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Effect of temperature on growth rates and organogenesis in the larvae of halibut (*Hippoglossus hippoglossus* L.)

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Growth of halibut larvae at 9°, 6°, and 4°C was monitored during the yolk-sac stage in three 15 m^3 silos with gentle upwelling. Jaw deformations and edema were most common at 9°C, while growth and yolk absorption were better at 4° than either 9° or 6°C. Fewer day degrees were necessary to reach end-of-yolk-sac-stage in cold water than in warm water. Scanning electron microscopy revealed the absence of a stomodeum but the presence of a pair of branchial pits with many pores at about 3 d after hatching. These cavities eventually expanded to expose a small part of the naked gill arches at about 20 d after hatching. Mouth development, normal and abnormal, was also followed using scanning electron microscopy. The behaviour of larvae at two densities is described from hatching onwards. Increases in duration of swimming period are apparent about 35 days after hatching, after the duration of resting periods has decreased. Mean swimming speed changes with development. Changes in behaviour are related to the developmental stages.

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Introduction

For successful mass production of halibut, with around 50 d from hatching to end-of-yolk-sac-stage (EYS), selection of a commercially feasible and biologically sound optimum temperature is imperative. Growth rates may slow at high temperatures while the time required to absorb the yolk decreases with increasing temperature (Petersen *et al.*, 1977). Low temperatures are associated with reduced growth rates and developmental abnormalities (Alderdice and Velsen, 1971; Peterson *et al.*, 1977). An optimal temperature would thus be one where growth is best, anomalies are few and the cost of production is justifiable.

Growth may also be affected by the duration of swimming periods and the average speed maintained during those periods. High levels of activity may indicate stress and will reduce the amount of energy which could otherwise be directed towards somatic growth.

In this experiment, temperatures of 4°C and 6°C were chosen to approximate the temperatures of the water masses where eggs are most abundant (Haug *et al.*, 1984), while 9°C was introduced to approximate the high temperatures used in another experiment (Dye and Brancker, 1987). The duration, frequency and speed of swimming throughout the yolk-sac stage was observed in relation to larval density.

Materials and methods

Egg source

Eggs were stripped from one female and milt was stripped from two males of the broodstock held at about 7.1°C at the Austevoll Aquaculture Station. There was 87% fertilization of the eggs, which were incubated in two 250-l incubators at a mean temperature of 7.06°C. The hatching was estimated to be 78%. There was no treatment with antibiotics either before or after incubation.

Experimental conditions

Three 15-m^3 silos at Sauaneset, a new land-based site belonging to the Austevoll Aquaculture Station, were used to hold the larvae throughout the yolk-sac stage (Fig. 1). They are about 4.5 m high and 3 m wide and are equipped with a flow-through system with the water inlet at the bottom. Sand-filtered water from 40 m depth

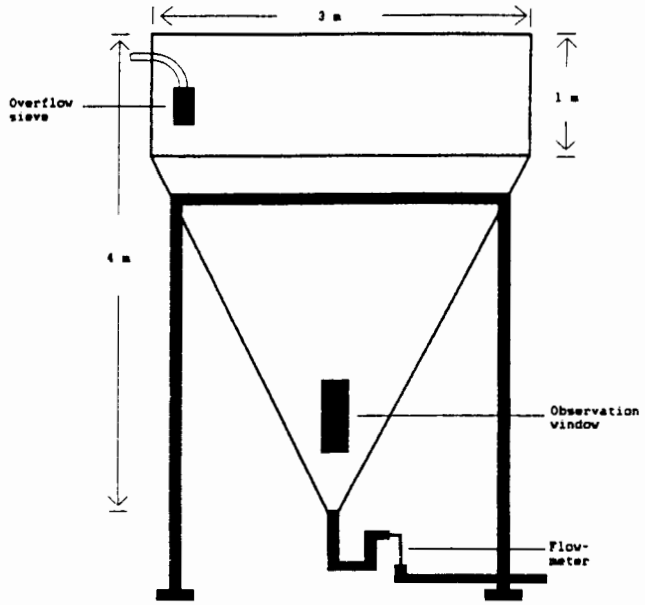

Figure 1. Diagram of a silo showing observation window and flow control.

was piped to the silos through mixing valves (Satchwell Linear Actuator, type ALM 1601) for direct coupling to a Satchwell C-type control valve. The flow monitors were Rotameter HRV-4DA (0–15 l min^{-1}) installed below the silos. Flow was generally kept at 10 l min^{-1}.

One litre of eggs (approximately 35 000 eggs) was transferred to each of three 15-m^3 silos just prior to hatching. During the first week after hatching, the temperature was the same in all silos (about 6.5°C) due to technical problems. After the first week, water temperature was adjusted up to 9°C (silo 3) or down to about 4°C (silo 1) over two days or maintained at 6°C (silo 2).

Larvae were kept in total darkness until morphological examination confirmed full pigmentation of the eyes. Potential prey (cultivated Rotatoria and *Artemia*, and natural zooplankton from 180–500 µl) were added prior to the EYS at days 33, 39, and 49 for 9°, 6°, 4°C, respectively.

Sampling

Temperature was measured daily from surface to 4 m at depth intervals of 1 m, using an Aanderaa temperature probe. Temperature control was automatically performed using a temperature probe installed on the mixing valve of the incoming water. Day degrees were estimated by taking the average of the temperatures recorded.

Live larvae were removed from the silos using a beaker or a modified plankton net with a small cod end. The net could be lowered to about 2.5 m and slowly drawn upward. Larvae were then rinsed out of the cod end and examined at up to 50× while still alive under a Wild-Heerbrug dissecting microscope. Measurements were taken of the standard length, yolk-sac length, eye diameter and myotome height with a calibrated ocular. The condition and development of each larva was described.

Larvae selected for examination by scanning electron microscopy were preserved in a fixative containing 1 ml 25% glutaraldehyde and 9 ml of a cacodylate buffer (0.1 M sodium cacodylate, 4 %wt polyvinyl pyrrolidone and 0.044 %wt calcium chloride plus distilled water). The larvae were then stained using 1% osmium tetroxide (100 mg OsO_4 in 9 ml buffer), dehydrated in acetone, dried to critical point and coated in palladium. Samples were examined using a JEOL JSM-35 scanning electron microscope at the Elektronmikroskopisk Felleslaboratorium of the University of Bergen.

A time series of larvae from each group, from hatching to beyond EYS, was prepared for examination by scanning electron microscope. The results reported here concern a portion of the series, covering 25 to 195 day degrees or 3–31 d.

Dead larvae were removed almost every second day from the conical bottom of the silos by stopping the flow for about 20 min, inserting a 50-l salt plug of about 50 ppt and then filtering out about 35 l. These were preserved in formalin and later examined for total number and incidence of deformities. The larvae were categorized according to their condition – decomposed; OK, where the larvae are clear and normally developed; and deformed, where the larvae have obvious deformities, especially of the jaw.

Behaviour

Behavioural studies of larvae were conducted using a computer-aided video system, allowing three-dimensional registration of the larvae in 70-l observation chambers (system described in Huse and Skiftesvik, 1985). Temperatures were maintained at 5–6°C. The larvae in the behaviour observation chambers were kept in total darkness, except for the hour prior to and during recordings.

Two densities, 1 larvae and 5 larvae l^{-1}, were used. Larvae were observed from day 0 to day 58 for behaviour and swimming speed. Each larva was tracked for 5 min. Position and behaviour was logged each second. Their behaviour was divided into swimming and non-swimming periods, where the length of a period is taken as the mean time for 10 larvae.

Results

Temperature

Temperatures in the silos varied close to the desired mean (Fig. 2) with the largest vertical variation occurring in the coldest silo. The temperature was gradually increased as the larvae in this silo approached EYS, for

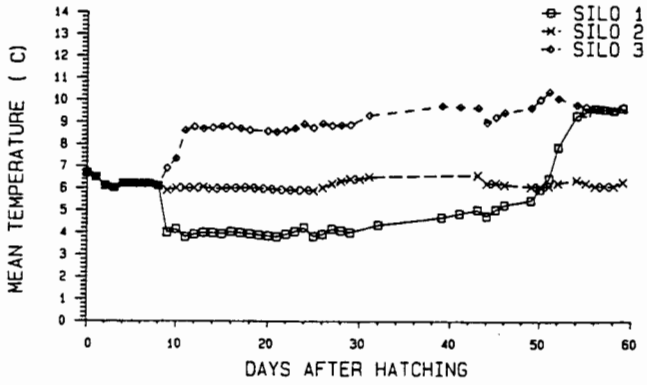

Figure 2. Mean temperatures in the three silos. Temperature control began on Day 7.

acclimatization to the temperature used in an outdoor mesocosm where subsequent feeding trials with natural zooplankton were conducted.

Growth

After the introduction of temperature-controlled water (day 7), growth in standard length seemed initially better in the group receiving warmest water (Fig. 3). There was generally little difference up till day 46. There were, however, significant differences in standard length at EYS, which was reached about day 46, day 52, and day 59 for the 9, 6, and 4°C groups respectively. Standard length at this stage was significantly different from each group, the best length of 15 mm having been obtained using the coldest water (one-way ANOVA, $p = 0.0022$). Approximately 395 day degrees were necessary for larvae raised at 9°C to reach EYS, whereas only 305 day degrees were required for those raised at 4°C.

The yolk-sac length of all three groups was approximately equal during the first three weeks after hatching (Fig. 4). Differences began to appear about 30 d after hatching when the yolk sacs of the 9°C group were much smaller than in the two other groups.

Deformities and mortality

The number of dead larvae and the number displaying deformities collected from the bottom water of the silos are listed in Tables 1–3. This excludes about 6000 larvae transferred from the coldest silo around day 50. The percentage of deformed larvae found in the bottom water vs. the total mortality was greatest at the highest temperature, where 18% of those examined had jaw deformities. This group also had the highest percentage of edema (swelling of the peritoneal cavity), but since the larvae float until decomposing, very few were found in the bottom water.

Scanning electron microscopy

Three-day-old larvae are quite primitive, having no stomodeum, no pectoral fins, and the head confluent with the yolk sac. A stomodeum may be present from about day 8, but this is still inconclusive.

While there was no stomodeum at day 3, there were external pits in the branchial area adjacent to the largest neuromasts at the posterior margin of the head. The pits measured about 86 µm × 30 µm and were surrounded by small pores. The time series shows the gradual growth and transformation of the branchial pit to an opercular cleft, in which can be seen two naked gill arches of the branchial basket. At day 8 the branchial pit was about 140 µm at the broadest, with what appeared to be a narrow extension proceeding anteriorly. The pores at this time were only about 4 µm in diameter with some internal structure. By day 16, the primitive operculum had formed as a fold extending backwards covering the branchial clefts. A primitive pectoral fin appeared as a constricted elongation on the dorsoanterior portion of the yolk sac about 16 d after

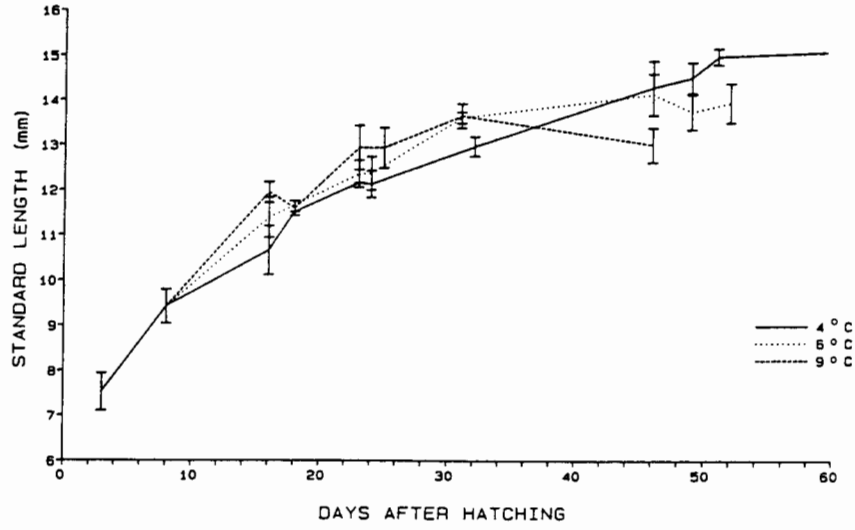

Figure 3. Mean standard length of halibut larvae grown at three temperatures to EYS.

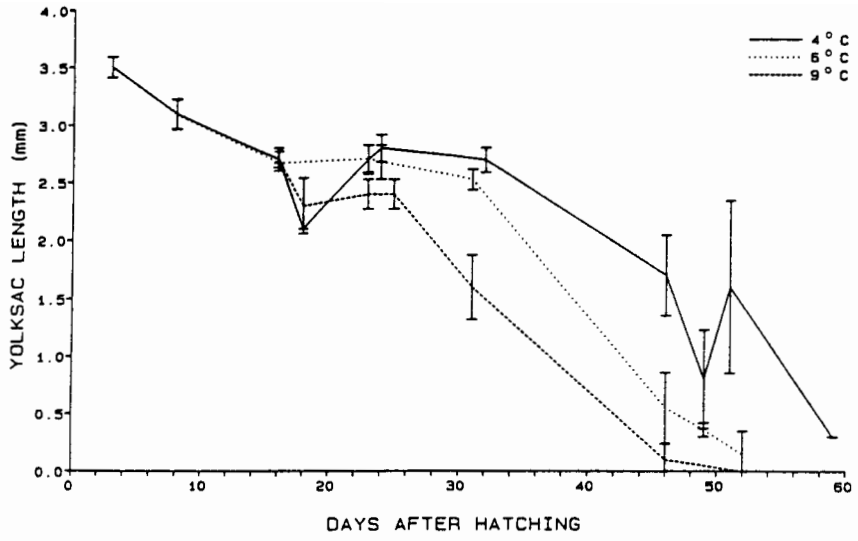

Fig. 4. Mean yolk-sac length of halibut larvae grown at three temperatures to EYS.

hatching. This was already a paddle-shaped fin on larvae of the same age which had experienced 9°C water.

The mouth was open but not functional by day 16 at 9°C. Many of the larvae in this group became "gapers" with jaws locked open, where it seems that the articulation of the maxillaries and the dentary is compromised before ossification sets in. The buccal valve, which prevents the forward flow of water when the buccal cavity is contracted, is often deteriorated or absent in larvae with jaw deformations. Scanning electron micro-

Table 1. Mortalities in silo 1 (4°C) as determined by bottom water sample counts. Decomposed larvae were those which were reduced before being extracted; deformed refers generally to jaw deformities as those with edema did not sink until decomposed; and OK were those larvae which were clear and normal. Excludes 5000 larvae transferred to another system on day 49 and 1280 transferred on day 51. Cum. Tot. is cumulative total mortality.

Day	Decomposed	OK	Deformed	Total	% Def.	Cum. Tot.
1	1642			1642 (egg)		1642
2	1551			1551		3193
4	164			164		3357
7	130	1	1	132 (19)	0.75	3489
9	46			46 (1)		3535
11	304			304		3839
14	1040	2		1042		4881
16	2044	12		2056		6937
18	1060			1060		7997
21	562	5		567		8564
23	231			231		8795
25	24		1	25	4	8820
28	12			12		8832
30	9	1		10		8842
32	21	1	5	27	19	8869
35	15	1	6	22	27	8891
39	14			14		8905
42	8	6	9	23	39	8928
44	3		2	5	40	8933
46	16	3	13	32	41	8965
49	312	163	106	581	18	9546
51	161	249	62	472	13	10 018
53	259	66	74	399	19	10 417
56	116	168	74	358	21	10 775
58	581	534	132	1247	11	12 022
62	36	18	10	64	16	12 086
67	3	3		6		12 092
70	4	4		8		12 100
75		1		1		12 101
	10 368	1238	495		4	

Table 2. Mortalities in silo 2 (6°C) as determined by bottom water sample counts. Decomposed larvae were those which were reduced before being extracted; deformed refers generally to jaw deformities as those with edema did not sink until decomposed; and OK were those larvae which were clear and normal. Cum. Tot. is cumulative total mortality.

Day	Decomposed	OK	Deformed	Total	% Def.	Cum. Tot.
1	2229			2229 (egg)		2229
2	849			849		3078
4	1705			1705		4783
7	295			295 (153)		5078
9	125			125 (4)		5203
11	60	2		62 (1)		5265
14	1523			1523		6788
16	708			708		7496
18	236	17		253		7749
21	106		1	107	1	7856
23	30			30		7886
25	30		2	32	6	7918
28	32		3	35	9	7953
30	19		3	22	14	7975
32	14			14		7989
35	64	3	7	74	9	8063
39	1017	1563	807	3387	24	11 450
42	5895	734	1280	7909	16	19 359
44	201	52	11	264	4	19 623
46	86	33	9	128	7	19 751
49	140	90	12	242	5	19 993
51	82	39	8	129	6	20 122
53	317	242	15	574	3	20 696
56	885	127	41	1053	4	21 749
58	79	25	9	113	8	21 862
67	4			4		21 866
	16 731	2927	2208		10	

scopy did not reveal evidence of bacterial activity on the surface, although it was obvious that some form of tissue decomposition was occurring. Decomposition affected both the margins of the jaws and the area around the olfactory papillae, exposing the underlying cartilage.

Six pairs of neuromasts posterior to the yolk sac were photographed on an 8-day-old larva. The large neuromasts posterior to the eye were found to persist from hatching until at least day 31. In addition to this and the neuromasts along the lateral line, another pair were found along the ventral margin of the eye. A particularly well-preserved suborbital neuromast was found on a 16-day-old larva, while the bases of these could be identified on larvae from day 23 and day 31.

Behaviour

The mean length of the swimming period (4.9 s) is equal for days 1–14 and days 15–30 (Fig. 5, Mann-Whitney U-test), but is slightly longer for those larvae in density of $5\,l^{-1}$ (Wilcoxon Signed Rank test). After days 25–30, the length of the swimming period increases to a maximum of about 1 min. The duration of the passive or non-swimming periods decreases from day 0 to day 30 (Fig. 6; trend test from Lehman, 1975) and is longer for larvae in the lowest density (Wilcoxon Signed Rank test). After day 30, the duration of the passive periods varied between 5 and 25 s.

The average swimming speed (Fig. 7) for larvae in the high density was significantly faster than for larvae in the low density (Wilcoxon Signed Rank test). Speeds of up to $3\ mm\ s^{-1}$ were found between days 26–36 and thereafter the speed decreased to around $1.5–2\ mm\ s^{-1}$.

In the late yolk-sac stage the larvae were attracted to light. When presented with a white aquarium wall, the larvae pressed their snouts against the wall while swimming. When the colour was switched to black, the larvae returned to swimming normally in the water.

It was observed that approximately 20 d before EYS, the larvae began to display the "S" shape typical of preying activity. Often they assume the "S" only to relax within 1–2 s. A horizontal "C" shape, where the larva vibrates with its tail, was also observed at this time.

General survival

The survival of halibut larvae raised in the three silos during the 1988 season is given in Table 4. Mean survival until start-feeding, the date at which prey items were

Table 3. Mortalities in silo 3 (9°C) as determined by bottom water sample counts. Decomposed larvae were those which were reduced before being extracted; deformed refers generally to jaw deformities as those with edema did not sink until decomposed; and OK were those larvae which were clear and normal. Cum. Tot. is cumulative total mortality.

Day	Decomposed	OK	Deformed	Total	% Def.	Cum. Tot.
1	3507			3507 (egg)		3507
2	1008			1008		4515
4	339			339		4854
7	332			332 (36)		5186
9	195			195 (4)		5381
11	126	7		133 (1)		5514
14	948			948		6462
16	321			321		6783
18	154	1	20	175	11	6958
21	496	2	78	576	14	7534
23	340	35	125	500	25	8034
25	453	22	210	685	31	8719
28	704	23	346	1073	32	9792
30	764	13	306	1083	28	10 875
32	568	8	215	791	27	11 666
35	558	15	153	726	21	12 392
39	120	9	18	147	12	12 539
42	92	52	59	203	29	12 742
43			550[1]	550		13 292
44	446	156	382	984	39	14 276
46	1187	92	463	1742	27	16 018
49	49	46	29	124	23	16 142
51	21	15	11	47	23	16 189
53	4	3	3	10	30	16 199
56	10		1	11	9	16 210
58	5	7		12		16 222
67	7	3		10		16 232
70		7	1	8	13	16 240
75		1		1		16 241
	12 754	517	2970		18	

[1] Skimmed off the surface, all suffering from edema.

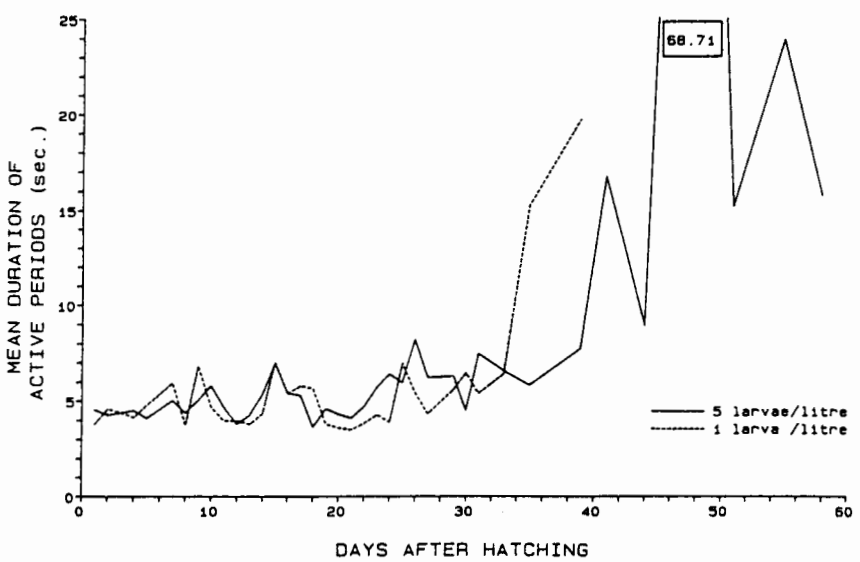

Figure 5. Mean duration of the swimming periods for halibut larvae held at two densities.

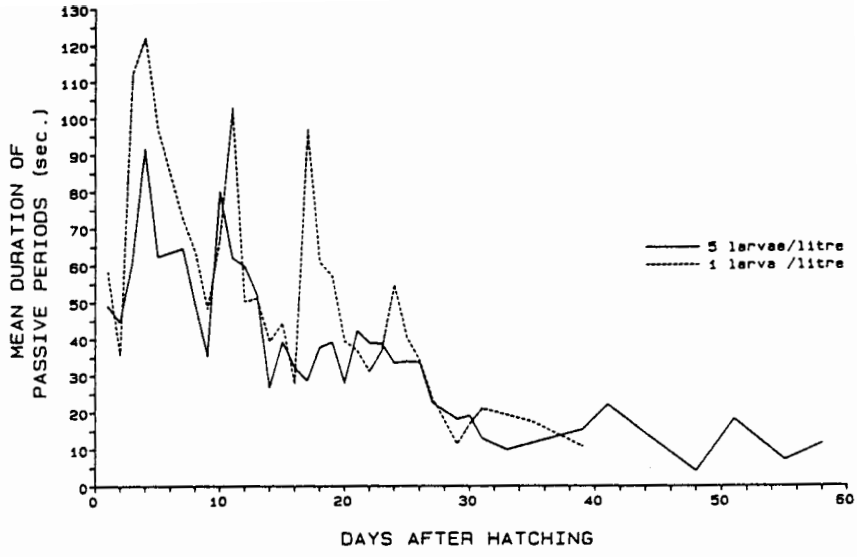

Figure 6. Mean duration of resting period for halibut larvae held at two densities.

introduced, was about 24%. However, the nature of the deformities in the groups (lockjaw and edema) allows some larvae to survive until EYS.

About 300 halibut raised at 4°C survived beyond metamorphosis, accounting for about 75% of the station's production. These had been transferred in a group of about 6000 from the silo to a mesocosm on days 49 and 51 for feeding on natural zooplankton. About 70 larvae survived to EYS in the 9°C and some were observed to feed, although they did not reach metamorphosis. An electrical short-circuit during the installation of lights in the 6°C silo was quickly followed by the mortality of about one third of the group. None of the larvae raised at 6°C survived to metamorphosis.

Discussion

Growth and temperature

The growth curves appear similar to those in the literature for Pacific and Atlantic halibut (Forrester and Alderdice, 1973; Blaxter *et al.*, 1983). The standard length at EYS varied inversely with temperature, while the time necessary to complete absorption of the yolk was shortest for the group receiving 9°C water. This seems to concur with the observations of Peterson *et al.* (1977) that, in salmon (*Salmo salar*), growth rate slows at higher temperatures and the time required to absorb the yolk decreases with increasing temperature. As with salmon eggs and alevins, fewer day degrees are necessary to produce halibut larvae of a given size when there is a longer time from hatching to yolk absorption.

Significantly larger larvae at EYS were produced using water of 4°C. However, the first half of the yolk-sac stage of all the groups was presumably affected by the initial temperature of 6°C. This may be the reason for the similarity of the growth curves during the beginning of the experiment, which gradually changed as the temperature effects could alter metabolic rates and enzyme affinities (Hochachka and Somero, 1971). It is

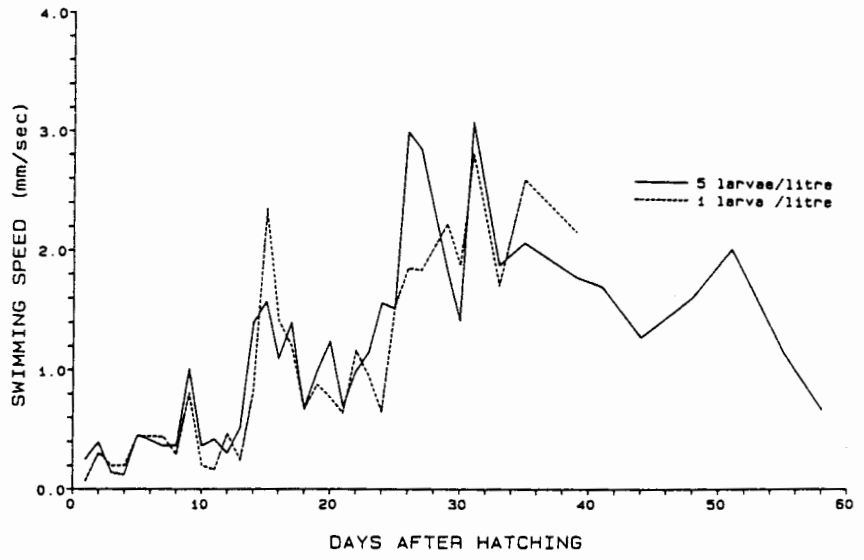

Figure 7. Mean swimming speed of halibut larvae held at two densities.

Table 4. General survival during the experiment. The day of prey addition is considered the point of start feeding, but the number of larvae feeding is not given. Total mortality is based on bottom water counts and estimated percent loss is the difference between the estimated initial number of larvae and the number accounted for. Percent survival is the amount surviving until start-feeding.

Mean silo temp °C	Day of prey addition	Estimated initial number of larvae	Mortality at day of prey addition	Total mortality	Estimated %loss	% survival
4	49	35 000	9546	12 101	65	21[1]
				18 000	49	47[1]
6	39	35 000	11 450	21 866	38	48[2]
	42		19 359			11[2]
9	33	35 000	12 392	16 241	54	24

[1] About 6000 larvae were transferred from this silo on days 49 and 51. Most of these died. If this estimate is included in the calculations, then the amount of larvae for which we cannot account becomes 49%, and the percent surviving until the addition of prey items is 47%.
[2] High mortality rates coincided with the addition of light (the system short-circuited) and prey. The first prey items were introduced on day 39, but 3 d later mortality had increased by about 8000. No feeding was observed during these 3 d.

conceivable that if 4°C is near an optimal temperature for the early stages of egg and larval development, low temperatures in both the hatchery and the early yolk sac stage should be used. The advantages of this are balanced by the disadvantages of the time factor.

It may be significant that 4°C closely resembles the temperature of the water where the station's broodstock used in this experiment are assumed to spawn in nature. Stock differences in optimal temperatures occur in other species and the amenability of fish to various manipulations clearly depends on the natural history.

Deformities

A significant number of the larvae raised at 9°C were deformed, displaying both edematous conditions and jaw deformities. Such anomalies in association with temperature experiments have been described previously by Alderdice and Velsen (1971) and Peterson *et al.* (1977). In Pacific herring larvae, *Clupea pallasi*, groups raised at low temperatures of 4.7°C and 4.0°C had jaw deformities consisting of either the absence of a lower jaw or retarded development and continual gaping (Alderdice and Velsen, 1971). The larvae in their Figure 3 (b and c) bear a striking resemblance to the jaw deformities experienced in the present experiment, to the inclusion of what appears to be an edematous condition in one of the pictured larvae. The edematous condition of salmon larvae subjected to large temperature decreases (Peterson *et al.*, 1977) is proposed as associated with osmotic failure at low temperatures.

Mortalities

Early and high mortalities were experienced in all silos, with the usual peak in larval mortality around 15 d after hatching (Pittman *et al.*, 1987). However, a period of high mortalities between 18 and 40 d after hatching at 9°C contrasted with fairly low mortalities during that period in the other two silos. The high losses of the 6° group (10 000 larvae over a four-day period) about days 39–42 coincided with the installation of the lighting system and the introduction of Rotatoria as prey items, as well as some short-circuiting of the lighting system.

All our mortality figures are actually the number of larvae found in the bottom water, extracted about every second day. This is not equivalent to the mortality at a given time, since a fraction of the larvae may decompose within the system or float for a period prior to sinking into the bottom water. Larvae with edema were almost never found in the bottom water because they float until decomposing. Dead larvae may also settle on the sloping walls of the rearing unit and contribute to the general fouling, rather than settle on the bottom. The deformities, lockjaw and edema, will allow the larvae to survive until yolk exhaustion, after which their inability to capture prey will lead to mortality. This may be misinterpreted as failure to start-feed, while the real cause of the problem may lie as far back in time as the egg stage. Examination of the dead larvae may give some insight into the problems posed by certain rearing systems. However, survival to start-feeding is not the same as ability to start-feed successfully.

Scanning electron microscopy

The branchial cavities of the early larval *Hippoglossus hippoglossus*, in their initial posthatching form, bear a strong resemblance to the embryonic cavity and branchial chamber of the embryonic killifish, *Fundulus heteroclitus*, as described by Guggino in 1980. He states that the 10-day-old killifish has a pair of branchial chambers which communicate anteriorly with the phar-

ynx through the embryonic gill slits and posteriorly with the perivitelline space by means of pores, such that a channel is available for water to enter the pharynx and the rest of the alimentary system. Although not conclusive, the presence of similar branchial chambers on Atlantic halibut would seem to support and provide a mechanism for Tyler and Blaxter's (1988) finding of drinking ability in halibut larvae from 3 to 7 d old.

There was no SEM evidence of bacterial growth on, or bacterial decomposition of, a buccal valve. Examination revealed tissue damage like that caused by enzyme activity, which seemed not to affect the cartilage underlying the afflicted tissue.

Behaviour

Halibut larvae are passive during the early part of the yolk-sac stage, and generally lie still in the water column with the head downward. When disturbed, the larvae swim for about 5 s and then lie passively again for about 2 min. Swimming was observed from hatching onward. The length of the swimming period remains constant throughout the first 30 d of the yolk-sac stage, and is longer at higher larval densities. As the larva develops both a streamlined form and larger pectoral fins, the swimming becomes more frequent, faster, and lasts longer. The passive periods are longer during the early yolk-sac stage.

Swimming was often a response to a disturbance such as contact with the walls or other larvae and the sudden exposure to light. The behavioural observations of the early yolk-sac stage needed a certain amount of light to see the larvae. The larvae do not have completely pigmented eyes until the latter half of this stage, so that swimming in the early stage may have been a response to stress by the illumination, although this is difficult to ascertain. In general, changes in oxygen or salinity levels, noise, and the effects of transport may also be expected to disturb swimming frequency and speed.

The "S" shape assumed by the larvae is interpreted as an attack position for feeding activity. A horizontal "C" shape where the tail vibrates rapidly may be interpreted as a stress reaction in the larvae, since it is often the response to sharp light, such as a flashlight. This has been stated by our own and other workers, but is unpublished.

Growth and activity

The energy devoted to swimming is least during the early yolk-sac stage, when the length data show that growth at different temperatures is approximately equal. This indicates that energy is not limiting at this time, and that different activity levels within a limited range will not have much influence on growth during the early yolk-sac stage.

There is a progressive increase in the frequency of the active periods as larvae develop, and the growth curves of the different temperature groups begin to separate around day 20; thus the larvae seem to be entering a phase where energy is a limiting factor. Increased metabolism at this point, whether due to high activity or other factors, will produce a measurable difference in the size of halibut larvae at yolk absorption. It is therefore suggested that, during the latter part of the yolk-sac stage, larval density should be as low as possible, the temperature cold, and the disturbances minimal to give large and healthy halibut larvae.

Conclusions

The group of yolk-sac halibut (*Hippoglossus hippoglossus*) grown at an average temperature of 4°C were significantly larger at EYS than the groups raised at 6 and 9°C. The number of deformities was also less in this group whether measured on dead or live larvae. Use of relatively cold water in the hatchery and early larval stages may improve overall survival in future experiments.

A pair of external cavities in the branchial area was found and followed on larval halibut through days 3–31. It is suggested that, in the absence of a stomodeum, halibut larvae may be capable of drinking through these structures even earlier than a few days after hatching.

The frequency and duration of active periods increase as larvae absorb the yolk supply and energy concurrently becomes a limiting factor. It is therefore suggested that larval density and temperature be kept low and disturbances minimal during the latter half of the yolk-sac stage in order to produce large and healthy halibut larvae at start-feeding.

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