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Digestive enzyme profiles of spiny lobster *Jasus edwardsii* phyllosoma larvae

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ABSTRACT: Digestive enzyme activities of cultured (Stage I to VI) and wild (Stage V to XI) phyllosoma larvae of the spiny lobster *Jasus edwardsii* were investigated over progressive stages of development to provide an indication of their digestive capabilities and nutritional requirements and to help identify the characteristics of their natural prey. Protease, trypsin, amylase, α-glucosidase, chitinase and lipase were detected in all cultured and wild specimens, suggesting phyllosoma can readily digest dietary protein, lipid and carbohydrate, including chitin at all stages of development. Protease and lipase activities were considerably higher than amylase and α-glucosidase, indicating that dietary protein and lipid is more important than carbohydrate and thus suggests a carnivorous diet. Total digestive enzyme activities (Units larva^{-1}, units defined as the amount of enzyme that catalysed the release of 1 µmole of product per minute) increased significantly with larval development, reflecting the considerable increase in digestive capacity that is required to meet the metabolic requirements of increasing larval body mass. Relatively constant specific enzyme activity (Units mg^{-1}) in cultured larvae fed the same diet suggests that specific activity variations evident in wild larvae may reflect changes in natural diet or feeding abilities. A large increase in protease, trypsin and amylase specific activity between wild phyllosoma Stages VI and VII may be driven by an increase in food availability or processing efficiency that precedes a large increase in phyllosoma size. Enzyme profiles for both cultured and wild *J. edwardsii* phyllosoma suggest that spiny lobster phyllosoma are capable of digesting a wide range of zooplankton prey, but they make best use of dietary items that are high in protein and lipid.

KEY WORDS: Spiny lobster · Phyllosoma · Larvae · Digestive enzymes · Diet · Ontogeny · Nutrition

INTRODUCTION

Spiny lobsters are of great economic and ecological significance throughout much of the world; However, their larval biology is poorly understood (Booth 2002). In particular, the natural prey of the larvae, or phyllosoma, are unknown (Cox & Johnston 2003a). Increasing global demand, a high market value, and concern for the sustainability of wild stocks have created significant interest in the development of spiny lobster aquaculture (Jeffs & Hooker 2000). The lack of knowledge about the natural diet and nutritional requirements of spiny lobster phyllosoma has been a major impediment to the development of larviculture, with unsuitable diets and feeding regimes being blamed for the consistent occurrence of high mortalities (Phillips & Sastry 1980, Kittaka 1994, 1997, Cox & Johnston 2003a). Brine shrimp *Artemia* sp. are currently used in most hatcheries attempting to culture phyllosoma (Tong et al. 1997, Moss et al. 1999, Ritar et al. 2002), but a wide variety of inert and live foods including fish larvae, jellyfish, polychaetes and mussel flesh have also been

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tried with variable success (Ritar et al. 2002, Cox & Johnston 2003a). A range of approaches to identify the natural diet of phyllosoma has had very limited success, including using biochemical dietary markers (Nichols et al. 2001, Jeffs et al. 2004), gut contents (Phillips & Sastry 1980), digestive morphology (Johnston & Ritar 2001, Cox & Bruce 2002, Nelson et al. 2002, Cox & Johnston 2003b), natural prey-choice experiments (Mitchell 1971) and laboratory trials (Kittaka et al. 2001).

The analysis of digestive enzyme activities has proven to be an effective approach in understanding the crustacean digestive process and determining the nutritional characteristics of natural diets (Lovett & Felder 1990, Fang & Lee 1992, Kamarudin et al. 1994, Jones et al. 1997, Hammer et al. 2000, Johnston 2003). Crustacean larval digestive physiology is a reflection of diet and feeding strategy (Jones et al. 1997, Le Vay et al. 2001), and ontogenetic changes in digestive enzyme expression during larval development may be used to identify natural developmental feeding transitions (Hammer et al. 2000). Adjusting larval artificial feed formulations to coincide with any natural feeding transitions would result in greater productivity by improving the health, maximising growth, and reducing waste of feed in an aquaculture setting (Tengjaroenkul et al. 2002).

The development of digestive function by early life history stages of lobsters is largely unstudied, with only a few published studies on the larval stages of *Homarus americanus*, *H. gammarus* and *Procambarus clarkii* (Biesiot & Capuzzo 1990, Kurmaly et al. 1990, Hammer et al. 2000). However, the biology of spiny lobster phyllosoma is unique, and they are likely to have a totally different feeding and digestive capacity. Spiny lobster phyllosoma have a long larval period of up to 2 yr, and in the case of *Jasus edwardsii* the phyllosoma pass through 11 developmental stages (Lesser 1978, Lipcius & Eggleston 2000). Early stages (I to III) are predominantly found in coastal waters, whereas later stages (V to XI) inhabit oceanic waters where markedly different potential prey species are present at generally far lower abundance (Bruce et al. 1997). Phyllosoma development involves a phenomenal increase in body mass (several orders of magnitude; Ritar et al. 2003), and the accumulation of sufficient energy stores in the final developmental stages to power the non-feeding but highly active post-larval or puerulus stage (Jeffs et al. 1999).

While there are no data on digestive enzymes in spiny lobster phyllosoma, one study has quantified developmental changes in enzymology of puerulus, juvenile and adult *Jasus edwardsii* (Johnston 2003). Enzyme profiles of these later stages of the lifecycle revealed that protein and lipid are important energy sources. The non-feeding puerulus was found to rely primarily on stored lipid reserves, and although lobsters were carnivorous, there appeared to be a selection of carbohydrate-rich prey by early juvenile lobsters (Johnston 2003). Mouthpart and foregut structure has been examined in *J. edwardsii* phyllosoma and minimal change in mouthpart structures indicated that ingestive capabilities and mastication are well developed from hatch (Johnston & Ritar 2001). Digestive gland volume increases significantly during development and enzyme secreting F-cells are present post-hatch, suggesting that digestive capabilities may be developed from the time of first feeding, and increase in late-stage phyllosoma (Johnston et al. 2001).

This study examines digestive enzyme profiles from early and mid-stage cultured *Jasus edwardsii* phyllosoma fed a diet of known composition to determine their digestive capacity and identify whether it changes as the larvae develop. The digestive enzyme profiles from mid- and late-stage wild phyllosoma are also examined to determine changes in their digestive capacity and nutritional requirements and to help identify the characteristics of their natural prey. Our understanding of phyllosoma digestive capacity and the nature of the wild diet gained from the wild samples will contribute to the development of culture diets tailored to meet the digestive capabilities of spiny lobster phyllosoma at each larval stage.

MATERIALS AND METHODS

Wild-caught larvae. Wild caught phyllosoma were sampled at 8 to 14 approximately equidistant stations along each of 5 transects extending 1 to 318 km offshore from the south-east coast of the North Island of New Zealand (Jeffs et al. 2001). An Engel fine-meshed (12 mm) mid-water trawl plankton net was towed at approximately 3 nautical miles h^{-1} by the 70 m RV 'Tangaroa' for 10 min at the 100, 60, and 20 m depth horizons at night. The phyllosoma were sorted by size initially into mid- and late stage larvae and the individual stages identified according to Lesser (1978). Larvae were frozen in liquid nitrogen, stored at –80°C, and individuals at each stage (n = 5 to 18 depending on number available) were subsequently thawed and measured for total length (from the anterior tip of the cephalic shield between the eyestalks to the posterior tip of the abdomen) and width (left and right extremes of the cephalic shield) on a Nikon 6C Profile Projector (Japan) prior to enzyme analyses.

Cultured larvae. Newly hatched phyllosoma larvae were collected in July 2001 from a female held at the Marine Research Laboratories, Taroona, Tasmania, for 2 yr after capture. The female had previously been

exposed to an altered phototherm regime to mate and hatch out of season (Smith et al. 2003), and had previously been fed on fresh mussels, squid and commercial prawn pellets. The temperature at the time of hatch was 17.5°C. After disinfection with 25 ppm formaldehyde in sea water for 30 min, phyllosoma were dispensed into 18 culture vessels (35 l plastic vessels containing 10 l water) at approximately 1600 larvae per vessel. The culture vessels were supplied with seawater at 18°C, filtered to 1 µm, and disinfected with ultraviolet radiation (Ritar 2001). There was partial recirculation of seawater through the entire system at a rate of approximately 6 complete exchanges daily. Decapsulated *Artemia* cysts (E.G. grade, Artemia Systems, INVE) were hatched and cultured to ≥1.5 mm. Juvenile *Artemia* were enriched for 6 h with DHA Selco oil enrichment (INVE) and *Isochrysis* sp. (Tahitian strain) and fed daily to phyllosoma at a density of 3 ind. ml^{-1}. At approximately 5 d after the peak of moulting at each stage, larvae (n = 15) were measured for length and width before being returned to the culture vessel.

Enzyme analyses. ***Larval samples:*** Cultured larvae were sampled in triplicate with each sample consisting of pooled animals ranging from 1000, 500, 300, 150 and 100 for Stages I, II, III, IV, and V and VI, respectively. Wild-caught larvae samples were either pooled or individual, with replicates varying depending on numbers available. Two replicates of 4 pooled animals were used for Stage V, 3 replicates of 6 pooled animals for Stage VI, 4 replicates of 3 pooled animals for Stage VII and 5 replicates of individual animals for Stages VIII to XI. There were insufficient wild-caught larvae to examine α-glucosidase and chitinase activity. *Artemia* samples (n = 1000 × 3 replicates) were also assayed for enzyme activity at the beginning and end of the culture period to compare with phyllosoma activity.

Enzyme extraction: Cultured larvae samples were homogenised for 5 min in 1 ml of chilled 50 mM Tris, 10 mM $CaCl_2$, 20 mM NaCl buffer pH 7.5 using an electric Ultraturrax disperser (IKA Works). Wild-caught larvae samples were homogenised in 2 ml of buffer. The homogenate was centrifuged at 10 000 × *g* for 10 min at 4°C and 200 µl aliquots of supernatant transferred to microfuge tubes and stored at –20°C.

Enzyme assays: One enzyme unit was defined as the amount of enzyme that catalysed the release of 1 µmole of product per minute, and was calculated using the appropriate molar extinction coefficient (ε) in the assay conditions or a standard curve. Specific activity was defined as enzyme activity per mg of larval protein (Units mg^{-1}) and total activity was defined as enzyme activity per larva (Units $larva^{-1}$). Protein concentration was determined by the method of Bradford (1977) using bovine serum albumin as the standard. Spectrophotometric enzyme assays (200 µl micro-assays) were performed in duplicate at 37°C in IWAKI flatbottom microplates and absorbances read in a Tecan Spectro Rainbow Thermo microplate reader. Appropriate controls were included with each analysis. Tests confirmed that enzyme activities were linear with incubation time.

Proteases: Total protease activity was measured by casein hydrolysis (modified by Walter 1984). Each assay consisted of 100 mM Tris, 50 mM NaCl buffer pH 8.0 and 1% casein (w/v) dissolved in 100 mM Tris, 50 mM NaCl buffer pH 8.0. The reaction commenced with the addition of enzyme extract, incubated for 60 min at 37°C, and stopped by adding 8% (w/v) trichloroacetic acid. Reaction tubes were placed immediately on ice for 30 min, centrifuged at 1200 rpm (968 × *g*) for 10 min and the absorption of the supernatant read at A_{280}. One unit of total protease activity was calculated from a tyrosine (0.25 mg ml^{-1} stock solution) standard curve that was generated by diluting aliquots of the tyrosine stock solution with 100 mM HCl.

Trypsin was assayed using N-α-benzoylarginine-ρ-nitroanalide (BAPNA) dissolved in dimethylformamide (DMF) as substrate. Each assay contained a final concentration of 1.25 mM BAPNA in 200 mM Tris, 200 mM NaCl, 10 mM $CaCl_2$ and 0.2% (w/v) polyethylene glycol 6000 pH 8. Assays were initiated by the addition of enzyme extract and the release of ρ-nitroanalide measured at $A_{400-410}$. Under these assay conditions the molar extinction coefficient was 9300 M^{-1} cm^{-1} for ρ-nitroanaline (Stone et al. 1991). A positive control of 3 mg ml^{-1} porcine pancreas trypsin in 1 mM HCl was used.

Carbohydrases: Amylase activity was assayed using a Sigma micro-kit. The assay contained Infinity Amylase Reagent and enzyme extract. Change in absorbance was monitored over a 2 min period at A_{405} and activity calculated using the molar extinction coefficient 10 130 M^{-1} cm^{-1}.

α-Glucosidase and chitinase activities were determined using the substrates ρ-nitrophenyl α-D-glucopyranoside and ρ-nitrophenyl N-acetyl β-D-glucosaminide, respectively. Each assay contained a final concentration of 4 mM substrate in 200 mM Tris, 200 mM NaCl, 10 mM $CaCl_2$, and 0.2% (w/v) polyethylene glycol 6000 pH 5 (for glucosaminidase) or pH 4.5 (for glucosidase). Assays were initiated with the addition of enzyme extract. Aliquots of assay mixture were then removed at time intervals and added to 1 M Na_2CO_3 pH 11, to terminate the reaction. Liberation of ρ-nitrophenol was measured at A_{400}. The molar extinction coefficient is 18 300 M^{-1} cm^{-1} for ρ-nitrophenol at pH > 9 (Erlanger et al. 1961).

Lipases: Lipase activity was determined using a method modified from Gjellesvik et al. (1992) using

4-nitrophenyl caproate (4-NPC) dissolved in ethanol as substrate. Each assay contained a final concentration of 2.5 mM 4-NPC in 6 mM sodium taurocholate, 500 mM Tris, 100 mM NaCl buffer pH 8.5. Assays were initiated by the addition of enzyme extract and the release of nitrophenol was measured at A_{405}. Under these assay conditions the molar extinction coefficient was 19 800 M^{-1} cm^{-1} for nitrophenol (Gjellesvik et al. 1992).

Statistical analyses. Mean values from duplicate assays for each pooled or individual larval sample were compared with a 1-way ANOVA to identify significant changes in specific and total activities of enzymes between stages (significance level $p < 0.05$). Data from cultured larvae and wild-caught larvae were analysed separately to allow each analysis to be balanced and with sufficient replication. For each analysis the assumptions of ANOVA were checked using residual plots. Tukey's HSD post hoc test was used to identify differences between means for different developmental stages.

RESULTS

Growth

For phyllosoma cultured to Stage VI over 73 d, survival from hatch to Stage II was 63%, from Stage II to III was 65%, Stage III to IV was 32%, Stage IV to V was 22% and Stage V to VI was 63%. Larvae moulted to Stage II between Days 11 and 13, Stage III between Days 21 and 23, Stage IV between Days 32 and 37, Stage V between Days 45 and 51, and Stage VI between Days 59 and 69. Growth increments in total length and carapace width between each developmental stage were 0.60 to 1.90 and 0.30 to 1.31 mm, respectively (~3.5 times increase in length and ~4.5 times increase in carapace width) (Fig. 1A). However, wild-caught Stage V and VI phyllosoma were between 44 and 60% longer and 53 and 68% wider than their cultured counterparts. The size of wild-caught phyllosoma increased markedly between Stages VII and VIII, and total length increased more than carapace width (Fig. 1A). Phyllosoma protein content ranged from 00.007 mg $larva^{-1}$ for Stage I to 20.89 mg $larva^{-1}$ for Stage XI. Protein content increased significantly between Stage VII and X, and is consistent with the marked increase in phyllosoma size (Fig. 1B).

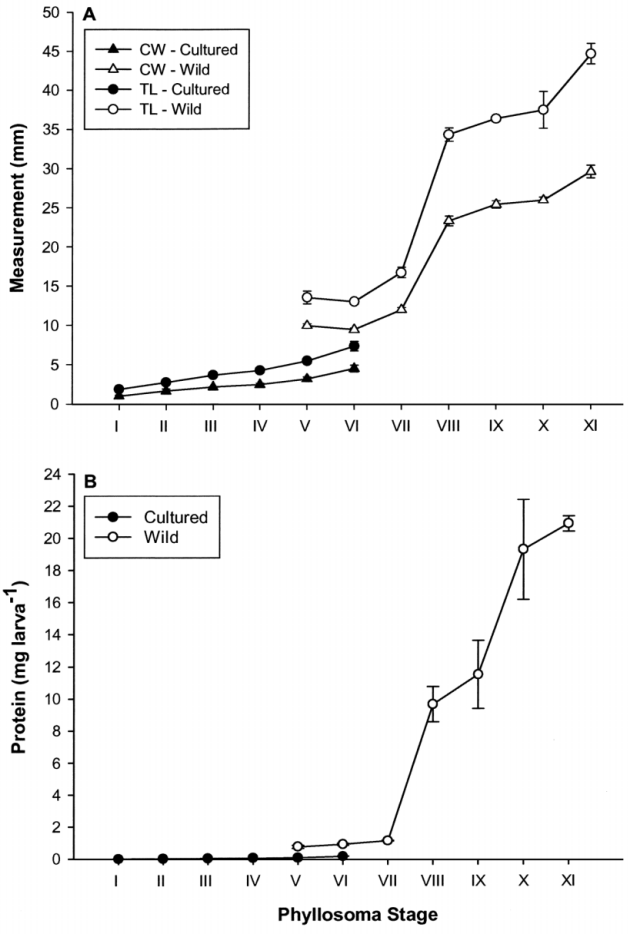

Fig. 1. *Jasus edwardsii*. (A) Growth of cultured and wild caught phyllosoma larvae. CW: carapace width; TL: total length. (B) Relationship between cultured and wild phyllosoma stage and protein content. Data presented as mean ± SE

Enzyme activity

All enzymes analysed were present post-hatch (Days 0 and 1) and in all stages of cultured (I to VI) and wild caught (V to XI) phyllosoma. Protease and lipase total and specific activities were higher than carbohydrases in cultured larvae (Table 1). α-Glucosidase activities were lowest (Table 1). Total enzyme activities in the juvenile *Artemia* fed to the cultured phyllosoma were generally low and at the lower end of the range exhibited by cultured phyllosoma (Table 1).

Proteases

Protease total activity increased significantly between Stages I and IX for both cultured and wild-caught phyllosoma ($F_{1,7} = 47.6$; $p < 0.001$; $F_{1,6} = 34.5$; $p < 0.001$), with marked increases between Stages III and VI of cultured larvae, and Stages VII and VIII of wild larvae (Fig. 2A). Enzyme activity was constant between Stages VIII and XI in wild larvae. Trypsin total activity also increased significantly during development ($F_{1,7} = 36.6$; $p < 0.001$; $F_{1,6} = 5.2$; $p = 0.002$),

Table 1. *Jasus edwardsii.* Total and specific activities of digestive enzymes in cultured phyllosoma and juvenile *Artemia* fed during culture. Phyllosoma data are the mean activities between Stages I and VI and *Artemia* data are the mean activities at the beginning and end of the culture period. Total activity given as Units larva^{-1} or Units *Artemia*$^{-1}$; specific activity given as Units mg^{-1}

Enzyme	Cultured phyllosoma total activity (range)	Cultured phyllosoma specific activity (range)	*Artemia* total activity (mean)
Protease	0.009–0.28	0.61–2.47	0.003
Trypsin	0.0002–0.002	0.011–0.057	0.0006
Amylase	0.00008–0.0025	0.024–0.03	0.00022
α-Glucosidase	0.000003–0.00013	0.0008–0.0015	0.0000001
Chitinase	0.00023–0.012	0.063–0.07	0.000025
Lipase	0.001–0.21	0.027–1.24	0.001

with a marked increase from Stage V to XI in wild-caught larvae (Fig. 3A). Protease total activity was significantly higher in wild-caught larvae than cultured larvae of an equivalent stage, whereas trypsin total activity was similar (Figs. 2A & 3A).

Protease specific activity was constant between cultured Stages I and VI (Fig. 2B), but increased significantly between Stages VI and VII in wild larvae then decreased to Stage XI ($F_{1,6}$ = 22.2; $p < 0.001$) (Fig. 2B). Trypsin specific activity decreased significantly between day-old and Stage III larvae,

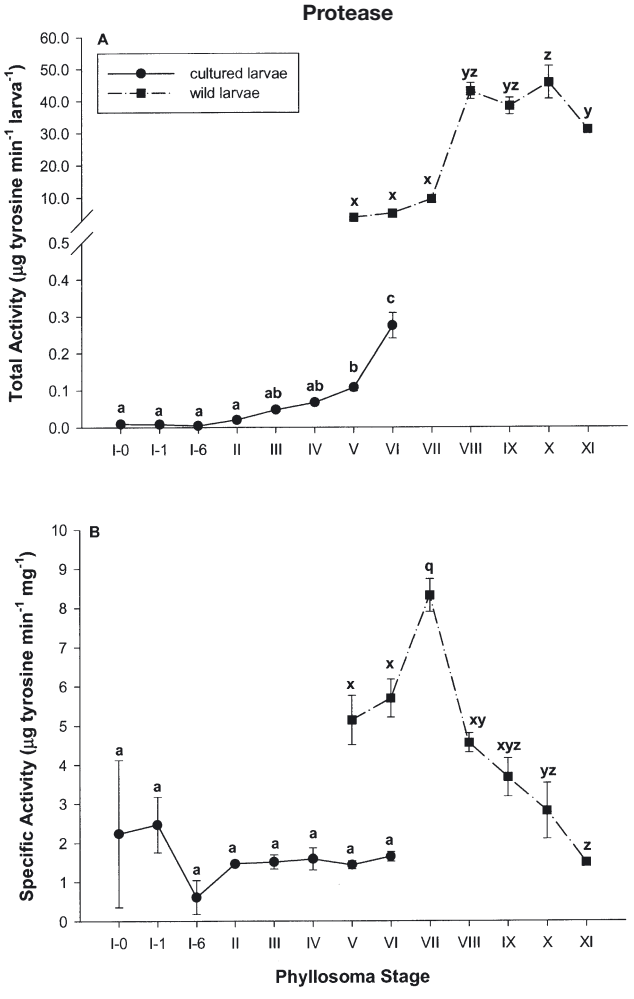

Fig. 2. *Jasus edwardsii.* Protease (A) total activity and (B) specific activity during development of cultured and wild phyllosoma larvae. Data are means ± SE. Stages with different superscripts are significantly different. The 2 sets of superscripts indicate 2 separate analyses. I-0, I-1, I-6 indicate Stage I larvae sampled at Days 0, 1, and 6, respectively

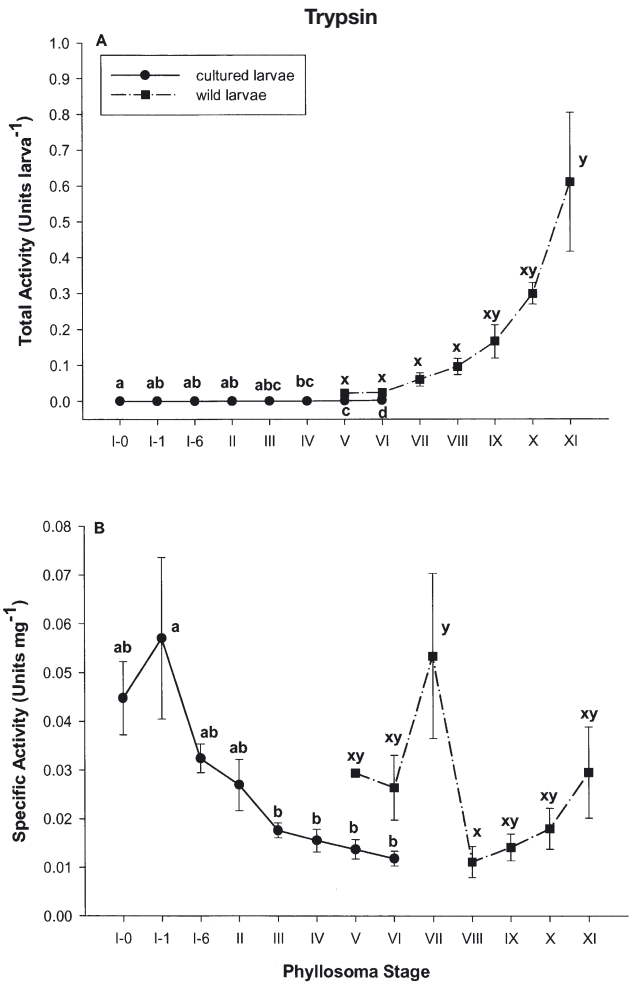

Fig. 3. *Jasus edwardsii.* Trypsin (A) total activity and (B) specific activity during development of cultured and wild phyllosoma larvae. Data are means ± SE. Stages with different superscripts are significantly different. The 2 sets of superscripts indicate 2 separate analyses. I-0, I-1, I-6 indicate Stage I larvae sampled at Days 0, 1, and 6, respectively

and remained low until Stage VI ($F_{1,7}$ = 5.1; p = 0.002). Trypsin specific activity in wild-caught phyllosoma had no consistent trend except a peak in activity at Stage VII ($F_{1,6}$ = 3.1; p = 0.024) (Fig. 3B). Protease specific activity was considerably higher in wild-caught than cultured larvae of equivalent stage, whereas trypsin specific activity was similar between cultured and wild-caught phyllosoma at equivalent stages (Figs. 2B & 3B).

Carbohydrases

Amylase, α-glucosidase and chitinase total activity increased significantly in cultured larvae between Stage I and VI ($F_{1,7}$ = 22.7; p = 0.0; $F_{1,7}$ = 1604.2, p < 0.001; $F_{1,7}$ = 35.0, p < 0.001) (Figs. 4A, 5A & 6A).

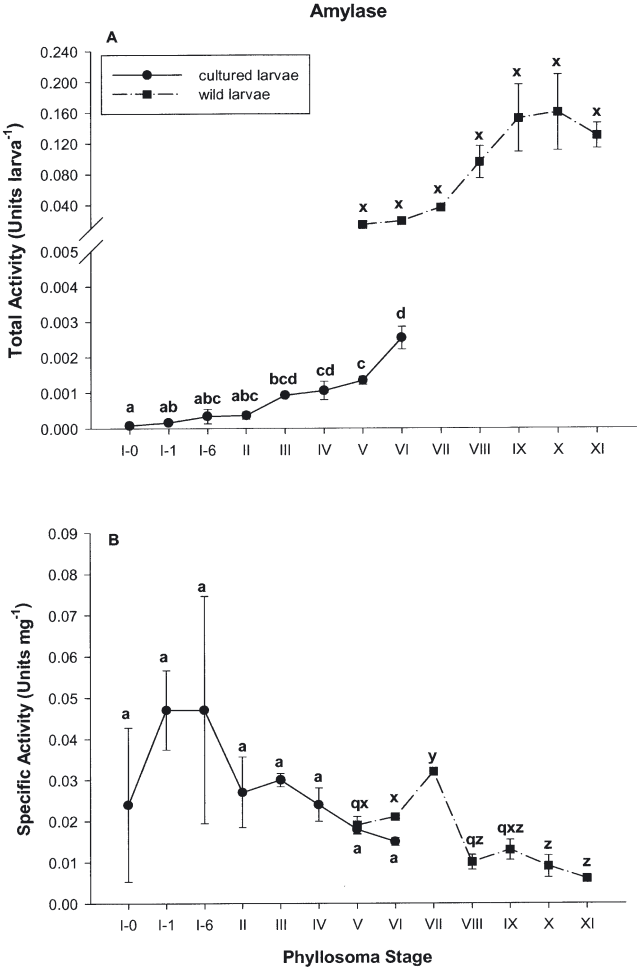

Fig. 4. *Jasus edwardsii.* Amylase (A) total activity and (B) specific activity during development of cultured and wild phyllosoma larvae. Data are means ± SE. Stages with different superscripts are significantly different. The 2 sets of superscripts indicate 2 separate analyses. I-0, I-1, I-6 indicate Stage I larvae sampled at Days 0, 1, and 6, respectively

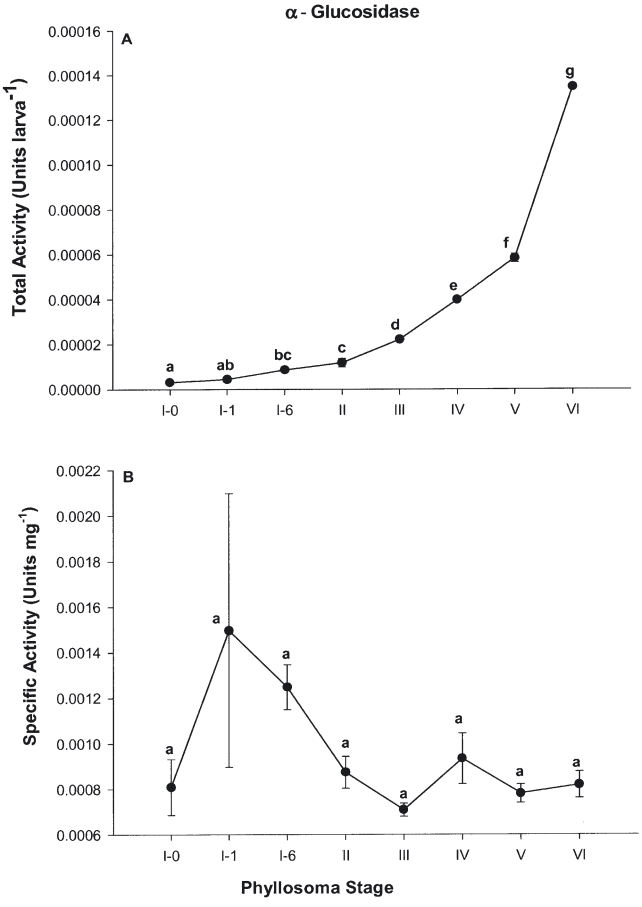

Fig. 5. *Jasus edwardsii.* α-Glucosidase (A) total activity and (B) specific activity during development of cultured phyllosoma larvae. Data are means ± SE. Stages with different superscripts are significantly different. I-0, I-1, I-6 indicate Stage I larvae sampled at Days 0, 1, and 6, respectively

Amylase total activity increased with age in wild caught larvae, but the increase was not significant (Fig. 4A). Specific activity of amylase, α-glucosidase and chitinase was relatively constant during larval development (Figs. 4B, 5B & 6B), although amylase specific activity increased between wild-caught phyllosoma Stages V and VII and then decreased between Stages VII and VIII before remaining constant until Stage XI ($F_{1,6}$ = 20.0; p < 0.001) (Fig. 4B). Amylase specific activity was similar in wild and cultured larvae of the same stage, V and VI (Fig. 4B).

Lipases

Lipase total activity increased significantly with development of both cultured and wild-caught larvae ($F_{1,7}$ = 93.3; p < 0.001; $F_{1,6}$ = 21.1; p < 0.001), with significant changes between cultured Stages IV to VI and

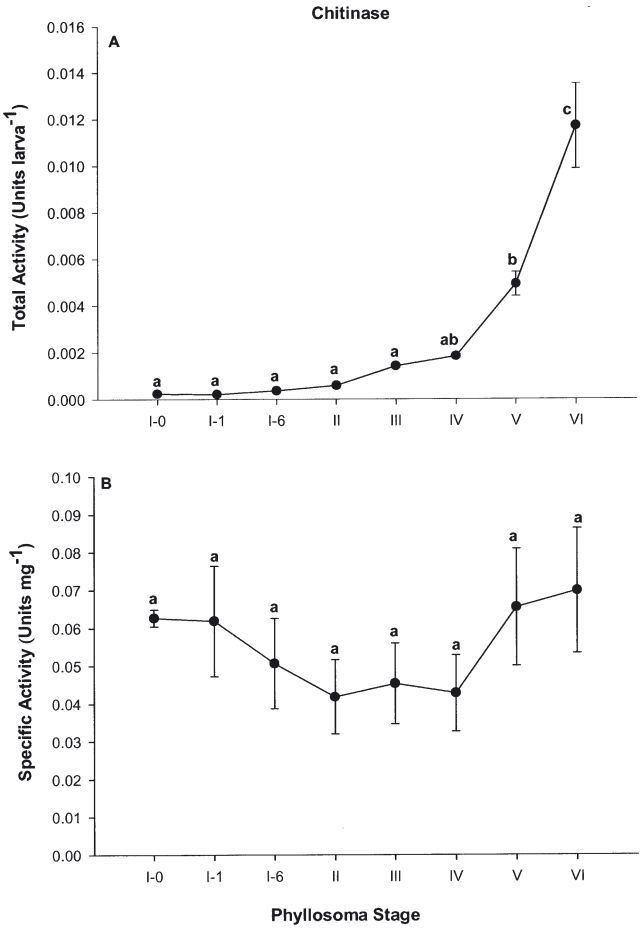

Fig. 6. *Jasus edwardsii.* Chitinase (A) total activity and (B) specific activity during development of cultured phyllosoma larvae. Data are means ± SE. Stages with different superscripts are significantly different. I-0, I-1, I-6 indicate Stage I larvae sampled at Days 0, 1, and 6, respectively

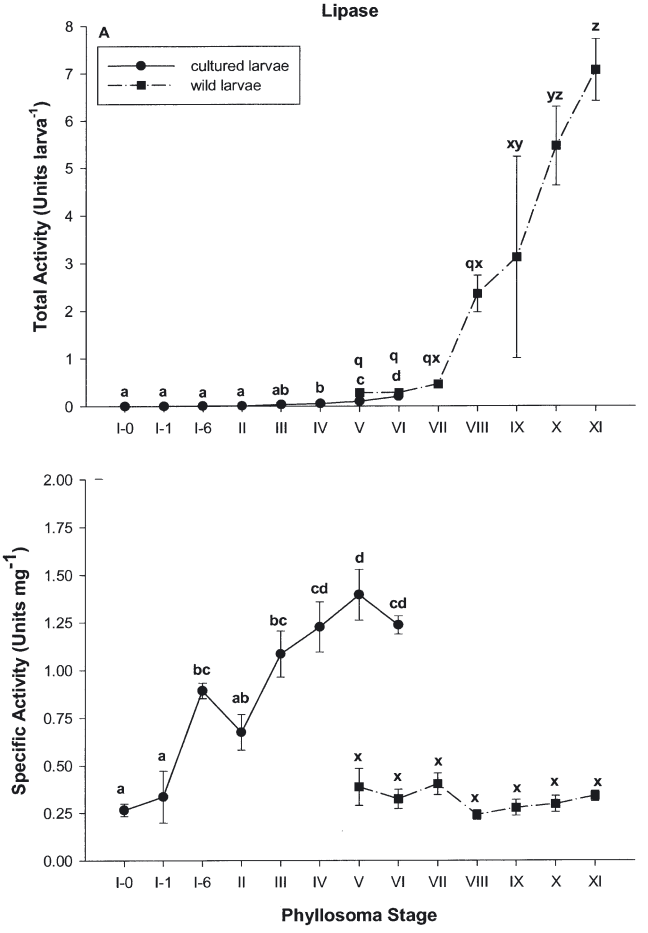

Fig. 7. *Jasus edwardsii.* Lipase (A) total activity and (B) specific activity during development of cultured and wild phyllosoma larvae. Data are means ± SE. Stages with different superscripts are significantly different. The 2 sets of superscripts indicate 2 separate analyses. I-0, I-1, I-6 indicate Stage I larvae sampled at Days 0, 1, and 6, respectively

wild Stages VII to XI (Fig. 7A). Lipase total activity was similar between cultured and wild-caught larvae of equivalent stage (Fig. 7A). Lipase specific activity increased significantly between cultured larval Stages I and VI ($F_{1,7}$ = 17.7; p < 0.001), whereas it remained constant between wild-caught larval stages (Fig. 7B). Lipase specific activity was significantly lower in wild caught larvae than cultured larvae (Fig. 7B).

Amylase:protease ratio

There were no significant ontogenetic trends in the amylase:protease ratio between Stage I and VI cultured larvae, nor between Stage V and XI wild-caught larvae (Fig. 8). Amylase:protease ratios were generally lower in wild-caught larvae than cultured larvae of equivalent stage.

DISCUSSION

Growth and survival

The survival of phyllosoma in culture was lower than some previous reports for this species grown under similar conditions (Illingworth et al. 1997, Tong et al. 1997), but similar to others (Kittaka 1994). The relatively high mortality rate may have been due to a reduction in egg quality related to phototherm manipulation and the long period in captivity for the broodstock (Smith et al. 2003), or microbial infection (Handlinger et al. 2001). Growth of the phyllosoma under culture conditions was consistent with previous results for this species. In this study, cultured phyllosoma reached Stage VI in 59 to 69 d compared to other findings of 70 to 78 d (Illingworth et al. 1997, Tong et al. 1997). Estimates from wild phyllosoma indicate that

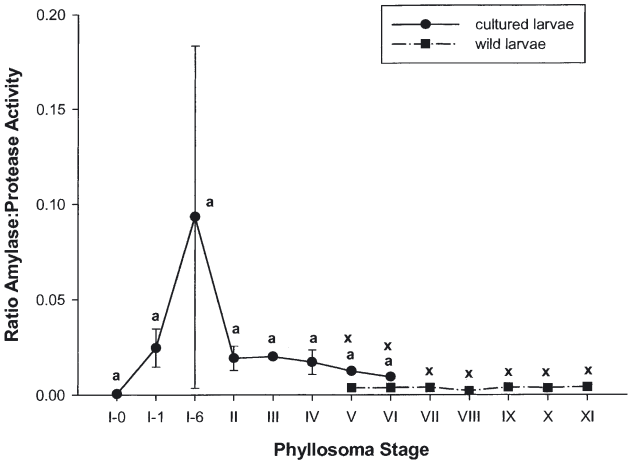

Fig. 8. *Jasus edwardsii*. Ratio of amylase activity to total protease activity for cultured and wild phyllosoma larvae. Data are means ± SE. Stages with different superscripts are significantly different. The 2 sets of superscripts indicate 2 separate analyses. I-0, I-1, I-6 indicate Stage I larvae sampled at Days 0, 1, and 6, respectively

it takes substantially longer to reach Stage VI between 126 and 145 d (Bruce et al. 1997). Although cultured phyllosoma progress through the developmental stages more quickly, they do so at a smaller size than their wild counterparts. By Stages V and VI the cultured phyllosoma were between 44 and 60% shorter and 53 to 68% narrower than the wild larvae. It is possible that these differences in growth and development are due to disparities in the nutritional value of natural versus *Artemia* culture diets. Moulting in some crustacean larvae is believed to be triggered by reaching a critical nutritional point (McConaugha 1982, 1985) and high lipid levels have been implicated as a possible cue in advancement of metamorphosis in spiny lobster phyllosoma (McWilliam & Phillips 1997, Jeffs et al. 2001). Wild phyllosoma feeding on natural prey contain up to 35% of body mass as lipid (Phleger et al. 2001), whereas cultured larvae fed enriched *Artemia* contain less than 21% lipid (Nelson et al. 2003, Ritar et al. 2003). It is possible that an inability to sequester sufficient lipid whilst being fed *Artemia* has adversely affected the development of cultured phyllosoma. This growth phenomenon of advanced development but at a smaller size has, however, been observed in other decapod larvae when cultured on natural zooplankton diets (Anger 2000). Therefore, it may also be possible that the relatively fast development but smaller size of cultured phyllosoma may be attributed to their confined culture conditions at high densities and relatively high temperatures (18°C), as phyllosoma in their natural environment are found at much lower densities and are known to frequent cooler waters at times during the year (Lesser 1978, Booth 1994).

Enzyme profiles

It is possible that the digestive enzymes from *Artemia* consumed by cultured phyllosoma may have contributed to the enzyme activity measured in this study, although the contribution of *Artemia* enzymes in the gut of prawns has been found to be minimal (Lovett & Felder 1990, Kamarudin et al. 1994). In most cases we recorded low total enzyme activities in *Artemia* compared to the cultured phyllosoma, suggesting that it is very unlikely that a substantial proportion of the enzyme activity measured in *Jasus edwardsii* phyllosoma originated from ingested *Artemia* (Table 1). Furthermore, although *J. edwardsii* phyllosoma ingest 15 to 25 *Artemia* d^{-1} (unpubl. data), they are not ingested whole but are torn apart and small pieces of tissue are ingested (Cox & Bruce 2002). So it is unlikely that the enzymes contained within the gut of the *Artemia* are ingested in an active form. In 2 cases (trypsin and amylase) the concentration of enzymes within the gut of the *Artemia* is within the lower range of those measured in the phyllosoma. It is not possible to quantify the contribution of *Artemia* to the digestive enzyme compliment in these cases because *J. edwardsii* phyllosoma do not ingest whole *Artemia*. We suggest that the results from the 2 cases where the concentration of digestive enzymes in the gut of the *Artemia* are low, but not negligible in comparison to the magnitude of those in the gut of the phyllosoma, should be interpreted with some caution.

All of the digestive enzymes analysed (protease, trypsin, amylase, α-glucosidase, chitinase and lipase) were detected in every stage of cultured (I to VI) and wild-caught (V to XI) larvae, revealing that *Jasus edwardsii* phyllosoma are capable of digesting protein, carbohydrate (including chitin) and lipid at all stages of development. Protease and lipase activities were significantly greater than carbohydrases (up to 3 orders of magnitude) in both cultured and wild-caught larvae, indicating *J. edwardsii* phyllosoma rely more heavily on protein and lipid than carbohydrate for their early nutrition. Similarly high protease, but low carbohydrase, activity has been found in larval stages of clawed lobsters *Homarus americanus*, *H. gammarus* and *Procambarus clarkii* (Biesiot & Capuzzo 1990, Kurmaly et al. 1990, Kumlu & Jones 1997, Hammer et al. 2000), as well as in juvenile and adult stages of spiny lobsters *J. edwardsii* (Johnston 2003) and *J. lalandii* (Barkai et al. 1996), the slipper lobster *Thenus orientalis* (Johnston et al. 1995, Johnston & Yellowlees 1998) and freshwater crayfish *Cherax quadricarinatus* (Figueiredo et al. 2001). Lipase levels were generally higher in wild caught *J. edwardsii* phyllosoma than in other crustacean larvae such as clawed lobster (Biesiot & Capuzzo 1990, Kurmaly et al. 1990, Jones et al. 1997,

Kumlu & Jones 1997), suggesting that lipid is a very important nutritional component in their diet. Pelagic zooplankton generally have high protein and lipid, and low carbohydrate content (Le Vay et al. 2001), so the enzyme profiles of *J. edwardsii* phyllosoma are evidence of a carnivorous zooplankton diet.

High protease and trypsin activity has been observed in some crustacean species that consume a low protein diet, and it is thought to occur to maximise assimilation efficiency of the rare metabolic substrate (Jones et al. 1997). However, this relationship does not appear to be ubiquitous. A recent study comparing the digestive enzyme activities of a number of species of crabs occupying different dietary niches showed that although one herbivorous species did display high protease activity, in general protease activity increased in proportion to the importance of protein in the diet (Johnston & Freeman unpubl.). This apparent lack of fidelity between protease activity and protein in the diet demonstrates that while protease activity is indicative of dietary protein, it may not unambiguously define the nature of the diet. As such, we examine other digestive enzymes, in addition to the proteases, to obtain an accurate picture of dietary composition in this study.

The low amylase and α-glucosidase activities are indicative of low carbohydrate consumption and suggest that *Jasus edwardsii* phyllosoma are almost exclusively carnivorous. Amylase activity is 2 to 3 orders of magnitude lower than in omnivorous species such as *Penaeus setiferus* larvae (Lovett & Felder 1990), but similar to *Fennero Penaeus indicus* larvae fed a carnivorous diet of *Artemia* and mussel flesh (Ribeiro & Jones 2000). Chitinase activity, although quite low in the early stages, suggests that *J. edwardsii* phyllosoma have some limited capacity for digesting the chitinous exoskeleton of crustaceans such as copepods, amphipods and krill that are frequently abundant potential prey items in coastal and oceanic zooplankton.

Ontogenetic changes

The presence of all enzymes in newly hatched, 1 and 6 d old phyllosoma indicates that they are capable of digestion from the onset of feeding. This is consistent with the observed presence of enzyme secreting F-cells in epithelial tissues of digestive glands of Stage I *Jasus edwardsii* phyllosoma (Johnston et al. 2001). Other crustacean larvae such as *Penaeus setiferus*, *Macrobrachium rosenbergii* and *P. clarkii* are also known to produce digestive enzymes at hatch and prior to feeding (Lovett & Felder 1990, Kamarudin et al. 1994, Hammer et al. 2000). The early digestive competence of phyllosoma will make it unnecessary to include digestive enzyme supplements into early larval formulated diets to facilitate the rapid development of digestion, as is the case for many post-hatch fish larvae (Kolkovski 2001).

Significant increases in total activities of protease, trypsin, amylase, α-glucosidase, chitinase and lipase in cultured larvae, along with significant increases in protease, trypsin and lipase in wild-caught larvae, reflects an increasing digestive capacity during larval development that is required to meet the metabolic and structural requirements of larger animals. These increases in total activity in mid- and late-stage larvae are facilitated by the proliferation of digestive gland lobes, from 6 in Stage I phyllosoma to more than 20 in Stage X and XI phyllosoma (Johnston et al. 2001). Similar increases in total activity with size have been reported for other crustacean larvae (Biesiot & Capuzzo 1990, Kamarudin et al. 1994, Hammer et al. 2000) and have been associated with the branching of the digestive gland lobes (Lovett & Felder 1990), gland maturation and increased gland volume (Hammer et al. 2000).

Although there were significant increases in total activities of most enzymes, there were no significant changes in specific activities of protease, amylase, α-glucosidase and chitinase in cultured larvae, suggesting that phyllosoma did not change their capacity to digest dietary protein and carbohydrate between Stages I and VI. Lipase was the only enzyme that increased in specific activity in cultured phyllosoma, suggesting that lipid is utilised to a greater extent in mid- than early-stage larvae, possibly as a response to increased consumption of lipid-rich *Artemia* with age. This level of lipase activity in cultured *Jasus edwardsii* phyllosoma is considerably higher than in other species of crustacean larvae fed enriched *Artemia* diets (Lovett & Felder 1990, Hammer et al. 2000, Figueiredo et al. 2001), suggesting that they are capable of making opportunistic use of lipid when it is available. The depressed total activities of protease and amylase, and specific activities of protease and trypsin in cultured phyllosoma compared to wild larvae of the same developmental stages, may reflect the increased reliance on dietary lipid as an energy source over protein and carbohydrate alternatives.

The specific activity of chitinase did not increase with advancing development of cultured phyllosoma in the same manner as lipase, despite the importance of the chitinous *Artemia* diet. Previous observations of the feeding behaviour of these early-stage phyllosoma indicate that they tear apart prey externally using the pereiopods and maxillipeds, followed by mandibular biting and ingestion via suctorial movements of the foregut (Cox & Bruce 2002). This feeding morphology and behaviour is well suited to soft prey items, but for

chitin encased *Artemia* it is difficult for the phyllosoma to consume anything other than small pieces of flesh dislodged from the exoskeleton during manipulation of the *Artemia.* This feeding behaviour is likely to result in a very high lipid diet, as enriched *Artemia* contain up to 30% body mass of lipid (Smith et al. 2002). These differences in dietary intake would explain patterns in the digestive enzymes observed in cultured phyllosoma during this period, i.e. the depressed protease, amylase and low chitinase-specific activities, and the increasing lipase specific activity. By contrast, a mixed zooplankton diet in the wild, including soft and fleshy prey items, would be more likely to supply a dietary intake rich in both protein and lipid.

The lack of marked shifts in specific enzyme activity (with the exception of lipase and trypsin) in cultured larvae fed a constant diet suggests that variations in enzyme profiles of the wild larvae are more likely to be in response to changes in natural diets and nutritional requirements. In wild phyllosoma, the specific activity of protease increased from Stage V to peak at Stage VII, and then decreased quite substantially in subsequent developmental stages (Fig. 2B). Trypsin and amylase specific activities also peaked at Stage VII, but were generally low at other stages (Figs. 3B & 4B). This sharp increase in protease, amylase and trypsin specific activity immediately precedes a large increase in larval size (Fig. 1A), suggesting that increased protein and carbohydrate in the diet help to fuel this growth. Also, between Stages VI and VII the foregut of phyllosoma becomes more complex, greatly improving the mechanical processing of coarser and more proteinaceous dietary material (Johnston & Ritar 2001). This improved processing could be expected to release more carbohydrate and protein for digestion, requiring correspondingly more digestive enzymes. The marked increase in phyllosoma size at Stage VII is also associated with the development of an abdomen with swimming pleopods, which allows for the start of directional swimming and presumably increased prey capture capabilities. This morphological shift is also associated with a dramatic metabolic shift, especially for the aerobic metabolism of phyllosoma (Wells et al. 2001). Stage VII also marks the completion of the shift of phyllosoma distribution from coastal waters rich in potential prey to oceanic waters with generally lower available prey biomass (Bruce et al. 1997, Bradford-Grieve et al. 1999). These ecological and morphological changes reflect digestive enzyme profiles in the same manner that changes in digestive enzymes of other crustacean larvae have been associated with changes in trophic level, and reflect adaptations to variability in prey density (Le Vay et al. 2001).

For development beyond Stage VII, the specific activity of protease, trypsin and amylase remains lower than at Stage VI. For all of the digestive enzymes, except protease, total and specific activities continue to scale in concentration with the increasing body mass of the phyllosoma beyond Stage IV, presumably in order to maintain growth. Lipase also follows this pattern of scaling, although it is well known that during the late stages of development large amounts of lipid are accumulated by the phyllosoma (up to 34% of total body mass) to fuel the non-feeding post-larval, or puerulus stage (Jeffs et al. 2001, Phleger et al. 2001). By contrast, protease does not scale with increasing body mass in wild phyllosoma, suggesting that dietary protein becomes less important in these later stages. These trends are consistent with suggestions by Nichols et al. (2001) and Jeffs et al. (2004) that the later stage phyllosoma may be consuming lipid-rich prey such as krill. The capture and consumption of these fast-moving prey would be made possible by their greatly increased physical size, prey handling abilities and digestive tract, as well as improved swimming and sensory abilities (Johnston & Ritar 2001, Cox & Johnston 2003b).

The similar amylase:protease ratio throughout development of wild phyllosoma (Fig. 8) suggests that, although there were these large changes in the specific activity of protease and amylase, the actual ratio of dietary protein and carbohydrate remained constant.

CONCLUSIONS

Jasus edwardsii phyllosoma hatch with and retain a suite of digestive enzymes (including proteases, trypsin, amylase, α-glucosidase, chitinase and lipase) that enables them to utilise many different dietary components as sources of energy and materials for growth. They also appear to be capable of adjusting their digestive enzymes to suit the available carnivorous diet. Such a flexible and generalist digestive capacity may help *J. edwardsii* exploit a wide array of zooplankton prey and maximise the chances of survival in the early stages. This generalised digestive ability is consistent with randomly encountering a wide variety of potential prey through the tumbling, swimming and grasping feeding behaviour that characterises early developmental stages (Cox & Bruce 2002, Nelson et al. 2002). The presence of digestive enzymes in newly hatched *J. edwardsii* phyllosoma suggests that enzyme supplements do not need to be added to an artificial diet. The apparent suboptimal performance of phyllosoma reared on oil-enriched *Artemia* suggests that alternative artificial dietary sources should be used to better supply the protein and carbohydrate needs of the developing phyllosoma. Prey that is fleshier than

Artemia may better suit the feeding behaviour and morphology of phyllosoma in culture and in the wild, provided it delivers both high levels of protein and lipid to the diet. Options may include fish larvae which have been readily ingested by phyllosoma during laboratory studies (Macmillan et al. 1997, Kittaka et al. 2001), or krill, amphipods or copepods which have been found to possess lipid signatures consistent with wild phyllosoma (Jeffs et al. 2004).

The analyses of digestive enzymes in wild phyllosoma highlight some key developmental and ecological changes, especially around Stage VI and in the late-stage larvae. These changes suggest that from Stage VI, phyllosoma are capable of tackling and consuming more active prey that continue to provide a high return of protein and lipids. In late-stage phyllosoma, prey containing higher levels of lipid such as krill may become more important in providing lipids that are digested and stored in preparation for the non-feeding puerulus stage.

This investigation has helped to elucidate the larval biology of spiny lobster phyllosoma through a better understanding of the digestive process and determination of the characteristics of natural diets which are currently undescribed.

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LITERATURE CITED

Anger K (2001) The biology of decapod crustacean larvae. Swets and Zeitlinger B.V., Lisse

Barkai A, Davis CL, Tugwell S (1996) Prey selection by the South African Cape rock lobster *Jasus lalandii*: ecological and physiological approaches. Bull Mar Sci 58:1–8

Biesiot PM, Capuzzo JM (1990) Changes in digestive enzyme activities during early development of the American lobster *Homarus americanus* Milne Edwards. J Exp Mar Biol Ecol 136:107–122

Booth JD (1994) *Jasus edwardsii* larval recruitment off the east coast of New Zealand. Crustaceana 66:295–317

Booth JD (2002) Early life history, recruitment processes and settlement of spiny lobsters. Fish Sci 68:384–389

Bradford MM (1977) A rapid and sensitive method for the quantitation of microgram quantities of proteins utilising the principle of protein-dye binding. Anal Biochem 72:248–254

Bradford-Grieve JM, Boyd PW, Chang FH, Chiswell SM and 5 others (1999) Pelagic ecosystem structure and functioning in the Subtropical Front region east of New Zealand in austral winter and spring 1993. J Plankton Res 21:405–428

Bruce B, Bradford R, Griffen D, Gardner C, Young J (1997) A synthesis of existing data on larval rock lobster distribution in southern Australia. Final report to the Fisheries Research and Development Corporation. Project 96/107, CSIRO Marine Laboratories, Hobart

Cox SL, Bruce MP (2002) Feeding behaviour and associated sensory mechanisms of stage I–III phyllosoma of *Jasus edwardsii* and *Jasus verreauxi*. J Mar Biol Assoc UK 82: 4134/1–5

Cox SL, Johnston DJ (2003a) Feeding biology of spiny lobster larvae and implications for culture. Rev Fish Sci 11:89–106

Cox SL, Johnston DJ (2003b) Developmental changes in the structure and function of mouthparts of phyllosoma larvae of the packhorse lobster, *Jasus verreauxi* (Decapoda: Palinuridae). J Exp Mar Biol Ecol 296:35–47

Erlanger BF, Kokowsky N, Cohen W (1961) The preparation and properties of two chromogenic substrates of trypsin. Arch Biochem Biophys 95:271–278

Fang LS, Lee BN (1992) Ontogenetic change in digestive enzymes in *Penaeus monodon*. Comp Biochem Physiol 103B:1033–1037

Figueiredo MSRB, Kricker JA, Anderson AJ (2001) Digestive enzyme activities in the alimentary tract of redclaw crayfish, *Cherax quadricarinatus* (Decapoda: Parastacidae). J Crustac Biol 21:334–344

Gjellesvik DR, Lombardo D, Walther BT (1992) Pancreatic bile salt dependent lipase from cod (*Gadus morhua*): purification and properties. Biochim Biophys Acta 1124: 123–134

Hammer HS, Bishop CD, Watts SA (2000) Activities of three digestive enzymes during development in the crayfish *Procambarus clarkii* (Decapoda). J Crustac Biol 20:614–620

Handlinger JH, Carson J, Ritar AJ, Crear BJ, Taylor DP, Johnston DJ (2001) Disease conditions of cultured phyllosoma larvae and juveniles of the southern rock lobster (*Jasus edwardsii*, Decapoda; Palinuridae). In: Evans LH, Jones JB (eds) Proc Int Symp Lobster Health Management. Curtin University Press, Perth, p 75–87

Illingworth J, Tong LJ, Moss GA, Pickering TD (1997) Upwelling tank for culturing rock lobster (*Jasus edwardsii*) phyllosomas. Mar Freshw Res 48:911–914

Jeffs A, Hooker S (2000) Economic feasibility of aquaculture of spiny lobsters *Jasus edwardsii* in temperate waters. J World Aquacult Soc 31:30–41

Jeffs AG, Willmott ME, Wells RMG (1999) The use of energy stores in the puerulus of the spiny lobster *Jasus edwardsii* across the continental shelf of New Zealand. Comp Biochem Physiol 123A:351–357

Jeffs AG, Nichols PD, Bruce MP (2001) Lipid reserves used by pueruli of the spiny lobster *Jasus edwardsii* in crossing the continental shelf of New Zealand. Comp Biochem Physiol 129A:305–311

Jeffs AG, Nichols PD, Mooney BD, Phillips KL, Phleger CF (2004) Identifying potential prey of the pelagic larvae of the spiny lobster *Jasus edwardsii* using lipid signatures. Comp Biochem Physiol 137:487–507

Johnston DJ (2003) Ontogenetic changes in digestive enzymology of the spiny lobster, *Jasus edwardsii* Hutton (Decapoda, Palinuridae). Mar Biol 143:1071–1082

Johnston DJ, Ritar A (2001) Mouthpart and foregut ontogeny in phyllosoma larvae of the lobster *Jasus edwardsii* (Decapoda: Palinuridae). Mar Freshw Res 52:1375–1386

Johnston DJ, Yellowlees D (1998) Relationship between the dietary preferences and digestive enzyme complement of the slipper lobster *Thenus orientalis* (Decapoda:Scyllaridae). J Crustac Biol 18:656–665

Johnston DJ, Hermans JM, Yellowlees D (1995) Isolation and

characterisation of a trypsin from the slipper lobster *Thenus orientalis* Lund. Archiv Biochem Biophys 324: 35–40

Johnston DJ, Ritar A, Hart P (2001) Mouthpart and foregut ontogeny in phyllosoma larvae of the lobster *Jasus edwardsii* (Decapoda: Palinuridae). In: Crear B, Hart P (eds) FRDC Final Report — The development of rock lobster propagation techniques for aquaculture in Australia PN1999/315. Marine Research Laboratories, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Hobart

Jones DA, Kumlu M, Le Vay L, Fletcher DJ (1997) The digestive physiology of herbivorous, omnivorous and carnivorous crustacean larvae: a review. Aquaculture 155: 285–295

Kamarudin MS, Jones DA, Le Vay L, Abidin AZ (1994) Ontogenetic change in digestive enzyme activity during larval development of *Macrobrachium rosenbergii.* Aquaculture 123:232–333

Kittaka J (1994) Culture of phyllosomas of spiny lobster and its application to studies of larval recruitment and aquaculture. Crustaceana 66:258–270

Kittaka J (1997) Culture of larval spiny lobsters: a review of work done in northern Japan. Mar Freshw Res 48:923–930

Kittaka J, Kudo R, Onoda S, Kanemura K, Mercer JP (2001) Larval culture of the European spiny lobster *Palinuris elephas*. Mar Freshw Res 52:1439–1444

Kolkovski S (2001) Digestive enzymes in fish larvae and juveniles — implications and applications to formulated diets. Aquaculture 200:181–201

Kumlu M, Jones DA (1997) Digestive protease activity in planktonic crustaceans feeding at different trophic levels. J Mar Biol Assoc UK 77:159–165

Kurmaly K, Jones DA, Yule AB (1990) Acceptability and digestion of diets fed to larval stages of *Homarus gammarus* and the role of dietary conditioning behaviour. Mar Biol 106:181–190

Lesser JHR (1978) Phyllosoma larvae of *Jasus edwardsii* (Hutton) (Crustacea: Decapoda: Palinuridae) and their distribution off the east coast of the North Island, New Zealand. NZ J Mar Freshw Res 12:357–370

Le Vay L, Jones DA, Puello-Cruz AC, Sangha RS, Ngamphongsai C (2001) Digestion in relation to feeding strategies exhibited by crustacean larvae. Comp Biochem Physiol 128A:623–630

Lipcius RN, Eggleston DB (2000) Ecology and fishery biology of spiny lobsters. In: Phillips BF, Kittaka J (eds) Spiny lobsters: fisheries and culture. Fishing News Books, Oxford, p 1–41

Lovett DL, Felder DL (1990) Ontogenetic change in digestive enzyme activity of larval and postlarval white shrimp *Penaeus setiferus* (Crustacea, Decapoda, Penaeidae). Biol Bull 178:144–159

Macmillan DL, Sandow SL, Wikeley DM, Frusher S (1997) Feeding activity and the morphology of the digestive tract in stage-I phyllosoma larvae of the rock lobster *Jasus edwardsii*. Mar Freshw Res 48:19–26

McConaugha JR (1982) Regulation of crustacean morphogenesis in larvae of the mud crab *Rhithropanopeus harrisii*. J Exp Zool 223:155–163

McConaugha JR (1985) Nutrition and larval growth. In: Wenner AM (ed) Larval growth. Balkema, Rotterdam, p 127–154

McWilliam PS, Phillips BF (1997) Metamorphosis of the final phyllosoma and secondary lecithotrophy in the puerulus of *Panulirus cygnus* George: a review. Mar Freshw Res 48: 783–790

Mitchell JR (1971) Food preferences, feeding mechanisms and related behavior in phyllosoma larvae of the California spiny lobster, *Panulirus interruptus* (Randall). MSc thesis, San Diego State College

Moss GA, Tong LJ, Illingworth J (1999) Effects of light intensity and food density on the growth and survival of early-stage phyllosoma larvae of the rock lobster *Jasus edwardsii*. Mar Freshw Res 50:129–34

Nelson MM, Cox SL, Ritz DA (2002) Function of mouthparts in feeding behavior of phyllosoma larvae of the packhorse lobster, *Jasus verreauxi* (Decapoda: Palinuridae). J Crustac Biol 22:595–600

Nelson MM, Crear BJ, Nichols PD, Ritz DA (2003) Feeding southern rock lobster, *Jasus edwardsii* Hutton, 1875, phyllosomata in culture: recent progress with lipid-enriched *Artemia*. J Shellfish Res 22:225–234

Nichols P, Mooney B, Jeffs A (2001) The lipid, fatty acid and sterol composition of potential prey items of the southern rock lobster *Jasus edwardsii*: an aid to identification of food-chain interactions. Report CMR/MP2, CSIRO Marine Research, Hobart, p 1–11

Phillips BF, Sastry AN (1980) Larval ecology. In: Cobb JS, Phillips BF (eds) The biology and management of lobsters. Academic Press, New York, p 11–57

Phleger CF, Nelson MM, Mooney BD, Nichols PD, Ritar AJ, Smith GG, Hart PR, Jeffs AG (2001) Lipids and nutrition of the southern rock lobster, *Jasus edwardsii*, from hatch to pureulus. Mar Freshw Res 52:1475–1486

Ribeiro FALT, Jones DA (2000) Growth and ontogenetic change in activities of digestive enzymes in *Fennero Penaues indicus* postlarvae. Aquacult Nutr 6:53–64

Ritar AJ (2001) The experimental culture of phyllosoma larvae of southern rock lobster (*Jasus edwardsii*) in a flow-through system. Aquacult Eng 24:149–156

Ritar AJ, Thomas CW, Beech AR (2002) Feeding *Artemia* and shellfish to phyllosoma larvae of southern rock lobster (*Jasus edwardsii*). Aquaculture 212:183–194

Ritar AJ, Dunstan GA, Crear BJ, Brown MA (2003) Biochemical composition during growth and starvation of early larval stages of cultured spiny lobster (*Jasus edwardsii*) phyllosoma. Comp Biochem Physiol 136:353–370

Smith GG, Ritar AJ, Phleger CF, Nelson MM, Mooney B, Nichols PD, Hart P (2002) Changes in gut content and composition of juvenile *Artemia* after oil enrichment and during starvation. Aquaculture 208:137–158

Smith GG, Ritar AJ, Carter CG, Dunstan GA, Brown MR (2003) Morphological and biochemical characteristics of phyllosoma after photothermal manipulation of reproduction in broodstock of the spiny lobster, *Jasus edwardsii*. Aquaculture 220:229–311

Stone ST, Betz A, Hofsteenge J (1991) Mechanistic studies on thrombin catalysis. Biochemistry 30:9841–9848

Tengjaroenkul B, Smith BJ, Smith SA, Chatreewongsin U (2002) Ontogenetic development of the intestinal enzymes of cultured Nile tilapia, *Oreochromis niloticus* L. Aquaculture 211:241–251

Tong LJ, Moss GA, Paewai MM, Pickering TD (1997) Effect of brine-shrimp numbers on growth and survival of early-stage phyllosomal larvae of the rock lobster *Jasus edwardsii*. Mar Freshw Res 48:935–940

Walter HE (1984) Proteinases: methods with haemoglobin, casein and azocoll as substrates. In: Bergmeyer HU (Ed) Methods of enzymatic analysis, Vol 5. Verlag Chemie, Weinheim, p 270–277

Wells RMG, Lu J, Hickey AJR, Jeffs AG (2001) Ontogenetic changes in enzyme activities associated with energy production in the spiny lobster, *Jasus edwardsii*. Comp Biochem Physiol 130B:339–347

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