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**Effects of continuous flow rate on development and
mortality of halibut yolk sac larvae**

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ABSTRACT

Halibut (Hippoglossus hippoglossus L.) yolk sac larvae were raised in incubators with different rates of continuous flow. Most larvae died between Days 10 and 29 after hatching. Rate of mortality increased with increasing rate of flow, but in a stagnant incubator there were no surviving larvae beyond Day 12 after hatching. Larval dry weight decreased with increasing rate of flow, whereas yolk sac dry weight did not differ significantly. Yolk sac utilization efficiency was higher with lower flow.

INTRODUCTION

Halibut larvae have an endogen development stage from hatching until first feeding at about 150 day degrees (Skiftesvik et al. 1990). This is a very long period compared with other marine fish larvae investigated in the North-East Atlantic (Russel 1976). During the yolk sac stage, the mortality is extremely high and a large fraction of the larvae develop deformities (Pittman et al. 1989, 1990 a,b). The energy of the yolk sac is used for growth, development and activity. If the larvae through stress use too much of the energy for activity it will be at the expense of growth and morphological development. Pommeranz (1974) found that spraying newly hatched plaice larvae with water led to heavy mortality. Rosenthal and Alderdice (1976) have reviewed how stress in form of unfavourable salinity, temperature, and pollution leads to deformities and high mortality. Temperatures experienced by the primitive halibut larvae during the yolk sac stage have been shown to affect growth and yolk conversion efficiencies as well as rates of deformity

(Pittman et al. 1989, 1990a). Taranger et al. (1985) showed that physical stress on salmon yolk sac larvae led to lower growth rate and lower RNA/DNA ratio. Physical stress is also believed to increase the vulnerability of the larvae to attacks by opportunistic pathogens (Bergh et al. 1990, Pittman et al. 1990a).

Halibut yolk sac larvae at Austevoll Aquaculture Research Station, as well as in several commercial fish farms, are kept in incubators with continuous water flow as described by Jelmert and Rabben (1987) The objective of the present study was to explore correlations among rate of water flow, mortality, bacterial growth, larval growth, jaw development, yolk conversion efficiency, and content of RNA and DNA.

MATERIALS AND METHODS

Egg source and incubation

One female and two males from the broodstock of the Austevoll Aquaculture Research Station were stripped for eggs and sperm, fertilised, and incubated in incubator systems modified from Jelmert and Rabben (1987). The temperature was kept between 6 - 7 °C, and the salinity was 32 +/- 1 ‰. The percentage of fertilization was 97 % . The diameter of the eggs were 3.26 +/- 0.04 mm. The eggs were surface disinfected with Prefuran. Rate of survival during incubation was 93 %. The eggs were transferred from the egg-incubators the day before hatching.

Raising of larvae

Water were piped from a depth of 55 m, sandfiltered and microfiltered (5 µm) and UV-radiated. A total of 10 tanks were used, 3 tanks for each group, except the stagnant, control group. Each tank was a semiconical, 250 l fibreglass unit, painted black inside as described by Pittman et al. (1990a). A split inlet valve on the bottom for introduction of new water and extraction of sedimented dead larvae, and a sleeve filter for overflowing water at the top. A lid prevented light from reaching the larvae. The temperature was 6.4 +/- 0.4 C, and the salinity was 32 +/- 1 ‰. The tanks were divided into four groups, according to rate of water flow (Table 1). In each tanks there were about 2000 larvae.

Table 1. Rate of water flow in the tanks of the different groups.

Group no.	Rate of flow * min ⁻¹	No. of tanks
1. group -	350 ml * min ⁻¹	3
2. group -	700 ml * min ⁻¹	3
3. group -	1050 ml * min ⁻¹	3
4. group-	(stagnant)	1

Larval samples

Day 0 of larval development were defined as when 50% of the eggs had hatched. 20 - 30 larvae were taken from each incubator for morphometric measurement on Days 1, 5, 12, 19, 22, 26 and 29 after hatching. Notochord length was measured on live larvae, then the larvae were washed in distilled water and frozen. The yolk sac and larval body were separated after being freeze-dried, and weights were measured on a Metler M 3 electrobalance weight. The calculation of yolk conversion efficiencies was done according to Blaxter (1969). In addition 6 group of 8 larvae were taken for measurement of RNA and DNA contents, according to procedures described by Raae et al. (1988). Statistical testing was done by Student's t-test and the Kolmogoroff-Smirnoff test for normal distribution.

Mortalities

Dead larvae were removed from the incubators every second or third day by stopping the flow and introducing ten liters of salted water (about 40 ppt) for about 10 minutes. Dead larvae sank and were removed via bottom valve along with the salted water. They were then filtered and preserved in 4% phosphate-buffered formaldehyde for counts of mortalities and jaw deformities. The water flow was restarted and adjusted.

Enumeration of Bacteria

Total counts of bacteria in incubator water was recorded every third or fourth day throughout the experiment. Water samples from each incubator were fixed with formaldehyde to final concentration of 2%, filtrated on 0.2 um Nuclepore filters and stained with 4'6-diamidino-2-phenylindole (DAPI) (Porter and Feig 1980). Support filters were soaked in a 10 ug/ml DAPI solution, according to Hoff (1988). Counting was carried out using a Nikon epifluorescence microscope, operated at 600x. At least 200 cells were counted in each sample.

RESULTS AND DISCUSSION

Mortality

The cumulative mortality in the tanks with different flow is shown in Figure 1. In the control group (Group 4), there were no surviving larvae beyond Day 12 after hatching. In the incubators with flow the larvae started to die on Day 12 and the rate of death was higher with higher rate of flow. In the incubators of Group 2 and 3, the last surviving larvae were sampled on Days 29 and 22, respectively. In the tanks of Group 1, the survival was about 1 % on day 29.

Halibut larvae start to sink passively when the head lifts from the yolk sac to

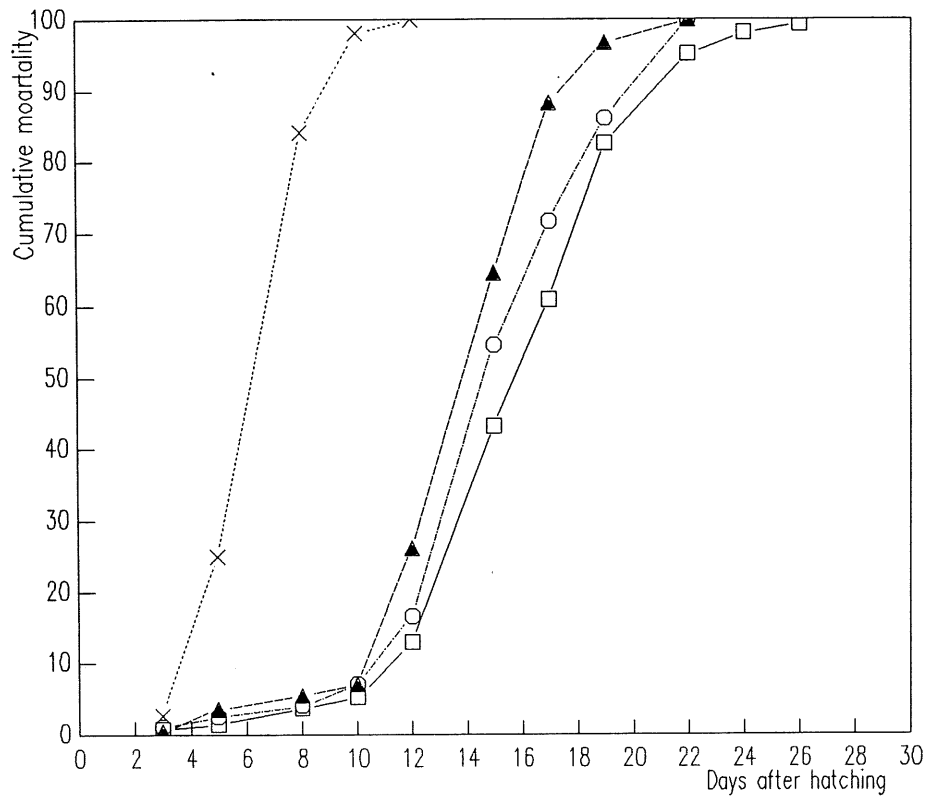


Figure 1. Cumulative mortality (in %). Symbols are: Squares (Group 1), circles (Group 2), filled triangles (group 3) and crosses (group 4).

mid-eye and there is a groove for the mouth (Pittman et al 1990b).

In many systems and experiments (Blaxter et al 1983, Pittman et al 1989, 1990a,b) this period corresponds with the highest mortalities during the entire yolk sac stage. In the incubators with water flow (Groups 1-3), the rates of death are higher with higher flow. However, in the stagnant incubator (Group 4), the rate of death is much higher. The halibut larvae start to sink passively on Day 3 after hatching (Pittman et al 1990b). Most probably, they are concentrated in the cone at the bottom of the incubators. The activity of the larvae increases with increasing number of larvae per volume (Pittman et al. 1990b). This might lead to injuring of larvae, infection by bacteria, and subsequently to death.

In the incubators with flow the larvae started to die around Day 12. The water flow most probably kept the larvae in the water-column a longer period, thus preventing them from sinking into the cone at the bottom of the incubators.

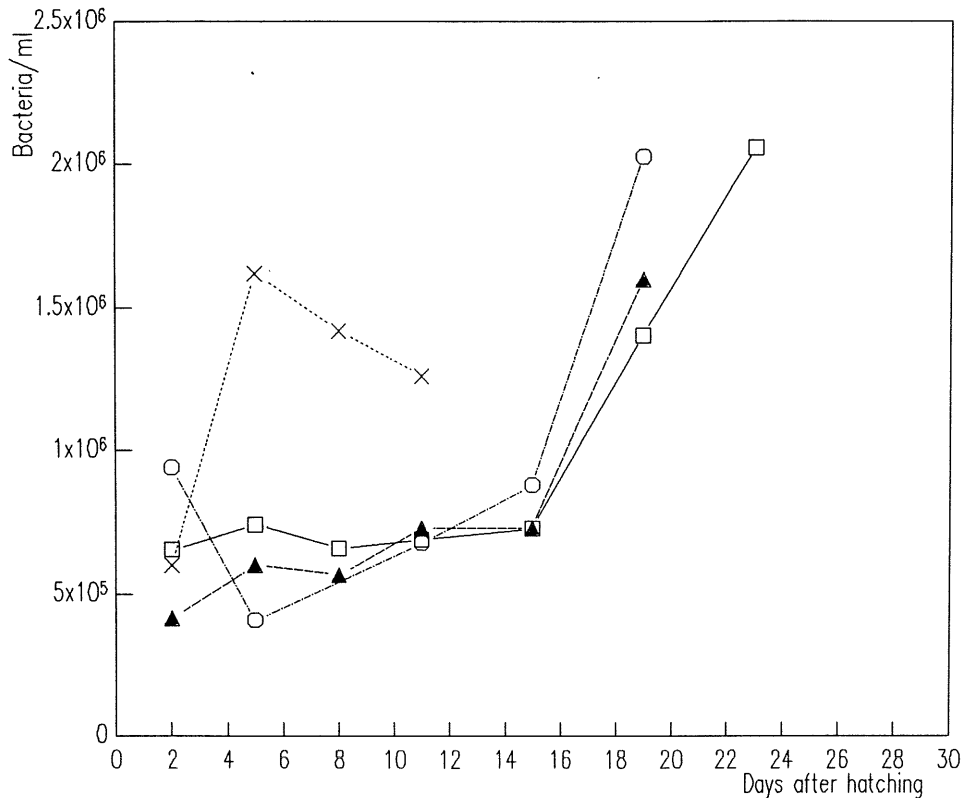


Figure 2. Total counts of bacteria in the incubators (mean values). Symbols are: Squares (Group 1), Circles (Group 2), filled triangles (Group 3) and crosses (Group 4).

Bacteria

The numbers of bacteria in incubator water are shown in Figure 2. In the stagnant incubator (Group 4), the high mortality from Day 3 onwards was preceded by a rapid accumulation of bacteria. In Groups 1-3, bacterial growth was delayed, and numbers did not exceed 10^6 until after Day 16. Also common to Groups 1-3 was that accumulation of large numbers of bacteria did not occur until after larval mortality curve had begun to rise. Bacterial numbers never exceeded $2.5 \cdot 10^6$ cells \cdot ml⁻¹ in any group, thus bacterial numbers *per se* could not be an explanation of larval mortality in this experiment. However, as all samples were taken from the upper part of the water-column, we do not know whether bacterial numbers were higher in the cone at the bottom of the incubators. The results implies that the rate of larval mortality is the major determining factor of bacterial growth. This should be expected, as dead larvae is the major supply of reduced carbon to the bacteria in the incubators. Thus, frequent removal of dead larvae from incubators should be an efficient way of preventing high bacterial numbers. The delayed accumulation of bacteria in Groups 1-3 compared to Group 4 shows that water exchange may efficiently reduce accumulation of bacteria in the incubators, but this should be carefully weighed against physical stress on the larvae caused by the water flow.

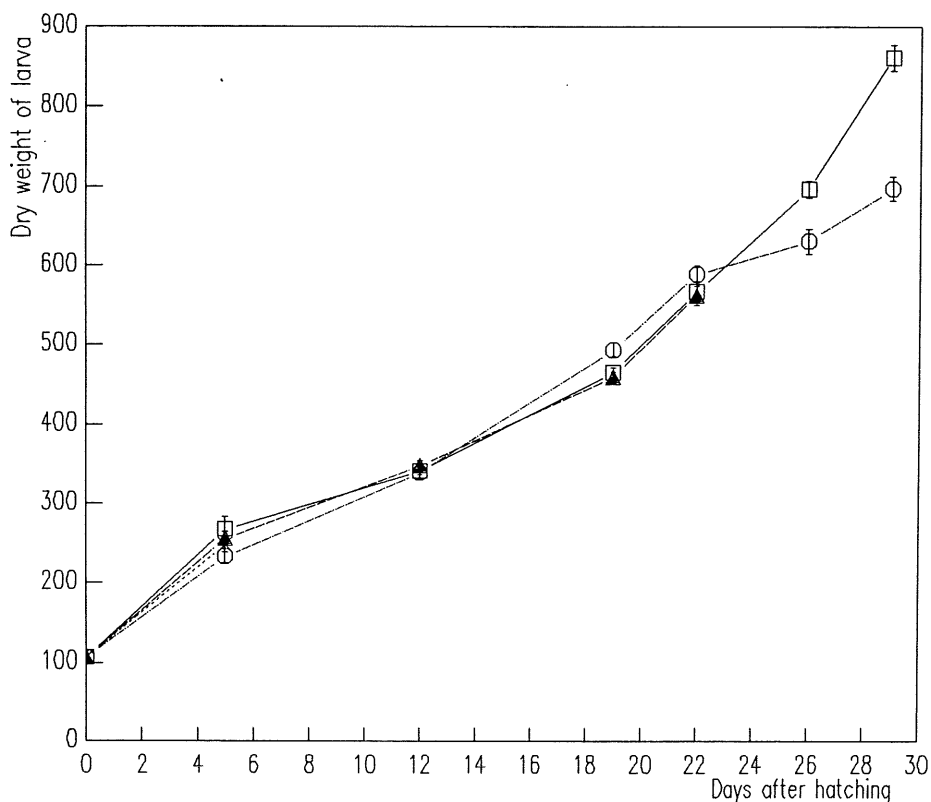


Figure 3. Dry weight of larval body (mean value, with standard errors). Symbols are: Squares (Group 1), Circles (Group 2), filled triangles (Group 3) and crosses (Group 4).

Jaw deformities

Table 2. Percentage of larvae with jaw deformities in each group.

Days after hatching	19	22	24	26	29
1. group	54	50	40	21	67
2. group	54	51	43	30	20
3. group	59	42	-	-	-

Table 2 shows the percentage of larvae with jaw deformities from Day 19 onwards. On day 19 after hatching it is possible to see the jaw development (Pittman et al. 1990b). The percentage of larvae with jaw deformities was slightly higher in the tanks of Group 3 on day 19. On Day 22, there were 10 % fewer larvae with jaw deformities in same group. On Day 24, there was little difference between groups. Day 26 there was higher number of larvae with jaw deformities in Group 2 compared to Group 1. However on Day 29 the number with jaw deformities was higher in Group 1 than Group 2.

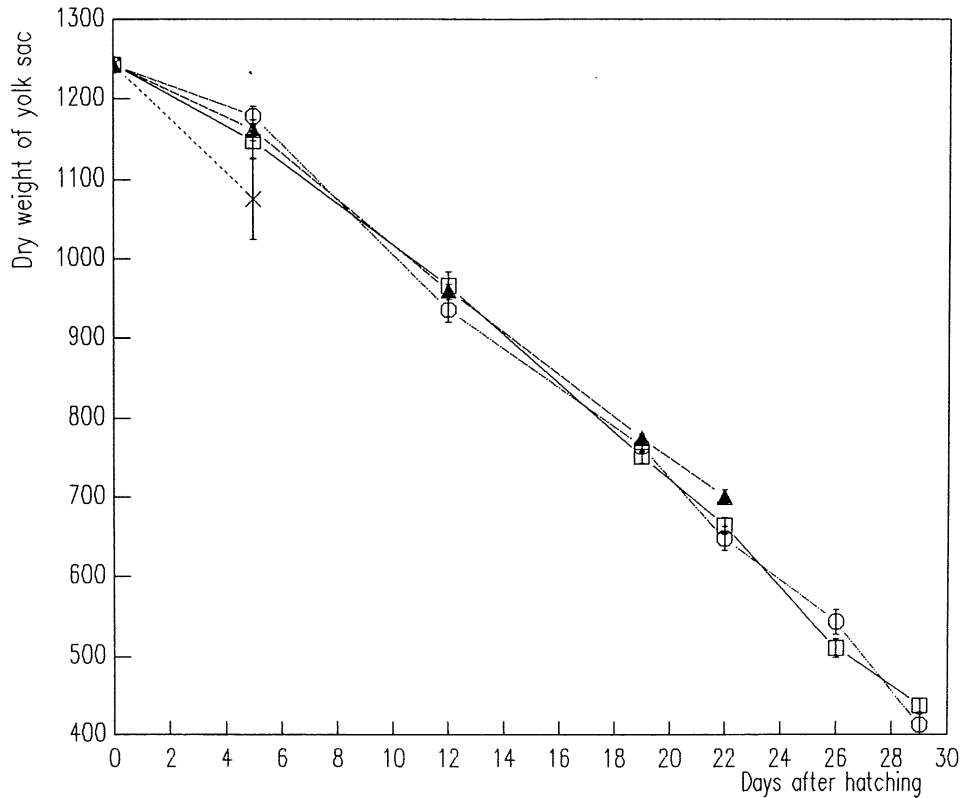


Figure 4. Dry weight of yolk sac (mean value, with standard errors). Symbols are: Squares (Group 1), circles (Group 2), Filled triangles (Group 3) and crosses (Group 4).

The reason why the number of larvae with jaw deformities is highest on Day 19 in group 3 and lowest on Day 22 might be that the larvae with deformities die at earlier stages. In Groups 1 and 2 the same pattern can be seen, as 90 % of the larvae are dead before Day 19 after hatching. If the larvae with deformities die at early stages when exposed to physical stressors, this might be the reason why we were not able to show correlation between rate of flow and number of deformed larvae. Physical stressors such as temperature, light and air bubbling have been shown to increase the number of deformities (Opstad and Raae 1986, Bolla and Holmefjord 1988, Pittman et al 1989, 1990a)

Growth and yolk sac utilization

Body dry weight (Figure 3), increased from 113.5 ug on Day 1 to 861.8 and 697.0 on Day 29 in Groups 1 and 2, respectively. In the same period the yolk weight (Figure 4) decreased from 1240.8 ug to 437.0 ug and 413.1 in Group 1 and 2, respectively. The yolk absorption rates were 28,7 ug dry weight * day⁻¹ in Group 1 and 29.6 ug dry weight * day⁻¹ in Group 2 in the period between Day 1 and Day 29 after hatching. From Day 26 after hatching onwards there was significantly

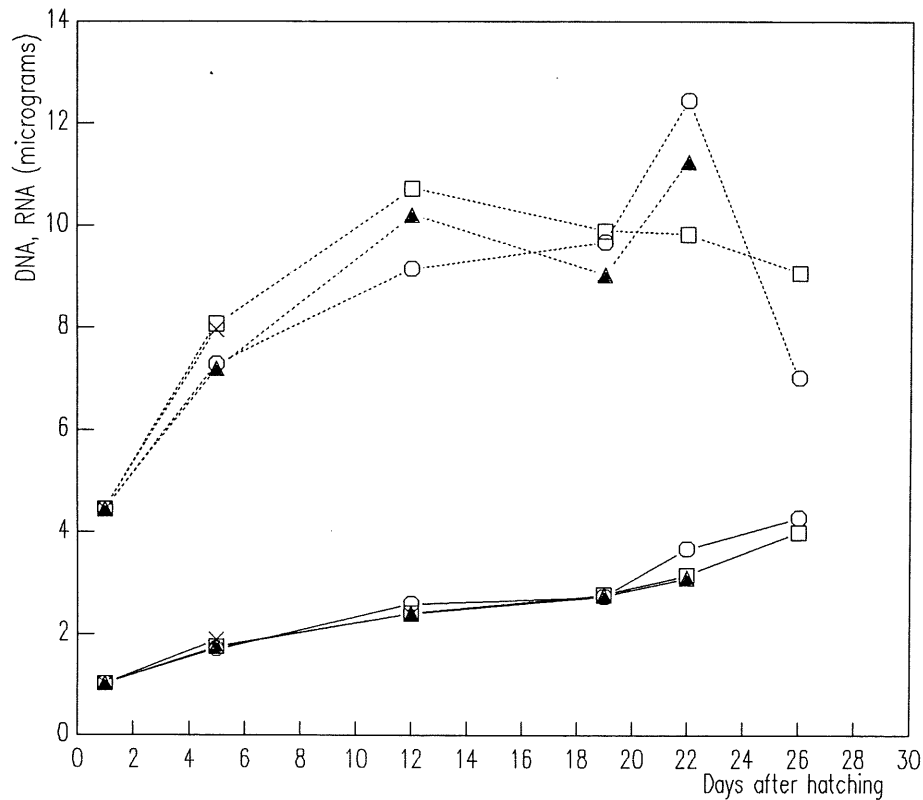


Figure 5. RNA (dotted lines) and DNA (solid lines) content per larva (in micrograms, mean values). Symbols are: Squares (Group 1), circles (Group 2), filled triangles (Group 3) and crosses (Group 4).

higher dry weight of larval body in Group 1 than in Group 2. There was no significant difference in yolk sac dry weight in the same period. The yolk conversion efficiencies between Day 1 and Day 29 were 93 % and 71 % in Groups 1 and 2, respectively.

Table 3. Growth rate (ug/day)

Days	Group 1.	Group 2.	Group 3.	Group 4.
1-5.	38.9	30.0	35.9	38.3
5-12.	10.3	15.1	15.9	-
12-19	17.7	22.0	15.9	-
19-22	34.1	32.2	35.3	-
22-26	32.2	10.4	-	-
26-29	55.2	22.0	-	-

The growth rate was high between Days 1-5 after hatching (Table 3), and the

difference among groups was small. Between Days 5-19, the rate of growth decreased to less than half compared to Days 1-5. From Day 19 onwards, the rate of growth increased to the same level as newly hatched larvae. In the period between Days 22-29, there was a large difference in the rate of growth between the two remaining groups (Groups 1 and 2).

In Group 2, the energy from the yolk sac has most probably been used by activity stress instead of being canalized to larval body growth. The yolk sac conversion efficiencies are 20 % less in Group 2 than in Group 1. This is supported by data from a stress experiment with halibut larvae by Opstad and Raae (1986).

Similar results have also been reported for salmon yolk sac larvae. Hansen and Møller (1985). Hansen and Torrissen (1984) and Taranger et al (1985). They found a difference in growth rate and hence weight between substrate reared and flat screen reared salmon larvae. The weight difference were in disfavor of flat screen reared larvae, probably due to high activity stress.

The amount of DNA (Figure 5) increased significantly from hatching to Day 12 after hatching, in all groups, except Group 4. This differs from Pittman et al. (1990a), who found no significant increase in the amount of DNA until Day 14. Between Days 12 and 19 there was no significant increase in the amount of DNA. Between Days 19 and 26, the DNA content increased, significantly in Groups 1 and 2. However, in Group 3, no significant increase was found between Day 19 and Day 22, when the last larvae were sampled.

Comparing the increase in DNA content with growth rate as measured by larval dry weight, there was high rate of growth the 5 first days after hatching, followed by a period with slow growth. After Day 19, the growth rate increases again. There are two ways of growing: either the individual cells become larger or the number of cells increases. The latter is the norm for embryonic development. Both ways will usually lead to an increase in biomass. The pattern might be: An increase in the number of cells the first days after hatching is followed by an increase in the size of a fairly constant number of cells in the period with slow growth. This is in turn followed by a period with increase in the number of cells.

The amount of RNA (Figure 5) increased significantly the 5 first days after hatching in Group 4 only. From Day 12 onwards there was no significant change in the amount of RNA per larva.

On day 1 after hatching the RNA/DNA ratio (Figure 6) was 4.3 and remained fairly constant until day 12, where it decreased. This differs from Pittman et al. (1990a), who found that the RNA/DNA ratio increased from hatching until Day

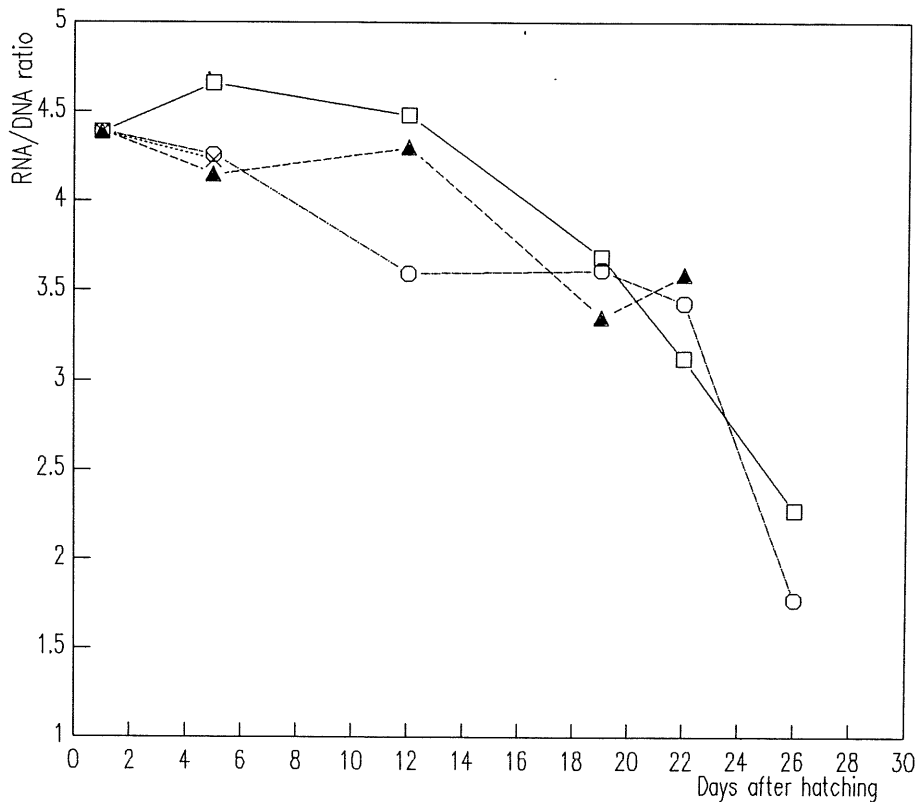


Figure 6. RNA/DNA ratio. Symbols are: squares (Group 1), circles (Group 2), filled triangles (Group 3) and crosses (Group 4).

and then decreased. There were no significant differences in the RNA/DNA ratio among the groups. Similar results were found by Taranger et al. (1985), who found little difference in RNA/DNA ratio in a stress experiment with salmon yolk sac larvae.

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