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The influence of essential fatty acids composition on growth of larval cod (Gadus morhua L.). Preliminary observations.

by
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Abstract.

An experiment on early post yolk-sac larval cod (Skagerrak stock) was carried out to examine the influence of dietary lipids (20:5(n-3)) and 22:6(n-3)) on growth during April 1995. During the experiment, larvae were maintained under 3 experimental regimes, two based on feeding *Acartia tonsa* N1 nauplii of different essential fatty acid compositions, while one group of larvae was starved. In order to vary the essential fatty acid composition of the *Acartia tonsa* nauplii, eggs were obtained from cultures of adult Acartia fed either a) the diatom *Thalassiosira weissflogii* (TW), or b) the flagellate *Rhodomonas baltica* (RHO). Larvae (1500 per tank) were maintained in three 176 litre tanks at 8.0 °C during the duration of the experiment.

Estimates of larval growth and condition were obtained from measurements of standard length, dry weight and storage lipid content as well as nucleic acid content. On the last day of the experiment (day 18 after hatch) no significant difference in dry weight, standard length nor nucleic acid content per larvae was observed between the two larval groups fed on the different essential fatty acid diets (t-test, $p>0.05$). The starved larvae survived until day 11, at which time standard length was significantly lower than that of the larvae fed on the RHO or TW diets (ONEWAY, $p<0.05$), and individual dry weight was lower than that of Rho fed larvae (ONEWAY, $p<0.05$). No significant differences were observed in RNA/DNA-ratios of starved and fed larval groups (ONEWAY, $p>0.05$), however starved larvae had a significantly (ONEWAY, $p<0.05$) reduced RNA/dry weight and DNA/dry weight ratios compared to the larvae from the two feeding regimes.

The lipid and fatty acid composition in the cod larvae was examined on day 5 and day 16, and compared to the dietary lipids reported in the phytoplankton. Lipid levels in the individual larvae increased from approximately 10 µg to approximately 15 (RHO-group) and 23 (TW-group) after feeding on copepods for 11 days. Polar lipids were highest (15 µg) in the TW group on day 16 but the percentage distribution of lipid classes were similar for both fed groups and for the different age groups. TAG levels were around 1 ug/larvae and were similar or slightly higher in the 16-day old larvae than in the 5 day-old. Percentage-wise, TAG levels were much lower in the TW group. Levels of 20:4(n-6) in individual larvae were higher in the 16-day old larvae fed TW (540 ng), than larvae fed Rho (270 ng). The level in 5 day old larvae was 371 - 468 ng. The percentage levels of 20:5(n-3) (EPA) and 22:6(n-3) (DHA) were higher compared to levels in the 5-day old larvae. EPA levels were furthermore higher in the TW fed groups (1224 ng) than in the RHO group (733 ng), whereas they in percentage of total lipid were similar (5.0 - 5.4%). DHA levels were higher in TW group (3507 ng) compared to the Rho group (2416 ng) on day 16, but percentage-wise the groups were similar (15.4 - 16.5). DHA/EPA rations remained stable at around 3.

The utility of these different estimates of growth and condition for identifying processes influencing larval fish survival and the implications of variations of essential fatty acid content of food will be discussed with respect to growth and condition of early post yolk-sac cod larvae.

INTRODUCTION

The high mortality most marine fish species experience during the larval stage is often ascribed to starvation and predation acting independently or in conjunction (e.g. Hjort 1914; Lasker 1975; Sinclair, 1988). Low food abundances and hence reduced feeding success reduces the energy available to the larvae for both growth and metabolism thereby impairing the condition of the larvae and it's ability to avoid predators. Hence, mortality can occur due to starvation *per se* or as a consequence of prolonged or increased susceptibility to size selective predators.

However, the condition of larval fish is influenced not only by the amount of food ingested, but also by its quality (e.g. Scott et al. 1979). One of the constituents determining the quality of the food organisms is their fatty acid composition, and for marine fish larvae especially the polyunsaturated fatty acids (PUFA) are important (Fraser et al. 1988). Copepod nauplii, the principal food of most marine fish larvae, primarily provide PUFA as the two essential fatty acids (EFA) eicosapentaenoic acid (EPA; 20:5(n-3)) and docosahexaenoic acid (DHA; 22:6(n-3)) (Klungsøyr et al. 1989). Supplied in varying levels and ratios EPA and DHA have been shown to influence the growth of marine fish larvae (Koven et al. 1993).

In order to assess the condition and growth of larval fish and thereby better understand the linkage between variability in recruitment success and environmental processes a number of indices have been developed to assess the growth and condition of larval fish. For example, it has been shown that the ratio of ribonucleic acid (RNA) to deoxyribonucleic acid (DNA) is a useful indicator of the nutritional condition. Nucleic acids play a major role in growth and development. The amount of DNA, the carrier of the genetic information is quasi-constant in somatic tissues and tissue concentrations therefore reflect cell numbers (Dortch et al., 1983, Regnault & Luquet, 1974) while the amount of RNA in the cell is directly proportional to the amount of protein synthesis occurring. The relationship between RNA and DNA is an index of the cells metabolic intensity and has been used to measure recent growth in fishes (see review by Bulow, 1987). It has proven a useful indicator of the nutritional condition as shown in several larval fish studies (e.g. Buckley 1980, Clemmesen 1987, 1994, Robinson & Ware 1988).

Lipid content especially the ratio of triacylglycerol (TAG) to structural components of larval fish (e.g. cholesterol, phospholipids as well as dry weight) has been utilized for a number of fish species to assess the individuals ability to withstand periods of low feeding success (e.g. Håkanson, 1989). Triacylglycerol has also been observed to be preferentially stored by successfully feeding larvae able to meet their lipid metabolic requirements (e.g. Fraser et al., 1987). Stored TAG has been proposed to be preferentially utilized in some fish species for short term energy requirements, and has been proposed as an index of condition when use in relation to structural components such as sterols (e.g. Fraser et al., 1987).

The aim of this study is to examine the effects of the two essential fatty acids (EFA) eicosapentaenoic acid (EPA; 20:5(n-3)) and docosahexaenoic acid (DHA; 22:6(n-3)) on growth and condition of larval cod. In an attempt to investigate EFA effects on growth we have cultured the copepod *Acartia tonsa* on two monocultures of algae with different EPA and DHA levels, and fed the nauplii to two batches of cod larvae. The growth and condition in the different feeding regimes has been estimated on the basis of length, dry weight, RNA/DNA-ratios and larval lipid composition and compared with unfed larvae.

MATERIALS AND METHODS

Cod eggs and sperm were obtained from running adult cod obtained in trawls off Gilleleje, in the southern Kattegat on March 6, 1995. The fertilized eggs from 5 females were incubated separately in 20 l. polyethylene tanks containing 27 ppt sea water treated initially with Penicillin and Streptomycin at concentrations of 50 i.u./ml. The fertilized eggs were incubated at 8 °C without light prior to transfer to rearing tanks 5 days after hatching. The larvae hatched on 29 March and larvae (from one female) were transferred to 3, 176 l. cylindrical black polyethylene (1 m dia., 1.5m height) rearing tanks (1500 larvae per tank). The rearing tanks were double walled allowing cooling water to be circulated inside the walls thus maintaining the water temperature inside the tanks at 8.2 (+/- 0.1) °C during the experiment. Filtered sea water (27 ppt) was continuously added to the tanks through five water jets placed throughout the water column insuring a homogeneous distribution of nauplii

prey items. Water was drained from the tanks through a central stand pipe fitted with a 20 µm mesh nitex filter. Cool-white fluorescent tubes were utilized to vary light levels at the surface of the tanks from 0 to 1000-1300 Lux over a 24 hr light-dark cycle (14.5 hrs light day-1).
During the experiment, larvae were maintained under 3 experimental feeding regimes based on feeding *Acartia Tonsa* N1 nauplii of different essential fatty acid compositions to two groups while one group of larvae was starved. In order to vary the essential fatty acid composition of the nauplli, cultures of adult Acartia were fed either diet of a) the diatom *Thalassiosira weissflogii* , or b) the flagellate *Rhodomonas baltica*. Semi-continuous cultures of these algae were raised under constant light and temperature (16.0± 0.2 °C) as food for the adult *Acartia tonsa* cultures. Copepod eggs were then collected and stored for between 1-5 weeks prior to the experiment. Prior to introduction to the cod rearing tanks, copepod eggs were transferred to hatching tanks (50 l) and held at 16 °C for two days to ensure hatching of the eggs. The Stage 1 nauplii from these hatching tanks were then collected, counted and added as required to the larval rearing tanks. After the removal of the larval cod samples from the tanks, Acartia nauplii abundance was determined at three depths in each tank and the abundance of nauplii in each tank (excluding starvation trial) was raised to 200 N1 Acartia nauplii l-1.

Larval Sampling Regime

A total of 1031 cod larvae were sampled during the experiment for determination of biochemical variables and morphometric analysis. Table 1 gives the details of numbers fish sampled per day and analysis performed.

Dry Weight and Morphometric Analysis

Between 10 and 20 larvae were sampled daily for measurement of total length, eye diameter and notochord height at the point above the anus. After morphometric analysis larvae were rinsed in distilled water, dried at 60 °C for 24 hours prior to storage in a dessicator. Larvae were later individually weighed on a Cahn electrobalance. Specific growth rates (SGR) were calculated as: SGR = 100 ((expG) - 1); where $G = (\ln DWt_1 - \ln DWt_0) / (t_1 - t_0)$.

Biochemical Analysis

Samples of between 3-10 larvae were taken during the study (Table 1) for identification of group fatty acid composition and nucleic acid analysis. Larvae were examined microscopically for evidence of feeding, measured for total length then individually preserved in 1 ml polyethylene Safelock tubes under nitrogen gas prior to storage at -80 °C.

Nucleic Acid Analysis

During this study nucleic acid content was performed on individual larvae following the sampling scheme outlined in Table 1. Prior to analysis larvae were thawed and the standard length and freezedried (24 hrs) dryweight (Cahn Microbalance) of each larvae was determined. RNA- and DNA-content of individual larvae was then determined according to the fluorescence technique previously described by Clemmesen (1993) using a volume of 300 µl. The analytical procedure involves the purification of larval tissue homogenates prior to fluorescence-photometric measurements using the specific nucleic acid fluorescent dye (Ethidium Bromid) (EB)- for DNA and RNA. In order to determine the DNA-content of a sample, RNA was enzymatically digested with RNase and the remaining DNA was determined with EB.

Cholesterol and Phospholipid Content

Analysis for lipid group composition was performed on the homogenized tissue of between 3 and 5 larvae combined with the internal standard n-hexadecan-3-one (for the sampling schedule see Table 1). The tissue homogenate + standard was extracted twice in 1 ml chloroform:methanol (2:1 v/v) for 24 hours at -20 °C following the procedure outlined in Bligh and Dyer (1959). The tissue/solvent solution was then centrifuged for 10 minutes at 5000 rpm, and the extracting solvent removed. The solid fraction was then washed again with chloroform:methanol (2:1 v/v) and centrifuged for 5 min at 5000 rpm and the extracting solvent removed. The two solvent extracts were then combined, evaporated under N_2 gas and the remaining lipid fraction dissolved in hexane. Lipid group determinations on this lipid extract were performed by Thin Layer Chromatography/Flame Ionisation Detection (TLC/FID) on an Iatrascan Mk-5 following procedures outlined in Ackman(1981) and Ohman (1988). Throughout the study the flame-ionization detector was operated at a hydrogen pressure of 160 ml. min-1 and an air flow of 2000 ml. min-1. Individual sample runs were performed on 1.4 µl of the sample extract which was spotted onto type S-III Chromarods using

an automated sample spotter (Model SES 3200/IS-01). The spotted rods were humidified before development in a supersaturated NaCl solution, then developed once for 35 min. in a solvent composed of hexane/diethyl ether/ formic acid (82:18:1, v/v/v). The developed rods were then dried at room temperature, before being scanned on the TLC/FID system for 1 uninterrupted pass of 35 seconds. Analysis and quantification of lipid profiles from these samples was performed on an IBM computer using Chromastar Light software version 3.24S for windows (Bruker Fransen Analytik & SCPA). Triplicate analyses were performed on each tissue subsample from each female and the reported mass is the mean of these three estimates. Calibration of the Iatroscan was performed on standards of Phospholipids (PL; L- -phosphatidylcholine); Sterols(ST; Cholesterol); Free Fatty Acids(FFA; palmitic acid); triacylglycerols (TAG; tripalmitin); wax esters(WE; palmitic acid palmityl ester) obtained from Sigma. Identification of the specific lipid classes in the samples were performed by comparison to the retention times of these known standards. The mass of each lipid class was then estimated by integrating the area under the curve at the known retention time for this class and comparison to the estimated mass of the internal standard. The mass of each lipid component was then corrected for its specific mass response factor relative to that of the internal standard.

Fatty Acid Composition

Samples of 5 day-old unfed and 16-day-old larvae from the two previously described feeding regimes were taken for lipid analysis at Stirling University, UK. Larval samples were immediately extracted in a methanol/ethanol solution containing 0.01% (w/v) BHT as antioxidant and stored at -20 °C prior to analysis. Total lipid was separated into classes by thin-layer chromatography following procedures outlined in (Fraser et al., 1987)

RESULTS.

Morphometric Analysis.

The mean standard length of the larvae at the start of the feeding experiment (day 5) was 4.52 mm ± 0.2 (s.d.). No significant change in length of the starved group was observed between day 5 and day 11. A significant difference in total length in fish from the two feeding regimes was not evident until after day 12, due to large day to day variations. At the end of the experiment (day 18) total lengths were 5.1±0.23 mm and 5.1±0.14 mm of larvae fed either the *Thalassiosira* and *Rhodomonas* diets respectively. The mean starting weight (day 5) of the cod larvae was 38.3±3.5 µg. Larvae from the starvation trial gradually lost weight and at day 11 weight was significantly lower than on day 5 (31.3±1.8 µg; t-test, $p<0.05$). The group fed Rho increased weight from day 9, and reached 60.1±9 µg day 18. Significant gain in weight was first seen on day 18 in the TW fed group (66.6±9 µg; t-test, $p<0.05$). Specific growth rates from day 5 to day 12 were 0.01% d-1 , 3.53% d-1 and -2.84% d-1 for the TW, Rho and starved group, respectively. From day 12 to 18 specific growth rates of the TW and Rho groups were 9.66 % d-1 and 3.54 % d-1.

RNA and DNA-Analysis

DNA-content of fed larvae increased from day 6 up to day 16 in both fed groups (Fig. 2). For some unknown reason DNA- content per larvae decreased between day 16 and day 18 in both feeding regimes. No significant differences in DNA content were observed between the two fed groups during the study. Larvae from the starving group had similar DNA-content between day 6 and day 10 as that observed in the fed larvae whereafter a decrease in the DNA- content per larvae was observed in comparison to the fed larvae. A significant difference between DNA content of the starved and fed groups was evident on day 11. The DNA/dryweight ratios of individually measured larvae is given in Fig. 3. The ratio decreased during the course of the experiment indicating that the amount of cytoplasma increases in relation to DNA. The values in both feeding groups follow the same trends showing no significant differences. However, in the starved group an overshoot of DNA/dryweight between day 7 and day 8 can be seen followed by a decrease in the DNA/dryweight ratio until the end of the experiment at day 11.

A comparison of the RNA-content of fed and starved cod larvae is given in Fig. 4. The RNA-concentration increased from day 6 to day 18 showing no significant difference between larvae from the two feeding regimes. The RNA-content of the starved group was similar to the values found in the fed groups up to day 9. After this time RNA-content decreased and was significantly lower than in the fed groups. The RNA/DNA ratios during the course of the experiment are given in Fig. 5.

The RNA/DNA ratios varied around a value of 2 in both feeding groups and showed high variability. No significant differences between both feeding groups could be seen. The RNA/DNA ratios in the starved group overall showed a trend to lower ratios compared to the feeding groups, but could not be significantly separated. The RNA/dryweight ratios are presented in Fig. 6. Both feeding groups follow the same trend starting with high values at day 6 and then levelling of at a ratio of about 40. No significant differences in both feeding groups can be seen. The RNA/dryweight ratio of the starved cod larvae starts to separate from the feeding groups on day 9 and decreases until the end of the experiment.

Lipid Composition

The lipid content in the 5-day old larvae was around 10 ug and increased to 14.6 ug in the larvae fed Rho and to 22.7 ug in those fed TW (Table 3). The neutral lipids were dominated by cholesterol and triacylgylcerol (TAG) levels ranged from 5 - 10% of the total lipids. Expressed in absolute values, ug /larvae, TAG levels were relatively similar in the 5-day old (0.7 to 0.9 ug/larvae) and in the TW and Rho fed groups respectively (1.1 and 1.3ug/larvae). Polar lipids constituted 60-66% of total lipids, the major groups being phosphatidylcholine (PC) and phosphatidylethanolamine (PE). HUFA levels expressed as percentage of larval dry weight increased from 5.4-5.7% in the 5 day-old to 6% in the 16-day old larvae fed Rho and to 12.5% in those fed Rho. Levels of EPA increased from 1.1% of the dry weight in 5-day old to 2.7% in those fed TW as compared to 1.2% in Rho-fed larvae. Levels of DHA increased from around 2.9-3.0% in 5-day old cod to 4% in those fed Rho and 7.7% in those fed TW.

DISCUSSION.

Morphometric Analysis.

The growth trajectories found in this study are comparable to those of other experiments carried out on Atlantic cod (Neilson et al. 1986, Raae et al. 1988, Otterå, 1993). Typically (especially in laboratory experiments) minor changes in length and weight are observed in larval cod prior to day 10 after. A low feeding incidence reported for nauplii feeding cod larvae younger than 8-10 days (Ellertsen et al. 1980, Otterå 1993) could explain this. After day 10 to 12 increases in dry weight was observed in both fed groups indicating adequate feeding conditions. However, the specific growth rates observed in this study were low compared to other studies (van der Meeren et al. 1993, Pedersen et al. 1989). The differences in the diets of the two fed groups did not reflect in any significant differences in weight or length at day 18. However the mean dry weight of the fish from the TW regime was 10% higher than those from the RHO regime with higher specific growth rates evidenced in the TW group than in the Rho during the last part of the experiment.

RNA/DNA-analysis

No differences in the condition of cod larvae fed on the two different Acartia diets could be found neither with DNA- content, RNA -content, RNA/DNA ratio or DNA/dryweight or RNA/dryweight ratio. The difference in the fatty acid composition of the diets had no discernible effect using this technique on growth and performance on cod larvae in the life stage analyzed. In this study the nutritional situations was best reflected by the RNA-content. In contrast to other studies by Clemmesen (1987, 1994) and Buckley (1984) the RNA/DNA ratio could not separate between the feeding and starving larvae convincingly. In this study, larval DNA content was used as an indicator of cell number, reflecting the growth of the larva. During yolk absorption the DNA- content was stable and increased with age starting on day 7. The DNA- content surprisingly seemed to be affected by the feeding situation. This was most obvious when comparing the DNA/ dryweight ratios in the starved larvae where a decrease in DNA/dryweight ratio could be seen. Since the methodology was sensitive enough to measure the dryweight and the DNA content on the same larvae new insights into DNA metabolism have been made possible.

Lipid Composition

Lipid content in the 5-day old larvae in this experiment were 1-4 ug higher than levels reported in 5-day old cod larvae by Rainuzzo et al. (1992). During starvation these lipid levels were observed to drop to 5 ug/larvae during the first 13 days after hatching Fraser et al., (1988). Lipid levels in larval

cod (< 1 cm) in nature have not been reported, but the increase in levels during the experiment suggest that the larvae were feeding well with an approximate doubling of lipid content in larvae raised in the TW regime and a 60 % increase in the lipid content of the larvae from the Rho regime. Cod have been reported to catabolise phospholipids, primarily PC during the first week after hatching (Fraser et al., 1988) providing lipid-bound phosphorus and choline which has been shown to be essential for larval growth. PC levels in 5-day-old fish were found to vary from 38-42% of total lipids (Rainuzzo et al., 1992). In this experiment levels varied from 24-26% in the 5-day and 16-day old cod. Individual levels were higher in the feeding larvae (16-day-old) suggesting that this resource was being depleted during feeding.

PC was suggested to be the main source of essential fatty acids (EFA) for developing embryos (Fraser et al., 1988) and this was further supported by Rainuzzo et al. (1988) for eggs and larvae of cod, implicating a nutritional and physiological role. The nutritional importance of EPA and DHA has been reported for several marine larval species (Watanabe, 1982). Although quantitative requirements have not been established for cod, HUFA levels in this experiment represent 12 and 6% of the larval dry weight for TW and Rho fed larvae respectively, and consist mainly of EPA and DHA. In the fish fed TW, DHA and EPA levels, in terms of percentage larval dry weight, was doubled. However, in larvae from the Rho feeding regime, levels of these 2 fatty acids were not very different from those in the 5-day old. EPA and DHA levels in mature cod eggs were reported to represent 3.5% of the dry weight (Tocher and Sargent, 1984) and an estimated dietary requirement of 1.3% DW was reported for these EPAs by Le Milinarie et al. (1983) for turbot larvae. In this experiment, HUFA levels in the Rho-fed larvae were lower than in those fed TW, but were very high in both treatments. Larvae fed Rho were larger by day 16 than those fed TW. EPA levels in this study compare well with levels reported for newly-hatched cod (2.3-2.6 ug DHA/larvae and 0.8-0.9 ug EPA/larvae) by Rainuzzo et al. (1992). The levels found in this study suggest that levels in the copepods met with the cod larval dietary requirements for these EPAs. Unfortunately, it was not possible for us to compare dietary lipids with those in the larvae in this experiment. The cryptophyte, *Rhodomonas baltica* contained slightly lower DHA levels with a DHA/EPA ratio of 0.7 (Norsker & Støttrup, 1994), whereas *Thalassiosira weissflogii* contained relatively high levels of EPA, with a DHA/EPA ratio 0.22. In the cod larvae, ratios were around 3 (2.6 - 3.3), and correspond to ratios found in nauplii of the harpicticoid, Tisbe holothuriae (1.8) (Norsker & Støttrup, 1994) and in turbot larvae fed calanoid copepods from outdoor tank cultures (1.5-3.2) (Witt et al., 1984).

TAG is most likely the most important source of metabolic energy during the larval stage. This was shown to be the case during embryogenesis in cod (Fraser et al., 1988), a finding which was at variance to the suggestion by Fyhn & Serigstad (1987), that free amino acids (FAA) were important energy substrates during embyrogenesis and during the yolk-sac stage and very important in the first food items for cod larvae. TAG levels in the cod in this experiment varied between 5-10% and were similar to levels in previously observed in newly-hatched cod of 6-9% (Rainuzzo et al. 1992).

Conclusion

The results of this study are restricted due to the fact that an evaluation of feeding success and thus nutritional condition in early larvae is influenced by the process of internal yolk absorption. Hence, differentiating condition between larvae learning to capture food items and starving larvae in the first days after hatching is difficult and a general problem in condition analysis independent of the technique used (see Clemmesen 1994). Future studies will examine the these relations more closely in older larvae in order to further evaluate the results observed here.

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Table 1. Numbers of cod larvae analyzed.

Analysis	Morphometrics			RNA/DNA			Cholesterol/Phospholipid			Lipid composition		
Feeding regime	TW	Rho	Starved	TW	Rho	Starved	TW	Rho	Starved	TW	Rho	Starved
Age	Sampled larvae			Sampled larvae			Sampled larvae			Sampled larvae		
5	30 x 1 larvae were sampled prior to splitting into feeding regimes			-	-	-	-	-	-	2 x 200 larvae were sampled prior to splitting into feeding regimes.		
6	20 x 1	20 x 1	20 x 1	5 x 1	6 x 1	-	2 x 5	1 x 3	1 x 5	-	-	-
7	20 x 1	20 x 1	20 x 1	9 x 1	8 x 1	6 x 1	-	1 x 5	1 x 5	-	-	-
8	20 x 1	20 x 1	20 x 1	11 x 1	8 x 1	7 x 1	2 x 5	1 x 4 1 x 5	1 x 5	-	-	-
9	20 x 1	20 x 1	20 x 1	10 x 1	8 x 1	10 x 1	1 x 4 1 x 5	2 x 5	1 x 5	-	-	-
10	20 x 1	20 x 1	20 x 1	10 x 1	10 x 1	9 x 1	2 x 5	1 x 4 1 x 5	2 x 5	-	-	-
11	20 x 1	20 x 1	20 x 1	10 x 1	10 x 1	8 x 1	2 x 5	1 x 4 1 x 5	1 x 5	-	-	-
12	20 x 1	20 x 1	-	10 x 1	10 x 1	-	1 x 5 1 x 3	2 x 5	-	-	-	-
13	-	-	-	-	-	-	-	1 x 5	-	-	-	-
14	10 x 1	10 x 1	-	5 x 1	5 x 1	-	1 x 5	1 x 5	-	-	-	-
15	-	-	-	5 x 1	5 x 1	-	2 x 5	2 x 5	-	-	-	-
16	10 x 1	10 x 1	-	5 x 1	5 x 1	-	1 x 5	1 x 5	-	1 x 49	1 x 50	-
17	-	-	-	-	-	-	-	-	-	-	-	-
18	10 x 1	10 x 1	-	10 x 1	10 x 1	-	2 x 5	1 x 5	-	-	-	-

Table 2. Cholesterol and phospholipid content of starved, TW or Rho fed cod larvae. Lipid determinations were performed by Thin Layer Chromatography/Flame Ionisation Detection. Dry weights are mean of 20 larvae.

Feeding regime	TW			Rho			Starved		
Age	Chol	PI	DW	Chol	PI	DW	Chol	PI	DW
6	0,31	7,65	35,10	0,31	7,65	37,60	0,31	7,65	34,03
7			35,45			35,48	0,40	8,87	34,80
8		9,12	38,15	0,64	8,49	34,22			32,67
9	0,65	9,48	37,67	0,89	11,63	43,98			30,85
10	0,58	10,10	35,30	0,72	11,43	41,54	0,73	10,67	31,32
11	0,82	11,26	33,07			43,94			
12	0,71	12,30	38,32	0,91	12,60	48,80			
13				1,01	11,86				
14			48,43			56,21			
15	0,67	13,27		1,37	11,35				
16									
17									
18	0,93	13,88	66,64	1,47	12,30	60,13			

Table 3. Fatty acid composition of larvae before start-feeding and larvae fed TW and Rho.

	5d	5d	TW(16d)	Rho(16d)
Nr. larvae	196	198	49	50
Lipid (µg/larvae)	10,9	9,3	22,7	14,6
Total polar (%total lipids)	63,8	59,3	66	63,6
PE (µg/larvae)	2,2	1,7	4,9	3,6
PC (µg/larvae)	2,8	2,2	5,8	3
Total neutral (% total lipid)	34,8	38,7	34,2	36,3
Cholesterol (µg/larvae)	2,3	1,9	5,3	3
TAG (µg/larvae)	0,7	0,9	1,1	1,3
Fatty Acid Composition	**5d**	**5d**	**TW(16d)**	**Rho(16d)**
12:00	0,030	0,026	0,067	0,046
14:00	0,060	0,045	0,173	0,116
16:00	1,560	1,250	2,688	1,675
18:00	0,641	0,479	1,156	0,749
Total saturates	2,290	1,800	4,085	2,586
16:1n-7	0,289	0,232	0,626	0,309
18:1n-9	0,724	0,598	1,166	0,888
18:1n-7	0,372	0,292	0,626	0,371
20:1n-9	0,106	0,091	0,164	0,085
22:1n-11	0,070	0,060	0,145	0,077
24:1	0,027	0,020	0,048	0,031
Total monoenes	1,586	1,293	2,775	1,760
18:2n-6	0,056	0,088	0,241	0,185
18:3n-6	0,000	0,011	0,058	0,000
20:2n-6	0,020	0,017	0,029	0,023
20:3n-6	0,013	0,000	0,029	0,000
20:4n-6	0,468	0,371	0,540	0,270
22:5n-6	0,023	0,020	0,106	0,039
Total n-6	0,581	0,507	1,002	0,517
18:3n-3	0,066	0,017	0,029	0,116
18:4n-3	0,013	0,000	0,000	0,039
20:3n-3	0,000	0,000	0,000	0,000
20:4n-3	0,030	0,011	0,029	0,031
20:5n-3	0,428	0,417	1,224	0,733
22:5n-3	0,123	0,116	0,260	0,124
22:6n-3	1,095	1,134	3,507	2,416
Total n-3	1,756	1,695	5,049	3,458
Sum Lipids	6,213	5,295	12,911	8,322
Total PUFA	2,337	2,203	6,051	3,976
Total HUFA	2,200	2,086	5,723	3,636
%HUFA	35,417	39,400	44,328	43,692
%HUFA/DW	5,745	5,447	12,496	6,000
%DHA/DW	2,860	2,961	7,657	3,987
%EPA/DW	1,118	1,088	2,672	1,210
DHA/EPA	2,558	2,721	2,866	3,295

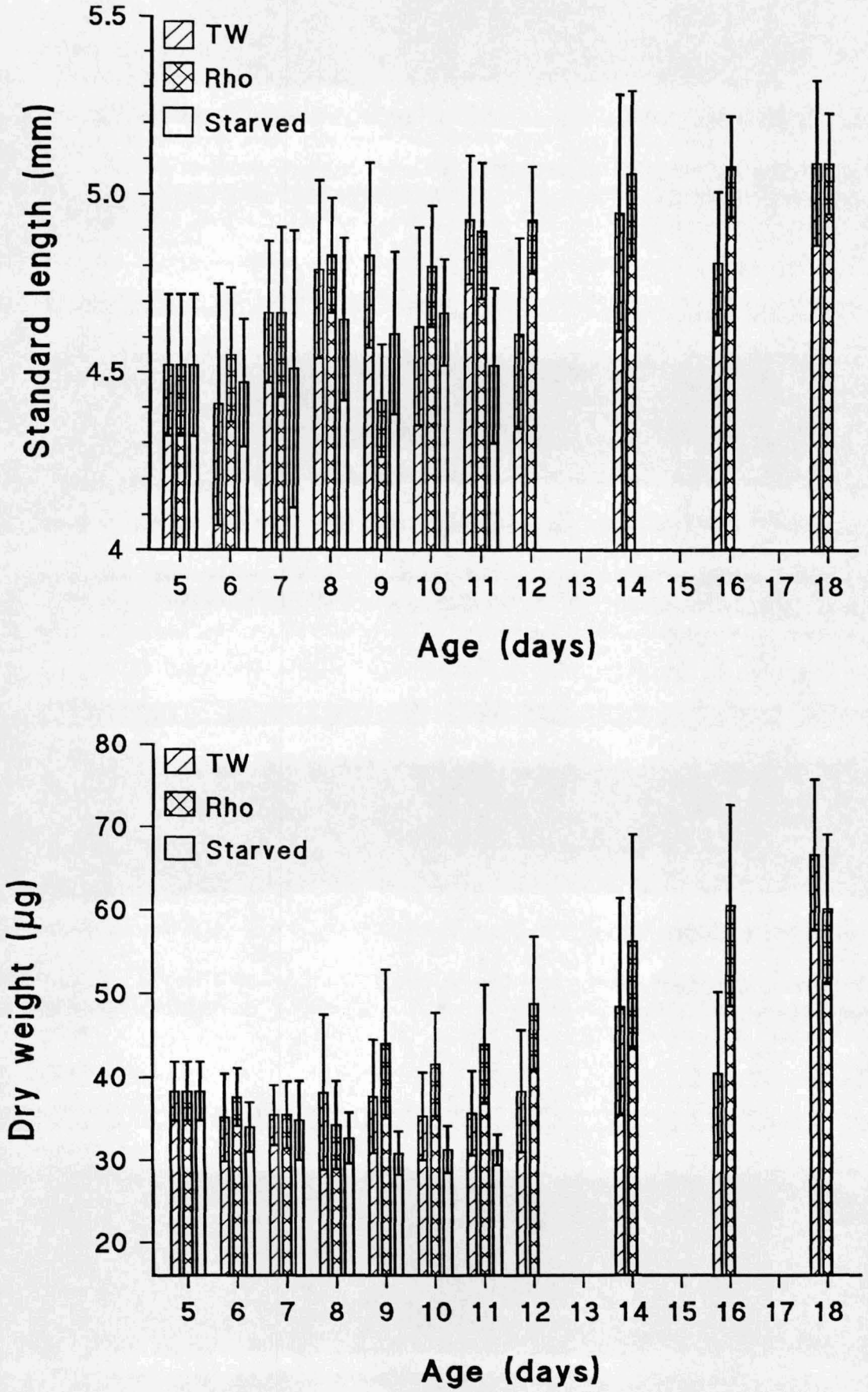
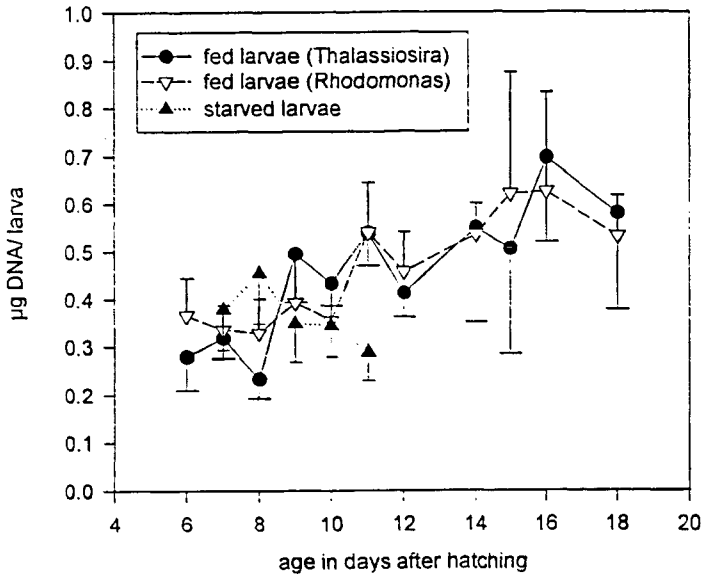


Fig. 1. Mean standard length and dry weight (± s.d.) of 20 larvae.



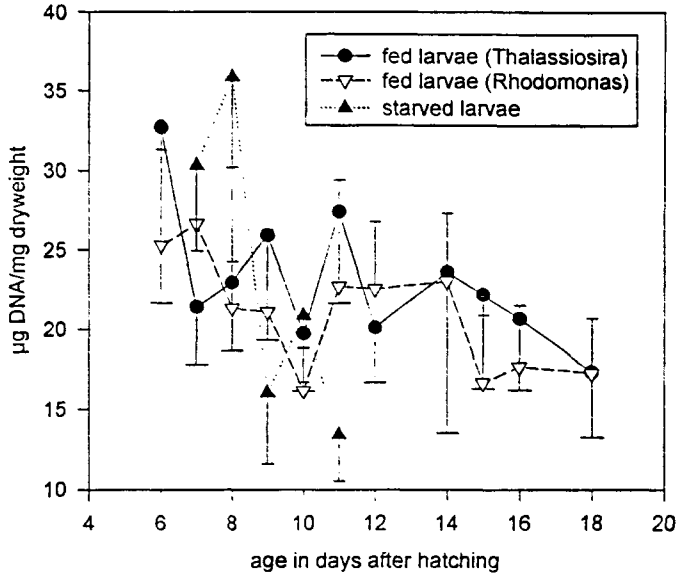


Fig. 2. DNA- content per larva in relation to larval age of fed and starved cod larvae. Data points are means of 5-10 individually analysed cod larvae. Error bars are shown only one-sided.

Fig. 3. DNA/dryweight ratio per larva in relation to larval age of fed and starved cod larvae. Data points as fig. 2.

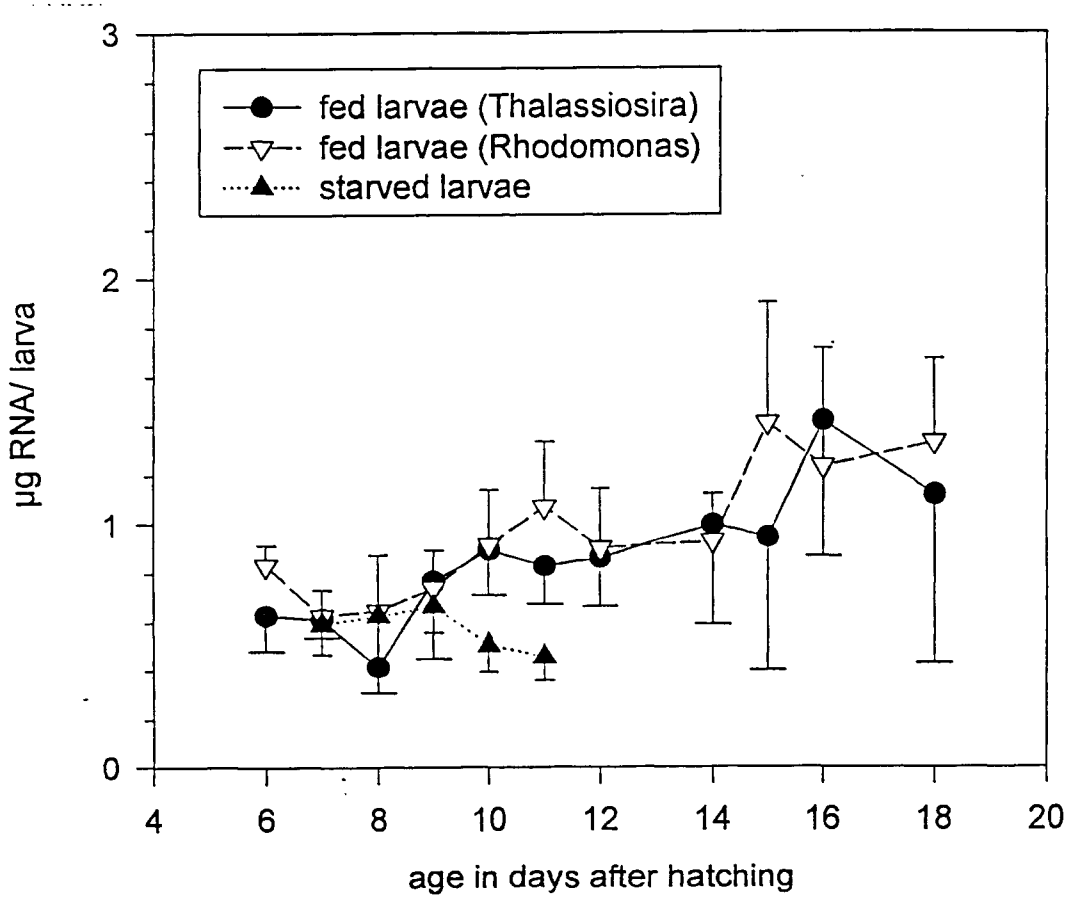


Fig. 4. RNA- content per larva in relation to larval age of fed and starved cod larvae. Data points as fig. 2.

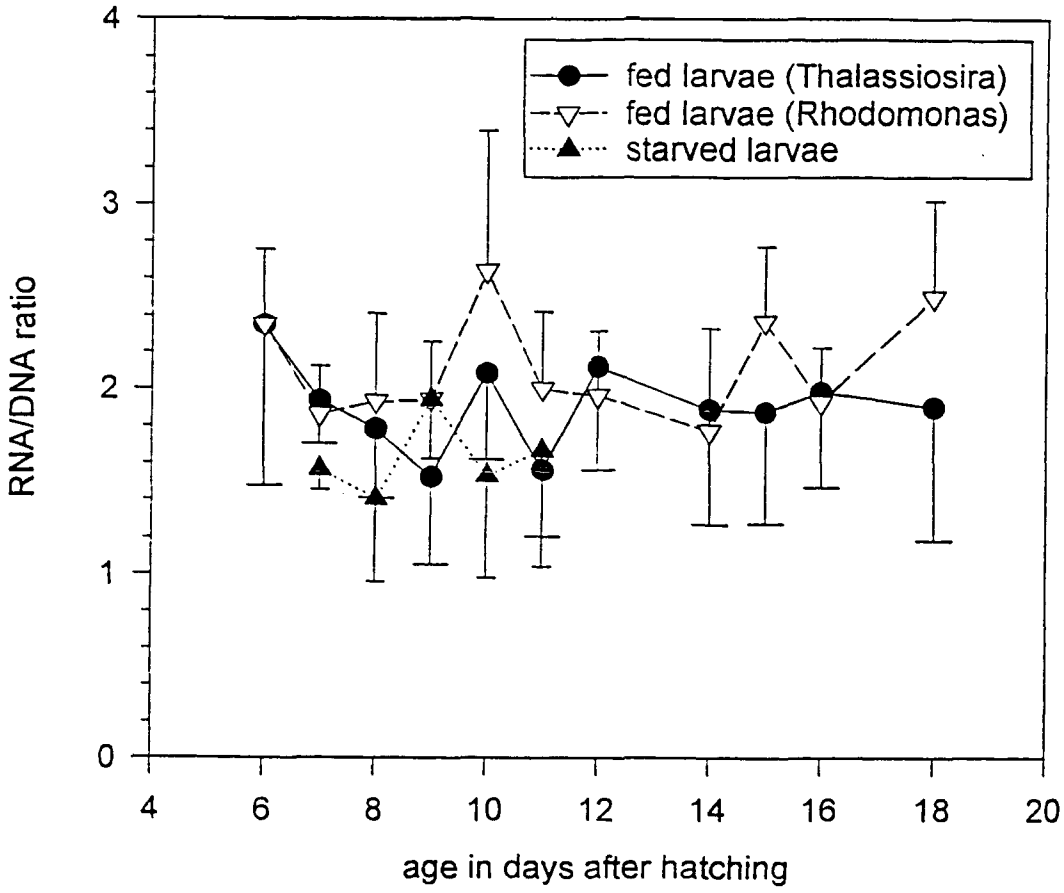


Fig. 5. RNA/DNA ratio in relation to larval age of fed and starved cod larvae. Data points are means of 5-10 individually analysed cod larvae. Error bars are shown only one-sided.

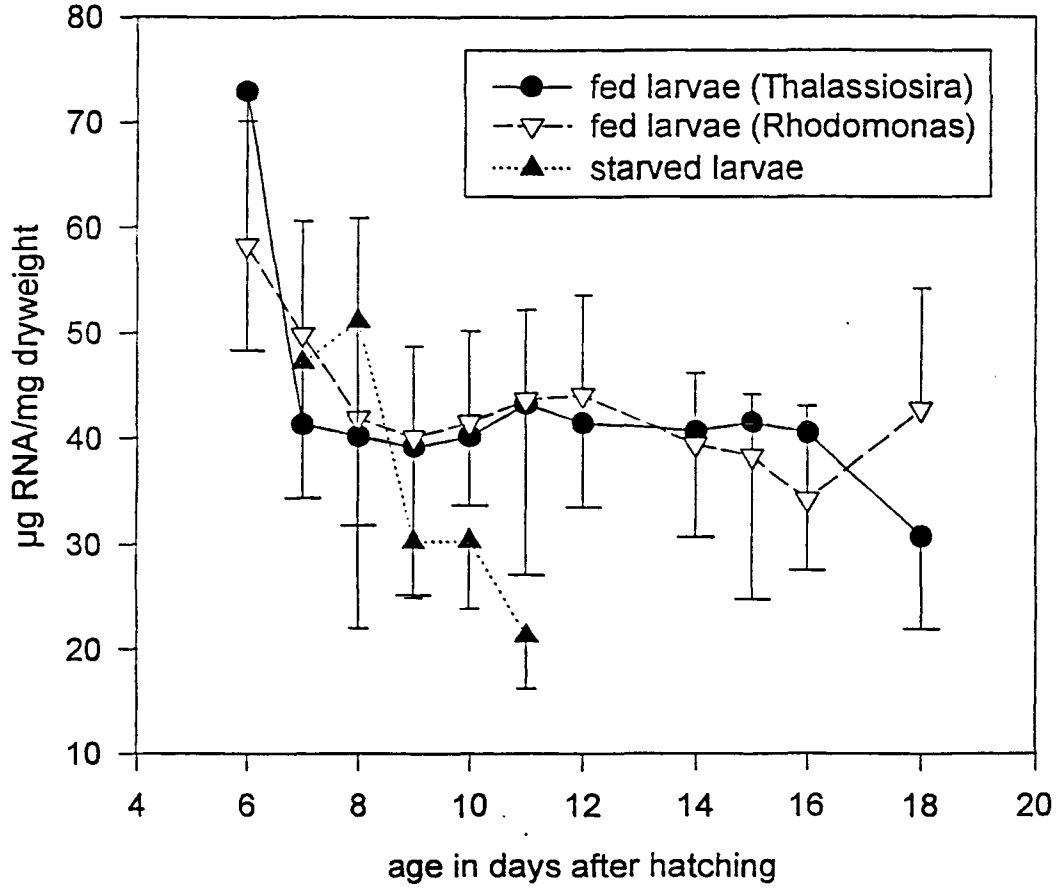


Fig. 6. RNA/dryweight ratio per larva in relation to larval age of fed and starved cod larvae. Data points are means of 5-10 individually analysed cod larvae. Error bars are shown only one-sided.