Marine Biology (1999) 133: 621–633

F. M. L. D'Souza · N. R. Loneragan

Effects of monospecific and mixed-algae diets on survival, development and fatty acid composition of penaeid prawn (*Penaeus* spp.) larvae

Received: 22 April 1998 / Accepted: 3 December 1998

Abstract Four species of microalgae (*Chaetoceros muelleri*, *Tetraselmis suecica*, Tahitian *Isochrysis* sp. (T-*iso*) and *Dunaliella tertiolecta*) with distinctly different fatty acid profiles were grown in continuous culture and fed to prawn larvae (*Penaeus japonicus*, *P. semisulcatus* and *P. monodon*) as monospecific diets. The best two diets (*C. muelleri* and *T. suecica*) were also fed as a mixed diet. Experiments were run until the larvae fed the control diet of *C. muelleri* metamorphosed to Mysis 1. The survival and development (i.e. performance) of the larvae were affected by algal diet, and the diets were ranked in the order of decreasing nutritional value: *C. muelleri* ≥ *T. suecica* > T-*iso* > *D. tertiolecta*. Larvae fed a mixed diet of *C. muelleri* and *T. suecica* (2:3 by dry weight) performed as well or better than those fed *C. muelleri*, and the performance of both these groups of larvae was better than those fed *T. suecica*. The lipid and carbohydrate compositions of the algae had little or no effect on the lipid and carbohydrate compositions of the larvae or their performance. However, the larvae that performed best (i.e. those fed *C. muelleri*) had significantly more lipid and carbohydrate than those that performed worst (i.e. those fed *D. tertiolecta*). Larvae fed *C. muelleri* or the mixed-algae diet had higher proportions of the essential fatty acids eicosapentaenoic acid [EPA, 20:5(*n*-3)] and arachidonic acid [ARA, 20:4(*n*-6)] than the larvae fed on other diets. Furthermore, the larvae fed *T. suecica*, which showed intermediate performance between larvae fed *C. muelleri* and T-*iso* or *D. tertiolecta*, also had higher proportions of EPA and ARA. Both *C. muelleri* and *T. suecica* contained EPA and ARA, but T-*iso* and *D. tertiolecta* did not, except for trace amounts of EPA in T-*iso*. The fatty acid ARA appears to be much more important in the diet of larval prawns than has so far been considered. The level of the essential fatty acid docosahexaenoic acid [DHA, 22:6(*n*-3)] in the algal diet and the larvae was not related to the performance of the larvae; only *C. muelleri* and T-*iso* contained DHA. However, the nauplii contained large proportions of DHA, suggesting that these were sufficient to meet the larval requirements for DHA during their development to Mysis 1. Mixed-algae diets could improve the performance of larvae by providing a more comprehensive range of fatty acids.

Communicated by G.F. Humphrey, Sydney

F.M.L. D'Souza
Cooperative Research Centre for Aquaculture,
PO Box 120, Cleveland, Queensland 4163, Australia

F.M.L. D'Souza (✉) · N.R. Loneragan
CSIRO Marine Research,
PO Box 120, Cleveland, Queensland 4163, Australia
Fax: 0061 (0)73826 7222
e-mail: frances.dsouza@marine.csiro.au

Introduction

Phytoplankton are the main food of larval stages of some crustaceans (Preston et al. 1992), of all the growth stages of bivalves (e.g. Frankish et al. 1991), and of the early growth stages of some fishes (Reitan et al. 1994).

Detailed studies have been carried out on the nutritional value of microalgae to bivalve larvae (e.g. Thompson and Harrison 1992). Differences in the composition of protein, lipid, and particular fatty acids in the algal diet were associated with different growth rates and different biochemical compositions of the larvae.

However, little work has been done on the nutritional requirements of prawn larvae. There are large differences between the survival and growth of prawn larvae fed different species of algae (Chu and Lui 1990; Naranjo et al. 1995). For example, both are high when the larvae are fed *Chaetoceros gracilis* (Simon 1978), but low when they are fed *Dunaliella tertiolecta* (Kurmaly et al. 1989). Some mixed-algae diets have resulted in higher survival and faster development of larvae than the component species alone (Kurmaly et al. 1989). Assuming that the toxicity, size, shape, and digestibility of the cells are the same or are not a contributing factor, the difference has

been attributed to the nutritional compositions of the algae (Webb and Chu 1983). To determine which components in an alga provide nutritional value to prawn larvae, three major parameters should be measured: the simultaneous biochemical compositions of algae and of larvae, and the growth response of the larvae. Several studies have measured one or two of these parameters, but not all three (e.g. Tobias-Quinitio and Villegas 1982).

The gross biochemical composition (protein, lipid and carbohydrate) of the diets has, alone, not explained the growth performance of prawn larvae (Villegas and Kanazawa 1979; Tobias-Quinitio and Villegas 1982). Therefore, the quality (amino acid, monosaccharide and fatty acid composition) rather than the quantity of gross biochemical constituents may be more significant.

Unlike later development stages, little is known about the essential fatty acid requirements of prawn larvae (Jones et al. 1979). Four fatty acids are believed to be essential for juvenile penaeid prawns: 18:2(*n*-6), 18:3(*n*-3), 20:5(*n*-3) and 22:6(*n*-3) (Kanazawa and Teshima 1977; Kanazawa et al. 1977, 1978, 1979 b,c). The larvae have a greater ability than the juveniles to convert 18:3(*n*-3) to highly unsaturated fatty acids (HUFAs) (Teshima et al. 1992), indicating differences in the metabolism of larvae and juveniles. A fifth fatty acid, arachidonic acid [20:4(*n*-6)], may be essential for adult penaeid prawns (Xu et al. 1994a).

The aim of this study was to assess the nutritional value of four species of algae (*Chaetoceros muelleri*, *Tetraselmis suecica*, *Isochrysis* sp., *Dunaliella tertiolecta*) fed as monospecific diets to penaeid prawn larvae. The biochemical compositions of the larvae and of their algal diets were measured and related to the larvae's survival and development. The two best algal diets (in terms of survival and development of the larvae) were also fed to the larvae in combination. We explored the possibility that the nutritional value of the microalgae is related to the content of lipid, carbohydrate and the fatty acids 18:2(*n*-6), 18:3(*n*-3), 20:4(*n*-6), 20:5(*n*-3) and 22:6(*n*-3). As far as we know, this is the first study to simultaneously analyse the prawn larvae and their algal diets as a means of comparing the nutritional value of different diets.

Materials and methods

Algal culture

Cultures of microalgae were obtained from the CSIRO Microalgae Culture Collection, CSIRO Marine Research, Hobart, Tasmania. The digestibility of all of the species was assumed to be high because they have been fed to prawn larvae (Sánchez 1986; Kurmaly et al. 1989; Jackson et al. 1994; D'Souza 1998) and bivalves (Ukeles and Wikfors 1988) with reasonable success. Axenic continuous cultures of *Tetraselmis suecica* (Kylin) Butcher (CS-187), *Isochrysis* sp. (T-*iso*) (CS-177), *Dunaliella tertiolecta* Butcher (CS-175) and non-axenic *Chaetoceros muelleri* Lemmermann (CS-176) [previously known as *C. gracilis* in all publications by M.R. Brown, e.g. Brown (1991)] were established in 20-litre carboys containing Medium *f* (Guillard and Ryther 1962). Natural seawater for this medium (from 10 m depth, ~35‰ *S*) was filtered through a sand filter (~1 mm gravel, giving 100 μm effective pore size), followed by a 1 μm and a 0.2 μm cartridge filter. The cultures were maintained at 18 °C and diluted with seawater at a rate of 20% d^{-1}. Nutrients were dosed hourly at a rate that produced an average incoming nutrient concentration of 67% that of the standard medium *f*. Illumination (12 h light: 12 h dark) of both sides of the culture was provided by two racks of four fluorescent lights (Osram Fluora), providing 180 μmol photons m^{-2} s^{-1} (average of top, bottom and middle measurements on the outside of the culture, Licor 4π PAR spherical sensor). The culture was aerated and the pH was regulated electronically between 7.8 and 8.0 by a pH controller dosing with food-grade CO_2. Cell density in the cultures was monitored with a Neubauer haemocytometer. The same continuous culture of each of the species of alga was used for Trials 1 and 2. The same *C. muelleri* culture was also used for Trial 5. A second *C. muelleri* culture was used for Trials 3 and 4. Two more *T. suecica* cultures were established: one for Trials 3 and 4 the other for Trial 5.

Larval culture

Adult females from three species of penaeid prawn were collected by commercial fishers from the coast of northern Queensland, Australia, and flown to the laboratory where they spawned. *Penaeus japonicus* Bate were trawled offshore of Mackay (21° 9′S; 149° 9′E) in January and February 1995; *P. semisulcatus* de Haan from Albatross Bay (12° 40′S; 141° 43′E) in May 1994; and *P. monodon* Fabricius offshore of Cairns (16° 55′S; 145° 44′E) in April 1995.

The nauplii were stocked at a density of 250 larvae l^{-1} in 3-litre glass tubes in temperature-controlled environmental cabinets. The larvae were grown in the glass tubes with 0.8 μm-filtered seawater at 27.3 °C ± 0.3 C°, and fed to excess with the appropriate alga at the following densities: *Chaetoceros muelleri* at 1×10^5 cells ml^{-1} [density used by Villegas and Kanazawa (1979) and by Australian prawn hatcheries]; *Tetraselmis suecica* at 3×10^4 cells ml^{-1} [density determined in a preliminary trial by D'Souza (1998)]; T-*iso* at 1×10^5 cells ml^{-1} and *Dunaliella tertiolecta* at 5×10^4 cells ml^{-1} [densities chosen by comparing their cell volumes (Smayda 1978) with those of *C. muelleri* and *T. suecica*]. The two species forming the mixed-algae diet were fed at half of their densities in the single-alga diet: *C. muelleri* (0.5×10^5 cells ml^{-1}) and *T. suecica* (1.5×10^4 cells ml^{-1}). The cell densities in the larvae-rearing vessels were adjusted to the desired level every 24 h. The tubes were aerated from the base to provide mixing as well as oxygen. The guts and faeces of the larvae were examined microscopically to confirm that the different alga cells were being ingested and digested. The experiment was stopped when the larvae fed *C. muelleri* metamorphosed to Mysis 1 (M1) in ~4 to 5 d. Three control tubes containing starved larvae were also monitored in each trial to confirm that nutritionally useful particles had been removed from the seawater: the starved individuals did not metamorphose to Protozoea 2 (PZ2).

At the end of the experiments, larvae from three tubes were concentrated into one tube for each replicate. Estimates of survival and development were made by taking 3 × 200 ml subsamples from these pooled larvae and preserving them in a neutral buffered (i.e. boric acid-saturated) solution of 10% formaldehyde in seawater. Later, the larvae were counted under a stereomicroscope and their stage of development (as described in Dall et al. 1990) was recorded.

The development of the larvae was followed in more detail during Trials 1, 2 and 5. The stage of development of the larvae from each alga treatment was recorded when the larvae fed *Chaetoceros muelleri* moulted to each successive stage. A 200 ml subsample was taken from a rearing vessel, and the larvae were concentrated on a mesh screen, gently washed into a glass petri dish, and staged under a stereomicroscope. A development index was calculated by the following formula (Villegas and Kanazawa 1979): development index = $A \div$ total number of larvae staged, where A = stage value × number of larvae at that stage. The stage values increase as the larvae moult through the protozoeal

substages and metamorphose to M1 with values for PZ1 = 1, PZ2 = 2, PZ3 = 3 and M1 = 4. Therefore, the greater the proportion of later-stage larvae in a treatment, the higher the development index.

To obtain samples for dry weight and biochemical analysis, the remaining larvae from the pooled tubes were concentrated on 142 μm Nytal mesh screen, rinsed briefly with distilled water to remove external salts, and stored in glass vials at −80 °C.

Experimental design

The experiments were designed to investigate the effect of different alga diets and different spawnings on the survival, growth and biochemical composition of the larvae. Although different species of prawns were used for some of the trials, previous work had shown that there is more variability is survival of larvae between spawnings than between species (D'Souza 1998).

Two series of experiments were undertaken: in the experiment with the single-alga diet, larvae were fed one of four species of algae; in the experiment with the mixed-algae diet, larvae were fed one of two species of algae alone (as a control) or in combination (Table 1). In the single-diet experiment, *Chaetoceros muelleri*, *Tetraselmis suecica*, T-*iso* and *Dunaliella tertiolecta* were fed to *Penaeus japonicus* larvae from single spawnings of different female, in two trials (Trials 1 and 2), i.e. Trial 1 = larvae from one female, Trial 2 = larvae from a second female. In the mixed-diet experiment, *C. muelleri*, *T. suecica* or a mixture of the two (equivalent to 2:3 by dry wt), were fed to larvae from single spawnings of different females, in three trials (Trials 3 and 4 with *P. semisulcatus* and Trial 5 with *P. monodon*). *C. muelleri* and *T. suecica* were chosen for the mixed diet because survival and development were greatest in larvae fed these two species.

There were three larval replicates per treatment, except in Trial 1 where only two replicates were used because of a small spawning (Table 1). The prawns from three larval rearing tubes were pooled for each replicate at the end of the trials. One sample per replicate was taken for larval biochemical analyses. Three samples per replicate were used to estimate survival and development at the end of the trials. In addition, two samples per treatment were taken more frequently during Trials 1, 2 and 5 to record the development of the larvae in more detail (see preceding subsection "Larval culture").

During the trials, three samples were collected for biochemical analyses of each of the continuous alga cultures on Days 2 and 4, except in Trial 1 for *Dunaliella tertiolecta*, where there was insufficient culture to sample on Day 4. The mean of the three samples from each day was treated as a replicate for each alga within a trial.

Biochemical analyses of algae and larvae

Samples of the algae for biochemical analysis (carbohydrate, lipid and individual fatty acids) were taken every 2 d, starting on the first day that the prawn larvae were fed. It was assumed, on the basis of Thompson and Harrison's (1992) work, that there was little or no change in the biochemical composition of the algal cells over a 24 h period. The dry weight of the algae determined by filtering a known volume of algal culture (~100 ml) onto precombusted (450 °C, 2 h), tared glass-fibre filters (Whatman GF/C, 4.7 cm diam) and washing with 0.5 *M* ammonium formate to remove residual salts from the seawater medium. The filters were then dried at 60 °C for 24 h, cooled under vacuum for 1 h, and weighed to the nearest 0.1 mg. Carbohydrates and lipids were extracted from the same sample of alga; the same volumes and method of collection as for dry weight analysis were used, except that the filtered algae were not dried.

Before each trial, a sample of the nauplii was collected for fatty acid analysis only, since the spawnings were too small to measure all the biochemical components.

A relationship was established between wet weight and and dry weight of larvae for a given sample. The was done by taking a known wet weight of larvae and then drying them on polycarbonate filters (Nuclepore, 10 μm pore size), following the same washing and drying protocol as for algae dry weights. This relationship was used to calculate the dry weights of larvae from wet weights of subsamples taken for biochemical analyses. Carbohydrate and lipid were extracted from the same sample of larvae.

Lipids were extracted on ice with chloroform:methanol (2:1 v/v; 3 × 5 ml extractions) by Folch et al.'s (1957) method. Filter papers were sonicated with the addition of 0.2 ml dimethyl sulfoxide to aid the disruption of the cells (Brown 1991), and 0.1 mg butylated hydroxytoluene to prevent oxidation of the lipids. The dried lipid was weighed to the nearest 1 μg.

Fatty acid methyl esters were prepared from the extracted lipid by Morrison and Smith's (1964) method and an internal standard (21:0, Nu-Check Prep, Minnesota) was included in each sample.

The fatty acid methyl esters (FAMEs) were separated on a Hewlett-Packard (HP) 6890 capillary gas chromatograph with a split/splitless injection system, flame ionisation detector, and HP Chemstation integrator. Separations were carried out on a J and W Scientific DB-23 fused silica capillary column (30 m × 0.25 mm internal diam × 0.25 μm coating), with nitrogen as a carrier gas, at a flow rate of 0.9 ml min^{-1} and a split ratio of 50:1. The injector and detector temperatures were 250 °C and the oven temperature was programmed to start at 160 °C (hold for 10 min) and rise at 3 °C min^{-1} to 210 °C, where it was held for 7 min. Peaks were identified from retention times relative to the internal standard (21:0). Standards for 31 other FAMEs (Sigma) were run in combination with the internal standard to obtain relative retention and calibration-curve data.

The fatty acids were grouped in the tables according to the number of double bonds present: saturated fatty acid (SFA) = no double bonds, monounsaturated fatty acid (MUFA) = one double bond, polyunsaturated fatty acid (PUFA) = two or three double bonds, highly unsaturated fatty acid (HUFA) = four or more double bonds.

Table 1 *Penaeus* spp. Summary of experiments to investigate effects of different algal diets on survival, development and biochemical composition of penaeid prawn larvae (*Cm* = *Chaetoceros muelleri*; *Ts* = *Tetraselmis suecica*; *T-iso* = *Isochrysis* sp.; *Dt* = *Dunaliella tertiolecta*; *n alg* algal replicates per treatment; *n larv* larval replicates per treatment; *Tubes* tubes pooled per replicate)

Experiment No.	Trial No.	Species	*n* alg	*n* larv	Larval samples per replicate:		Tubes (*n*)
					biochemical analyses	survival/ development	
1: Single-alga diet	1	*P. japonicus*	2*	2	1	3	3
(*Cm*, *Ts*, T-*iso*, *Dt*)	2	*P. japonicus*	2	3	1	3	3
2: Mixed-algae diet	3	*P. semisulcatus*	2	3	1	3	3
(*Cm*, *Ts, Cm*+*Ts*)	4	*P. semisulcatus*	2	3	1	3	3
	5	*P. monodon*	2	3	1	3	3

* $n = 1$ for *Dt*

Carbohydrates were analysed by the method of Dubois et al. (1956). Only the polysaccharide fraction was analysed because the monosaccharide fraction was <10% of the carbohydrate of both larvae and algae and highly variable. Fibre was not measured.

Data analysis

Data on biochemical composition are presented on a percentage dry weight basis since weights of larvae differed significantly between trials and algal diets. Two-way analyses of variance (ANOVA) were used to test whether the survival, development and biochemical composition of the larvae differed between algal diets and trials. Proportion data were arcsine $\sqrt{x}$-transformed before analysis. Differences in biochemical composition between species were not examined in detail because (1) these differences were generally much smaller than those due to diet, and (2) there was inadequate replication across species. If there were no differences between trials within an experiment, the values from each trial were pooled and the means are presented. Differences between the means were tested by an a posteriori Tukey's test (SAS Institute Inc. 1989). Note that the fatty acid data for the nauplii were not included in any analyses because they were not replicated within each trial.

Canonical discriminant analysis was used to examine graphically whether firstly the algae could be grouped according to their fatty acid profiles (i.e. proportion of each fatty acid), and secondly, whether the larvae fed the various algal diets could also be categorised this way (SAS Institute Inc. 1989). Canonical correlation analysis was then used to see whether there was any relationship between the proportion of fatty acids in the larvae and survival and development of the larvae (SAS Institute Inc. 1989). Proportion data were arcsine $\sqrt{x}$-transformed before analysis.

Results

Survival, development and growth of *Penaeus* spp. larvae

Single-alga diets

The survival of prawn larvae was significantly higher when they were fed *Chaetoceros muelleri* than with any of the other three alga diets in both trials. (Fig. 1). Based on survival, the other diets could be ranked in the following order: *Tetraselmis suecica* > T-*iso* > *Dunaliella tertiolecta*. The differences in survival of the larvae fed *T. suecica,* T-*iso* and *D. tertiolecta* were significant for larvae in Trial 1 (from one *Penaeus japonicus* spawning) but not for those in Trial 2 (from another *P. japonicus* spawning; Fig. 1). Overall, the survival of larvae was higher in Trial 2 than in Trial 1, with almost double the proportion of larvae surviving with each algal diet in Trial 2.

In both trials, larvae developed fastest ($p < 0.05$) when fed *Chaetoceros muelleri* or *Tetraselmis suecica*, with no significant difference between the two treatments ($p > 0.05$); and slowest when fed *Dunaliella tertiolecta* ($p < 0.05$; Fig. 2). The larvae fed T-*iso* developed more slowly than those fed *C. muelleri* but faster than those fed *D. tertiolecta* ($p < 0.05$). Significant differences in development rates betweem different algal diets were detected from the second moult (PZ2 to PZ3) in Trial 2,

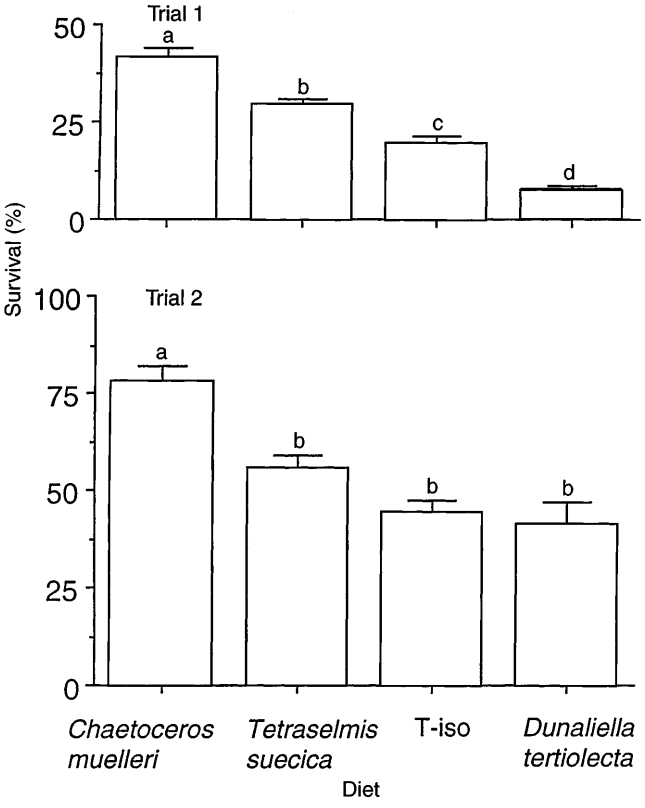

Fig. 1 *Penaeus japonicus*. Mean (+1SE) percentage survival [to time of moult of control (*Chaetoceros muelleri*-fed) to M1] of larvae fed one of four single-alga diets in two separate trials (*T-iso* = *Isochrysis* sp.). For each mean, $n = 6$ in Trial 1 and $n = 9$ in Trial 2. Means with same superscripts are not significantly different ($p > 0.05$)

and in the third moult in Trial 1 (PZ3 to M1) (Fig. 2). Overall, the larvae developed faster in Trial 2 than in Trial 1.

Mixed-algae diet

In Trials 3 and 4, the survival of larvae was the same (Trial 3) or significantly higher (Trial 4) for the mixed diet compared with the *Chaetocecos muelleri* diet (Fig. 3). However, in Trial 5, the survival of the larvae was significantly higher for the *C. mulleri* diet than for the mixed diet (Fig. 3). In all three trials, the lowest survival of larvae was recorded with the *Tetraselmis suecica* diet. Overall, the survival of larvae was higher in Trials 3 and 4 than in Trial 5.

The larvae developed significantly slower when fed *Tetraselmis suecica* than when fed the other two diets in Trials 3 and 4 ($p < 0.05$; Fig. 4). Although the results were not always statistically significant, the larvae developed faster when fed the mixed-algae diet than when fed the single-alga diets in all three trials (Fig. 4). The *Chaetoceros muelleri* diet also generally resulted in faster-developing larvae. Of the three mixed-algae diet trials, the development of the larvae was followed in

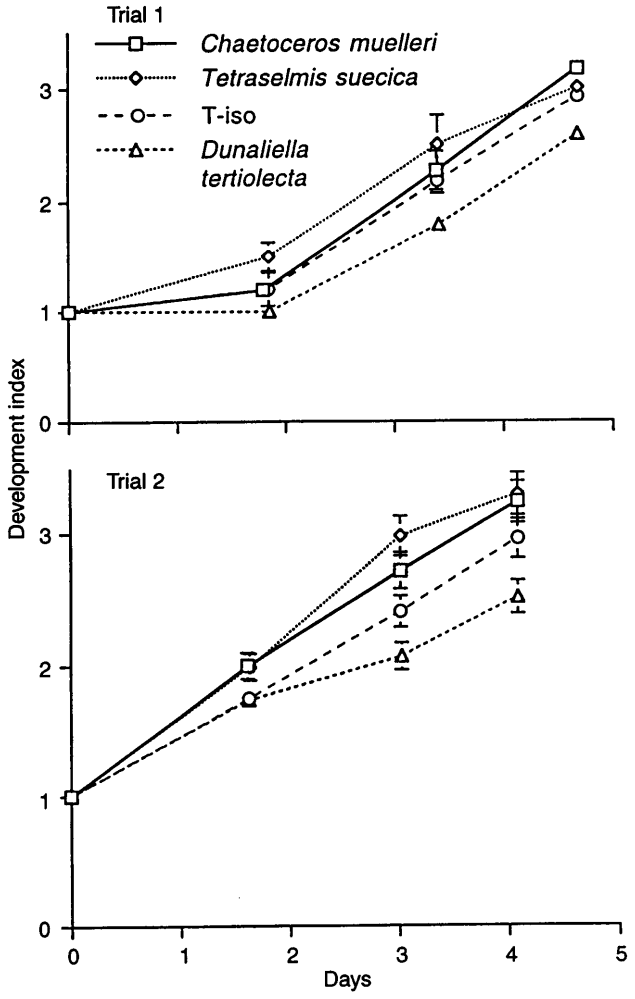

Fig. 2 *Penaeus japonicus*. Change in mean (±1SE) development index over time for larvae fed one of four single-alga diets in Trials 1 and 2 (*T-iso* = *Isochrysis* sp.); $n = 2$ for each mean

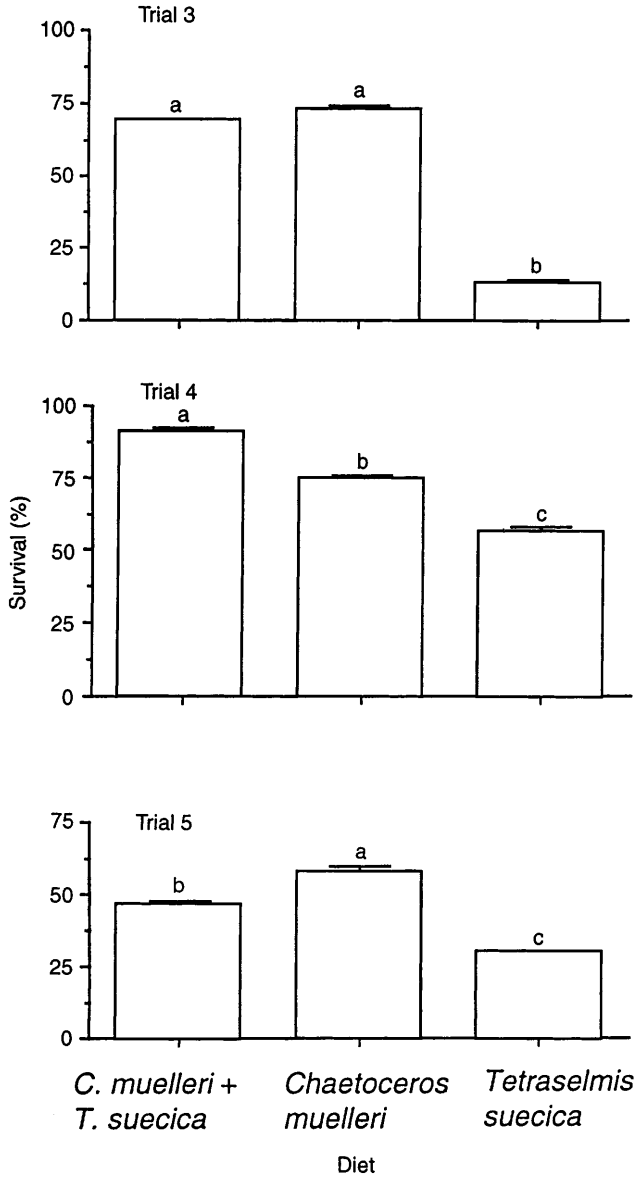

Fig. 3 *Penaeus semisulcatus* (Trials 3 and 4) and *P. monodon* (Trial 5). Mean (+1SE) percentage survival [to time of moult of control (*Chaetoceros muelleri*-fed) to M1] of larvae fed one of two single-alga diets or a mixed-algae diet; $n = 9$ for each mean. Means with same superscripts are not significantly different ($p > 0.05$)

detail in Trial 5 only (Fig. 4). Significant differences in rates of development were detected only during the third moult from PZ3 to M1, similar to that described for Trial 1 in first subsection of "Results". Although the development of the larvae in Trial 5 was significantly faster when fed the mixed-algae diet than when fed *C. muelleri* alone, the larvae fed *T. suecica* metamorphosed at a rate that was not significantly different from that of larvae on either of the other two diets ($p > 0.05$; Fig. 4). Overall, the differences in the diets were less distinct in Trial 5 ($p > 0.05$), and the slopes of the lines (Fig. 4) show that overall development was also much slower than in the other two trials.

Biochemical composition of algae and larvae

In the experiment with the single-alga diets the proportion of lipid in *Chaetoceros muelleri* and T-*iso* was significantly higher than that in *Tetraselmis suecica* and *Dunaliella tertiolecta* cells (Table 2). However, the proportion of polysaccharide in *C. muelleri* and *D. tertiolecta* did not differ significantly, and was less than in T-*iso* and more than in *T. suecica* (Table 2).

In the prawn larvae, there was a significantly higher proportion of lipid and polysaccharide in those fed *Chaetocecos muelleri* than in those fed *Dunaliella tertiolecta* (Table 2).

In the mixed-diet experiment, the proportion of lipid and polysaccharide in *Chaetoceros muelleri* was significantly greater than in *Tetraselmis suecica*, as observed above for the single-alga diet (Table 2).

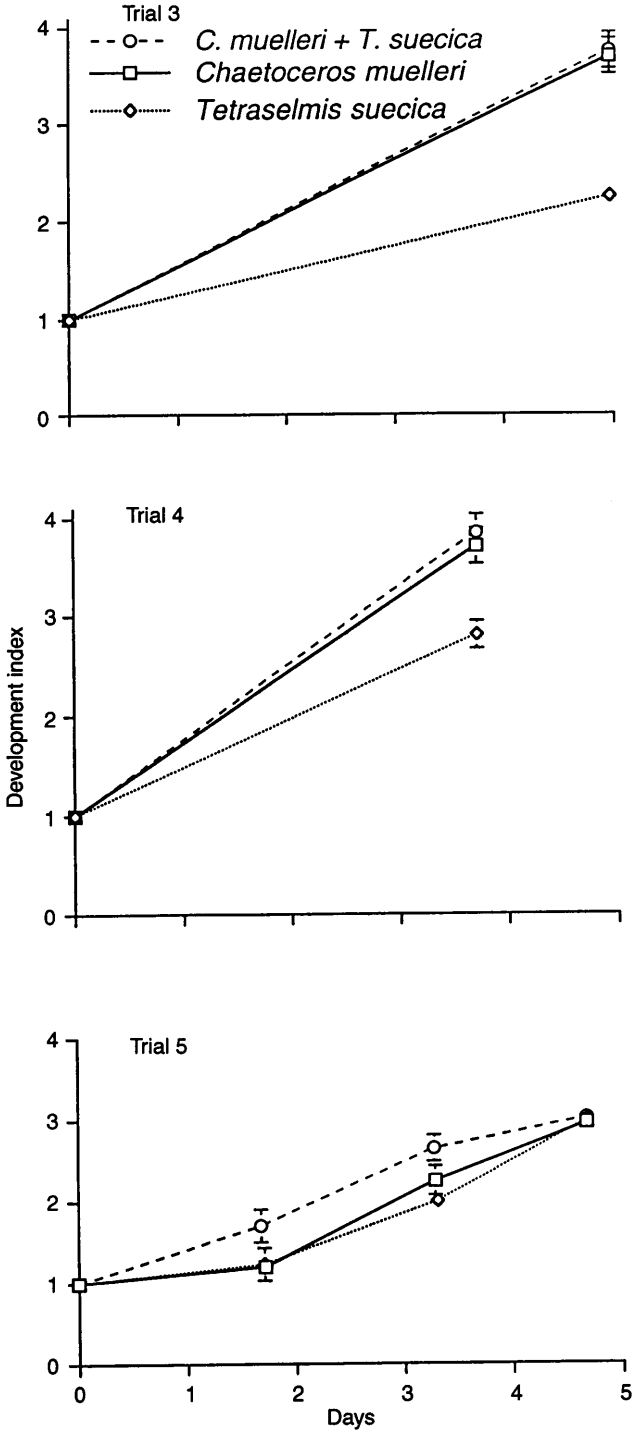

Fig. 4 *Penaeus semisulcatus* (Trials 3 and 4) and *P. monodon* (Trial 5). Change in mean (±1SE) development index over time for larvae fed one of two single-alga diets or a mixed-algae diet; $n = 2$ for each mean

However, there were no significant differences in the proportion of lipid or polysaccharide in prawn larvae fed *Chaetoceros muelleri*, *Tetraselmis suecica*, or the mixed-algae diet (Table 2).

Fatty acids in algae and larvae

Algae

Chaetoceros muelleri had high percentages (>50%) of total fatty acids in the total lipid pool in both sets of experiments (Tables 3, 4). When examined by major groups of fatty acids, all four algae species had similar proportions of SFAs and MUFAs, but *Dunaliella tertiolecta* had the highest proportion of PUFAs and T-*iso* the highest proportion of HUFAs. Of the three important HUFAs [20:4(*n*-6), 20:5(*n*-3), 22:6(*n*-3)], all three were found only in *C. muelleri*. All the algae, except *D. tertiolecta*, had 20:5(*n*-3). *Tetraselmis suecica* also had 20:4(*n*-6), but it was not detected in T-*iso* or *D. tertiolecta* (Tables 3, 4). Similarly, T-*iso* contained 22:6(*n*-3), but this was not found in *T. suecica* or *D. tertiolecta*.

Chaetoceros muelleri contained higher proportions (≥4.5% each) of 16:1(*n*-7), 16:2(*n*-4), 16:2(*n*-7), 16:3(*n*-4) and 20:5(*n*-3) than the other algae species. In contrast, it had lower proportions (≤5.8% each) of 16:0, 18:1(*n*-9), 18:2(*n*-6), 18:4(*n*-3), and unmeasurable amounts of 16:1(*n*-9) and 18:3(*n*-3) (Tables 3, 4).

Dunaliella tertiolecta had higher proportions (≥3.5% each) of 16:0, 19:0, 17:1(*n*-7), 18:1(*n*-7) and 18:3(*n*-3), but lower proportions (≤1% each) of 14:0, 16:1(*n*-9) and 18:4(*n*-3) than the other algae species (Table 3). The following fatty acids were not present in measurable amounts: 16:2(*n*-4), 16:2(*n*-7), 20:1(*n*-9), 20:4(*n*-6), 20:5(*n*-3) and 22:6(*n*-3).

The species of alga could be clearly separated by canonical discriminant analysis on the basis of their fatty acid compositions (Fig. 5). The scores for *Chaetoceros muelleri* were clearly separated from those of the other three species on Canonical Variable 1 (Fig. 5). This variable was highly significant ($p < 0.001$), and accounted for 58% of the variance. The scores on Canonical Variable 2 separated the other three species, *Tetraselmis suecica*, *Dunaliella tertiolecta* and T-*iso* (Fig. 5). Together, the Canonical Variables 1 and 2 accounted for 95% of the total variance explained by the first three canonical variables.

Larvae

The prawn nauplii had the largest proportion of fatty acids (>78%) in their total lipid (Tables 3, 4). The HUFAs were the most prevalent, followed closely by the SFAs and then the MUFAs. The PUFAs comprised a very small proportion of the total fatty acids. The fatty acid composition of the nauplii was dominated by 16:0, 18:1(*n*-9), 20:5(*n*-3), 20:4(*n*-6) and 22:6(*n*-3) (together accounting for ~60% of the fatty acids). The proportions of 18:2(*n*-6) and 18:3(*n*-3) were lower (<3%) than in most of the algae.

The protozoeae fed *Chaetoceros muelleri* had a higher proportion of total fatty acids in their total lipid than those fed the other algal diets (Table 3). The proportions

Table 2 Microalgae and *Penaeus* spp. Mean biochemical composition (% ± 1SE) of algae and prawn larvae in two separate experiments: single-alga diet experiment ($n = 4$ for each algal mean, $n = 5$ for each larval mean) and mixed-algae diet experiment ($n = 6$ for each algal mean, $n = 9$ for each larval mean). Means with same superscript within an algal or larval row are not significantly different ($p > 0.05$) (– not tested)

Biochemical composition (% dry wt)	Alga				Larvae fed:				
	Chaetoceros muelleri	*Tetraselmis suecica*	*Isochrysis* sp.	*Dunaliella tertiolecta*	*Chaetoceros muelleri*	*Tetraselmis suecica*	*C. muelleri* + *T. suecica*	*Isochrysis* sp.	*Dunaliella tertiolecta*
Single-alga diet experiment									
Lipid	21.0 ± 0.2^{a}	17.0 ± 0.4^{b}	19.3 ± 0.9^{a}	10.0 ± 0.2^{c}	24.0 ± 1.2^{ab}	20.4 ± 4.8^{bc}	–	17.5 ± 2.8^{bc}	13.4 ± 2.9^{c}
Polysaccharide	12.3 ± 0.5^{b}	9.8 ± 0.4^{c}	14.8 ± 0.6^{a}	13.1 ± 2.1^{b}	8.5 ± 0.2^{ab}	7.2 ± 1.7^{bc}	–	5.1 ± 1.3^{bc}	3.0 ± 0.8^{c}
Mixed-algae diet experiment									
Lipid	23.8 ± 0.9^{a}	20.7 ± 0.5^{b}	–	–	23.9 ± 2.0	18.2 ± 2.1	20.2 ± 1.1	–	–
Polysaccharide	16.9 ± 0.6^{a}	10.5 ± 0.3^{b}	–	–	8.2 ± 0.7	9.1 ± 2.1	9.5 ± 2.0	–	–

Table 3 Microalgae and *Penaeus* spp. Mean percentage fatty acid composition (>1% total fatty acids ± 1SE) of algae and prawn larvae fed one of four single-alga diets in two separate trials ($n = 4$ for each algal mean, $n = 5$ for each larval mean). Means with same superscript within an algal or larval row are not significantly different ($p > 0.05$) [*SFAs* saturated fatty acids; *MUFAs* monounsaturated fatty acids; *PUFAs* polyunsaturated fatty acids] *HUFAs* highly unsaturated fatty acids; *nd* not detected; *tr* trace; *FA* fatty acid (nd and tr were treated as 0 for statistical purposes)]

Fatty acids	Alga				Nauplii	Larvae fed:			
	C. muelleri	*T. suecica*	*Isochrysis* sp.	*D. tertiolecta*		*C. muelleri*	*T. suecica*	*Isochrysis* sp.	*D. tertiolecta*
SFAs									
14:0	10.3 ± 0.3^{b}	0.4 ± 0.1^{c}	13.6 ± 0.3^{a}	0.1 ± 0.1^{d}	1.5 ± 0.2	3.9 ± 0.6^{b}	tr^{c}	5.8 ± 0.7^{a}	tr^{c}
16:0	5.8 ± 0.2^{c}	17.5 ± 0.1^{a}	9.9 ± 0.2^{b}	18.3 ± 1.0^{a}	20.0 ± 0.2	16.8 ± 0.2^{b}	22.4 ± 1.0^{a}	20.1 ± 0.4^{a}	21.7 ± 1.2^{a}
17:0	nd	nd	nd	nd	1.7 ± 0.1	nd	nd	nd	nd
iso 18:0	nd	nd	nd	nd	1.3 ± 0.2	2.6 ± 0.2	1.9 ± 0.5	1.0 ± 0.4	2.4 ± 1.2
18:0	0.8 ± 0.0^{a}	nd^{c}	tr^{c}	0.3 ± 0.1^{b}	7.4 ± 1.5	8.9 ± 0.6^{b}	7.3 ± 0.3^{c}	5.8 ± 0.2^{d}	11.6 ± 0.8^{a}
19:0	0.4 ± 0.0^{c}	1.6 ± 0.1^{b}	0.1 ± 0.1^{d}	3.5 ± 0.2^{a}	0.4 ± 0.0	0.9 ± 0.1^{a}	0.3 ± 0.1^{ab}	0.1 ± 0.1^{b}	0.5 ± 0.5^{ab}
$\sum$	17.2	19.6	23.5	22.3	32.3	33.2	31.9	32.9	36.2
MUFAs									
16:1*n*-7	17.1 ± 0.7^{a}	2.8 ± 0.1^{c}	4.0 ± 0.1^{b}	4.4 ± 0.4^{b}	4.0 ± 0.5	11.7 ± 1.0^{a}	0.4 ± 0.2^{c}	1.9 ± 0.5^{b}	tr^{c}
16:1*n*-9	nd^{b}	1.1 ± 0.1^{a}	tr^{b}	tr^{b}	0.1 ± 0.1	nd^{b}	0.4 ± 0.2^{a}	tr^{b}	nd^{b}
17:1*n*-7	nd^{c}	1.4 ± 0.0^{b}	nd^{c}	3.7 ± 0.1^{a}	0.9 ± 0.0	nd	nd	nd	nd
18:1*n*-7	3.5 ± 0.0^{b}	2.8 ± 0.2^{c}	1.3 ± 0.1^{d}	6.3 ± 0.9^{a}	3.4 ± 0.2	7.0 ± 0.2^{a}	2.8 ± 0.3^{b}	3.5 ± 0.2^{ab}	2.7 ± 1.4^{b}
18:1*n*-9	0.5 ± 0.0^{d}	8.3 ± 0.2^{b}	14.0 ± 0.2^{a}	4.6 ± 0.12^{c}	11.5 ± 1.8	1.5 ± 0.1^{d}	10.4 ± 0.2^{b}	11.8 ± 0.2^{a}	7.3 ± 0.3^{c}
20:1*n*-9	nd^{b}	1.8 ± 0.0^{a}	nd^{b}	nd^{b}	1.2 ± 0.1	0.2 ± 0.1^{b}	1.5 ± 0.4^{a}	0.7 ± 0.3^{c}	tr^{b}
$\sum$	21.1	18.2	19.2	19.0	21.1	20.5	15.4	17.9	10.0
PUFAs									
16:2*n*-4	7.5 ± 0.1^{a}	0.3 ± 0.1^{c}	1.0 ± 0.0^{b}	nd^{d}	nd	0.6 ± 0.2^{a}	nd^{b}	tr^{b}	nd^{b}
16:2*n*-7	4.5 ± 0.2^{a}	nd^{b}	tr^{b}	nd^{b}	nd	0.5 ± 0.2^{a}	nd^{b}	tr^{b}	nd^{b}
16:3*n*-4	17.3 ± 0.2^{a}	nd^{b}	tr^{b}	0.1 ± 0.1^{b}	nd	1.3 ± 0.4^{a}	nd^{b}	tr^{b}	nd^{b}
18:2*n*-6	0.5 ± 0.0^{d}	10.9 ± 0.3^{a}	3.6 ± 0.1^{c}	5.0 ± 0.5^{b}	2.5 ± 1.3	1.5 ± 0.1^{d}	14.9 ± 0.8^{a}	5.6 ± 0.4^{c}	9.6 ± 0.6^{b}
18:3*n*-3	nd^{d}	15.0 ± 0.2^{b}	8.0 ± 0.1^{c}	36.8 ± 0.6^{a}	0.5 ± 0.0	0.1 ± 0.1^{d}	7.6 ± 0.4^{b}	3.9 ± 0.3^{c}	20.2 ± 0.5^{a}
20:2*n*-6	nd	nd	nd	nd	1.0 ± 0.1	0.2 ± 0.1^{b}	1.7 ± 0.4^{a}	0.2 ± 0.2^{b}	tr^{b}
20:3*n*-3	nd	nd	nd	nd	0.4 ± 0.0	nd^{c}	1.2 ± 0.3^{b}	0.1 ± 0.1^{c}	4.3 ± 0.4^{a}
$\sum$	29.8	26.2	12.6	41.9	4.4	4.1	25.3	9.8	34.1
HUFAs									
16:4*n*-3	nd^{c}	17.6 ± 0.2^{a}	nd^{c}	14.4 ± 0.3^{b}	nd	nd	tr	nd	tr
18:4*n*-3	0.5 ± 0.0^{c}	8.0 ± 0.1^{b}	23.6 ± 0.5^{a}	1.0 ± 0.2^{c}	nd	0.1 ± 0.1^{c}	0.5 ± 0.2^{b}	5.2 ± 0.7^{a}	tr^{c}
20:4*n*-6	1.5 ± 0.1^{a}	1.5 ± 0.0^{a}	nd^{b}	nd^{b}	5.7 ± 0.3	8.0 ± 0.5^{a}	5.6 ± 0.1^{a}	2.0 ± 0.5^{b}	2.5 ± 1.2^{b}
20:5*n*-3	23.3 ± 1.2^{a}	6.4 ± 0.1^{b}	0.3 ± 0.1^{c}	nd^{c}	11.5 ± 0.6	22.1 ± 0.4^{a}	13.2 ± 0.5^{b}	7.0 ± 0.4^{c}	8.6 ± 0.5^{c}
22:6*n*-3	1.8 ± 0.1^{b}	nd^{c}	13.7 ± 0.2^{a}	nd^{c}	16.8 ± 0.2	5.8 ± 0.3^{c}	3.7 ± 0.5^{d}	21.3 ± 0.5^{a}	8.0 ± 0.4^{b}
$\sum$	27.1	33.5	37.7	15.5	34.0	35.9	22.9	35.5	18.1
$\sum$ total FA	95.2	97.5	94.7	98.7	91.8	94.2	95.6	98.0	98.4
Total *n*-3	25.6	47.0	45.6	52.4	29.2	28.0	26.1	37.6	41.1
Total *n*-6	2.0	12.5	3.6	5.0	9.2	9.7	22.1	7.7	12.1
n-3:*n*-6	12.8	3.8	12.6	10.5	3.2	2.9	1.2	4.9	3.4
% total FA/lipid	53.7 ± 0.6^{a}	51.0 ± 0.7^{b}	41.3 ± 0.4^{c}	41.3 ± 2.3^{c}	78.3 ± 4.2	66.2 ± 1.5^{a}	55.5 ± 6.3^{ab}	51.3 ± 7.4^{b}	41.2 ± 4.0^{b}

Table 4 Microalgae and *Penaeus* spp. Mean percentage fatty acid composition (>1% total fatty acids ±1SE) of algae and prawn larvae fed one of two single-alga diets or a mixed-algae diet, in three separate trials ($n = 6$ for each algal mean, $n = 9$ for each larval mean) Means with same superscript within an algal or larval row are not significantly different ($p > 0.05$); *SFAs* saturated fatty acids; *MUFAs* monounsaturated fatty acids; *PUFAs* polyunsaturated fatty acids; *HUFAs* highly unsaturated fatty acids; *nd* not detected; *tr* trace; *FA* fatty acid (nd and tr were treated as 0 for statistical purposes)

Fatty acids	Algae		Nauplii	Larvae fed:		
	C. *muelleri*	*T. suecica*		*C. muelleri*	*T. suecica*	*C. muelleri* + *T. suecica*
SFAs						
14:0	15.2 ± 0.3[a]	0.6 ± 0.1[b]	1.8 ± 0.1	2.8 ± 0.1[a]	tr[c]	1.0 ± 0.0[b]
iso 16:0	nd	nd	1.0 ± 0.1	1.4 ± 0.0[a]	0.6 ± 0.4[b]	1.6 ± 0.1[a]
16:0	9.5 ± 1.0[b]	17.3 ± 0.1[a]	20.7 ± 0.2	17.1 ± 0.1	20.2 ± 2.9	18.9 ± 0.2
17:0	nd	nd	1.6 ± 0.2	tr	tr	tr
*iso*18:0	nd	nd	1.1 ± 0.1	3.3 ± 0.1[a]	2.3 ± 0.7[b]	3.3 ± 0.1[a]
18:0	0.6 ± 0.0[a]	0.1 ± 0.0[b]	8.0 ± 0.4	11.8 ± 0.1	10.6 ± 1.7	10.6 ± 0.1
19:0	1.4 ± 0.1	1.5 ± 0.0	0.3 ± 0.0	1.1 ± 0.0[a]	tr[c]	0.5 ± 0.1[b]
∑	26.8	19.3	34.5	37.4	33.7	35.9
MUFAs						
16:1*n*-7	21.8 ± 1.5[a]	2.4 ± 0.1[b]	4.1 ± 0.2	8.3 ± 0.3[a]	tr[c]	3.5 ± 0.1[b]
16:1*n*-9	nd[b]	1.4 ± 0.1[a]	tr	tr	tr	tr
17:1*n*-7	nd[b]	1.4 ± 0.0[a]	0.9 ± 0.1	nd	nd	nd
18:1*n*-7	2.3 ± 0.2	2.3 ± 0.1	3.1 ± 0.1	8.4 ± 0.2[a]	1.1 ± 0.5[c]	4.8 ± 0.1[b]
18:1*n*-9	0.8 ± 0.1[b]	9.5 ± 0.2[a]	11.8 ± 0.5	1.7 ± 0.1[b]	8.4 ± 1.2[a]	5.9 ± 0.1[a]
20:1*n*-9	nd[b]	1.9 ± 0.0[a]	1.2 ± 0.1	0.1 ± 0.1[b]	0.2 ± 0.2[b]	1.4 ± 0.0[a]
∑	24.9	18.9	21.1	18.6	9.7	15.5
PUFAs						
16:2*n*-4	4.8 ± 0.1[a]	0.6 ± 0.0[b]	nd	tr	nd	tr
16:2*n*-7	2.2 ± 0.1[a]	nd[b]	nd	tr	nd	tr
16:3*n*-4	9.7 ± 0.8[a]	nd[b]	nd	tr	nd	tr
18:2*n*-6	0.7 ± 0.1[b]	10.8 ± 0.2[a]	2.1 ± 0.1	1.8 ± 0.1[b]	13.7 ± 2.1[a]	7.5 ± 0.3[a]
18:3*n*-3	nd[b]	13.9 ± 0.1[a]	0.3 ± 0.1	tr[b]	5.5 ± 0.8[a]	2.7 ± 0.1[a]
20:2*n*-6	nd	nd	0.9 ± 0.0	0.2 ± 0.1[b]	1.1 ± 0.5[b]	2.3 ± 0.1[a]
20:3*n*-3	nd	nd	0.3 ± 0.0	nd	0.2 ± 0.2[b]	1.4 ± 0.1[a]
∑	17.3	25.3	3.6	2.1	20.4	13.7
HUFAs						
16:4*n*-3	nd[b]	14.2 ± 1.5[a]	nd	nd	tr	nd
18:4*n*-3	1.0 ± 0.0[b]	7.8 ± 0.1[a]	nd	tr	tr	tr
20:4*n*-6	3.5 ± 0.1[a]	1.4 ± 0.0[b]	6.2 ± 0.9	10.2 ± 0.2[a]	6.4 ± 1.0[b]	7.7 ± 0.1[a]
20:5*n*-3	21.7 ± 1.8[a]	6.3 ± 0.1[b]	9.2 ± 0.5	21.5 ± 0.4[a]	15.1 ± 2.3[b]	20.2 ± 0.2[a]
22:6*n*-3	2.2 ± 0.2[a]	nd[b]	18.3 ± 0.6	7.7 ± 0.2[a]	2.2 ± 1.1[c]	5.4 ± 0.3[b]
∑	28.4	29.7	33.7	39.3	23.7	33.3
∑ total FA	97.4	93.3	92.9	97.3	87.5	98.4
Total *n*-3	24.9	42.2	28.1	29.2	23.0	29.6
Total *n*-6	4.7	12.2	9.2	12.2	21.2	17.5
n-3:*n*-6	5.3	3.5	3.0	2.4	1.1	1.7
% total FA /total lipid	63.2 ± 2.5[a]	53.5 ± 0.5[b]	86.7 ± 3.8	59.2 ± 1.4[a]	32.6 ± 5.8[b]	61.7 ± 2.4[a]

of each of the groups of fatty acids were almost identical to those in the nauplii. The PUFAs constituted a much smaller proportion of the total fatty acids of *C. muelleri*-fed larvae than of larvae fed the other algal diets. The former also contained >7% of each of 16:1(*n*-7), 18:1(*n*-7), 20:4(*n*-6) and 20:5(*n*-3), and <1.5% or undetectable levels of 18:1(*n*-9), 18:2(*n*-6), 18:3(*n*-3) and 20:3(*n*-3).

Conversely, the protozoeae fed *Dunaliella tertiolecta* contained low proportions of total fatty acids, MUFAs and HUFAs (Table 3). Furthermore, these larvae had low proportions of 20:5(*n*-3) and 20:4(*n*-6). The *D. tertiolecta*-fed larvae had the highest proportion of 18:3(*n*-3) (as had the alga) and high proportions of 20:3(*n*-3).

In both series of experiments (single- and mixed-algae diet experiments) the larvae fed *Chaetoceros muelleri* or *C. muelleri* + *Tetraselmis suecica* had high proportions of total fatty acids, MUFAs and HUFAs and low proportions of PUFAs (with larvae fed *C. muelleri* + *T. suecica* having a proportion of PUFAs intermediate between larvae fed *C. muelleri* or *T. suecica* alone) (Tables 3, 4). In terms of individual fatty acids, the larvae fed the diets containing *C. muelleri* contained higher proportions of 20:5(*n*-3) and 20:4(*n*-6) than the larvae fed the other diets.

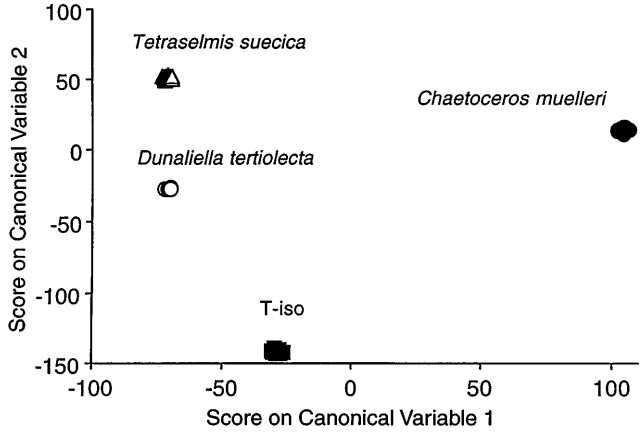

Fig. 5 Microalgae. Canonical discriminant scores of algae for Canonical Variables 1 and 2 based on their fatty acid composition (*T-iso* = *Isochrysis* sp.)

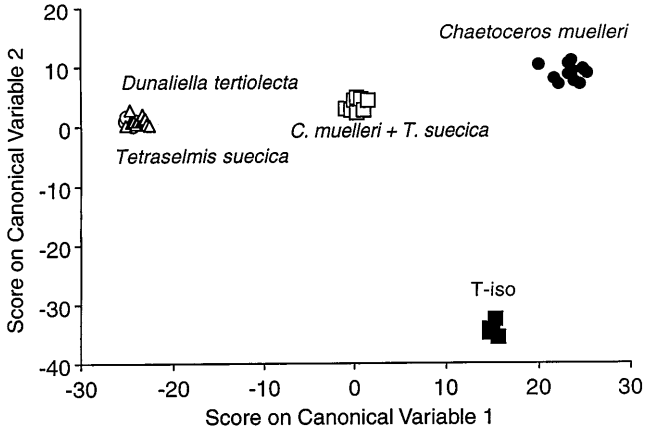

Fig. 6 *Penaeus* spp. Canonical discriminant scores of larvae for Canonical Variables 1 and 2 based on their fatty acid composition. Different symbols represent different diets (O larvae fed *D. tertiolecta*: Δ larvae fed *T. suecica*: *T-iso* = *Isochrysis* sp.)

The percentage composition of a few fatty acids followed the same trend in the algae as in the larvae feeding on them: 14:0, 18:1(*n*-9), 18:2(*n*-6), 18:3(*n*-3), 20:4(*n*-6) and 20:5(*n*-3) (Tables 3, 4).

The larvae could be separated into dietary groups on the basis of their fatty acid composition (Fig. 6). There was clear separation of the scores on Canonical Variable 1 obtained for those larvae fed *Chaetoceros muelleri* from those fed *Tetraselmis suecica* and *Dunaliella tertiolecta*. The scores of those larvae fed the mixed diet were intermediate between those fed *C. muelleri* alone and those fed *T. suecica* alone. On Canonical Variable 2, larvae fed with T-*iso* were separated from those fed all the other diets. Larvae fed *T. suecica* and *D. tertiolecta* were not well separated on either variable. Canonical Variable 1 was highly significant ($p < 0.001$), and accounted for >70% of the variance. Canonical Variable 2 accounted for a further 28% of the variation. Together, Canonical Variables 1 and 2 accounted for 98% of the total variance explained by the first four canonical variables.

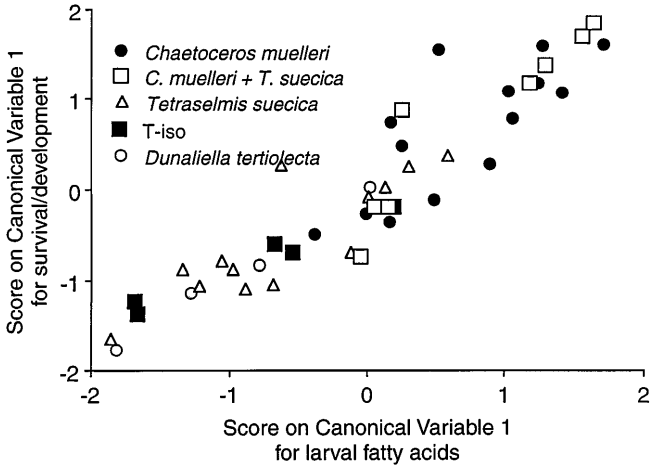

Fig. 7 *Penaeus* spp. Canonical correlation scores of larvae for first canonical variable based on their fatty acid composition, and based on their survival and development (*T-iso* = *Isochrysis* sp.)

Relationship between survival/development and fatty acid content of larvae

Canonical correlation analysis revealed that 68% of the variation in the first canonical variable for the survival and development of the larvae was explained by the fatty acid composition of the larvae (Fig. 7). A further 15% of the variation was accounted for by the second canonical variable for their survival and development. The fatty acids in the larvae that were most strongly correlated (r) with survival alone were 20:4(*n*-6) ($r = 0.71$), 20:5(*n*-3) ($r = 0.65$), *iso* 18:0 ($r = 0.64$), 16:1(*n*-7) ($r = 0.58$), 18:1(*n*-7) ($r = 0.56$) and 18:0 ($r = 0.55$). The correlations between fatty acids in the larvae and development were generally lower ($r \leq 0.48$) than those for survival.

Discussion

This study showed that prawn larvae (*Penaeus* spp.) do as well, or better, on a diet of C*haetoceros muelleri* alone than on a diet of either of the other three algae. However, their survival and development may be better on a mixed diet of *C. muelleri* and *Tetraselmis suecica*. The survival of larvae fed *C. muelleri* or the mixed diet was always higher than that of larvae fed *T. suecica,* T-*iso* or *Dunaliella tertiolecta* alone. Furthermore, the development of larvae fed *C. muelleri* or the mixed diet was always at least as fast as those fed *T. suecica*, and always faster than that of larvae fed the other diets.

The poorest algal diet in terms of survival and development of the larvae was *Dunaliella tertiolecta*, which had previously been found to be inadequate for *Penaeus monodon* larvae (Kurmaly et al. 1989).

The differences in the composition of fatty acids in the algal diets seemed to be the factor most likely to explain the differences in larval survival and development. The gross lipid and carbohydrate compositions of the algal diets did not explain the observed differences in growth of the larvae nor did they correspond to the gross composition of the larvae. Although protein levels were not presented here due to problems with the assay (D'Souza 1998), those that were measured in this study were similar (30 to 40% dry wt) for the different species of algae; always high, and presumably of very similar amino acid composition (Brown 1991). Previous studies have also found that the gross composition of algae alone could not explain differences in the survival and growth of prawn and bivalve larvae (e.g. Tobias-Quinitio and Villegas 1982; Webb and Chu 1983). However, in bivalve larvae, once the requirements for fatty acids have been met, the gross composition (such as carbohydrate level) has more of an effect on survival and growth (Enright et al. 1986).

Other nutritional components such as vitamins and sterols, which were not measured in this study, may affect the growth of prawn larvae. Another factor is the rate of digestion of the algal cells, which may differ between algae species in respect to palatability, cell density or nutritional value. The rate of digestion of the cells was not measured in this study.

Fatty acids in algae and larvae

This is the first study to show a connection between the fatty acid compositions of algae and prawn larvae and the growth performance (i.e. survival and development) of larvae: the fatty acid profiles of larvae fed *Chaetoceros muelleri* and the mixed diet (*C. muelleri* + *Tetraselmis suecica*) had higher proportions of 20:4(*n*-6) and 20:5(*n*-3) (as well as resulting in better survival and development) than the profiles of larvae fed the other diets. Furthermore, the larvae fed *T. suecica*, which had proportions of 20:5(*n*-3) and 20:4(*n*-6) intermediate between those fed *C. muelleri* and those fed T-*iso* or *Dunaliella tertiolecta*, also showed intermediate survival and development. The proportions of these fatty acids in the larvae reflected their presence in the algal diets.

In contrast, the proportion of 22:6(*n*-3) in *Chaetoceros muelleri*, *Tetraselmis suecica* and *Dunaliella tertiolecta* was not reflected in the larval tissue and was not related to the survival or development of protozoeae or the Mysis 1 stage. However, T-*iso*, which contained a very large proportion of 22:6(*n*-3) compared to the other species, produced larvae with even higher proportions of this HUFA, but again this did not translate to high survival or development. This is probably because T-*iso* either lacked or had low proportions of the other important fatty acids [20:4(*n*-6) and 20:5(*n*-3)]. The high values of 22:6(*n*-3) found in the nauplii in this study support the suggestion that this fatty acid plays a role in the development of egg to nauplius (Xu et al. 1994a).

The only fatty acid groups to follow the same pattern in the larvae as in the algae were the SFAs and the HUFAs. The SFAs were present in similar proportions in all the algal species, and also in the larvae fed the different algal diets. The lowest proportion of HUFAs was in *Dunaliella tertiolecta* and in the larvae feeding on it. However, larvae feeding on *D. tertiolecta* were supplied with high proportions of the essential fatty acids 18:2(*n*-6) and 18:3(*n*-3). Studies with ^{14}C-labelled fatty acids have shown that larval and juvenile *Penaeus japonicus* convert 18:3(*n*-3) to the longer-chain HUFAs 20:5(*n*-3) and 22:6(*n*-3) (Kanazawa and Teshima 1977; Jones et al. 1979; Kanazawa et al. 1979a; Teshima et al. 1992). However, the rate of desaturation and elongation to the HUFAs is probably too slow to meet their requirements for these fatty acids (Kanazawa et al. 1979a). This would explain why supplements of HUFAs improve growth in juvenile prawns and why *D. tertiolecta*, deficient in these fatty acids, results in poor growth of larvae.

The proportion of 18:3(*n*-3) in the larvae was always at least half of that in their algal diets, and was undetectable in larvae within the first 12 h of starvation (D'Souza 1998). Therefore, this fatty acid is probably actively catabolised. Furthermore, its absence from *Chaetoceros muelleri* and its near-absence from larvae fed this diatom, suggest that it is not an essential fatty acid for prawn larvae, especially when HUFAs are present. A radiotracer study of labelled 18:3(*n*-3) injected into *Penaeus japonicus* protozoeae supports this hypothesis: 18:3(*n*-3) was converted to shorter-chain fatty acids such as the SFAs 14:0 and 16:0 and the MUFAs 16:1(*n*-9) and 18:1(*n*-9), as well as *n*-3 HUFAs (Teshima et al. 1992). The PUFA 20:3(*n*-3) was only present in larvae that were fed algae containing 18:3(*n*-3), providing evidence that 18:3(*n*-3) is converted to 20:3(*n*-3). The data of Xu et al. (1994b) also support this conversion pathway of 18:3(*n*-3) to 20:3(*n*-3) in juvenile *P. chinensis*. In addition, our study suggests that 18:2(*n*-6) is accumulated when it is in excess in the diet, because it increased in proportion in larvae fed algal diets rich in 18:2(*n*-6) but stayed at low levels in starved larvae (D'Souza 1998) and in larvae fed an alga (*C. muelleri*) with small amounts of this fatty acid.

Chaetoceros muelleri had very low levels of the PUFA 18:2(*n*-6) and undetectable levels of the PUFA 18:3(*n*-3) but, nonetheless, of the four algal species tested, it produced the highest survival and development of prawn larvae. These PUFAs were also in very low proportions in the nauplii. However, the presence of HUFAs in the diet may reduce the requirement for these PUFAs. For example, although 18:3(*n*-3) was not detected in *C. muelleri*, it was detected in small amounts in the larvae that fed on this alga. The *n*-3 HUFAs 18:4(*n*-3), 20:5(*n*-3) or 22:6(*n*-3) may have been metabolised to produce 18:3(*n*-3). Alternatively, the small amount of 18:3(*n*-3) in larvae fed on *C. muelleri* may have originated from the nauplii.

High levels of 20:4(*n*-6) were found in prawn larvae feeding on algae with high 20:4(*n*-6) (i.e. *Chaetoceros*

muelleri and *Tetraselmis suecica*). These larvae displayed better survival and development than other larvae. There was little evidence that the larvae can convert 18:2(*n*-6) to 20:4(*n*-6) at an appreciable rate. For example, although *Dunaliella tertiolecta* had moderately high levels of 18:2(*n*-6), it produced larvae with low levels of 20:4(*n*-6). Whilst some research has concluded that 18:2(*n*-6), 18:3(*n*-3), 20:5(*n*-3) and 22:6(*n*-3) are essential for prawn larvae and juveniles, nothing is known of the role of 20:4(*n*-6) in larvae, and only recently has 20:4(*n*-6) been investigated in juveniles (e.g. Xu et al. 1994b). Arachidonic acid is a precursor to prostaglandins, which are involved in reproduction in mammals and insects (Stanley-Samuelson 1987; Brenner and Bernasconi 1989). Prostaglandins have not been identified in penaeids as yet, but some appear to be involved in the control of moulting (development) in adult prawns (Koskela et al. 1992). Perhaps the faster development of prawn larvae fed *C. muelleri* or *T. suecica* is due, in part, to the presence of 20:4(*n*-6) in their diets.

The SFAs 16:0 and 18:0 were present in high proportions in the larvae regardless of their proportions in the diet, suggesting that the two fatty acids are actively synthesised. This was especially true for 18:0, which was present in very low levels in the diets (≤0.8%). One possible explanation for the high relative content of 16:0 and 18:0 is that they are produced by chain elongation of 14:0, which was present in high proportion in two of the algae (*Chaetoceros muelleri* and T-*iso*) but at lower levels in the larvae feeding on them. The other two algae had 14:0 in only trace amounts, but had high levels of 16:0. For each alga, the sum of 14:0 plus 16:0 appeared adequate to support the synthesis of 18:0 by the larvae. Alternative sources of 18:0 for the larvae include the de novo synthesis of 18:0 from acetate, or the conversion to 18:0 from 16:3(*n*-4), 16:4(*n*-3) or 18:3(*n*-3), which were far more plentiful in the algae than in the larvae.

The MUFA 16:1(*n*-7) was found in very high proportions in *Chaetoceros muelleri* compared to the other algae, and was also present in high proportions in the larvae feeding on this alga, which suggests the direct uptake of this fatty acid. There may also have been some conversion of 16:1(*n*-7) to 18:1(*n*-7), since 18:1(*n*-7) was high in the *C. muelleri*-fed larvae but not in their diet.

Biochemical composition of algae and larvae

The proportions of lipid and polysaccharide in the algae were not reflected in the proportions in the prawn larvae, and neither were they related to the survival and development (=performance) of the larvae. However, larvae that performed best (i.e. those fed *Chaetoceros muelleri*) had significantly more lipid and polysaccharide than those that performed worst (i.e. those fed *Dunaliella tertiolecta*). Similar findings were reported when *Penaeus monodon* larvae were fed *C. calcitrans* and *Tetraselmis chuii* (Tobias-Quinitio and Villegas 1982). In their study, the algae differed greatly in their protein content [*C. calcitrans* (24% protein) and *T. chuii* (49%)], but had a similar lipid and carbohydrate content; and yet the *C. calcitrans*-fed larvae, which performed better in the protozoeal stages, had three times the proportion of lipid than the *T. chuii*-fed larvae. The results from the study of Tobias-Quinitio and Villegas and the present study suggest that the proportion of lipid in the larvae may be an indicator of their nutritional condition.

Variability in survival and development of larvae

The survival and development of prawn larvae varied greatly between spawnings (trials). Larvae from, spawnings in which survival was low overall (e.g. Trials 1 and 5) also developed more slowly and often showed greater differences in survival rates when fed different diets than larvae from spawnings with high overall survival. Similar variations in survival and development of the larvae from different spawnings fed the same diet have been recorded for *Metapenaeus ensis* larvae (Chu and Lui 1990). Most of this variation is probably due to differences between the quality of the nauplii from different spawnings. Crocos and Coman (1997) have shown that when the diet of *Penaeus semisulcatus* broodstock is constant, the survival of the PZI stage varies with such factors as age of the broodstock and season of spawning. The nutritional condition of the broodstock also appears to influence reproductive performance: the fatty acids, 20:5(*n*-3) and 22:6(*n*-3), in the eggs of *P. chinensis* were correlated with fecundity and the hatch rate of eggs (Xu et al. 1994a).

Influence of size of algal cells on larval survival and growth

Some authors have attributed the poor performance of prawn larvae to the large size of the algal cells in the diet (Tobias-Quinitio and Villegas 1982; Sánchez 1986). *Chaetoceros muelleri* and T-*iso* are small and very similar in size (3 to 5 μm in length), while *Tetraselmis sucecia* and *Dunaliella tertiolecta* are larger and similar in size (5 to 12 μm in length). In the current study, the larvae of both the small *C. muelleri* and the large *T. suecica* displayed high survival and development. Furthermore, the natural diet of *Penaeus merguiensis* PZI larvae includes algae as large as 1 mm long (*Rhizosolenia* sp.), and algae >20 μm long are often ingested (Preston et al. 1992). It is therefore unlikely that the differences in larval performance between algal diets were due to the differences in size of the algal cells alone.

Future research should focus on feeding different proportions of *Chaetoceros muelleri* and *Tetraselmis suecica* (2:3 by dry weight in the current study) to try and optimise the mixed diet for prawn larvae. Increasing the proportion of *C. muelleri* should improve the diet, since *C. muelleri* alone performs well. The *T. suecica* component may improve the mixed diet because it contains high proportions of 18:2(*n*-6) and 18:3(*n*-3).

A mixture of *C. muelleri* and T-*iso* might also provide a diet encouraging high survival and fast development because of the presence of 18:2(*n*-6), 18:3(*n*-3) and 22:6(*n*-3) in the T-*iso* cells. These latter two species are part of the standard AQUACOP protocol for rearing *Penaeus vannamei* larvae, but there are no survival and growth comparisons to other species of algae to confirm their superiority as a diet (AQUACOP 1983).

The results of the current study also suggest that more research is warranted on 20:4(*n*-6) in penaeid prawn larvae to establish whether it is an essential fatty acid and to define its role in larval nutrition. Similarly, the "essential" status of 18:2(*n*-6) and 18:3(*n*-3) needs to be examined. Future research could examine the effects of T-*iso* and *Dunaliella tertiolecta* diets supplemented with microencapsulated 20:4(*n*-6) on growth, survival and fatty acid composition of prawn larvae. Both algal diets would probably require supplements of 20:5(*n*-3).

Acknowledgements This research was part of the senior author's doctoral studies at the Queensland University of Technology. FMLD was supported by an Australian Postgraduate Award and CSIRO Postgraduate Project Award. G. Coman and J. Henderling maintained the *Penaeus japonicus* and *P. semisulcatus* broodstock, and G. Marsden and M. Bourke provided the *P. monodon* larvae. Drs P. Rothlisberg, K. Williams, M. Brown and G. Kelly improved earlier drafts of this manuscript. All experiments complied with the current laws in Australia.

References

AQUACOP (1983) Penaeid larval rearing in the Centre Océanologique du Pacifique. In: McVey JP (ed) Handbook of mariculture. Vol. 1. Crustacean aquaculture. CRC Press, Boca Raton, Florida, pp 123–127

Brenner RR, Bernasconi A (1989) Prostaglandin biosynthesis in the gonads of the hematophagus insect *Triatoma infestans*. Comp Biochem Physiol 93B(1): 1–4

Brown MR (1991) The amino-acid composition of 16 species of microalgae used in mariculture. J exp mar Biol Ecol 145: 79–99

Chu KH, Lui LK (1990) Survival and development of *Metapenaeus ensis* larvae reared on algal and artificial diets. In: Hirano R, Hanyu I (eds) The Second Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines, pp 391–394

Crocos PJ, Coman GJ (1997) Temporal and age variability in the reproductive performance of *Penaeus semisulcatus* broodstock: optimising broodstock selection. Aquaculture, Amsterdam 155: 55–67

Dall W, Hill BJ, Rothlisberg PC, Staples DJ (1990) The biology of the Penaeidae. In: Advances in marine biology. Vol. 27. Academic Press, London

D'Souza FML (1998) The nutritional value of microalgae to penaeid prawn larvae. PhD thesis, Queensland University of Technology, Queensland, Australia

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances, Analyt Chem 28: 350–356

Enright CT, Newkirk GF, Craigie JS, Castell JD (1986) Growth of juvenile *Ostrea edulis* L. fed *Chaetoceros gracilis* Schütt of varied chemical composition. J. exp mar Biol Ecol 96: 15–26

Folch J, Lees M, Sloane Stanely GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J biol Chem 226: 497–509

Frankish KR, Goard LJ, O'Connor WA (1991) The development of hatchery rearing techniques of the Sydney rock oyster (*Saccostrea commercialis*). NSW Fisheries, Brackish Water Fish Culture Research Station, Salamander Bay, New South Wales

Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms. 1. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. Can J Microbiol 8: 229–239

Jackson CJ, Rothlisberg PC, Pendrey RC (1994) The larvatron: an example of computer-based automation in experimental larval culture. In: Chou LM, Munro AD, Lam TJ, Chen TW, Cheong LKK, Ding JK, Hooi KK, Khoo HW, Phang VPE, Shim KF, Tan CH (eds) Proceedings of the Third Asian Fisheries Forum, 26–30th October 1992, Singapore. Asian Fisheries Society, Manila, Philippines, pp 102–105

Jones DA, Kanazawa A, Ono K (1979) Studies on the nutritional requirements of the larval stages of *Penaeus japonicus* using microencapsulated diets. Mar Biol 54: 261–267

Kanazawa A, Teshima S (1977) Biosynthesis of fatty acids from acetate in the prawn. *Penaeus japonicus*. Mem Fac Fish Kagoshima Univ 26: 49–53

Kanazawa A, Teshima S, Endo M, Kayama M (1978) Effects of eicosapentaenoic acid on growth and fatty acid composition of the prawn, *Penaeus japonicus*. Mem Fac Fish Kagoshima Univ 27: 35–40

Kanazawa A, Teshima S, Ono K (1979a) Relationship between essential fatty acid requirements of aquatic animals and the capacity for bioconversion of linolenic acid to highly unsaturated fatty acids. Comp Biochem Physiol 63B: 295–298

Kanazawa A, Teshima S, Ono K, Chalayondeja K (1979b) Biosynthesis of fatty acids from acetate in the prawns, *Penaeus monodon* and *Penaeus merguiensis*. Mem Fac Fish Kagoshima Univ 28: 21–26

Kanazawa A, Teshima S, Tokiwa S, Kayama M, Hirata M (1979c) Essential fatty acids in the diet of prawn. II. Effect of docosahexaenoic acid on growth. Bull Jap Soc scient Fish 45: 1151–1153

Kanazawa A, Tokiwa S, Kayama, M, Hirata M (1977) Essential fatty acids in the diet of prawn. I. Effects of linoleic and linolenic acids on growth. Bull Jap Soc scient Fish 43: 1111–1114

Koskela RW, Greenwood JG, Rothlisberg PC (1992) The influence of prostaglandin E_2 and the steroid hormones, 17a-hydroxyprogesterone and 17b-estradiol and moulting and ovarian development in the tiger prawn, *Penaeus esculentus* Haswell, 1879 (Crustacea: Decapoda). Comp Biochem Physiol 101A: 295–299

Kurmaly K, Jones DA, Yule AB, East J (1989) Comparative analysis of the growth and survival of *Penaeus monodon* (Fabricius) larvae, from protozoea 1 to postlarva 1, on live feeds, artificial diets and on combinations of both. Aquaculture, Amsterdam 81: 27–45

Morrison WR, Smith LM (1964) Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J Lipid Res 5: 600–608

Naranjo J, Porchas MA, Robles M, Magallon FJ, Valdez J, Salinas C, Villareal H (1995) Survival, metamorphosis and growth of brown shrimp *Penaeus californiensis* larvae, fed with different microalgae. In: Aquaculture '95 Conference. February 1–4 San Diego, California. World Aquaculture Society, Baton Rouge, Louisiana, p. 235 (Abstr. No. A503)

Preston NP, Burford MA, Coman FE, Rothlisberg PC (1992) Natural diet of larval *Penaeus merguiensis* (Decapoda: Penaeidae) and its effect on survival. Mar Biol 113: 181–191

Reitan KI, Bolla S, Olsen Y (1994) A study of the mechanism of algal uptake in yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus*). J Fish Biol 44: 303–310

Sánchez R (1986) Rearing of mysid stages of *Penaeus vannamei* fed cultured algae of three species. Aquaculture, Amsterdam 58: 139–144

SAS Institute Inc. (1989) SAS/STAT user's guide. Version 6. SAS Institute Inc., Cary, North Carolina

Simon CM (1978) The culture of the diatom *Chaetoceros gracilis* and its use as a food for penaeid protozoeal larvae. Aquaculture, Amsterdam 14: 105–113

Smayda TJ (1978) From phytoplankters to biomass. In: Sournia A (ed) Phytoplankton manual. UNESCO, Rome, pp 273–279

Stanley-Samuelson DW (1987) Physiological roles of prostaglandins and other eicosanoids in invertebrates. Biol Bull mar biol Lab, Woods Hole 173: 92–109

Teshima S, Kanazawa A, Koshio S (1992) Ability for bioconversion of n-3 fatty acids in fish and crustaceans. Océanis 18: 67–75

Thompson PA, Harrison PJ (1992) Effects of monospecific algal diets of varying biochemical composition on the growth and survival of Pacific oyster (*Crassostrea gigas*) larvae. Mar Biol 113: 645–654

Tobias-Quinitio E, Villegas CT (1982) Growth, survival and macronutrient composition of *Penaeus monodon* Fabricius larvae fed with *Chaetoceros calcitrans* and *Tetraselmis chuii*. Aquaculture, Amsterdam 29: 253–260

Ukeles R, Wikfors GH (1988) Nutritional value of microalgae cultured in the absence of vitamins for growth of juvenile oysters, *Crassostrea virginica*. J Shellfish Res 7: 381–387

Villegas CT, Kanazawa A (1979) Relationship between diet composition and growth rate of the zoeal and mysis stages of *Penaeus japonicus* Bate. Fishery Res J Philipp 4: 32–40

Webb KL, Chu FE (1983) Phytoplankton as a food source for bivalve larvae. In: Pruder GD, Langdon CJ, Conklin DE (eds) Proceedings of the Second International Conference on Aquaculture Nutrition: biochemical and physiological approaches to shellfish nutrition. October 27–29, 1981, Lewes/Rehoboth Beach, Delaware. World Mariculture Society, Louisiana, pp 272–291

Xu XL, Ji WJ, Castell JD, O'Dor RK (1994a) Influence of dietary lipid sources on fecundity, egg hatchability and fatty acid composition of Chinese prawn (*Penaeus chinensis*) broodstock Aquaculture, Amsterdam 119: 359–370

Xu XL, Ji WJ, Castell JD, O'Dor RK (1994b) Essential fatty acid requirement of the Chinese prawn, *Penaeus chinensis*. Aquaculture, Amsterdam 127: 29–40