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Large-scale rearing of Atlantic halibut, *Hippoglossus hippoglossus* L., yolk sac larvae: effects of flow rate on growth, survival and accumulation of bacteria

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Abstract

The role of flow rate in large-volume production units (2800 L silos) for Atlantic halibut, *Hippoglossus hippoglossus* L., larvae was studied. Correlations between flow rate, bacterial numbers (a measure of water quality), the larval growth and development rates, and mortality were assessed. The experiment included a total of six silos, two each at three different flow rates. Flow rate and mortality were positively correlated: the number of dead larvae on day 30 was highest (i.e. 2200 and 2000) in the silos with highest flow rate ($8\,L\,min^{-1}$) and lowest (i.e. 1300 and 1200) in the silos with the lowest flow rate ($2\,L\,min^{-1}$). Larval weight was negatively correlated with flow rate: on day 30, the mean dry weight was 968 µg in the silo with the lowest flow rate and 820 µg in the silo with highest flow rate. Yolk sac utilization efficiency was 92% in the silo with the lowest flow rate and 72% in the silo with the highest flow rate. The number of bacteria were highest ($2.7–10^6\,mL^{-1}$) in the incubators with the lowest flow rate.

Introduction

The yolk sac stage in Atlantic halibut, *Hippoglossus hippoglossus* L., lasts for 265 degree-days. During this lengthy prefeeding stage, Atlantic halibut reared in intensive culture systems exhibit elevated and highly variable mortality (20–50%), and a large fraction of larvae develop deformities (Pittman, Skiftesvik & Harboe 1989; Pittman, Bergh, Opstad, Skiftesvik, Skjolddal & Strand 1990a; Pittman, Skiftesvik & Berg 1990b). Atlantic halibut hatch at an early and relatively undeveloped embryonic stage (Pittman *et al.* 1990b). These fish are highly sensitive to changes in the rearing environment, such as handling (mechanical stress), or alterations in temperature or salinity (Opstad & Raae 1986; Bolla & Holmefjord 1988; Pittman *et al.* 1989). The larvae are also susceptible to infections by opportunistic bacteria, which appear to underlie at least some of the observed mortality (Bergh, Hansen & Taxt 1992). Such bacteria tend to accumulate in the incubation vessels of intensive rearing systems (Bolinches & Egidius 1987).

At Austevoll Aquaculture Research Station, Storebø, Norway, Atlantic halibut yolk sac larvae are cultured in large (2800 L) silo-style incubators with a slow up-welling water flow (Rabben, Jelmert & Huse 1987; Harboe, Tuene, Mangor-Jensen, Rabben & Huse 1994). Results from smaller silo-style upwelling incubators (250 L) have indicated that larval mortality is positively related to flow rate, while the relationship between the rate of yolk sac utilization and flow rate is negative (Opstad & Bergh 1993). Flow rate also influences bacterial numbers and water quality, and this, in turn, may affect larval mortality and quality. Therefore, careful evaluation of flow rates is of central importance to working out protocols for the large-scale intensive rearing of Atlantic halibut larvae.

The purpose of the present study was to investigate the effect of different flow rates on larval

survival and development, and on the accumulation of bacteria in large-volume, silo-style production units (2800 L silos).

Materials and methods

Egg source and incubation

Fertilized Atlantic halibut eggs were obtained by stripping one male and one female from the broodstock maintained at the Austvoll Aquaculture Research Station. Approximately 94% of the eggs were fertilized. Eggs were incubated in 250-L up-welling incubators, as described by Jelmert & Rabben (1987). The water in the incubation system was pumped from a depth of 55 m and passed through sand and microfilters (5 μm). The temperature was 7.5 ± 0.5 °C and the salinity was 32 ± 1‰. The mean diameter of the fertilized water-hardened eggs was 3.0 mm. The eggs were transferred from the egg incubators to the yolk sac larval experimental units 2 days before 50% of the eggs had hatched (day 0). The hatching success was 97%.

Experimental conditions

The experimental silos were semiconical, 2800-L fibreglass units with black interior walls (Harboe *et al.* 1994). The water temperature was 7.5 ± 0.2 °C and salinity was 32 ± 1‰. Each tank was stocked with 0.2 L of eggs (i.e. ≈7000 eggs). The larvae were kept in darkness until day 30, when the experiment was terminated. The six silos were divided into three groups, with two silos in each group, according to the flow rate of water being up-welled through them. The flow rates were 2, 4 and 8 L min^{-1} for units A1 and A2, B1 and B2, and C1 and C2, respectively.

Mortality

Dead larvae were removed from the silos every day according to the method described by Harboe *et al.* (1994). The water flow was stopped, and 50 L of saline water (≈42‰) was introduced from the bottom and left there for ≈10 min. Dead larvae sedimented out at the bottom and were removed, along with the high salinity water, using a valve assembly. The water flow was then immediately resumed and adjusted to the previous flow rate. This procedure took ≈30 min. Water removed from the silos in this way was carefully filtered and the number of dead larvae were counted on the same day.

Counts of bacteria

Water samples (20 mL) from each silo, drawn from a depth of ≈40 cm, were fixed with formaldehyde at a final concentration of 3.7%. In order to evaluate the bacterial load in the water source for the six silos, samples were also taken from the common header tank. These samples were filtered onto 0.2-μm Nuclepore filters which were prestained with Irgalan Black. Thereafter, the bacteria were stained with 4'6 diamidino-2-phenylindole (DAPI) following the methods of Porter & Feig (1980). Bacterial counts were made using a Nikon epifluorescence microscope at × 600 magnification and a minimum of 200 cells were counted from each sample.

Dry weight and jaw deformities

On day 1, 48 newly hatched larvae were sampled randomly from each silo for the measurement of dry weight (freeze-dried individuals). Another 48 newly hatched larvae were sampled randomly from each silo on day 30 after hatching. These larvae were washed in distilled water and frozen (–20 °C). The yolk sac and larval body were dissected and weighed separately using an electrobalance (Mettler M 3, Mettler Instrumente, Greifensee, Switzerland, with an accuracy of ± 1 μg). The yolk conversion efficiency (YCE) was calculated from these data following the method of Blaxter (1969) and using the formula:

dry weight increment of body/dry weight decrement of yolk × 100

On day 30, the larvae were randomly sampled from each silo (Table 1) for determination of jaw deformities according to Pittman *et al.* (1990b).

Statistical methods

Between-silo differences in the YCE and in the percentage of larvae with jaw deformities were tested using Student's *t*-test after arcsine transformation of the data. The mean dry weight of whole larvae, larval body and yolk sac were

compared by using Student's *t*-test (Sokal & Rohlf 1981). The significance level applied was $P < 0.05$ in all tests.

Results

Mortality

Mortality was positively related to flow rate: the number of dead larvae on day 30 was highest (i.e. 2200 and 2000) in the silos with highest rate of flow ($8\,L\,min^{-1}$) and lowest (i.e. 1300 and 1200) in the silos with lowest flow ($2\,L\,min^{-1}$) (Fig. 1). The highest mortality occurred between days 3 and 16 in all groups. After day 16, the mortality was negligible.

Table 1 Silo number, rate of flow and percentage yolk conversion efficiency (YCE) in each silo, and percentage of larvae with jaw deformities in each silo on day 30

Silo	Rate of flow ($L\,min^{-1}$)	YCE (%)	Jaw deformities (%)
A1	2	69 ($n=46$)	36 ($n=87$)
A2	2	93 ($n=47$)	30 ($n=93$)
B1	4	87 ($n=47$)	34 ($n=76$)
B2	4	81 ($n=47$)	49 ($n=79$)
C1	8	72 ($n=47$)	13 ($n=72$)
C2	8	73 ($n=47$)	22 ($n=68$)

Bacteria

The highest total counts (2.7×10^6 bacteria mL^{-1}) were found on day 16 in water from the A silos, which had the lowest rate of flow (Fig. 2). Except from this peak, the water in the silos showed relatively small variations with respect to bacterial numbers during the experiment. The number of bacteria in the inlet water for all the silos varied between 2.7×10^5 and 7.4×10^5 bacteria mL^{-1}. During most of the experiment, the inlet water had less bacteria than the water of the silos.

Jaw deformities

The lowest percentages of larvae with jaw deformities on day 30 were observed in silos C1 and C2 (Table 1). Silo B2 had a significantly higher number of larvae with jaw deformities than silos A2, C1 and C2 ($P < 0.05$). The numbers of larvae with jaw deformities in silos A1 and B1 were significantly higher than for silo C1 ($P < 0.01$).

Dry weight

Larval dry weight was inversely related to flow rate. The mean (± SD) body dry weights were $138.7 \pm 20.4\,\mu g$ (10% of total dry weight) on day 1. On day 30, the mean dry weight of the larvae in group A2 ($2\,L\,min^{-1}$) had increased to $968.0 \pm 96.7\,\mu g$ (76% of total dry weight) (Fig. 3). This was the highest mean dry weight of all the groups. Silo A2 had significantly higher body dry weight than B1 and B2 (Student's *t*-test, $P < 0.02$). Both silos B1 and B2 had

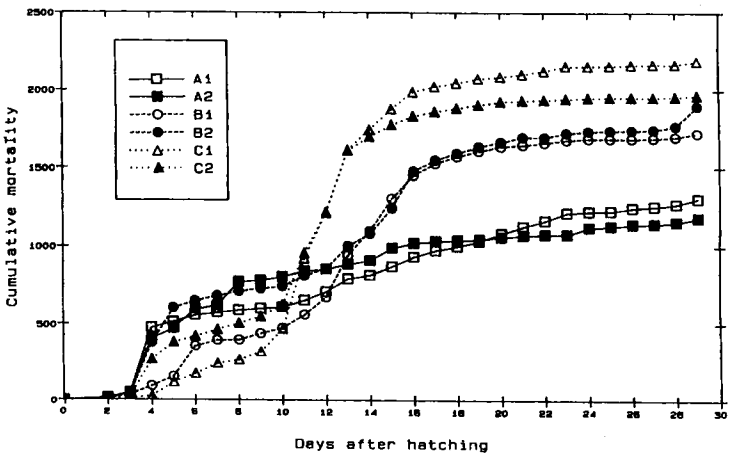

Figure 1 Cumulative mortality of Atlantic halibut yolk sac larvae (number of dead larvae) in the silos with different rates of flow from days 0 to 30. The flow rates were $2\,L\,min^{-1}$ (silos A1 and A2), $4\,L\,min^{-1}$ (silos B1 and B2) and $8\,L\,min^{-1}$ (silos C1 and C2).

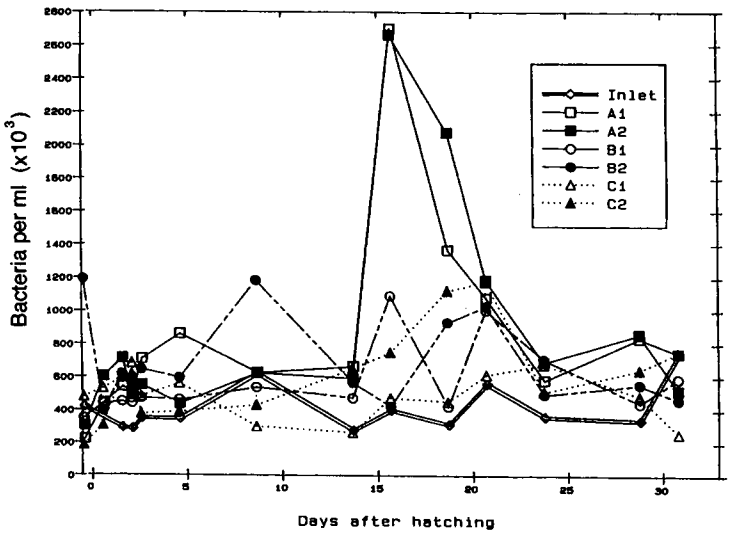

Figure 2 Total counts of bacteria in each of the silos (bacteria $\times 10^3$ mL^{-1}) with different rates of flow and in the inlet water from days 0 to 30. The flow rates were 2 L min^{-1} (silos A1 and A2), 4 L min^{-1} (silos B1 and B2) and 8 L min^{-1} (silos C1 and C2).

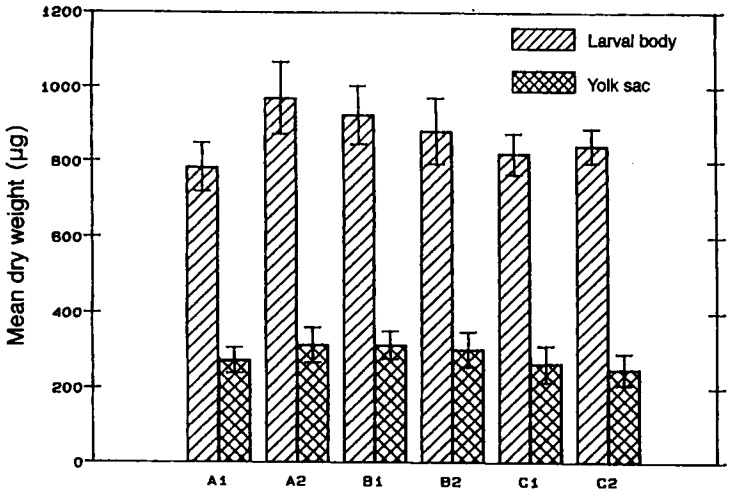

Figure 3 Dry weight (µg) of larval bodies and the yolk sac of larvae from each of the silos on day 30. The values are the mean ± SD. The flow rates in the silos were 2 L min^{-1} (silos A1 and A2), 4 L min^{-1} (silos B1 and B2) and 8 L min^{-1} (silos C1 and C2).

significantly higher body dry weight than silos C1 and C2 (Student's *t*-test, $P < 0.007$). Silo A1 was an exception and had the lowest dry weight of all groups (782.7 ± 63.6 µg).

Yolk sac dry weight also appeared to be inversely related to flow rate. The dry weight of the yolk decreased from 1208.6 ± 75.3 µg on day 1 to 247.4 ± 41.1 µg on day 30 in silo C2. This was the lowest yolk dry weight of all silos. Yolk sac dry weights in silos B1 and B2 were significantly higher than in silos C1 and C2 ($P < 0.0002$). Silo A2 had significantly higher yolk dry weight than silos C1 and C2 ($P < 0.006$). The yolk sac dry weight in silo A1 was significantly lower than the yolk dry weights in silos A2, B1 and B2 (Student's *t*-test, $P < 0.01$).

With the exception of silo A1, all YCEs were generally inversely related to flow rate. The highest YCE (93%) was found in the silo with the lowest flow rate (A2) (Table 1), and was significantly higher than the YCEs in silos C1, C2 and A1 ($P < 0.004$). The lowest YCE was found in silos C1 and C2 (72% and 73%), which had the highest flow rates. The silos with moderate flow rates (B1 and B2) had intermediate YCEs. The YCE for silo A1 could not be distinguished from that of silo B2. Silo A1 had significantly lower YCE than silo B1 ($P < 0.03$).

Discussion

The present results demonstrate a direct relationship between flow rate and mortality in large-scale rearing of Atlantic halibut yolk sac larvae. The larval groups reared under the highest flow rates (groups B and C) exhibited the highest mortality between days 10 and 16. This is consistent with results obtained from a smaller-scale experiment in which Atlantic halibut larvae were reared in up-welling incubators (Opstad & Bergh 1993). During the early life history of Atlantic halibut, the period between 10 and 16 days post-hatch is associated with high mortality (Blaxter, Danielsen, Moksness & Øiestad 1983; Pittman *et al.* 1990a,b; Bergh *et al.* 1992; Opstad & Bergh 1993). This has also been described as a sensitive period in the development of Atlantic halibut larvae; during this period, the fish are especially sensitive to any kind of stress (Pittman *et al.* 1990a; Kjørsvik & Reiersen 1992).

With the exception of silo A1, the YCEs were inversely related to flow rate. A decrease in the rate of yolk absorption is a typical stress response in marine fish larvae (Rosenthal & Alderdice 1976), suggesting that increases in flow rate stress Atlantic halibut larvae. The direct relationship between mortality and flow rate is consistent with this interpretation.

The larval body dry weight and yolk sac dry weight were inversely related to flow rate. These results are similar to those from a previous experiment conducted in 250-L incubators (Opstad & Bergh 1993). However, no significant differences in the dry weight of the yolk sac were found in the earlier study. This might be explained by the different range of flow rates used in the two series of experiments and/or the different incubator systems used. For larvae reared at higher flow rates, it is likely that a larger proportion of yolk sac energy reserves is used for swimming activity.

Bacterial numbers *per se* could not explain the mortality observed in silos B1, B2, C1 and C2 since the counts in these silos never exceeded 1.2×10^6 bacteria mL^{-1} (which is below the bacterial cell counts that result in infection (Bergh *et al.* (1992). However, in silos A1 and A2, bacterial numbers exhibited a sharp peak near day 15, rising to $\approx 2.7 \times 10^6$ bacteria mL^{-1}. This exceeds the bacterial cell counts which resulted in 60–100% mortality in the pathogen challenge experiments reported by Bergh *et al.* (1992). Thus, if the bacterial community was comprised of a high number of opportunistic pathogens, it is likely that the larvae could have been affected by such numbers of bacteria. However, the bacterial species composition in the incubators was not examined and this would have been necessary to confirm this hypothesis.

Conclusion

From the results of this study, the present authors will recommend the intermediate flow rate of $4\,L\,min^{-1}$. Flow rate and mortality were positively correlated, but larval weight and yolk sac utilization were negatively correlated with flow rate. However, the number of bacteria were highest in the incubators with the lowest flow rate.

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