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# THE EFFECT OF AMBIENT SALINITY ON THE BUOYANCY OF EGGS FROM THE ATLANTIC HALIBUT HIPPOGLOSSUS HIPPOGLOSSUS.

by

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ABSTRACT

The effect of ambient salinity on buoyancy and the formation of perivitelline fluid in eggs from the Atlantic halibut Hippoglossus hippoglossus have been investigated. The results clearly demonstrate that the water balance of the eggs are independant of the ambient salinity the first days after fertilization. The water loss from eggs fertilized in 17 ppt saline sea water was not less than from eggs fertilized in 34 ppt sea water in spite of a reduced osmotic gradient. Neither was the egg buoyancy altered by fertilization in low saline sea water. For proper fertilization, the eggs from the Atlantic halibut need concentrations of calcium near the concentration found in sea water.

INTRODUCTION

Eggs from marine teleosts are most commonly divided into two separate groups depending on their specific weight. Demersal eggs are shed near the bottom where they usually stick to the substrate. The free floating pelagic eggs maintain buoyancy in sea water due to a larg volume of diluted yolk The hyposmolality of the marine egg is secured passivly by an extremely low water permeability over the vitelline membrane (Mangor-Jensen, 1986). Any stress that increases the passive osmotic water loss from the embryo will therefore presumably cause loss of buoyancy. Although the pelagic eggs from the cod are buoyant in 34 ppt salinity throughout development, significant changes in neutral buoyancy can be monitored. These changes are closely related to the volume of the embryo (Mangor-Jensen, 1986). Still this regulation of specific gravity is dependent on the initial buoyancy of the newly fertilized egg, as the possibilities for an egg to reduce its initial specific weight are very limited.

In 1985 Solemdal showed that eggs from flatfish Pleuronectes platessa, and the cod Gadus morhua shed in the low saline waters of the Baltic had a reduced specific weight compared to the eggs from the same species shed in full strength sea water. The eggs shed in the Baltic were thus neutral buoyant in their natural environment.

The Atlantic halibut is known as a stenohaline fish living under very constant environmental conditions in the deep waters of the Atlantic. In contrast to most other fishes that produces pelagic eggs, the eggs from the halibut are not immediately buoyant in 34 ppt salinity. Several net surveys around the natural spawning grounds of the halibut have revealed that halibut eggs are not found in the upper layers of water. The present theory therefore concludes that the halibut eggs are either bathypelagic or preadjusted to fit the specific weight of the phycnocline between heavy bottom water and lighter surface water.

The egg of the halibut posess all the characteristics of a pelagic egg, except for being pelagic in 34 ppt salinity (Riis-Vestergaard, 1983). The hypothesis for this investigation was that the eggs are adjusted in buoyancy to fit the external salinity. This may be accomplished according to the following two models:

1. In the time just after fertilization in sea water the membrane permeability is reduced by a factor of 100. In the same period the perivitelline space develops as a combined result of embryonic volume decrease and increased total egg volume due to turgor pressure inside the chorion. In this process the osmotic loss of water from the embryo may be adjusted according to external salinity thus regulating its neutral buoyancy.

2. The osmotic loss of water that is found in relation to the activation process is constant regardless ambient salinity. The buoyancy of the fertilized eggs is determined by the plasma osmolality of the maternal fish that to a certain extent is dependent on ambient salinity.

In this investigation the first model was tested.

## MATERIAL AND METHODS

Eggs and sperm were stripped from ripe specimens of the atlantic halibut Hippglossus hippoglossus. The fish were kept in 40 $m^3$ tanks supplied with continuous flow (approx. 0.25 $m^3$/min) of sea water (34 ppt, 5 $^0$ C). The eggs split into 4 similar batches, each incubated in different salinity ranging from 17 to 34 ppt salinity and fertilized by addition of sperm. After 10 minutes the entire incubation medium was renewed to remove surplus sperm. The eggs were left for 2 days at constant temperature of 5 $^0$ C in a refrigerated room.

Fertilization rates in the different salinities were determined by use of a binocular dissection microscope. The presence of a morula was used as a criterion for fertilization.

Volume of the perivitelline space (PVS) was determined as a chloride space as described by Mangor-Jensen (1986).

Yolk osmolality was determined by freezing point depression in small samples of yolk material withdrawn from the eggs by micropuncture with a pointed glass capillary. The yolk sample was sealed within liquid praffin immediately after sampling, and frozen for later analysis in a Clifton Nanolitre Osmometer.

Neutral buoyancy was determined using a density gradient coloumn as described by Coombs (1981). The gradient were calibrated by density beads with known specific weight.

RESULTS

The eggs from the halibut showed reduced fertilization rates in diluted seawater. However, by addition $CaCl_2$ to 10 mM final concentration in the media, the fertilization rates was similar to fertilization rates in 34 ppt salinity (Table 1).

Table 1. Fertilization rates of halibut eggsin different salinities and water qualities.

| Salinity (ppt) | % fertilization |
|---|---|
| 17 | 5 |
| 23 | 20 |
| 27 | 84 |
| 34 | 92 |
| 17 + $Ca^{++}$ | 89 |
| 23 + $Ca^{++}$ | 94 |
| 27 + $Ca^{++}$ | 90 |

Measurements of the PVS volume, revealed that the initial development of this space is independent of the ambient salinity (Table 2). No significant difference in PVS volume was found between the groups.

Table 2. Volume of PVS in eggs fertilized at different salinities. All solutions containing 10 mM $CaCl_2$.

| Salinity | PVS (% of egg volume) | | n |
|---|---|---|---|
| 17 | 16.4 | 0.5 | 4 |
| 23 | 17.2 | 0.7 | 4 |
| 27 | 16.8 | 0.6 | 4 |
| 34 | 16.6 | 0.8 | 4 |

The yolk osmolality showed a marked increase in all the groups after activation compared to yolk osmolaity of unfertilized eggs (Table 3). This agrees well with previous reported data on pelagic cod eggs (Mangor-Jensen, 1986).

Table 3. Yolk osmolality of halibut eggs fertilized in different salinities ranging from 17 to 34 ppt.

| Salinity | Yolk osmolality (mOsm) |
|---|---|
| 17 | 470 |
| 23 | 467 |
| 27 | 480 |
| 34 | 473 |
| unfertilized | 396 |

The measurements of natural buoyancy of halibut eggs one day after fertilization in different salinities are given in table 4. There were no significant differences between the groups (student t-test). These findings are in agreement with the obtained results for PVS-volume and yolk osmolality.

Table 4. Neutral buoyancy of halibut eggs reared in different salinities. Values are mean ± SD.

| Salinity | neutral buoyancy as function of salinity | | n |
|---|---|---|---|
| 17 | 35.086 | 0.015 | 11 |
| 23 | 34.989 | 0.011 | 11 |
| 27 | 34.923 | 0.017 | 20 |
| 34 | 34.773 | 0.017 | 17 |

DISCUSSION

In a pelagic egg, a large volume of diluted yolk compensate for the relatively heavy chorion and embryo. Neutral buoyancy is thereby obtained. The formation of the PVS have been described as the result of increased osmotic pressure between the embryo and the chorion due to extrution of glycoproteins from the cortical vesicles located in the thin layer of cytoplasm that surrounds the yolk (Ginzburg, 1968). This leads to an osmotic influx of seawater into the PVS, and subsequent to a turgor pressure that keeps the chorion tense. The formation of a PVS therefore does not necessarily involve osmotic water loss from the embryo, but rather an active extrution of vesicular material.

The results from this investigation demonstrated that the water loss in the activation process was not due to osmosis, since alterations in the osmotic gradient between the embryo and the ambient sea water did not affect the initial water loss.

It should be investigated if the buoyancy of halibut eggs are governed by the ambient salinity to which the female broodstock is exposed.

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