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# REARING OF HALIBUT LARVAE (<u>HIPPOGLOSSUS</u> <u>HIPPOGLOSSUS</u> L.) TO METAMORPHOSIS AND BEYOND

by

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## ABSTRACT

A female halibut caught by gill-net was stripped and the eggs were imediately fertilized and incubated in the laboratory. About 50% of the eggs hatched when incubated in a refrigerator in stagnant water treated with antibiotics and with increased salinity. Of the about 1700 larvae kept in the refrigerator, about 35% were alive after 30 days, the temperature being about  $5^{\circ}$ C. The salinity of the water was about  $37^{\circ}$ /oo and was treated with antibiotics as before.

A functional mouth started to develop 25 days after hatching. The last larvae in the refrigerator died on 5 May at an age of 60 days.

At an age of 40 days larvae were transferred to a large basin two black plastic bags with 50 larvae in each enclosure. In one of the plastic bags two of the halibut larvae survived and reached metamorphosis at the end of May at an age of 80-90 days and at a length of about 3.0 cm.

## **INTRODUCTION**

Rearing experiments with halibut larvae have been carried out sporadically in different research laboratories, but without success (Rollefsen 1934, Forrester and Alderdice 1973, Solemdal et al. 1974). The most successful experiment so far was carried out in Norway in 1974 (Solemdal et al. 1974), when larvae were kept alive for 60 days. The peculiar development of this species during yolk resorbtion was then observed. In the present experiment different types of systems were tested for incubation of the eggs and for storage of the larvae during the long time period until the first feeding stage was reached. Feeding experiments were then started.

## MATERIAL AND METHOD

A gill-net fishery for spawning halibut was carried out in a small fiord north of Bergen in February 1979 at a depth of 600-700 m.

A female halibut was stripped on I4 February, and after fertilization the eggs were brought to Statens Biologiske Stasjon Flødevigen outside Arendal, where they were incubated in the laboratory 14 hours after fertilization.

Most of the 14 000 eggs were incubated in 25 one litre plastic beakers in a refrigerator at  $4.7^{\circ}$ C. The salinity of the water was increased to about  $37^{\circ}/00$  and antibiotics were added according to the dosage suggested by Shelbourne (1963). To the bottom water an additional quantity of salt was added to make . siphonation of dead eggs and frequent water exchange easy without removing live eggs.

Additional experiments were carried out in Hetofrig temperature regulators and in an open water circulation system described by Dannevig (1910), but these experiments will only be briefly referred to.

Some eggs were released (Table I) in the basin described by  $\emptyset$ iestad et al. (1976) and in black plastic bags with a volume of  $2m^3$ , described by  $\emptyset$ iestad and Moksness (1979). In the plastic bags the salinity close to the bottom was increased by addition of 1 kg NaCl to  $36.5^{\circ}/\circ o$ .

A rather large quantity of eggs and a few hundred larvae were sent to laboratories in Great Britain.

Transportation mortality of eggs was tested on 18 and 27 February. Transportation mortality was tested on larvae sent to Great Britain on 14 March.

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Of the about 14 000 eggs initially incubated in the refrigerator, a number equal to 8 000 eggs were transferred during incubation as described.

Most of the larvae that hatched in the refrigerator were transferred to two buckets each of 10 l seawater treated with antibiotics and with salinity gradients. They were kept in the refrigerator at 5,3<sup>°</sup>C. From 12 April only one bucket was in use.

Quantities of larvae were added to plastic bags and the basin (Table I). Larvae were regularly sampled from the refrigerator to test neutral buoyancy, swimming pattern and behaviour in relation to light. On 11 and 17 April groups of larvae were transferred to a stagnant 140 l jar for feeding. From 26 April the bucket in the refrigerator was placed in a water bath in the laboratory and the larvae feed with natural zooplankton.

Larvae hatched in the Hetofrigs were kept there until total mortality had occured (Danielssen pers. comm.). Larvae hatched in the open circulation system were partly transferred to an open circulation jar of 140 l. The others were kept in the system until total mortality (Moksness pers. comm.). All eggs and larvae were kept in darkness except those transferred to the basin and those in the feeding experiments. During cleaning operations light was used.

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## RESULTS

The average egg diameter was 3.12 mm and the neutral Eggs. buoyancy was 36.5°/oo. The mortality curve for the eggs in the refrigerator is indicated in Fig. 1, where about 50 % of the eggs hatched. Eggs removed from the refrigerator have been corrected for. Heavy mortality occurred from migration of germ ring (day 3) to closure of blastopore (day 10) and from then on negligible mortality occurred until hatching, when it increased for a while. Although antibiotics were added and rather frequent changes of water were carried out, the water smelled bad at the end of incubation. The chorion was also opaque, in contrast to the eggs incubated in the open water circulation. The mortality in that system was not measured, but seemed to be somewhat less than in the stagnant system (Moksness pers. comm.). Egg incubated without addition of antibiotics died before hatching (Danielssen pers. comm.). The incubation time was 20 days at 4.7°C. The transportation of eggs resulted in negligible mortality and the eggs hatched normally.

Larvae. The mean length at hatching was 6.4 mm and increased to 11.5 mm (all conserved lengths) on day 50 (Fig. 2). The neutral buoyancy was  $35.8^{\circ}/00$  at hatching,  $34.8^{\circ}/00$  on day 12, and increased to  $36.4^{\circ}/00$  on day 35. The mortality pattern of the larvae in the refrigerator is illustrated in Fig. 3, where larvae transferred are corrected for.

A steady mortality of 2 % and 6.8% per day for two long-term periods is most obvious. Two short periods with heavy mortality (11% and 22% per day) are also noticeable.

The heavy mortality from day 8 to 13 was partly due to an accident when changing water. The survival to day 30 was 35%. On that day about 65% had developed a functional mouth and all had a coiled gut (Fig. 4). The end of the yolk sac stage was reached at about day 50 (Figs. 2 and 5).

The eggs and larvae transferred to the plastic bags showed very

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low survival until the bags were terminated in April. (Table I). The temperature was initially rather low (Table I).

In one of the two bags started in April, two of the 50 halibut larvae survived beyond metamorphosis, Fig. 6. For further pictures of halibut larvae, see Thompsen and van Cleve (1934).

The larvae transferred to the feeding jars in the laboratory died within a few days. No larvae were observed with food in the gut among the laboratory groups.

All larvae in the open water ciculation died within few days (Moksness pers. comm.).

The larvae did not seem to react to light for the first 30 days. The eyes turned black after about 25 days (Fig. 4). A lateral line system was observed on larvae a week after hatching and onward. The system consisted of small bumps in the skin, probably being neuromasts.

The swimming pattern from day 20 was characterized by bursting when disturbed; otherwise by cruising and bending. The larvae could also be quiet for a few minutes. When bending the larvae formed a "U" shape and could maintain this position for minutes at a time, and then swimming further or bend in the other direction while the tip of the tail vibrated constantly. A larva sampled alive from a plastic bag on 28 April and being beyond the stage of first feeding (13 mm; Fig. 2 and 7) showed the same behaviour.

The metamorphosed halibut fry in the black plastic bag swam pelagically in an upright position and were not observed settling on the wall or bottom. A sigmoid body shape was observed when the fry approached a prey organism, followed by snapping. When one of them was transferred to the laboratory on 27 May on day 90, being 29 mm long (TL) it settled to the bottom, only occasionally swimming along the wall in the jar. The myotome height of the larvae in the refrigerator increased from 0.38 mm to 0.76 mm at end of yolk sac stage (EYS) and the largest diameter of the eye increased from 0.37 mm to 0.73 at EYS (Fig. 7). The observed myotome height and eye diameter of the larvae caught in the plastic bag on 28 April is also indicated.

## DISCUSSION

The egg incubation can be carried out in stagnant water with antibiotics added. An increase in water salinity might be necessary. An open water circulation might also be convenient.

The sensitivity to handling and transportation was not pronounced, in contrast to the observation made by Forrester and Alderdice (1973), which might indicate differences in egg quality in the two experiments.

The main problem in halibut rearing is a storage problem. How can one keep the larvae alive for 30-40 days until they can start feeding? Of the initial 1700 larvae in this experiment only 400 reached this stage.

The problem might be partly reduced by use of an elevated temperature and further work has to be done within that field.

Anyway, an open water circulation system seems to be inconvenient for larval storage. A stagnant system of some sort might therefore be best suited for storage purposes. A large volume of sea water with a salinity gradient and with no or scarce illumination might be an optimalization of larval demand. The observed failure of such a system in most cases might have been caused by the low temperature and by a degrading of the salinity layer. After all, the successful rearing of halibut larvae was reached in a large black plastic bag with a salinity gradient. REFERENCES

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Dat	te	Stage	Age	Nr.	Encl.	Temp. T=0	Term.	Dur.	Obs.
18 1	Feb.	Εσσ	4	200	DB-1		11 7	E 2	
29 F	Feb.	Eaa	-	350		1 0	II Apr.	55	0
29 F	Feb	Eaa	15	350		1.U 2 0	25 Apr.	50	0
23 I 1 N	Mar	Laruao	_1	100	FD-2	2.0	25 Apr.	51	0
4 1	Mar .		-1	100	PB-2	3.9	25 Apr.	51	0
4 N	Mar.	Larvae	-1	100	PB-2	3.9	22 Apr.	49	0
4 M	Mar.	Larvae	-1	100	PB-1	1.4	ll Apr.	37	l larva 🦕
10 M	Mar.	Larvae	5	200	PB-2	3.5	25 Apr.	46	0
10 №	lar.	Larvae	5	200	PB-2	3.5	22 Apr.	43	C
10 M	lar.	Larvae	5	200	PB-2	3.5	25 Apr.	46	0
10 M	lar.	Larvae	5	200	PB-1	1.7	25 Apr.	51	0
17 M	/ar.	Larvae	12	180	PB <b>-</b> 1	2.0	ll Apr.	37	2 larvae
10 A	Apr.	Larvae	36	50	PB-1	6.7	27 May	47	2 metam.
10 A	Apr.	Larvae	36	50	PB-1	6.7	27 May	47	0
21 F	'eb.	Egg	7	500	Basin ŀ	0.7 <sup>0</sup> C	l		
5 M	lar.	Larvae	0	200	Basin l	1.5 <sup>0</sup> C	Not t	ermina	ted
10 A	Apr.	Larvae	37	50	Basin l	6.7 <sup>0</sup> C		~	

Table 1. Transfer program of halibut eggs and larvae from refrigerator to plastic bags (PB) located in basin 1 (PB-1) and basin 2 (PB-2) and to basin 1.











Fig. 4. Organ development of halibut larvae in the refrigerator. A. Development of the gut. I: stright gut without any differentiation, II: stright gut with start of rectum formation, III: gut bended and with lumen, distinct rectum, IV: coiled gut.

> B. Development of the mouth. I: Mouth not open, II: mouth open or widely open, III: mouth shutted or shutable.

> C. Development of the eyes. I: Unpigmented eyes, II: pale pigment on edge of eyes, III: pale pigmentation of most of the eyes, IV: dark pigmentation of the eyes.



Fig. 5. Volume of yolk sac of halibut larvae from refrigerator according to  $4/3 \pi . L.H^2/6$  and coarsely corrected for deviation from an ellipsoid body.



Fig. 6. A picture of a 90 days old halibut fry from the Flastic bag experiment (photo  $\emptyset$ . Paulsen).



Mean myotom height measured behind anus ( $\bigtriangledown$ ) and mean largest Fig. 7. eye diametre (.) from halibut larvae in the refrigerator. Myotom height and eye diametre of halibut larvae from plastic bag 28 April is indicated.