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Control of light condition affects the feeding regime and enables successful eye migration in Atlantic halibut juveniles

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ABSTRACT

Incomplete eye migration is one of the major problems in intensive production of juvenile Atlantic halibut. More than 60% of an average juvenile population reared according to best practice suffers from this abnormality. In commercial production, these fish are discharged and represent a substantial economic loss and a large welfare problem. In the present investigation it is demonstrated that by controlling diurnal light and darkness periods together with a meal based feeding regime, incomplete eye migration can be dramatically reduced in production systems for Atlantic halibut.

Control groups were reared under continuous light conditions, whereas the experimental groups were given 7 h of darkness and 17 h of light during a 24 hour cycle, in a period lasting from 12 to 35 days post first-feeding. Otherwise both groups were reared under continuous light conditions. All larvae were fed short time enriched *Artemia* supplied two times daily.

The experimental conditions did not affect the overall growth or survival up to day 85 after first feeding. However, $27 \pm 3\%$ of the fry reared under continuous light conditions had complete eye migration, whereas in juveniles reared under shifting light and darkness conditions, complete eye migration was $85 \pm 7\%$. These results represent a major improvement in production systems for Atlantic halibut juveniles.

1. Introduction

Commercial juvenile production of marine cold water species such as Atlantic cod, *Gadus morhua*, and Atlantic halibut, *Hippoglossus hippoglossus* L., has improved considerably during the last years, principally due to improved zootechnology and food quality. Nevertheless, large variations in survival rates and larval quality are still common, and particularly during the initial stages when the larvae rely on a diet of live prey organisms. Systematic investigations have led to better understanding of the dynamics in larvae cultures (Mangor-Jensen et al., 2007), effects of physical factors such as light, turbidity and turbulence (Naas et al., 1992; Harboe et al., 1998) and biological factors such as time for first feeding and effects of prey density (Harboe et al., 1998; van der Meeren and Ivanikov, 2006). Recent studies have revealed that different feeding strategies during the live prey period play an important role in both larval survival and quality of juvenile halibut, where comparative studies showed significantly higher survival when the larvae were fed distinct meals compared with continuous feeding. The nutritionally incompleteness of the common prey organisms, *Artemia* and rotifers (Seikai, 1985; Hamre et al., 2007, 2008; van der Meeren et al., 2008), have for a long period attained research attention and development of enrichment diets has led to improvement of growth and survival, and pigmentation in flatfish. The results from investigations on both zootechnical and nutritional effects on fish larvae have successively been incorporated in production trials and full-scale production methods now being applied by the industry. However, impaired eye migration in hatchery produced Atlantic halibut has remained an unsolved issue.

There are very few reports attempting to understand eye migration in flatfish. Most of these studies have a nutritional approach. Hamre et al. (2002) showed that Atlantic halibut fed copepods had dramatically improved eye migration compared to larvae fed *Artemia*. Both groups were held under similar conditions in an intensive rearing system. This indicated that nutrition was an important factor in promoting eye migration, which was supported by studies showing that ongrown, compared to short term enriched *Artemia* and that improved energy status and lipid level in Atlantic halibut larvae enhanced eye migration (Olsen et al., 1999; Hamre and Harboe, 2008a,b). Gara et al. (1998) correlated eye migration success to juvenile size in Atlantic halibut and proposed energy supply as an important factor for eye migration. Furthermore, Estevez and Kanazawa (1996) found that turbot larvae fed diets deficient in $n-3$ HUFA had impaired eye migration, while two studies indicate that EPA (eicosapentaenoic acid, 20:5 $n-3$) and

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DHA (docosahexaenoic acid, 22:6 $n-3$) enhance, while ARA (arachidonic acid, 20:4 $n-6$) delays eye migration in flatfish (Copeman et al., 2002; Villalta et al., 2005). The remodelling of craniofacial structures participating in eye migration has been described by Schreiber (2006) and Sæle et al. (2006).

It is commonly known that *Artemia* may be incompletely digested after passing through the intestinal tract (Govoni et al., 1986), and in extreme cases survive a complete transit through the larval digestive system. This has also been observed in halibut cultures (personal observations by the authors). A phenomenon that is frequently seen when halibut larvae are fed short term enriched *Artemia* is extremely long faecal pellets consisting of half-digested *Artemia* that protrude from the anus of the larvae. In contrast, when larvae are fed a diet consisting of wild zooplankton instead of *Artemia*, the gut content is homogenous without recognizable structures from the ingested plankton, indicating that their natural prey organisms are far better digested than *Artemia*. If the incomplete digestion of *Artemia* leads to restricted energy access, this may also affect the thyroid hormone regulation. Van der Geyten et al. (1998) saw changes in deiodinase activity in fasted tilapia (*Oreochromis niloticus*), in particular deiodinase type II (DII), which led to altered thyroid hormone plasma levels. This may in turn affect the ongoing metamorphic processes in halibut larvae, including eye migration.

During the first-feeding period there is normally a relatively large mortality and survival rates exceeding 50% from egg to juvenile is considered more than acceptable for most species of marine fish larvae. For Atlantic halibut the highest mortality rates are found in the early larval period, before day 20 after onset of exogenous feeding, and often the moribund and newly dead larvae are found with the gut filled with *Artemia*. Despite a full gut it is hypothesize that deficiency of critical nutrients caused by incomplete digestion of the prey organism can occur and that this can lead to both mortality and deformities in cultures. Halibut larvae are visual feeders and rely on light to capture its prey. In nature, it has been demonstrated that marine fish larvae have a diurnal feeding rhythm due to vertical migration of the prey and light conditions. However, the predominant method in intensive larvae cultures is based on continuous illumination and frequent feedings. Under such conditions the larvae will continue to capture prey even if the gut already is filled. Earlier studies have demonstrated that gut evacuation changes in response to feeding conditions and that the gut transit time is reduced when prey availability is high (Canino and Bailey, 1995). In this manuscript it is hypothesized that high gut evacuation rates with resulting low digestion efficiency, may be an explanation for some of the early mortality and variation in quality and growth in juvenile culture systems. Further, by allowing the larvae distinct meals followed by periods of food deprivation where the gut transit rates may be slowed down, the digestion will be enhanced allowing absorption of slowly digested and critical or essential components in the ingested prey. The aim of this investigation was therefore to examine the relative differences in early mortality and eye migration between larvae under constant- and aborted feeding regimes.

2. Materials and methods

2.1. Eggs and yolk sac larvae

Eggs stripped from one female and fertilized by sperm from two males (Norberg et al., 1991) were kept in 300 L incubators as described by Mangor-Jensen et al. (1998) for 10 days at 6 °C. Thereafter the eggs were collected and surface disinfected by submersion in a diluted glutaric aldehyde solution and reincubated in large conical tanks (silos) where they hatched ca. 12 days after fertilization. The yolk sac larvae were reared in silos for 43 days (265 day degrees) according to Harboe et al. (1994), and then transferred to the first-feeding systems.

2.2. Experimental setup

The larvae were distributed in six first-feeding tanks (1500 L; 3500 ind./tank), three tanks for each treatment. In the periods between first feeding to day 11 and the period after day 36 all six tanks were constantly illuminated. During the experimental phase – days 12 to 35 after first feeding – three control tanks were given constant (24 h) illumination, whereas three tanks had a light regime that consisted of 17 h light and 7 h with shade (13.00 to 20.00 h).

Short term enriched *Artemia* was used as food for the larvae during the first feeding period.

Artemia cysts (EG-type, Great Salt Lake UT, USA) were incubated in 300 L tanks at 27 °C where they hatched into Instar I nauplii within 24 h. After the first moulting, the nauplii were transferred to a 600 L tank for another 24 h at 27 °C for enrichment. The enrichment (MultiGain, DANA feed) was administrated twice, the first 50% immediately after transfer, and the second half continuously from 12 to 17 h after enrichment started. After the enrichment period, the *Artemia* were filtered and washed thoroughly and kept in a holding tank at 7 °C ± 0, 2 until fed to the halibut larvae. The *Artemia* were administered to the larval tanks two times daily, (10.00 and 20.00 h), and the amount was adjusted every day so that amount of *Artemia* leftovers were approximately zero before the morning meal.

The first-feeding tanks were supplied with a central aeration to create a slow vertical water convection that enhanced food capture efficiency (Harboe et al., 1998). During the first 18 days of feeding, the water was made turbid by administration of microalgae (*Nannochloropsis* paste, Reed Mariculture, USA) twice daily to a concentration corresponding to 2 NTU. Overhead mounted fluorescent light tubes (OSRAM — daylight) gave light intensities of approximately 300 μW/cm^2 measured at the surface. To enhance tending procedures each tank was equipped with automatic bottom cleaners (Holm et al., 2004) that within 1 h swept the tank floor completely. Cleaning procedures were carried out every second day and dead larvae were counted. In addition to a central open aerator each water inlet had a separate vacuum degasser to avoid gas super saturation due to temperature elevation when the water passed through in the heat pump. Water flow was gradually increased from 1 to 6 L/min, and the temperature was kept at 12.5 ± 0.3 °C during the experiment.

2.3. Sampling and analysis

At four time points during the experiment (days 0, 15, 45 and 79) larvae were sampled from the tanks for determination of growth and lipid analyses. At the same time *Artemia* were sampled from the production unit for chemical analysis. The samples were stored in at −80 °C until analysis. Larvae samples for analysis of outer ring deiodinase activity were taken at days 0, 25 and 49 after first feeding and larvae analysed for expression of deiodinases I, II, and III were taken at days 0, 6 and 49 after first feeding. Observations of larval gut content were done prior to feeding during the experimental phase (days 12 to 35 after first feeding).

Number of larvae incubated in each tank was estimated at the start of the experiment when they were transferred from the yolk sac incubators to the start feeding tanks. At the end of the experiment, this number was compared and adjusted to the sum of larvae removed as dead larvae during the experiment, the live larvae at the end of the experiment and the larvae sampled for different analysis during the experiment.

2.4. Fatty acid composition

Fatty acid composition of total lipids was analysed according to Lie and Lambertsen (1991), using 19:0 as internal standard. Briefly, the lipid was extracted with chloroform: methanol 2:1, with internal standard added, and methylated in methanol/NaOH with BF3. The