International Council for Exploration of the sea C.M.1987/F:20

UPWELLING INCUBATORS FOR EGGS OF THE ATLANTIC HALIBUT (HIPPOGLOSSUS HIPPOGLOSSUS L.)

by

Anders Jelmert and Håvard Rabben
Institute of Marine Research
Austevoll Marine Aquaculture Station
N-5392 Storebø, NORWAY

ABSTRACT

Incubators with upwelling water for eggs of the Atlantic Halibut are described. Tending and fate of the eggs where monitored, with special emphasis on egg mortality and bacterial activity. Bacterial activity in the incubators was compared to bacterial activity in unfiltered and filtered(0.2um) seawater.

INTRODUCTION

The last decade there has been systematic attempts to produce juveniles of The Atlantic Halibut under controlled conditions. Rearing experiments have been carried out at different research laboratories without much succsess.(Rollefsen 1934; Solemdal et.al. 1974).
The lack of success is mainly due to gross mortality in the periods of hatching, yolk sack stage, and first feeding of larvae (Blaxter et.al.1983).
The last years several experiments has shown that the bacterial processes in the incubation water should be given more attention(Rabben and Jelmert 1986; Rabben et.al. 1986).

MATERIALS AND METHODS.

Eggs and fertilization.

Newly stripped and fertilized eggs from broodstock of the Atlantic Halibut were transferred to the described incubators. The eggs were wet fertilized by supplying

egg, milt and seawater in the ratio: 100:1:100, respectively.

Two batches of eggs were incubated, the first containing 2600 ml(aprox.104.000)egg, The second containing 2000 ml approx.(80.000)egg.
The fertilization rates for the the two batches were 86% and 41%, respectively.

With the above amount of gametes introduced to each of two incubators(volume approx. 250 l), the exess sperm (milt) was diluted approximately 10.000-fold.
The waterflow through the system did dilute the sperm further, reducing amount of bacteria growing on the dying sperm.

Water.

Seawater was pumped from 55m depth approximately 200 m off the Austevoll Marine Aquaculture Station. The water was sandfiltered (average 10um pore size), cartridge filtered(5 um pore size) and UV-treated(Katadyn Multus U3/PE, 725 W) before inlet to the incubators.

It should be noted that there was established a microbial ecosystem containing several species of bacteria and bacterial grazers(nanoflagellates and ciliates) within the seawater tubing system (after the UV-treatment) supplying the incubators.

The water temperature in the incubation period varied betwen 5.2 and 5.5 C

Bacterial activity.

The bacterial activity was monitored as described in Somville and Billen (1983) exept the following modifications: The kuvettes were rinsed with destilled water (2x2ml) and then 2 ml of sample water, before the 50 ul substrate was added to a 2 ml sample.
The substrate, 40 mM L-leucine- -Naphtylamide, were stored at -18 C and thawed before use.
The developed fluorescence was monitored on a Shimadzu RF-530 HPLC-Monitor and a Tarkan W+W 600 Recorder.
Developed fluorescence(relative fluorescence units, RFU) was calculated as the slope of the recorded line.
50 ul substrate was added to 2 ml of sterile destilled water and measured. The developed fluorescence(spontaneous hydrolysis) was subtracted as a background.

DESCRIPTION OF THE INCUBATORS.

The incubators are made of fiberglass reinforced polyester and are presented in figure 1. The incubators are moulded on the outside of a "plug", giving a smooth

inner surface.
The tubings and fittings were made of polyethylene.
The incubators rested on a framework of 25 mm plywood and 50 x 100 mm woodbars(Figure 1).

A cover of black plastic film was mounted on top of the incubators, and the use of light was minimized.

OPERATIONAL PARAMETERS

The waterflow in the incubators were set at approximately 3 l/min. This gives a holding time of approximately 1.4 h for the water in the incubators.

Maximum 31 of fertilized eggs are incubated in one vessel.

Tending.

Once a day the incubators were observed with a hand held flash-light supplied with a blue filter. The filter had a maximum absorbance of approx. 1 absorbance unit in the range 600-700 nm compared to air(Shimadzu UV-160 Spectrophotometer).

When a certain amount of dead(or heavy) eggs were observed, the incubator underwent the following routine.

1. The water-supply was closed.
2. Water above the upper sieve(B, figure 1) was removed with a siphon.
3. The upper sieve was removed and 10 l of 40 ppt saltwater was gently supplied in the bottom of the incubator with a siphon. The dead and heavy eggs were thus concentrated in the bottom of the incubator.
4. The dead(or heavy) eggs and a large fraction of the supplied heavy water was removed with a siphon.
5. The upper sieve was mounted, and the waterflow was adjusted to the normal level.

Sampling.

Once a day 10 ml water samples were withdrawn from the surface water with sterile pipettes.
The water samples were imidiately measured for bacterial activity as described above.

RESULTS

The removal of eggs and the bacterial activity are shown in figure 2 a and b.

DISCUSSION.

The treatment of the seawater was not intended to remove bacteria as such, but to minimize the possibility of introduction of eventually pathogen bacteria. It is likely that the water treatment produce substrate suitable for bacterial growth.
With the routines described, there are several routes for inoculation of bacteria in addition to the established microbial flora in the tubing system.

With measurements of bacterial activity once a day, and **ad hoc** - removal of dead eggs, it was difficult to infer what was cause and what was effect in this situation.

The results of the measurements of bacterial activity are shown in figure 2 a and 2 b.
The bacterial activity in the unfiltered 50 m water increased in the observed periode, probably due to sediments from a culminating spring bloom (2 a). The bacterial activity in the 50 m water had been low and stabile for a periode of 14 days before the experiment(data not shown).
The situation in the 50 m water is only moderately reflected in the inlet water and the 0.2 um-filtered water(2 a).
As seen in figure 2 b there was a good correlation between egg mortality and bacterial activity. Removal of eggs are marked by arrows. In general the bacterial activity increased steadily until a batch of dead/heavy eggs were removed. After the removal of an egg batch, the bacterial activity decreased markedly.

The first removal of eggs in incubator number 2 represent an anomality. The first removal of eggs was the large fraction of unfertilized eggs in this specific batch. With a low concentration of bacteria in the start, only limited amounts of bacteria could be produced in the incubator in this short periode of time. A large fraction of potential bacterial substrate was thus removed before utilization by the bacteria.

The two batches off eggs used in this study was of poor quality as can be seen from the relatively low fertilization percentages.
With measurements of bacterial activity once a day, and ad hoc - removal of dead eggs, it was difficult to decide if inherent mortality or bacterial activity was the most important cause of death.

In another experiment, Mangor-Jensen et. al. (1987) found that carbon and nitrogen content of halibut eggs averaged 0.73 0.015 mg and 0.185 0.005 mg, respectively. A dead egg releasing its content into the water represent a high amount of assumed well-suited bacterial substrate.

The substrate made available will rise the bacterial activity. High bacterial activity will eventually again lead to higher egg mortality, and bacterial activity might increase further.

If the incubators are considered to be continuous bacterial cultures the bacterial growth rate u will equal the dilution rate D.
The dilution rate= 1/holding time. The generation time(or doubling time) for a bacterium is ln2/u
With the given water flow, the bacteria in the incubators must have a generation time of maximum 58 min.
A longer generation time will eventually lead to a wash out from the system. This generation time is rather short, but absolutely possible, under the conditions given.
The walls and sieve of the incubators and the suface of eggs does on the other hand represent refuges where slower growing bacteria can prevail.
To avoid contamination from one incubator to another, it is nessescary to have one siphon for each incubator.

From the data it is obvious that the tending routines are of great importance. Any accumulation of dead or dying eggs should be avoided. The incubators described do not allow a hygenic and effective removal of eggs. A reconstruction of the incubators should be considered.

REFERENCES.

Blaxter,J.H.S., D.Danielssen, E.Moksness and V.Øiestad, 1983.
Description of the early development oof the Halibut (Hippoglossus hippoglossus L.) and attempts to rear the larvae past first feeding.
Marine Biology 73:99-107

Rabben and Jelmert 1986.
Hatching of Halibut(Hippoglossus hippoglossus L.) eggs under different light conditions.
Coun. Meet. int. Coun. Explor. Sea, 1986(F:17)(Mimeo.)

Rabben,H., T.O.Nilsen, I.Huse and A.Jelmert, 1986.
Production experiment of halibut fry in large enclosed water coloumns.
Coun. Meet. int. Coun. Explor. Sea, 1986(F:19)(Mimeo.)

Rollefsen,G., 1934.
The eggs and larvae of the Halibut(Hippoglossus

vulgaris L.).
K.Norske Vitensk.Selsk.Skr 7,20-23(1934)

Solemdal,P., S.Tilseth and V.Øiestad, 1974.
Rearing of halibut. I. Incubation and the early larval stages.
Int.Counc.Explor.Sea C.M. 1974/F:41 (1974).

Somville,M., and G.Billen, 1983.
A method for determining exoproteolytic activity in natural waters.
Limnol.Oceanogr. 28(1):190-193

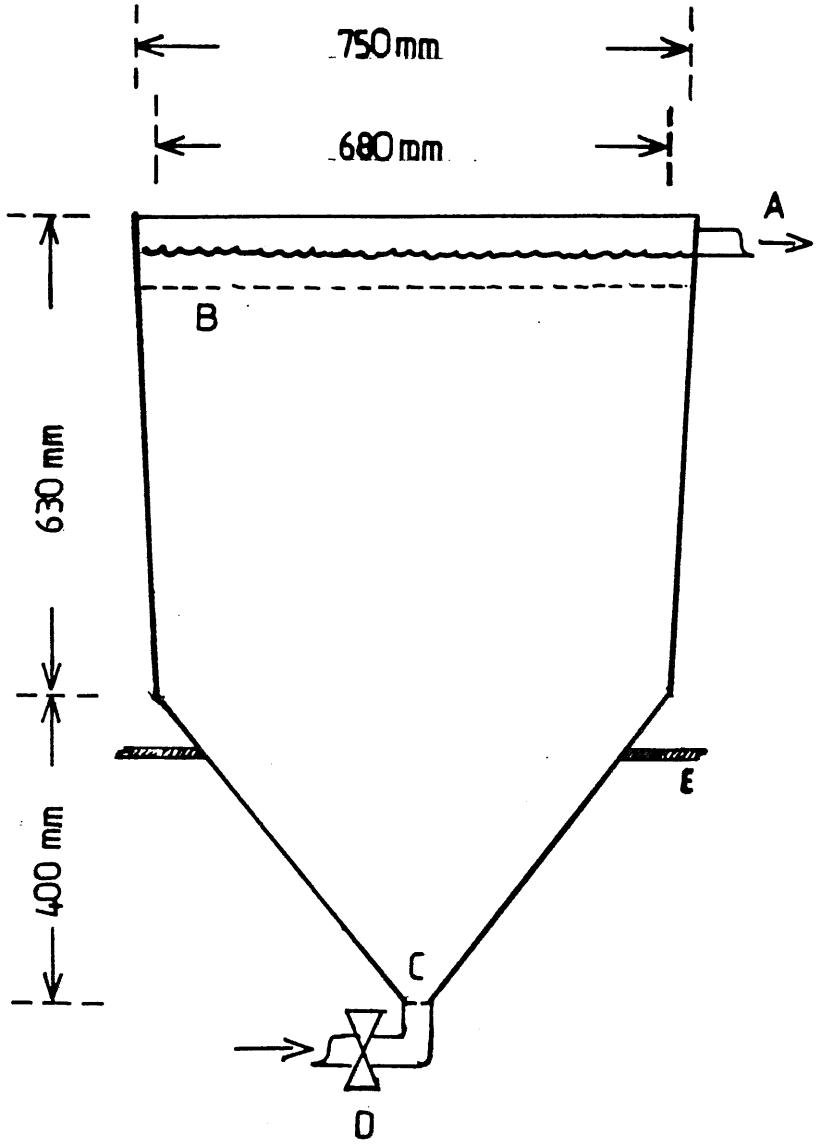

Figure 1. Incubator vessel (not drawn to scale). A: Water outlet, Inner diam. 55 mm., B: Sieve of plankton net 1000 um mesh., C: Water inlet, inner diam. 55 mm, supplied with 250 um sieve, D: Valve, water inlet. E: Support, 25 mm plywood.

2 A

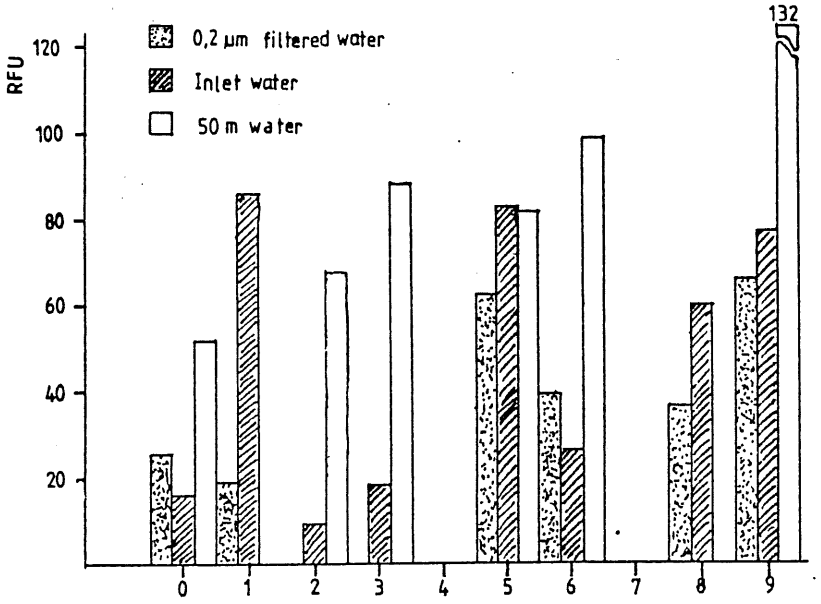

Figure 2 a: Bacterial activity in 50 m seawater, inlet seawater to the hatchery and 0.2 um -filtered seawater.

2 B

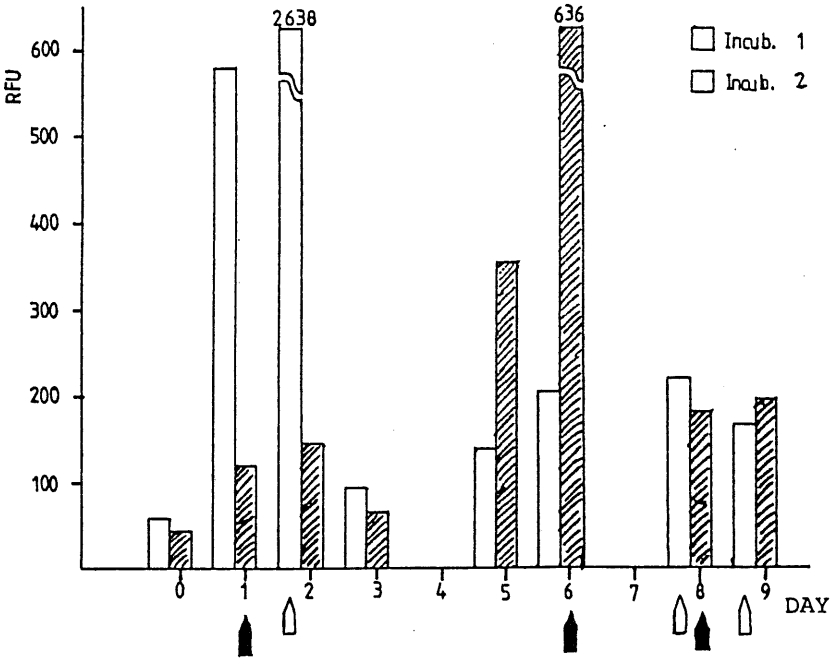

Figure 2 b: Bacterial activity and removal of eggs in incubator 1(white arrows) and 2(black arrows).