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Incubation of halibut yolk sac larvae improved by addition of freshwater and oxygen

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Halibut fry has been produced regularly by scientific and commercial institutions since late 80-ties. The yearly number of halibut fry produced have, however, not fulfilled the optimistic expectations. This could partly be explained by lack of appropriate yolk sac rearing methods. Halibut larvae has, compared to other marine fish species, a long-lasting yolk sac period. Newly hatched larvae are poor developed and are very sensitive to handling. Two main methods have been used for halibut yolksac rearing; small stagnant and large flow-through incubators. The flow-through incubator has recently been modified, resulting in higher survival rates and simpler operating procedures. The modifications include a flow-through salinity gradient and thereby exclusion of the traditional mechanical outlet sieve. Oxygen is added at the bottom of the incubators to promote an ideal free distribution of the larvae in the water column and to avoid low oxygen tensions.

INTRODUCTION

Halibut larvae has, compared to other marine fish species, a long yolksac stage. The period from hatching to preferred first feeding lasts approximately 44 days at 6 °C. Newly hatched larvae are poor developed (Pittman et al 1990) and are very sensitive to handling (Opstad and Raa 1986), and therefor the halibut eggs are normally transferred to the yolk-sac incubators before hatching. In a period (day 8-12 after hatching) the larvae tend to aggregate in the bottom of the incubator. Two main methods have been used for halibut yolk-sac rearing; small stagnant units (Holmefjord et al 1993) and large flow-through incubators (Harboe et al 1994).

When a halibut egg hatch, the larvae float towards the surface. In this very sensitive period, physical contact with the outlet sieve and also contact with the surface layer is fatal for the larvae. To avoid contact between the larvae and the surface, a layer of lower salinity water has traditionally been introduced in the top of the incubator (Fig 1B). Contact with the outlet sieve is however more difficult to avoid. The eggs are semipelagic and need a lift made by the waterflow or by other means in order to maintain position in the water column. Stagnant conditions is not recommended since the eggs will sink to the conical bottom of the incubator and contaminate. The traditional way to overcome this, is to lower the outlet sieve so that the larvae after hatching float into a stagnant layer under the lower salinity layer but above the outlet sieve (Fig. 1B, sieve position 1). Three to six days after hatching (varying between groups) the larvae distribute more evenly in the water column and the water flow can be re-established in the upper layer, by raising the sieve (Fig. 1B, sieve position 2). During this period the water quality in the stagnant layer is reduced. The oxygen content is reduced and ammonia level increased dependent on larvae density.

There is a reduction in specific weight during transition from egg to larvae. This reduction corresponds to a salinity of 1,5 to 2 ppt (Mangor-Jensen and Huse 1991). A newly hatched halibut larvae is neutrally buoyant at a salinity of approximately 32 ppt. By adding freshwater gently and continuously at a certain depth (30 - 50 cm) in the incubator in a constant mass relation with up-flowing seawater, one can decide the salinity of the outlet water (Fig. 1). If the water that surround the outlet sieve has a salinity lower than 32 ppt, the vulnerable larvae will not get in physical contact with the sieve. This principle was investigated in the following experiment.

In order to re-distribute the larvae in the water column in the period when they are located in the conical bottom of the incubator, addition of water with higher salinity and addition of oxygen bubbles are tested.

The current paper presents a flow-through system with total renewal of water without outlet arrangements that harm the larvae (flow-through salinity gradient). Also a method to distribute the larvae in the water column is described.

MATERIAL AND METHODS

Experimental design

The egg material originated from a photomanipulated broodstock (Næss et al.1997). The females were relative small and produced egg-batches of 1 to 1.3 litre. To achieve a certain larvae density in the incubators, incubator 1 and 2 were incubated with eggs from one female and incubator 3 and 4 with eggs from another. Both egg batches were fertilised with sperm from two males. The eggs were kept in an upstream egg incubator in 11 days at 6°C. Than they were disinfected with glutaraldehyde according to Harboe et al 1994, split into two parts, each of 22000 eggs (0.55 litre) and transferred to the yolk-sac incubators (silos).
Two of the incubators had a continuously renewed salinity gradient and two had outlet sieves. To redistribute the larvae in the period when they were located near the bottom, both oxygen and higher saline water was investigated (Table 1).

Description of the incubators

The upstream incubators used is made by fibreglass and consists of a cylindrical upper part and a conical lower part (Harboe et al. 1994, Fig. 1). The incubator is 4,7 m^3, 150 cm in diameter by 400 cm high. The inlet diameter is 15 cm. The outlet sieves are of polyethylene covered with 500 µm-mesh plankton netting. In the period from incubation of eggs, through hatching and until the larvae are evenly distributed in the water column, the outlet sieve were placed in a position 1.5 m below the surface (Fig. 1B). Thereafter the sieve is located below the freshwater layer (Fig. 1B). The silos have lids with access ports on the top. There are complete darkness in the silos except during daily observation of the larvae.

Freshwater was continuously added through a 40 mm pipe. The pipe is blinded in the bottom and has three bands of orifices. The diameter of the orifices are 2 mm and the distance between the bands are 150 mm. The flow rate of both sea and freshwater is adjusted with valves according to the readings of flowmeters.

Water quality

Seawater was taken from 55 m depth, pumped through a sand filter and heat pump and than into column aerators and header tanks. Water temperature was 6.0 ± 0.5 C. Two of the incubators received water with 0.6 ppt higher salinity than the two other incubators. The salinity was 34.1 ± 0.2 and 34.7 ± 0.1 ppt respectively.

Oxygen was added trough ceramic diffusers placed in the bottom of the incubators. Addition of oxygen was in the range of 5 to 15 ml per min.

Operating procedures

Once a day the larvae were observed by use of a flashlight. The volume of the incubator is divided into 5 parts (0-30 cm, 30-100 cm, 1-2 m, 2-3m and near the inlet, Fig. 1.), and an visual evaluation of the distribution between the different parts was given in percentage. Water and oxygen flow rates were controlled twice a day. Salinity measurements were also done daily by use of salinometer and corrected twice a week by the Winkler method. Dead eggs and larvae were removed and counted daily by flushing 30-50 litre of water from the bottom of the incubators.

RESULTS

Salinity and temperature

There were at all measurements significant difference in salinity between the increased and ambient water (Fig. 2). At the time of shift from ambient to increased salinity the ambient water had a salinity of 33.67 ppt and the increased salinity was 34.69 ppt. The temperature in all 4 incubators were identical through the entire experiment (6.0 ± 0.5 C).

Water flow-rate

Inlet flow rate for incubator 1 and 4 (with outlet sieve), was 2 litre per min from hatching to day 7. From day 8 and for the rest of the yolk sac period, the flow was 4 litre per minute. For incubator 2 and 3 (addition of freshwater) the seawater flow was the same as for incubator 1 and 4, and freshwater flow was 2/3 of seawater flow.

Egg stage and hatching

The mortality from fertilisation to transfer to yolk-sac incubators were 21 and 15 % for the egg-batch going to incubator 1, 2 and 3, 4, respectively. The eggs in incubator 1 and 2, had an evenly distribution in the water column. Hatching was completed within 2 days. The eggs in incubator 3 and 4, were located in the conical part and kept dispersed in the deepest sector by the water current. Hatching in these incubators lasted for 3 days.

Survival during yolk-sac period

The larvae in incubator 2 and 3 (salinity gradient) had lower mortality compared to incubator 1 and 4 (Fig. 3). At the end of the period survival rates were 65 % and 59 % in incubator 2 and 3. Incubator 1 and 4 had 28 % and 26 % survival, respectively. Mortality occurred during hatching and in the period when the larvae were located deep in the incubator. The mortality from day 12 was low in incubator 3 and 4 (oxygen addition) compared to incubator 1 and 2 (increased salinity).

Egg and larvae distribution

The distribution of the larvae in the water column varied during the incubation period (Fig. 4). The eggs in incubator 1 and 2 were distributed evenly in the water column, whereas the eggs in incubator 3 and 4 were located deep. After hatching, the larvae were located high in all

incubators, and gradually dispersed deeper in the water column. At day 11 after hatching, the larvae were located deep in all incubators. Water with increased salinity was then added incubator 1 and 2. The day after the larvae were redistributed in the water column, but the effect of increased salinity gradually disappeared. After oxygen was added to incubator 3 and 4, the larvae redistributed into the water column and kept that distribution throughout the period (Fig. 4).

Larval quality

At the end of the yolk-sac period, proportion of jaw-deformed larvae was 42 and 66% for incubator 1 and 2, 15 and 1 % for incubator 3 and 4.

The size (dry-weight) of the larvae at 270 day-degrees after hatching were 1.2 mg (±0.5), 1.2 mg (±0.6), 1.1 mg (±0.5) and 1.1 mg (±0.5) for incubator 1, 2, 3 and 4, respectively.

DISCUSSION

Larval survival was significantly improved in the incubators with a flow-through salinity gradient, compared to traditional outlet sieve. Addition of freshwater in the water column creates horizontal density gradients. Water movement / turbulence is mainly a function of flow rate, and larval movement was observed in the period close after hatching when the larvae were located in the salinity gradient. However, a salinity decrease down to 31 ppt is enough to avoid halibut larvae from the water outlet. A low freshwater flow rate is then needed and hence little freshwater and thereby little larval movement is created. By use of a flow-through salinity gradient there are no physical contact with any outlet arrangements, and good water quality is maintained in the entire incubator throughout the rearing period. Operating procedures are also easier, compared to the traditional way of running the incubators (Rabben, Jelmert and Huse 1987; Harboe et al 1994). This is supported by the reduced incidence of damages on the dead larvae removed by tending.

Larval distribution in the water column has been a subject of investigations for several years. The reasons for an altered distribution can be a change in specific weight and active swimming of the larvae. To achieve an evenly distribution of the larvae in the water column increased salinity was compared to addition of oxygen. The day after the salinity was increased, the

larvae had an evenly distribution. However, already the next day the larvae had aggregated into a deeper position (Fig. 4). In the incubators were oxygen was added, the larvae distributed evenly in the water column and maintained the distribution throughout the yolk-sac period. Halibut larvae are positive rheotactic, and it is therefor possible that the reason for the deep location in the period from approximately day 9 after hatching, is active swimming towards the water inlet. By adding water with higher density, the waterflow become more laminar for a period. When the entire volume of the incubator is filled with more saline water, the laminar flow is lost. By addition of small oxygen bubbles, water turbulence is created and the larvae cannot orient and swim towards the same direction.

A serious problem in halibut larvae culture is occurrence of jaw-deformities. Larvae suffering of this deformity are moribund. Temperature (Lein et al 1997), oxygen (Jelmert 1996), abrasion of the head (Morrison and MacDonald 1995) and salinity (Lein et al. 1998; Ottesen and Bolla 1998) are parameters reported to affect occurrence of this deformity. In this experiment the incubators with a flow-through salinity had a higher occurrence of deformed larvae. Other experiments (own observations) indicate that salinity's below 30 ppt should be avoided. This is in agreement with Lein et al (1998).

By use of this method one take advantage of the lacking ability of the larvae to float in lower saline water. Flow-rate of freshwater is determined of the specific weight of the eggs and larvae. Hatching of halibut eggs can easily be synchronised by use of light (Helvik and Walter 1991), but light exposure of halibut eggs affects their specific weight (Mangor-Jensen and Huse 1991). The eggs become heavier, and therefore need water up-flow prior to hatching. The combination of high flowrate and outlet sieve leads to clogging of newly hatched larvae on the sieve. This are overcomed by use of a flow-through salinity gradient, and this improved method opens therefor for the use of light synchronised hatching.

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Table 1. Experimental setup. Numbers refer to period of treatment (days).

Outlet sieve refer to the traditional incubator with stagnant layer and **Salinity gradient** to the flow-through layer of brackish water.

	Incubator			
	1	**2**	**3**	**4**
Outlet sieve	(0-43)			(0-43)
Salinity gradient		(0-43)	(0-43)	
Oxygen			(10-43)	(10-43)
Increased salinity	(10-43)	(10-43)		

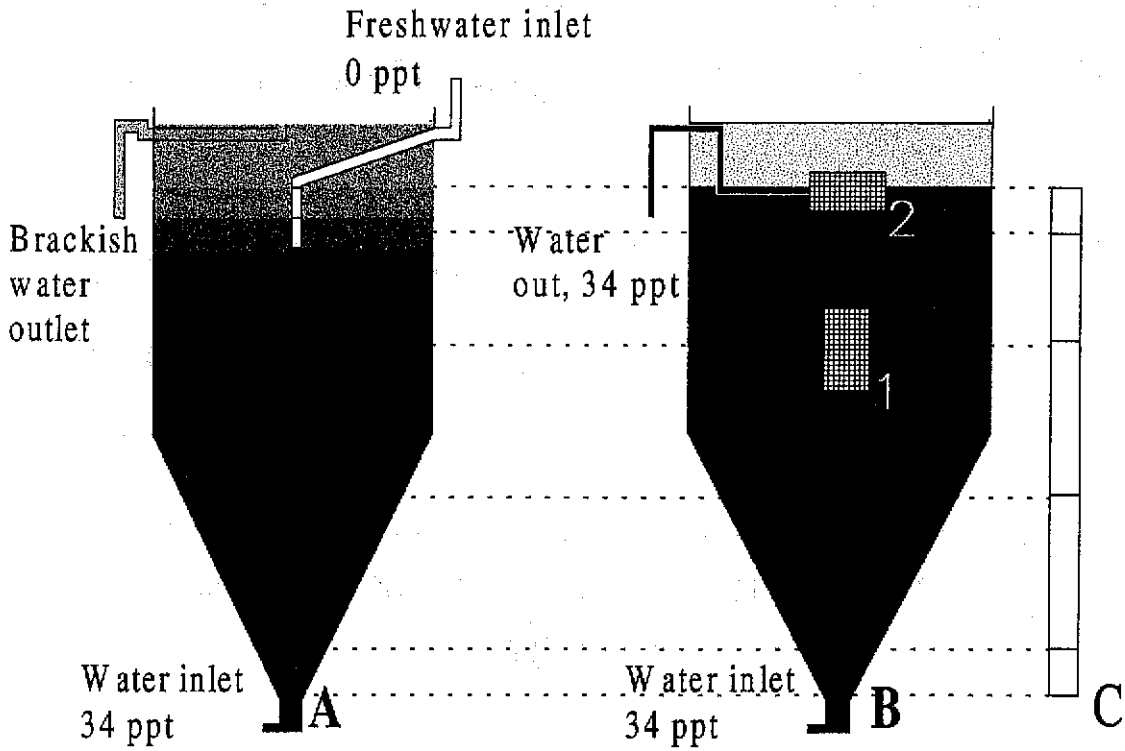

Fig. 1. Silo design and operation principle. Type A with flow-through salinity gradient (incubator 2 and 3). Type B is the traditional silo. Sieve position 1 was operated days - 2 to 3, position 2 days 3 to end of experiment. Freshwater is indicated above position 2. The stacked bars indicate the sectors for larval distribution registrations. A, B and C are in scale. See text for further details.

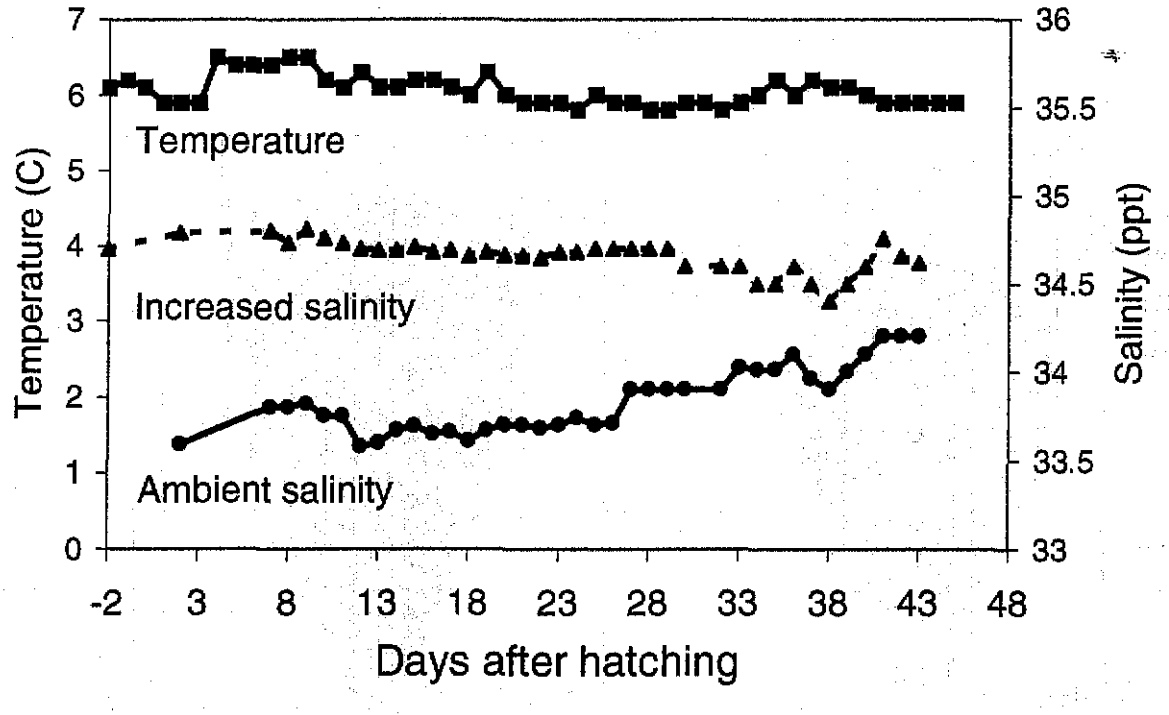

Fig. 2. Temperature (upper curve, left abcisse) and salinities (right abcisse) during the experiment.

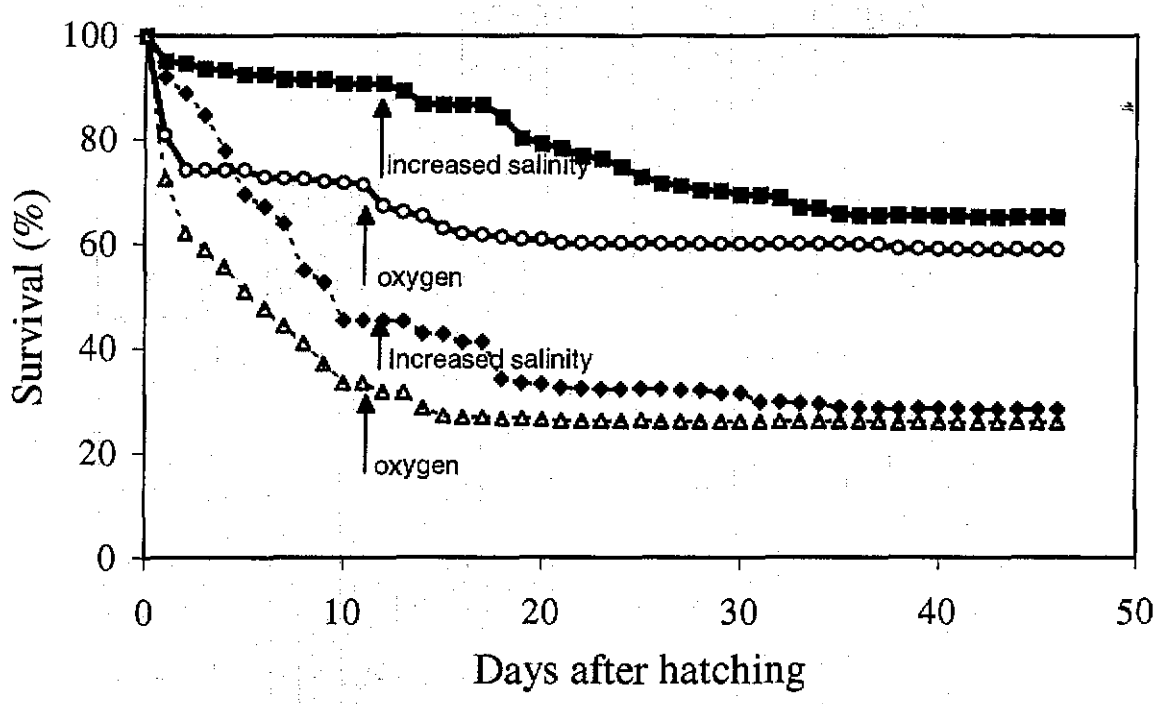

Fig. 3. Survival in the experiment. Incubator 1 (filled diamonds, dotted line), 2 (filled squares, solid line, 3 (open circles, solid line) and 4 (open triangles, dotted line). Filled symbols denote increased salinity from day 10 (arrow), open symbols addition og diffused oxygen from day 10 (arrow). Dotted lines are incubators with traditional outlet sieves (Fig. 1B) while solid lines are incubators with a flow-through salinity gradient (Fig. 1A).

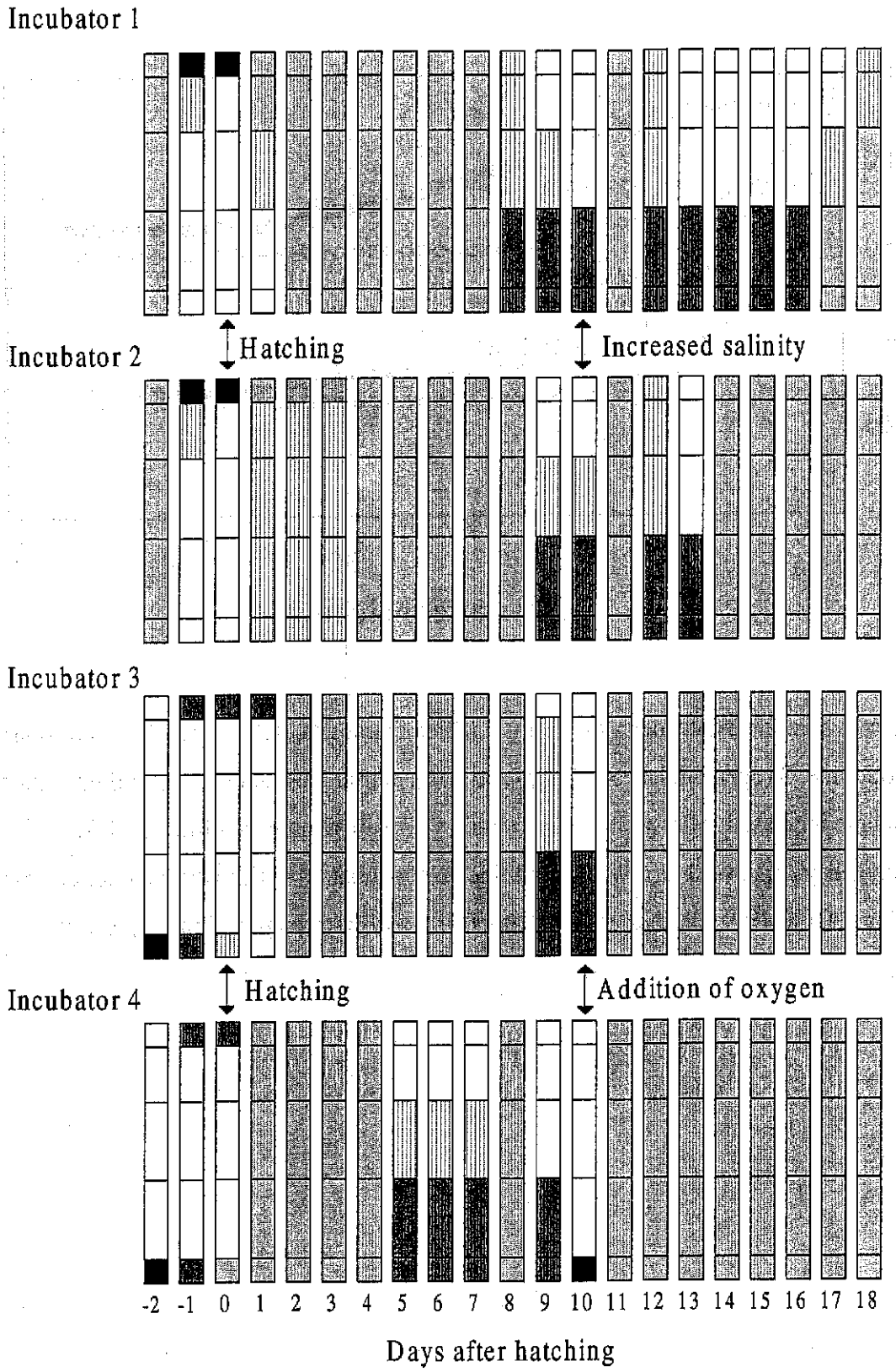

Fig. 4. Vertical distribution of larvae in the incubators. Sector boundaries are shown in Fig. 1. Hatching changed water quality (increased salinity and addition of oxygen) is indicated. = 0-9 %, = 19-10, = 20 %, = 21-60 % and = 61-100 % of the larvae