Marine Biology 118, 45–51 (1994)

Marine Biology
International Journal on Life in Oceans and Coastal Waters
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Biochemical composition and digestive enzyme activity in larvae and postlarvae of *Penaeus japonicus* during herbivorous and carnivorous feeding

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Received: 13 July 1993 Accepted: 12 August 1993

Abstract. The growth, survival, digestive enzyme activity and biochemical composition of *Penaeus japonicus* (Bate) larvae and postlarvae were measured under three feeding regimes. Larvae were reared through the protozoeal stages using *Chaetoceros gracilis*. From the first mysis stage, three feeding regimes were used: (A) *C. gracilis* plus *Artemia* sp. nauplii, (B) *Artemia* sp. nauplii alone or (C) *C. gracilis* alone. No significant difference was found in growth, survival, protein content or lipid content of postlarvae from the treatments receiving the single-feed type, despite the low protein (7%) and highly unsaturated fatty acid content of the alga. Growth of larvae receiving the mixed diet was significantly higher than in the other treatments. Trypsin activity was more strongly influenced than amylase activity by dietary treatment, and differences in the ratio of these enzymes between treatments suggest independent control of their secretion. Trypsin activity recorded in larvae feeding on *C. gracilis* was up to six time higher than in larvae feeding on *Artemia* sp. nauplii, apparently in response to the low protein content of the alga. Larvae receiving the mixed diet exhibited an intermediate level of trypsin activity: it is suggested that the ingestion of algae is necessary for optimal assimilation of the zooplankton component of the diet.

Introduction

After five or six non-feeding nauplius stages, penaeid larvae develop through three herbivorous filter-feeding protozoeal (PZ_{1-3}) moults before the final mysis (M_1–M_3) stages prior to metamorphosis to postlarva. In the wild, penaeid larvae are found in coastal waters where the co-occurrence of both phytoplankton and zooplankton prey is typical. These conditions are mimicked in most commercial culture systems, where microalgal feeds are supplied at least until the end of the mysis stages, with the addition of zooplankton, usually *Artemia* sp. nauplii, between PZ_3 and M_3 (Jones et al. 1987, Hirono and Leslie 1992, Liao 1992, Smith et al. 1992). Under laboratory conditions, raptorial feeding on zooplankton usually begins at the last protozoeal (PZ_3) stage, with a gradual decline in filter-feeding through the mysis stages to complete carnivory during the first post-larval moults (Emmerson 1980, 1984 for *Penaeus indicus*, Yufera et al. 1984 for *P. kerathurus*). However, *P. aztecus* has been shown to feed successfully on phytoplankton throughout the post-larval period (Gleason and Zimmerman 1984). Laboratory studies have shown several penaeid species to be capable of attaining metamorphosis whilst feeding exclusively on phytoplankton (Gopalakrishnan 1976 for *P. marginatus*, Kuban et al. 1985 for *P. aztecus*, *P. setiferus*, *P. vannamei* and *P. stylirostris*). In general, although survival rates are comparable, growth is superior in larvae receiving *Artemia* sp. nauplii from the M_1 stage (Kuban et al. 1985).

While the complex larval development of penaeid prawns has hampered research into their nutritional requirements, their free-swimming larval development has allowed detailed description of the ontogeny of their digestive anatomy and physiology (Lovett and Felder 1989, 1990 a, b, Abubakr and Jones 1992, Jones et al. 1993 a, b). The ability to grow and survive on such different food as phytoplankton or zooplankton implies a high degree of flexibility in digestive physiology to meet nutritional needs. Evidence of such flexibility in digestive enzyme activity has only recently been described in *Penaeus monodon* larvae feeding on live and artificial diets (Jones et al. 1993 a, b). In contrast, previous studies emphasise the importance of diet-independent development of digestive enzyme secretion (Lovett and Felder 1990 a).

Since the development of effective artificial diets for penaeid larvae (Jones et al. 1979 b), complete replacement of algae is now routine in some commercial hatcheries (Avale and Rothius 1991, Ottogalli 1991, 1993). However, it is apparent from recent reviews that, despite the widespread use of formulated feeds in commercial culture of penaeid larvae, there is urgent need for information on nutritional requirements for even the major dietary components, protein, lipid and carbohydrate, during larval life (Guillaume 1990, Kanazawa 1990, Liao and Liu 1990,

Jones et al. 1933 b). Virtually nothing is known of the relative importance of phytoplankton and zooplankton in fulfilling such requirements.

The present results describe the separate and interactive influences of herbivorous and zooplanktivorous feeding on the biochemical composition of *Penaeus japonicus larvae*, and the adaptive response of digestive enzyme activity to diet. *Chaetoceros gracilis* was selected as a phytoplankton feed as this species is widely used in hatcheries and because penaeid larvae are known to feed readily on *Chaetoceros* spp. in the wild (Preston et al. 1992). *Artemia* sp. nauplii were chosen as the zooplankton prey due to their universal use in commercial penaeid hatcheries.

Materials and methods

Algal culture

Chaetoceros gracilis was grown in UV-irradiated, well-source sea water filtered to 1 μm (32‰S) and supplemented with 0.2 ml l^{-1} commercial plant fertiliser (Nutrileaf, Agrocross S.A.) and 1 ml l^{-1} sodium silicate solution (5.8% w/v). Culture densities reached 6×10^6 cells ml^{-1} during semi-continuous batch culture. A concentration of 50 cells μl^{-1} was initiated in *Penaeus japonicus* (Bate) larval cultures, although further growth raised densities to up to 125 cells μl^{-1}. Samples were collected for biochemical analysis by centrifugation, washed, frozen in liquid nitrogen, lyophilized and stored at −80 °C (Mourente et al. 1990).

Artemia sp. nauplii

Cysts of a parthenogenetic *Artemia* sp. strain, collected from the saltmarshes of Cádiz, Spain (Roman and Rodríguez 1989) were hatched overnight in 1 μm-filterd, UV-irradiated, well-source water (32‰S). Fresh nauplii were added to larval cultures each day after water exchange. Samples of freshly hatched nauplii were also collected for biochemical analysis.

Culture of *Penaeus japonicus* larvae

Penaeus japonicus larvae were obtained from a commercial hatchery (CUPIMAR S.A., San Fernando, Cádiz, Spain) during the spawning season, May–June 1992. Larval stages were identified following Hudinaga (1942). Larvae were reared from PZ_1 to PZ_3 using *Chaetoceros gracilis* at a density of 50 to 125 cells μl^{-1}. From M_1, feeding regimes were: (A) *C. gracilis* at 50 to 125 cells μl^{-1} plus freshly hatched nauplii of *Artemia* sp. at 3 to 7 μl^{-1}; (B) freshly hatched *Artemia* sp. nauplii at 3 to 7 ml^{-1}; (C) *C. gracilis* at 50 to 125 cells μl^{-1}. Rearing containers were 25-litre clear acrylic tubes fitted with conical bases and lowest-point aeration. Sea water was supplied from a well source, filtered to 1 μm and UV-irradiated. Water renewal was 50% d^{-1}. Temperature and salinity during the experiment were 26 to 27 °C and 32 to 33‰, respectively. A photoperiod of 16 h light:8 h dark was maintained throughout the experiment. Larvae were stocked at an initial density of 100 l^{-1}. Samples of larvae were collected a each mysis stage and immediately frozen and stored at −80 °C prior to enzyme analysis. When more than 50% of the larvae had reached the first post-larval stage (PL_1), cultures were harvested and counted. Samples of PL_1 were sorted and collected for dry weight estimation and biochemical analysis.

Dry weight determination

Samples of 15 to 25 postlarvae were washed in distilled water and oven-dried at 110 °C for 24 h. After cooling in vacuo for at least 1 h, samples were weighed using a Mettler UM3 microbalance.

Total lipid extraction and quantification

Lipids were extracted after homogenization in chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene (BHT) (Folch et al. 1957) and dried in oxygen-free nitrogen. Total lipid content was determined gravimetrically using a Mettler UM3 microbalance.

Fatty acid analysis

Fatty acid methyl esters were prepared by acid-catalysed transmethylation for 16 h at 50 °C, using nonadecanoic acid (19:0) as internal standard (Christie 1989). Methyl esters were extracted and purified following the method of Tocher and Harvie (1988). The fatty acid methyl esters were analysed in a Hewlett-Packard 5890 A Series II gas chromatograph equipped with a chemical bonded (PEG) Omegawax 320 fused silica wall coated capillary column (30 m × 0.32 mm i.d., Supelco Inc., Bellefonte, USA) using hydrogen as the carrier gas with a thermal gradient from 185 to 235 °C. Individual fatty acid methyl esters were identified following the methods of Tocher and Harvie and quantified using a Hewlett-Packard 3394 recording integrator.

Compositional analysis of feeds and postlarvae

Elemental composition (C, N) analyses of postlarvae and feeds were performed in an elemental analyser Carlo Erba 1106, using cyclohexane 2,4-dinitrophenylhydrozone as standard. Protein content was obtained by multiplying nitrogen content by 6.25. Carbohydrate was determined by the phenol-sulphuric acid method (Kochert 1978). Energy values for *Chaetoceros gracilis* and *Artemia* sp. nauplii were obtained by multiplying carbohydrate, protein and lipid contents by 17.14, 23.42 and 39.31 J, respectively (Winberg 1971).

Trypsin activity

Samples of larvae and postlarvae were homogenised in ice-cold Tris-HCl buffer (46 m*M* Tris, 11.5 m*M* $CaCl_2$, pH 8.1). Homogenates were centrifuged at 12000 × *g* for 5 min and the supernatant was assayed for trypsin-like activity at 25 °C using 1 m*M* *N*α-p-toluene-sulphonyl-L-argenine methyl ester in the same buffer (Hummel 1959). The rate of hydrolysis of the substrate was recorded as increase in absorbance at 247 nm in a Perkin-Elmer Lambda UV/vis spectrophotometer. Activity per larva was calculated, and expressed in units of μmoles of substrate cleaved per minute, based on an extinction coefficient of 0.54 cm^2 μmol^{-1} for the product of the reaction (Rick 1984).

Amylase activity

Samples of larvae and postlarvae were homogenized in ice-cold phosphate buffer (20 m*M* phosphate, 10 m*M* NaCl, pH 6.9), centrifuged for 5 min at 12000 × *g*, and samples of the supernatant were incubated with 1% w/v starch in the same phosphate buffer. Production of reducing sugars was quantified colorimetrically following addition of dinitrosalicylic acid reagent (1% 3.5-dinitrosalicylic acid, 30% w/v potassium sodium tartrate) (Rick and Stegbauer 1984). A standard curve was prepared using maltose solution instead of enzyme sample, and results are expressed as μmol of maltose equivalents released per larvae per minute.

Statistical analysis

Except where indicated, results are presented as means ± standard deviation. Differences between treatments were analysed by one-

Table 1. *Chaetoceros gracilis* and *Artemia* sp. Composition (% dry wt), gross energy (J mg^{-1} dry wt) and fatty acid content (µg fatty acid mg^{-1} dry wt) of diatom *C. gracilis* and nauplii of branchiopod crustacean *Artemia* sp. (parthenogenetic strain from salt marshes of Cádiz, Spain) used for rearing *Penaeus japonicus* larvae. Data are means ± SD ($n = 3$); SD of 0.0 implies SD < 0.05; nd: not detected. HUFA: highly unsaturated fatty acids (≥ C_{20}, with at least 3 double bonds)

Component	*C. gracilis*	*Artemia* sp.
Protein	6.9 ± 0.5	56.2 ± 1.5
Lipid	4.1 ± 0.7	17.5 ± 1.1
Carbohydrate	15.2 ± 0.8	16.5 ± 0.3
Ash	73.8 ± 0.2	9.8 ± 0.4
Energy content	5.8 ± 0.2	21.3 ± 0.3
Fatty acid		
14:0	1.5 ± 0.1	2.2 ± 0.2
15:0	0.4 ± 0.1	2.4 ± 0.3
16:0	1.0 ± 0.1	18.9 ± 0.4
16:1*n*-9	1.1 ± 0.1	nd
16:1*n*-7	3.8 ± 0.4	22.2 ± 0.3
16:2	1.6 ± 0.2	1.9 ± 0.2
16:3	3.6 ± 0.2	2.1 ± 0.2
16:4	0.5 ± 0.1	0.5 ± 0.2
18:0	0.2 ± 0.0	6.2 ± 0.3
18:1*n*-9	0.1 ± 0.0	26.1 ± 0.6
18:1*n*-7	0.1 ± 0.0	17.8 ± 1.1
18:2*n*-6	0.1 ± 0.0	6.0 ± 0.5
18:3*n*-3	0.2 ± 0.0	4.2 ± 0.3
18:4*n*-3	0.1 ± 0.0	1.3 ± 0.1
20:1*n*-9	0.1 ± 0.0	0.3 ± 0.2
20:2*n*-6	0.1 ± 0.0	0.4 ± 0.0
20:3*n*-6	0.1 ± 0.0	0.3 ± 0.1
20:4*n*-6	0.3 ± 0.0	2.6 ± 0.2
20:4*n*-3	0.1 ± 0.0	0.5 ± 0.1
20:5*n*-3	4.6 ± 0.1	14.6 ± 0.6
22:6*n*-3	0.3 ± 0.0	nd
Total saturates	3.2 ± 0.2	30.5 ± 0.3
Total monoenes	5.3 ± 0.1	66.9 ± 2.1
Total polyunsaturates	11.9 ± 0.2	35.1 ± 0.3
n-6 HUFA	0.3 ± 0.0	2.6 ± 0.3
n-3 HUFA	5.1 ± 0.4	15.4 ± 0.5

way analysis of variance, followed when pertinent by a multiple-comparison test (Tukey's for equal sample sizes, Scheffé's method for unequal sample sizes). Differences are reported as statistically significant when $p < 0.05$ (Zar 1984).

Results

The proximate composition, energy content and fatty acid content of *Chaetoceros gracilis* and *Artemia* sp. nauplii are shown in Table 1. The differences in protein, lipid and energy content of the feeds are largely accounted for by the high ash content of *C. gracilis* (73.8%) compared to that of *Artemia* sp. nauplii (9.8%). Protein content of *C. gracilis* is still substantially lower (26.3%) than that of *Artemia* sp. nauplii (61.6%) when expressed as percentage of ash-free dry weight. Differences in highly unsaturated fatty acid (HUFA) content are also largely accounted for by the difference in ash content, although only *C. gracilis* contained measurable amounts of 22:6*n*-3. In contrast, the similar levels of carbohydrate found in the two feed species reflect a higher content in the diatom compared to the

Table 2. *Penaeus japonicus*. Protein, lipid and carbohydrate contents (µg mg^{-1} ash-free dry wt), dry wt and ash content (µg postlarva^{-1}), length (mm) and survival (%) of postlarvae reared on *Chaetoceros gracilis* throughout all stages *plus Artemia* sp. nauplii during mysis and postlarval stages (Diet A); *C. gracilis* through protozoeal stages only, and thereafter *Artemia* sp. nauplii *alone* for mysis and postlarval stages (Diet B); or *C. gracilis alone* throughout all stages (Diet C). Data are means ± SD ($n = 3$); Values within any given row *not* bearing same superscript are significantly different at $p < 0.05$; values bearing no superscript are not significantly different

Component	Diet A	Diet B	Diet C
Protein (µg mg^{-1})	695.7 ± 12.2^a	620.8 ± 29.3^b	594.5 ± 4.1^b
Lipid (µg mg^{-1})	263.2 ± 14.4^a	334.8 ± 10.2^b	349.3 ± 7.9^b
Carbohydrate (µg mg^{-1})	44.6 ± 1.7^a	43.3 ± 1.6^a	52.5 ± 3.5^b
Dry weight (µg postlarva^{-1})	115.4 ± 6.8^a	76.3 ± 7.2^b	71.3 ± 6.7^b
Ash (mg postlarva^{-1})	23.6 ± 3.1	16.4 ± 2.5	22.0 ± 2.3
Length (mm)	5.0 ± 0.2^a	4.3 ± 0.2^b	3.8 ± 0.1^b
Survival (%)	75.7 ± 5.3^a	46.0 ± 2.7^b	46.2 ± 3.8^b

Artemia sp. nauplii when expressed as percentage of ash-free dry weight.

Length, weight, survival and proximate analysis for *Penaeus japonicus* at postlarval Stage I (PL_1) are shown in Table 2. There was no significant difference in dry weight, length or survival between the two treatments receiving either *Chaetoceros gracilis* or *Artemia* sp. nauplii as sole feed from M_1 to PL_1. However, postlarvae reared on the mixed diet were significantly greater in dry weight, length and survival compared to both other treatments ($p < 0.05$).

Postlarvae from the two treatments receiving *Chaetoceros gracilis* or *Artemia* sp. nauplii as sole food from M_1 did not differ significantly in body protein or lipid content. Carbohydrate content was significantly higher for the treatment receiving only *C. gracilis* than for the other treatments ($p < 0.05$). Postlarvae from the treatment receiving both *C. gracilis* and *Artemia* sp. nauplii had significantly higher body protein content and lower lipid content than the treatments receiving only one feed type ($p < 0.05$).

The fatty acid contents of postlarvae are shown in Table 3. Comparison of the treatments receiving *Chaetoceros gracilis* or *Artemia* sp. nauplii exclusively shows that the fatty acid content of the postlarvae reflects the content of these feeds. The *n*-3 HUFA content of postlarvae reared on the mixed feed was intermediate to that of individuals reared on either feed exclusively. However, the 20:5*n*-3 content of individuals from the mixed-feed treatment did not differ significantly from that of larvae reared on *Artemia* sp. nauplii alone. Despite the lack of a measurable quantity of 22:6*n*-3 in *Artemia* sp. nauplii, 1.2 µg mg^{-1} of this fatty acid was recorded in postlarvae feeding on *Artemia* sp. nauplii exclusively during the mysis stages. In contrast, the presence of low levels of 22:6*n*-3 in *C. gracilis* resulted in significantly more of this fatty acid accu-

Table 3. *Penaeus japonicus*. Total lipid fatty acid contents (µg fatty acid mg^{-1} dry wt) of postlarvae fed on same diets as detailed in legend to Table 2. Data are means ± SD ($n = 3$); SD of 0.0 implies SD < 0.05. Values within any given row *not* bearing same superscript are significantly different a $p < 0.05$; values bearing no superscript are not significantly different. Totals include some minor components (< 0.1%) not listed separately. HUFA: highly unsaturated fatty acids (≥ C_{20}, with at least 3 double bonds)

Fatty acid	Diet A	Diet B	Diet C
14:0	1.8 ± 0.2	1.6 ± 0.9	2.4 ± 0.6
15:0	3.3 ± 0.4	2.9 ± 0.8	4.8 ± 0.7
16:0	8.3 ± 0.2^{a}	9.9 ± 0.4^{b}	7.8 ± 0.3^{b}
16:1*n*-7	3.8 ± 0.2^{a}	4.9 ± 0.6^{a}	2.0 ± 0.1^{b}
16:2	0.7 ± 0.1	0.9 ± 0.5	0.5 ± 0.1
16:3	1.2 ± 0.1^{ab}	0.9 ± 0.1^{a}	1.5 ± 0.3^{b}
16:4	0.9 ± 0.0	1.2 ± 0.1	0.9 ± 0.0
18:0	6.5 ± 0.3	7.9 ± 0.5	5.8 ± 0.3
18:1*n*-9	7.9 ± 0.4^{a}	11.7 ± 0.8^{b}	2.6 ± 0.6^{c}
18:1*n*-7	8.2 ± 0.5^{a}	11.6 ± 0.6^{b}	2.5 ± 0.4^{c}
18:2*n*-6	2.1 ± 0.1^{a}	3.6 ± 0.1^{b}	0.8 ± 0.2^{c}
18:3*n*-3	0.4 ± 0.1	0.9 ± 0.5	1.0 ± 0.2
18:4*n*-3	0.8 ± 0.3	1.1 ± 0.5	0.3 ± 0.1
20:0	0.3 ± 0.0^{a}	0.3 ± 0.1^{ab}	0.5 ± 0.1^{b}
20:1*n*-9	0.5 ± 0.0^{a}	0.7 ± 0.1^{a}	0.9 ± 0.1^{b}
20:1*n*-7	0.3 ± 0.0	0.4 ± 0.1	0.4 ± 0.1
20:2*n*-6	0.3 ± 0.1	0.3 ± 0.0	0.5 ± 0.2
20:3*n*-6	0.2 ± 0.1^{a}	0.4 ± 0.0^{a}	0.7 ± 0.1^{b}
20:4*n*-6	2.1 ± 0.1^{a}	2.5 ± 0.1^{b}	0.9 ± 0.1^{c}
20:3*n*-3	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:4*n*-3	0.2 ± 0.0^{a}	0.4 ± 0.0^{ab}	0.6 ± 0.2^{b}
20:5*n*-3	12.5 ± 0.7^{a}	15.8 ± 1.1^{a}	7.4 ± 1.4^{b}
22:3*n*-3	0.2 ± 0.0	0.2 ± 0.1	0.5 ± 0.3
22:5*n*-6	0.2 ± 0.1	0.2 ± 0.0	0.3 ± 0.1
22:5*n*-3	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.1
22:6*n*-3	1.2 ± 0.2^{a}	1.2 ± 0.0^{a}	2.5 ± 0.4^{b}
Total saturates	21.3 ± 0.3	23.1 ± 1.3	22.0 ± 0.8
Total monoenes	20.9 ± 1.0^{a}	29.5 ± 2.1^{b}	9.0 ± 0.8^{c}
Total polyenes	24.5 ± 1.1^{a}	31.4 ± 0.7^{b}	20.5 ± 1.0^{c}
n-6 HUFA	2.5 ± 0.1^{a}	3.1 ± 0.1^{b}	1.9 ± 0.1^{a}
n-3 HUFA	14.6 ± 0.7^{a}	18.1 ± 1.1^{b}	11.3 ± 1.2^{c}

mulating in individuals feeding on the alga alone. Surprisingly, there was no difference between the 22:6*n*-3 content of larvae feeding on the mixed diet and those feeding only on *Artemia* sp. nauplii.

The results of the trypsin analysis are shown in Fig. 1. After a steady rise in trypsin activity from PZ_1 to M_1 in larvae feeding on *Chaetoceros gracilis*, there were significant differences in trypsin activity between treatments during the mysis stages. Activity in mysis larvae and postlarvae feeding on *C. gracilis* was substantially higher than that of larvae feeding on *Artemia* sp. nauplii, with activity in M_2 to M_3 larvae feeding on *C. gracilis* being over five times higher than in larvae feeding on *Artemia* sp. nauplii. Trypsin activity in larvae feeding on the mixed diet followed an intermediate pattern to those observed in larvae feeding on *C. gracilis* or *Artemia* sp. nauplii alone.

The results of the amylase analyses are shown in Fig. 2. There were significant differences in amylase activity between dietary treatments in the mysis stages, but these were not as large or as consistent as those observed for trypsin. At M_1 and M_2, amylase activity in larvae feeding

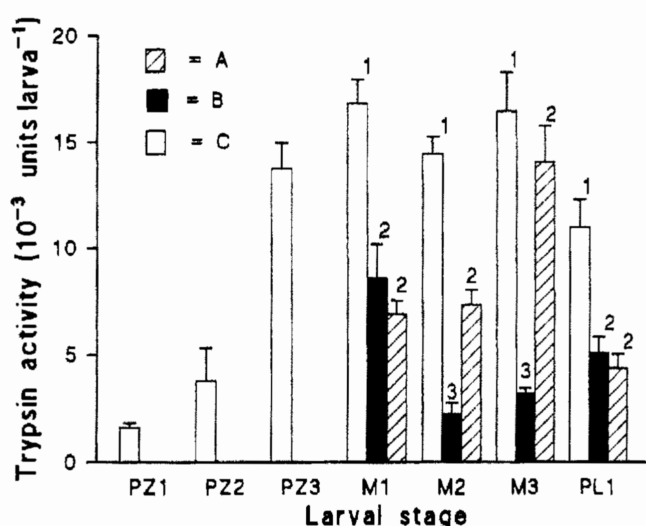

Fig. 1. *Penaeus japonicus*. Trypsin activity (U $larva^{-1}$) in larvae and postlarvae fed *Chaetoceros gracilis* throughout all stages *plus Artemia* sp. nauplii during mysis and postlarval stages (A); *C. gracilis* through protozoeal stages only, and thereafter *Artemia* sp. nauplii *alone* for mysis and postlarval stages (B); or *C. gracilis alone* throughout all stages (C). $n = 3$ to 6 for each data point. Treatments bearing different superscripts are significantly different at $p < 0.05$. PZ: protozoea; M: mysis; PL: postlarva. Subscripts denote specific stages

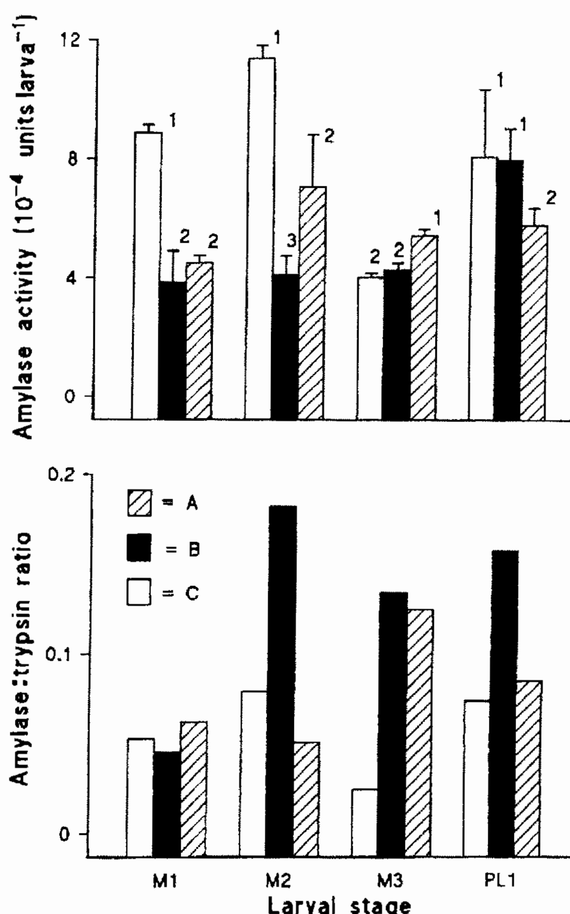

Fig. 2. *Penaeus japonicus*. Amylase activity (10^{-4} U $larva^{-1}$) and amylase:trypsin ratio in larvae and postlarvae fed same diets as detailed in legend to Fig. 1. $n = 5$ to 6 for each data point. All other details as in legend to Fig. 1

on *Chaetoceros gracilis* was significantly higher than that measured in larvae feeding on *Artemia* sp. nauplii or the mixed diet ($p < 0.05$). At M_3, activity in larvae feeding on *C. gracilis* fell to the same level as that observed in larvae feeding on *Artemia* sp. nauplii alone, with the mixed diet producing significantly higher activity ($p < 0.05$). Amylase activity in individuals from the treatment feeding on *Artemia* sp. nauplii alone did not rise substantially until PL_1, where levels were equivalent to those for larvae feeding on *C. gracilis*, both these treatments giving higher values than the mixed diet.

Discussion

The proximate analysis of *Artemia* sp. nauplii used in the present study is within the range of values for nauplii used in penaeid larval culture (Jones et al. 1993 b). The high ash content of *Chaetoceros gracilis* is surprising, but repeated analyses of new cultures from the same stock subculture used in this study have yielded identical results to those presented here and the proximate composition of the organic component of *C. gracilis* agrees with results of Ben-Amotz et al. (1987).

In general, successful feeds for penaeid larvae have ranged from 23 to 55% protein content and HUFA content of > 1% (Table 4). In the present study on *Penaeus japonicus*, an algal diet of less than 7% protein content and low HUFA content supported growth and survival through the mysis stages equal to that achieved with a zooplankton diet of higher HUFA and protein content. Given that prior to the experiment all larvae had been raised from PZ_1 exclusively on the same algal feed, it is clear that the absolute requirements of *P. japonicus* larvae for protein and HUFA may be substantially lower than suggested in Table 4. However, while growth and survival through the mysis stages is possible on either *Chaetoceros gracilis* or *Artemia* sp. nauplii alone, results with these feeds used singly are clearly sub-optimal. In contrast, growth and survival on the mixed-free regime are equivalent to the best results achieved by Hudinaga (1942).

It is remarkable that the large difference in gross nutritional content of *Chaetoceros gracilis* and *Artemia* sp. nauplii and consequent differences in energy and protein intake is not reflected in the growth, survival or body composition of the *Penaeus japonicus* larvae feeding on them. The explanation for this may lie in the digestive response of the larvae to the two feeds. As compensation for feeding on an algal species of low protein content, the high level of trypsin activity observed in *P. japonicus* larvae presumably acts to maximise assimilation of this scarce nutrient, as has been suggested for *Calanus helgolandicus* (Harris et al. 1986). Furthermore, Jones et al. (1993 a, b) report a stimulation of trypsin activity in *P. monodon* larvae by microalgae which does not appear to be related to gross dietary protein content. The higher levels of trypsin activity recorded in larvae feeding on *Chaetoceros gracilis* in the present study may be due to the combination of a general response to low dietary protein availability and a direct stimulation of secretion by an active component of the alga. The relationship between diet and amylase activity is less clear, particularly as the carbohydrate content of *C. gracilis* and the *Artemia* sp. nauplii were similar. It is not known to what extent algal carbohydrates are available for digestion by crustacean larvae (Lovett and Felder 1989). Thus, the high levels of amylase activity observed in early mysis larvae feeding on *C. gracilis* might also reflect low substrate availability, although the ability to vary amylase secretion with diet is markedly reduced by M_3 and PL_1. The differences in the amylase:trypsin ratio between dietary treatments (Fig. 2) support the contention of Hirche and Anger (1987) that decapod larvae capable of herbivorous feeding should exhibit independant regulation of these enzymes.

The superior larval growth, survival and protein retention observed in larvae receiving a mixture of *Chaetoce-*

Table 4. *Penaeus* spp. Summary of reported dietary protein and lipid levels which have supported good growth and survival in *Penaeus japonicus* and *Penaeus monodon* larvae (adapted from Jones et al. 1993 b). PZ: protozoea; M: mysis; PL: postlarva. Subscripts denote specific stages

Nutrient	Level	Comment	Source
Protein			
P. japonicus	45–55%	Given 15–25% carbohydrate	Kanazawa (1990)
P. monodon	48–52%	Best growth, live food	Kurmaly et al. (1989)
P. monodon	51–56%	Best growth, encapsulated diets	Kurmaly et al. (1989)
P. monodon	30%	Microparticulate diet	Khannapa (1979)
P. monodon	23%	PZ_1-M_1, live food	Tobias-Quinitio and Villegas (1982)
Lipid			
P. japonicus	Dietary requirement for HUFA		Jones et al. (1979 a)
	1% HUFA requirement		Guillaume (1990)
	3% phospholipid requirement		Kanazawa (1990)
	1% cholesterol requirement		Teshima et al. (1983)
P. monodon (live food)	4.3% total lipid, 3.4% HUFA (PZ_{1-3})		Kurmaly et al. (1989)
	16% total lipid, 12.4% HUFA (M_1-PL_1)		Kurmaly et al. (1989)
P. monodon (encapsulated feed)	23.5% total lipid, 26.1% HUFA (PZ_{1-3})		Kurmaly et al. (1989)
	18% total lipid, 12.4% HUFA (M_1-M_3)		Kurmaly et al. (1989)

ros gracilis and *Artemia* sp. nauplii may be due in part to elevated trypsin levels which would have contributed to improved digestion of the *Artemia* sp. component of the diet. Thus, while growth of larvae feeding on *C. gracilis* alone was very probably directly limited by nutrient availability, for larvae feeding on *Artemia* sp. nauplii assimilation and retention of dietary protein may have been reduced in the absence of the digestive response induced by the algal co-feed. Although *Penaeus japonicus* larvae are known to have an essential dietary requirement for n-3 HUFA, the ability of *P. japonicus* larvae feeding exclusively on *Artemia* sp. nauplii to synthesise some 22:6n-3 is in accordance with the findings of Jones et al. (1979 a). However, it is not known to what extent supplementation with trace levels of dietary 22:6n-3, such as those present in *C. gracilis* in the present study, might also have contributed to the improved growth and survival of the mixed feed treatment.

A peak in digestive enzyme production has been reported in PZ_3 to M_1 larvae of several penaeid species (Laubier-Bonichon et al. 1977, Galgani and Benyamin 1985, McDonald et al. 1989, Lovett and Felder 1990 a, Jones et al. 1993 a, b, Le Moullac et al. 1993). This period of development coincides with both the maximal volume of the anterior midgut diverticulae (AMD) (Lovett and Felder 1989, 1990 b, Abubakr and Jones 1992), and the commencement of carnivorous feeding. Thus, the decline in digestive enzyme activity from the early mysis stages of penaeid larvae has been interpreted either as an adaption to change in feeding requirements (Laubier-Bonichon et al. 1977) or as a consequence of regression of the AMD in the ontogenetic development of the digestive secretory apparatus (Lovett and Felder 1990 a, c). In the present study, there is evidence of great flexibility in digestive enzyme response to diet through the later larval stages, and it is clear that the patterns of trypsin and amylase activity for at least mysis-stage penaeid larvae reported in the literature are likely to reflect an adaptation to the particular laboratory feeding regime used rather than the actual secretory capacity of the species in question.

The degree to which the modulation of digestive enzyme activity in response to diet observed in this study extends through the "critical" early postlarval period (Wickins 1976, Bages and Sloane 1981), when the AMD becomes vestigial, remains to be examined. Ingestion of benthic microalgae is known to persist through this period for some species (Gleason and Zimmerman 1984, Gleason 1986), and Griffith et al. (1992) have recently reported beneficial effects on growth and survival of *Penaeus vannamei* postlarvae feeding on benthic diatoms in addition to *Artemia* sp. nauplii.

Acknowledgements. This work was supported by the Comision Interministerial de Ciencia y Tecnologia, Spain (CICYT project MAR 90-0767-C04-02) and by Acciones Intergradas Hispano-Britanicas No. 175A. D. A. Jones and L. Le Vay acknowledge the support of an SERC (Marine Technology Directorate) cooperative research grant. We thank S. Gonzalez and A. Arjonilla for technical assistance and CUPIMAR S. A. for the supply of *Penaeus japonicus* larvae.

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Communicated by J. Mauchline, Oban