 cells $\mu L^{-1}$ in the mixed diets, whilst the cell density of *S. costatum* varied between 25 and 55 cells $\mu L^{-1}$. All other experimental procedures were indentical to the first experiment.

### Experiment 3

The effect of algae, together with live *Artemia* or without *Artemia,* on the growth and survival of *P. indicus* in the mysis stages was investigated in this experiment. The PZ3/M1 larvae, previously reared on live mixed algae (25 cells $\mu L^{-1}$ *T. chuii* and 35 cells $\mu L^{-1}$ *S. Costatum)* were stocked in 2-L experimental round-bottom glass flasks at a density of 75 larvae $L^{-1}$. Larvae were fed using three feeding regimes of mixed algae, mixed algae plus five *Artemia* $mL^{-1}$*, and five* Artemia $mL^{-1}$ only, from the PZ3/M1 to PL stages. The algal-cell density was increased from 60 cell $\mu L^{-1}$ (Z3/M1) to 70 (M2/M3) and finally to 80 cells $\mu L^{-1}$ (PL1) by increasing the cell density of *S. costatum* to ensure that there was sufficient food in the culture medium at all times without causing larval fouling. Complete water changes were performed in the flasks every day, when the staging, growth and survival measurements were carried out on two replicates of each feeding regime.

### Experiment 4

This experiment was conducted to test again the suitability of the red algae *R. reticulata* as food for *P. indicus* larvae. The growth and survival of the larvae were assessed from two replicates in 2-L flasks on *R. reticulata* at 50 cells $\mu L^{-1}$ and mixed *T. chuii/S. costatum* used as the control at 60 cells $\mu L^{-1}$ from the PZ1 to the M1 stage. PZ1 stage larvae were also starved in two flasks to determine how long they could survive without food. Two 2-L flasks were also set up without larvae to determine the settlement rate of the cells of *R. reticulata* (50 cells $\mu L^{-1}$) over a 24 h period. The water of the culture changed completely every day, when the larvae were measured for total length and counted. Larvae were observed continuously to ascertain whether they were ingesting and digesting the algae through examination of the guts and faeces under a microscope at each protozoeal stage.

### Statistical analysis

In the first and second experiments, the data for larval survival and growth according to algal feed at various algal-cell concentrations (10-50 cells $\mu L^{-1}$ and 50-80 cells $\mu L^{-1}$) were analysed with two-way ANOVA together with one-way ANOVA in separate analyses for algal species and cell concentrations at the PZ3/M1 and PL1 stages. One-way ANOVA was used in the statistical analysis of the data obtained in the third and fourth experi-

ments. Appropriate multiple pairwise comparison tests (Turkey's for equal sample sizes and Scheffé's method for unequal sample sizes) were carried out to determine any significant effects ($P \leq 0.05$). Before the statistical analysis, the data was checked for normality and homogeneity of variances using Bartlett's box test (18). All statistical analyses were conducted using Minitab statistical software.

## Results

### Experiment 1

### Growth and survival at M1 stage

Densities of 10 and 20 cell $\mu L^{-1}$, except for the combination of *T. chuii/S. costatum,* did not support survival and growth up to the M1 stage (see Table 2a). At 30, 40 and 50 cells $\mu L^{-1}$, the combination of flagellate and diatom *(T. chuii/S. costatum)* produced the highest survival and growth rates between the PZ1 and M1 stages ($P<0.05$). Irrespective of the cell densities used in this study, *R. reticulata* did not sustain larval survival up to the M1 stage. The results indicate that this algal species, either alone or in combination with the other algal species, is not suitable for *P. indicus.*

Table 2a. Survival (%) and growth of *P. indicus* larvae reared on algal feeds at cell concentrations of between 10 and 50 cells $\mu l^{-1}$ from the PZ1 to M1 stages. Each value is a mean ± s.d. (n=2). Means marked with different superscripts differ significantly with respect to each other ($P<0.05$).

| | Larval survival (%) ± s.d. (at mysis 1) | | | | |
|---|---|---|---|---|---|
| Algal species/ Cell concentration | 10 cells $\mu l^{-1}$ | 20 cells $\mu l^{-1}$ | 30 cells $\mu l^{-1}$ | 40 cells $\mu l^{-1}$ | 50 cells $\mu l^{-1}$ |
| *T. chuii* | 0 | 0 | $15.00^{b}$±1.41 | $38.50^{b}$±3.54 | $39.00^{c}$±2.8 |
| *R. reticulata* | 0 | 0 | 0 | 0 | 0 |
| *S. costatum* | 0 | 0 | $14.00^{b}$±2.83 | $39.50^{b}$±2.12 | $50.25^{b}$±3.18 |
| *T.chuii/R. reticulata* (1:1) | 0 | 0 | 0 | 0 | 0 |
| *S. costatum/R. reticulata* (1:1) | 0 | 0 | 0 | 0 | $10.50^{d}$±3.54 |
| *T. chuii/S. costatum* (1:1) | 0 | 28.00±2.83 | $50.50^{a}$±2.12 | $58.50^{a}$±4.95 | $79.75^{a}$±5.30 |
| | Larval growth (mm) ± s.d. (at mysis 1) | | | | |
| Algal species/ Cell concentration | 10 cells $\mu l^{-1}$ | 20 cells $\mu l^{-1}$ | 30 cells $\mu l^{-1}$ | 40 cells $\mu l^{-1}$ | 50 cells $\mu l^{-1}$ |
| *T. chuii* | 0 | 0 | $2.54^{b}$±0.02 | $2.7^{c}$±0.02 | $3.59^{c}$±0.01 |
| *R. reticulata* | 0 | 0 | 0 | 0 | 0 |
| *S. costatum* | 0 | 0 | $2.59^{b}$±0.04 | $3.33^{b}$±0.08 | $3.92^{b}$±0.03 |
| *T.chuii/R. reticulata* (1:1) | 0 | 0 | 0 | 0 | 0 |
| *S. costatum/R. reticulata* (1:1) | 0 | 0 | 0 | 0 | $2.85^{d}$±0.07 |
| *T. chuii/S. costatum* (1:1) | 0 | 2.58±0.08 | $3.14^{a}$±0.06 | $3.70^{a}$±0.05 | $4.08^{a}$±0.01 |

Table 2b. Survival (%) of *P. indicus* PL1 larvae reared on algal feeds at cell concentrations of between 10 and 50 cells $\mu l^{-1}$ together with five *Artemia* $ml^{-1}$ between the M1 and PL1 stages. Each value is a mean±s.d. (n=2). Means marked with different superscripts differ significantly with respect to each other (P<0.05).

| | Larval survival (%) ± s.d. (at postlarva 1) | | | | |
|---|---|---|---|---|---|
| Algal species/ Cell concentration | 10 cells $\mu l^{-1}$ | 20 cells $\mu l^{-1}$ | 30 cells $\mu l^{-1}$ | 40 cells $\mu l^{-1}$ | 50 cells $\mu l^{-1}$ |
| *T. chuii* | 0 | 0 | $10.00^{b}$±2.83 | $24.50^{b}$±2.12 | $27.00^{c}$±1.41 |
| *R. reticulata* | 0 | 0 | 0 | 0 | 0 |
| *S. costatum* | 0 | 0 | $6.50^{b}$±2.12 | $30.75^{b}$±5.30 | $41.50^{b}$±4.95 |
| *T.chuii/R. reticulata* (1:1) | 0 | 0 | 0 | 0 | 0 |
| *S. costatum/R. reticulata* (1:1) | 0 | 0 | 0 | 0 | 0 |
| *T. chuii/S. costatum* (1:1) | 0 | 11.00±3.54 | $44.50^{a}$±2.12 | $55.00^{a}$±7.07 | $76.50^{a}$±0.71 |
| | Larval growth (mm) ± s.d. (at postlarva 1) | | | | |
| Algal species/ Cell concentration | 10 cells $\mu l^{-1}$ | 20 cells $\mu l^{-1}$ | 30 cells $\mu l^{-1}$ | 40 cells $\mu l^{-1}$ | 50 cells $\mu l^{-1}$ |
| *T. chuii* | 0 | 0 | $4.72^{b}$±0.14 | $4.82^{c}$±0.14 | $5.13^{b}$±0.11 |
| *R. reticulata* | 0 | 0 | 0 | 0 | 0 |
| *S. costatum* | 0 | 0 | $4.64^{b}$±0.09 | $4.96^{b}$±0.13 | $5.19^{b}$±0.12 |
| *T.chuii/R. reticulata* (1:1) | 0 | 0 | 0 | 0 | 0 |
| *S. costatum/R. reticulata* (1:1) | 0 | 0 | 0 | 0 | 0 |
| *T. chuii/S. costatum* (1:1) | 0 | 4.95±0.06 | $5.05^{a}$±0.13 | $5.33^{a}$±0.13 | $5.65^{a}$±0.13 |

Higher growth and survival were supported as the algal-cell concentrations increased from 30 to 50 cells $\mu L^{-1}$. The highest growth and survival were obtained when the larvae were fed at 50 cells $\mu L^{-1}$, whereas the lowest growth and survival were observed at 30 cells $\mu L^{-1}$. The total length of larvae fed *S. costatum* was significantly greater (3.92 mm) than that of those fed on *T. chuii* (3.59 mm) at the cell concentration of 50 $\mu L^{-1}$ (Table 2a). The greatest growth (4.08 mm TL), however, was obtained with the mixed algae at 50 cells $\mu L^{-1}$. When *P. indicus* larvae were fed *R. reticulata* at the cell density of 50 cells $\mu L^{-1}$, 23% of the larvae developed into PZ2/PZ3 stages, but never reached the M1 stage. This algal species in combination with *S. costatum* realised only 10.5% survival up to the PZ3/M1 stages.

### Growth and survival at PL1 stage

The highest larval survival (77%) the and growth (5.65 mm TL) were obtained with the mixed algae (*T. chuii/S. costatum)* at cell density of 50 $\mu L^{-1}$ with five *Artemia* $mL^{-1}$ (P<0.05) at the PL1 stage (Table 2b). The was no significant difference (P>0.05) between the mean larval survival and growth on *T. chuii* and *S. costatum* in unmixed form. An increase in the algal-cell densities, regardless of the algal species, from 30 to 40 and 50 cells $\mu L^{-1}$ increased larval survival and larval growth (see Table 2b). There was no sig-

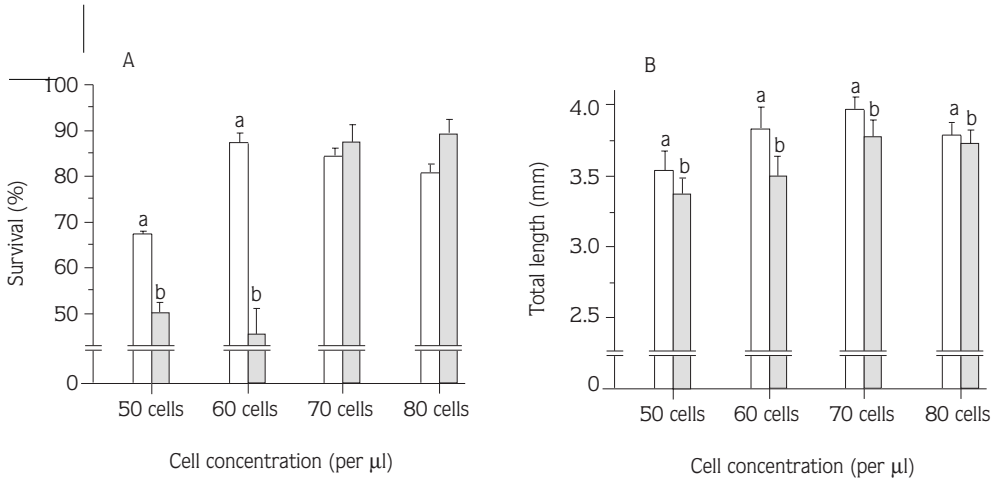


Figure 1. (a) Survival (%) and (b) total length (mm) of *P. indicus* larvae fed from PZ1 stage to M1 on *T.chuii/S. costatum* (open bar) and *S. costatum* only (hatched bar). All bars representing a mean ± s.d. marked with different superscripts differ significantly with respect to each other (P<0.05).

nificant difference (P>0.05) between the larval growth produced by different single algal diets with added *Artemia*. However, the larvae fed *S. costatum* exhibited higher survival than those fed *T. chuii* at metamorphosis.

### Experiment 2

### Growth and survival at M1

Greater total length and higher survival were obtained with mixed algae (*T. chuii/S. costatum*) than with the single diatom (*S. costatum*) at 50 and 60 cells $\mu L^{-1}$ in the M1 stage (P<0.05) (Fig. 1a, b). Higher cell densities (70-80 cells $\mu L^{-1}$) produced better larval survival and growth than lower cell densities (50-60 cells $\mu L^{-1}$) (P<0.05). The mixed algae (*T. chuii/S. costatum*) gave 67% survival and 3.54 mm TL at 50 cells $\mu L^{-1}$ compared to 50% survival and 3.37 mm TL with unmixed *S. costatum* (P<0.05). Similary, at 60 cells $\mu L^{-1}$, the larvae fed the mixed algae showed significantly better growth (3.84 mm TL) and a survival rate (87.50%) almost twice as high (P<0.05) as that obtained with the single algal diet which produced only 45.5% survival and 3.51 mm TL. Although the survival rates at 70 and 80 cells $\mu L^{-1}$ were not significantly different from each other, larval growth prouced by the mixed algae (*T. chuii/S. costatum*) was significantly better than that on *S. costatum* (P<0.05) (Fig. 1a, b). *S. costatum*, at cell densities of both 70 and 80 cells $\mu L^{-1}$, tended to aggregate in the larval-culture water, causing larval fouling. It was observed that a number of larvae were stuck together by their faecal strings. The feeding density of *S. costatum* at 50 and 60 cells $\mu L^{-1}$ was not sufficient for good larval growth and survival.

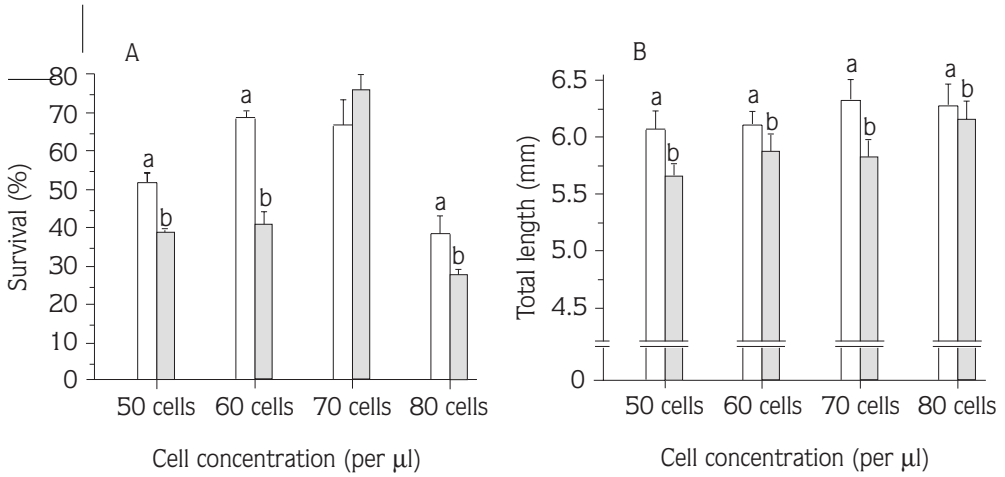


Figure 2. (a) Survival (%) and (b) total length (mm) of PL1 *P. indicus* larvae fed from the PZ1 stage on *T. chuii/S. costatum* (open bar) and S. costatum (hatched bar) plus 5 *Artemia* nauplii $ml^{-1}$ from M1 onwards. All bars representing a mean±s.d. marked with different superscripts differ significantly with respect to each other (P<0.05).

### Growth and survival at PL1

The mixed algae (*T. chuii/S. costatum*) with *Artemia* gave a higher survival rate and greater size at PL1 than *S. costatum* with *Artemia* (P>0.05) (Fig. 2a, b). The larvae fed the mixed algae (*T. chuii/S. costatum*) exhibited significantly better growth /6.07 mm TL) and survival (52%) (P<0.05) than *S. costatum* (5.65 mm TL, 39% survival) at 50 cells $\mu L^{-1}$. Larval survival (69%) and growth (6.1 mm TL) on the mixed algae at 60 cells $\mu L^{-1}$ were again significantly better than on *S. costatum* (41%, 5.87 mm TL) in the PL1 stage (p<0.05). At 70 cells $\mu L^{-1}$, survival with the mixed algae and single algal diet did not differ significantly (P>0.05). However, larval growth on the mixed algae was significantly better (6.33 mm TL) than on the diatom (5.82 mm TL) (P<0.05). At the highest experimental cell density, the mixed algal feed gave significantly better larval survival (58.5%) and growth (6.28 mm TL) than the single algal diet (28%, 6.16 mm TL) (P<0.05) (see Fig. 2a, b). As in the protozoeal stages, feeding *S. costatum* to the larvae at 70 and 80 cells $\mu L^{-1}$ fouled the appendages of the larvae, which subsequently caused mortality.

### Experiment 3

The larvae reared on algae from the M1 to the PL1 stage without *Artemia* displayed the lowest growth (5.37 mm TL) and survival (74%) on day 10 (Fig. 3a, b). The growth (6.64 mm TL) of algae/*Artemia*-fed larvae was not significantly different to that (6.72 mm TL) of larvae fed with *Artemia* alone. Presenting only the prey, *Artemia,* to the mysis larvae resulted in 98% survival compared to 92% in those receiving algae/*Artemia* (p>0.05). The larvae fed with *Artemia* and algae/*Artemia* started to metamorphose into the PL1

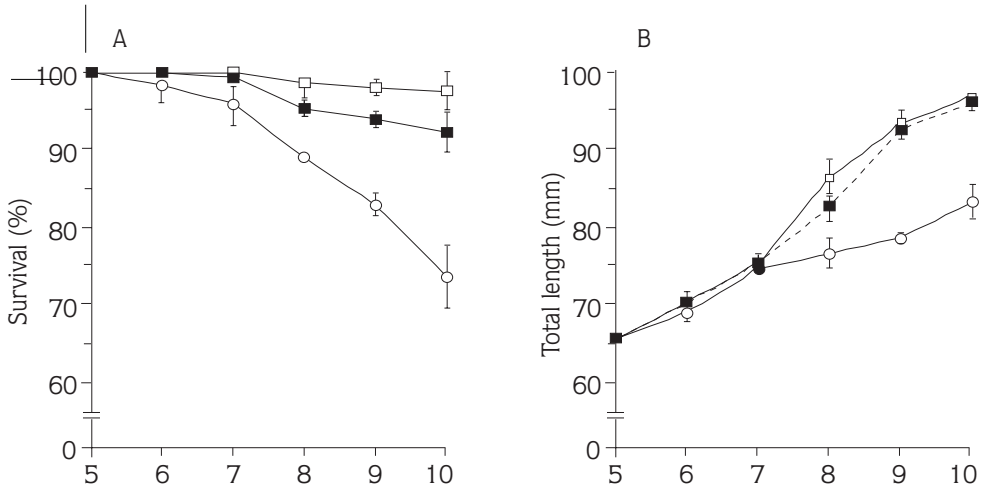


Figure 3. (a) Survival (%) (b) total length (mm) of *P. indicus* larvae fed from the M1 PL1 stage with the mixed algae (○), mixed algae plus *Artemia* nauplii (■) and only *Artemia* nauplii (□). Each symbol represent a mean±s.d. (n=2).

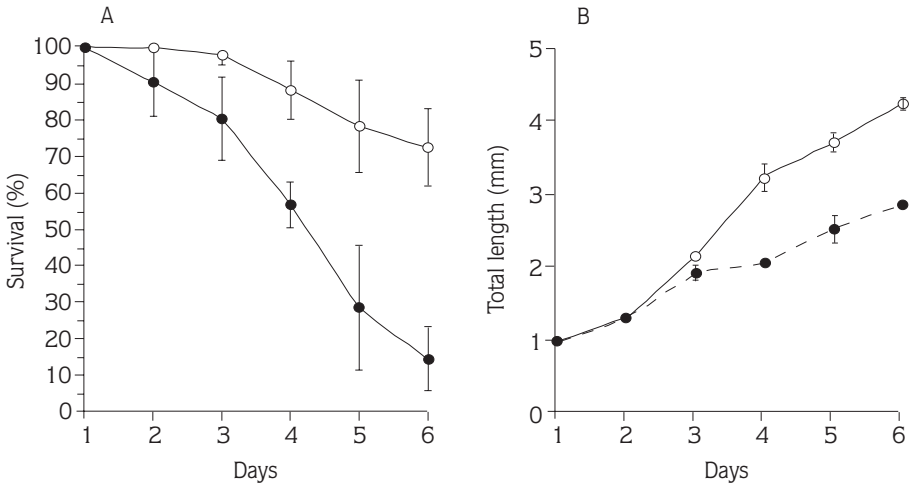


Figure 4. (a) Survival (%) and (b) total length (mm) of *P. indicus* larvae fed from the PZ1 to M1 stage with *T. chuii/S. costatum* (○) and *R. reticulata* (●). Each symbol represent a mean ± s.d. (n=2).

stage on day 8, whereas larvae fed with algae alone reached this stage on day 10.

### Experiment 4

From the first day of rearing, PZ1 larvae fed with *R. reticulata* exhibited lower survival and growth compared to those fed with the mixed algae. Larval survival with the mixed algae and *R. reticulata* on day 6 were 73% and 15%, respectively (Fig. 4a, b). Larvae fed with the mixed algae grew to 4.26 mm TL, whereas those fed on the single algae reached

only 2.87 mm TL. On day 6, the larval stages on *R. reticulata* were 95% PZ3 and 5% PZ2. All the larvae on the mixed algae were, however, at the M1 stage. Starved larvae (PZ1) completely perished 24 h after they were stocked in the experimental flasks.

Observations of the larval guts and faeces showed that the larvae ingested and digested the cells of *R. reticulata*. When PZ1, PZ2 or PZ3 larvae on *R. reticulata* were sampled, the guts full of the red algae were clearly visible. No undigested cells were observed in the larval faeces at any stage during the protozoeal stages. The cells of *R. reticulata*, in control flaks without larvae, slightly increased from 50 cells $\mu L^{-1}$ to 57 cells $\mu L^{-1}$ over 24 h.

**Discussion**

The data above indicate that low cell concentrations of either single algal feeds or mixed algal diets do not produce satisfactory larval growth and survival in *P. indicus*. The flagellate *T. chuii* and diatom *S. costatum* fed in unmixed form at 10-20 cells $\mu L^{-1}$ did not enable survival further than the PZ1/PZ2 stages, suggesting that this penaeid species cannot be reared successfully at such low cell densities using these rearing methods. However, when the mixed algal (*T. chuii/ S. costatum*) diet was given at 20 cells $\mu L^{-1}$, 28% survival was obtained in the M1 stage. Emmerson (11) reports very good survival (96%) and normal larval development using *T. weissflogii* (10.7 μm in diameter) at 7 cells $\mu L^{-1}$ in the culture of *P. indicus*, but at a stocking density of only 35 larvae $L^{-1}$ in large (70-L) culture vessels at 26°C. It appears that the higher larval stocking density (100 larvae $L^{-1}$) used in the study presented here produces a grazing demand which cannot be met at low cell densities. Amjad (19) reports total mortality in *P. monodon* larvae at a cell density of 10 cells $\mu L^{-1}$ and low survival at 20 cells $\mu L^{-1}$ (*T. chuii/R. reticulata*) in similar culture conditions to those described here. Aquacop (5) suggests that a cell density of 100 cells $\mu L^{-1}$ of mixed algae is required at high larval stocking densities (100-120 larvae $L^{-1}$), whereas Galgani and Aquacop (12) recommend 30-40 cells $\mu L^{-1}$ algae at a larval stocking density of 100 $L^{-1}$ during the protozeal culture of *P. indicus.* As cell density was increased from 30 to 40 and then 50 cells $\mu L^{-1}$, the larval survival and growth of *P. indicus* progressively increased. This indicates that a low algal-cell density cannot provide sufficient nutrients or energy at 100 larvae $L^{-1}$ for *P. indicus.* Kurmaly et al., (4) suggest that *P. monodon* PZ1 larvae at this stocking density require 45 cells $\mu L^{-1}$ per $day^{-1}$ for optimal survival and growth. In the experiments described in this paper, this level is reached at 60-70 cells $\mu L^{-1}$ per $day^{-1}$. Hence, levels below these are inadequate for larval growth, survival and development. Low survival and growth obtained during the protozoeal stages affected the subsequent results at metamorphosis, despite the fact that the larvae were fed with *Artemia* nauplii from PZ3/M1 onwards.

The evaluation of algae as live feeds for penaied larvae is generally based on the selection of species that sustain the maximum growth, survival and development. This study suggests that, of the three unicellular algal species tested, the diatom *S. costatum* promotes better larval growth, survival and development throughout all larval stages than the flagellate *T. chuii* (10). It is known that *S. costatum* is one of the most suitable live diets

for penaied larvae during the protozoeal stages (20) and is therefore commoly used in hatcheries (21). The results of the second experiment also confirm that is true for *P. indicus* larvae. However, it has been observed that *S. costatum* at high cell concentrations (70-80 cells $\mu L^{-1}$) causes mechanical fouling that may hamper the feeding and respiration of penaeid larvae (21, 17). Liao et al. (22) report that the exclusive use of *S. costatum* may be harmful to penaeid larvae if the alga is harvested in or after the stationary growth phase. The different growth and survival reponses of penaeid larvae to the algal feeds may be due to variations in the nutritive value, cell size, digestibility or chemical composition of the algal species used (10). In this study, it is unlikely that the cell size of the algal feeds was inappropriate for the larvae (see Table 1).

Some algal species, such as *Chorella* sp. *Pavlova* sp., are known to be nutritionally poor for most aquatic species (19, 23). The nutritive value of microalgae may vary greatly even within the same species, depending on culture conditions and the time of harvest (24). The macronutrient content and fatty-acid profile of the algal species used in this study are summarised by Kurmaly et al. (17). The quantities of these macronutrients in all the algal species are within the range required for penaeid larvae (17, 25). It is not always correct to give the nutritional contents of algae as the only reason for poor larval performance with algal feeds. For example, when Rodriguez et al. (25) fed *P. japonicus* mysis larvae on *Chaetoceros gracilis* containing only 7% protein and separately on *Artemia* with a much higher protein content, they obtained no significant differences in the growth, survival, protein or lipid content of the postlarvae. Hence, rearing penaeid larvae on more than one algal species with a wide diversity of macronutrients and micronutrients, such as vitamins, has a better chance of meeting nutritional requirements (19). In this study, the combination of *T. chuii* and *S. costatum* consistently produced significantly superior larval growth and survival compared to single algal feeds ($P<0.05$) in both the first and second experiments. Hence, these results confirm that using mixed algal species helps to ensure good results (12, 17).

The results show that *R. reticulata,* even at 50 cells $\mu L^{-1}$, did not lead to good survival and growth further that the PZ3/M1 stages. However, Kurmaly et al., (17) and Amjad (19) report that this alga produced higher survival and better growth in *P. monodon* than *T. chuii* and/or *S. costatum* when fed in unmixed form. On the evidence of these results and those of Kurmaly et al. (17) and Amjad (19), it is difficult to see why *R. reticulata* is such a suitable food for *P. monodon*, but is inadequate for *P. indicus*. Observations of the guts of the larve fed with *R. reticulata* and their faeces under a microscope revealed that this alga was ingested and digested. In addition, this alga, either unmixed or in combination with *T. chuii*, induces high larval trypsin activity when fed to *P. indicus* larvae (26). The results of the fifth experiment also showed that the cell numbers of this algal species remain approximately constant for a period of 24 h in 2-L flasks without larvae. Moreover, when high larval mortality was observed in the PZ2/Z3 stage with *R. reticulata* at 50 cells $\mu L^{-1}$, the larval guts were full of algae, and larval faeces were also clearly visible. Heavy larval mortality using *R. reticulata*, both unmixed and in combination with other algae, suggests that this alga may be nutritionally inadequate for *P. indicus* larvae. Therefore, this

study demonstrates that penaeid larvae show species-specific differences between algae.

Data from the third experiment suggest that there may not be any benefit to *P. indicus* larvae in feeding algal diets during the mysis stages as larvae fed only with *Artemia* from the PZ3/M1 to PL stages displayed equal growth and better survival than those fed with algae and *Artemia.* The results of the second experiment have confirmed this with *P. indicus* larvae. Studies with other penaeid larvae, such as *P. marginatus* (27), *P. aztecus, P. setiferus* and *P. vannamei* (9), have also shown that the exclusive use of algae throughout all the larval stages results in lower growth and delayed metamorphosis, although comparable survival rates can be achieved. Rodriguez et al. (25) obtained significantly higher growth and survival when they fed *P. japonicus* larvae with alga (*C. gracilis*) throughout the larval stages together with *Artemia* during the mysis stages, as opposed to larvae fed with only the alga. In their study, the survival and growth of larvae receiving either alga or *Artemia* during the mysis stages did not differ significantly.

The study presented here demonstrates that *Penaeus indicus* can be reared successfully from the PZ1 to the PL1 stage within only 6-7 days at 25 ppt salinity and 27-28°C using a mixed algal diet of *Tetraselmis chuii* (25 cells $\mu L^{-1}$) and *Skeletonema costatum* (35-45 cells $\mu L^{-1}$) with five *Artemia* $mL^{-1}$ after the PZ3/M1 stage.

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