

**ONTOGENY OF DIGESTION IN LARVAL ATLANTIC COD
(*Gadus morhua*) AND HADDOCK (*Melanogrammus aeglefinus*)**

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EXTENDED ABSTRACT ONLY-DO NOT CITE

Introduction

Two major problems faced in the culture of non-salmonid fish are high mortality during the larval stages and the requirement for use of live feeds such as rotifers and *Artemia*. High mortality during the larval stage may be due to feeding problems such as poor nutrition. Commercial scale production of live feeds is difficult and expensive and is considered to be a bottleneck for successful fish production. The development of a formulated diet for larval haddock and cod would simplify and reduce production costs. Information on what the larvae are capable of ingesting and digesting during ontogeny is important for the development of such diets. We used biochemical and molecular biological techniques to examine the patterns of digestive enzyme activity throughout development in these species.

Methods

Biochemical Analysis of Enzymes Activities

Triplicate pooled samples of 100-300 non-fed haddock and cod larvae were collected from hatch through to 45 days post hatch (DPH). Larvae were raised

using standard larval rearing protocols for these species. Average water temperature was 11.9° C for haddock and 10.6° C for cod. Upon collection the larvae were immediately frozen on dry ice and transferred to -80° C for long-term storage. Whole body homogenates were prepared by homogenizing the larvae on ice in 150 mM NaCl. These homogenized samples were aliquoted and stored at -80° C until use. Samples were analyzed for general protease (GP), trypsin, pepsin, α -amylase, general lipase and bile salt activated lipase (BAL) activity using different biochemical techniques (Gawlicka *et al.* 2000; Parent 1998; Iijima *et al.* 1998). Enzymes specific activities are reported as units of activity per mg of protein. Samples of live prey were analyzed for digestive enzyme activities and their contribution to larvae digestion was calculated.

Analysis of Haddock Bile Salt Activated Lipase Expression by RT-PCR

Replicate pools of 20 haddock larvae were taken from hatch through to 45 DPH, rinsed in RNALater (Ambion, Austin, TX, USA), transferred into 1.5 ml Eppendorf tubes containing 0.5-1.25 ml RNALater, and stored at -80° C until used. For each sampling date total RNA was isolated from the homogenate of a single larval pool using the RNA Wiz kit (Ambion, Austin, TX, USA). Samples were treated with a DNA Free Kit (Ambion, Austin, TX, USA). First strand cDNA was synthesized from 1.5 μ g of total RNA using the RetroScript kit (Ambion, Austin, TX, USA) and aliquots of the reaction products were subjected to PCR using rTaq polymerase (Amersham Pharmacia Biotech AB, Uppsala, Sweden) and specific primers designed for haddock BAL. Amplification of GAPDH mRNA was performed to confirm the level of expression of a housekeeping gene and provide an internal control. Amplification products were visualized on a 2.3 % agarose gel.

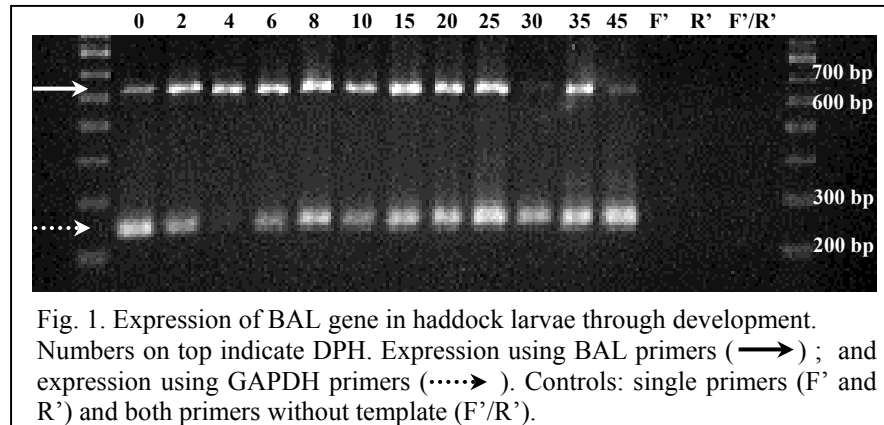
Results

Expression of BAL

Transcripts of BAL were detected at hatch and were present throughout larval development. Expression of BAL in haddock larvae was low at hatch, when compared to the GAPDH controls, and increased from 2 through to 25 DPH. Expression decreased from 30 to 45 DPH (Fig. 1).

Specific Activity of Digestive Enzymes

The specific activities of digestive enzymes in larval haddock and cod are shown in Fig. 2. Cod and haddock GP activities generally increased during early development (<15 DPH) and then decreased possibly due to increasing levels of protease inhibitors in the homogenates of larger larvae. General protease activity in both species showed an increase in the oldest larvae (>35 DPH).



Trypsin-like activity was present in both haddock and cod at hatch. Trypsin-like activity of cod generally showed an inverse pattern to that of haddock. Pepsin-like activity was evident in both species at hatch declining until 25 DPH and increasing thereafter. General lipase activity was evident in cod and haddock from hatch. The level of activity remained relatively constant over time with the exception of haddock that showed a marked increase in activity at 35 DPH. General lipase activity in haddock was higher than that of cod at all ages. Activity of BAL was evident in both species at hatch with the activity in haddock being higher than that in cod at all time points. Haddock BAL activity declined over time, whereas cod BAL activity remained relatively constant. There was no α -amylase activity detected in either species, except at 4 DPH for cod and 15 DPH for haddock. At these times large numbers of rotifers, having high levels of α -amylase activity, were present in the gut. With exception of α -amylase activity the enzyme contribution of live feed to the digestive ability of these species is estimated to be less than 17% for cod and 14% for haddock.

Conclusions

Biochemical assays demonstrated that larvae of both haddock and cod are capable of digesting proteins and lipids from hatch. The activity of trypsin-like

enzymes and BAL was present at hatch as reported for other species (Oozeki and Bailey 1995). Although pepsin-like enzyme activity was present at hatch, it is possible that much of the activity is the result of the measurement of other protease that function at low pH since the appearance of gastric glands first occurs at 30 DPH in both cod and haddock. The lack of α -amylase activity in

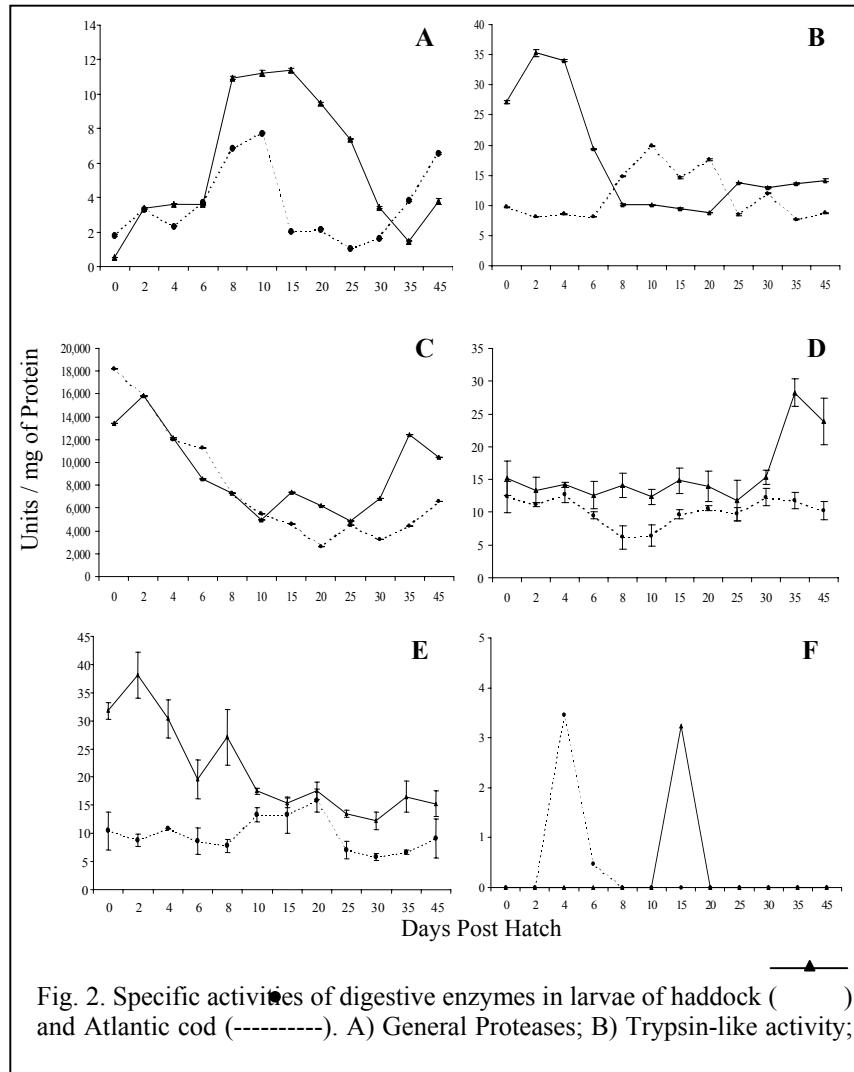


Fig. 2. Specific activities of digestive enzymes in larvae of haddock (—●—) and Atlantic cod (-----○-----). A) General Proteases; B) Trypsin-like activity;

C) Pepsin-like activity; D) General lipase; E) Bile Salt Activated Lipase and F) α -amylase. Values are means \pm SE of 3 samples.

cod and haddock was not unexpected as these species in their early life history stages feed primarily on zooplankton (Kane 1984).

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