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Morphological and behavioural development of halibut, *Hippoglossus hippoglossus* (L.) larvae

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Live yolk-sac halibut, *Hippoglossus hippoglossus* (L.) larvae from rearing experiments at Austevoll Aquaculture Station, Norway, were examined from hatching to past first feeding for developmental morphology and behaviour. The findings include development of the respiratory and circulatory organs, eye pigmentation, mouth formation, organs of the digestive system and the process of yolk absorption, as well as swimming speed and activity levels.

A stomodeum is not present at hatching although drinking is possible through a pair of branchial pits which gradually develop into the operculum and gill basket. The mouth normally opens slowly, the gape being restricted by a transverse septum until bones are formed. The amount of time spent swimming varies from less than 15% of the observation period during the first 2 weeks after hatching to between 70 and 100% around the seventh week after hatching, when individual differences become more apparent. Larvae generally react with a burst of swimming when two come into contact. Speed and duration of swimming seems to be correlated with development of eye pigment, heart size and fin formation. The yolk-sac period is divided into four stages.

Key words: *Hippoglossus hippoglossus*; larvae; behaviour; morphological development.

I. INTRODUCTION

In the approximately 50 days from hatching to end of the yolk-sac stage, the Atlantic halibut, *Hippoglossus hippoglossus* (L.) changes from a primitive, passive and unpigmented yolk-sac larvae to an actively swimming predator. The halibut larva emerges from the egg when the ring of hatching enzymes has broken down the zona radiata in a circle around the region of the larval head. This allows a cap of shell to be pushed out from within, the larva escaping through the hole (Helvik, 1988). When hatched at 6 to 7 mm S.L., the larva is completely unpigmented as pictured in Rollefsen (1934). The head is confluent with the yolk sac, there is no stomodeum (Pittman *et al.*, 1988) and a faint ring of hatching glands can be seen on the anterior yolk sac reaching to behind the otoliths. The gut is straight, the urinary bladder simple and the heart is a simple tube (Blaxter *et al.*, 1983). At this stage, the following organs are not seen: pelvic and pectoral fins, liver, gallbladder and gill filaments.

Attempts to describe the further development of the halibut larva have been hampered by high rates of mortality and deformation, although it is known that the halibut larvae develops organs and pigmentation during the yolk-sac stage that other species complete in the egg stage (Blaxter *et al.*, 1983; Senstad, 1984; Pittman *et al.*, 1987). However, knowledge of the natural history of the early life stages of this species is still limited (Haug *et al.*, 1989), while information on the normal rates and processes are essential for development of aquaculture systems.

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This paper describes the behaviour and morphology of the developing yolk sac larvae of *H. hippoglossus* at mean temperatures ranging from 4 to 9° C. It is based on both morphological measurements and observations including scanning electron microscopy, as well as behavioural observations over a total of three seasons of successful halibut rearing experiments at Austevoll Aquaculture Station in Norway. Some observations on post-larvae are also included.

II. MATERIALS AND METHODS

MORPHOLOGY

Larvae for the morphological measurements and observations were obtained, in 1987, from two cohorts raised from 50% hatching (hereafter referred to as day 0) in floating 11·5 m^3 plastic bags at a mean rearing temperature of 7° C (Berg *et al.*, 1987) and, in 1988, from one egg group raised from day 0 in three 14 m^3 silos with mean rearing temperatures of 4, 6 and 9° C (Pittman *et al.*, 1988).

Larvae from each group were sampled two to three times a week from day 0 to past first feeding. Larvae were captured live by gentle hauls with a modified plankton net or a closable tube. Sample size varied around a mode of five per group. They were kept alive in cool water and measured at up to 50 × on a dissecting microscope for standard length (snout to tip of notochord), myotome height (posterior to the urinary bladder), eye diameter (along the body axis) and yolk-sac length (the longest axis of the yolk). They were then placed in either (1) buffered 4% formaldehyde for storage or (2) a cacodylated 2·5% glutaraldehyde solution for further examination by scanning electron microscopy. Figures were drawn of live larvae using a camera lucida.

BEHAVIOUR

Three groups of larvae were observed for behaviour. Two of these (A and B) were from one egg batch, transferred from the station's hatchery to observation aquaria at 50% hatching. These were kept in the aquaria for the duration of the experiment. The third group (C) of 10 larvae was transferred, after first feeding, from large floating bags in a seawater pond at about 7–8° C (Berg & Øiestad, 1986) to an observation aquarium, where they were held for 1 week. The initial densities of 1 larva l^{-1} (A) and 5 larvae l^{-1} (B) were maintained only through the first 2 weeks by replenishing with larvae of the same egg batch kept in the hatchery. Live natural zooplankton was presented from day 20 in groups A and B, and during the week of observation in group C.

The observation aquaria measured 20 × 60 × 70 cm, holding 70 l of water at 34‰ S and 5° C. Water exchange rate was 15% per day. The behaviour observation system is outlined in Fig. 1. The main components were a light-sensitive video camera moved by a pair of servomotors, and precision potentiometers mounted on both the servomotors and the focal ring of the camera. A more detailed description is given in Huse & Skiftesvik (1985). Three-dimensional co-ordinates were logged onto a microcomputer at 1 s intervals along with records of the behaviour of the individual larvae.

Recordings were made almost daily for the first 5 weeks, and thereafter every 2 to 3 days. Each of 10 randomly chosen larvae from groups A and B were observed for 5 min during an observation period. Larvae were kept in darkness, except for 1 h prior to and during observation when a 300 lx light was used. Duration of swimming and resting periods were measured, as well as activity levels defined as the percent of swimming time during the observation time (100% is equal to continuous swimming). The position at the beginning and end of the rest periods was recorded for passive sinking or rising.

III. RESULTS

The following description is valid for 4–7° C, which encompasses the most common rearing temperatures. Relating development at different temperatures to ° days revealed a difference of about 100 ° days at end of yolk-sac stage (EYS), with

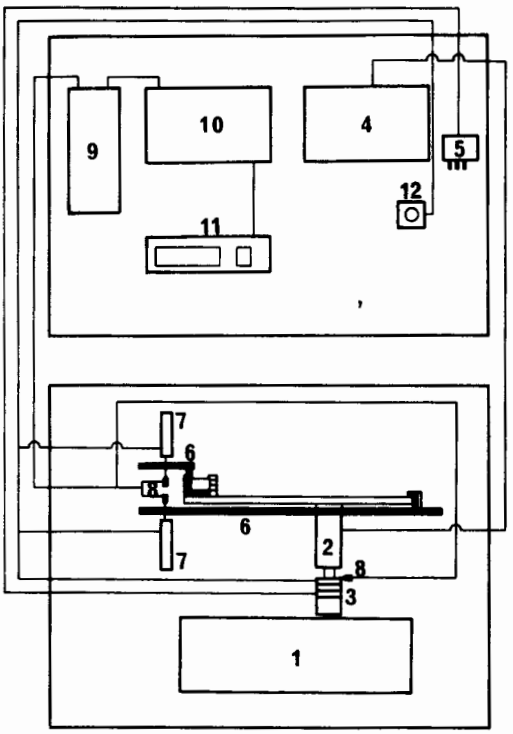

FIG. 1. Overview of the behaviour observation system. 1, Observation chamber; 2, video camera; 3, lens with motor focus and motor iris; 4, video monitor; 5, iris control; 6, belt drives; 7, servos; 8, potentiometers; 9, a.c./d.c. converter and disk drive; w, monitor; 11, keyboard; 12, joystick.

the group raised at 4° C taking about 300 ° days to reach EYS, while those raised at 9° C took about 400 ° days. Larvae reared at 9° C are discussed only with regard to deformations, as their development deviated from that of the other groups.

DEVELOPMENT OF ORGANS

Respiratory and circulatory organs

Red blood cells are not visible in the larval and post-larval halibut. The heart, which is initially a primitive tube, differentiates during the yolk-sac stage into a four-chambered heart consisting of a sinus venosus, an atrium, a ventricle and a bulbus arteriosus. Around day 20, the tube thickens and slowly forms the four chambers, while later on during weeks 5–6 the ventricle cardiac muscle thickens further. The larval halibut heart has only one visible entrance to the sinus venosus and has no distinct apex on the ventricle (Fig. 5). A valve is seen between the ventricle and the bulbus.

At hatching, there are a pair of external branchial pits, one on either side of the head posterior to the large neuromasts [Fig. 8(a)]. These expand from a diameter of 86 × 30 μm at 3 days post-hatching to about 90 μm in width near day 30 when the gill slits have broken through, forming the four naked gill arches [Fig. 8(d)].

Branchial respiratory movements were first noted around day 30 whereas primitive gill filaments appeared much later, around day 47, on the second and third gill arches. The gills appeared to be fully developed after day 60. There is no

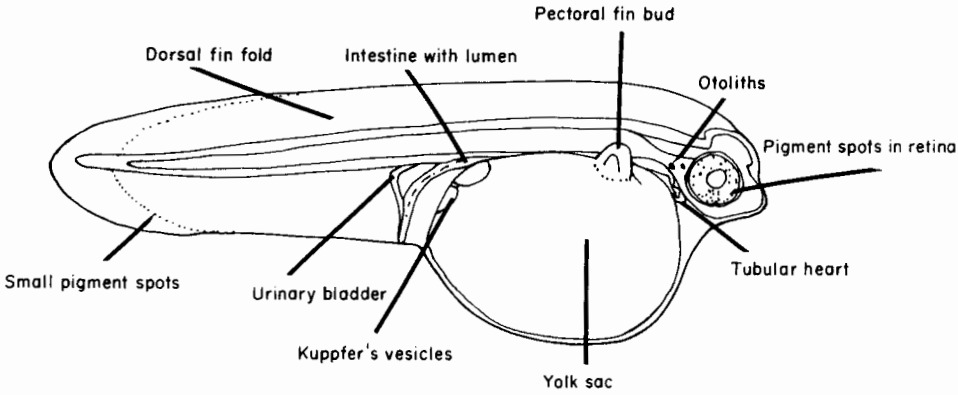

FIG. 2. A halibut larva about 13 days post-hatching and 8 mm S.L. The pectoral fin buds have developed and the eye pigmentation has begun, Stage 2.

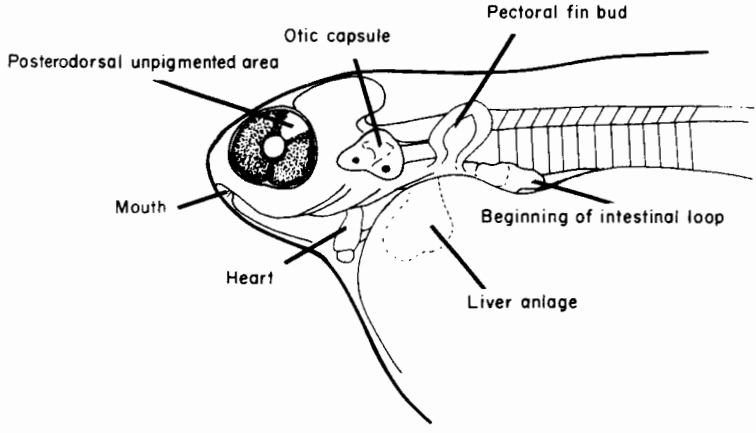

FIG. 3. At about 18 days post-hatching and 11·5 mm S.L., this larva had a faint outline of the presumptive liver on the anterior yolk sac, Stage 2.

colour in the lamellae. A pseudobranch begins to develop immediately behind each eye around 55 days post-hatching, after the onset of exogenous feeding (Fig. 6).

Eye development

The eyes are symmetrical on either side of the head during the entire yolk-sac stage. The eyes at hatching are semi-circular and pressed against the yolk sac [Fig. 8(a)]. Within a week, a constriction develops between the head and the yolk sac, lifting the head well above the yolk and allowing eye diameter to increase. A lens appears to be present from hatching and a foetal eye gap is visible.

The periphery of the eye cup develops small dots of pigment about 13–15 days post-hatching (Fig. 2). Between the inception of pigmentation and its completion at around day 25–30, all larvae have a dorsal unpigmented area on the retina (Fig. 3). While the rest of the eye may be darkly pigmented in the fourth week, there remains a well-defined, uncoloured area almost opposite the foetal eye gap which lasts for about 2–4 days. Due to the transparency of the larvae, it is necessary that

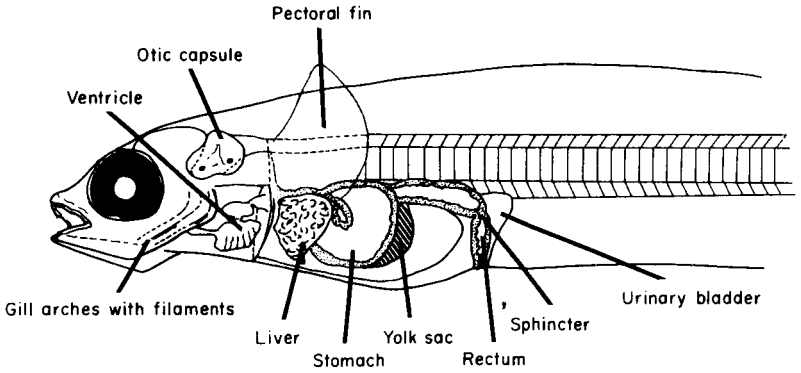

FIG. 4. At about 44 days, this larva (14·5 mm S.L., 0·95 mm myotome height) had begun to develop gill filaments along the arches, Stage 3.

the pigmentation of one eye does not mask the lack of colour in the other eye during examination.

Full pigmentation occurs after about day 30 and the eyes can be seen to move in response to visual stimuli. The left eye may begin to migrate at about day 80, well after yolk absorption, and be completely on the right side of the head by 110 days posthatching (Fig. 9).

Stomodeum, mouth and jaws

There is no stomodeum at hatching [Fig. 8(a)]. A small cleft, originating near the branchial pits develops on either side and allows the head to lift from the yolk sac [Fig. 8(b)]. About 7 to 10 days after hatching a stomodeum is present. Although the mouth opening as such is formed, a transverse skin septum, which will later give the transverse buccal valves, is present. We cannot say if this is perforated or not—if entire there is as yet no communication between mouth and buccal cavity. This septum maintains the mouth slightly agape for some time, while the skeletal framework of the jaws are being formed. The stomodeum gradually widens and begins to resemble a mouth surrounded by rudimentary jaws around days 15–20 [Fig. 8(c)]. About 1 month after hatching, the buccal valve can be seen to billow outward during branchial respiration. Small teeth have been observed on the anterior maxillary and dentary about 25 days after hatching.

Although not a part of the normal course of events, many halibut larvae have been observed with ' lockjaw ', a deformation of the jaw and joints which renders them incapable of closing the mouth. This begins to appear around day 15 after hatching and may be quite evident as gaping by the fourth week.

Digestive system

Initially the gut is straight and simple, its lumen developing around hatching and the gut beginning to form a loop by about day 14 (Fig. 3). This looping is accompanied by an apparent thickening of the anterior intestine. The first observations of peristaltic contractions were made at about day 35, when slow movements in the anterior intestine could be seen. Within the following fortnight the intestine had formed a sac-like loop on the left side of the yolk and the colon was almost perpendicular to the notochord (Fig. 4). The intestinal wall becomes gradually thicker and rhythmic contractions more obvious.

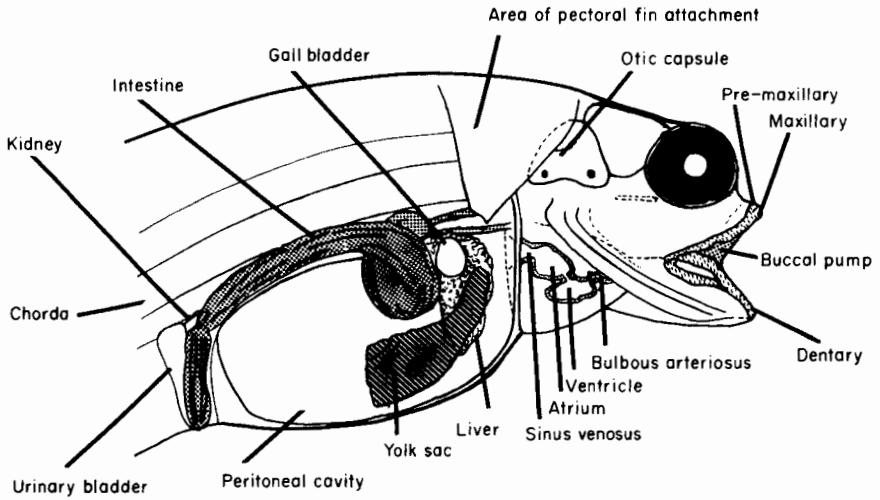

FIG. 5. At about 45 days, this larva was 12·6 mm S.L. and the yolk was quite small. The four chambers of the heart are drawn to include wall thickness, Stage 3.

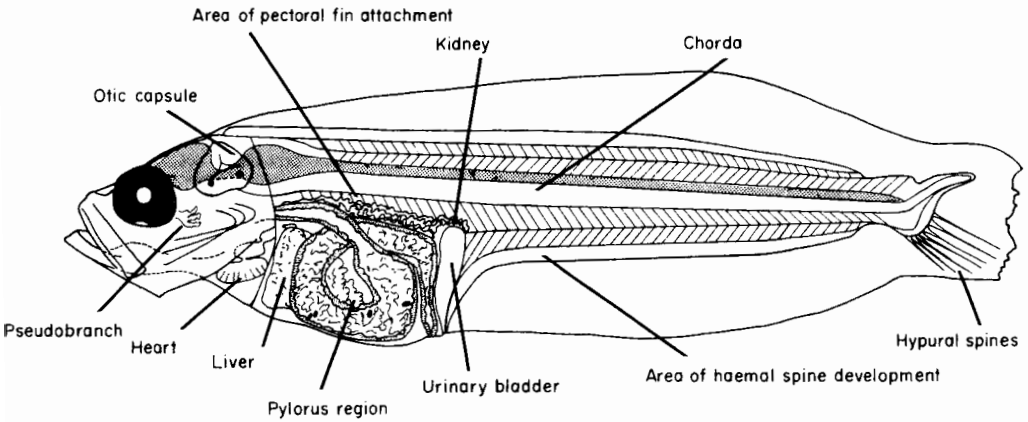

FIG. 6. A 60-day-old halibut larva (16·8 mm S.L., 2·4 mm myotome height) with food in gut.

The liver is not present at hatching. At about 15–18 days post-hatching, the liver anlage could be distinguished as a complex of cells on the left anterodorsal yolk sac (Fig. 3). Within a week this faint outline becomes more defined as an organ nestled on the yolk sac. As the yolk mass dwindles, the liver assumes a fairly conical form, and occupies about one-fifth of the peritoneal cavity. The gallbladder is noticed at about day 25 as a small globe mediolateral to the liver (Fig. 5) when it initially has a light yellowish green colour, maintained over several days around the fourth week after hatching after which transparency is regained.

The anal sphincter is seen in halibut larvae shortly after hatching but we cannot say if the anal opening is formed at this early stage. In moribund larvae at later stages, the sphincter relaxes to about the diameter of the rectum.

After the commencement of exogenous feeding, the gut continues to grow and form a complete loop (Fig. 6). Between days 80 and 90, three diverticula begin to

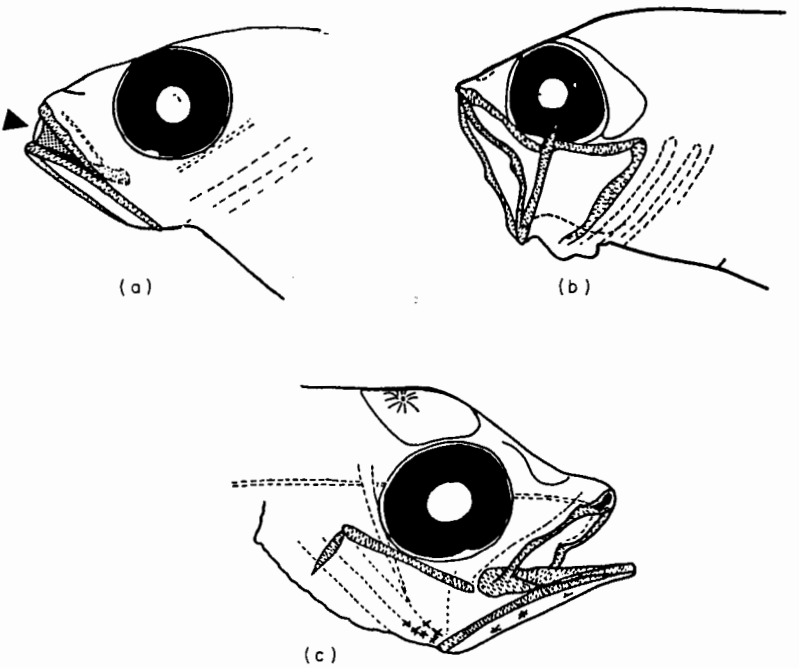

FIG. 7. Apparent mouth development. Stippled areas are cartilage. (a) Normal mouth development about 30 days post-hatching. Arrow indicates opening. (b) Abnormal development about 30 days post-hatching. (c) A functional mouth about 50 days after hatching.

develop within the loop; these are believed to be the forerunners of the pyloric caeca. As the post-larva takes on the typical flatfish form, the anus moves anteriorly relative to the growing body.

Fin development

The pectoral fins are not present at hatching. By the end of the first week after hatching, small lobes are faintly outlined and gradually constrict to fin buds at about day 12. Within another 10 days, the pectoral lobes having expanded to thick paddles, and can be used in synchronized swimming motions, although there appears to be a short period of unco-ordinated movements prior to 25 days (Figs 4, 5 and 7).

The pelvic fins appear much later and develop apparently much quicker. These fins were first seen as a pair of dark patches external to the anteroventral tip of the liver between days 75 and 80 (Fig. 9). They quickly become functional fins of about 1·5 mm length.

Hypural and epural fin rays seem to develop around the onset of exogenous feeding, following the hypural muscle becoming thicker and more complex (Fig. 6). The dorsal and ventral fin rays appear gradually after exogenous feeding, filling the area of the primordial fin lobe. The caudal end of the notochord begins to turn markedly upward about day 70.

Yolk absorption

The yolk sac is a little over 3 mm in length 1 day after hatching. As the liver and the intestine develop on the left side of the yolk, and as the pectoral fin movements

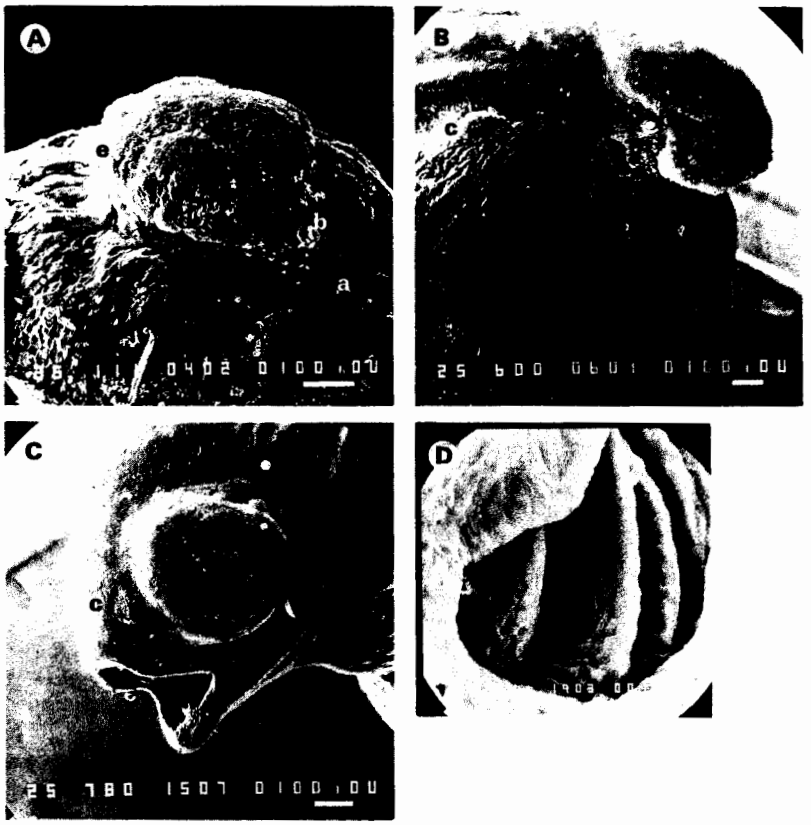

FIG. 8. Scanning electron micrograph of halibut larvae. (A) 3-day-old larva showing (a) branchial cavity (b) neuromast (c) lack of stomodeum (d) incompletely expanded eye and (e) olfactory papillae. (B) 6-day-old halibut larva showing (a) expansion of branchial cavity (b) neuromast and (c) developing pectoral fin. (C) 16-day-old larva raised at an average temperature of 9° C showing (a) new neuromasts (b) remains of the buccal pump (c) olfactory papillae and (d) opercular opening. (D) Close-up of a 24-day-old larva showing the naked gill arches through the opercular opening. Photographs: T. Rolfsen, E. Erichsen, K. Pittman.

restrict the form anterodorsally, the yolk becomes pear-shaped with the largest portion caudally. When the gut coils, the yolk is displaced back and to the right side. As absorption continues the yolk becomes elongate and thinner and is finally reduced to a small globule, posteroventral to the gallbladder (Fig. 5). Occasionally one or more such globules can be found with no apparent connection to other internal organs.

There are no visible blood vessels surrounding the yolk at any time during the yolk-sac stage. When the gut rotates, the yolk extends backward from a point near the conjunction of the liver and the intestine. Yolk absorption is apparently mediated through the liver, as a connection between the liver and the yolk was visible throughout the largest part of the yolk-sac stage (with the exception of the aforementioned globules).

Abnormal yolk absorption may be seen about 3 weeks after hatching, becoming more and more evident in the afflicted larvae. In addition to an irregular silhouette

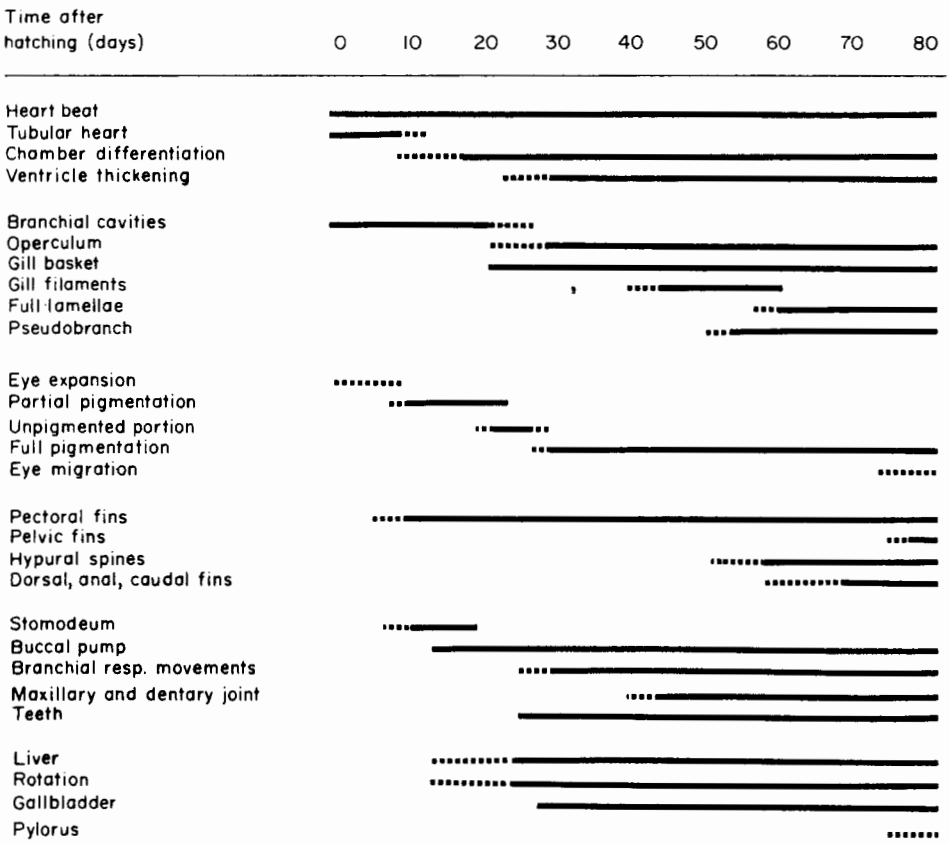

FIG. 9. Overview of the timing of developmental events in yolk-sac halibut larvae.

of the dwindling yolk sac, there is an oedema of the peritoneal cavity and the larva may either float upside down or swim in small circles.

DEVELOPMENT OF BEHAVIOUR

The behaviour of halibut larvae during the yolk-sac stage is limited to passive rising/sinking, resting and swimming.

Passive rising/sinking

The larvae float upwards, but with the head down, during the first few days after hatching, and begin to sink slowly by the end of the first week. In the third week larvae may be either negatively, positively or neutrally bouyant. A passive upward floating begins to occur around the fourth week after hatching (Fig. 12). Generally, the larvae swam in the opposite direction of their passive drift until about 3 weeks after hatching, when swimming became more horizontal.

Resting and swimming

During approximately the first 4 weeks after hatching there are long resting periods and short swimming periods. The fifth week was characterized by short

periods of both resting and swimming while the general activity level increases (Fig. 10). Contact with other larvae is associated with burst swimming, with two larvae swimming so rapidly away from each other that they elude video observation.

There is large variation in the activity levels (percent of time spent in swimming) around days 8–9. The activity level was less than 15% in the first 2 weeks post-hatching, varied in the third week and began to rise toward full activity after day 25 (Fig. 10). Most larvae had activity levels between 50 and 80% around day 40 (Fig. 10). For Group B (five larvae l^{-1}), the larvae exhibited activity levels between 70–100% around day 48, after which individual variation was more apparent. These larvae had been presented with natural zooplankton but it was not possible to distinguish differences between larvae with or without food in their guts.

An increase in activity level coincides with an increase in mean swimming speed (given in Pittman *et al.*, 1989); together these factors can be used to give a more informative picture of the energy used by the individual larva when active (Fig. 11). In Group A variation is greatest on the last observation day, day 39. Variation is even greater in Group B, although the last observation day, day 58, shows relatively little spread in the data.

Larvae from the rearing units in Hyltropollen, a seawater pond, at an age of 52–53 days, were active nearly 100% of the time, with swimming speeds around the fastest recorded for laboratory larvae near the same age (Table I). The halibut larvae from Hyltropollen were fed natural zooplankton in the laboratory, but the swimming speed decreased after a week of observation.

An attempt was made to determine swimming distance. The swimming speed and activity level of each larva in groups A and B were multiplied to find the possible distance covered during a given period of time. However, individual differences in this factor were so great that no clear trend could be observed during the yolk-sac stage.

IV. DISCUSSION

MORPHOLOGY

Temperature affects both the rate of development and the rate of growth in halibut larvae (Pittman *et al.*, 1988). Egg incubation temperatures of around 9° C have been associated with significantly smaller yolk sacs at hatching and with significantly more gaping at about 2 weeks after hatching (Pittman *et al.*, 1990).

The halibut larva has some unusual features in its developmental morphology, such as the shape of the heart, the pattern of eye pigmentation, the lack of visible blood and the late development of functional gills. The larval halibut heart has only one visible entrance to the sinus venosus and has no distinct apex on the ventricle but as in teleosts generally, there are no valves in the bulbus (Randall, 1970), although a valve is seen between the ventricle and the bulbus.

The branchial pits, which become the opercular opening, bear a strong resemblance to the embryonic cavity and branchial chamber of the killifish, *Fundulus heteroclitus*, as described by Guggino (1980). The pits are connected to the pharyngeal area by a tube at 2 days after hatching (E. Kjørsvik, University of Tromsø). The development of these pits provides a possible mechanism for larvae less than 1 week old to drink in the absence of a stomodeum (Tytler & Blaxter, 1988).

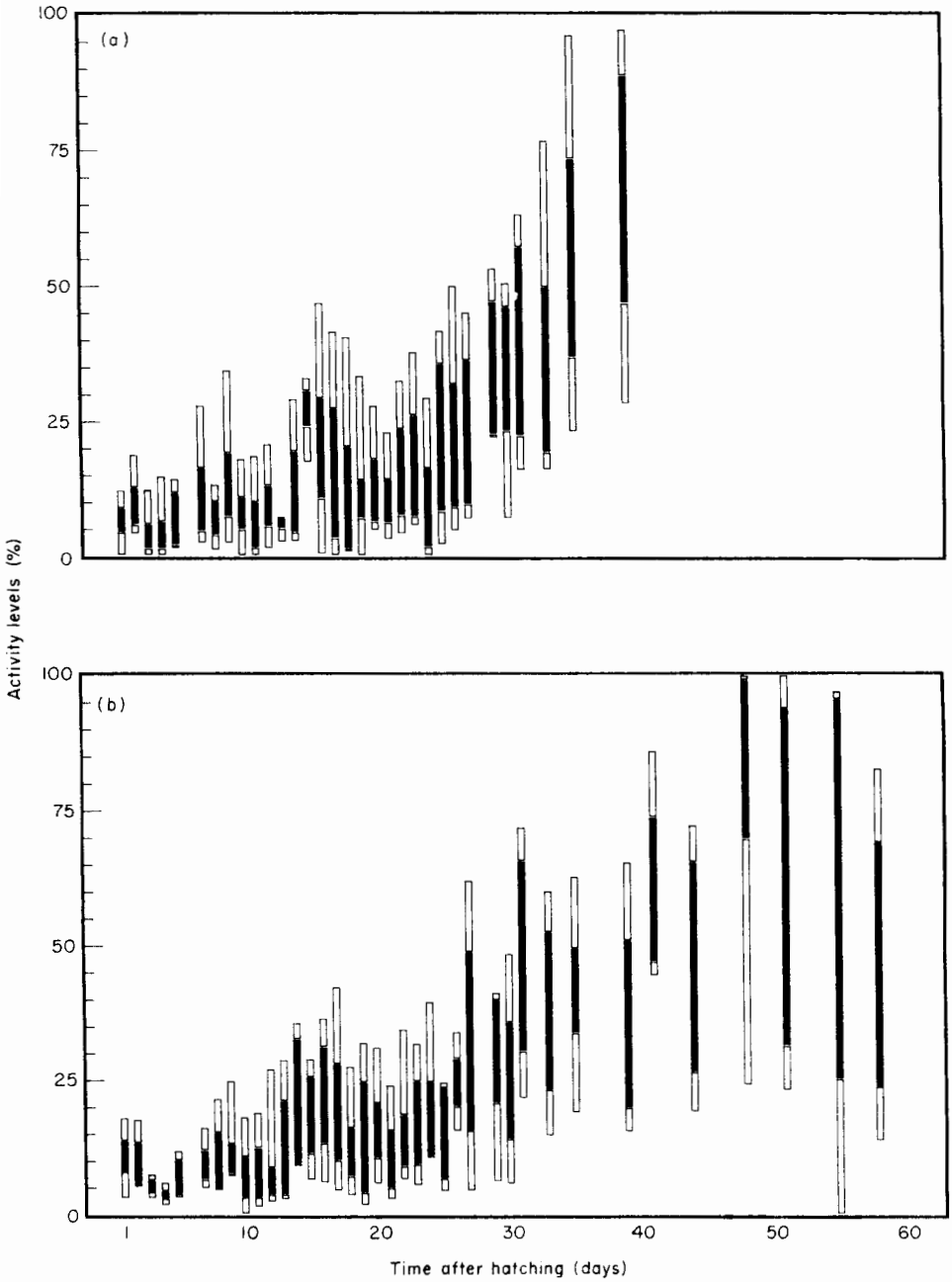

FIG. 10. Activity levels (percent of time in activity) of yolk-sac halibut larvae at initial densities of (a) 1 larva l^{-1} and (b) 5 larvae l^{-1}. The columns contain all observations, while the dark area contains the middle 60% of the values.

The periphery of the eye cup in our halibut larvae develops small dots of pigment about 13–15 days post-hatching (Fig. 2), although Senstad (1984) reports this as occurring around day 10. This can be seen to vary according to the temperatures

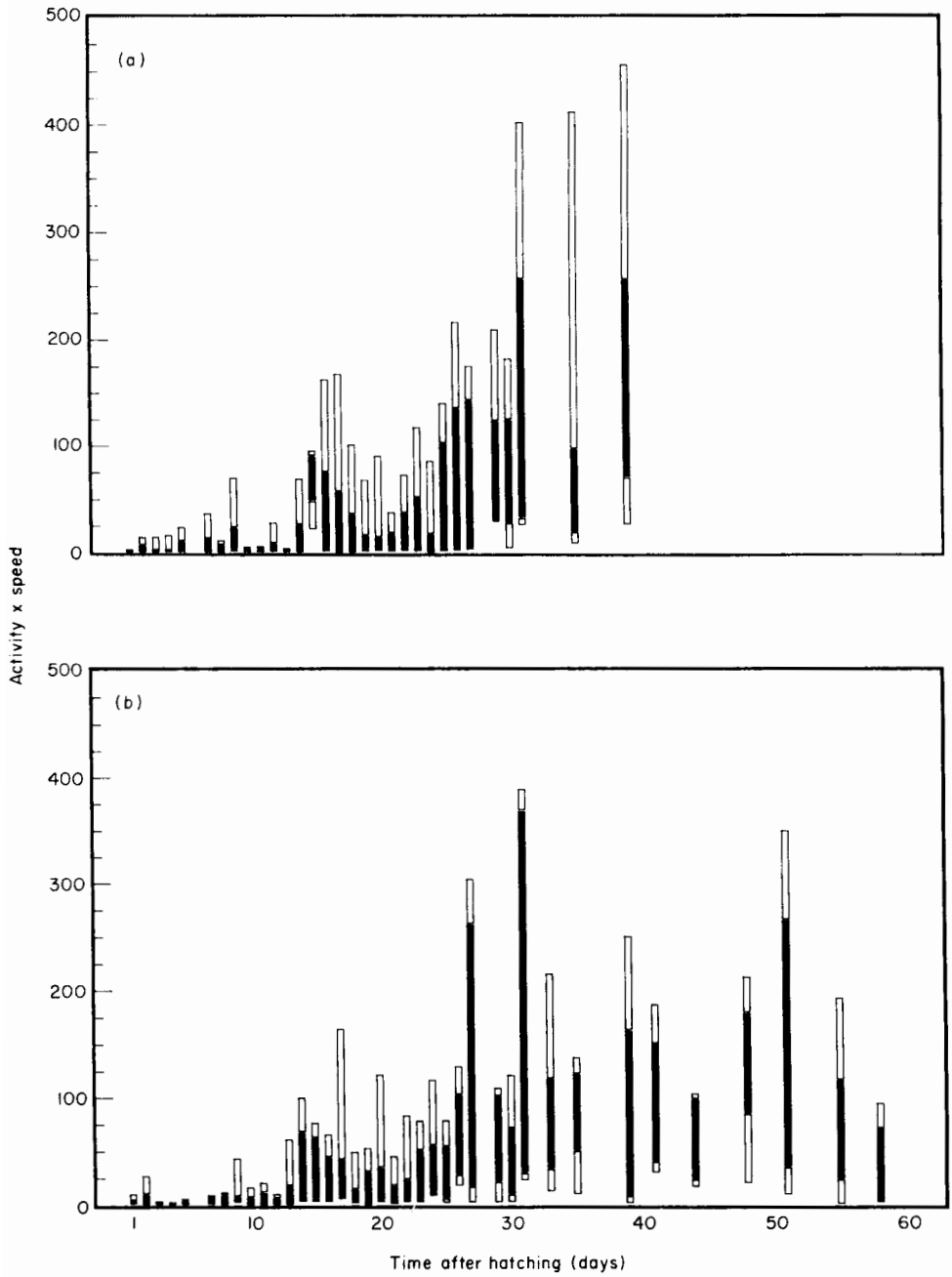

FIG. 11. Activity levels × speed of yolk-sac halibut larvae at initial densities of (a) 1 larva l^{-1} and (b) 5 larvae l^{-1}. The columns contain the values of all observations while the middle 60% are contained in the dark area.

during development, with partial pigmentation occurring as early as about day 7 after hatching when eggs and larvae have been incubated at 9° C (Pittman *et al.*, 1990). However, migration of the eye seems to be correlated with general body growth rather than the rearing temperature. Less developed larvae of the same

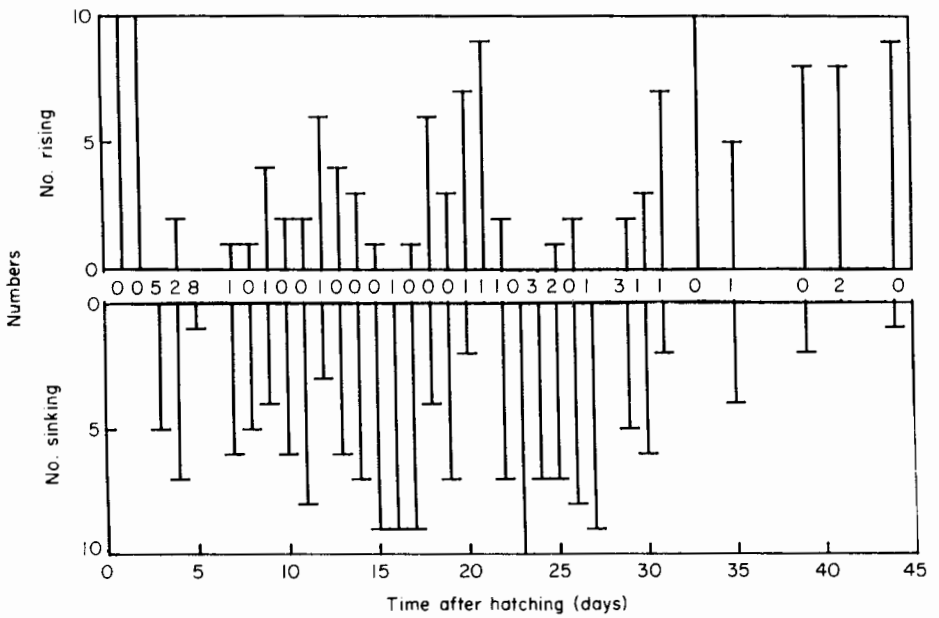

FIG. 12. Passive sinking and rising of yolk-sac halibut larvae. The number of neutrally buoyant larvae is given in the middle.

TABLE I. Activity levels and swimming speed of halibut larvae raised in floating bags in a pool

Day	Activity level %		Swimming speed	
	x	(S.D. n)	x	(S.D. n)
51	97·63	(1·46, 3)	4·58	(0·64, 3)
52	93·46	(4·40, 7)	7·62	(2·21, 6)
53	98·30	(0·93, 5)	10·13	(3·45, 3)
54	97·21	(2·47, 8)	9·91	(3·08, 8)
57	92·88	(6·63, 4)	6·09	(—, 2)

age maintain eye symmetry, while quickly growing larvae may be undergoing metamorphosis already 60 days after hatching. The caudal end of the notochord begins to turn markedly upward about day 70 but, as with eye migration, this is related to good general body growth.

About 1 month after hatching, the buccal valve can be seen to billow outward during branchial respiration. Small teeth are seen on the anterior maxillary and dentary about 25 days after hatching. This leads us to believe that the mouth may be functional at this time. However, we cannot yet say whether the jaws are ossified or remain hyaline cartilage throughout the yolk-sac stage.

In *H. hippoglossus* the presumptive buccal valve, or oral membrane, seems to be both a fragile structure and essential to the correct articulation of the jaw (Fig. 7 and Pittman *et al.*, 1988). It seems that if the buccal septum breaks down too early, the jaws may be permanently left agape (pictured in Blaxter *et al.*, 1983) thus

explaining the occurrence of the lockjaw condition. Gaping begins to appear around day 15 after hatching and may be quite evident as gaping by the fourth week (Senstad, 1984; Pittman *et al.*, 1990). Lockjaw is associated with degradation of the tissue of the snout and buccal valve, its prevalence in our material correlated with high temperatures (Pittman *et al.*, 1988, 1990; Bolla & Holmefjord, 1988) but may be due to other factors. This course of events in the development of Atlantic halibut larvae may be similar to that experienced by the Pacific halibut but Forrester & Alderdice (1973) stated that the mouth of the newly hatched *H. stenolepsis* was not as easily visible as in Thompson and van Cleve's Stage 1 for larvae of that species.

Yolk absorption is apparently mediated through the liver, as a connection between the liver and the yolk was visible throughout the largest part of the yolk-sac stage (with the exception of the aforementioned globules), as was observed by Blaxter *et al.* (1983). However, the liver arises after yolk absorption has begun (Fig. 3), so initial absorption may be more direct. The rate of yolk absorption at three temperatures is given in Pittman *et al.* (1988, 1990).

Abnormalities in yolk absorption are seen. In addition to an irregular silhouette of the dwindling yolk sac, there may be an oedema of the peritoneal cavity and the larva may finally either float upside down or swim in small circles (Senstad, 1984). Oedema in our material is mostly associated with high temperatures (Pittman *et al.*, 1988) but the causal mechanism is unclear.

Gallbladder coloration during a short but distinct period in the yolk-sac stage suggests the presence of digestive enzymes, although in our data this is not conclusive. The light green colour appears coincidental with the appearance of peristaltic contractions, functional pectoral fins and full pigmentation of the eyes. Observations of prey ingestion in yolk-sac larvae indicate that a functional digestive system may be established as early as about day 30, although histological descriptions of the digestive system are needed.

BEHAVIOUR

The early behaviour is limited to swimming periods and resting periods as well as sinking or rising passively according to developmental stage. During approximately the first 4 weeks after hatching there are long resting periods and short swimming periods. There is no aggressive behaviour in the yolk-sac stage and when two larvae come into contact, each reacts with a burst of swimming away, escaping with a speed too fast to be accurately recorded. Long-resting periods with a well-developed escape response seem to be a good strategy for fish larvae to avoid predation (Bailey & Yen, 1982), and to escape detection by predators, especially copepods.

There is large variation in the activity levels (percent of time spent in swimming) around days 8–9, coinciding with variations in the developing eye pigmentation and the start of pectoral fin formation. Around day 25, the larva increases its mean swimming speed from 0·5 to 3·0 mm s^{-1} (Pittman *et al.*, 1988) and its activity level gradually rises. About day 30 the behaviour was characterized by short periods of both resting and swimming while the general activity level and swimming speed increase (Fig. 10 and Pittman *et al.*, 1988). This period coincides with the development of fully pigmented eyes and co-ordinated pectoral fins, along with observations of branchial respiration and increased pumping capacity of the heart. An increase in activity and swimming speed also coincide with an increase in

passive rising. In the latter half of the yolk-sac stage, the larvae may swim for almost the entire observation period, but at slightly lower speeds than observed during mid-yolk-sac stage. This leads us to believe that they are either conserving energy or searching the water column or both and would seem to support this time period as one of natural start-feeding.

Most larvae had activity levels between 50 and 80% around day 40 (Fig. 10), agreeing with Senstad's (1984) observations. This coincides with the time when the yolk supplies are reduced and a streamlined form is attained. However, in Group A variation in activity level is greatest on the last observation day, day 39, perhaps reflecting variation in the general condition of the larvae.

The halibut larvae from Hyltropollen were fed natural zooplankton in the laboratory, but the swimming speed decreased after a week of observation, concurring with Westernhagen & Rosenthal's (1979) observation that the activity of herring larvae collected from the wild was lower than those still wild but not as low as laboratory-reared larvae.

The activity levels were measured in the presence of light levels at 300 lx at the surface. This may have affected the true activity levels, as some fish have exhibited higher activity rates in light than in darkness (Batty, 1987). However, the preferred light levels of halibut larvae have not yet been determined and this preference may change with different developmental stages.

V. CONCLUSIONS

The similarity in the early life stages of Atlantic (*H. hippoglossus*) and Pacific halibut (*H. stenolepis*) and the Pacific sablefish *Anoplopoma fimbria* (see Alderdice *et al.*, 1988) suggests a similarity in the bathypelagic ecological strategies of these species. It is possible that large unpigmented eggs, relatively large yolk supplies and yolk-sac stages of long duration and poorly developed larvae, along with flight reactions to tactile stimuli during the early yolk-sac stage, may be adaptive to solitary existence in waters of low productivity and low light intensity. The passive sinking and rising at various stages may be a strategy to reach first feeding and capture prey items where they occur, perhaps at the bottom of the photic zone.

Based on the results from 1987–88, the following divisions are proposed for separating the yolk-sac phase of Atlantic halibut into four stages. These represent a further division of Thompson & Van Cleve's (1936) stages 1 and 2 for Pacific halibut (*H. stenolepis*). A larva's development and placement into one of the four stages should generally be possible with the aid of low power magnification or by close observation of the larvae in an incubator. Not all larvae in a group will have the same degree of development (Blaxter *et al.*, 1983), and rearing temperature will affect the relative rate of development. The following divisions are assumed to be valid for mean rearing temperatures of 4 to 7° C. The variation in duration of each stage reflects both variation due to temperature changes and individual variation in growth rates.

STAGE 1

Morphology

Yolk present; notochord straight; no stomodeum; no chromatophores; eyes symmetrical and pressed against the yolk sac; a pair of external branchial pits posterior to head; intestine straight; no pectoral fins; simple heart.

Behaviour

Body generally lies passive and almost vertical in the water column with head downwards; infrequent swimming generally in the vertical plane, mean speed 0·5 mm s^{-1}; passive rising the first few days, thereafter sinking, duration 5–7 days.

STAGE 2

Morphology

Yolk present; yolk sac changing from an elliptical form to a pear-shaped form; notochord straight; mouth present as a small opening; eyes symmetrical, circular and lightly pigmented to pigmented with an unpigmented dorsal gap; branchial pits expand to short opercular flaps; intestine thickening and beginning to loop; pectoral fins develop from buds to paddle-like fins; heart differentiating into four chambers; liver and gallbladder develop; gallbladder may be coloured light green for a short period late in this stage.

Behaviour

Mean swimming speed increases from 0·5 to 3·0 mm s^{-1} when active, passive sinking generally, duration 17–20 days.

STAGE 3

Morphology

Yolk present; yolk sac reducing to tubular form on right side of intestine, connected to medial side of liver; notochord straight; mouth becomes functional; teeth observed; eyes symmetrical and fully pigmented; gill basket developing with some filaments on the arches; intestine fully looped; rectal sphincter develops; ventricular cardiac muscles thicken.

Behaviour

Mean length of active periods increases from about 5 s to over 1 min; swimming often horizontally, mean swimming speed between 2–3 mm s^{-1}; generally passive rising; larvae can assume ' C ' and ' S ' forms in the horizontal plane in this stage for a few seconds at a time, duration 15–20 days.

STAGE 4

Morphology

Yolk present; little or no yolk left; notochord straight; hypural thickening; hypural spines appear; mouth functional, buccal valve billows outward when buccal cavity contracts; eyes symmetrical; gill filaments on arches; some black melanophores along margin of ventral finfold and notochord; ratio of standard length to myotome height is still greater than 15.

Behaviour

Larva displays searching activity and attacks prey items but mean duration of active periods drops, duration 4–7 days.

Premature gaping, or 'lockjaw' (Pittman *et al.*, 1987, 1988), becomes apparent during Stage 2. Oedema is apparent in affected larvae during Stage 3, when they will float near the surface, but may begin much earlier. In aquaculture systems, potential prey may be added and light turned on during Stage 3–4. Stage 3 and Stage 4 may coincide with startfeeding, but are distinguished by the lack of growth in myotome height. When exogenous feeding has taken place, the ratio of standard length to myotome height drops well below 15 as the larva assumes a typical flatfish form. Exogenous feeding may coincide with or precede considerably, the complete absorption of the yolk, and there are indications that 'good' larvae may begin feeding as early as 4 weeks before end of the yolk-sac stage.

Further development of the Atlantic halibut does not seem to differ significantly from Thompson & Van Cleve's description of the post-larval Pacific halibut until Stage 10 when the pigmentation pattern is different. It may be added that the pre-metamorphic post-larvae attack their prey from an upright (dorsoventral) position while the eyes are symmetrical (Thompson & Van Cleve's Stages 4 and 5, 1936) and that the gills develop fully during this time.

Our data on development, growth, activity levels and swimming speed all indicate a crossroad at 25–30 days post-hatching. It may be that their reactions are less restricted, with a wider spectrum of reaction possibilities after this period. It may also be that the environment experienced in the early yolk-sac stage contributes to or partly determines the condition of the late larval stage, or that energy then becomes the limiting factor (Pittman *et al.*, 1988).

The time of first feeding is an important event which we have not been able to define further. The extremes vary with temperature and range as early as 23 to 25 days after hatching (L. Berg, unpublished data). Factors such as egg size and incubation temperature, genetic stock differences, epigenetic influences on critical periods in development, optimal prey size and nutritional content all remain to be clarified.

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