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Effect of Starvation on Growth and Hepatocyte Nuclear Size of Larval Haddock, *Melanogrammus aeglefinus*

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ABSTRACT : Early growth, the rate of yolk sac absorption, and nucleus size in liver parenchymal cells were correlated with the nutritional status of first feeding larval haddock, *Melanogrammus aeglefinus*. Larvae that successfully began exogenous feeding maintained high growth rates, delayed yolk sac resorption, and had larger hepatocyte nuclear sizes than starved larvae. At 10 days post hatch (DPH) the cumulative mortality in the starved larval haddock group was 100%. The area of the hepatocyte nuclei in starved larvae gradually decreased, reaching its lowest value by 9 DPH. Our results support the current practice of providing the first food supply at 3 DPH. Hepatocyte nuclear size can be used to assess larval haddock nutrition status, and may be a good criterion for assessing the success of transition from endogenous to exogenous feeding.

Key words : Early growth, hepatocyte nuclear size, larval haddock, starvation

INTRODUCTION

Haddock (*Melanogrammus aeglefinus*) is an economically valuable species withenormous potential in terms of commercial culture in Atlantic Canada (Litvak, 1998; Kim &Lall, 2001; Castell et al., 2003). The principal obstacle to haddock culture is poor survival (mean ~2%; range 0%~33%) from the first feeding larval stage, which requires live food, to weaning and metamorphosis (Castell et al., 2003).

For this reason the success of haddock culture is contingent upon refining hatchery techniques to improve larval growth and survival. A critical factor in hatchery rearing of haddock is accurate estimation of the appropriate time for transition from endogenous to exogenous feeding (Downing & Litvak, 1999). Although the commercial potential of haddock for aquaculture has been well documented, there have been no comprehensive studies focusing on the nutritional condition of larval haddock, or its relationship to successful transition from endogenous to exogenous feeding (Hamlin et al., 2000; Kim & Lall, 2001; Castell et al., 2003).

The purpose of this study was to investigate the effects of starvation on growth and hepatocyte nuclear size of larval haddock. As restricted food availability is known to cause a reduction in the dimensions of the nuclei in liver parenchymal cells (Storch & Juario, 1983; Strüssmann & Takashima, 1989, 1990), we used this morphological feature as an indicator of nutritional status in haddock larvae.

MATERIALS AND METHODS

M. aeglefinus, larvae were hatched and reared at the Aquaculture Research Station of the National Research Council, Sandy Cove, Nova Scotia, Canada. The larvae (initial density 15 individuals L^{-1}) were reared for 10 days in 500 L tanks in a flow-through system. Three replicate tanks were established for each of the starved and fed

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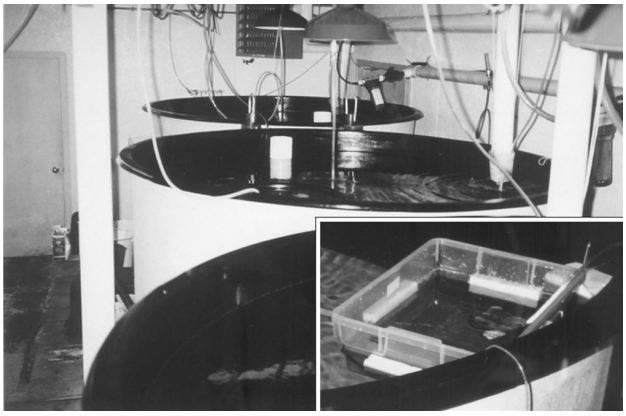

Fig. 1. Rearing tank and water temperature control (inset) used in experimentsinvolving haddock larvae, *Melanogrammus aeglefinus*, from 1 to 10 days post hatch.

groups (Fig. 1). The tanks were supplied at a rate of 0.2 L min^{-1} with filtered seawater (1.0 μm) at ambient salinity (31 ppt), and were equipped with water temperature control (Fig. 1, inset). Throughout the study the mean water temperature was10±0.5℃ and the dissolved oxygen level was 11±2.0 mg^{-1}.

The larvae were maintained under constant illumination (240 lux), and the microalgae *Pavlova* sp. was added twice daily to "green" the water and be used as a food source. From the time of mouth opening at 3 days post hatch (DPH) to the end of the experiment, the fed group received enriched (DHA Selco™, Inve Aquaculture, Inc. Grantsville, UT, USA) rotifers three times daily (9, 15, 20 h), with the aim being to maintain a concentration in the tanks of five rotifers mL^{-1}. Larval age was transformed from DPH to degree-days, following the method of McCormick and Molony (1995).

Each day 50 larvae from each tank were removed and euthanized with an anesthetic overdose (300 ppm lidocaine-HCl/$NaHCO_3$). These larvae were used for growth measurements and histological analysis. The larval total length (mean±standard error), yolk length (mean±standard error), and yolk height (mean±standard error) were measured using a PC-based image analysis system (Stemi DV4, Zeiss, Germany). The body wet weight of larvae in each replicate was estimated to the nearest 0.01 mg. Samples for histological examination were fixed in Bouin's fixative, dehydrated through a graded ethanol series, cleared with xylene, infiltrated with paraffin, and embedded for both sagital and frontal sectioning. Serial sections (approximately 4-6 μm thick)were cut and stained with hematoxylin and eosin-phloxine B. The mean area of nuclei in hepatocytes in the sections examined was calculated by computer-assisted planimetry (Axiostar plus, Zeiss, Germany) (Strüssmann & Takashima, 1990). The hepatocyte nuclear area (S) was calculated as $S=\pi a \times b/4$, where a and b are the major and the minor axes of the cell and the nucleus, respectively (Seol et al., 2008).

Differences in mean nuclear area between serial sections from the same individual were analyzed using Duncan's analysis of variance. Following arc transformation, morphometric data were analyzed by analysis of variance (ANOVA; p values<0.05 indicated statistical significance) and Tukey's post-hoc test, using SYSTAT 10 (SYSTAT Software Inc., USA).

RESULTS AND DISCUSSION

Evaluation of nutritional status during the transition from endogenous to exogenous feeding can be used to assess early larval survival rates for both cultivated and wild fish (O'Connell, 1976; Theilacker, 1978; Strüssmann & Takashima, 1989, 1990; Lee et al., 1998; Park et al., 1998). In this study no significant difference in survival rates within the fed group were found, although there was a high level of variability among the replicates (0.5%-10.8%). However, at 10 DPH (100 degree-days: DD) all larvae in the starved group had died. When the data were standardized to DD, the time of death of the starved haddock larvae was significantly different from that reported for larvae of other marine fish. Complete mortality induced by starvation has been reported inpejerrey (*Odontesthes bonariensis*) within 5 DPH (100-105 DD), in rock fish (*Sebastes schlegeli*) within 5 DPH (70-80 DD), in spotted sea bass (*Lateolabrax* sp.) within 9 DPH (171-189 DD), and

in red spotted grouper (*Epinephelus akaara*) within 5 DPH (136-150 DD) (Strüssmann & Takashima, 1990; Lee et al., 1998; Park et al., 1998).

There was no significant difference found in the larval body length between fed and starved larvae at 1 DPH (Fig. 2a). The mean total length at 1 DPH was not significantly different (p>0.05) between the fed (5.23±0.44 mm) and starved (5.21±0.49 mm) larvae. Throughout the experiment the fed larvae continued to grow, reaching a mean length of 6.36±0.51 mm at 10 DPH (p>0.05) (Fig. 2a).

The starved larvae increased in length until 6 DPH, after which their length remained relatively constant. At 10 DPH the starved larvae had a mean length of 5.81± 0.39 mm. The mean weight of larvae within the starved group was 0.86±0.08 mg at 1 DPH, and there was no significant change in larval weight within this group until 7 DPH (p>0.05) (Fig. 2b).However, from 5 DPH to the end of the experiment the starved larvae lost weight, and at 10 DPH the mean weight was 0.49±0.05 mg (Fig. 2b). At 10 DPH the fed larvae had a mean weight of 1.59± 0.11 mg. Although the fed group began to feed on rotifers at 3 DPH, significant differences in larval length and weight were not evident for several days. This demonstrates that the yolk sac is capable of meeting the growth requirements of haddock during early development. A similar result was reported by Kim et al. (2004), who showed that as haddock grows and the total length increases, the yolk sac reduces in size. It was unknown what effects delayed feeding would have, if any, on the success of transition to exogenous feeding, and long-term somatic growth and survival. In rockfish a significant reduction in length was reported following 2 days of starvation, and a reduction in body weight was observed following 1 day of starvation (Park et al., 1998), while in the spotted sea bass growth in total length declined following 3 days of starvation (Park et al., 1998). However, the effects of starvation in these two species were evident earlier because of their higher rearing temperature.

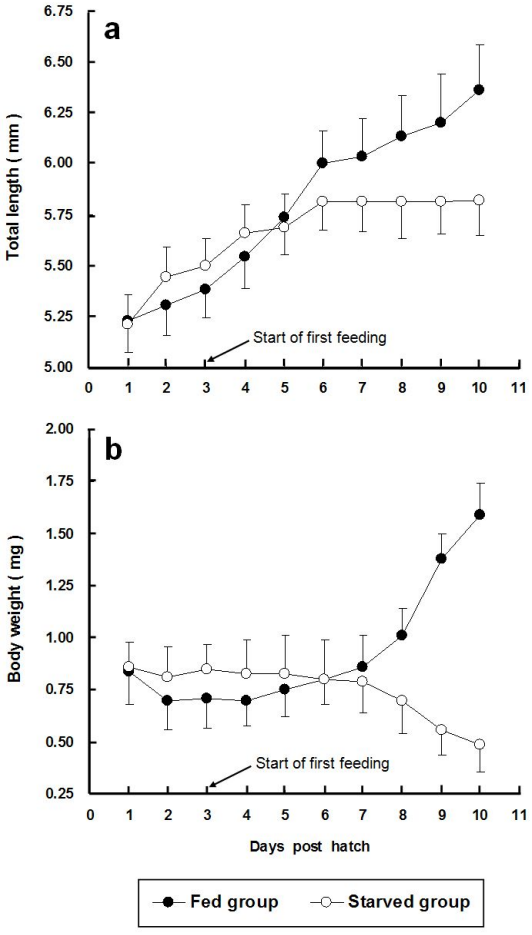

Fig. 2. Total length (a) and body weight (b) of fed and starved haddock larvae, *Melanogrammus aeglefinus*, from 1 to 10 days post hatch. Values are the mean±SE (n=150) of triplicate tanks. Asterisks denote significantly different at P=0.05.

The mean yolk sac length and height in the larval haddock at hatching were 1.00±0.01 mm and 0.69±0.06 mm, respectively. As shown in Figure 3, within the first 3 DPH there were no significant differences in yolk sac length or height between the fed and starved groups (p>0.05). However, from 4 to 6 DPH there were significant differences in the yolk sac dimensions between the two groups, with starved larvae having smaller yolk sacs (p<0.05). Although yolk sac size declined in both groups throughout the experiment, the rate of yolk sac decrease was higher in the starved group. By 7 DPH most of the yolk in each group had been used, and during this period differences in total length and body weight between the

groups were becoming evident. At this time liver degeneration may have commenced in the starved group. In most fish the size and shape of the hepatocyte nucleus is constant if adequate nutrition is supplied. In the absence of adequate nutrition the contents of the hepatocyte nucleus is converted to non-chromosomal protein, resulting in a change to the size and shape of the nucleus (Alvarez & Cowden, 1966; Storch & Juario, 1983). In marine fish, starved larvae begin to show liver degeneration following complete absorption of the yolk. This degeneration can lead to a marked increase in mortality (O'Connell, 1976; Theilacker, 1978; Wang & Takashima, 1984; Strüssmann & Takashima, 1989; Park et al., 1998).

Storch and Juario (1983) first suggested that the dimensions of nuclei in liver parenchymal cells (hepatocytes) could be used as an indicator of larval nutritional status. Strüssmann and Takashima (1989, 1990) also demonstrated a decrease in the dimensions of hepatocyte nuclei in starved pejerrey larvae, and reported that this was related to rearing temperature, as the reduction in the size of the nucleiwas more pronounced at higher temperatures. In this study we investigated use of the area of hepatocyte nuclei as an indicator of nutritional status in haddock larvae. At 1 DPH there was no significant difference in the hepatocyte nuclear area between the starved and fed groups (p>0.05) (Table 1). In the fed group the mean hepatocyte nuclear area remained relatively constant throughout the study, ranging from 16.25 to 17.55 μm^2 (Fig. 4c). In the starved group there were significant declines in hepatocyte nuclear area from 5 to 9 DPH (Fig. 4b); a rapid decrease occurred from 5 to 7 DPH, which coincided with the end of the yolk sac absorption period (Fig. 3). At 9 DPH the size of hepatocyte nuclear area in starved larvae was approximately 51% less than at 1 DPH (Table 1). In a study of red spotted grouper that investigated hepatocyte nuclear size and shape, larval survival rates, and the period taken for larval absorption of the yolk sac (Lee et al., 1998), hepatocyte nuclear size was not found to decline until 72-84 h after hatching, when absorption of the yolk sac

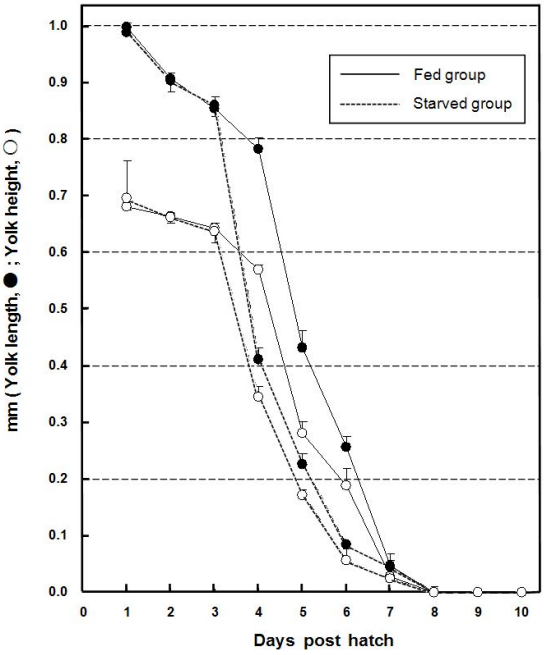

Fig. 3. Changes in yolk length and yolk height of fed and starved haddock larvae, *Melanogrammus aeglefinus*, from 1 to 10 days post hatch. Values are mean±SE (n=150) for triplicate tanks. Asterisks denote significantly different values (P<0.05).

Table 1. Hepatocyte nuclear area in fed and starved haddock, *Melanogrammus aeglefinus*, larvae from 1 to 10 days post hatch*

Group	Days post hatch									
	1	2	3	4	5	6	7	8	9	10
Initial	16.31±0.89^a	-	-	-	-	-	-	-	-	-
Fed	16.25±0.97^a	16.35±0.56^a	17.55±1.21^a	16.92±1.32^a	16.24±1.59^a	17.14±1.42^a	16.98±2.15^a	16.93±1.82^a	17.41±1.63^a	17.52±1.34^a
Starved	16.38±0.97^a	16.29±1.20^a	16.99±1.10^a	15.12±1.42^a	13.23±0.68^b	11.21±1.12^c	9.62±0.95^d	8.23±1.40^d	8.03±0.99^e	8.03±0.86^f

***The values (μm^2) are the mean±SE (n=150) for triplicate tanks. Within each group the means with the same letter are not significantly different (p>0.05).**

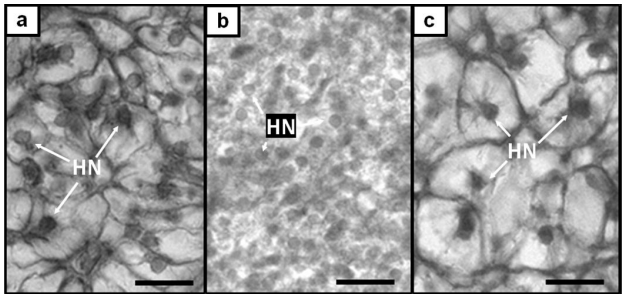

Fig. 4. Hepatocyte nucleus and contiguity in larvae of *Melanogrammus aeglefinus* at initiation of the experiment (a), and in the starved (b) and fed (c) groups. Bars indicate 20 μm; HN=hepatocyte nucleus.

was complete, and neither the total length nor the survival rate of starved larvae was different from that in the fed group. As mentioned above, until 5 DPH there was no significant difference in growth between the starved and fed haddock larvae, and the hepatocyte nuclear size was not significantly different between the two groups. Thus, if feed is provided at 5 DPH, haddock survival should be similar to that of red spotted grouper.

Hamlin et al. (2000) described liver development in larval haddock. At hatching the liver is well differentiated, with hepatocytes arranged in a chord-like pattern between the sinusoids. By 2 DPH the liver begins to elongate and conform to the body cavity. The hepatocytes are arranged loosely around a central vein, and are not divided into distinct lobules. No vacuoles are present at these early stages. By 9 DPH the liver has grown considerably and the hepatocytes have become much more contiguous. In this study the formation of vacuoles was evident after 3 DPH, and in the fed group we observed a similar pattern of liver development to that described above, with liver cells being well separated by large irregularly-shaped lipid storage vacuoles. In the liver of starved haddock larvae the size and number of storage vacuoles declined throughout the study. This resulted in a tighter distribution of nuclei in starved fish, and an irregular pattern in their distribution by 5 DPH. These changes were very similar to those reported for other fish species by Strüssmann & Takashima (1990) and Lee et al. (1998).

In this study, we investigated that starvation during development effect growth and hepatocyte nuclear size of larval haddock. But additional studies are required to further clarify the degenerative process occurring in liver cells of starved haddock larvae. In other fish species electron microscopy and biochemical tools have been used to examine this process (Haines, 1973; Buckley, 1979; Senger, 1985; Lee et al., 1999). It has been reported that the RNA: DNA ratio is highly correlated with feeding and growth rates (Haines, 1973; Buckley, 1979). This technique could also be used to study the feeding and growth of haddock under different nutritional conditions.

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