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PHYSICAL STRESS ON HALIBUT LARVAE.

by

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ABSTRACT

Halibut larvae were exposed to physical stress in the form of different levels of airation. Survival rate, development, dry weight, RNA, DNA and protein were measured. At the end of the experiment the larvae exposed to gentle airation had the highest survival rate, total dry weight, dry weight of the yolk sac, content of RNA and RNA-DNA ratio. However, the group without airation had the highest dry weight of larval body, growth rate and yolk conversion efficiency. The functional jaw development had the same value and were highest in these two groups.

INTRODUCTION

Halibut larvae have an endogen development stage from hatching until first feeding of about 30 days. This is a very long period compared with other marine fish larvae investigated in the North - East Atlantic (Russel, 1976).

In rearing experiments with halibut larvae, the mortality is high and many of the larvae end up with deformities such as bent notochord, deformed mouth and swollen yolk sac (Senstad 1984). The energy in the yolk sac should be used to growth, development and activity. If the larvae through fysical stress use to much of the energy for activity it will most probably be at the expence of growth and development. Rollefsen (1930, 1932) showed that fysical stress on cod eggs led to deformities. Pommeranz (1974) found that spraying newly hatched plaice larvae with water led to heavy mortality. He also exposed plaice eggs to air bubbling, waterstreams and waves respectivily, and they showed a great vulnerability to these stress factors. Rosenthal (1976) rewieved how stress in the form of unfavourable salinity, temperature, and pollution leads to deformities and high mortality in marine eggs and larvae. The RNA/DNA ratio has also been reported to respond to environmental stress such as high temperatures (Spigarelli and Smith 1976) and heavy metal stress (Kearnes and Atchingson 1979).

It is well demonstrated that artificial hatching substrate enhances growth, yolk conversion efficiency and reduces mortality of alevins of alantic salmon, probably due to reduced stress (Hansen and Møller 1985, Taranger et al.,1985).

In the present experiment, halibut larvae were exposed to fysical stress in the form of different amounts of air bubbling from hatching until first feeding. During development, observations were made of mortality, growth, jaw development, yolk conversion effiency, RNA/ DNA ratio, protein content and deformities.

MATERIALS AND METHODS

Artificially fertilized halibut eggs (Hippoglossus hippoglossus L.) were obtained from the spawning population kept at the Institute of Aquaculture Research, N-6600 Sunndalsøra. They arrived at Austevoll Marine Aquaculture Station ca. 70 day degrees old. The eggs were incubated in 200 l open sirculation tanks, and kept in darkness.

Experimental conditions

When the eggs started to hatch, they were transferred to 6 l plastic tanks, 120 eggs in each. The tanks were kept in water baths. They were covered with plastic plates and kept in darkness. The temperature was 7 ± 0,5 °C. The salinity was 32 ± 2 0/00. Oxytetracyklin (0.025 g/l) was added to the water in the tanks. The seawater was UV -irradiated and filtered (0,2 µ). As much as possible of the water was changed every 4. day. Dead larvae were removed every second day in the beginning of the experiment, later on every 4. day. Five groups with six parallels in each group were set up:

1. group without airbubbling
2. group with 4 ml air/min in each tank
3. group with 10 ml air/min in each tank
4. group with 1,5 l air/min in each tank
5. group with 5 l air/min in each tank

The air was filtered (0,22 µ Millipore).

Sampling and measurements

The mortality in each group was recorded as mean value of dead larvae in the parallels. Every 7. day all larvae in a tank were sampled in each group. 15 - 30 larvae were preserved in 4 % formaldehyde and dissected into yolk sac and body. Dry weight measurements were made to an accuracy of 1 µg after 48 h. in an oven at 60 °C.

Growth rate was recorded as daily increase in yolk sac free body. Yolk absorbtion rate was calculated as daily decrease in yolk weight. Yolk conversion efficiency was calculated from the data using the dry weight method given by Blaxter (1969).

Determination of DNA, RNA and protein

From each group 8 larvae x 4 were pipetted into 1,5 ml Eppendorf tubes, rinsed with destilled water, and immediately frozen and stored in liquid nitrogen.

The frozen vials, containing 8 larvae in about 250 µl destilled water were thawed and centrifuged. The larvae were then homoenized with a sonicator for 20 seconds and centrifuged. The volume was adjusted to 500 µl with Tris HCL pH 7,8.

The nucleic acids were assayed essentially according to the procedure of Boer (1975). Total RNA and DNA was determined by adding 100 µl of halibut larvae homogenate to 3 ml of phosphate buffered saline (PBS); (0.01 % $CaCl_2$, 0.02 % KCl, 0.02 % KH_2PO_4, 0.01 % $MgCl_2$, 0.8 % NaCl and 0.11 % Na_2HPO_4 adjusted to pH 7.5 with NaOH) containing 2.5 µg/ml Ethidiumbromide. The readings were carried out on a Perkin-Elmer spectrofluorometer LS 5 with excitation at 360 nm and emission at 590 nm.

DNA was assayed by the same procedure after RNA had been digested by RNase. To 100 µl halibutlarvae extract, was added 100 µl phosphate buffered saline containing 0,5 µg RNase. The reaction was carried out at 37 °C for 30 minutes. The reaction mixture was then added to 3 ml PBS containing 2.5 µg/ml ethidiumbromide and the DNA content was determined. DNA standards were prepared from a stock solution of 3 mg/ml DNA from herring sperm. Protein was determined according to Lowery et al (1951).

RESULTS

Mortality

The mortality pattern of the larvae in the 5 different groups is shown in Fig 1. The development in the period between day 0 and day 6 did not show any clear corelation with the quantity of airbubbling, although the mortality is highest in groups 4 and 5. From day 6 to day 28, however, the mortality increased with increasing amount of airbubbling with the exception, of group 1. From day 24 to day 28 there was a heavy mortality in group 1.

Survival on day 28.

1. group 65 %
2. group 81 %
3. group 61 %
4. group 36 %

The rest of the larvae in group 5 were sampled on day 21. The survival at day 28 was highest in group 2, and lowest in group 4. In group nr. 1 and 3 there was only a small difference in survival.

Dry weight development

Table 1. gives the total dry weight development. From day 7 to day 14 there was an increase in total dry weight in groups 1 and 5. From day 14 to day 28 the total dry weight decreased in all groups. The decrease was lowest in group 2 and highest in group 4.

The dry weight development of the larval body and the percentage of the total body weight are given in table 1. Group 1 had the highest dry weight of the larval body from day seven to day 28. The lowest larval dry weight had group 5 on day 7 and day 14. On day 21 had group 4 and 5 the lowest and on day 28 group 3 and group 2 the lowest.

Growth rate

Between day 7 and 14 the growth rate (Table 2) was highest in group 1 (14,7 µg/day), and lowest in group 2 (9,7 µg/day). The growth rate was also highest in group 1 the seven next days (15,4 µg/day). It was now however lowest in group 4 (8,7 µg/ day). From day 21 to day 28 it was highest in group 4 (31,7 µg/day) and lowest in group 3 (18,6 µg/day).

Yolk absorbtion (YAR)

The period between day 7 and 14 the YAR (Table 3) was lowest in group 5 (9 µg/day) and highest in group 3 (18,9 µg/day). The seven next days there was little difference in the YAR between the 5 groups. Between day 21 and 28 it was highest in group 4 (46,5 µg/day), closly followed by group 1. There was little difference between the two other groups.

Yolk conversion efficiency (YCE)

The YCE was extremely high in groups 1, 4 and 5 the period between day 7 - 14 (Table 4). The seven next days it was lowest in group 4 (37,3 %). Between the other groups there was little difference. Between day 21 and 28 the YCE was highest in groups 2 and 4 (68 %) and lowest in groups 1 and 3 (55 %).

RNA and DNA development

On day 21 the DNA content (Fig. 2) was lowest in group 1 (0,27). Between the four other groups there was little difference in DNA content. The RNA content was highest in group 1 (1,7 µg) and decreased with increased "physical stress". In group 5 it was (0,7 µg). The RNA/DNA ratio showed the same pattern: highest in group 1, 6,4 and 2,2 in group 5.

One week later the DNA (Fig. 3) content was highest in group 4 (2,5 µg) and lowest in group 2 (0,7 µg). The RNA content, however, was highest in group 2 (8,4 µg). Between the other three groups there was little difference in the RNA content. The RNA/DNA ratio was highest in group 2 (11,5) and lowest in group 4 (3,5).

In the period from day 21 to day 28 the content of both DNA and RNA increased in all groups. The DNA content in group 4 increased most from day 21 (0,38 µg) to day 28 (2,5 µg). The RNA content increased most in group 2 from 1,2 (day 21) to 8,4 (day 28). Also the RNA/DNA ratio increased most in group 2 from 3,5 (day 21) to 11,5 (day 28).

Protein content

On day 21 the protein content (Table 5) was highest in group 4 and lowest in groups 1 and 5. One week later it was highest in group 1. The protein content inreased from day 21 to day 28 except in group 4.

Jaw Development

Percentage with functional jaw on day 28:

Group 1: 69%
Group 2: 68%
Group 3: 33%
Group 4: 6%

The results show a clear inverse correlation between jaw development and amount of "physical stress" in the form of airbubbling.

DISCUSSION

From day six to day twentyeight the mortality rate was positively correlated with the quantity of airbubbling, except for group 1. This period the growth rate was very high and there was a great increase in the content of DNA and RNA, which indicates a high metabalic rate with a correspondingly high demand of oxygen and a high excretion of waste products. The heavy mortality in group 1 between day 24 and day 28 might therefore have been related to bad water quality. Blaxter et al. (1983) reported a similar mortality in stagnant systems with antibiotics.

In two groups there was an increase in total dry weight from day seven to day fourteen. An increase in total dry weight from hatching to day 15 post - hatching was also found by Blaxter et al. (1983).

From the beginning of the experiment to day 21 the larvae in the tanks without airbubbling (group 1) had the highest growth rate and the highest efficiency of yolk utilization. The dry weight of larval body, the percentage larval body dry weight of total dry weight, the content of RNA, and the RNA/DNA ratio were also highest in this group, and the mortality was low. These results support the theory that halibut larvae are very sensitiv to mechanical stress.

Similar results have been reported for salmon larvae. Hansen and Møller (1985), Hansen and Torrissen (1984) and Taranger et al. (1985) found a difference in growth rate and hence weight between substrat reared and flat screen reared salmon larvae. The weight difference was in disfavor of flat screen reard larvae, probably due to high activity stress. Taranger et al. (1985) found a lower content of RNA, DNA and RNA/DNA ratio in the flat screen reared larvae than the substrat reard larvae, also due to activity stress in the flat screen reared larvae.

From day 21 to day 28 the growth rates and the absorbtion rates of the yolk sac were highest in group 4 wich correlates with the highest increase in the DNA content.

Airbubbling seems to become a positive growth factor in this period, most probably due to an increased O_2 - content in the water. The larvae might have a great demand of oxygen because of the increased metabolic activity in this period. Blaxter et al. (1983) found a sharp increase in the metabalic rate after day 25. It reached a level almost double the routine metabalic rate of larval plaice. This is the period when halibut larva become fully developed to feed from the aspect of its sens organs, mouth and gut (Blaxter et al. 1983).

At the end of the experiment (day 28) group 2 (4 ml air/min) had the highest survival rate. The total dry weight, the dry weight of the yolk sac, the content of RNA and the RNA/DNA ratio were also highest in this group. The group without airation (group 1), however, had the highest larval dry weight.

The degree of mouth development on day 28 is lowest in group 4 (6 %) and highest in groups 1 and 2 (69 and 68 %). These results supports the theory that fysical stress may create malformation.

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Table 1.

Dry weight changes with age (in days from hatching) of the total body (A), the larval body, % of total (B) and the yolk sac (C) in the 5 different groups.

A. Total dry weight (µg).

	Group nr.				
Age(d)	1	2	3	4	5
7	1068	1132	1134	1096	1058
14	1097	1094	1085	1090	1067
21	1020	1012	1020	987	985
28	892	943	911	883	-

B. Dry weight larval body (µg) and % of total.

	Group nr.									
	1		2		3		4		5	
Age(d)	larval body	% of total	larval body	% of total	larval body	% of total	larval body	% of total	larval larval	% of total
7	200	19	196	17	195	17	193	18	164	16
14	303	28	264	24	279	26	271	25	236	22
21	411	40	358	35	376	37	332	34	346	35
28	575	64	510	54	506	56	554	63	-	-

C. Dry weight yolk sac (µg)

	Group nr.				
Age(d)	1	2	3	4	5
7	868	936	939	903	894
14	794	830	807	818	831
21	609	654	644	655	638
28	318	433	405	329	-

Table 2. Growth rate (µg dry weight/day).

	Group nr.				
Age(d)	1	2	3	4	5
7 - 14	14,7	9,7	12,0	11,2	10,3
14 - 21	15,4	13,4	13,9	8,7	15,7
21 - 28	23,4	21,6	18,6	31,7	-

Table 3. Yolk absorbtion rate (µg dry weight/day).

	Group nr.				
Age(d)	1	2	3	4	5
7 - 14	10,6	15,1	18,9	12,1	9,0
14 - 21	26,4	25,1	23,3	23,3	27,5
21 - 28	41,6	31,6	34,1	46,5	-

Table 4. Yolk conversion efficiency (%).

	Group nr.				
Age(d)	1	2	3	4	5
7 - 14	139	64	64	92	114
14 - 21	58	53	60	37	57
21 - 28	55	68	55	68	-

Table 5. Protein content (mg 18 larver).

	Group nr.				
Age(d)	1	2	3	4	5
21	3,0	3.3	3,7	4,3	3,0
28	4,1	3,8	3,5	3,8	-

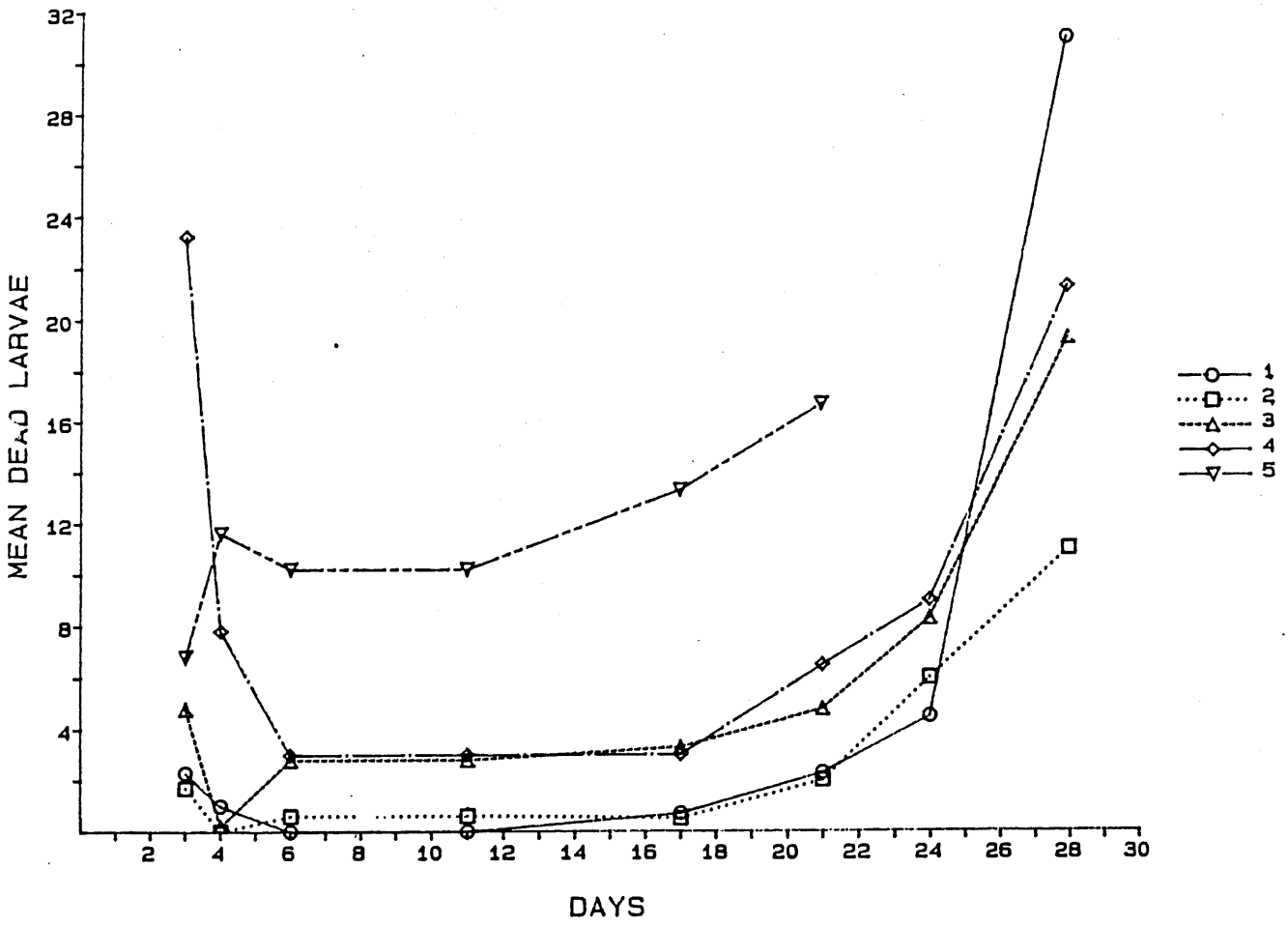

Fig. 1. Mean dead larvae in the groups 1 - 5 from hatching to day 28.

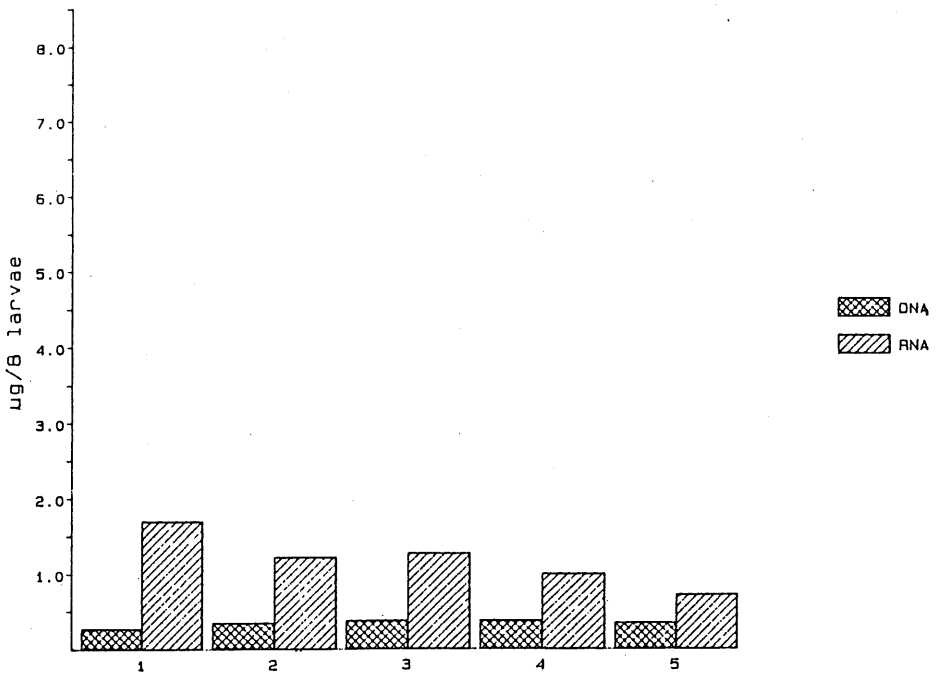

Fig. 2. DNA and RNA content in the groups 1 - 5 on day 21.

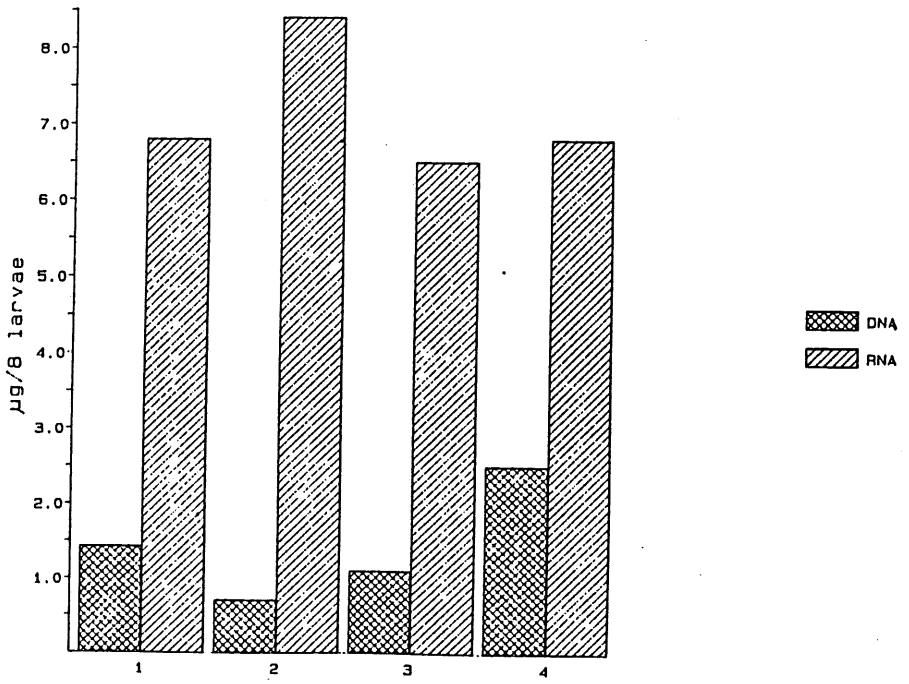

Fig. 3. DNA and RNA content in the groups 1 - 4 on day 28.

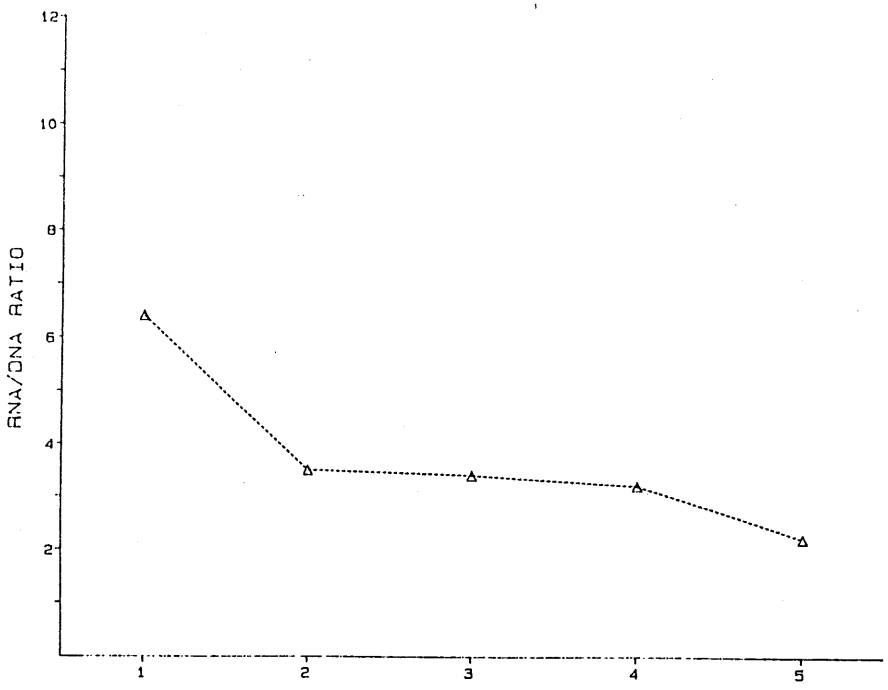

Fig. 4. RNA - DNA ratio in the groups 1 - 5 on day 21.

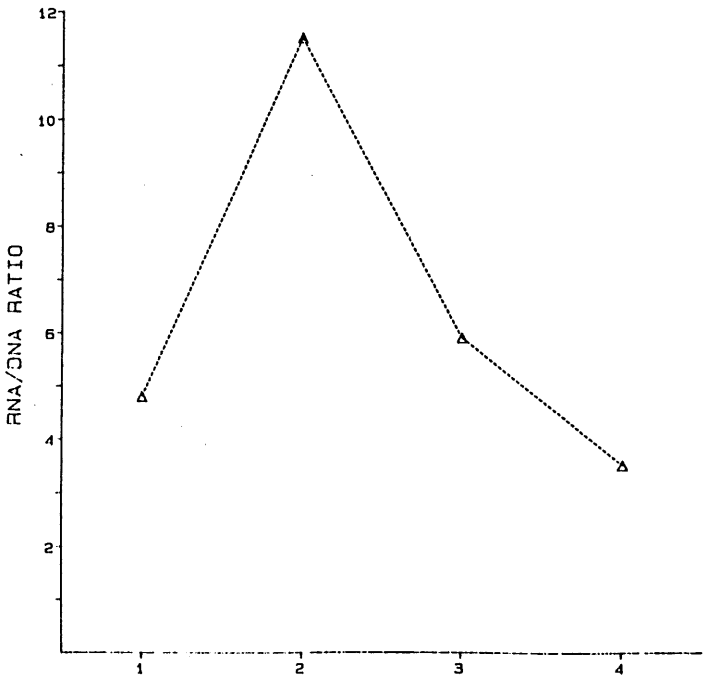

Fig. 5. RNA - DNA ratio in the groups 1 - 4 day 28.