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HATCHING OF HALIBUT (HIPPOGLOSSUS HIPPOGLOSSUS L.) EGGS UNDER DIFFERENT LIGHT CONDITIONS

by

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ABSTRACT

Halibut eggs were hatched in total darkness and in illumination (60 lux light). A simoultanous hatching to a final hatching of 99%, and positive buoyant larvae in the dark hatching jars were experienced. In the illuminated group the hatching was postponed, hatching curve progressed more slowly to a final hatching of 72 and 80% in two experiments. Both eggs and larvae developed a slightly negative buoyancy.

INTRODUCTION

The last decade there have been systematic attempts to domesticate the Atlantic halibut (Hippoglossus hippoglossus L.). If juveniles of a species are not readily available from natural sources it is of crucial importance in the domestication process to be able to produce enough juveniles under controlled conditions.

Rearing experiments have been carried out at different research laboratories without much success (Rollefsen 1934; Solemdal et al., 1974).

The lack of success is mainly due to mortality experienced in the periods of hatching, yolk sac stage and first feeding of larvae (Blaxter et al., 1983).

The last three years established broodstocks of Atlantic halibut have provided egg material for a wide range of experiments.

It is known that hatching of halibut eggs takes place at depths of 150 to 200 m (Haug et al., 1984). This is well below the photic zone in the actual waters.

This experiment examined some effects of light in the latest stage of egg development and especially on the hatching process. A microbiological survey was carried out to examine how the effects of light exposure on eggs did influence the microbial society surrounding the eggs.

MATERIALS AND METHODS

Egg material

Eggs were obtained by stripping one mature female and two males of the halibut broodstock kept at Austevoll Marine Aquaculture Station.

The stripped eggs were fertilized wet. Eggs, milt and the inflowing water to the broodstock tank, were mixed in the ratio 100:1:100, respectively. The fertilized eggs were incubated in slowly upstreaming seawater in the hatchery. The upstreaming water should prevent the slightly negative buoyant eggs to sediment in the available water (34 ppt).

When the eggs had reached a late embryonic stage they were transferred to the experimental jars.

The first experiment was carried out with eggs transferred January 20th 1986 after 82 day-degrees. No bacteriological survey was made in the first experiment.

The second experiment was carried out with eggs transferred April 10th 1986 after 80 day-degrees.

Water

Sea water was pumped from 55 m depth approximately 200 m off the station. The water was sand filtered (10 µm pore size), cartridge filtered (5 µm pore size), UV-treated and cartridge filtered (0.45 µm pore size). At the time of the experiment the water had a salinity of 34.5 ppt and a temperature of 6.5 ^{0}C.

Experimental conditions. Jars made of plexiglass (6 l vol.) were filled with 5 l of sea water (see above) and eggs (27-34) were added to each jar. Five jars were kept illuminated and 5 jars were kept covered in a thermostatted waterbath in a thermostatted room

at 6±1 ^{0}C. Two light tubes (Osram 140W/32 Sa) at a distance of 2 m gave a light intensity of 60 lux. The cover of the unilluminated group was a tent made of black plastic film. The oxygen tension of the water was measured daily (Winkler titration). The two groups of eggs will later be named light-group and dark-group, respectively.

Samples and measurements

Samples for counting were fixed with formaldehyde to final concentration 2.5% and stored in darkness at 6 ^{0}C until counted.

Bacterial numbers were counted by an epifluorescence method with the fluorochrome DAPI (4'6-diamidino-2-phenylindole, Porter & Feig (1983)). Two ml of the fixed samples were filtered on prestained (irgalan black) Nuklepore polycarbonate filters (0.2 µm). The filtered bacteria were counted with a Zeiss standard microscope equipped with a high pressure mercury lamp HBO 100 W/2, and filter set number 02.

Bacterial activity was measured by a method described by Somville and Billen (1983). Activity in the water was measured by adding 50 µl substrate (L-leucyl-β-naphtylamine) to a 2 ml sample. The increase in fluorescence (exitation wavelength 360 nm, emmission wavelength 410 nm) was followed for approx 5 minutes when linearity was established. The fluorescence was compared to the fluorescence from 1-naphtylamine (p.a., Merck) solved in 30% (v/v) ethanol, diluted to 3% (v/v) with destilled water. The production of naphtylamine was then calulated from the slope of the linear part of a plot.

Activity on surface of eggs and larvae was measured by immersing 4 eggs or 4 larvae in 2 ml of prefiltered sea water, adding 50 µl substrate, and incubating for 4 min. The eggs or larvae were removed, and the developed fluorscence was detected as described by Jelmert and Mangor-Jensen (1986). Fluorescence was detected on a Shimadzu RF-530 HPLC monitor and a Tarkan W+W recorder 600.

RESULTS

1. experiment (January 1986)

The dark-group showed a simultanous hatching the day after transfer to the experimental jars. The hatching was completed (50% to final hatching percentage 99.6%) within 12 hours and the larvae were all alive and buoyant 5 days after hatching.

The light-group developed quite differently. There was a lag-phase in hatching of 48 h compared to the dark-group. The last 40 h before hatching, some 50% of the eggs in the light-group showed a negative buoyancy. The hatching in this group reached a maximum of 72% four days after the start of hatching. The following days the unhatched fraction died, probably due to the increased microbial activity near the bottom of the jars.

The larvae in the light-group were buoyant for a few hours post-hatching, but after 12 h 50-70% showed a negative buoyancy.

2. experiment (April 1986)

The hatching followed the same pattern as in the first experiment. In the dark-group a simultanous hatching started the day after the transfer. The hatching was completed (99% final hatching percentage) within 48 h. The hatched larvae were buoyant and alive 5 days after hatching.

The light-group had a lag-phase of 24h and reached a maximum hatching of 81% the fifth day (4 days after hatching). The fifth day 76% of the larvae were negatively buoyant.

A plot of the hatching data is shown in Figure 1.

The oxygen was 6.94 ml/l at the start of experiment, 6.81 ml/l at 50% hatching(dark group) and 6.70 ml/l at the end of experiment

which corresponds to 99%, 98% and 96% saturation respectively. There was no difference between the two groups.

After 4 days of exposure, the bacterial count and activity was measured in 2 jars kept in light and 2 jars kept in the dark. In the same jars bacterial activity on the surface of egg and larvae was measured.

The water in the incubator from which the eggs to the experiment were taken, had a bacterial count of 2.2 E06 bacteria/ml. The bacterial activity of the water in the incubator was 0.015 nmol β-naphtylamine (BNA)/(min x ml), and the bacterial activity associated with eggs from the incubator was 0.200 nmol BNA/(egg x min) CV=8 %, n=6.

The water in the jars at time of transfer (0 days), had a bacteria count of 2.3 E05 bacteria/ml and a bacterial activity of 0.006 nmol BNA/(min x ml). There were no significant differences between the bacterial counts from the two experimental conditions.

The bacterial activity in the water in the control group was higher than the activity in the experimental group. At time = 4 days, all the eggs in the control group had hatched, and thereby probably released a larger amount of proteinaceous material than the experimental group where only 75% had hatched.

The bacterial activity on larvae was significant higher in the light-group than in the dark-group. The remaining moribund eggs in the light-group had a twentyfold increase in bacterial activity compared to the activity on the eggs taken from the incubator at the start of the experiment (4.650 and 0.200 nmol BNA/(min x egg), respectively).

DISCUSSION

The response to light was a lowered buoyancy after few hours, a prolonged hatching and a lowered hatching percentage. The light-group showed a higher mortality one week after the experiments were started.

It is unlikely that bacteria should represent serious stress on the larvae the first 2 days of the experiments where clear effects of light were demonstrated. It is also difficult to realize how the light intensity employed should result in different partial pressure of oxygen in these expriments.

In the first part of the experiments it is assumed that bacterial growth was based on easy utilizable substrates (Newell et al., 1981). Such substates were probably present in approximately equal amounts in the light-group and the dark-group the first days of experiment 2 (cf. the bacterial numbers and activity in Fig. 2).

It is reasonable to explain the results as effects on light directly on the eggs rather than effects of microbial activity.

Triggering mechanisms for release of hatching enzymes from buccal and pharyngeal cavities in fish embryos are reviewed by Ishida (1985). It has been shown in various experiments to be regulated by the respiratory center of the brain.

Our results indicate clearly that light of this intensity and wavelengths effects hatching processes.

A reasonable explanation to this phenomenon can be sought in an altered respiration activity as a light response.

The increased bacterial activity on surface of the light-group larvae compared with dark-group-larvae (Figure 2), does indicate that the exposure to 60 lux yields stressed larvae more vulnerable to microbial attack later in the posthatching period.

The lowered buoyancy indicates that the permeability of chorion and/or the vitelline-membrane are possible sites for action of the light.

Some caution has to be stressed on the measurements of the bacterial activity. The method is based on the assumption that a large part of natural occuring bacteria populations posesses proteolytic enzymes on the cell surface. The hatching enzyme chorionase is also known to be proteolyttic. For a rewiew of the matter consult Ishida (1985). The specificity of the halibut chorionase is not known, but the enzyme could interfere with the measurements of the activity by also yielding naphtylamine from the added substrate. It has been shown (Jelmert and Mangor-Jensen 1986) that proteolyttic activity associated with halibut eggs show a dose response to concentration ranges of two different antibiotics. It was thus concluded that the measured proteolyttic activity was of microbiological origin.

Ecologically a prolonged hatching combined with a negative buoyancy will allow the eggs to sink and thus avoid the light zone at the time of hatching and in early yolk sac stage. In accordance with studies on halibut eggs and larvae in natural waters (Haug et al., 1984) and rearing experiments presented by Berg and Øiestad (1986) and Rabben et al., (1986), the light zone is definitely not the place for yolk sac larvae of the Atlantic halibut.

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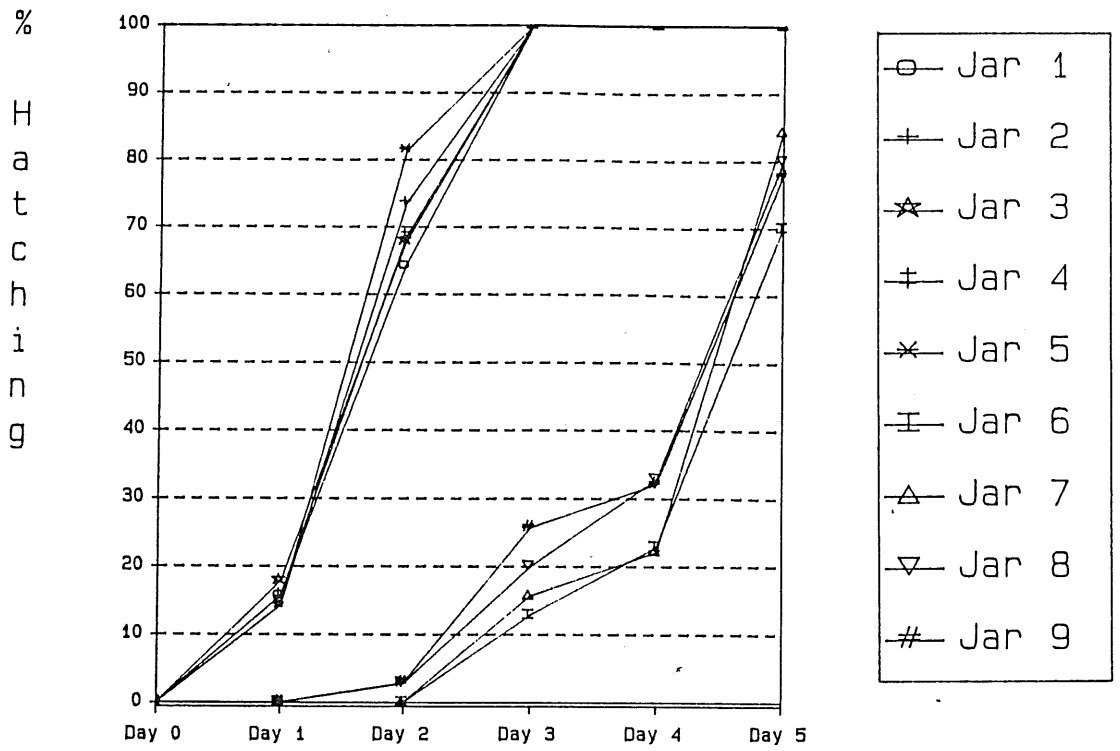

Figure 1. Hatching of halibut eggs in light (1 - 5) and in dark (6 - 9)

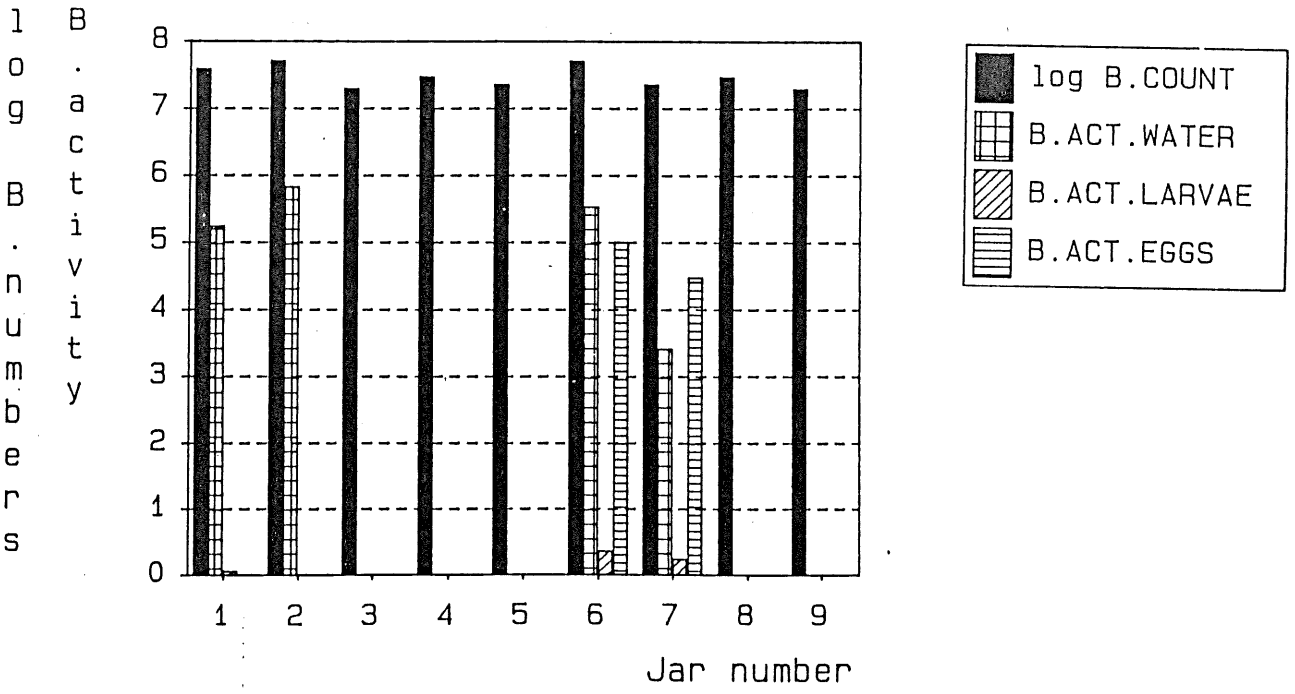

Figure 2. Bacterial counts and bacterial activity in light (1 - 5) and dark (6 - 9)