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ARTIFICIAL HATCHING SUBSTRATE, EFFECT ON RNA/DNA RATIO  
AND PROTEIN RETENTION DURING THE YOLK-SAC PERIOD OF  
ATLANTIC SALMON (Salmo salar).

by

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### ABSTRACT

Genetical groups of Atlantic salmon (Salmo salar) were hatched in a Californian hatching system with and without an astro-turf artificial hatching substrate, and were later transferred to separate feeding units.

The substrate reared (ATR) alevins grew faster, absorbed the yolk faster and more efficient, and absorbed protein from the yolk faster and more efficient, than flat screen reared (FSR) alevins. Protein synthesis, RNA content, DNA content, and the RNA-DNA ratio were higher in favour of the substrate reared alevins. The differences in disfavour of the FSR alevins are probably due to high activity stress.

After commencement of first feeding the ATR fry grew faster, and RNA content and DNA content were higher than in the FSR fry. However a difference in specific growth rate in favour of the FSR fry was observed at the end of the experiment. The RNA-DNA ratio was in favour of the ATR fry immediately after commencement of first feeding, but in favour of the FSR fry at the end of the experiment.

### INTRODUCTION

RNA and DNA play key roles in regulation of growth and development in the early life history of fishes. RNA content is correlated to protein synthesis rate (Munro, 1969, 1976; Millward, 1976) and consequently growth (Sutcliffe, 1969). DNA is present in a constant amount per cell for a given species and can be used as a count of cell number (Regnault and Luquet 1974; Zeitoun et al., 1977) and a indicator of cell size. The RNA/DNA ratio reflects the amount of RNA per cell (Hotchkiss, 1955).

This ratio has been demonstrated to be a reliable indicator of growth rate and condition in fish (Bulow, 1970; Haines, 1973; Bulow et al., 1981; Buckley, 1977, 1980, 1981; Thorpe et al., 1982; Wilder and Stanley, 1983). The RNA/DNA ratio is thought to be more sensitive to changes in growth rate than dry weight development and hence more sensitive to environmental changes. 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). Barton and Adelman (1984) observed changes in RNA, DNA, protein content and RNA-DNA ratio in fish larvae after exposure to sublethal doses of various toxicants.

It is well demonstrated that artificial hatching substrate enhances growth, yolk conversion efficiency (YCE), prevents yolk sac constrictions and reduces mortality of alevins of Atlantic salmon, this is reviewed by Hansen and Møller (1985).

Previous chemical and biochemical investigations in Atlantic salmon are done on flat screen reared alevins and fry. Since rearing of alevins on a flat bottom have severe effect on growth and mortality, probably due to high activity stress (Hansen and Møller, 1985), we assumed a decreased RNA-DNA ratio, RNA content, DNA content, protein synthesis rate, protein conversion efficiency, and protein absorption rate in the flat screen reared alevins compared to substrate reared alevins.

#### MATERIALS AND METHODS

Eggs from five females from the genetical program at Matre Aquaculture Station were used in this experiment. These females were selected because of their great amount of eggs which made each of these groups suitable for division into two fairly large subgroups. For the moment one of these groups has been worked up and is presented in this paper.

### Experimental conditions.

The eggs were shocked mechanically at the eyed stage, and the dead eggs were sorted out by an egg sorting machine. The eggs from each of the females were divided into two equally sized groups for further incubation. One of the groups were incubated in standard Ewos hatching trays; the other group were incubated in trays modified according to Hansen and Møller (1985). This modification consists of sewing backless astro-turf to the perforated aluminium bottom and a plastic screen with 3 x 20 mm perforations which hold back dead eggs and, therefore, prevent them from fouling the substrate.

The water input in the hatchery was 10 litres per minute per tray. The temperature varied between 5.4°C and 8.2°C, with a mean of 6.6°C. The pH varied between 6.1 and 6.9 with a mean of 6.4.

The alevins were transferred to startfeeding in separate feeding tanks (1 m<sup>2</sup>) 47 days after hatching. The fry were fed on EWOS ST 40 starfeed nr. 1 in surplus with automatic feeders.

### Sampling and measurements

During the hatchery period, 50 to 100 alevins were sampled with a dip net each week from each hatching tray. The alevins were anesthetized with benzocain, 30 individuals were dissected into yolk-sac and body. The alevins were then frozen, freeze-dried, and weighted on an electronic microbalance ( $d=\pm 0.1$  mg).

Growth rate was recorded as daily increase in yolk-sac free body. Specific growth rate was calculated according to Thorpe *et al.* (1984), using dry-weight of yolk-sac free body. Yolk conversion efficiency (YCE) was calculated from the data using the dry weight method of Blaxter (1969). Yolk absorption rate (YAR) was calculated as daily decrease in yolk weight.

After commencement of first feeding 20 individuals were collected from each feeding tank with a dip-net, first each week, later less frequent. The fry were anesthetized, frozen, freeze-dried and weighted on an electronic microbalance ( $d=\pm/_{-}$  0.1 mg). Growth rate was calculated as daily increase in total dry-weight. Specific growth rate was calculated from dry weight of whole fry.

### Chemical analysis

#### Determination of DNA and RNA.

Dry body of alevins and later fry were weighed on an electronic microbalance ( $d=\pm/_{-}$  0.1 mg), homogenized in a teflon Potter-Elvehjelm homogenizer in ice-cold phosphate buffered saline (PBS) (approx 6 mg dry tissue/ml PBS) (Karsten and Wollenberger, 1972).

RNA was detected by a modification of the Schmidt-Thannhauser method (Munro and Fleck 1966). An aliquot (2,5 ml) of the homogenate was acidified with 2,5 ml of 0.4 N ice-cold perchloric acid (PCA) and mixed with a Vortex mixer. The tubes were allowed to stand for 10 - 30 minutes on ice before they were centrifugated at 12 000 o/min for 20 min at 2°C. The precipitate was washed with 5 ml 0.2 N PCA and centrifugated as above, the supernatants were discarded. The precipitate was then dispersed and digested in 4 ml 0.3 N KOH for 1-1,5 h. at 37°C. The contents were chilled on ice, acidified with 1,5 ml 2 N PCA, allowed to stand on ice for 10 minutes and centrifugated as above. The supernatant was collected and the precipitate was washed with ice-cold 0.2 N PCA and centrifugated twice. The supernatant and washings were mixed and made up to a total volume of 20 ml with 0.2 N PCA. Digested RNA was detected spectrofotometric at 260 nm assuming that 32 ug/ml RNA gives an absorbance of 1.0.

DNA was detected by fluorometry in the presence of ethidium-bromide (Le Perq and Paoletti, 1966). An aliquot (500 ul) of the homogenate was incubated at 37°C in 30 min. in the presence of protease and RNase (Karsten and Wollenberger, 1972). The tubes were then centrifugated at 6000 o/min for 10 min at 2°C to remove interfering particles. The supernatant was immediately removed and analysed. Fluorescence was detected using an autoanalyser and a fluorimeter with flow cell (Ex:360 nm, Em: 590 nm). DNA from Salmon testis in PBS was used as standard.

### Protein

Crude protein was analysed by the Micro-Kjeldahl method. Dry whole alevins and alevins with removed yolk-sac were weighted to the nearest 0.1 mg and digested in sulfuric acid at 375°C in the presence of a catalyst (Kjeltabs). Ammonia was detected by photometry at 660 nm at 37°C with a modified Berthelot reaction (Crooke & Simpson, 1971) using an autoanalyser and a spectrophotometer with flow cell. (NH<sub>4</sub>)<sub>2</sub>-SO<sub>4</sub> was used as standard. Total protein was calculated as total nitrogen X 6.25.

Protein retention was calculated as increase in yolk-sac free body protein / decrease in total protein. Protein synthesis rate was calculated as daily increase in protein content in body of the alevins. Protein absorption rate was calculated as daily decrease in protein content in the yolk-sac.

## RESULTS

### Dry weight development during the hatchery period

Body dry-weight (Fig. 1), increased from 7.7 mg to 27 mg and 17 mg, in ATR and FSR alevins respectively between days 4 and 46. In the same period the yolk weight (Fig. 2) decreased from 54 mg to 28 mg and 31 mg, in ATR and FSR alevins respectively. The yolk absorption rate was 619 ug dry-weight/day and 548 ug dry-weight/day in ATR and FSR alevins respectively.

### Growth rate during the hatchery period

The growth rate in the ATR alevins increased from 186 ug dry-weight/day between days 4 and 11, to 621 ug dry-weight/day between days 18 and 32, and decreased to 500 ug dry-weight/day between days 32 and 46. In the FSR alevins the growth rate decreased from 243 ug dry-weight/day, between days 4 and 11, to 179 ug dry-weight/day between days 32 and 46 (Fig. 9).

The specific growth rate in the ATR alevins increased from 2.23 % increase/day between days 4 and 11, to 4.22 % increase/day between days 18 and 32, and decreased to 2.19 % increase/day between days 32 and 46. In the FSR alevins the specific growth rate decreased from 2.85 % increase/day between days 4 and 11, to 1.10 % increase/day between days 32 and 46 (Fig. 10).

#### Total dry-weight development

Total dry weight decreased from 62 mg at day 4, to 55 mg and 49 mg at day 46, in ATR and FSR alevins respectively. The ATR fry reached a minimum weight of 54 mg. at day 52, the FSR fry reached a minimum weight of 45 mg at day 60. This was followed by a rapid increase in total weight to 140 mg and 103 mg at day 103 in ATR and FSR fry respectively. The total dry weight was nearly identical in ATR and FSR alevins until day 25 where the ATR alevins was slightly heavier. This difference increased in favour of the ATR alevins during the yolk-sac period and maintained to increase after commencement of first feeding (Fig. 3).

#### Growth rate after commencement of first feeding

The growth rate in ATR fry increased from - 200 to 3200 ug dryweight/day between days 46 and 74, with a subsequent drop to 1662 ug dry-weight/day between days 74 and 103. In FSR fry the growth rate decreased from -150 to -388 ug dry-weight/day between days 46 and 60 with a subsequent rise to 1493 ug dry-weight/day between days 60 and 103 (Fig. 9).

The specific growth rate in ATR fry increased from -0.31 to 4.11 % increase/day, between days 46 and 74 with a subsequent drop to 1.45 % increase/day, between days 74 and 103. In FSR fry the specific growth rate decreased from -0.34 to -0.80 % increase/day, between days 46 and 60, with a subsequent rise to 2.32 % increase/day, between days 60 and 74, and a drop to 1.86 % increase /day, between day 74 and 103 (Fig. 10).



### Yolk conversion efficiency

The yolk conversion efficiency between days 4 and 32 was 76 % and 46 % in ATR and FSR alevins respectively, and 71 % and 34 % between days 32 and 46 in ATR and FSR alevins respectively.

### Protein development

The protein content in the yolk sac decreased from 33 mg to 8 mg and 16 mg protein/yolk-sac in ATR and FSR alevins respectively, during the hatchery period. This corresponds to 29 % protein (of dry yolk weight) and 51 % protein (of dry yolk weight) in ATR and FSR alevins respectively at time of transfer. The protein content in the body increased from 5.7 mg to 25 mg and 15 mg protein/body in ATR and FSR alevins respectively during the hatchery period.

The protein retention (protein conversion efficiency) during the hatchery period was 77.6 % for ATR alevins and 53.1 % for FSR alevins. Mean protein synthesis rate from day 4 to day 46 was 470 ug protein/day and 220 ug protein/day for ATR and FSR alevins respectively. Mean protein absorption rate from day 4 to 46 was 605 ug protein/day and 412 ug protein/day for ATR and FSR alevins respectively.

### RNA and DNA development

Both RNA (Fig. 5) and DNA (Fig. 6) content increased during the whole experiment, with the exception of a decrease in RNA from day 74 to 103 in ATR fry. The RNA content increased most after the commencement of first feeding. At day 18 ATR and FSR alevins contained equal amounts of RNA and DNA, during the rest of the experiment ATR alevins and fry contained more RNA and DNA than FSR alevins and fry. From day 74 to 103, however, the difference in RNA content between ATR and FRS fry decreased.

RNA (Fig. 7) and DNA (Fig. 8) concentration (% of body dryweight) decreased during the experiment. This trend, however was interrupted by an increase in RNA concentration from day 18 to 32 in ATR alevins, and from day 60 to 74 in both groups. An increase in DNA concentration from day 74 to 103 in FSR fry was also observed. The decrease was nearly similar for both ATR and FSR alevins and fry, however, FSR fry had a higher RNA and DNA concentration at day 103.

#### RNA-DNA ratio

The RNA-DNA ratio increased between days 18 and 32 from 1.5 to 2.2 and 1.9, in ATR and FSR alevins respectively. The ratio increased from 2.0 to 2.4 between days 60 and 74, and decreased to 1.5 between days 74 and 103, in the ATR fry. In FSR fry the ratio increased from 1.9 to 2.6 between days 60 and 74, and decreased to 1.7 between days 74 and 103 (Fig. 4).

### DISCUSSION

#### Growth and RNA-DNA ratio of alevins

The weight difference and growth rate in favor of ATR alevins are in accordance with earlier results from Atlantic salmon (Hansen and Møller 1985; Hansen and Torrissen, 1985) and also in accordance with the difference in RNA-DNA ratio in favor of ATR alevins at day 32. The difference in RNA-DNA ratio was however less than expected.

The reduction in specific growth rate in ATR alevins at the end of the hatchery period is in accordance with the decreased RNA-DNA ratio. Buckley (1980), observed a similar decrease in the RNA-DNA ratio of winter flounder (Pseudopleuronectes americanus) larvae at the end of the yolk-sac stage.

### Yolk absorbtion rate

The decreased yolk absorbtion rate (YAR) in disfavour of FSR alevins, supports the hypothesis of Hansen and Møller (1985). They suppose that the difference in growth rate and hence weight in disfavour of FSR alevins and fry is due to high activity stress. Hansen (1985) mention different stressors which is known to reduce yolk absorbtion rate (YAR) in fish larvae. Stress due to acid aluminium rich water is also known to reduce YAR (Skogheim and Rosseland, 1984).

The activity stress hypothesis is confirmed by Nortvedt et al. (1985). They found a large difference in respiration rate in disfavor of FSR alevins at early stages of yolk absorbtion. This assumption is also supported by the low RNA content, DNA content, RNA-DNA ratio, protein synthesis rate (PSR) and protein absorbtion rate (PAR) of FSR fry during the hatchery period.

The reduced yolk conversion efficiency in FSR alevins is probably due to the high swimming activity of the FSR alevins which lead to increased catabolic demands.

### Protein retention

The reduced protein retention (protein conversion efficiency) in FSR alevins compared ATR alevins is probably due to an increased catabolism of protein for energy metabolism in the FSR alevins.

The higher nonprotein content in the yolk of the ATR alevins compared to the FSR alevins at time of transfer, are probably due to higher fat catabolism in FSR alevins, since carbohydrates constitute a insignificant part of the yolk reserves (Smith, 1957). This is probably due to catabolism of phospholipids since the triglycerid fat mostly is retained to after commencement of first feeding and nonglycerid fat are used during the prefeeding period (Smith, 1957).

Growth and RNA-DNA ratio during feeding

The growth rate, and specific growth rate in favor of ATR fry immediatly after commencement of first feeding is in contrary to earlier results from Atlantic salmon (Leon and Bonney, 1979; Hansen and Møller, 1985), they observed a difference in favor of FSR fry. Leon and Bonney (1979) assumed that this was due to the large yolk reserves of FSR fry at the time of transfer. The lack of growth rate in favor of FSR fry immediatly after transfer may be due to a successfull first feeding in ATR fry and hence a lack of the common first feeding depression in growth.

The results are however difficult to intrepret since the fry were weigted without removing the yolk-sac. The negative growth rate of both groups immediatly after transfer may be due to increased yolk absorbtion rate and hence decreased total weigth.

Leon (1975), Leon and Bonney (1979), Hansen and Møller (1985) and Hansen and Torrissen (1985), however, observed a difference in growth rate in favour of ATR fry later in the feeding period. They assumes that this is due to a bigger and stronger fry and more advanced morphological development and concequently improved swimming and feeding ability.

The reduction in specific growth rate between day 75 and 103 in both ATR and FSR fry is in accordance with the reduction in the RNA-DNA ratio. Thorpe et al.(1984) found a similar pattern of specific growth rate and RNA-DNA ratio in first feeding fry of Atlantic salmon.

The drop in specific growth rate may be due to size dependent relative growth rate, since the smaller ATR and FSR fry both seemed to have a higher RNA-DNA ratio than the bigger ones on day 103 (Taranger, unpublished data). The specific growth rate in favor of FSR fry on day 103 may also be due size dependent relative growth. This is supported by the RNA-DNA ratio sligthly in favor of FSR fry on day 74 and 103.

Consequently the main difference between ATR and FSR fry after commencement of first feeding is a more advanced development and size. However the FSR fry does not seem to reach the same relative growth rate as ATR fry despite a slightly higher RNA-DNA ratio at the end of the experiment.

#### RNA and DNA development

The high DNA content in ATR larvae compared to FSR larvae indicate a higher cell number in favour of the ATR larvae and support the assumption of advanced morphological development (Hansen and Møller, 1985). The lower DNA content in the FSR larvae reflects reduced mitosis rate, this was also reported by Barron and Adelman (1984) in larvae of fathad minnows (Pimephales promelas) exposed to sublethal doses of various toxicants.

The reduction in DNA concentration with time reflects increasing cell size. Consequently a part of the growth is due to increasing cell volume. This trend is nearly similar in both ATR and FSR larvae.

The difference in RNA content in favor of the ATR larvae between days 18 and 103 reflects a larger protein synthesis capacity in favor of ATR fry. Barron and Adelman (1984) reported that the decrease in RNA content in fish larvae was highly responsible to the stress of various toxicants, consequently the low RNA content in the FSR fry are probably due to stress.

The large increase in RNA content between days 60 and 74 are in accordance with the high growth rate in the ATR fry, the decrease in RNA content between days 74 and 103 is in accordance with the decreased growth rate in this period. In the FSR fry the increased RNA content between days 46 and 60 is in contrary to the low growth rate, however, the increase in RNA content is prior to a large increase in growth rate between days 60 and 74.

The reduction in RNA concentration during the experiment reflects increasing cell size. The interrupt in this trend between days 60 and 74 is in accordance with the increased relative growth rate in this period. The FSR fry has a lower RNA concentration than the ATR fry at day 60, this is also reflected by the low specific growth rate between days 52 and 60. This is probably due to a lower feeding ability in the FSR fry compared to the ATR fry in this period. An increase in the RNA concentration is observed in ATR alevins from day 18 to 32, this is in accordance with a high relative growth rate and a high RNA-DNA ratio.

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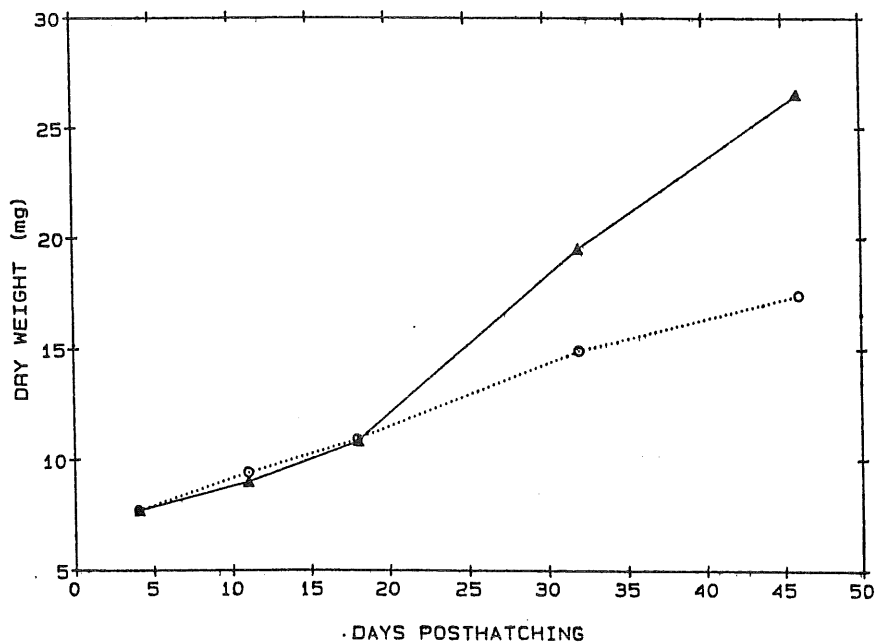


Fig. 1. Dry weight development of yolk-sac free alevins. Symbols: —▲—▲— ATR alevins, - - - O - - - FSR alevins.

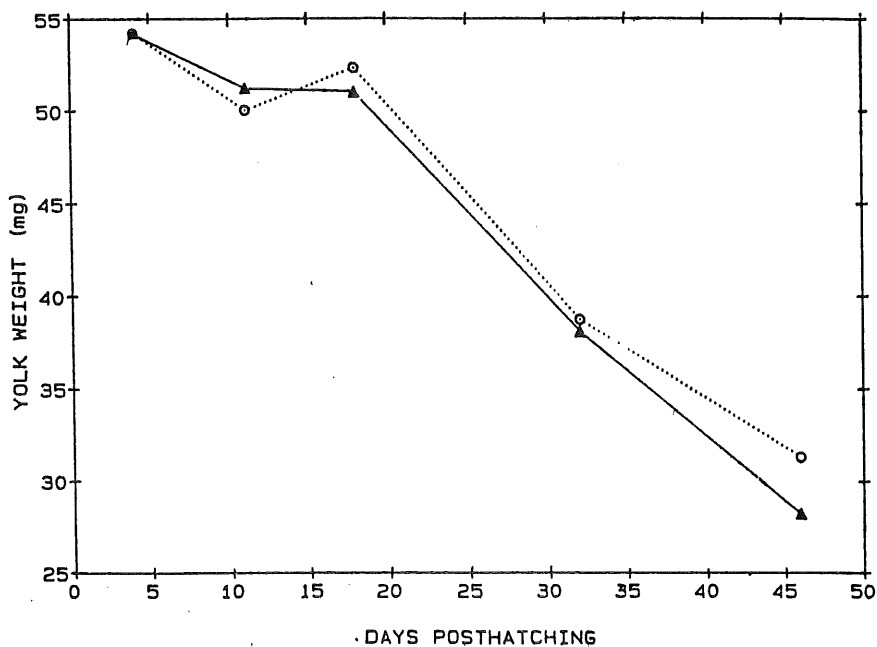


Fig. 2. Dry weight development of yolk during the hatchery period. Symbols as above.

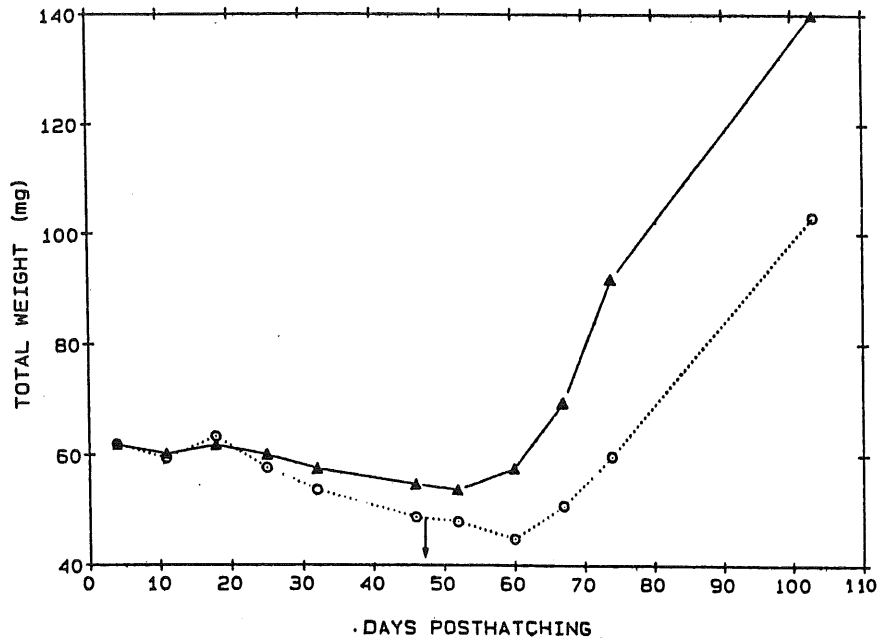


Fig. 3. Dry weight development of whole alevins and fry. Arrow indicate transfer to first feeding, symbols as above.

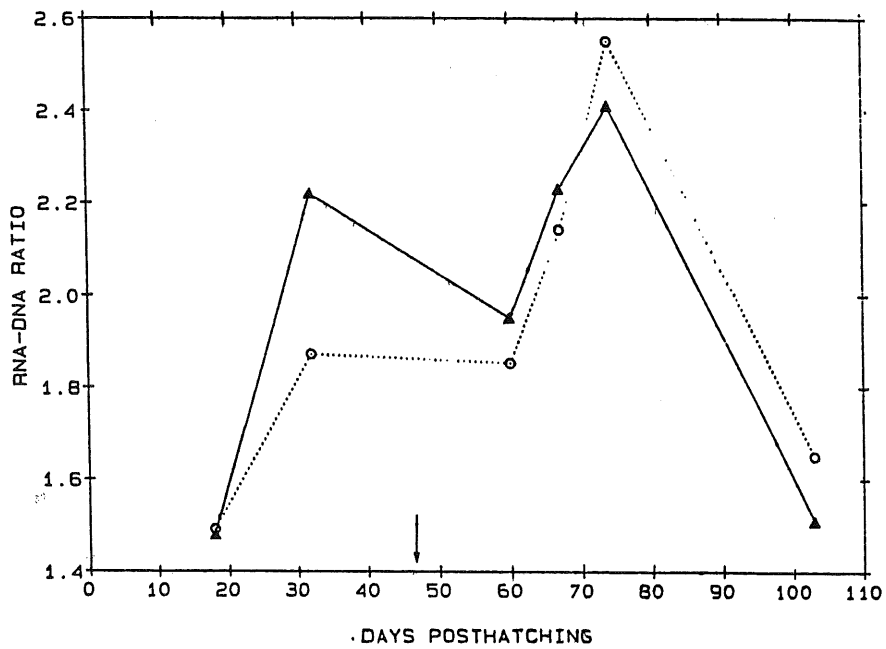


Fig. 4. RNA-DNA ratio of alevins and fry, symbols as above.

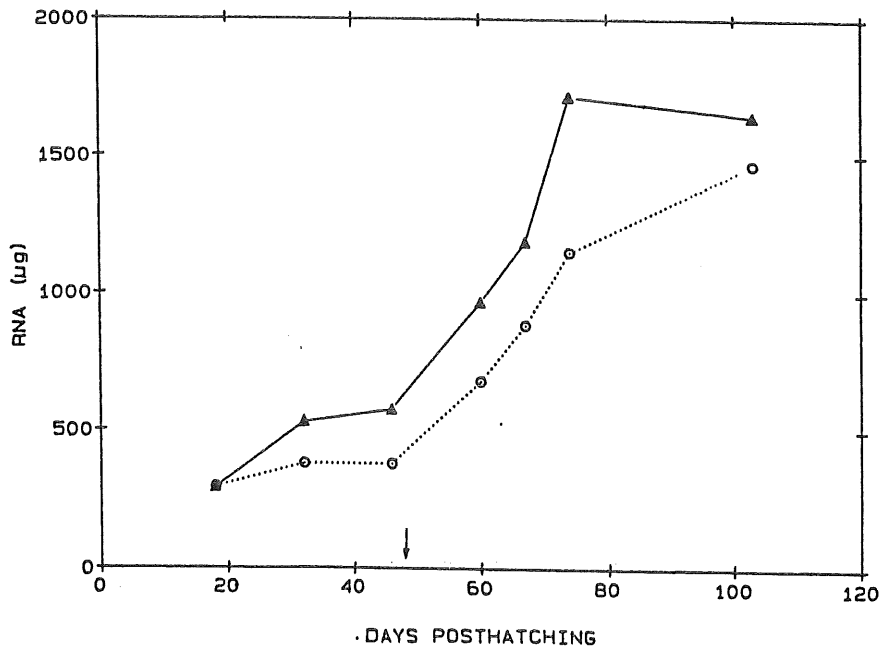


Fig. 5. RNA content in alevins and fry. Symbols as above.

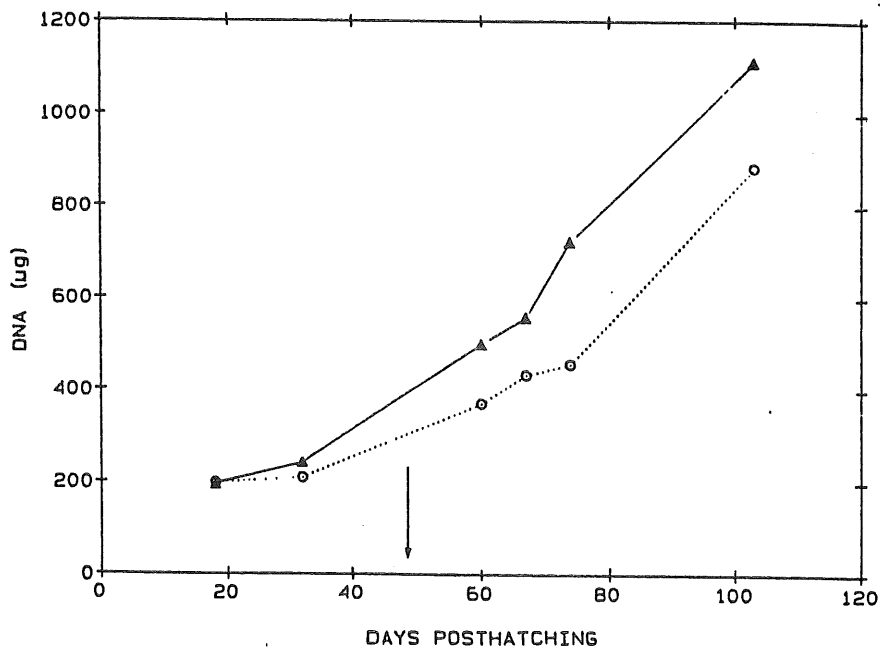


Fig. 6. DNA content in alevins and fry. Symbols as above.

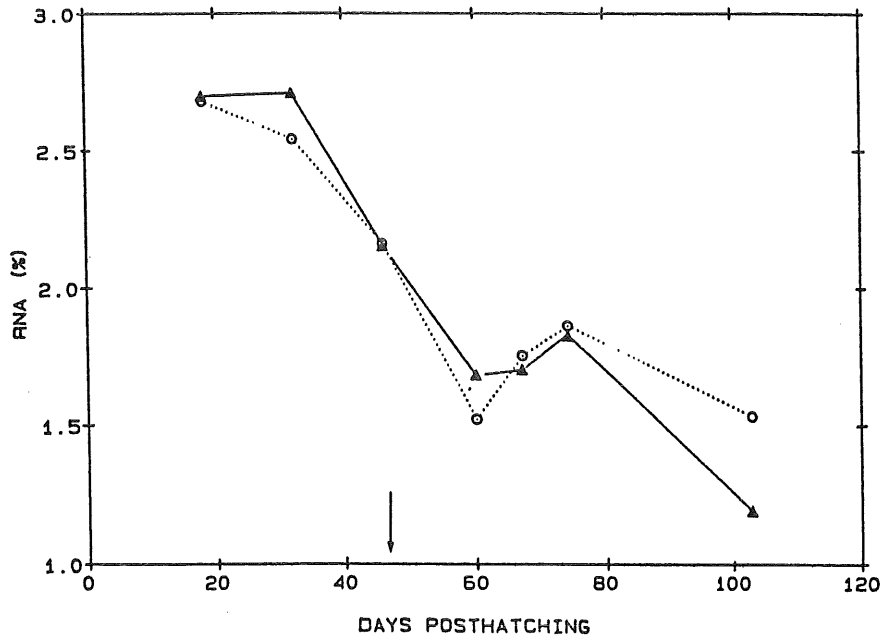


Fig. 7. RNA concentration (% RNA of dry-weight) in alevins and fry. Symbols as above.

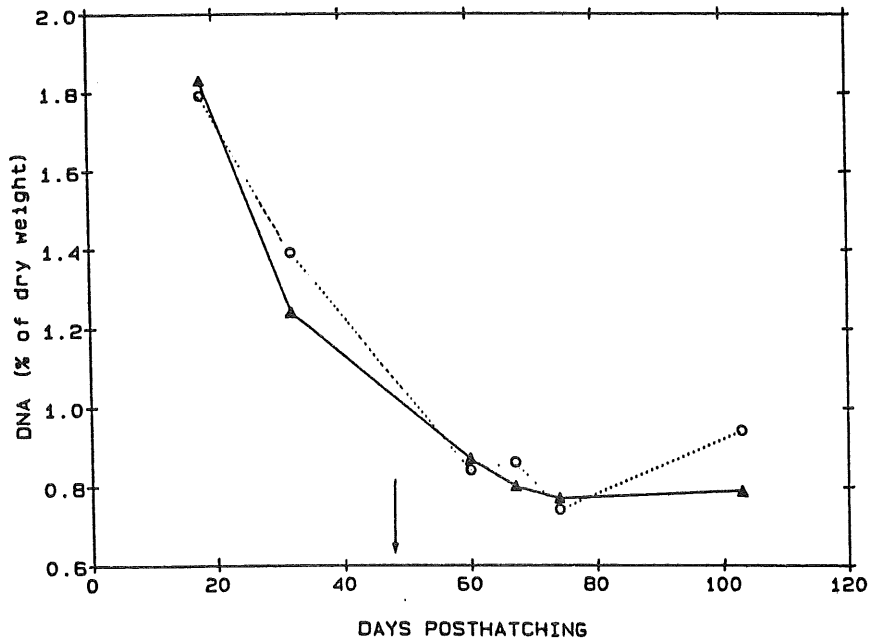


Fig. 8. DNA concentration (% DNA of dry-weight) in alevins and fry. Symbols as above.

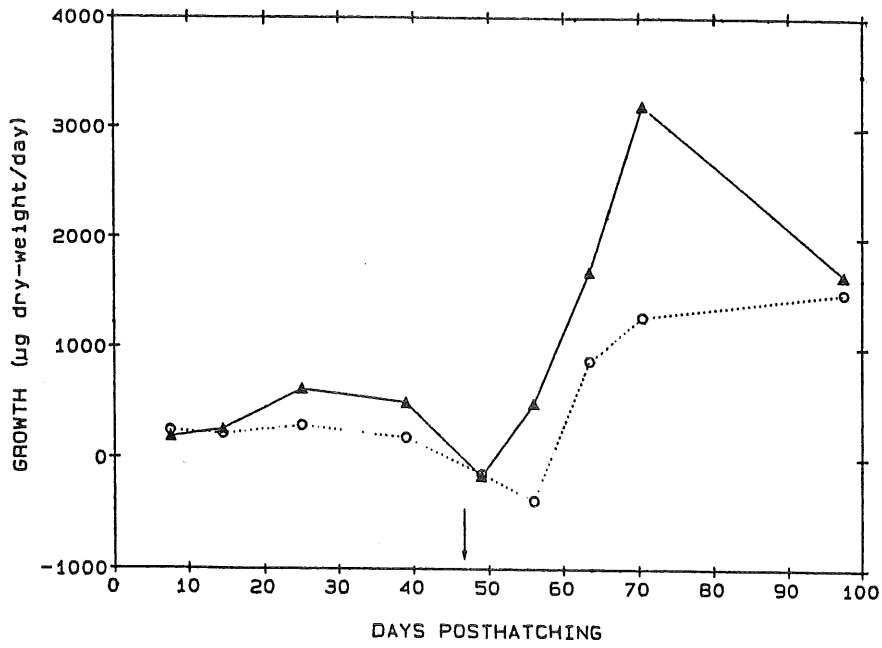


Fig. 9. Growth rate in alevins and fry. Symbols as above.

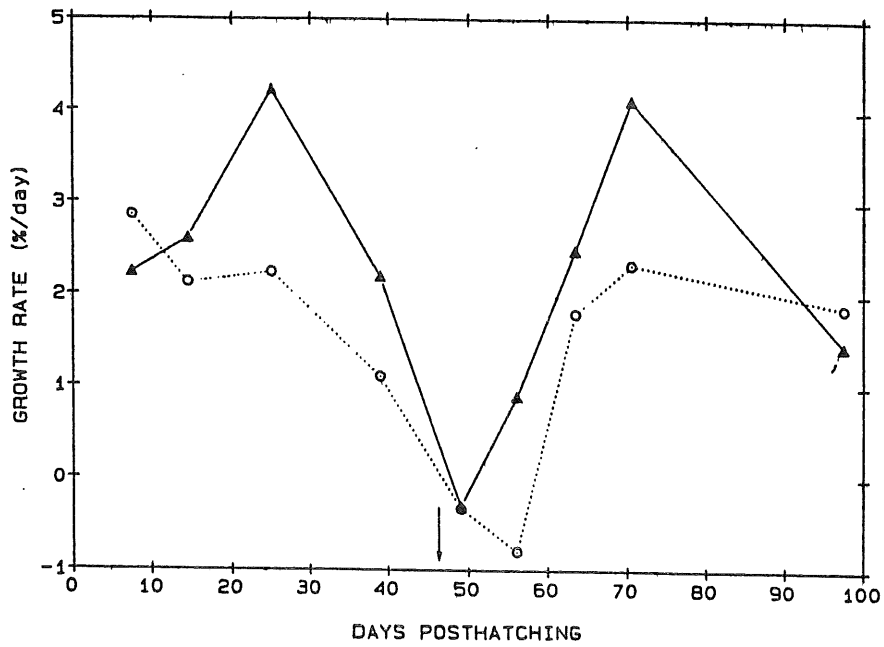


Fig. 10. Specific growth rate in alevins and fry. Symbols as above.

