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A pilot study of halibut larvae (Hippoglossus hippoglossus L.) reared from start feeding to metamorphosis on diets of wild zooplankton and Artemia.

by

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ABSTRACT

Halibut larvae ready to start feed were placed in two outdoor tanks of 7 m^3. The larvae in one tank were fed wild zooplankton throughout the whole period, and the larvae in the other tank were fed wild zooplankton from day one to day seven and Artemia thereafter. Measurements of growth, gut content and content of fatty acids were made of the larvae. Number and species of phytoplankton and zooplankton, as well as abiotic parameters were measured during the experiment.

Larval myotome height and dry weight were significant higher for the group supplied wild zooplankton and Artemia than for the group supplied only wild zooplankton, at day 22 after first feeding. For the larval group supplied only wild zooplankton the myotome height and length, at day 43 (the end of the experiment), were significant higher than the group supplied both wild zooplankton and Artemia.
Fatty acids analysis did not show significant differences between the larvae in the two feeding groups. There was not observed differences in larval pigmentation either.

INTRODUCTION

Halibut larvae have been reared to metamorphosis every year since 1985 at Austevoll Aquaculture Station (Berg and Øiestad 1986, Rabben et al. 1986, Naas et al. 1987). Different rearing systems have been used for both the yolk-sac stage and start feeding (Pittman et al. 1989). However, the main diet for start feeding experiments have been wild zooplankton, either collected from the sea or a pond. Amount and appearance of species in a wild zooplankton community are strongly varying during the season. Wild zooplankton is therefor not a reliable food source for artificial rearing of marine fish larvae. Cysts of Artemia are however commercially available, and Artemia would therefore be an alternative diet. Cultivated and enriched rotifers (Brachionus plicatilis) and Artemia have been used as food for halibut larvae (Lein and Holmefjord 1989). Growth and survival has so far been lower for larvae fed rotifers and Artemia than larvae fed wild zooplankton. Mal-pigmentation of halibut larvae occurs more frequently when fed rotifers and Artemia than wild zooplankton.

The present study was conducted in 1989 to see if Artemia could replace wild zooplankton as food for halibut larvae after these had been fed wild zooplankton for a short period.

MATERIALS AND METHODS

Eggs & larvae

Eggs were stripped from one female, and fertilized with sperm from one male. After 9 days in hatchery the eggs were transferred to 5 m^3 silos (Rabben et al. 1987). The mean water temperature during the yolk sac period was 7 °C. After 35 days (250 day-degrees) the larvae were collected from the silos, and transferred to two outdoor tanks, after sunset.

The system

Two flatbottomed tanks (3 m diameter, 1 m height) placed outdoor were used. Both tanks were filled with water taken from 50 m dept. Fertilizer (a N-P-K complex fertilizer 21-4-10, Norsk Hydro) were added 10 days before larvae were introduced, to create a phytoplankton bloom. The fertilizer was added three times during the experiment to reach a concentration of 20 μM nitrogen. The water was kept stagnant throughout the experiment except at two occasions where half the volume of the water were replaced with corresponding volume of 50 m water. The tanks were covered with a black polyethylene net (70 % light reduction) immediately after larvae were introduced and kept there for the hole experiment.

Live feed

Wild zooplankton (80 μm < x < 249 μm the first 20 days, x > 249μm from day 21) were collected from a pond by using a wheel filter (Unik Filtersystems A/S, Norway). The wild zooplankton was then administered to both tanks from day one and to day seven, giving final concentration of 300 - 500 individuals/l. Thereafter supply of wild zooplankton continued in tank A, while tank B was offered Artemia instar II at the same concentration. Artemia was hatched in accordance with Sorgeloos et al. (1986). A Schindler watersampler (15 l) was used to collect zooplankton samples and a Ruttner (2 l) watersampler to collect phytoplankton samples, at 0.5 m in the middle of the tanks, every 3.rd day. Zooplankton samples were fixed in acid Lugol's solution. Phytoplankton samples were fixed in 4 % formaldehyde.

Environmental measurements

Temperature, oxygen and salinity were monitored every 3.rd day and nutrients every 5.th. day. Water for analysis of nutrients were collected using a 2 l water sampler (Ruttner).

Larvae samples

Net samples of larvae were collected at day 8, 14, 22, 29, 36 and 43. Examination of growth (length, myotome height, wet- and dry weight), gut content and fatty acid content were done. Samples for size, weight and gut content were conserved on 4% formaldehyde and stored one month before further analyses in a dissecting microscope. Samples for fatty acid analysis were conserved as described below (analysis). At the end of the experiment (day 43) all of the remaining larvae were counted and photographed (in vivo) on millimeter paper for examination of growth (length and myotome height) and pigmentation. The number of larvae sampled for growth data is given in table 1.

Table 1. Number of larvae sampled and examined in wild zooplankton tank (A) and in wild zooplankton/Artemia tank (B).

	Day8	Day14	Day22	Day29	Day36	Day43
Tank A	12	10	10	6	7	207
Tank B	14	9	5	6	5	169

Analysis

Nutrients (NO_3^-, PO_4^{2-}, Si and NH_4^+) were analyzed on a Shimadzu UV-160 spectrophotometer. Chlorophyll a was analyzed using a Perkin-Elmer LS-3B Fluorescence Spectrophotometer. Phytoplankton was examined under a Wild M40 inverted face contrast microscope.

For fatty acid analysis the larvae sampled from the tanks were washed with freshwater to remove salt. The larvae were then stored in a Sovirel tube with chloroform : methanol (2:1) and 0.05 % butylated hydroxy toluene (BHT) as a antioxidant. The

samples were frozen at -27 °C until extraction of the fatty acids could be done.

For extraction of the fatty acids a method described by Folch et al. (1957) was used. The larvae were grounded in a Potter/Elvehjelm homogenizer with chloroform : methanol (2:1/v:v). Methylation of the fatty acids was done with 2 % sulfuric acid (H_2SO_4) in dry methanol as described in Christie (1982). The methylesters were extracted the next day after adding 5 % salt (NaCl) solution.

The methylesters were analyzed on a Carlo Erba Vega 6000, on-column gas chromatograph with a flame ionization detector (FID) and a fused silica capillary column (30 m * 0.32 cm i.d.) DB-23 from J & W. Gas chromatographic conditions were as follows:

Detector temperature : 250 °C

Temperature programme: Step1: 1 min 60 °C
" 2: 30 °C/min to 170 °C
" 3: 1 min 170 °C
" 4: 2 °C/min to 210 °C
" 5: 10 min 210 °C

RESULTS AND DISCUSSION

Environmental parameters

There were no differences between the temperatures in the two tanks. However, the temperature was varying between 11 and 19 °C during the experiment (fig. 1). Temperature is regarded as important in stimulating feeding behavior in fish larvae (Hunter 1972, 1977). The variation in temperature these larvae experienced, is probably not optimal for growth and survival for halibut larvae.

The salinity in both tanks were varying from 31 to 32 ppt at 0.5 and 1 m depth. At two occasions the salinity dropped to 5 ppt in the surface in both tanks, due to heavy rainfall. These drops in salinity have probably not affected the larvae, since they were located deeper in the tanks.

Variations in concentrations of nitrate and ammonium (fig. 2 a, b) were close to parallel in both tanks. High values of nutrients salts at day 1, 20 and 30 corresponds with the addition of new fertilizer. Both nitrate and ammonium were efficiently removed by phytoplankton. Concentration of ammonium never exceeded 8 μM, and was not regarded to be toxic to the larvae.

Oxygen measurements showed saturated water in the whole period. In periods with high light intensities, the oxygen content reached 19 ppm, due to high phytoplankton production.

"Green water", which was created by adding nutrients salts, is reported to have positive effects on larval growth and survival (Houde 1975, 1978). This is later examined by Næss et al. (1990). Chlorophyll a (fig. 3) showed increasing concentrations during the first 12 days of the experiment. For the rest of the experiment the concentrations of chlorophyll a were between one and three μg/l in both tanks. The phytoplankton society was dominated in numbers by flagellates less than 5 μm during the whole experiment. Diatoms and coccolitophorids were also present.

Live feed

There were little differences in the number of wild zooplankton in the two tanks during the experiment (tab. 2). Only at day 1 (first day of feeding), day 12 and at day 43 (the end of the experiment), there was a distinct difference in the wild zooplankton number. At day 1, the difference was due to the nauplii, 620 individuals/l in tank A and 400/l in tank B. At day 12 the wild zooplankton number was 19/l in tank A and 77/l in tank B. At day 43, the wild zooplankton number was 47/l in tank A and 9/l in tank B (fig. 4 a,b).

When Artemia was introduced in tank B at day 7, the wild zooplankton supply was terminated. This implies that the wild zooplankton later found in tank B water samples, either was original supply not eaten, intrinsic reproduction in the tank, or both.

In the water-column samples, Artemia was found only at day 29 and represented then 0.3 individuals/l. Even though Artemia often are patching in the upper part of the waterbody it should have been found in the samples, unless it was underfeed, quickly eaten or dying. The water temperature was relative high during the first part of the experiment, varying between 14 to 19 °C from day 7 to day 19. In this period phytoplankton <40 μm in diameter was present (feed for Artemia). Therefore, death due to temperature or starvation are not likely. After the experiment was terminated by total sampling of the larvae, a lot of large Artemia were observed in the tank.

Larvae, gut content

In tank A the food intake decreased from day 14 to day 29 (tab. 3). The same trend was also visible for the larvae in tank B. This may indicate that the larvae were under fed, and that the larvae in tank B were forced to eat Artemia.

The low frequencies of larvae which had eaten at day 8, were due to amounts deformed larvae unable to take food. Death due to starvation occurred between day 8 and day 14.

Artemia was supplied tank B at day 7. The larvae sampled at day 8 had no Artemia in the gut (tab. 3). At the next sampling (day 14), 2 of 9 larvae (22 %) had eaten Artemia (tab. 4). These two larvae had also the lowest growth (length, myotome height and dry weight).

The mean number of wild zooplankton in the larvae gut, were only slightly lower for larvae in tank B than in tank A (tab. 3). At day 36 it was distinct lower.
At day 29 and 36 all the larvae which had eaten, had consumed Artemia in large numbers. From day 29, the larvae in tank B, had switched to Artemia as main feed. The wild zooplankton found in the larvae gut, at least in the posterior part, were completely digested, i.e. only the colorless, transparent shell were left. We have never found Artemia digested in such way. There are always much content left in Artemia when they are passing the posterior part of the larvae gut.

Larvae growth and survival

From day 8 to day 29 we found an increase in growth for both groups. There was only a minor increase in length from day 8 to 14 and from day 22 to 29 for the larvae in tank B (fig. 5 a). Only a minor increase in myotome height and dry weight were also found for these larvae group from day 22 to 29 (fig. 5 b, c).
For the later period the specific growth rate was only 0.6 % based on dry weight (Houde & Schekter 1981). From day 22 to 29 the larvae had changed to Artemia as main food (tab. 3). Changes in prey organisms often give a decrease in larval growth due to the capture learning process. From day 29 to 36 larval growth in tank B was decreasing, while the specific growth rate for tank A was a modest 2.3 % based on dry weight.
We believe this decrease to be a result of erroneous sampling. 36 days after first feeding, the larger larvae had settled on the bottom, and the samples was biased towards the smaller larvae caught in the water column.
For the last sampling point, day 43, there were again an increase in the myotome height and length. The measurements on the larvae at day 43 were made on living individuals in contrast the earlier measurements made on fixed larvae. It is known that fish larvae shrink when they are fixed in formaldehyde (Hay 1981, 1984). Even when calculating a 10 % shrinkage of the length, there was an increase compared with day 29 for tank B and day 36 for tank A.
At day 22 the myotome height and dry weight for the larvae in tank B, were significant higher than for the larvae in tank A ($p<0.05$, t-test). The myotome height

and length were significant higher for the larvae in tank A, at day 43 (respectively $p < 0.0005$ and $p < 0.05$, t-test). Otherwise there were no significant differences ($p < 0.05$, t-test).

In figure 6 all the growth data from the larvae in the two tanks are presented as

two regression curves. The relationship between the variables are expressed in length * myotome height and dry weight, with the function $f(x) = k1 * x^{k2}$.

Survival through the experiment can not be established exactly because the larvae were not counted when administered to the tanks. However approximate number of larvae incubated in the silos, and mortality during the yolk sac stage are known. The frequency of deformed larvae (non-reduced yolk sac and jaw-deformities) was also measured. when those factors are taken into account, number of functional larvae ready for start feeding administered to each tank was approximately 1300. At the end of the experiment, 207 metamorphosed larvae were collected from tank A and 173 from tank B. 75 larvae were sampled from each tank during the experiment. All of the metamorphosed larvae had normal pigmentation.

Fatty acids

The analysis of fatty acids show that there were no significant differences between the two tanks, in content of unsaturated fatty acids in the larvae (fig. 7). The comparison of the fatty acid content in table 4 show no significant differences either. This could suggest that the different food of the two groups of the larvae have not induced any difference in the larvae lipid.

Naas et al. 1987, have done similar analysis on halibut larvae fed wild zooplankton. The content of 22:6w3 is 30% in Naas et al. to 15% in the present study. The level of monounsaturated corresponds in the two studies.

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Table 2. Wild zooplankton content in the water (ind./l) of tank A and tank B.

	Days after first feeding											
	1	5	8	12	15	19	22	26	29	33	36	43
A:	834	24	20	19	48	25	52	81	79	13	30	47
B:	517	26	16	77	34	61	45	97	54	26	35	9

Table 3. Mean number of prey organisms in the larval gut. Non-feeding larvae omitted.

	Days after first feeding				
	8	14	22	29	36
Tank A (wild zooplankton):	5.9	14.2	11.5	8.3	24.9
Tank B (wild zooplankton):	5.5	9.0	7.8	7.6	0.2
" B (Artemia):	0	9.5	0	296.0	207.0

Table 4. Frequency of larvae which had eaten.

	Days after first feeding				
	8	14	22	29	36
Tank A (%):	67	100	100	67	100
Tank B (%):	43	89	100	100	100
" B (w.zpl./Art.):	43/0	89/22	100/0	67/100	20/100

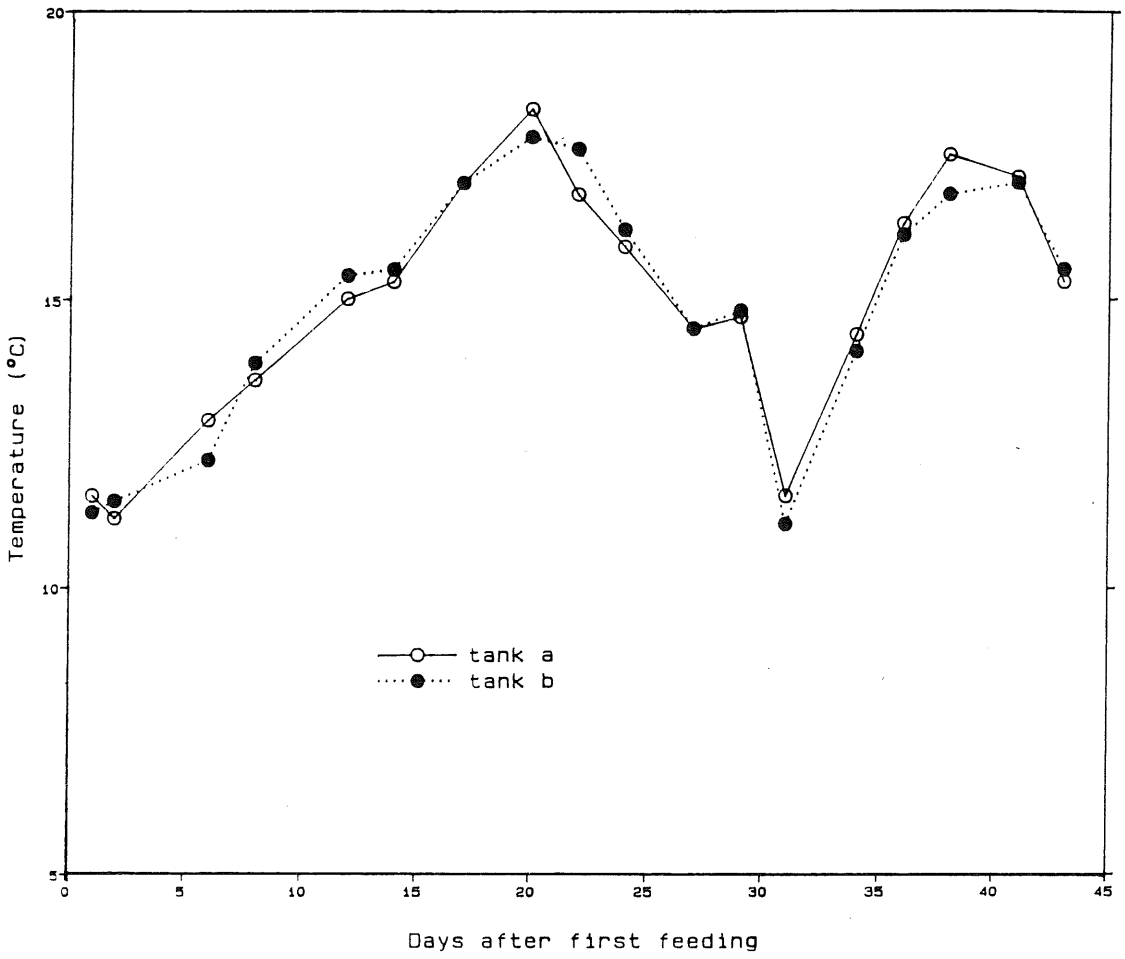

Figure 1. Temperature in tank A and B during the experiment.

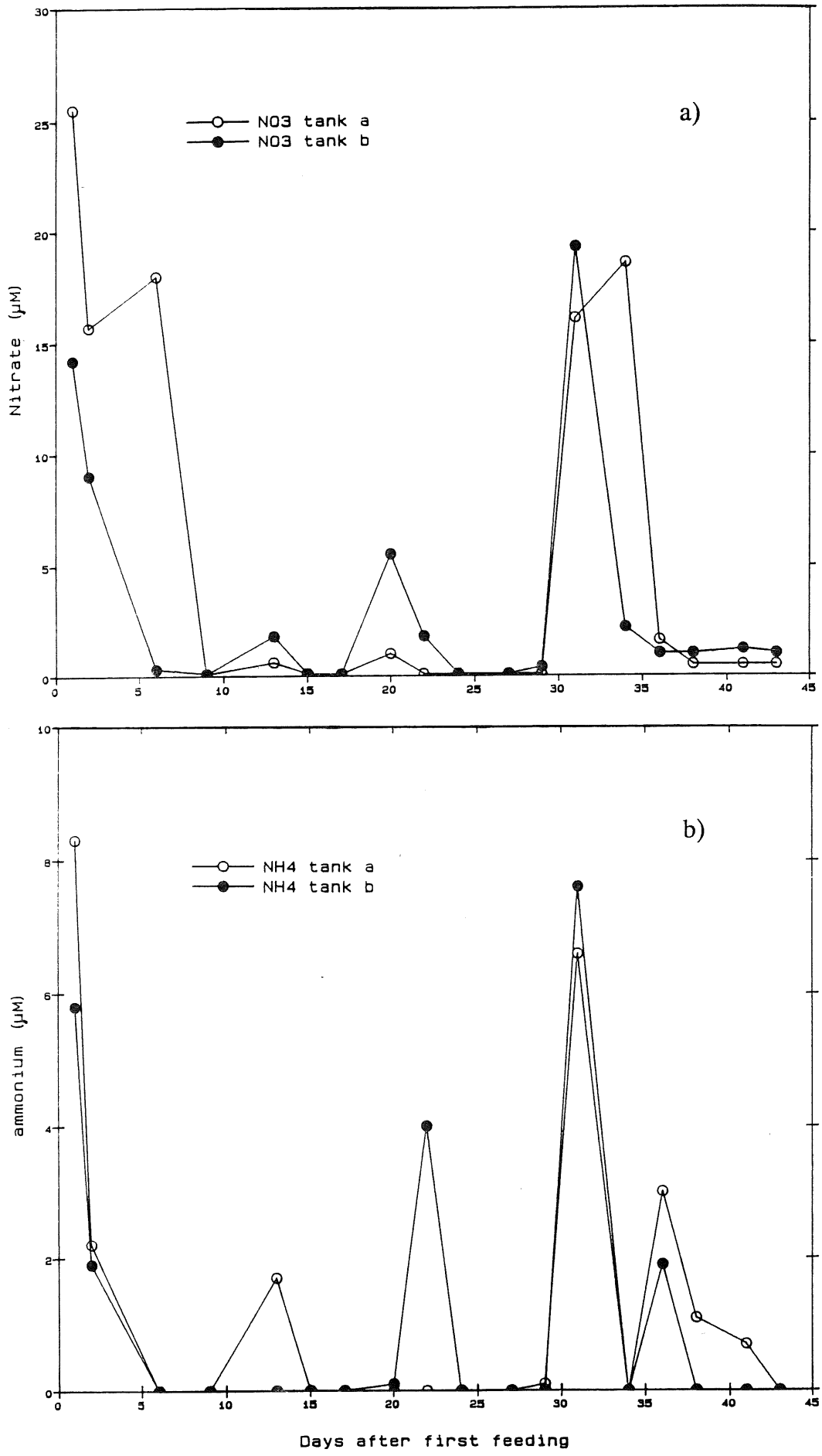

Figure 2 a, b. The nitrate and ammonium concentrations (μM) in tank A and B.

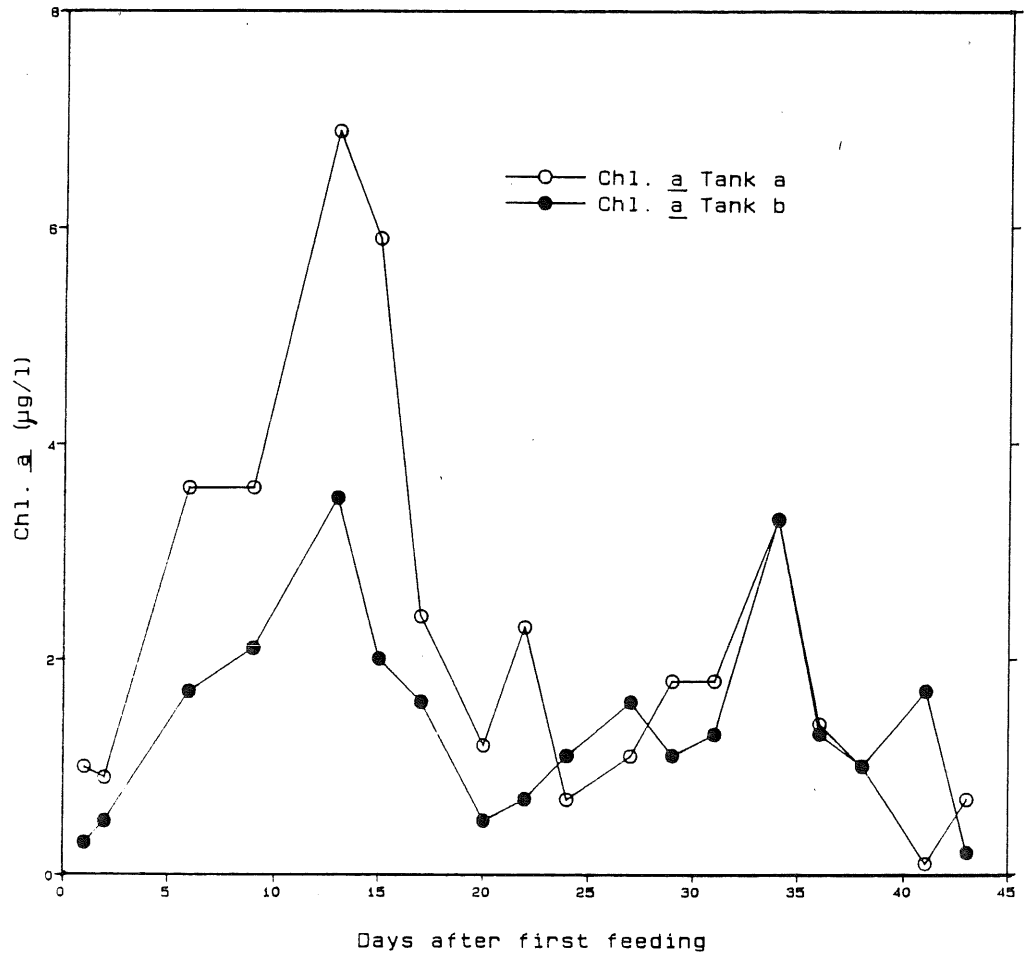

Figure 3. The chlorophyll a concentrations in tank A and B.

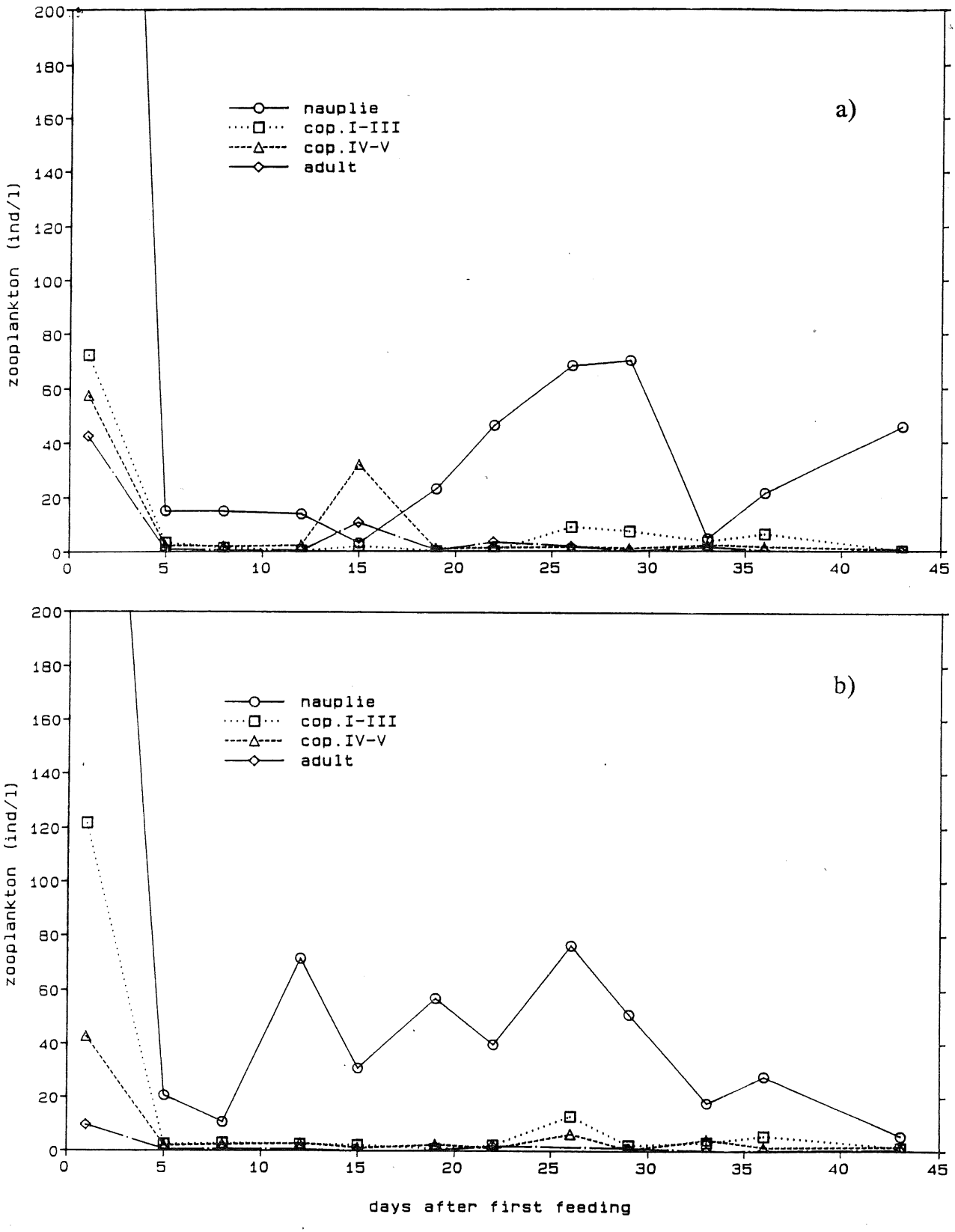

Figure 4 a, b. Number of zooplankton in tank A and B during the experiment.

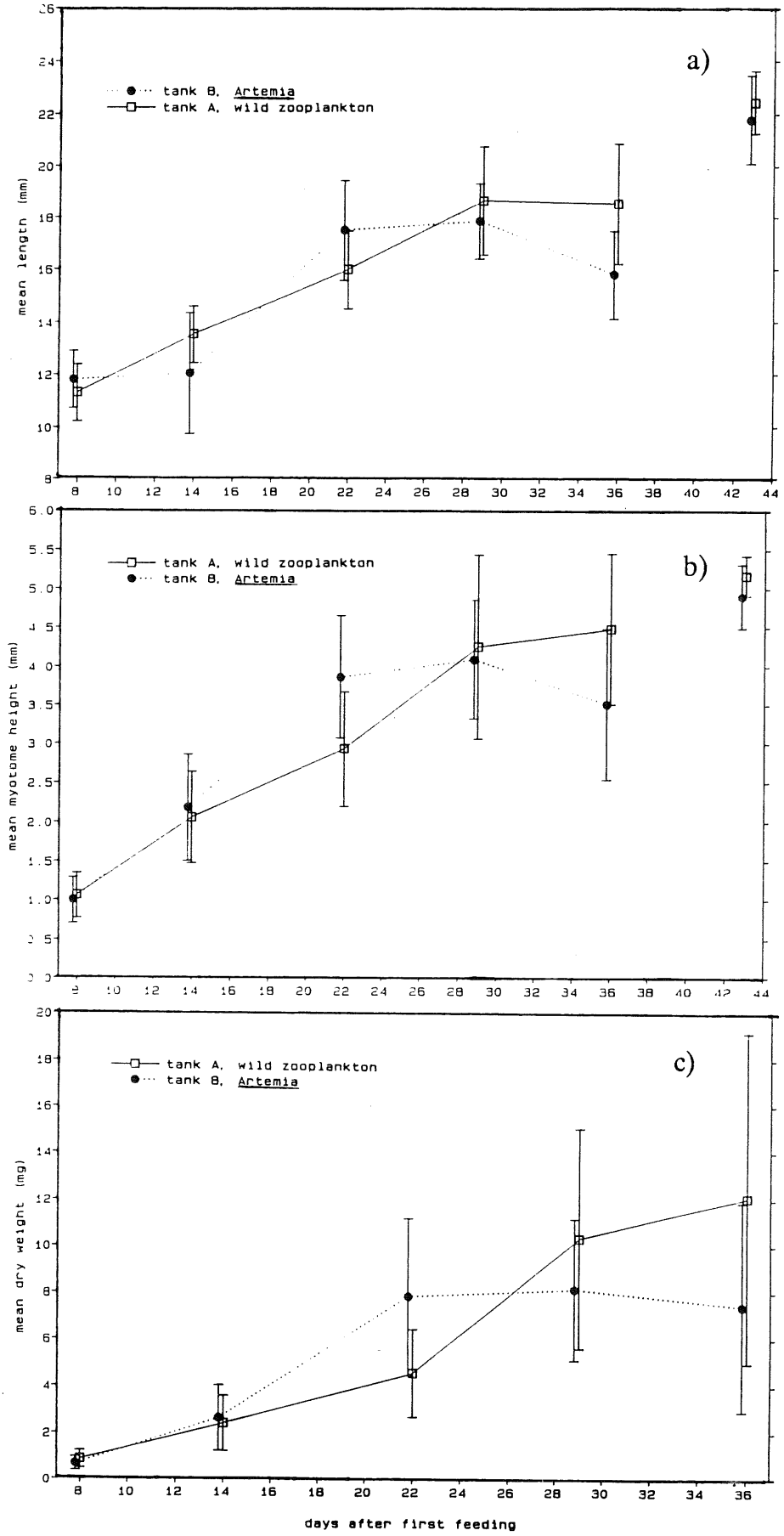

Figure 5 a, b, c. Length (a), myotome height (b) and dry weight (c) of halibut larvae in tank A and B. The curve points (tank B) are moved slightly to left to give a better resolution of the standard deviation bars.

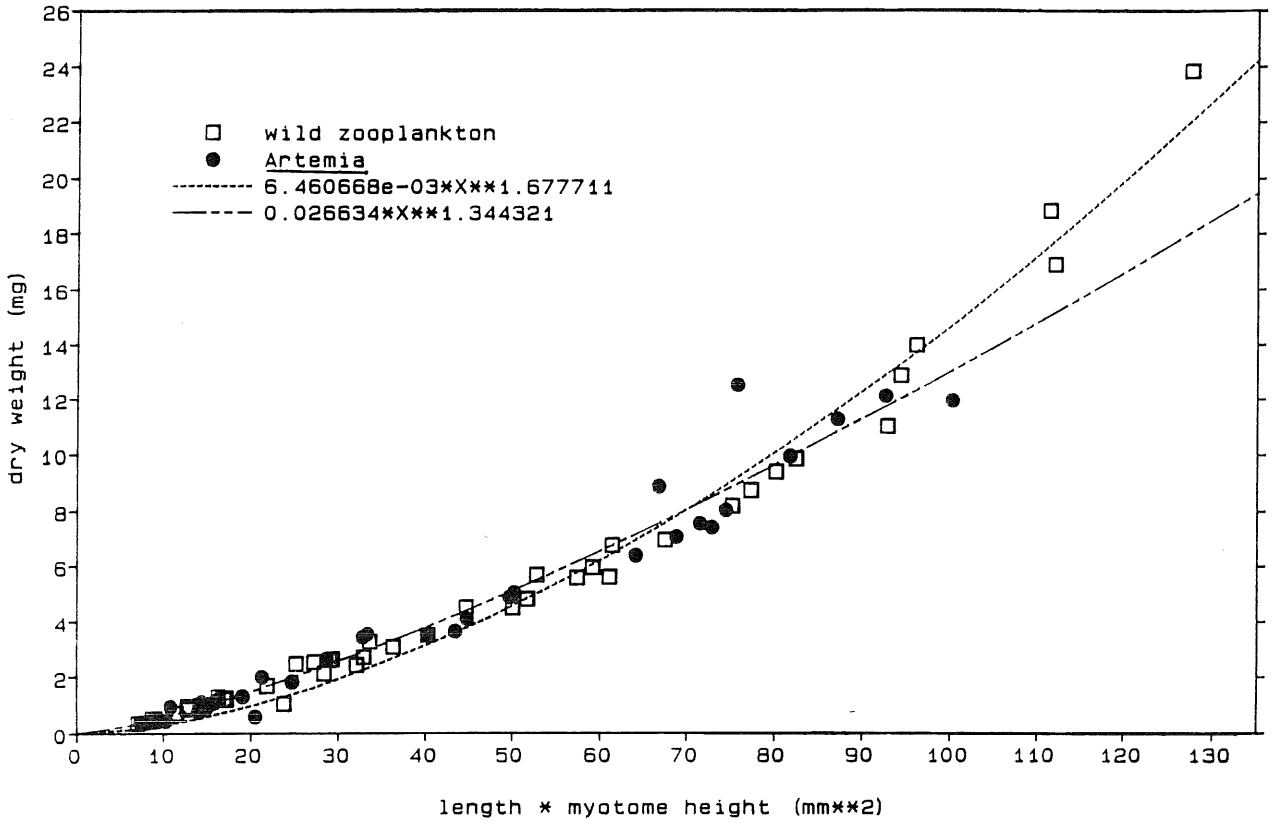

Figure 6. Regression between dry weight and myotome height * length of halibut larvae.

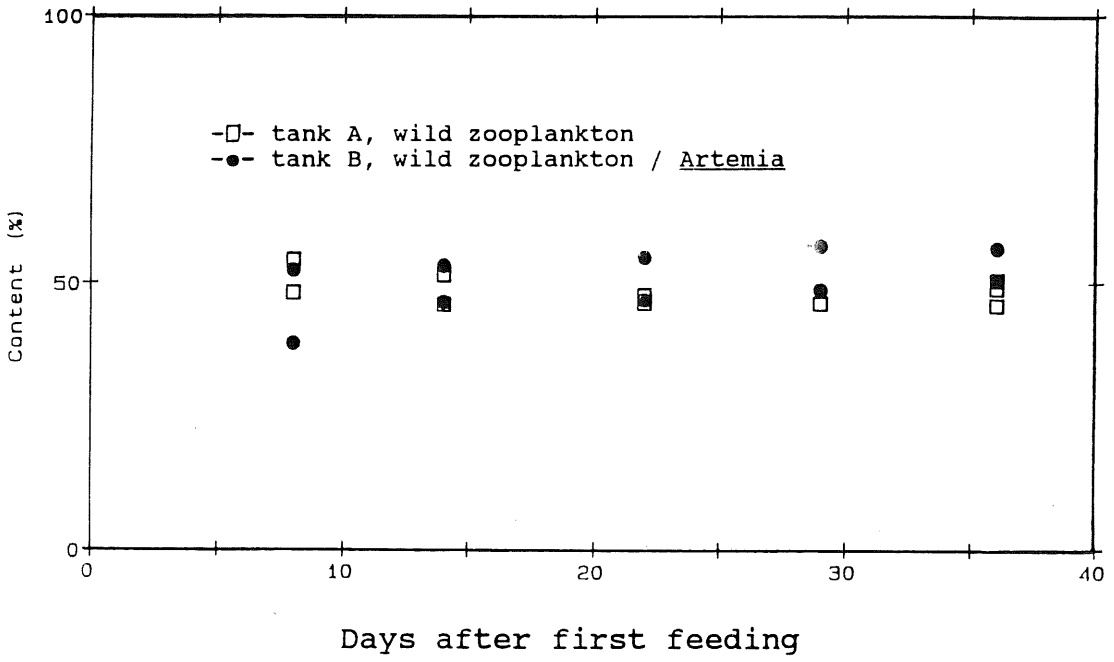

Figure 7. Content of unsaturated fatty acids in halibut larvae in tank A and B.

Table 5. Fatty acid composition in halibut larvae of two different feeding regimes.

Fatty acid composition of total lipid in halibut larvae fed wild zooplankton

	Day 8	Day 14	Day 22	Day 29	Day 36
14:0	3.95	3.45	3.05	5.61	3.76
16:0	11.22	9.82	8.61	9.08	9.53
16:1	7.53	7.14	6.95	7.29	7.29
18:0	7.38	7.08	6.85	7.10	7.44
18:1	10.09	9.61	8.93	9.37	9.62
18:2w6	2.43	1.95	2.56	1.98	1.24
18:3w6	0.93	0.93	0.86	0.50	1.11
20:0	0	0	0	0	0.09
20:1	1.15	0.47	1.01	0.57	0.98
20:2w6	0.33	0.34	0.92	0.67	0.67
20:3w6	0	0	0.19	0	0.13
20:3w3	0	0	0.61	0.52	0.56
20:4w6	1.75	1.16	2.00	2.02	1.95
20:5w3	9.63	9.72	7.78	8.46	8.58
22:0	0	0	0	0	0
22:1	0.32	0.40	0.36	0	0.41
22:6w3	17.22	17.26	15.07	14.66	16.17
24:0	1.92	2.76	2.95	3.51	3.47
% mono-saturated	19.08	17.63	17.26	17.24	18.30
% un-saturated	51.05	48.58	46.90	46.02	48.29

Fatty acid composition of total lipid in halibut larvae fed Artemia

	Day 8	Day 14	Day 22	Day29	Day 36
14:0	3.87	3.72	2.89	3.04	3.04
16:0	14.71	8.45	7.16	9.18	7.52
16:1	6.93	7.82	7.04	8.20	8.24
18:0	10.01	6.47	6.42	6.84	6.35
18:1	11.09	10.63	10.61	11.98	12.43
18:2w6	2.14	3.01	4.48	3.82	4.19
18:3w6	0.22	1.12	2.29	2.12	2.82
20:0	0	0	0.21	0	0
20:1	1.02	0.89	1.90	1.23	1.60
20:2w6	0.31	0.80	0.87	0.40	0.45
20:3w6	0	0	0.33	0.18	0
20:4w6	1.42	2.49	4.30	5.17	5.33
20:3w3	0	0.19	0.33	0.23	0.19
20:5w3	7.24	8.20	7.02	7.29	7.44
22:0	0	0	0	0	0
22:1	0	0.22	0.38	0.16	0
24:0	0.84	1.87	2.35	2.26	1.59
22:6w3	15.029	14.55	11.59	12.09	10.65
% mono-saturated	19.04	19.57	19.93	21.57	22.27
% un-saturated	45.40	49.69	50.76	52.72	53.34