

The effect of light on activity and growth of Atlantic halibut, *Hippoglossus hippoglossus* L., yolk-sac larvae

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Abstract

The growth and activity of Atlantic halibut, *Hippoglossus hippoglossus* L., yolk-sac larvae exposed to light of differing intensities and wavelengths were investigated every fifth day. The experiments were conducted at 6°C from day 1 until day 34 post hatch. Four intensities of constant white light (2.0, 0.3, 0.03 and 0.005 $\mu\text{E m}^{-2} \text{s}^{-1}$, λ_{max} 590 nm), and constant coloured light of equal intensities (0.03 $\mu\text{E m}^{-2} \text{s}^{-1}$) in the blue, green and red spectrums (λ_{max} 450, 560 and 670 nm, respectively) were used. In addition to a control treatment in constant darkness, one treatment was incubated in a 12:12 h light:dark photoperiod. The light treatments did not have any discernible effect on the total length, myotome height, dry weights or yolk conversion efficiencies. The most intense white light resulted in an increased activity on days 24 and 30 post hatch, resulting in a temporarily reduced length and myotome height for the larvae in these groups compared to the other treatments. Larvae from all treatments were of the same size 34 days post hatch. The dry weights of the larvae and yolk-sacs were unaffected by light treatment. The activity increased independently of light treatment until 120 degree-days, and thereafter, the strongest white light resulted in an temporarily increased activity. The distribution of activity changed independently of light regime in the beakers during development.

Introduction

Atlantic halibut, *Hippoglossus hippoglossus* L., have become a promising species for commercial aquaculture during the past decade. A large number of studies have been carried out to describe and understand the fundamental processes which are important for a successful culture of this marine flat fish. Much of the knowledge about the early life stages of Atlantic halibut originates from these experimental studies. In nature, Atlantic halibut eggs are spawned bathypelagically (Haug, Kjørsvik & Solemdal 1986), and are found between 100 and 250 m (Haug, Kjørsvik & Solemdal 1984). Despite significant effort, Atlantic halibut yolk-sac larvae are virtually absent from the net samples (Haug, Kjørsvik & Pittman 1989). The youngest specimens found had reached the end of the yolk-sac stage (North-Norway: Haug *et al.* 1989; Skagerrak: Bergstad & Gordon 1993). Thus, the natural ecology of the long yolk-sac stage of Atlantic halibut is poorly understood.

The Atlantic halibut hatches at a very early stage of development with a huge yolk-sac and a primitive larval body (Rollefsen 1934; Lønning, Kjørsvik, Haug & Gulliksen 1982), and it takes more than a month before the larvae have developed all vital organs necessary for becoming a functional predator. In comparison, cod, *Gadus morhua* L., and herring, *Clupea harengus* L., are able to feed less than

a week after hatching. Much of the development which takes place during the yolk-sac phase in Atlantic halibut occurs during the embryonic phase in cod and herring. The difference is obvious when one compares the development of the light sensory organs (Helvik, Utne, Steinevik, Forsell, Johnsson, Ekström & Holmqvist 1997) and the brain in these species (Holmqvist, Helvik & Forsell 1996).

The light-sensitive pineal organ in Atlantic halibut is developed prior to hatching (Forsell, Holmqvist, Helvik & Ekström 1997). Photodetection through the retina is probably not possible before day 25 post hatch, when the photoreceptor cells with outer segment are observed (Kvenseth, Pittman & Helvik 1996) and optomotoric behaviour occurs (Helvik & Karlsen 1996). The development of Atlantic halibut yolk-sac larvae can be separated into a pre-visual period, in which the larvae register the environmental light through the pineal organ, and a visual period, in which the retina is functional and the larvae also gets visual information. The Atlantic halibut larvae also have sensory inputs from the neuromast system (Blaxter, Danielssen, Moksness & Øiestad 1983; Holmqvist *et al.* 1996) and olfactory system (Holmqvist *et al.* 1996) during the yolk-sac period.

An obvious progression in larval functionality occurs between 16 and 23 days post hatch. The oral cavity is open (Pittman, Berg & Naas 1987; Pittman, Bergh, Opstad, Skiftesvik, Skjolddal & Strand 1990a; Kjorsvik & Reiersen 1992), the first branchial capillaries and primary lamellae are seen by about 150 degree-days (d°) (Kjorsvik & Reiersen 1992), and branchial respiratory movements are seen around 120 d° and onwards (Pittman, Skiftesvik & Berg 1990b). These observations indicate a shift from cutaneous to branchial respiration (Finn, Rønnestad & Fyhn 1995). Kidney tubules seem functional from about 100 d° and it has been suggested that a change in the pattern of passive sinking could be caused by a change from a non-specific osmoregulation to osmoregulation through the kidneys (Kjorsvik & Reiersen 1992).

Light is an important environmental stimulus for the early life stages of Atlantic halibut. It affects egg buoyancy after the blastopore is closed (Mangor-Jensen & Waiwood 1995), arrests the hatching process if the eggs are exposed to light (Helvik & Walther 1992) and affects the behaviour of the yolk-sac larvae (Naas & Mangor-Jensen 1990; Skiftesvik, Opstad, Bergh, Pittman & Skjolddal 1990; Mangor-Jensen & Naas 1993).

Atlantic halibut yolk-sac larvae are normally reared in darkness in up-welling incubators until the animals are transferred to start feeding systems (Harboe, Tuene, Mangor-Jensen, Rabben & Huse 1994). Earlier studies have indicated that light may influence the development of Atlantic halibut yolk-sac larvae (Helvik & Pittman 1990; Skiftesvik *et al.* 1990), either directly on a cellular level (e.g. retina development; Helvik & Karlsen 1996), or indirectly by stimulating activity, and thereby, changing the partitioning of nutrients between yolk used for growth and metabolism.

The correlation between light, activity and growth of marine flatfish larvae has received little attention in the literature. Atlantic halibut yolk-sac larvae may be a good model for such studies since the larvae is an enclosed system during this period which allow us to disregard effects from exogenous feeding. During this period, Atlantic halibut larvae develop from a very immature stage to a functional predator, which implies that daily loss of energy as a result of unnecessary activity may be critical. The aim of the present study was to evaluate whether light conditions during the yolk-sac period had any effect on the activity, growth and yolk-sac utilization of Atlantic halibut.

Materials and methods

Eggs and larvae

Two full-sibling egg groups were obtained from a brood stock of mature Atlantic halibut kept at Austevoll Aquaculture Research Station, Storebø, Norway. For each group, one female was manually stripped of ovulated eggs and the eggs were fertilized with sperm from one male. The eggs were incubated in 250-L up-welling incubators (Jelmert & Rabben 1987) at a temperature of 5–6 °C. Close to the first observed hatching, the eggs were transported to a temperature-controlled room, where the eggs were incubated in four 3-L glass jars containing 0.5 µm of filtered water from 55 m depth which was held for 2 weeks in a 250-L tank supplied with aeration before use.

The hatching of the eggs was synchronized to within 2 h by transfer of light-arrested eggs to darkness (cf. Helvik & Walther 1992). About 40 newly hatched larvae were placed in each of ten replicate 0.5-L plastic beakers for each treatment with static water where they were kept throughout the experimental period. The temperature was

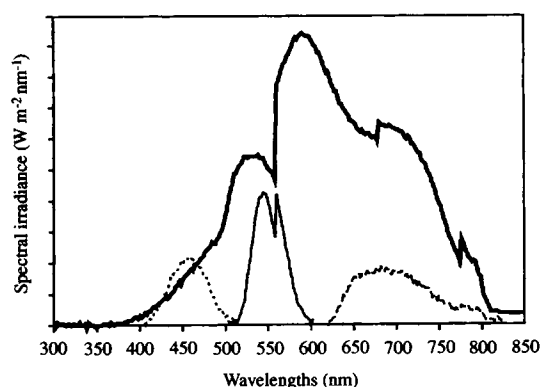


Figure 1 The characteristics of the lights employed in the study measured using a Li 1800 UW Underwater Spectroradiometer (Li-Cor, Lincoln, USA): (white) thick solid line; (blue) dotted line; (green) solid thin line; and (red) dashed line. All groups receiving white light followed the same pattern as shown. See text for

maintained at 6 °C (range = 5.5–6.4 °C) for all groups. Water was not changed and dead larvae were not removed during the experiment since this could not be done in the dark group without the use of light. Mortality was determined when the groups were measured every fifth day, and therefore, represents the cumulative mortality for larvae which had not totally disintegrated. In the first egg-group, mortality was less than 5% until 20 days post hatch, but then increased rapidly, and constituted between 13% and 31% at the end of the experiment at day 34 post hatch. In the second egg group, mortality was less than 7% until day 20 post hatch, but near total mortality was observed at day 25 post hatch. Therefore, only data until day 20 post hatch is available for this group. Therefore, the main analysis will focus on group A and the results will be compared with group B only where this is possible. The age is given as degree-days (d°), calculated as the product of days post hatch and the mean temperature in °C.

Light regimes

For each treatment, 10 replicate plastic beakers were placed in a light-proof black tent, one tent for each treatment, giving a total of 90 beakers, each containing about 40 larvae. The larvae were exposed to various light conditions by supplying the tents with light 80 cm above the floor through quartz fibreoptic cables from a metal halogen bulb (Philips 14.5 V 90 W EPX EPV, Phillips, Hamburg,

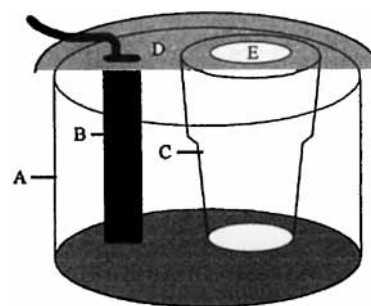


Figure 2 The ultrasonography system used to measure activity of Atlantic halibut yolk-sac larvae: (a) the observation chamber in transparent Plexiglas filled with sea water (the bottom is covered with black cloth); (b) ultrasound transducer; (c) beaker containing the larvae; (d) lid of transparent Plexiglas to keep beaker stationary, with perforation (e) to avoid filtering of light. The remaining parts of the ultrasonographic apparatus (i.e. computer and monitor) are not shown.

Germany). Four different intensities of constant 24-h white light (2.0, 0.3, 0.03 and 0.005 $\mu\text{E m}^{-2} \text{s}^{-1}$) and constant 24-h coloured light (0.03 $\mu\text{E m}^{-2} \text{s}^{-1}$) in the blue-, green- or red-spectrums (Kodak Wratten gelatine No. 98, 99 or 92 for blue, green and red, respectively, Kodak, New York, USA) were used (Fig. 1). In addition to a control treatment kept in 24-h darkness, one treatment was incubated in 12-h light from 0800 h and 12-h darkness from 2000 h (12:12 h light:dark photoperiod) at 0.23 $\mu\text{E m}^{-2} \text{s}^{-1}$.

Activity

The activity of the larvae were measured by use of ultrasonography (Fig. 2). By using this system, the present authors were able to recognize larvae which penetrated a cross-section of the beaker and the coordinates where the larvae penetrated. The cross-section was placed in the middle of the beaker and continuously monitored using a real-time linear array ultrasonographic scanner (PIE Medical 450 VET, B-mode, class I, PIE Medical Maastricht, The Netherlands) supplied with a 3.5-MHz transducer. The power output was 3 mW cm^{-2} (Anonymous 1982).

Activity was defined as the number of times larvae passed through a cross-section of the beaker during the observation period, divided by number of larvae in the beaker. Activity was measured inside the tent with the light (or darkness) the larvae were developed under by moving the observation system

inside the tent and then gently moving the beaker containing the larvae into the observation system. No extra light was used while measuring activity since the ultrasonographic method is independent of light. The 12:12 h light:dark photoperiod group were analysed in the light period, 2 h after the lights were turned on. The activity was measured for 60 min in one beaker for each treatment on days 1–16 and for 30 min on days 20–30. The activity was later analysed in six equal time series of 10 min (days 1–16) or 5 min (days 20–34) from videotapes. If the larvae penetrated the cross-section in the upper or lower half of the beaker, this was registered.

After the activity was measured inside the tents under the light (or darkness), the larvae were developed under the whole observation system, and the beaker containing the larvae was moved out of the tent and the activity was measured during 15 min in darkness. Immediately after this dark period, a light source placed 80 cm above the beaker supplying white light of $0.27 \mu\text{E m}^{-2} \text{s}^{-1}$ was turned on and the activity was measured for 30 min (days 1–16) or 15 min (days 20–34).

Larval size and dry weight

The beakers used for the analysis of activity were also used for analysis of growth and mortality. Twenty-four larvae were randomly sampled from each beaker in the nine treatments, and the notochord length and myotomal height were measured on living larvae using a binocular supplied with an ocular ruler (Wild M3, Wild, Heerbrugg, Switzerland). These larvae were washed in distilled water and frozen individually. The yolk sac and larval body were mechanically separated after freeze-drying the larvae, and the weights of these components were measured (Mettler M3, Mettler, Greifensee, Switzerland). The conservation of yolk matter was calculated according to Blaxter (1969): increase in larval body dry weight/decrease in yolk-sac dry weight $\times 100\%$.

At the end of the experiment, the dark and all the white light treatments were analysed in triplicate for the biometric and gravimetric data.

Statistics

Analysis showed that the biometric data could be analysed by using analysis of variance models on

log (x) or log (x + 1) transformed data. The changes in size or dry weights during the experimental period were not compared using an analysis of covariance (ANCOVA) because of the lack of homogeneity of slopes. Consequently, the data were compared using a non-replicate, two-way analysis of variance (ANOVA) with age and treatment as the factors. Unless otherwise stated, a significance level (*P*-value) of 5% was assumed.

Results

Biometric and gravimetric measurements

The length of the larvae reared in continuous darkness increased from an initial length of 6.5 ± 0.1 mm at hatching to 11.5 ± 0.4 mm at 204 d° (Table 1). The myotome height increased from 0.33 ± 0.00 to 0.80 ± 0.02 mm during the same period. The dry weight of the larval body in the dark group increased from 0.12 ± 0.03 mg at hatching to 0.59 ± 0.05 mg at 204 d° (Table 1). During the same period, the dry weight of the yolk-sac declined from 1.07 ± 0.06 to 0.26 ± 0.04 mg. The various light groups followed a similar pattern.

The different light treatments employed did not result in any major effect on larval length, myotome height or dry weight for the whole experimental period. Even though the statistical analysis showed that both larval age and light treatment significantly affected larval length, myotome height, and dry weights of larvae and yolk sacs in both groups (Table 2), a significant interaction between the factors indicates that the observed differences between the light treatments were not stable during the experimental period. The rank of the treatments according to the size of the larvae varied with age, and between groups A and B. The 12:12 h light: dark photoperiod group showed high total dry weights, and dry weights of larval body and yolk sac at 30 d°, and this treatment had the highest total dry weight at 120 d° in both groups. This was caused by a relatively large remaining yolk sac. The strongest white light and the 12:12 h light:dark photoperiod groups produced the smallest larvae at 144 and 180 d°, and these were significantly smaller than the largest groups (Tukey HSD, $P < 0.05$). The dark group had the longest larvae (Tukey HSD, $P < 0.05$), although there was no consistent pattern in myotome

height. At the end of the experiment (204 d°), these differences had disappeared.

At the end of the experiment on 204 d°, the dark and all the white light treatments were analysed in triplicate. The replicates were significantly different for all the biometric and gravimetric data (nested ANOVA with replicates nested under treatment, $P < 0.01$, $n = 248$). Analysis showed that the largest differences in length and myotome height between replicates within a treatment were 0.47 and 0.04 mm, respectively, which corresponds to 4.0% and 4.4% of the mean for this treatment. The largest differences in length between any of the treatments were 0.55 and 0.04 mm, corresponding to 4.7% and 4.8% of the mean for length and myotome height, respectively. Therefore, the variations between the replicates are of the same magnitude as the differences between the means of the treatments.

During the 34-day-long experimental period, the length nearly doubled, while the myotome height increased by nearly 2.5 times (Table 1). The increase in length and myotome height for pooled groups followed a power relationship with age in degree-days (Fig. 3). The growth represents a five-fold increase in larval body weight and a four-fold decrease in yolk-sac dry weight. The dry weight curve of the growing larvae crosses the dry weight curve of the declining yolk-sac around 150 d°, when larval body weight is about 0.50 mg (Fig. 4). From hatching until 204 d°, the dry weight of the larvae increased linearly with age, giving a daily increase in body dry weight of $0.0139 \text{ mg ind}^{-1}$.

Yolk conversion efficiency

The yolk conversion efficiency was calculated on every fifth day during the experimental period (Table 1). Until 30 d°, several treatments showed efficiencies better than 100%, particularly the 12:12 h light:dark photoperiod group. In the next period, until 66 or 94 d°, the efficiency declined for most groups down to a level comparable with the efficiencies during the whole experimental period. The dark treatment had a yolk conversion efficiency of around 58% during the whole period, with a trend towards higher efficiency in the first part of the experiment than in the latter part. This pattern seemed to be consistent for all treatments. The different light treatments did not have any major effect on the yolk conversion efficiency.

Activity

The larval activity was located in the upper part of the beaker, i.e. almost at the surface, for the first 30 d° (Fig. 5). Then the activity was distributed progressively lower in the incubator up to 96 d°. This was mainly caused by an increased activity in the lower part of the beaker. After a slightly higher distribution around 120 d°, the larvae were mainly located in the lower part of the incubator until 180 d°, and thereafter, the majority of the activity was in the upper part once again. Until 180 d°, the activity decreased in all treatments. From 180 to 204 d°, the activity in the lower part of the incubator decreased in all treatments and increased in the upper part. The activity in the 12:12 h light:dark photoperiod group was the highest of all treatments in the upper part of the incubator from 144 d° and onwards.

The activity at hatching was around $0.13 \text{ penetration min}^{-1} \text{ ind}^{-1}$ and this increased nearly linearly to approximately $0.43 \text{ penetration min}^{-1} \text{ ind}^{-1}$ around 120 d° in both groups A and B (Fig. 5). The activity increased significantly with larval age in both groups (two-way ANOVA, group A: $P < 0.01$, $n = 216$; group B: $P < 0.01$, $n = 108$). The treatments differed significantly in both groups ($P < 0.01$) and the interaction was significant in both groups ($P < 0.01$). In both groups A and B, the dark treatment had the highest activity and the green light the lowest at 120 d°. Only data from group A were available after 120 d°. In this group, the activity stabilized and declined in the dark treatment, and in the treatments receiving the lowest white light intensities and light of various colours. In the groups incubated in the highest white light intensities, the activity continued to increase up to a level of approximately $0.6 \text{ penetration min}^{-1} \text{ ind}^{-1}$ at 180 d° (Fig. 5), but dropped to the same level as the other treatments at 204 d°. The activity in the 12:12 h light:dark photoperiod treatment showed the highest activity at 204 d°.

Effect of light stimulation on activity

When the larvae were exposed to light after the acclimatization period in darkness, the activity was slightly suppressed compared to the activity inside the tents before 120 d° (Fig. 5). This was because of a depressed activity in the upper part of the beaker.

Table 1 Developmental changes in length, myotome height, dry weight of yolk-sac and larvae, and yolk conversion efficiencies (i.e. increase in larval body dry weight divided by decrease in yolk-sac dry weight $\times 100\%$) for group A raised under different light treatments from hatch until 204 d°. The larvae were raised in the light treatment from the time of hatching. Refer to the text for details about the spectrum of the light

Age after hatching (d°)	Light groups								
	Dark group	White (0.005 μE)	White (0.025 μE)	White (0.287 μE)	White (1.990 μE)	Blue (0.025 μE)	Green (0.025 μE)	Red (0.025 μE)	12:12 h (0.230 μE)*
Length (mm)									
6	6.5 ± 0.1								
30	8.2 ± 0.1	8.1 ± 0.2	8.1 ± 0.1	8.3 ± 0.2	8.2 ± 0.2	8.4 ± 0.2	8.3 ± 0.1	8.2 ± 0.1	8.0 ± 0.2
66	9.5 ± 0.3	9.3 ± 0.3	9.1 ± 0.2	9.6 ± 0.2	9.3 ± 0.4	9.7 ± 0.3	9.3 ± 0.2	9.6 ± 0.3	9.4 ± 0.2
94	10.9 ± 0.2	10.0 ± 0.2	10.4 ± 0.2	10.3 ± 0.4	9.9 ± 0.2	9.9 ± 0.2	10.4 ± 0.2	10.3 ± 0.4	9.9 ± 0.2
120	10.4 ± 0.3	10.2 ± 0.3	10.3 ± 0.3	10.1 ± 0.3	10.3 ± 0.2	10.7 ± 0.3	10.5 ± 0.3	10.8 ± 0.2	10.7 ± 0.2
144	11.0 ± 0.4	10.6 ± 0.3	10.6 ± 0.4	10.7 ± 0.3	10.4 ± 0.7	10.7 ± 0.4	10.6 ± 0.3	10.7 ± 0.3	10.5 ± 0.3
180	11.4 ± 0.2	11.3 ± 0.4	11.4 ± 0.3	11.3 ± 0.2	11.0 ± 0.6	11.2 ± 0.4	11.1 ± 0.4	11.1 ± 0.5	11.1 ± 0.3
204	11.5 ± 0.4	12.0 ± 0.4	11.3 ± 0.4	11.4 ± 0.3	11.5 ± 0.6	11.6 ± 0.3	11.3 ± 0.4	11.5 ± 0.4	11.4 ± 0.5
Myotome height (mm)									
6	0.33 ± 0.00								
30	0.49 ± 0.02	0.48 ± 0.01	0.48 ± 0.02	0.49 ± 0.01	0.49 ± 0.01	0.50 ± 0.01	0.49 ± 0.01	0.49 ± 0.01	0.49 ± 0.02
66	0.56 ± 0.02	0.56 ± 0.02	0.56 ± 0.02	0.56 ± 0.02	0.58 ± 0.02	0.57 ± 0.02	0.55 ± 0.02	0.56 ± 0.01	0.56 ± 0.03
94	0.64 ± 0.02	0.63 ± 0.02	0.63 ± 0.02	0.63 ± 0.02	0.61 ± 0.02	0.63 ± 0.02	0.63 ± 0.02	0.63 ± 0.02	0.62 ± 0.02
120	0.67 ± 0.02	0.67 ± 0.03	0.66 ± 0.03	0.67 ± 0.02	0.66 ± 0.02	0.68 ± 0.03	0.67 ± 0.02	0.69 ± 0.02	0.66 ± 0.02
144	0.74 ± 0.03	0.71 ± 0.02	0.69 ± 0.03	0.71 ± 0.03	0.69 ± 0.03	0.69 ± 0.03	0.72 ± 0.04	0.71 ± 0.03	0.70 ± 0.03
180	0.80 ± 0.02	0.80 ± 0.03	0.82 ± 0.02	0.79 ± 0.02	0.78 ± 0.03	0.79 ± 0.03	0.79 ± 0.03	0.81 ± 0.02	0.78 ± 0.02
204	0.80 ± 0.02	0.78 ± 0.04	0.84 ± 0.04	0.84 ± 0.03	0.79 ± 0.03	0.82 ± 0.02	0.80 ± 0.02	0.82 ± 0.03	0.80 ± 0.02
Dry weight yolk sac (mg)									
6	1.07 ± 0.06								
30	0.96 ± 0.09	1.00 ± 0.06	1.01 ± 0.05	0.97 ± 0.04	1.01 ± 0.05	1.01 ± 0.04	1.00 ± 0.05	0.99 ± 0.05	1.04 ± 0.06
66	0.85 ± 0.06	0.88 ± 0.04	0.86 ± 0.04	0.81 ± 0.08	0.85 ± 0.09	0.86 ± 0.04	0.84 ± 0.04	0.83 ± 0.07	0.87 ± 0.04
94	0.72 ± 0.04	0.68 ± 0.06	0.72 ± 0.05	0.70 ± 0.07	0.71 ± 0.05	0.70 ± 0.07		0.73 ± 0.08	0.70 ± 0.05
120	0.57 ± 0.07	0.57 ± 0.06	0.63 ± 0.04	0.58 ± 0.05	0.62 ± 0.07	0.55 ± 0.09		0.51 ± 0.09	0.62 ± 0.07
144	0.48 ± 0.05	0.48 ± 0.08	0.50 ± 0.07	0.44 ± 0.07	0.52 ± 0.04	0.48 ± 0.09	0.52 ± 0.04	0.50 ± 0.05	0.51 ± 0.04
180	0.38 ± 0.06	0.38 ± 0.04	0.37 ± 0.04	0.40 ± 0.03	0.39 ± 0.06	0.39 ± 0.04	0.37 ± 0.04	0.40 ± 0.04	0.38 ± 0.05
204	0.26 ± 0.04	0.27 ± 0.04	0.23 ± 0.04	0.29 ± 0.04	0.24 ± 0.04	0.24 ± 0.04	0.23 ± 0.04	0.28 ± 0.03	0.26 ± 0.04

Table 1 Continued

Light groups									
Age after hatching (d°)	Dark group	White (0.005 µE)	White (0.025 µE)	White (0.287 µE)	White (1.990 µE)	Blue (0.025 µE)	Green (0.025 µE)	Red (0.025 µE)	12:12 h (0.230 µE)*
Dry weight larvae (mg)									
6	0.12 ± 0.03								
30	0.21 ± 0.05	0.18 ± 0.02	0.20 ± 0.02	0.20 ± 0.03	0.20 ± 0.03	0.21 ± 0.03	0.19 ± 0.02	0.20 ± 0.04	0.23 ± 0.04
66	0.30 ± 0.03	0.29 ± 0.02	0.29 ± 0.03	0.29 ± 0.04	0.30 ± 0.04	0.29 ± 0.02	0.30 ± 0.03	0.28 ± 0.04	0.28 ± 0.02
94	0.38 ± 0.03	0.35 ± 0.03	0.34 ± 0.04	0.37 ± 0.06	0.36 ± 0.02	0.37 ± 0.04		0.31 ± 0.05	0.37 ± 0.03
120	0.44 ± 0.04	0.40 ± 0.02	0.41 ± 0.03	0.45 ± 0.04	0.44 ± 0.05	0.46 ± 0.04		0.46 ± 0.06	0.44 ± 0.03
144	0.49 ± 0.03	0.45 ± 0.04	0.44 ± 0.07	0.49 ± 0.05	0.44 ± 0.05	0.44 ± 0.04	0.46 ± 0.03	0.46 ± 0.04	0.45 ± 0.05
180	0.55 ± 0.03	0.56 ± 0.03	0.55 ± 0.04	0.52 ± 0.04	0.54 ± 0.05	0.57 ± 0.04	0.54 ± 0.03	0.55 ± 0.04	0.52 ± 0.05
204	0.59 ± 0.05	0.62 ± 0.05	0.60 ± 0.06	0.58 ± 0.03	0.60 ± 0.05	0.59 ± 0.04	0.54 ± 0.03	0.58 ± 0.04	0.61 ± 0.04
Yolk conversion efficiency (%)									
30	76	82	116	73	115	152	92	87	431
66	80	87	78	64	81	78	76	68	81
94	75	58	63	66	64	65	53	57	66
120	63	57	65	67	70	65	78	61	70
144	62	55	55	58	57	54	61	60	58
180	61	63	60	60	61	65	60	64	57
204	58	62	56	58	58	57	50	58	60

*12:12 h light:dark photoperiod.

Table 2 F-values, P-values and numbers tested with a two-way ANOVA with age and treatment as factors for the biometric and gravimetric data from groups A and B. Group B did not survive past 120 d°. The analysis is separated for the period when the retina is not functional (30–120 d°) and the period when the retina is functional (144–204 d°)

Variable	Group	Treatment		Age		Interaction		Number
		F-value	P-value	F-value	P-value	F-value	P-value	
Pre-visual period								
Length	A	26	0.000	4017	0.000	17	0.000	864
	B	11	0.000	1634	0.000	12	0.000	432
Myotome height	A	5	0.000	3758	0.000	3	0.000	864
	B	7	0.000	1894	0.000	1	0.423	432
Total dry weight	A	8	0.000	342	0.000	5	0.000	836
	B	2	0.036	99	0.000	2	0.132	365
Body dry weight	A	7	0.000	1753	0.000	6	0.000	813
	B	4	0.001	205	0.000	2	0.060	300
Yolk-sac dry weight	A	6	0.000	1672	0.000	4	0.000	801
	B	3	0.007	157	0.000	5	0.000	291
Visual period								
Length	A	7	0.000	252	0.000	3	0.000	645
Myotome height	A	11	0.000	839	0.000	6	0.423	645
Total dry weight	A	4	0.003	195	0.000	6	0.000	636
Body dry weight	A	3	0.003	476	0.000	6	0.000	608
Yolk-sac dry weight	A	2	0.044	1241	0.000	6	0.000	600

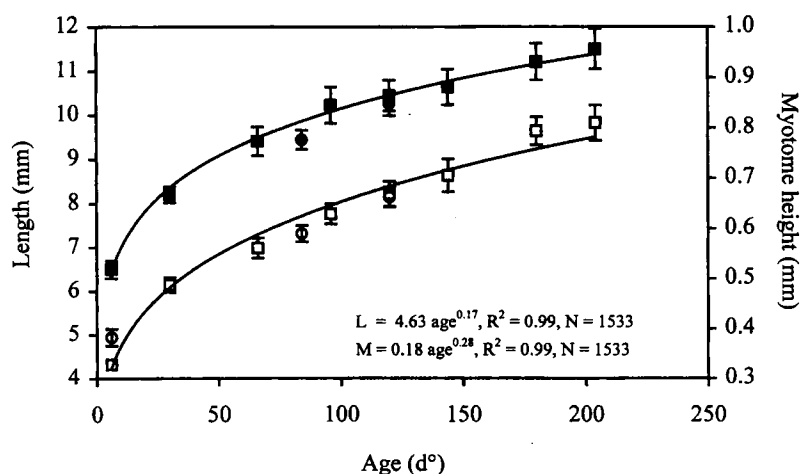


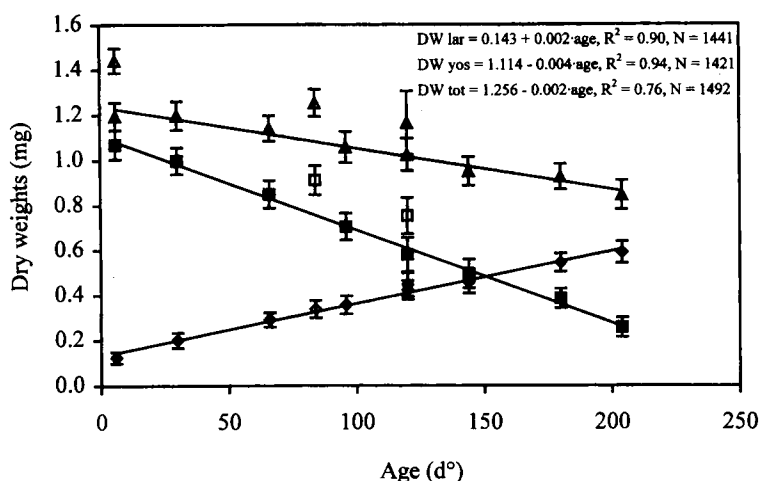
Figure 3 Age-related change in length (closed symbols) and myotome height (open symbols) from hatch until 204 d° for two groups of Atlantic halibut larvae from hatch until 204 d° as a mean of all treatments: (A) squares, solid line; and (B) circles, no line. The lines represent significant least squares fitted to the power equation for individual data from pooled treatments from group A for length (L) and myotome height (M).

The activity increased when stimulated with light for most groups between 120 and 148 d°, especially in the groups incubated under the lowest light intensity. The activity continued to increase compared to the activity inside the tents at 180 and 204 d°.

Effect of activity on growth

The initial specific growth rate (SGR: exponential growth model of Ricker 1958) in larval body dry weight in the newly hatched larvae was approximately 10% day⁻¹, and as the larvae developed, the SGR decreased to 2% around 120 d°. The SGR

Figure 4 Age-related change in dry weight (triangles), and dry weight of the yolk-sac (squares) and larval body (diamonds) for two groups of Atlantic halibut larvae from hatch until 204 d° as a mean of all treatments: (A) filled symbols, solid line; and (B) open symbols, no line. The lines represent least squares fitted to the linear function for individual data from pooled treatments from group A for dry total weights (DW tot), and separated into larvae body (DW lar) and yolk-sac larvae (DW yos).



stabilized at about this level during the remaining experimental period. The increased activity during the first half of the yolk-sac period occurred in the same period as larval growth rate decreased.

The activity until 120 d° was not correlated with larval size (length, myotomal height, larvae dry weight or yolk-sac of the larvae) on any day (Spearman rank-correlation R , $n = 9$, $P > 0.05$), nor to the change in these values from either measurement to measurement or from hatching using the mean of the nine treatments. The conservation of the yolk-sac was not correlated to activity in the present experiment. There are some extreme cases where there seems to be a correlation between growth and activity. Shortly after hatching, the 12:12 h light:dark photoperiod group grew the least and had of the highest activity. Since the dark group had as high a level of activity but showed medium growth, the correlation between activity and growth is not clear at this age. At 144 and 180 d°, the group with the highest activity had a reduced growth in notochord length, but as high a use of the yolk-sac as the other groups. At 144 d°, a shorter length and lower myotome height in the most active treatment was correlated with the high activity of this treatment. At 180 d°, the activity of the white $2.0 \mu\text{E m}^{-2} \text{s}^{-1}$ was highest and this group grew the least.

Discussion

Atlantic halibut yolk-sac larvae were incubated in various light conditions for 34 days. The experimental set-up, which used an array of 0.5-L plastic beakers, was selected to avoid larval disturbance and to allow the measurement of total activity in

the beakers without moving the larvae. This also gave the advantage of total sampling of the incubator so that the problem with obtaining representative sampling could be ignored. The use of the ultrasonic scanner gave us the opportunity to measure the activity in the dark group without light disturbance.

The majority of the larvae survived (69–87%) the 34-day-long experimental period in group A. During this period, the larval body increased five-fold in dry weight and the distribution of the larvae during development followed a similar pattern to that observed in studies in larger systems. This implies that small incubators can be used to analyse the effects of environmental factors on development of Atlantic halibut yolk-sac larvae. The use of incubator water which had been stabilized by air bubbling for 2 weeks to select for slow growing bacteria, and the transference of the larvae after hatching to avoid organic input from the eggshell, may be critical to a successful incubation in small systems without the use of antibiotic.

The light treatments had no consistent effect on the biometric growth, the dry weights or the yolk conversion efficiency until 120 d°. The growth in length was temporarily reduced in the treatments reared under the strongest white light compared with the others from 144 to 180 d°, while no effect on the dry weights or yolk-sac conversion were observed. This effect of light is in accordance with earlier results from cod, where yolk-sac larvae developed in darkness were longer than larvae developed under different intensities of white light, while the development in dry weights were independent of the light conditions which the larvae

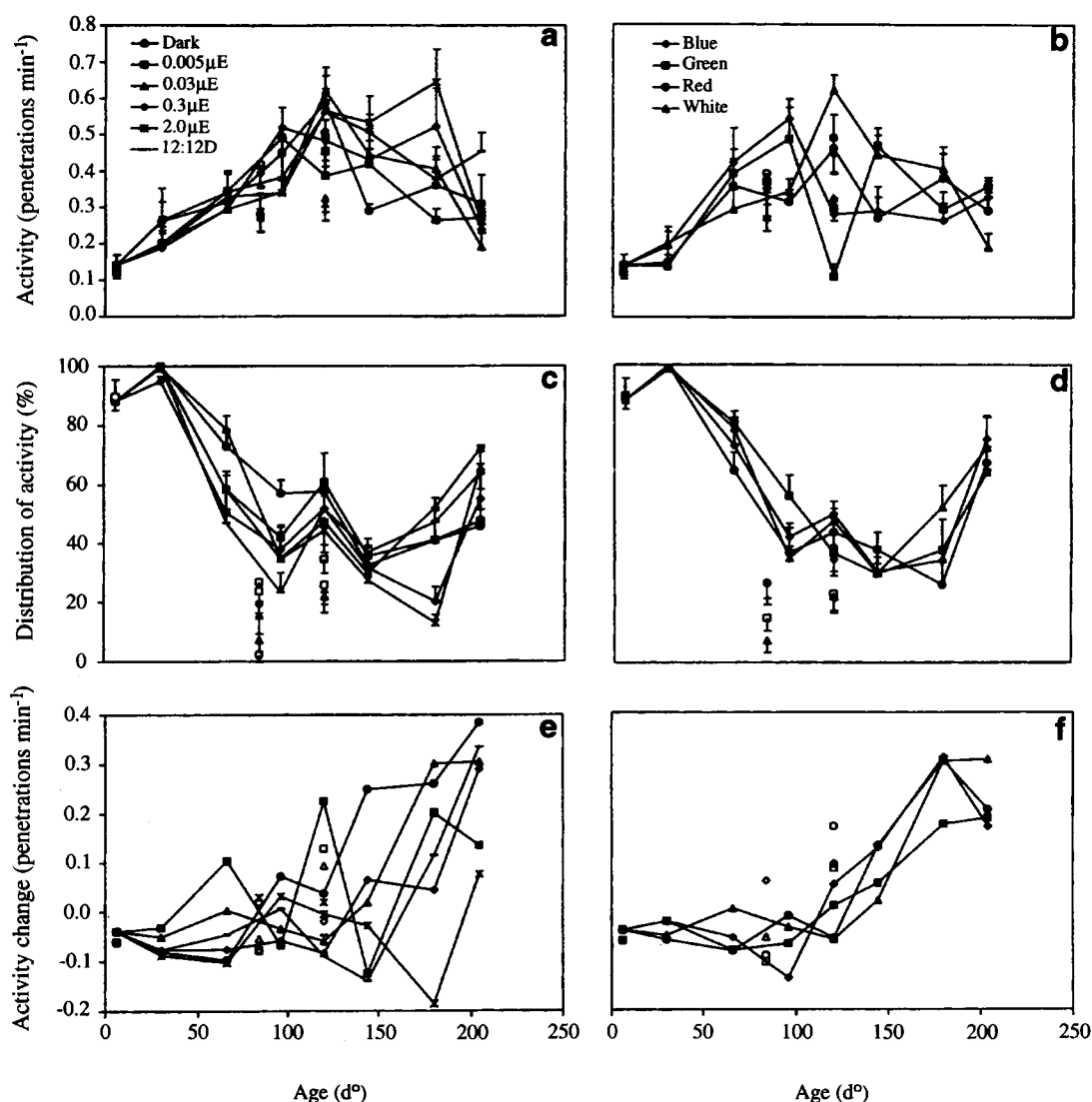


Figure 5 Age-related change in (a, b) the activity, (c, d) the percentage of total activity measured in the upper part of the beakers, and (e, f) the change in activity when stimulated with light after a period in darkness for the different light treatments of Atlantic halibut yolk-sac larvae from hatch until 204 d°. Filled symbols and solid lines represents group A, while open symbols without lines represents group B. The symbols for the different treatments are shown in the

were developed under (Solberg & Tilseth 1987; Skiftesvik 1994).

The yolk conversion efficiency declines during the yolk-sac period, and this may be because gradually more of the yolk nutrients are used for maintenance and energetic purposes as the larvae increase in size and acquire functional muscles (Rønnestad, Groot & Fyhn 1993; Rønnestad, Finn, Lein & Lie 1995). A change in the preferred substrate for energy metabolism from the yolk occurs early in development. Until ≈ 100 d°, the larvae are dependent on

nitrogenous substances, while later the animals begin to catabolize greater amounts of non-nitrogenous fuels (Rønnestad *et al.* 1993; Finn *et al.* 1995; Rønnestad *et al.* 1995).

Since the mass-specific oxygen consumption is directly related to the activity of the larvae (Dabrowski 1986), measurement of oxygen consumption during development in different light environments can be used to get information about activity. Finn *et al.* (1995) have compared oxygen consumption during development in Atlantic hali-

but yolk-sac larvae incubated in darkness and larvae adapted to light (4–6 h prior to measurement). The two groups follow more or less the same oxygen consumption from hatching until day 26 (≈ 160 d°). Thereafter, the two curves diverge and the oxygen consumption in the light-adapted larvae increases compared to the dark-adapted larvae (Finn *et al.* 1995). They assumed that this was a result of a higher level of activity caused by the phototactic behaviour of the larvae. Compared to the larval activity in the present experiment, there are some similarities to the treatment receiving highest light intensity. The activity in the first period in all treatments increased nearly linearly from hatching to about 120 d° without relation to light treatment. This indicates that the light treatments in this period did not have any major effect on the behaviour or growth of the larvae, which corresponds with Finn *et al.* (1995), who found that the dark and light group have similar rates of oxygen consumption. After 150 d°, the activity declined until 204 d° for most treatments, except for the two groups receiving highest light intensities where the activity stabilized until 180 d°, before it dropped to the level of the other groups at 204 d°. This implies that larvae receiving a light intensity higher than $0.3 \mu\text{Em}^{-2}\text{s}^{-1}$ have a higher level of activity than the other groups for several days after the eyes have become functional (Fig. 5). The drop in activity at 204 d° is difficult to explain, but could be a result of adaptation to the light environment. Even though the present authors found effects of light on activity in the group receiving the highest intensities, in general, there is no correlation between observed activity and any measure of growth or yolk conversion efficiency.

The distribution of larval activity within the incubator changes dramatically during development and all groups follow more or less the same pattern. For the first 30 d°, the activity was almost all at the surface, then the activity was distributed lower and lower in the incubator up to 96 d°. After a slightly higher distribution around 120 d°, the larvae were mainly located in the lower part of the incubator until 180 d°, thereafter most of the activity was in the upper part again. This pattern closely resembles the direction of passive sinking and rising found earlier (Pittman *et al.* 1990b) in which a temporarily higher distribution occurred around 120 d°. The temporarily higher distributed activity at 120 d° is probably not a result of the phototactic behaviour which develops from about

this time since this pattern was also found in the dark treatment. Kidney tubules seem functional from about 100 d° and it has been suggested that this change in pattern of passive sinking could be caused by a change from a non-specific osmoregulation to osmoregulation through the kidneys (Kjørsvik & Reiersen 1992). The distribution of activity declined at 144 d°, but then increased at 180 and 204 d° for all treatments except the two receiving strongest white light, where the distribution also decreased at 180 d° before it increased to the same level as the other treatments. It seems that the distribution of the larvae is independent of light since the dark group follows the same pattern as the light group. Before 120 d°, the pineal organ accounts for larval light detection (Forsell *et al.* 1997). From these data, it can be argued that the pineal organ is not involved in the regulation of the vertical location and that location is a passive process related to osmoregulation. Mangor-Jensen & Waiwood (1995) have shown that light affects egg buoyancy after the blastopore is closed. It seems from the present experiments that this opportunity is lost after hatching or that the larvae have already released the maximum amount of water.

The changes in the activity and distribution of larvae are related to the changes in behaviour. During the long yolk-sac period of Atlantic halibut, several major changes in behaviour are observed, particular around 150 d°. The behaviour the first 3 weeks is mostly confined to short bursts of vertical movement in the opposite direction to passive sinking or rising (Pittman *et al.* 1990b). The amount of time spent active is about 10% until 2 weeks after hatching and then increases, but is still below 20% until 100 d° (Pittman *et al.* 1990b; Skiftesvik, Bergh & Opstad 1994). The swimming speed in the active periods increases steadily from 2 to 10 mm s^{-1} during this period (Skiftesvik *et al.* 1994). After 120 d°, the larvae start to move more horizontally (Pittman *et al.* 1990b). The percentage of time spent active increases to 50% at 200 d°, while the swimming speed in the active periods decreases from 10 to 3 mm s^{-1} at about 120 d° (Skiftesvik *et al.* 1994). The pectoral fins have developed into paddles which can be used in synchronized swimming motions (Pittman *et al.* 1990b). These changes and the change in body shape could allow for the changed activity.

Light seems to not have any stimulating effect on the activity from hatching to about 120 d°. This is prior to retinal development (Kvenseth *et al.* 1996;

Helvik *et al.* 1997) and photodetection occurs through the pineal organ (Forsell *et al.* 1997). This result implies that the pineal organ may not interact with the centres stimulating swimming responses. Only after the retina are developed does light stimulation affect the larval activity. This confirms earlier results from Mangor-Jensen & Naas (1993), who showed that positive phototactic behaviour in Atlantic halibut larvae did not occur before 160 d°.

Considering this large growth and the long yolk-sac period, the present results indicate that the light treatments employed in the present study only created minor effects on the growth and the partitioning of energy for the larvae. Therefore, Atlantic halibut yolk-sac larvae seem to be little influenced by homogenous environmental light. In nature, the larvae may experience varying light conditions. It cannot be ruled out that light conditions different from those employed in the present study can have an effect on the growth and development of Atlantic halibut larvae. In future studies, one should consider using an experimental set-up which allows the larvae to choose between different environments. This may clarify to which extent the light environment are important for the development of the Atlantic halibut yolk-sac larvae.

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