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MORPHOLOGICAL DEVELOPMENT OF HALIBUT (HIPPOGLOSSUS HIPPOGLOSSUS) LARVAE WITH SPECIAL REFERENCE TO MOUTH DEVELOPMENT AND METAMORPHOSIS

by

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ABSTRACT

Halibut (*Hippoglossus hippoglossus*) larvae were raised from hatching to beyond metamorphosis in 11.5 m^3 plastic bags with a temperature stable around 6 ^{0}C. Standard length, myotome height, eye diameter and yolk sac length were measured on live or freshly preserved material. Mouth development and functionality, pectoral and pelvic fin development, intestinal development and the initiation of peristaltic contractions, and eye pigmentation and migration were observed from live material. The gape of the jaws was limited by an oral membrane which appeared coincident with the developing mouth and which persisted in either full or remnant form up to around the time of first feeding. The phenomena of yolk sac vesicles and the Non-reducing Yolk Sac Syndrome are discussed.

INTRODUCTION

Atlantic halibut, *Hippoglossus hippoglossus*, spawn at great depths and release bathypelagic eggs with no pigment and a very small perivitelline space (Rollefsen, 1935 and Tåning, 1936). The larvae have been described by Rollefsen (op. cit.) who was unable to keep them alive until the yolk sac was absorbed, and postlarvae have been described and drawn by Schmidt (1904) and Tåning (1936). Blaxter et al. (1983) describe the developing halibut larva up to Day 61, but with some differences from this paper's observations, particularly regarding the development of the mouth and the pigmentation of the eye. The development of the larva from startfeeding to beyond metamorphosis has been poorly documented due, in large part, to the scarcity of material.

Several thousand halibut larvae, from the broodstock at Austevoll Aquaculture Station, were reared in large plastic bags according to Naas et al., 1987. Although there were large mortalities, samples could be taken at fairly regular intervals from two cohorts up to and beyond metamorphosis. This report describes the general development of halibut larvae, with illustrations and photographs from live material.

MATERIALS AND METHODS

Two cohorts of larvae of Atlantic halibut were kept in twelve 11.5 m^3 plastic bags from a few hours after hatching to metamorphosis (Berg et al., 1987) and startfed on live zooplankton (Naas et al., 1987). Food density was monitored and the species composition of the offer and the preferred food was recorded (Naas et al., op. cit.).

The bottom water was renewed about twice a week. Oxygen levels in the water column were generally kept above 7 mg/l, except for the first four weeks for Cohort 1 when the initial filling used oxygen-poor water, resulting in levels as low as 4 mg/l. The temperature was fairly stable around 6^0C (Berg et al., op. cit.).

Samples of the two cohorts were captured live from the upper meters of water by pouring into a container, by gentle hauls with a modified plankton net or by capture in a closable tube. Larvae were kept alive in cool water until examination.

Measurements were made using a dissecting microscope with a calibrated ocular. Standard length, myotome height (immediately posterior to the anus), eye diameter and yolk length on the longest axis of the yolk were recorded. Diagrams were made using a camera lucida. Photographs were taken of live material through a dissecting microscope. After examination, larvae were preserved in 4% formalin.

Due to the varying catchability of the larvae and their varying distribution in the water column, the sample size was not constant and ranged from 1 to 32, with a mode of 5.

Larval age is given in daydegrees from 50% hatching.

All results are based on mean results except where otherwise stated.

RESULTS AND DISCUSSION

It is stressed that variation is apparent from hatching and increases with age.

1. Larvae

After hatching from approx. 3 mm eggs, the larvae were about 6.6 mm long and completely unpigmented. The head was not fully separated from the yolk sac, the gut was straight and without a lumen and there was no mouth. Neuromasts were visible under 25x, particularly the large pair posterior to the eyes. These appeared to be fairly robust and tolerated stimulation and manipulation.

The larvae are about 12.7 mm long when the yolk is absorbed (about 300 daydegrees) and are about 16 mm long when metamorphosis begins (after 520-560 daydegrees, with large individual variation). Myotome height is about 0.8 mm when the yolk is absorbed and may be about 3.0 mm when entering metamorphosis. The eyes increase from approx. 0.76 mm to 1.1 mm during this same period.

Growth in standard length was fairly linear during the observation period, whereas myotome height seemed to be constant near 0.77 mm between 150-300 daydegrees, until the yolk was almost fully absorbed. At this point, if the larva fed, there was rapid growth in myotome height, such that the ratio of myotome height to standard length increased from about 0.05 to 0.09 and up to 0.3.

Growth in standard length in mm was calculated to be 0.03 mm per daydegree from hatching to about 150 daydegrees, 0.01 mm per daydegree from 150-300 daydegrees, and 0.02 from 300-670 daydegrees. Growth from hatching to metamorphosis was about 0.02 mm per daydegree for both cohorts.

1.i. Yolk Sac Vesicles

Vesicles within the yolk sac were seen both pre- and posthatching. Irregular groups of 1-15 vesicles proximate to the anus, were seen on so many larvae that it is thought to be either a common phenomenon or an artifact of our rearing method. These vesicles can also be seen on Rollefsen's (1935) photographs of newly hatched and 6-day-old larvae and are photographed and discussed in Lønning et al.,1982, as Kuppfer's vesicles. By 86 daydegrees posthatching (Figs. 1 and 3) it was apparent that the vesicles were separated from the yolk by a surrounding membrane. Neither their function nor their origin is as yet understood. They were no longer apparent by the end of the fourth week (or near 160-170 daydegrees).

The observations of the early stages of larval development agree with Rollefsen (1935) and with Blaxter et al. (1983).

1.ii. Eye Development

About 110 daydegrees posthatching, the periphery of the eye develops small dots of pigmentation. The eye is fully pigmented by about 180 daydegrees. However, between the inception of pigmentation and its completion, all larvae have an unpigmented area of the eye, almost directly opposite to the constriction, which persists until the time of full coloration (Figs. 1-3). This area was well defined and always present in the same position on all larvae. It must be noted that, due to the transparency of the larvae, it is necessary to ensure that the pigmentation of the lower eye does not mask the lack of color in the upper eye during examination. This area can also be seen on preserved larvae.

The left eye may begin migrating about 550 daydegrees. The larva at this point still searches for prey in an upright position, which gradually changes to the horizontal, although they continue to attack the prey from an upright position. Between 630-667 daydegrees, there is about 0.5 mm difference between the dorsal edges of the eyes, or less than one third of the eye diameter. On some larvae, the eye has migrated halfway around by 795 daydegrees.

1.iii. Gut Development

By about 115 daydegrees, the lumen was developing in the rectum and the gut starting to bend (Figs. 3a,b). There was also an apparent thickening in the anterior intestine where the gut will rotate. The first observations of peristaltic contractions were made at about 200 daydegrees, when slow movements in the anterior half of the intestine could be seen. Within the next twelve days (about 80 daydegrees), the intestine had formed a sac-like loop on the left side of the yolk and the colon was at right angles to the intestine (Fig. 6). By 315-320 daydegrees, the yolk sac was fully absorbed and startfeeding was begun. The gut continues to grow and rotate one and a half times, filling the gut cavity (Fig. 8). Between 590-630 daydegrees, the pylorus muscle begins to differentiate as an elongated appendage on the interior circumference of the intestinal loop, from which will arise three pyloric caeca.

1. iv. Liver and Gall Bladder Development

Already at 115 daydegrees, the liver could be distinguished as a collection of complex cells on the left anterodorsal yolk sac (Figs. 3a,b). Within a week (about 40 daydegrees) this faint outline becomes more defined and is well nestled in the yolk sac by 150 daydegrees. By about 180 daydegrees, the gall bladder has formed as a small globe mediolateral to the liver. In more than half of the twenty larvae examined, the gall bladder was a light yellowish green, a color which persisted over several days of sampling.

1.v. Pectoral and Pelvic Fin Development

The pectoral fin is a faint outline on either side of the yolk sac by the end of the first week of life. It gradually constricts and forms a darkish bud by around 86 daydegrees. Within ten days (about 60 daydegrees) the fin is expanded and can be used in controlled swimming motions, although there appears to be a short period of uncoordinated movements prior to around 150 daydegrees (Figs. 1-3).

The pelvic fins appear much later and develop apparently much quicker. These were first seen as a dark patch anterior to the ventral tip of the liver between 540-580 daydegrees, and quickly became functional fins approximately 1.5 mm in length.

1.vi. Mouth development

The jaws begin to form as a small cleft at the end of the third week. The mouth appears to be open to the surrounding water already at about 115 daydegrees, as no separating membrane could be seen (Fig. 3b). The jaws appear to form normally and attain the typically pointed form of the lower jaw (Fig. 2). There seems normally to be no period of wide gape, in contrast to the findings of Blaxter et al., 1983.

By about 180 daydegrees, the jaws appear to be fully developed. However, it is apparent that, from the time of mouth opening to the time of startfeeding, the lateral areas of the mouth are enclosed by an oral membrane (Fig. 5a,6). This membrane can easily be seen by placing the forceps tip into the oral cavity - the gape is obviously restricted. Although water may enter through the original opening, and branchial respiration is visible at about this time, the oral membrane persists in either full form or in remnants until yolk absorption(Figs. 5a-c).

There is much variation in the disappearance of the oral membrane. Some larvae have functional mouths by about 245 daydegrees, whereas others have threads, remnants, one side or a full membrane up to startfeeding (Fig. 6). One larva was found with food in its gut and a remnant of the membrane on the right side of the mouth. However, many larvae were seen to gape, apparently to wear away the tissue. The gape could be restricted to as little as one third of the potential opening by an anterolateral membrane thread.

This membrane can be observed also in preserved larvae. However, it seldom appeared in larvae which were obtained by removal of the bottom water. These larvae were ostensibly dead or dying and were tapped out with the inherent bacterial fauna and detritus. This indicates that the membrane could possibly have been removed by bacterial degradation. Since Blaxter et al. (op. cit.) found no oral membrane on their larvae, which were raised in small (10 to 140 liter) containers without water renewal, the hypothesis of bacterial degradation of the oral membrane seems to be supported.

Larvae with mouths locked open and no oral membrane (Fig. 5b), as described by Blaxter et al. (op. cit.) were found but these were clearly in the minority. The first observation was at 178 daydegrees. The larva had branchial respiration but the mouth could not be closed, neither voluntarily nor by manipulation with forceps. The gape was such that the opening was high and narrow, and the bones appeared to have been "locked" in position. Such larvae were found in otherwise good condition until yolk sac absorption. It is therefore suggested that the oral membrane is necessary for the development of proper articulation in the jaws, and that the early degradation, by bacterial or other means, of the membrane may compromise both mouth functionality and larval viability.

As the jaws become more complex, the bones also thicken. Four small but proportionally correct teeth were seen on either side of the jaws on one large larva by 580 daydegrees.

1.vii. Respiration

Branchial respiratory movements were first noted at 180 daydegrees. Primitive lamellae had developed on the second and third gill arches around 280 daydegrees (Fig. 4a) and the gills appeared to be fully developed around 390-400 daydegrees. There is, however, no color in the lamellae. A pseudobranch begins to develop immediately behind the eye (Fig. 8) around 390 daydegrees.

2. Yolk Sac Absorption

The yolk sac is about 3.1 mm in length when the larva is one day old. There are no apparent blood vessels aiding in absorption, unlike salmon. As the liver and the intestine develop on the left of the yolk, and as the pectoral fins influence the formation anterodorsally, the yolk becomes both smaller and more elongated, with the largest portion being near the anus(Figs. 4b,6). When the gut coils, the yolk is pressed backward on the right side from a point near the conjunction of liver and intestine. As absorption continues, the elongation becomes thinner and reduces finally to a small globule posteroventral to the gall bladder. Occasionally, several such globules may be found with no apparent connection to other internal organs.

It appears that the yolk absorption is mediated through the liver, as a connection between the two was visible throughout (Fig. 6). However, the liver begins to develop after yolk absorption has begun. The mechanism has not yet been clarified.

3. Non-reducing Yolk Sac Syndrome

This has been the most apparent deformation and probable cause of mortality among the halibut larvae in Austevoll. The syndrome makes its first appearance around 210 daydegrees, most notably in larvae which float at the water surface and appear to have whitish yolk sacs. Upon closer examination, the yolk sac itself is not reduced, hence the floating, whereas the yolk is being absorbed although its outlines are not clear. The intestine is less than 75% percent of the normal diameter and the rectum is not at right angles to the notochord. In addition, the heart is often higher, thinner and less defined than a normal heart and the orbital bones are more than twice as wide, giving the larva the appearance of have "popeyes" (Fig. 7).

The immediate cause of the syndrome is that the yolk sac itself is not reduced, although the yolk is being absorbed. All the symptoms may be present in varying degrees, but common to all is the yolk sac, whose pressure limits the intestinal thickness and hinders the angling of the rectum. The more fundamental cause is open to speculation.

4. Functional Larvae

A functional larva must be one which is capable of capturing, ingesting and digesting prey. Our observations indicated that the eyes were fully pigmented around 180 daydegrees, by which time there is coordinated movement of the developed pectoral fins. Some digestive enzyme activity, as suggested by gall bladder coloration, was evident at around 180 daydegrees, but primitive peristaltic contractions were initiated some time later around 200 daydegrees. The jaws appear to be well formed around 180 daydegrees, whereas the oral membrane is not completely worn away on some larvae until as late as 300 daydegrees. The yolk sac is almost fully absorbed at this time, although some miniscule globules may remain until after 300 daydegrees. We therefore propose that functionality in halibut larvae occurs between 250-300 daydegrees.

Capturing prey requires familiarity with the movements of the prey object. Potential food animals were introduced to the halibut larvae between 190-215 daydegrees, when the eyes were fully pigmented and swimming motion was evident.

5. Mortality

Large losses were incurred, with two distinct periods of high mortality coinciding with the opening of the mouth (120-180 daydegrees) and with startfeeding (280-320 daydegrees). At the onset of startfeeding there was a little under 50% survival (Naas et al.,1987). A final source of mortality was an attack of vibriosis around 640-670 daydegrees, resulting in the death of many otherwise viable larvae (Figs. 9-10).

CONCLUSIONS

The concept of functional larvae has not been fully defined. In contrast to Blaxter et al. (1983), the mouth seems to be fully functional after the eyes are pigmented but shortly before the yolk is fully absorbed. This is due to the presence of the oral membrane. Evidence of the commencement of digestive enzymes is seen at about the beginning of the fifth week of life when the gall bladder exhibits a yellowish green color. Thus it seems that the larva is capable of capturing and digesting prey after about 250-300 daydegrees (or 40-48 days posthatching), even later than was suggested by Blaxter et al.

Variation in the rate of development was apparent from early on in the investigation. When some larvae were clearly approaching metamorphosis and had rapid growth in myotome height, others were as slim as at the end of yolk sac stage. Later when larvae were transferred to flat bottomed tanks, some were pigmented brown while others were still pink.

Production of halibut larvae may be accelerated if the following are clarified:

1. the structure and purpose of the oral membrane
2. the means by which the yolk is absorbed

3. the causes of the Non-reducing Yolk Sac Syndrome
4. the development of digestive enzymes
5. elucidating the relationship between light and food on morphological development
6. developing means to combat diseases in larval and postlarval halibut

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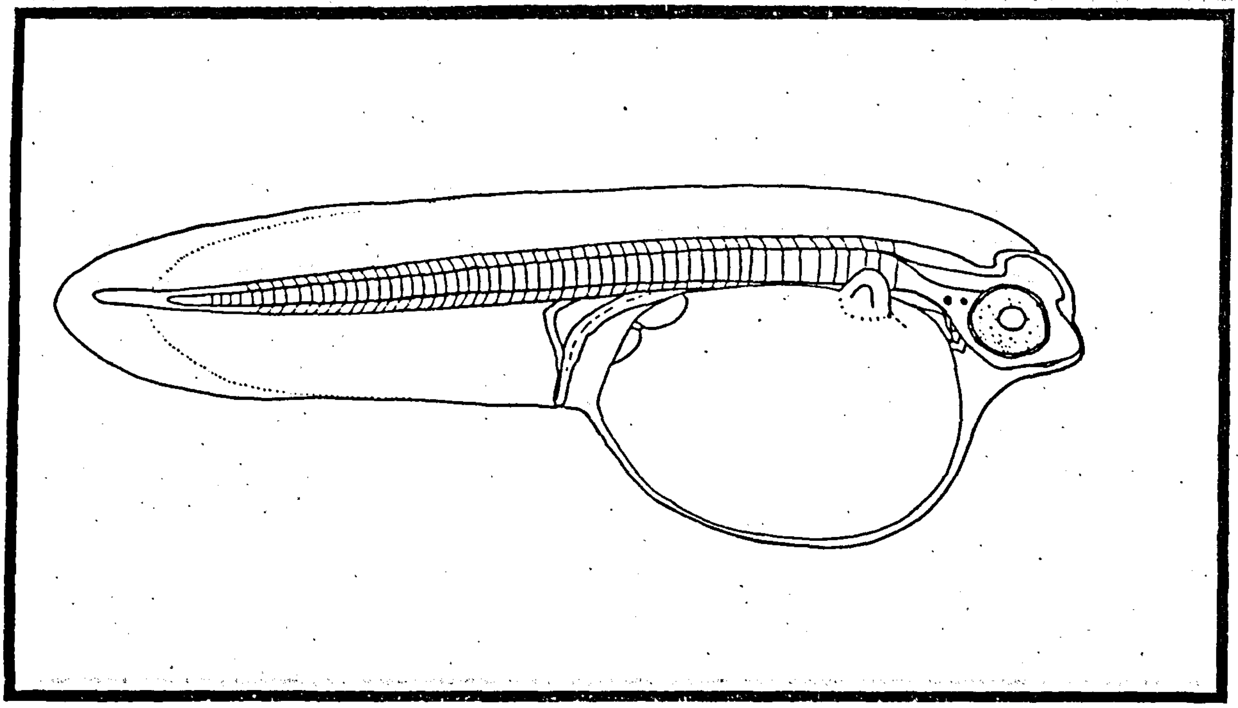


Fig. 1 A halibut larva at about 86 daydegrees. Note the vesicles in the yolk sac and the area of pectoral fin development. Pigmentation of the eye is beginning.

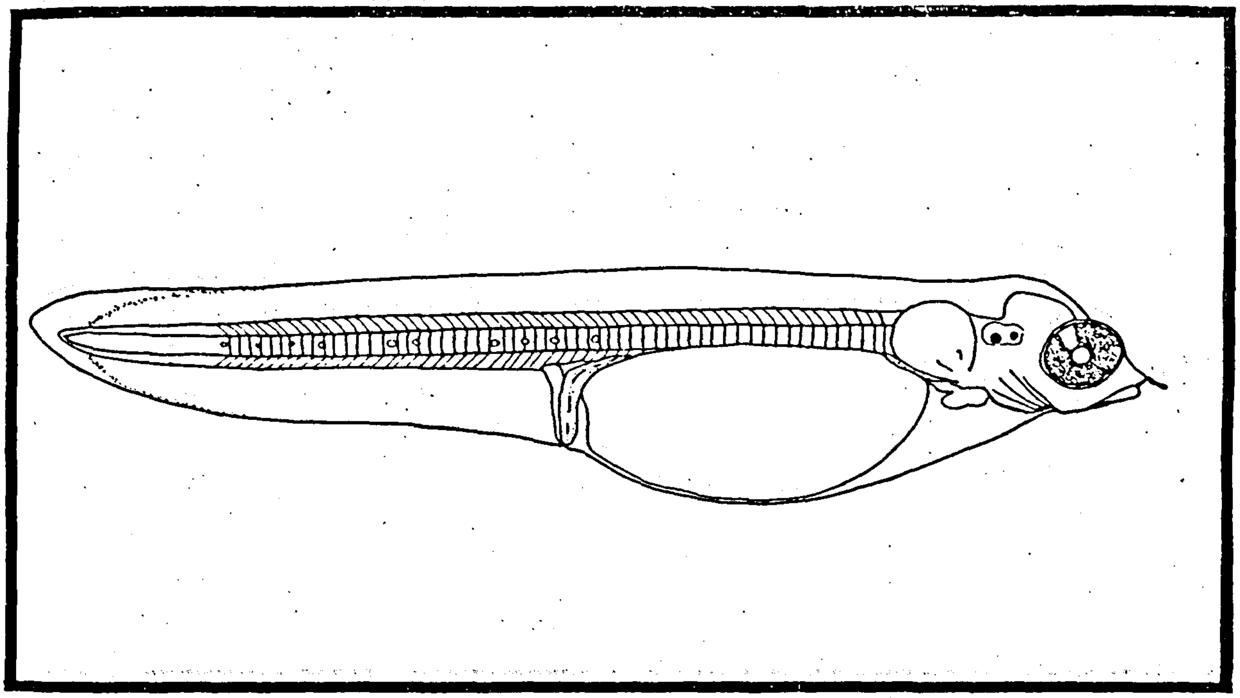


Fig. 2 Around 150 daydegrees, the unpigmented portion of the eye is still visible, as are many neuromasts (circles along notochord). The jaw has become more pointed but is laterally limited by the oral membrane, a fragment of which hangs from the upper tip. This larva was 12 mm long and had developed pectoral fins.

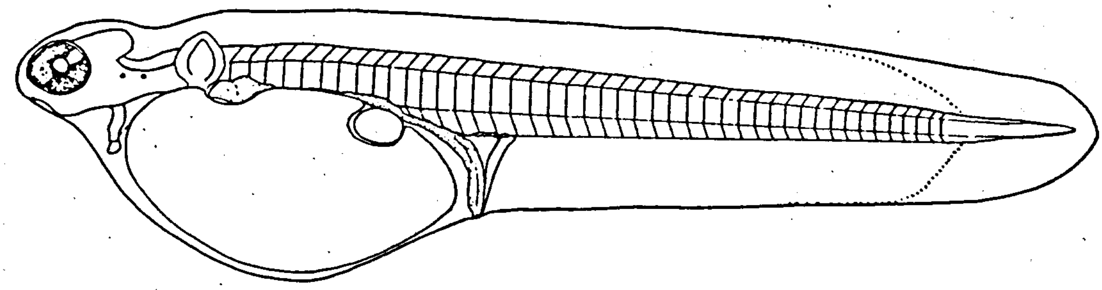


Fig. 3a By about 115 daydegrees, this larva was 11.5 mm long. The unpigmented portion of the eye is clearly seen, while there is a faint outline of the prospective liver on the yolk sac. The intestine has begun to rotate. The yolk sac vesicles are clearly separated from the yolk.

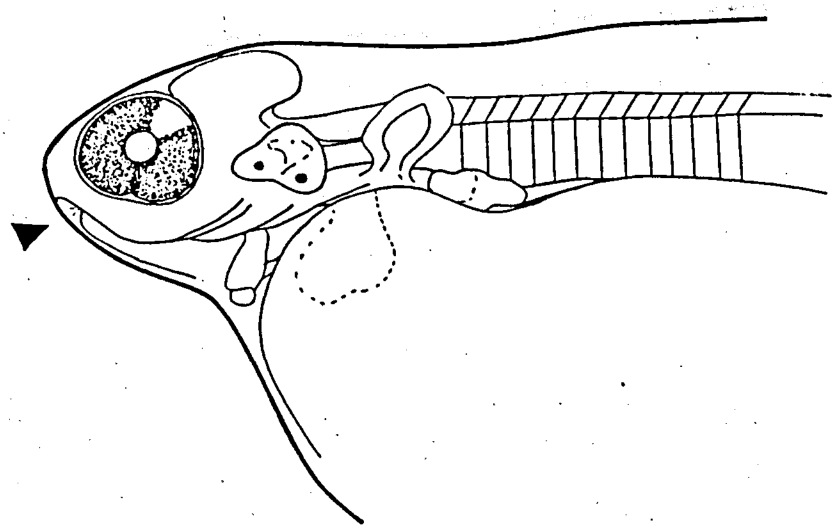


Fig. 3b A closeup of the head region. There was apparently no membrane to separate the oral cavity from the surrounding water. Arrow indicates opening.

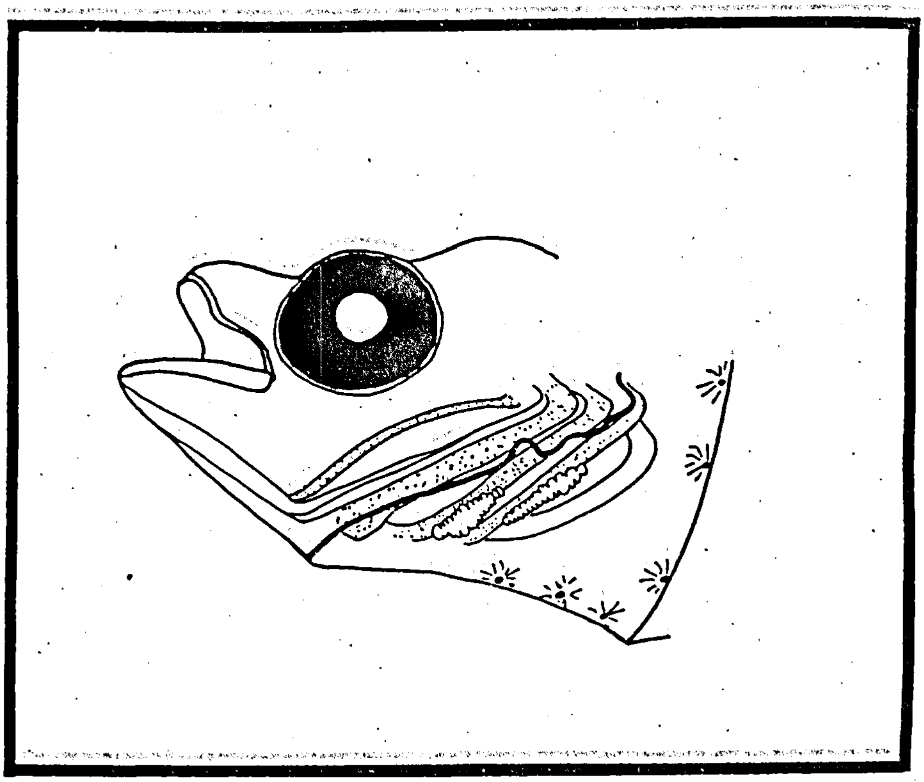


Fig. 4a Primitive colourless lamellae had developed on the second and third gill arches around 280 daydegrees. Branched melanophores are included ventral and posterior to the gill area. Dark line is operculum.

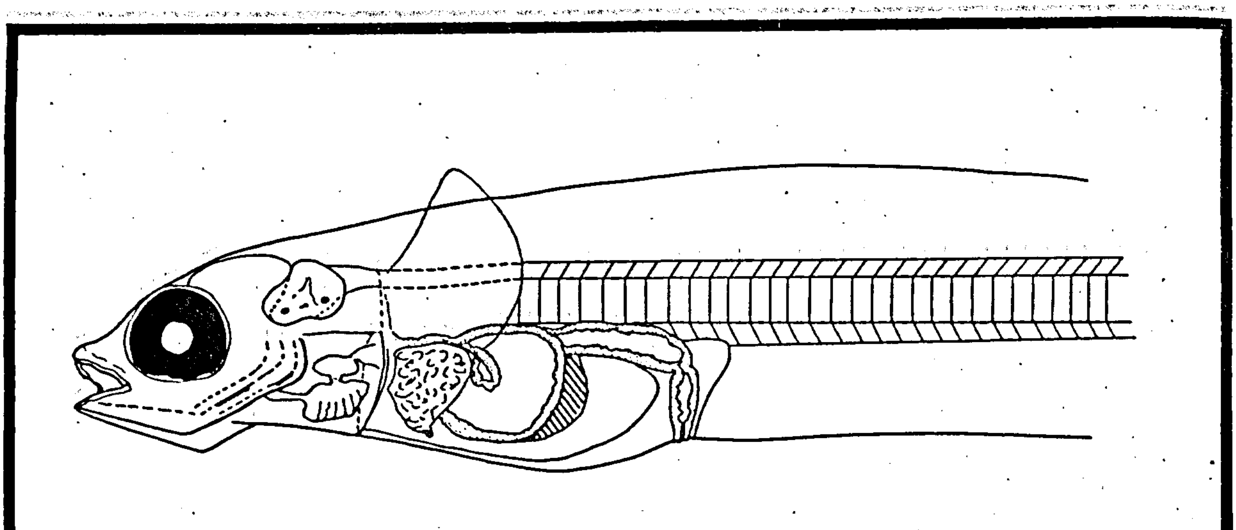


Fig. 4b Around 280 daydegrees, this larva was 14.5 mm long and had a myotome height of 0.95 mm. Striped area is yolk, behind the rotated intestine. Position of lamellae also included.

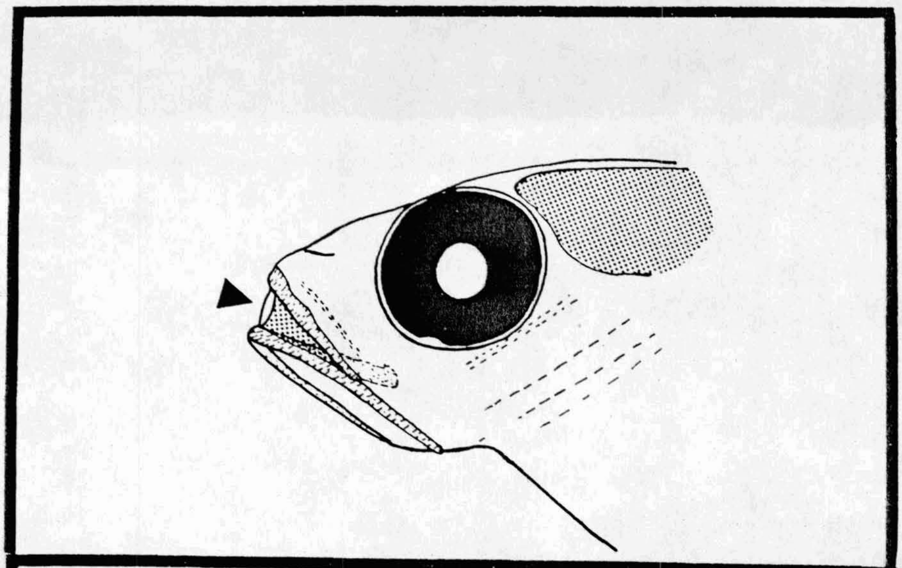


Fig. 5a

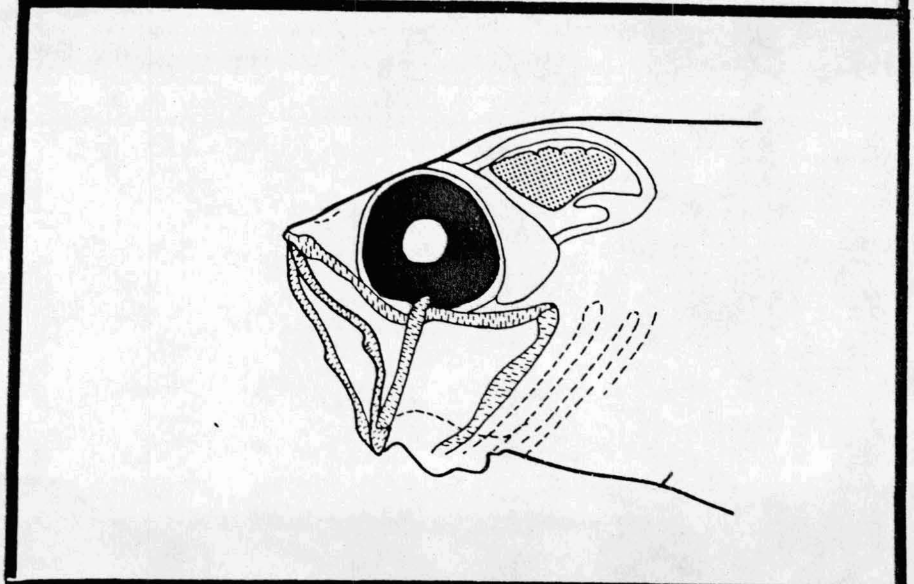


Fig. 5b

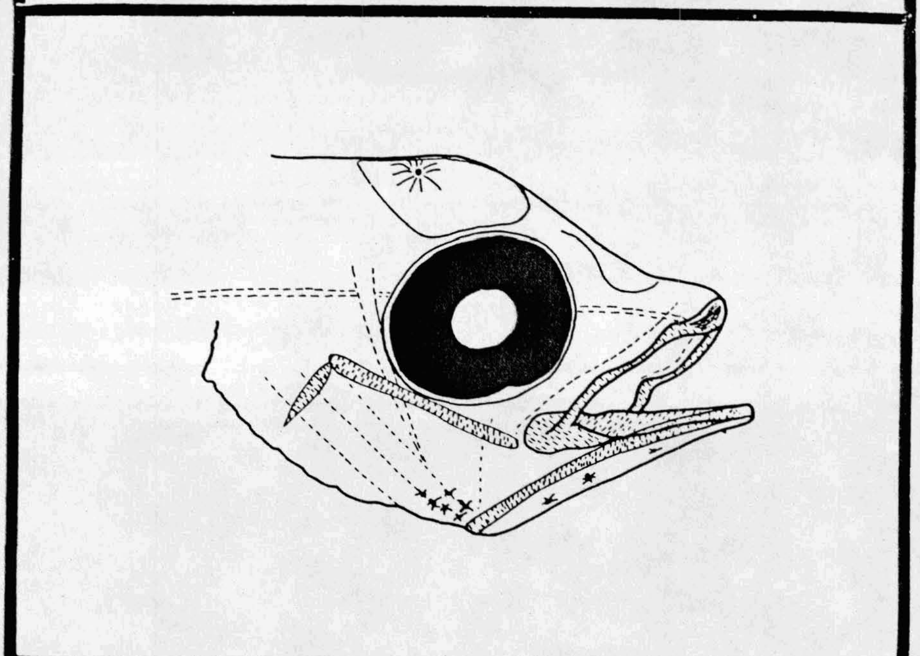


Fig. 5c

Fig. 5a Apparent bone structure of the mouth at about 180 daydegrees. At this time most larvae still have an oral membrane or remnants thereof. There is branchial movement and a light greenish tinge to the gall bladder. Arrow indicates opening.

Fig. 5b Some larvae do not have the oral membrane. This larva was the same age as the one in (a) but has different bone formation. The mouth appears to be locked open.

Fig. 5c Apparent bone structure in a functional mouth, around 300 daydegrees. This larva was 13 mm long and had an almost completely absorbed yolk. Melanophores are drawn on the brain and around the lower jaw.

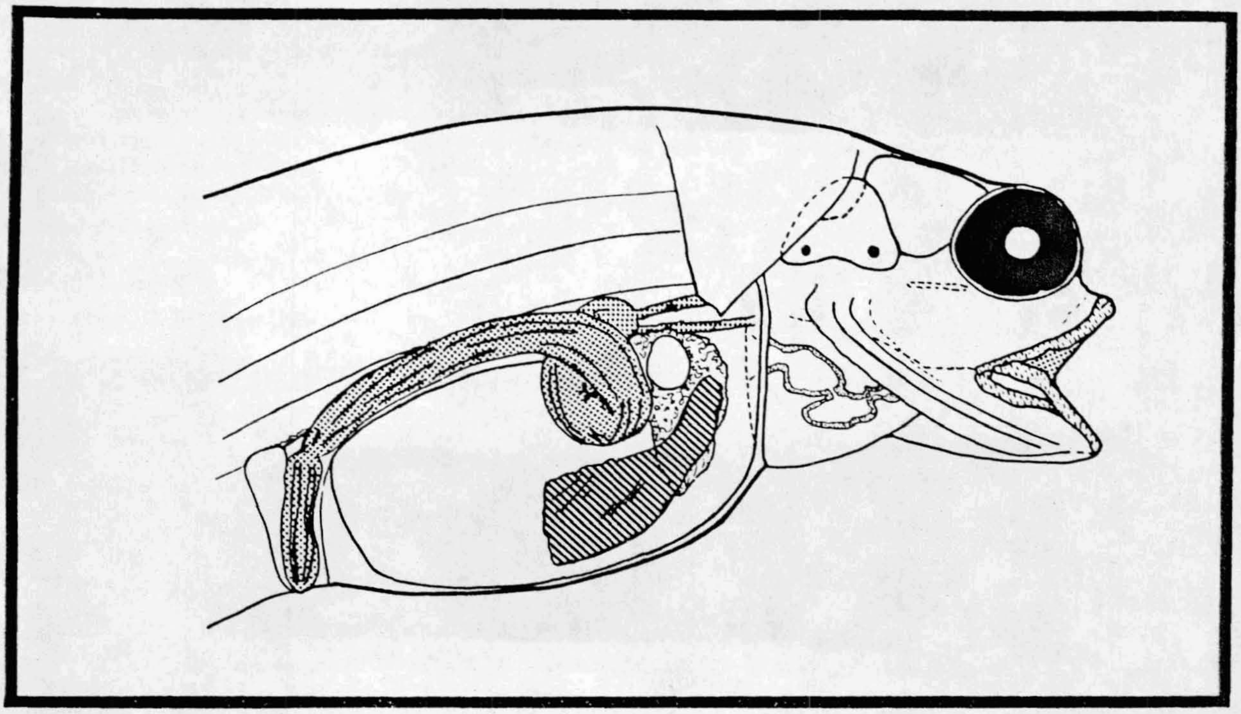


Fig. 6 Around 290 daydegrees, the intestine has become more developed and the yolk (striped) is nearly absorbed through the liver and the gall bladder is clearly visible on the liver. Dotted area in the mouth is remainder of the oral membrane. This larva was about 12.6 mm long.

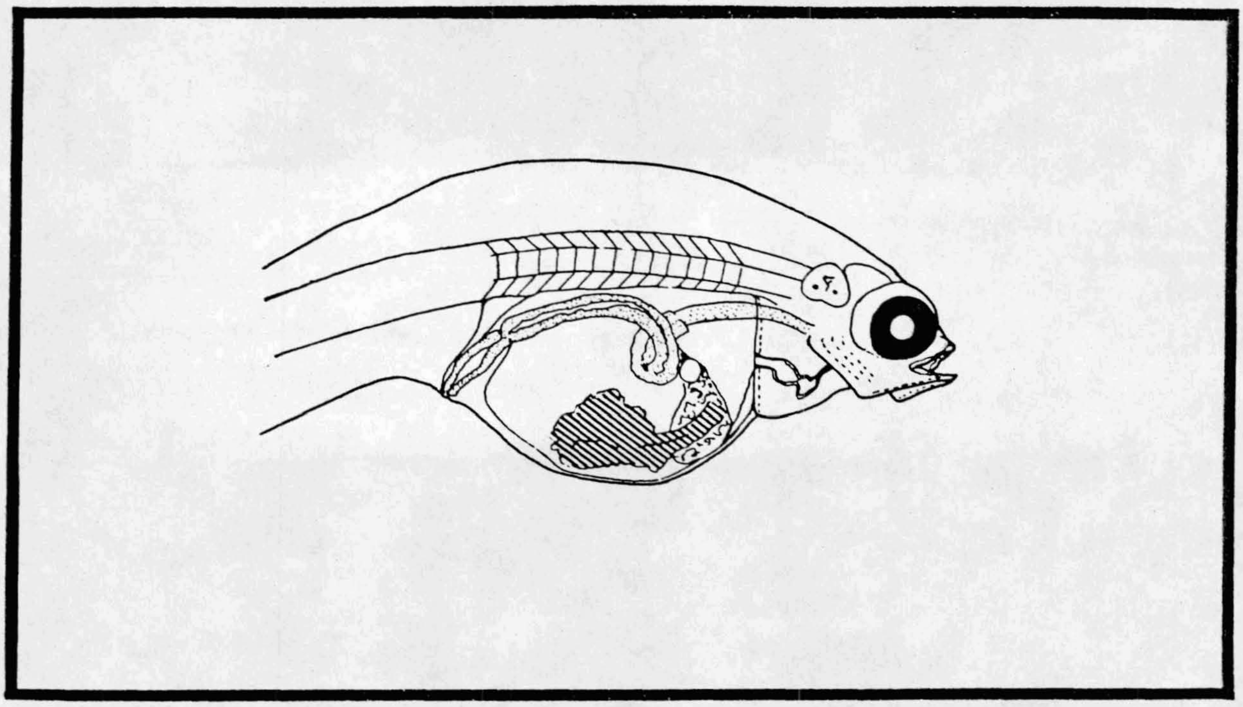


Fig. 7 The Non-reducing Yolk Sac Syndrome. From around 210 daydegrees, larvae with NYS can be seen floating near the water surface with an apparently white yolk sac. Upon closer inspection, the yolk (striped) is being absorbed but the sac is not reducing. The eyes appear to have large circumorbital bones and the heart is often smaller than normal. This larva was about 250 daydegrees.

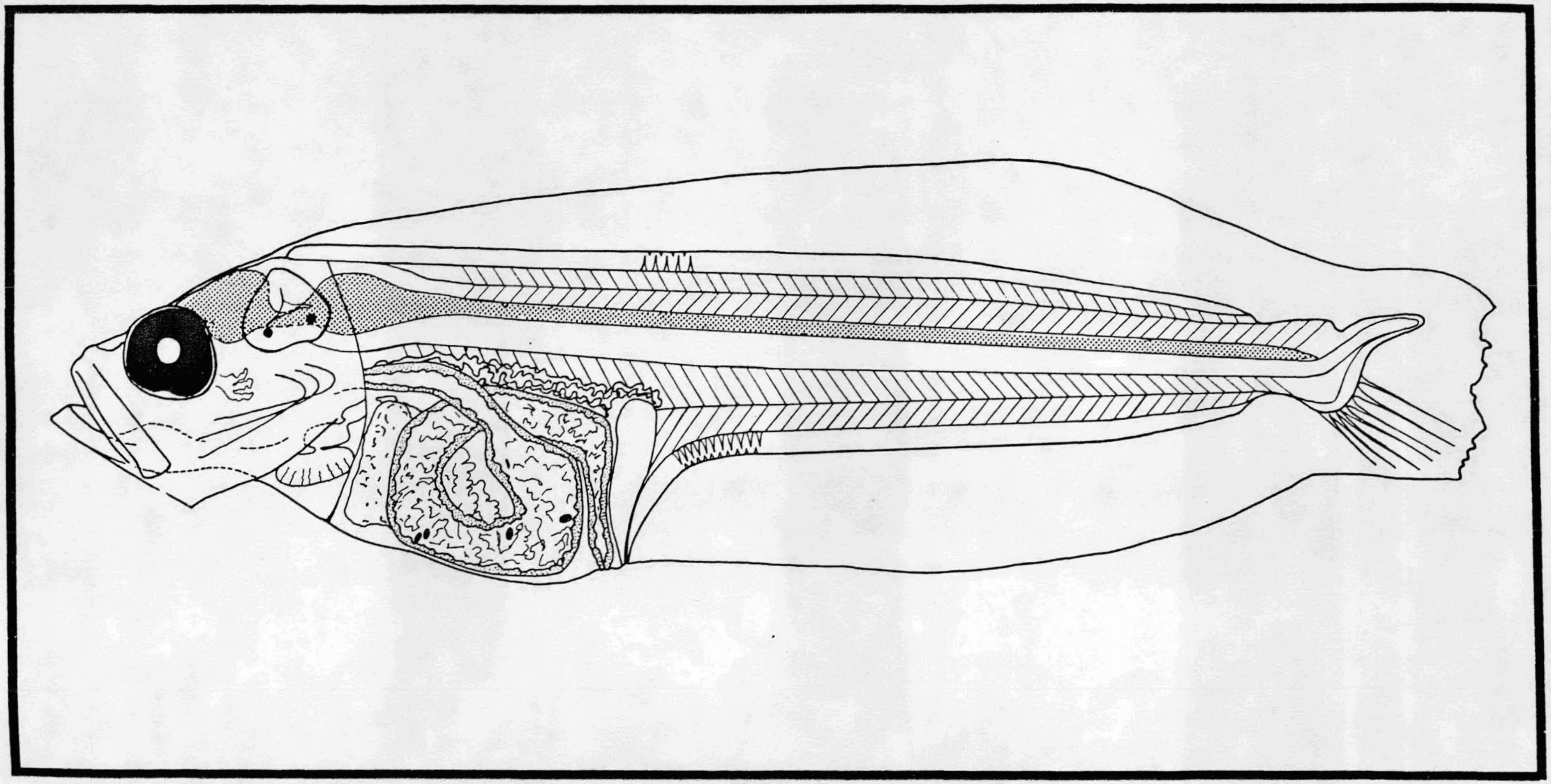
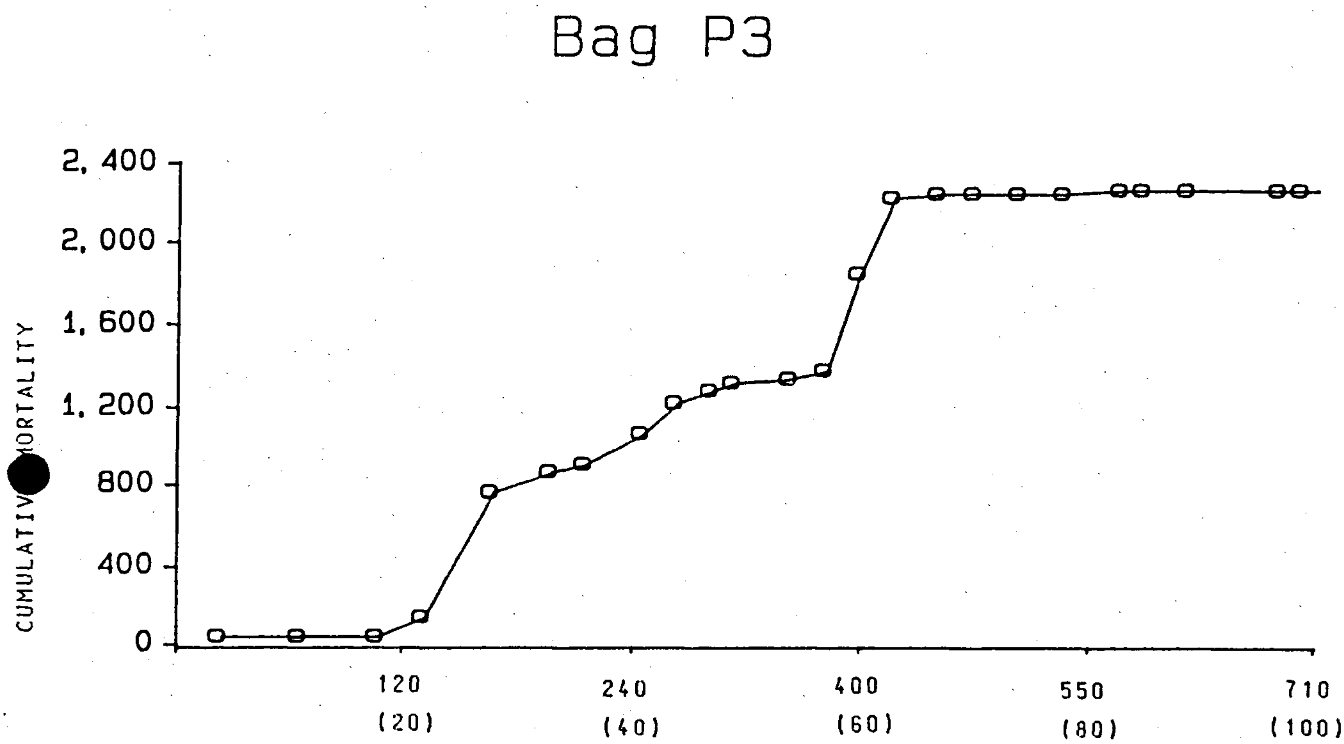


Fig. 8 A startfed larva at about 400 daydegrees, 16.8 mm standard length and 2.4 mm myotome height. Note the increasing complexity of the myomeres and the prospective pylorus (mass in the center of the rotated intestine), as well as the pseudobranch posterior to the eye, and the hemal and neural fin r[illegible] beginning in the outermost muscle [illegible]er.



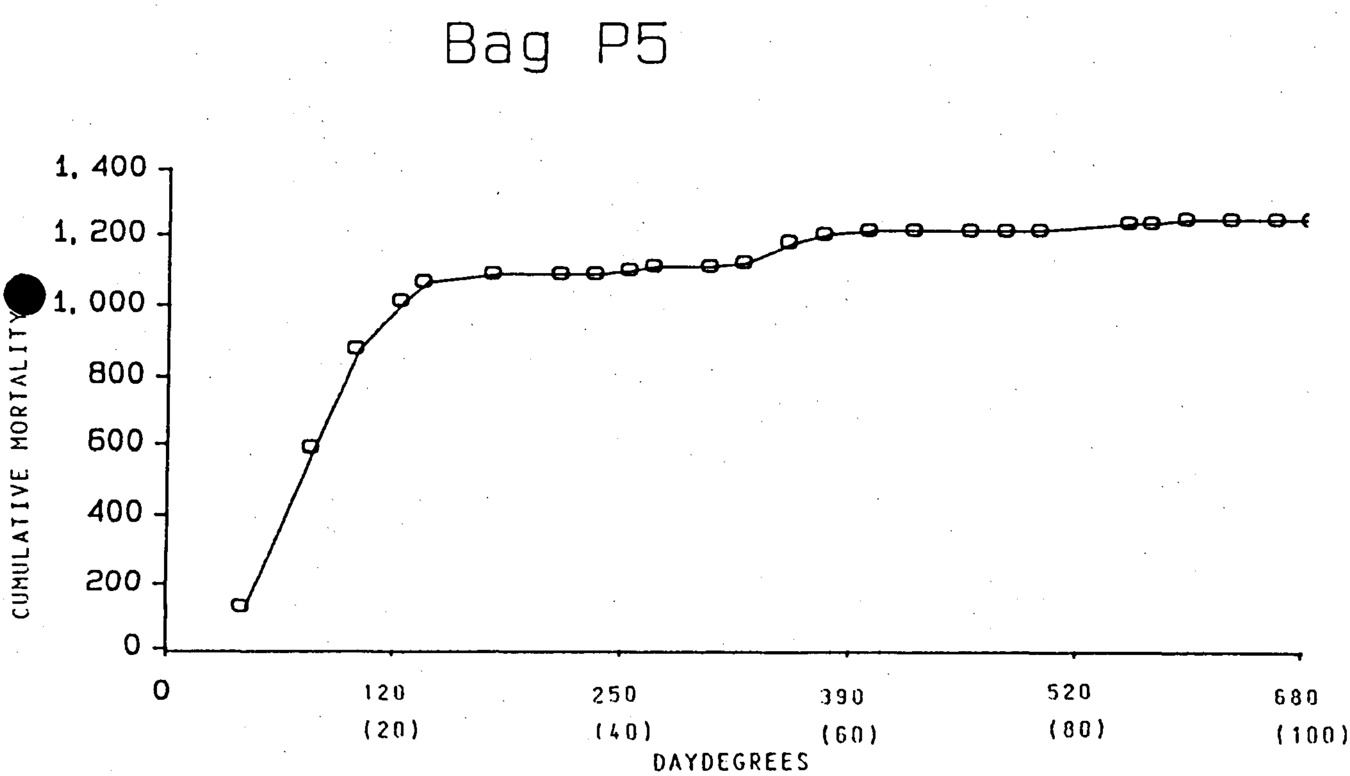


Fig. 10 Cumulative mortality in a group from Cohort 1 (top) and Cohort 2 (bottom) vs daydegrees.

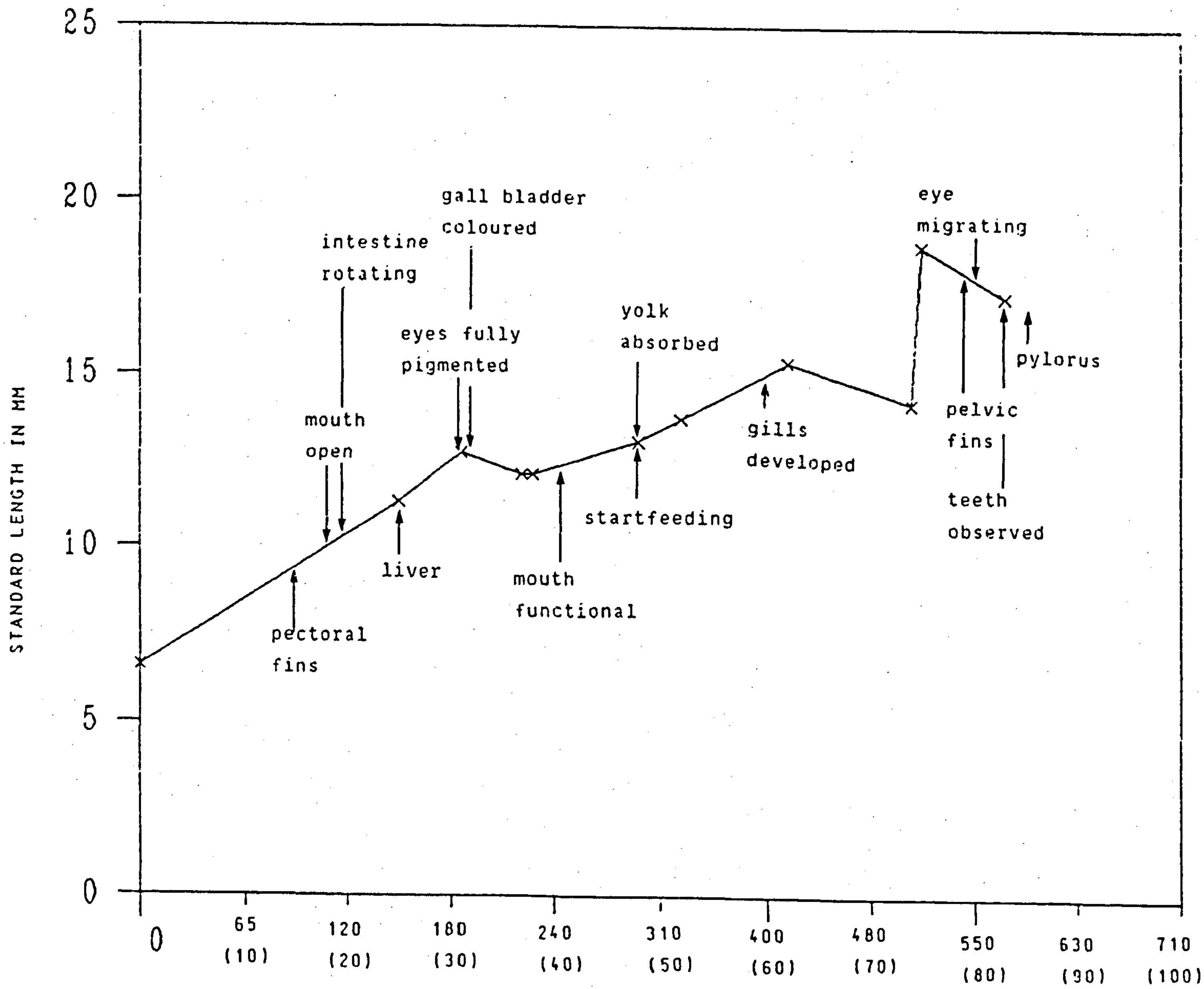


Fig. 9 Growth in mm of standard length of halibut larvae in Bag 3 vs daydegrees. Days are in parentheses. The appearance of important developmental features is indicated by arrows.

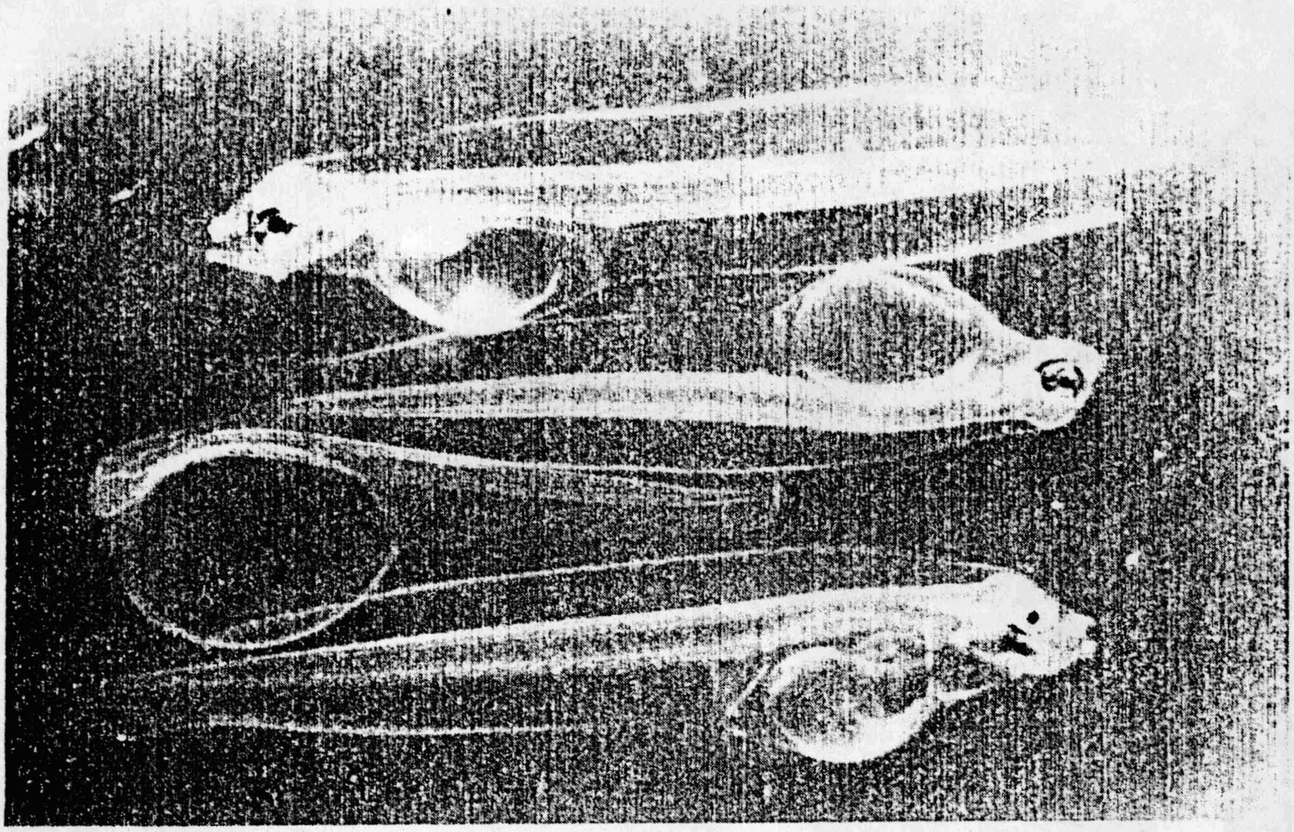


Fig. 11a. Halibut larvae from about 86 to 180 daydegrees. (Photo: Helge B. Botnen)

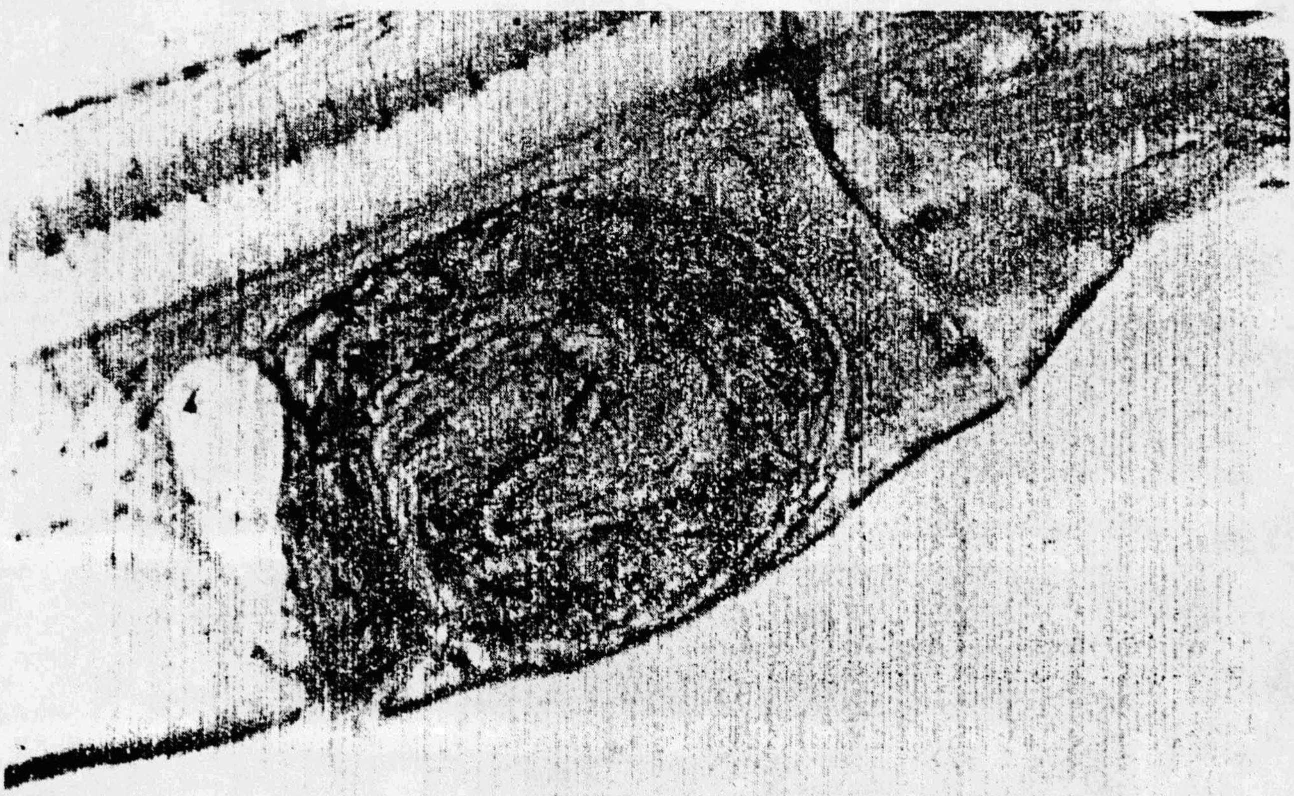


Fig. 11b Closeup of stomach of a startfed larva at about 400 daydegrees. (Photo: Helge B. Botnen)

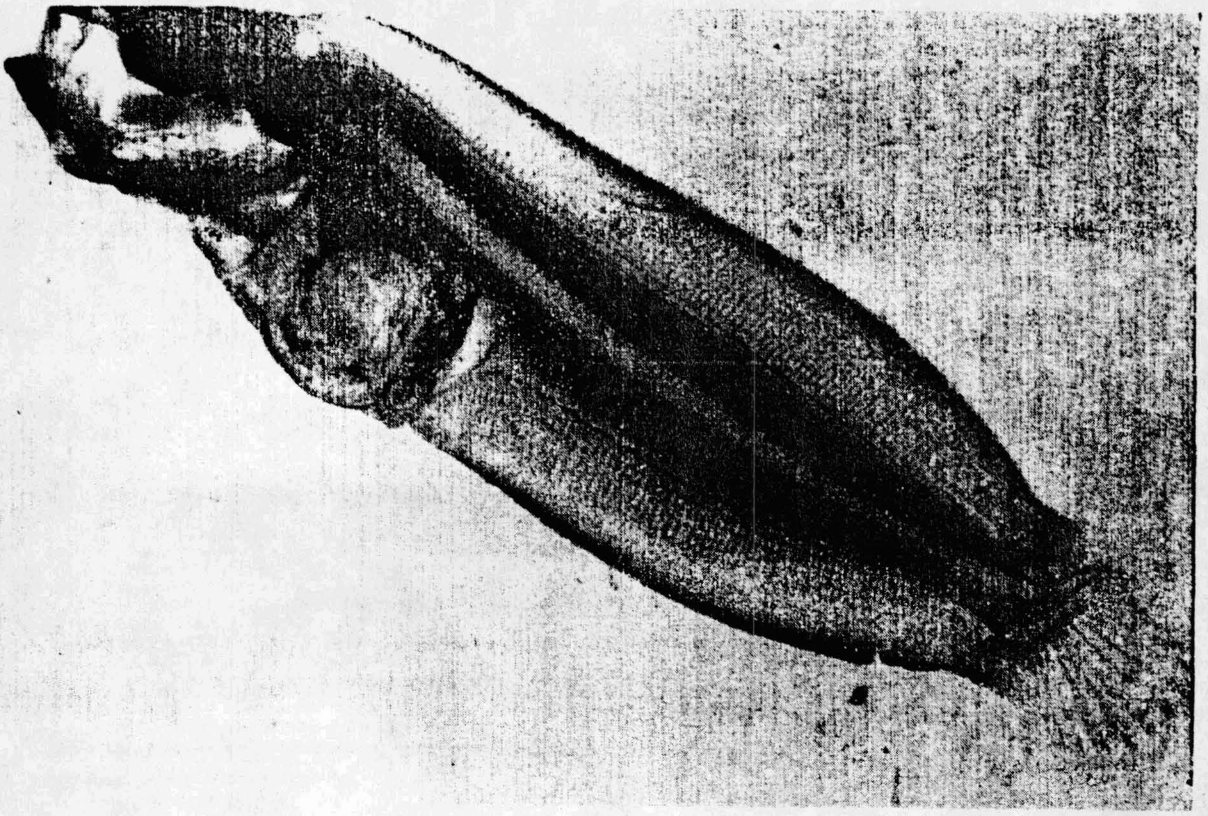


Fig. 11c Halibut larva at about 400 daydegrees. (Photo: Helge B. Botnen)

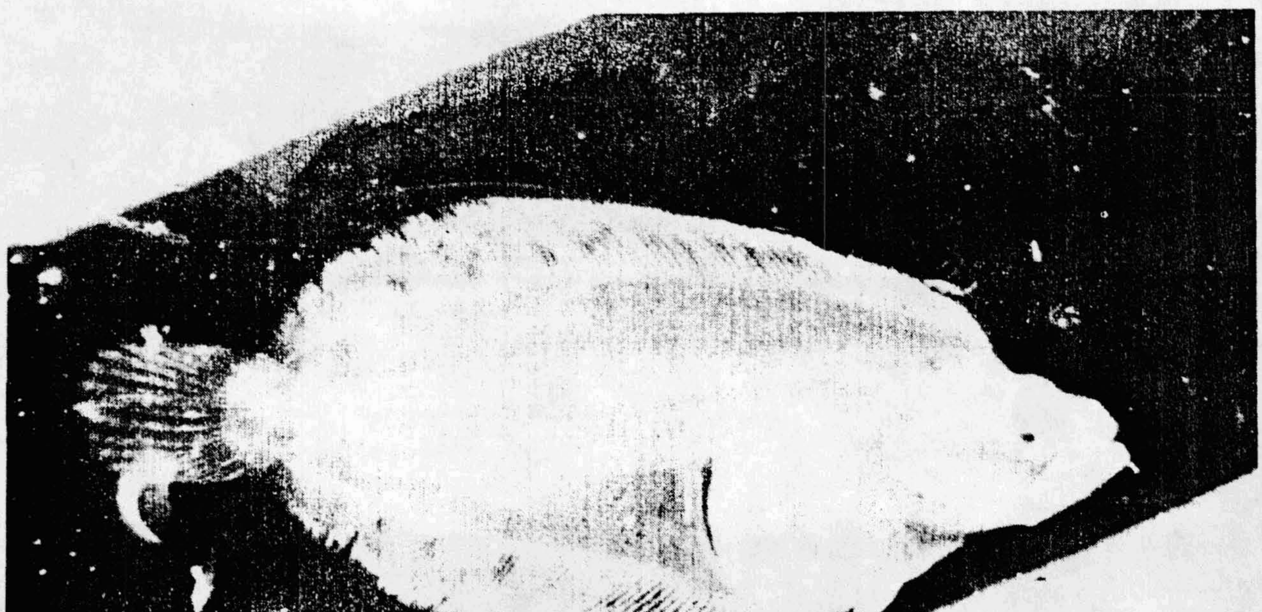


Fig. 11d Eye migrating on a halibut postlarva around 800 daydegrees. (Photo: Helge B. Botnen)

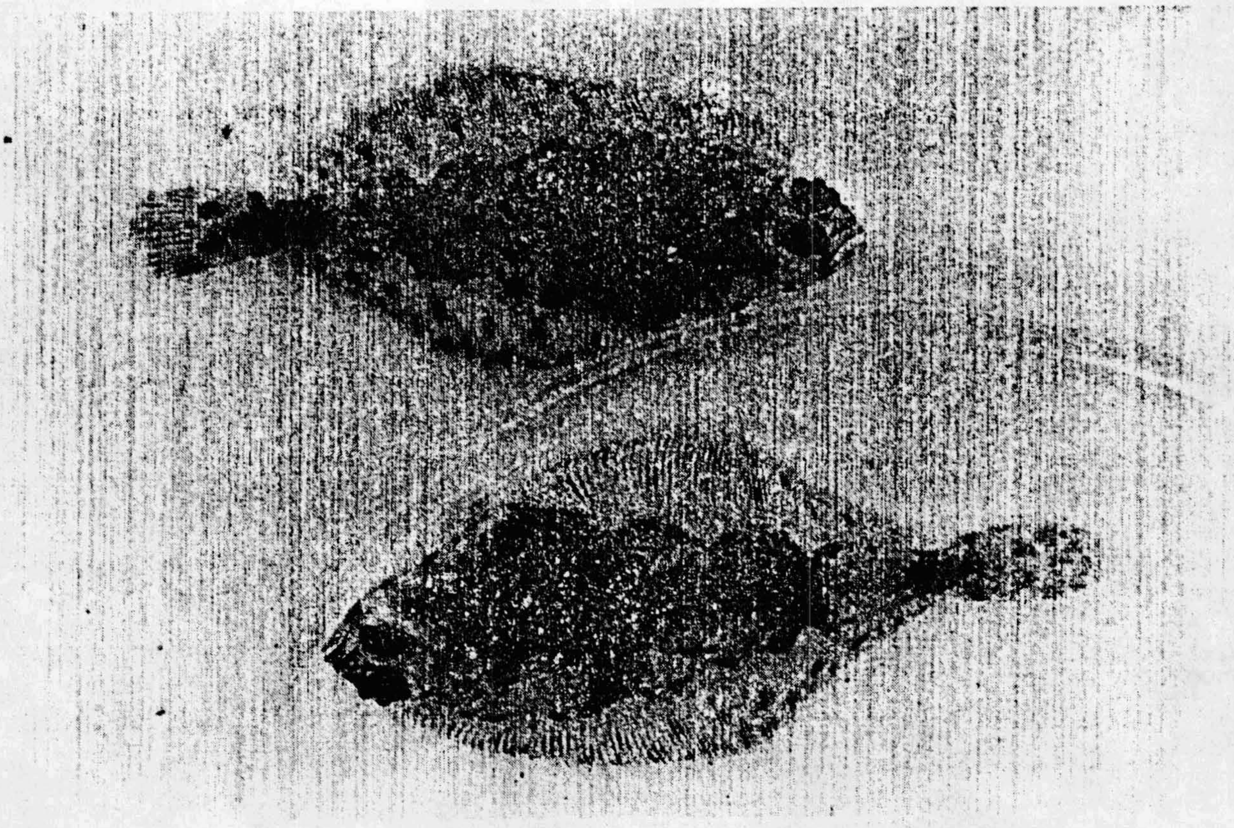


Fig. 11e Halibut fry at approx. 950 daydegrees. (Photo: Helge B. Botnen)