

The Activity of Digestive Enzymes in Larval Grouper and Live Feed

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Introduction

Low and inconsistent survival in the larval rearing of groupers is a major production bottleneck (Hussain and Higuchi 1980; Kohno et al. 1997; Toledo et al. 1999). The digestive tract of first feeding grouper larvae is rudimentary (G. Qunitio unpublished data; McBride unpublished data) and there is a short window of opportunity for the successful transition from endogenous to exogenous nutrition in comparison with many other marine species (Kohno et al. 1990; Ordonio-Aguilar et al. 1995). A better understanding of the digestive physiology of grouper larvae could assist in improving the quality and accessibility of nutrients from the diet. The aim of these studies was to investigate the ontogeny of digestive enzymes in larval groupers and assess the suitability of different live feeds.

Methods

Larvae of *Epinephelus coioides* were reared in a green-water semi-intensive system in five-tonne tanks at the Southeast Asian Fisheries Development Center (SEAFDEC) facility in Tigbauan (Iloilo, Philippines) as described by Toledo et al. (1999). Larvae of *Cromileptes altivelis* were reared at the Gondol Research Institute for Mariculture (Bali, Indonesia) in five-tonne concrete tanks in a green-water culture system as described by Sugama et al. (2001). Larvae of *E. fuscoguttatus* were reared in 300 L tanks in a clear-water recirculation system at the Northern Fisheries Centre (Cairns, Australia). Samples of live prey organisms (SS-strain rotifers *Brachionus rotundiformis* and the copepod *Acartia sinjiensis*)

were collected from standard cultures at the Northern Fisheries Centre.

Three to five replicates of pooled larvae (5–30 depending on age) were collected each sampling day except for *C. altivelis* where one or two replicates were collected. Samples of *E. fuscoguttatus* were only collected from three to six days post hatch (DPH) after which there was total mortality. A known number of live prey organisms were collected in triplicate. Samples were homogenised in a 10 mM Tris-HCl (pH 7.5) buffer, and centrifuged before the supernatants were collected for enzyme and protein analysis.

Concentration of soluble protein was determined using BioRad Protein Assay (Bradford) reagents (USA). Total protease and α -amylase activity were measured by sensitive fluorescent assays using casein and starch substrates respectively (Molecular Probes, USA). The activity of bile salt-dependent (bsd) lipase was estimated by an absorbance assay using the substrate 4-nitrophenyl caproate (4-NPC) (Gjellesvik et al. 1992). All enzyme assays were performed at 30°C. One unit of total protease activity was defined as the percentage change in fluorescence units from a negative control per hour. One unit of amylase activity was defined as the amount of enzyme required to liberate one milligram of maltose from starch in three minutes. One unit of bsd lipase was defined as nmoles 4-NPC hydrolysed per hour.

Differences in the emergence of digestive enzyme activity between *E. coioides* and *C. altivelis* were investigated using non-linear regression. A generalised logistic model was found the most appropriate with the enzyme activity modelled against age and grouped into species.

Results and Discussion

Generally the emergence of digestive enzyme activity in grouper larvae was characterised by three phases.

1. Low activities were detected in the three grouper species prior to nine DPH. An exception was bsd lipase activity, which was not detected in *E. coioides* or *E. fuscoguttatus* over this period.
2. The second phase occurred between 10 and 18 DPH in *E. coioides* and *C. altivelis*. Modulations in digestive enzyme activity were observed and corresponded with key developmental changes of the gastro-intestinal tract in *E. coioides* (G. Quintio unpublished data) and *C. altivelis* (McBride, unpublished data).
3. From 20 DPH, enzyme activity generally increased with age in both *E. coioides* and *C. altivelis* (Figs. 1 and 2).

The emergence of total protease and amylase activity with age in *E. coioides* was significantly different to the activities in *C. altivelis* ($P < 0.001$; adjusted $R^2 = 0.892$ and 0.960 respectively). The emergence of bsd lipase activity with age was similar between the two species ($P = 0.238$).

These findings suggest the two species may have different abilities to digest proteins and carbohydrates at the larval stage and this is likely to have implications for the development of artificial diets for larvae and juveniles.

Total protease activity in early feeding *E. coioides* larvae increased in response to initial feeding incidence (Fig. 3). In contrast, amylase activity was not correlated with feeding incidence (Fig. 3). Live food organisms may stimulate enzyme activity in the gut of early stage larvae either by their physical presence (Hjelmeland et al. 1988; Pedersen et al. 1987), the release of hormonal factors (Hjelmeland et al. 1988; Kamisaka et al. 2001; Srivastava et al. 2002) or by contributing an exogenous source of digestive enzymes (Dabrowski and Glogowski 1977; Lauff and Hofer 1984; Munilla-Moran et al. 1990; Oozeki and Bailey 1995; Pedersen et al. 1987).

Significant differences in digestive enzyme activities were observed between the live feed organisms (Fig. 4). The potential contribution from the live feed to the enzyme activity measured in a larva was estimated by multiplying the activity per individual prey item by the total number of prey items observed in the

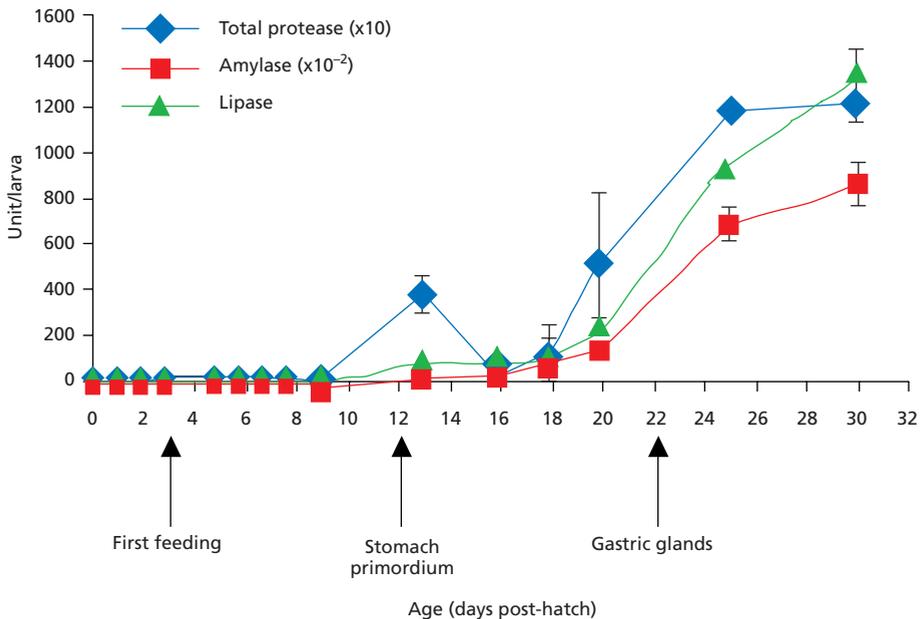


Figure 1. Emergence of digestive enzyme activities in *E. coioides* larvae with age. Arrows indicate major morphological changes in the gut development of larval *E. coioides*.

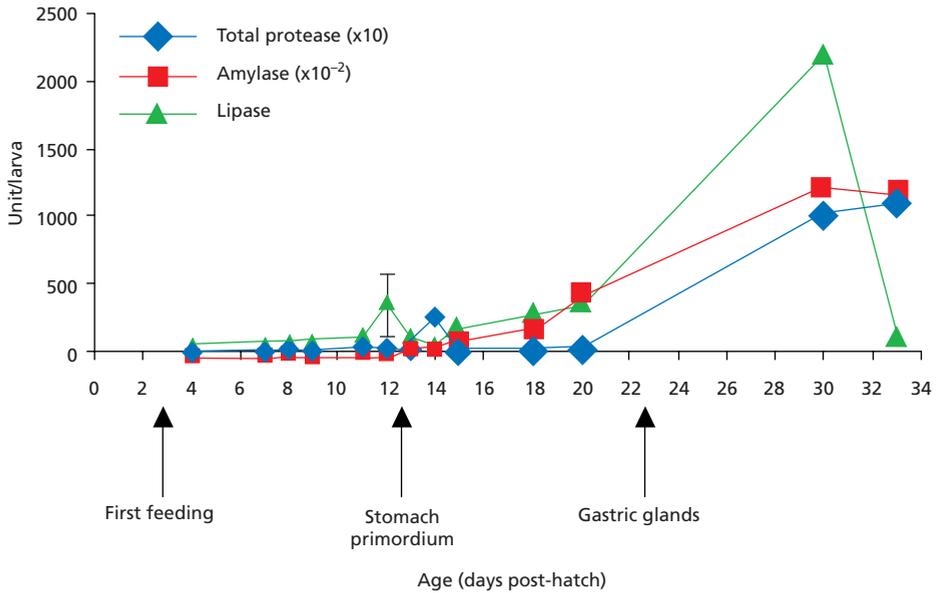


Figure 2. Emergence of digestive enzyme activities in *C. altivelis* larvae with age. Arrows indicate major morphological changes in the gut development of larval *C. altivelis*.

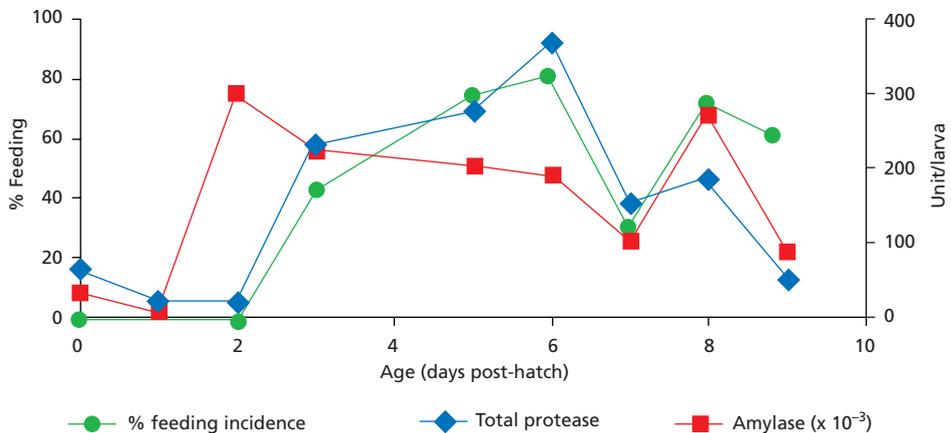


Figure 3. Correlation between feeding incidence in *E. coioides* larvae and the activity of total protease ($r = 0.791$, $P = 0.011$) and amylase ($r = 0.468$, $P = 0.204$).

gut for each age reported by Toledo et al. (1999). Rotifers contributed only 0.7% of total protease so it is unlikely that they make a significant contribution to larval digestion by providing exogenous protease enzymes. Non-feeding naupliar stages of *Arcartia* (n1–n2) contributed less than 2.5% of total protease activity and the feeding stages (n3–n4) contributed up

to 35.6% of total protease activity. These results indicate that n3–n4 copepod nauplii are potentially a significant source of exogenous proteases for the larvae. Surprisingly, the potential contribution of amylase from rotifers and copepod nauplii was relatively high (Fig. 5).

Copepod nauplii contained approximately twice the amount of soluble protein than

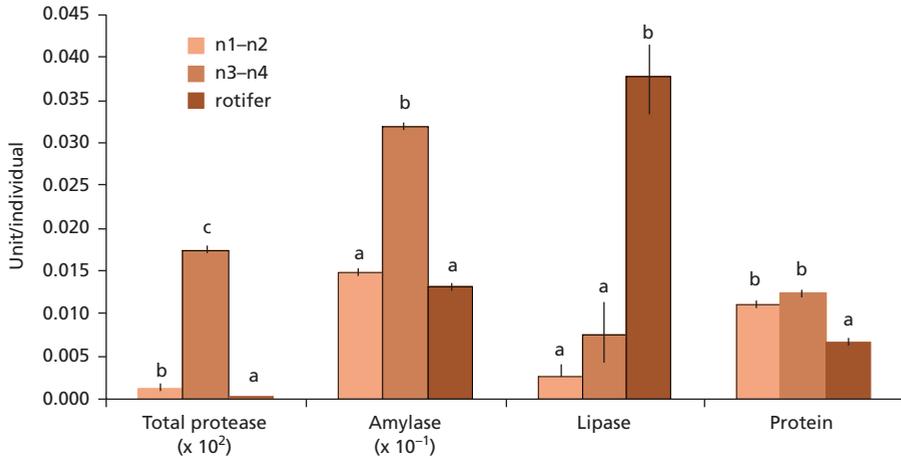


Figure 4. The digestive enzyme activities and protein content in rotifers, and n1-n2 and n3-n4 copepod nauplii. A unit/individual is a unit of enzyme activity/individual (total protease, amylase and lipase) or one µg of protein/individual (protein). Means within a category that are not significantly different share common superscripts (ANOVA; P > 0.01).

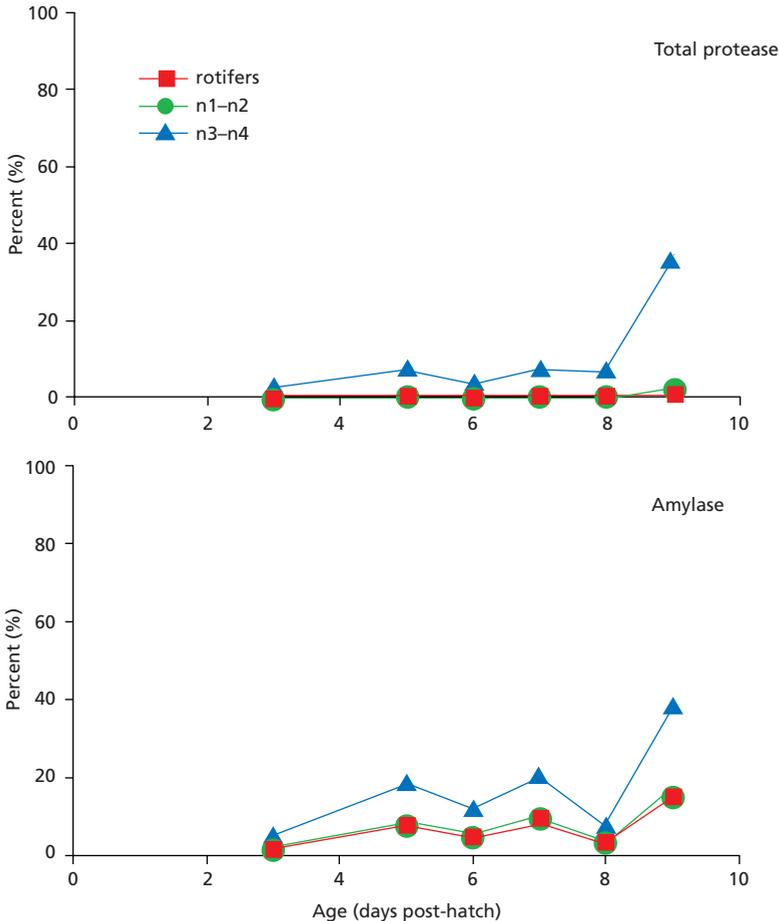


Figure 5. The estimated percent contribution of total protease and amylase activity from live feed to the respective activities measured in *E. coioides* larvae.

rotifers. The greater amount of soluble protein and protease enzymes in copepod nauplii indicates that they may provide a greater opportunity for access to protein than rotifers. This may have implications for the successful transition to exogenous feeding in grouper larvae (Ordonio-Aguilar et al. 1995). Improving nutrition during the initial feeding stages (3 to 9 DPH) may be a key to improving the quality of larvae, which are then able to undergo the major morphological changes between 10 to 20 DPH, faster and more successfully.

Conclusions

- Generally, digestive enzyme activities in larval *E. coioides* and *C. altivelis* were low prior to 18 DPH and then increased with age.
- Changes in the activity of digestive enzymes were associated with the morphological development of the digestive system.
- Total protease activity increased with feeding incidence in early feeding (3 to 9 DPH) *E. coioides* larvae.
- The emergence of total protease and amylase activity was different between *E. coioides* and *C. altivelis* larvae.
- n3–n4 copepod nauplii contained high total protease and amylase activities in comparison to n1–n2 nauplii and rotifers.

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