

## Early development of Northeast Arctic Greenland halibut (*Reinhardtius hippoglossoides*)

Anne Stene, Agnes C. Gundersen, Ole T. Albert, Per Solemdal and Kjell H. Nedreaas

### Abstract

The early development of the Greenland halibut (*Reinhardtius hippoglossoides*) has never been described in detail. This study contains some preliminary notes on the egg development of the Northeast Arctic Greenland halibut. Buoyancy and morphometric characteristics are compared with field sampled eggs, in order to identify these to species.

The eggs in the ovary of ripening females were large (about 4 mm), transparent, almost equal in size and stuck to the walls of the ovary. In ripe, artificially spawned females the ovulated eggs were all easily released in one batch. This may indicate that Greenland halibut has a comparatively long period between each egg batch delivered.

Artificially spawned eggs of the Greenland halibut were 3.3 – 4.2 mm and had neutral buoyancy in seawater of ca 33 ‰ during the first four days of development. During gastrulation the egg density increased until closure of blastopore. Eggs from field surveys had neutral buoyancy in seawater densities near 1.0279 g/cm<sup>3</sup>. This density is found at depths around 650 meters (34.9 ‰ S and 1.8° C) in the spawning area.

The eggs hatched after nearly two months incubation. The embryo seems to hatch at an apparently premature stage.

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## Introduction

The Greenland halibut (*Reinhardtius hippoglossoides* W.) has a wide subarctic distribution in the Atlantic and the Pacific oceans. According to Smidt (1969), spawning takes place around Greenland from December to April in deep waters of 800–1000 m. In the Northeast Arctic Hognestad (1969) reported spawning from April to July, while Fedorov (1971) indicated spawning mainly in October-January, and possibly year around.

Eggs and larvae of this species have been observed in plankton tows around West Greenland (Jensen, 1935; Smidt, 1969), West Iceland (Magnusson, 1977) and the Bering Sea (Bulatov, 1981). These observations indicate a bathypelagic distribution around 600 to 800 m depth, but the eggs have also been registered in plankton nets in the upper 50 m (Smidt, 1969). The eggs were identified according size, a reddish-brown vitelline membrane and the season. Eggs from the Northeast Arctic population were registered in the Barents Sea for the first time in December 1997 (Albert *et al.*, 1998).

Knowledge of vertical distribution is the first step in understanding the horizontal transport of fish eggs and larvae (Sundby, 1991). Attempts were made to establish a probable vertical distribution of eggs and larvae and to confirm their distribution in the watercolumn.

## Materials and methods

Adult Greenland halibut were caught using long line in the Norwegian Sea from August to December and shrimp trawl in the Barents Sea in the end of January. All fish were examined for maturity, in order to make egg cultures for development studies.

Only one ripe female (70cm/3088g) was found at a depth of 700 m in January. The ovary of the ripe female was removed and the eggs were artificially fertilised when brought onboard (male: 50cm/949g). The eggs were incubated in still water, which was changed every second day. No antibiotics were added.

Buoyancy studies indicated a development near the surface for the first four days, then further development in deeper, cooler water. The temperature was therefor 4-5°C for the first 5 days. Then the eggs were transferred to c. 2°C for further development. The development was studied and photographed in a light microscope, where egg diameter and larvae length was measured.

Plankton surveys were made in January with a MIK plankton sampler according to Albert *et al.* 1998. Eggs found in these surveys were kept alive at 4-5°C. Eggs from field surveys and 24 hours old artificially spawned eggs were transferred to seawater of different salinities for buoyancy studies. The seawater was salted with NaCl or diluted with distilled water. Twenty to thirty eggs were placed in graded glass columns of 500 ml for 24 hours in salinities of ca 31 to 36 ‰. The salinity of the surface water in the area was about 35 ‰.

Due to error in the CTD-probe onboard the research vessel, temperature, density and salinity were measured with a standard aerometer/densimeter. Hydrographical data referred to in the discussion are recorded with a CTD-probe in mid November 1997 and mid February 1998.

## Results and discussion

### Spawning

The eggs in the ovary of ripening females, just prior to spawning, were transparent, almost equal in size and stuck to the walls of the ovary (Figure 1 above). The eggs in the ovary of the ripe female were similar in size, but clearer and not attached to the ovary (Figure 1 below), and all came easily out when pressing the fish. Most female fish registered in the spawning period, were either prior to spawning or had just spawned.

During four expeditions in the spawning area from October to February only one ripe female was found. This can be due to sampling techniques and lack in appetite during the spawning period (longline). Difficulties in finding ripe females are also reported from others (Jensen, 1935; Boie, 1990).

It is not known whether Greenland halibut is a serial spawner, or deliver all eggs in one batch. Equal size of the eggs may however indicate that this species spawn fewer portions during the spawning season compared to for example cod. Few running females and even egg size in ripe and ripening fishes may indicate a long period between each batch spawned. In addition, spawning males of Greenland halibut have rather small testes compared to for example cod. This also may indicate fewer spawning events pr seasons.

In female Greenland halibuts, who seemed to have spawned recently, 50-100 transparent, loose eggs were usually observed in the ovary. These eggs had a buoyancy equal to newly fertilised eggs, but attempts to fertilise them did not succeed. In addition to these probably overripe eggs, small eggs (ca 0.5 mm), difficult to observe, were also found in the walls of the ovary. These would obviously not be ready for spawning until next spawning period.

Hognestad (1969) and Albert *et al.* (1998) indicate a spawning period both in the winter and in the spring. Fedorov (1971) on the other hand also discuss a possible spawning more or less year around. If Greenland halibut has more than one annual spawning period, this may be due to homogenous environmental conditions in deep waters where seasonal variations in light and temperature is less distinct.

### Early development

Eggs and larvae of Greenland halibut observed in plankton tows have been identified according to size, a reddish-brown vitelline membrane and the season. The mean diameter of eggs from the field survey in the Northeast Arctic in January 1998 was 4.4 mm (range 3.9 – 4.7 mm). This is somewhat larger than eggs from West Greenland: 4.0mm (range 3.8 – 4.3 mm) (Smidt, 1969), West Iceland: 4.01 mm (range 3.83 – 4,17 mm) (Magnusson, 1977) and

the Bering Sea: 3.84 mm (range 3.71-4.10 mm) (Bulatov, 1981). It may therefore be doubtful that the largest eggs sampled really were Greenland halibut. The largest eggs were late stages, but the embryo inside did resemble the artificially spawned eggs when reaching the same developmental stage (Figure 2A).

Regarding the diameter of the field eggs from the North Atlantic, the result is a mean of only seven eggs found. Other investigations have however shown that variation in egg size within and between populations can be large (Solemdal, 1970; Bagenal, 1971). In Atlantic halibut it is reported significant differences in egg diameter between different areas and between sampling years at the same spawning location (Haug *et al.*, 1984).

Magnusson (1977) found the egg diameter in ripe female Greenland halibut varying from 3.02 - 5.09 mm. There is fairly equal size between ripe unfertilised and fertilised eggs. The largest eggs from the North Atlantic may therefore be within the size range of Greenland halibut. However, more material needs to be examined before a conclusion can be drawn.

The egg diameter of artificially spawned eggs from the Northeast Arctic was 3.7 mm (range 3.2 - 4.2 mm). This is slightly smaller than measurements on field eggs from other areas. Smaller size of the artificially spawned eggs may be due to the fertilisation process and environmental conditions differing from that in the deep spawning area.

Eggs from field surveys and artificially spawned eggs from the Barents Sea did not have a reddish-brown membrane reported from other areas. This difference can be a population characteristic or may be related to diet. Colourless eggs (average diameter 3.9 mm) were also found in the Davis Strait spawning area of Greenland halibut in 1968. It was not decided whether these eggs were Greenland halibut or not (Smith, 1969).

The early development of Greenland halibut is not described in detail. Only one drawing of a late stage egg from the Greenland waters was found in the literature review (Smidt, 1969). Informative illustrations of Greenland halibut bathypelagic larvae are however made by Jensen (1935). The early development of Greenland halibut resembles in many ways that of other fishes with pelagic eggs. The egg and early larval development is therefore compared with other marine teleost, with special emphasis on Atlantic and Pacific halibut.

The unfertilised eggs were soft, with a wrinkled/striated surface. After contact with seawater and fertilisation, the chorion hardened. In contrast to Pacific and Atlantic halibut (Forrester & Alderdice, 1973; Lønning *et al.*, 1982), the striated appearance did not disappear until after gastrulation (Figure 3A). Fertilisation rate was 95%.

Like Pacific and Atlantic halibut, the eggs of Greenland halibut also have a rather small perivitelline space (Figure 3B), and cleave somewhat slower at 4-5°C than other pelagic fish eggs at the same temperature. The first cleavage of Greenland halibut eggs started 10 hours after fertilisation. Then they cleaved for every third hour reaching gastrulation after five days. Cleavage, the formation of the blastodisc with the surrounding periblast (Figure 3A), the gastrulation until closure of blastopore resembles that of other pelagic fish eggs (Figure 3C & D).

The tissue layers and the embryo starting to develop during gastrulation was very thin and rather difficult to observe and photograph. The embryo had no pigmentation and like the Atlantic halibut the tail was bent (Figure 3E). The structure observed at the back of the developing larvae was also present in late stage field eggs (Figure 3F and Figure 2B).

The eggs of Greenland halibut hatched 53 days after fertilisation. The newly hatched larvae were c. 6 m.m. long and seemed rather premature. Eyes and myotomes were visible but not pigmented. The heartbeats were difficult to observe (Figure 3G). The huge yolk sack left when hatching indicates a long period from hatching until first feeding. The larvae died 12 days after hatching probably due to bacterial infection (Figure 3H).

Artificially spawned Atlantic halibut was also reported to hatch at a seemingly premature stage, 18 days after fertilisation at 5°C (Lønning *et al.*, 1982). The low temperature can partly explain the rather long period between fertilisation and hatching in Greenland halibut. Hellvik & Pittman (1990) found that light exposure also inhibited and delayed hatching and affected eye pigmentation in Atlantic halibut. During examination the Greenland halibut eggs was exposed to light for short periods.

#### Vertical distribution and density

Plankton hauls with open trawl in the Northeast Atlantic (January 1998) resulted in 7 eggs of Greenland halibut. Two eggs were found in a haul from 800 m depth. Three eggs were found in a haul from 600 m depth, but in another location. One egg was found in a haul from 200 m. No eggs were found in a haul from 50 m depth (Albert *et al.*, 1998). To get more reliable data on vertical distribution of Greenland halibut eggs it is necessary to use a plankton sampler, which is possible to close in different depth.

In the Bering Sea eggs of Greenland halibut were reported above depth of 200-3000 m (Bulatov, 1981). West of Iceland, eggs were found in depth of about 850-1000 m (Magnusson, 1977). Smidt (1969) registered eggs in the upper 50 m, but the highest concentration was obtained with 600 m wire length. Actual depth is not reported in these investigations and it is therefor uncertain where the eggs aggregate in the water column.

The eggs of Greenland halibut have a small perrivelline space. This may indicate no subjection to sudden accelerates forces, and an adaptation to a bathypelagic distribution (Forrester & Alderdice, 1973). In contrast to pelagic eggs, the vertical spreading of bathypelagic eggs depends on their buoyancy alone and not on the vertical turbulence (Sundby, 1991). A study of vertical distribution is therefor the first step in understanding the horizontal transport of eggs and larvae in managing the fish stock.

The salinity of the surface water in the spawning area was measured to be 35.0 ‰ (c. 5° C, depth of 5 m). In this seawater artificially fertilised eggs had full buoyancy during the first days of development. Unfertilised eggs from ripe females would also float in this water. Eggs from the field survey sank.

Artificially spawned eggs had neutral buoyancy in salinities of 31.2 to 34.0 ‰ and sank to the bottom in salinities below 31 ‰. During gastrulation the egg density increased, stabilising at salinities of 35.5 ‰ (ca 2° C) after closure of blastopore (Figure 4). Neutral buoyancy salinity of eggs from the field survey was 35.2 ‰ (ca. 5 °C). Neutral buoyancy salinities determined in the laboratory upon artificially fertilised eggs of Atlantic halibut, were also higher than the buoyancy salinities of eggs from field surveys (Haug *et al.* 1982).

Magnor-Jensen & Waiwood (1995) also reported a steady decline in buoyancy of Atlantic Halibut eggs from 4 days after fertilisation independent of light exposure. Effects of light exposure were not evident during the first four days after development. Stripped Pacific halibut eggs would also increase their buoyancy salinity during gastrulation (Forrester & Alderdice, 1973). These investigations indicate that an increase in buoyancy during gastrulation is normal for both Pacific and Atlantic halibut eggs.

Kendall & Kim (1989) demonstrated how bathypelagic eggs changed their vertical distribution during the development. Availability to regulate buoyancy through water loss and perritelline space under varying environmental conditions starts during gastrulation. Light induced density regulation may be an adaptation to prevent the eggs from reaching the surface in their ascent from the deep spawning grounds (Magnor-Jensen *et al.*, 1995).

Judging from this, it is probably a large difference in the vertical distribution of the heaviest and lightest fraction of an egg population. Higher density of fish eggs reared in a laboratory may be due to bacterial infection of the chorion, light exposure and other diverging environmental conditions. It is therefore difficult to make direct comparison between the situation in the laboratory and in the sea.

The neutral buoyancy salinity of Greenland halibut eggs from field surveys was 35.2 ‰ at ca 5° C. This corresponds to a seawater density of ca 1.0279 g/cm<sup>3</sup>. This density is found at depths around 650 meters (34.9 ‰ S and 1.8° C) in the spawning area.

Atlantic halibut eggs from field surveys were found floating in salinities around 34.5 ‰ and seemed to aggregate between 1-200 m, at temperature of c. 6.5° C in seawater densities of 1.0254 -1.0266 g/cm<sup>3</sup> (Haug *et al.*, 1984). Higher density of Greenland halibut eggs corresponds with their expected distribution in cold water around 6-800 m depth (Smidt, 69).

## Acknowledgements

The material was sampled either by the Norwegian commercial vessel M/S Husby Senior conducted by Institute of Marine Research in Bergen and Møre Research in Ålesund or the research vessel R/V Jan Mayen, conducted by Institute of Fisheries and Aquaculture in Tromsø. We thank everybody that has been involved in the project.

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Figure 1. Ovaries of ripening (above) and ripe (below) Greenland halibut

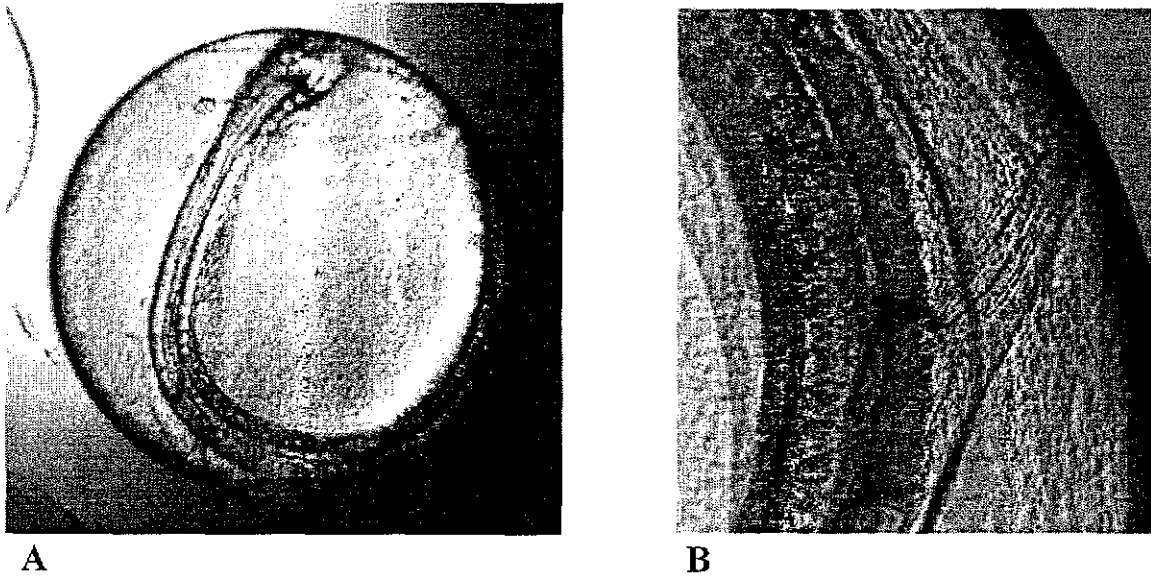
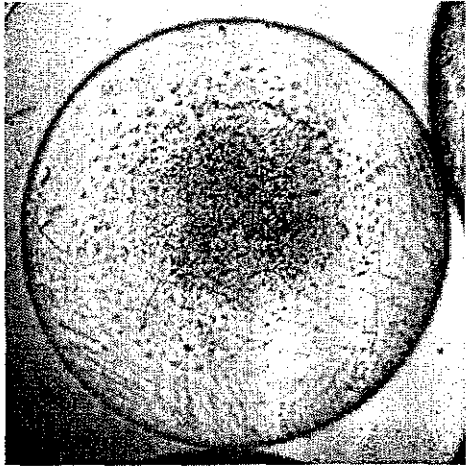
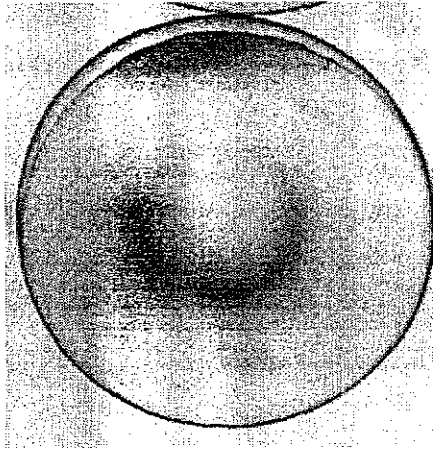


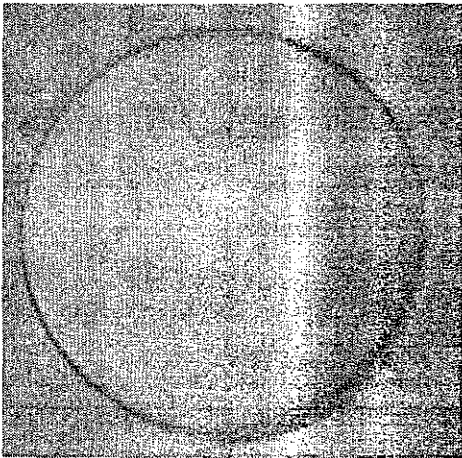
Figure 2: Late stage egg (dm. 4.7 mm) from field surveys in January 1998.  
A: The egg.  
B: The structure at the back of the developing embryo.



A



B



C



D



E



F

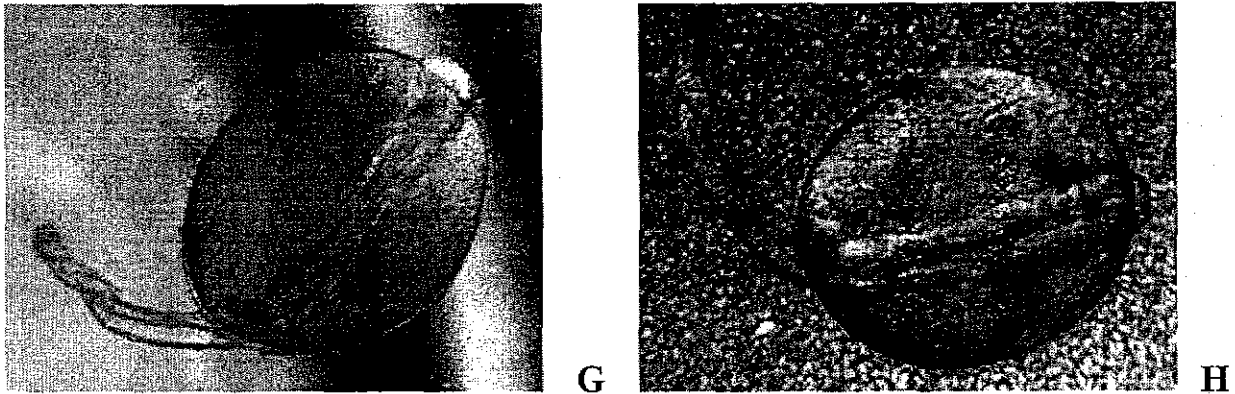


Figure 3: Early developmental stages of Greenland halibut eggs and yolk sac larvae.

- A: Late blastula stage (4 days) with the surrounding periblast
- B: Blastodisc with the surrounding perivitelline space
- C: Early gastrula stage (6 days).
- D: Gastrulation finished (24 days).
- E: Lacking pigmentation and bent body during the latter part of organogenesis.
- F: Late stage egg with the characteristic bending and structure at the back of the embryo.
- G: Newly hatched larvae (59 days old, 7 days after hatching).
- H: Larvae 10 days after hatching.

