International Counsil for
the Exploration of the Sea

C.M. 1987/F:17
Mariculture Committee

NATURAL AND CULTIVATED ZOOPLANKTON AS FOOD FOR HALIBUT (HIPPOGLOSSUS HIPPOGLOSSUS) LARVAE.

by

K. E. Naas 1), L. Berg 1), J. Klungsøyr 2) & K. Pittman 3)

ABSTRACT

Natural zooplankton were pumped into a collector and size-fractionated. The zooplankton smaller than 350 µm were fed on a diatom dominated algal suspension cultured in 3 m deep out-door plastic bags.

Halibut larvae were kept through the yolk sac stages in large temperature regulated bags, and when ready to start first feeding, they were offered both cultivated and natural zooplankton. The composition of fatty acids in growing larvae were analyzed to study the influence of dietary lipids.

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1) Institute of Marine Research, Austevoll Aquaculture station, N-5392 Storebø, Norway
2) Institute of Marine Research, N-5011 Nordnes Bergen, Norway
3) Institute of Fisheries Biology, University of Bergen, N-5011 Bergen, Norway

INTRODUCTION

Among Norwegian scientists the activity surrounding experimental rearing of halibut fry has increased in recent years. Successful startfeeding and survival beyond metamorphosis was achieved for the first time in 1980 (Blaxter et al. 1983). In 1986 more than 200 halibut fry were produced in black plastic bags located in Hyltropollen at Austevoll (Berg & Øiestad, 1986).

Successful startfeeding has until this year only been achieved by using concentrated natural zooplankton, and the only manipulation has been size-fractionating. The use of this kind of food source imposes two severe limitations to further development. First of all, production is limited by the variable availability of correct types of zooplankton in the natural surroundings and secondly by the variable nutritional status of natural zooplankton.

The aim of this study was to solve in a pilot scale these two problems by semi-intensive cultivation of prey organisms in mesocosms. Due to different technical problems and the size of the cultivation system, the comparative results from natural and cultivated plankton will be discussed only qualitatively.

MATERIALS AND METHODS

Halibut eggs were stripped from parent fish at the Austevoll Aquaculture Station, and hatched in incubators described by Jelmert & Rabben (1987). 50 % hatching, corresponding to Day 0 (D0), occured at about 78 daydegrees (March 7 and March 20 for cohort 1 and cohort 2 respectively). The two cohorts of halibut larvae were stored through the yolk sac stages in plastic bags surrounded by deepwater (Fig. 1) with almost constant temperature (Berg et al. 1987). Larvae of cohort 1 were stored in bags P1-4, and cohort 2 were stored in bags P5-12. The larvae were offered startfood in the same bags at Day 32 and 35 for cohort 1 and 2 respectively.

The cultivation system (Fig. 2), included four phytoplankton bags (Ph1 - Ph4), four zooplankton bags (Z1 - Z4), a nutrient reservoir, a dosing pump and a dosing tank. The nutrients were composed of Na_2SiF_6 and $NaNO_3$ dissolved in deepwater giving final concentrations of 40 µM nitrate and 20 µM silicate, and the deepwater itself contained about 2 µM phosphate. The rate of water exchange in the phytoplankton bags was approximately 25% per 24 hours. Whereas the four zooplankton bags each received about 0.5 l algal suspension per minute, which means a total water exchange every 16th day.

The phytoplankton bags were monitored two times a week with respect to nutrient concentrations, chlorophyll a concentrations and samples for identification. Samples for zooplankton identification were obtained by a tube sampler (10 l) in the zooplankton bags and in the larval rearing bags (P1 - P12) after first feeding. Occasionally bucket samples were used to study the zooplankton concentration in the surface layers.

The phytoplankton bags were inoculated with surface water filtered through a 120 µm filter in order to avoid larger zooplankton. The zooplankton bags were inoculated with concentrated natural zooplankton smaller than 350 µm, several times prior to April 20 and with an additional inoculation on May 4. The zooplankton collector is described in Jensen et al. (1979).

Due to a technical accident which caused total mortality in Bag P2 (cohort 1), this bag was refilled on May 6 (D47) with 400 larvae of cohort 2. These 400 larvae were fed exclusively on cultivated zooplankton from Z 1-4, while the larvae in the other bags were fed on natural zooplankton harvested from the seawater nearby.

Halibut larvae were sampled regularly for length- and weight measurements and morphological studies (Pittman et al. 1987). Samples for determination of fatty acid composition of total lipid were obtained of larvae receiving both natural and cultivated zooplankton. The samples were collected prior to and after observed start-

feeding. Single larvae were immediately placed in chloroform:methanol (2:1 v/v) and stored at -20^{0}C until final analysis. Lipid extraction was performed using the method described by Folch et al. (1957). Methyl esters of the fatty acids from the total lipid extracts were prepared by acid-catalysed transmethylation. The fatty acid methyl esters were analysed by capillary gas chromatography on a Hewlett Packard model 5890 instrument. Column used was a 30m x 0.32 mm ID fused silica capillary column coated with 0.25 µm DB-225 (J&W Scientific, inc.). Further details of the analytical procedure is described by Tilseth et al. (1987). Fatty acids (FA) were quantified by means of external standards of 20 of the authentic compounds.

Due to lack of time only results from selected phyto- and zooplankton bags will be presented in this report.

RESULTS AND DISCUSSION

Phytoplankton

The phytoplankton growth was probably light-limited during most of the experiment. Surplus concentrations of nutrients were measured except for one period at the end of May, and another period at the end of the study (Fig. 3). The two periods of low nitrate concentrations coincided with two chlorophyll a peaks (Fig. 4). No data on radiation is yet ailable, but observations indicated increased primary production due to clear weather and increased solar radiation in both periods.

The first chlorophyll a peak consisted of a bloom of unidentified pennate diatoms with cell numbers exceeding 15 million cells per litre (Table 1). On May 22, the diatoms contributed to 99.7 % of the estimated total cell volume, and were the dominating phytoplankton class during the whole experiment . This was confirmed by low silicate concentrations compared to the added amount of dissolved nutrients. By the end of June, small unidentified flagellates became very abundant (49 million cells per litre), but still diatoms

dominated the phytoplankton community in terms of volume (> 70 %).

Zooplankton

The zooplankton inoculated in bags Z1-4 consisted mainly of calanoid copepods. Copepodites (St.1-3) of Calanus finmarchicus were dominant, but also Pseudocalanus elongatus (ad. + juv.) and calanoid nauplii of various species occured in substantial numbers. During the experiment the inoculated cohort of C. finmarchicus copepodites gradually decreased in numbers as they grew to adult size, and they did not reproduce (Table 2).

On May 12, large numbers of P.elongatus nauplii were observed for the first time, indicating spawning and the possibility of P. elongatus to reproduce in such cultivation systems. Reproduction of Pseudocalanus has also been reported in different sized cultivation tanks (Breteler et al. 1982 and Davis, 1983). The population of P. elongatus occured with maximum concentrations on June 5 and declined towards the end of the month.

Larval food

The larvae in Bag P2 were offered food for the first time May 1, and although it was difficult to maintain sufficient concentrations, only cultivated zooplankton were transferred to this bag throughout the experiment. The zooplankton development in Bag P2 (Table 3) was therefore very similar to bags Z1-4, and the differences were probably due to predation by halibut larvae and to a possible oversampling of species aggregating in surface layers (i.e. Centropages hamatus and cladocerans). Zooplankton was transferred to Bag P2 by sampling surface water from the zooplankton bags. This might explain the peak in the concentration of cladocerans on June 5.

The zooplankton development in bags P3 and P5 (Table 4) reflects, to a large extent, the natural planktonic succession at the collecting site. The zooplankton communities in these bags were initially

dominated by copepopdite stages 1 to 3 of C. finmarchicus, but by the end of May and throughout the experiment, mainly the larger stages (4 and 5) were present. The concentration of other calanoid copepods was relatively constant during the study, but cladocerans (mainly Podon) increased substantially in numbers in the first part of May. Cladocerans were also very shallow distributed, as shown by the surface samples, corresponding with the observations by Berg & Øiestad (1986).

The most pronounced difference between Bag P2, receiving cultivated zooplankton, compared the other larval rearing bags, was the much lower total concentration of prey organisms in this bag. It is therefore impossible to compare growth and survival of halibut larvae in relation to type and nutritional composition of the food. However, the gut content of larvae in Bag P2 indicates a different diet compared to the other larvae, receiving natural zooplankton (Table 5). While the gut content of P2 larvae at D56 was totally dominated by calanaoid copepods and nauplii, the larvae receiving natural zooplankton were almost exclusively preying on Calanus finmarchicus (St. 1-3) from D46 - D66. It is also interesting to notice that despite the low concentration of prey organisms in Bag P2, the number of food organisms per larval gut was relatively high.

As the larvae fed natural zooplankton grew older, an increased gut content of calanoid copepods was observed, and the change in diet coincided with lower concentrations of smaller stages of C. finmarchicus (Table 4). Despite the very low concentrations of cladocerans in the larval bags (Table 4), they became the dominant prey organisms in all bags except P2, from D70. This was probably due to the shallow distribution of both the cladocerans and the halibut larvae observed at that time.

Larval survival

With an initial number of 2000 larvae per bag (coh.1) and 1500 larvae per bag (coh.2), the survival through the yolk sac stages was calculated to 20 % in Bag P1, approximately 40 % in bags P3-4, and 13 % for cohort 2 (bags P5-12). The estimated numbers of larvae taking part in the startfeeding experiment (surviving youlk sac stages) are shown in Table 6.

A significant difference in successful first feeding (% growing larvae) between the two cohorts may be due to different composition of the zooplankton offered at the time of first feeding. Positive observations of first feeding were observed for the first time April 26 (cohort 1) and May 5 (cohort 2). However, samples for gut content were not taken prior to May 6.

Symptoms of vibriosis and substantial mortality at about June 10, may be due to stress caused by high zooplankton concentrations. On June 5 and 10, Balanus nauplii occured with 115 and 270 organisms per litre respectively (not shown in table). At the same time cladocerans also became very abundant (Table 4). No substantial mortality occured in Bag P2 at that time, which may be explained by the absence of stress due to lower zooplankton concentration.

Larval growth

The halibut larvae of both cohorts grew rapidly after first feeding (Fig. 5). Mean specific growth rate from D0 to time of first feeding (D45) was 3.5 %, increasing to 6.1 % from D56 - D72. Due to the few larvae receiving cultivated zooplankton, no larvae were sampled for weight measurements in Bag P2, but observations indicate lower growth, probably due to lower concentration of prey organisms.

Fatty acid composition

It is accepted that the fatty acid composition of lipids in marine animals are dictated by the products of the metabolic activities in the animal and by the fatty acyl and fatty alkyl components of its diatary lipids (Ackman, 1980). In the present study the fatty acid composition of the total lipids were analysed in halibut larvae given different dietary regimes whose composition could be accurately monitored. This was performed to get information about the deposition in growing halibut larvae and to study the influence of dietary lipids on the fatty acid composition in developing fish.

Only data of the fatty acid composition from cohort 2 larvae (Bag P2, P9 and P11) are presented here. Larvae from Bag P11 were collected on April 21 (D32) before startfood was offered, larvae from Bag P9 were sampled on May 2 (D43) after being fed 9 days with natural zooplankton, and larvae from Bag P2 were sampled on May 10 (D51) after beeing fed 10 days with cultivated plankton. The amount of fatty acids (FA) increased in the larvae from 103 $\pm$ 11 µg (n=3) before startfeeding, to 380 $\pm$ 180 µg (n=3) and 510 $\pm$ 240 µg (n=3) in the larvae fed natural and cultivated zooplankton respectively. This indicates that large amounts of lipids are laid down in the halibut larvae after startfeeding. The relatively high standard deviations on the given mean weights of FA reflect the different growth of individual larvae seen in Fig. 5.

Table 7 shows that the three groups of larvae contained 28.0 - 28.6% saturated fatty acids (primarily 16:0), 14.5 - 17.6 % monounsaturated fatty acids (primarily 18:1 isomers) and 48.9 - 53.1 % polyunsaturated fatty acids (PUFA), especially 20:5 (n-3) and 22:6 (n-3). Their origin in the marine phytoplankton and their importance in the marine food web is well recognised (Sargent & Whittle, 1981). Differences in the relative abundances of saturated fatty acids were small between the the two groups of larvae fed on different diets. Both groups increased their relative abundance of 16:1 (n-7) and decreased their abundance of 16:1 (n-9), 20:1 (n-9), 22:1 (n-11) and 24:1 (n-9). Isomers of 18:1 varied in abundance in the

three groups. PUFA made up the major part of the fatty acids in the halibut larvae, and 20:5 (n-3) and 22:6 (n-3) were the individual compounds found at highest concentrations. Differences were noted in the compositions of PUFA between the larval groups. This was most clearly seen in the abundances of 20:5 (n-3) which varied from 10.9% to 17.1%. The (n-3)/(n-6) PUFA ratio was 12.9 before startfeeding and increased to 23.6 in the larvae given natural zooplankton and 18.6 in the larvae given cultivated zooplankton.

The fatty acid composition of the larvae prior to startfeeding (D32) was very similar to the fatty acid composition of ripe eggs of wild halibut (Falk-Petersen et al. 1986). This indicates that essential changes not occured during the yolk sac stages.

The gut content of the two groups of larvae indicated differences in the diets (Table 5). Further analysis will answer if there also were differences in the fatty acid composition of the different diets given to the larvae. Until these results are available it is impossible to compare the two diets, in terms of nutritional quality.

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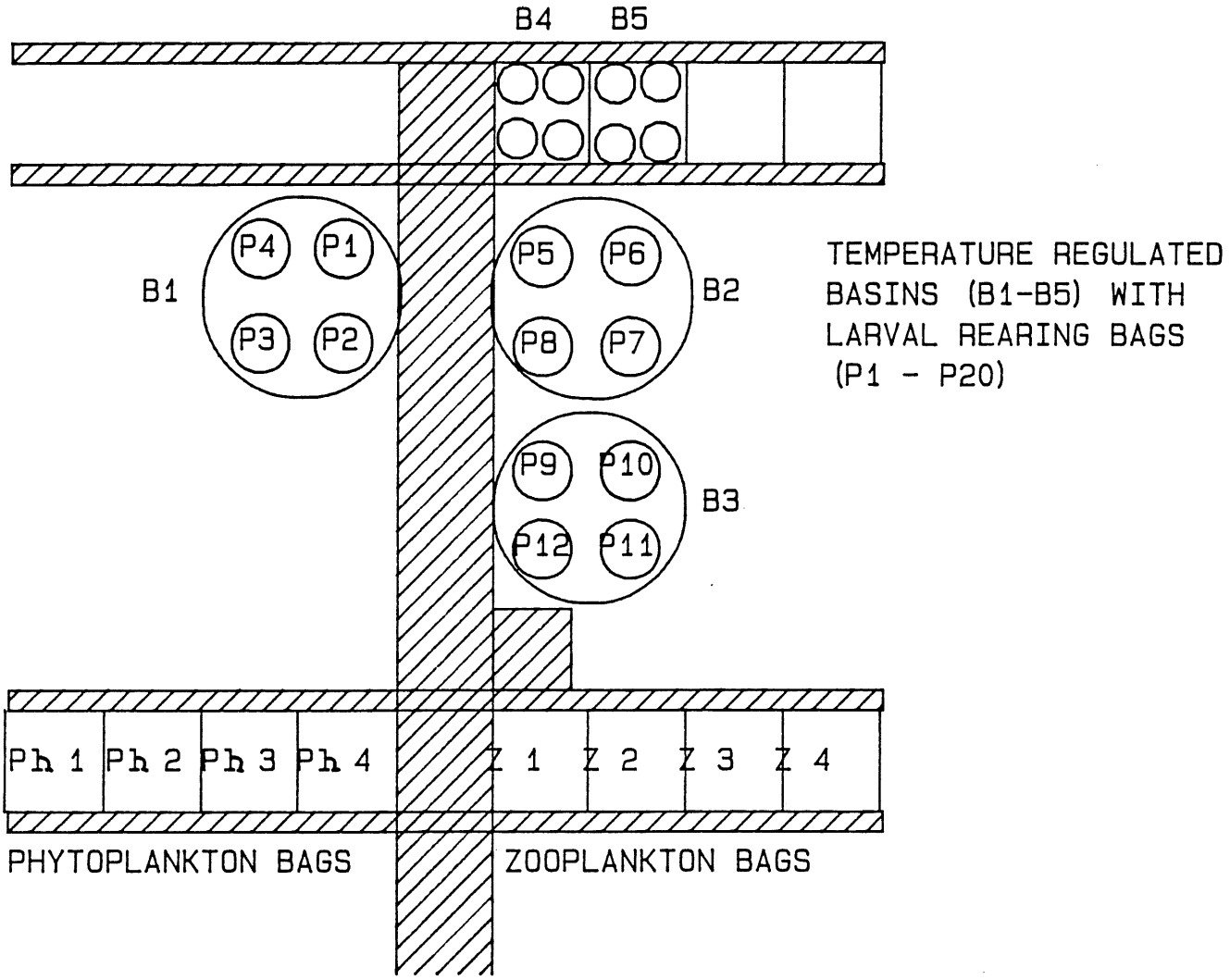

Figure 1. Map showing the larval rearing units (B), including phytoplankton (Ph) and zooplankton (Z) cultivation bags.

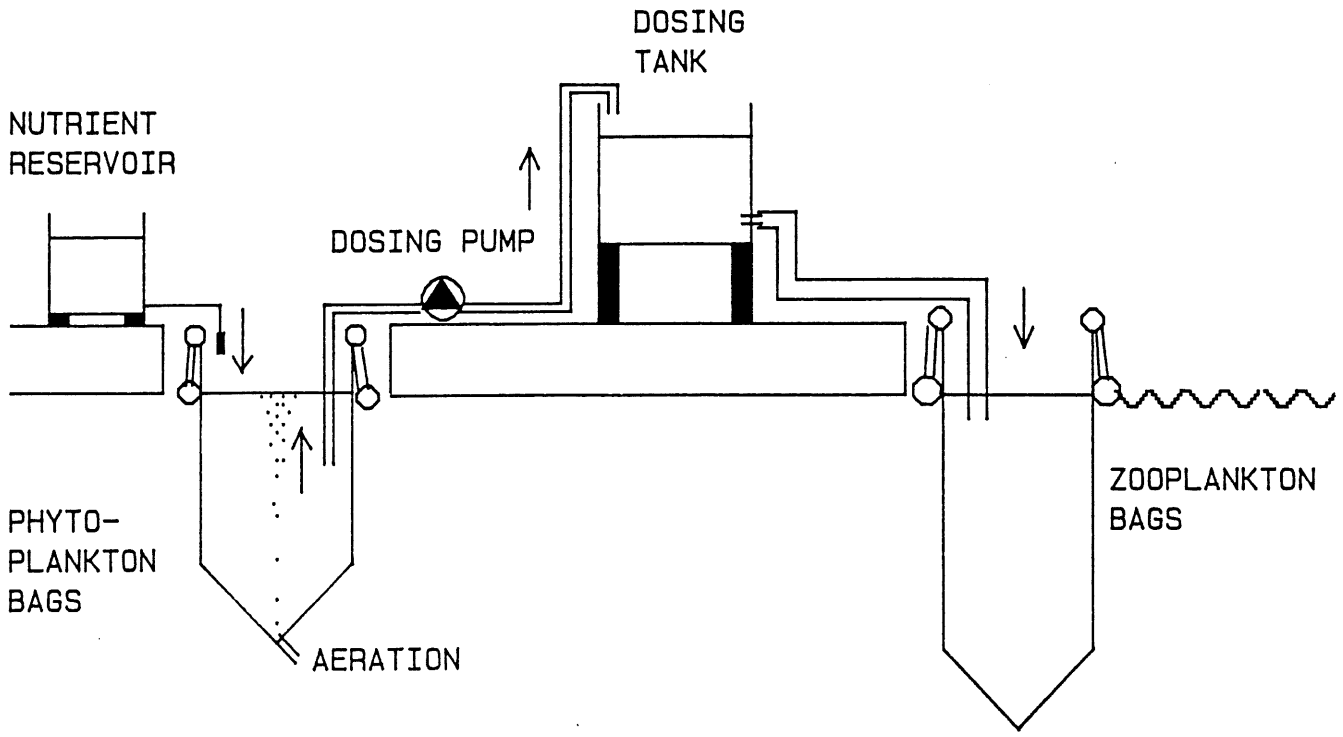

Figure 2. The outdoor system for cultivation of natural phyto-plankton and enrichment of natural zooplankton.

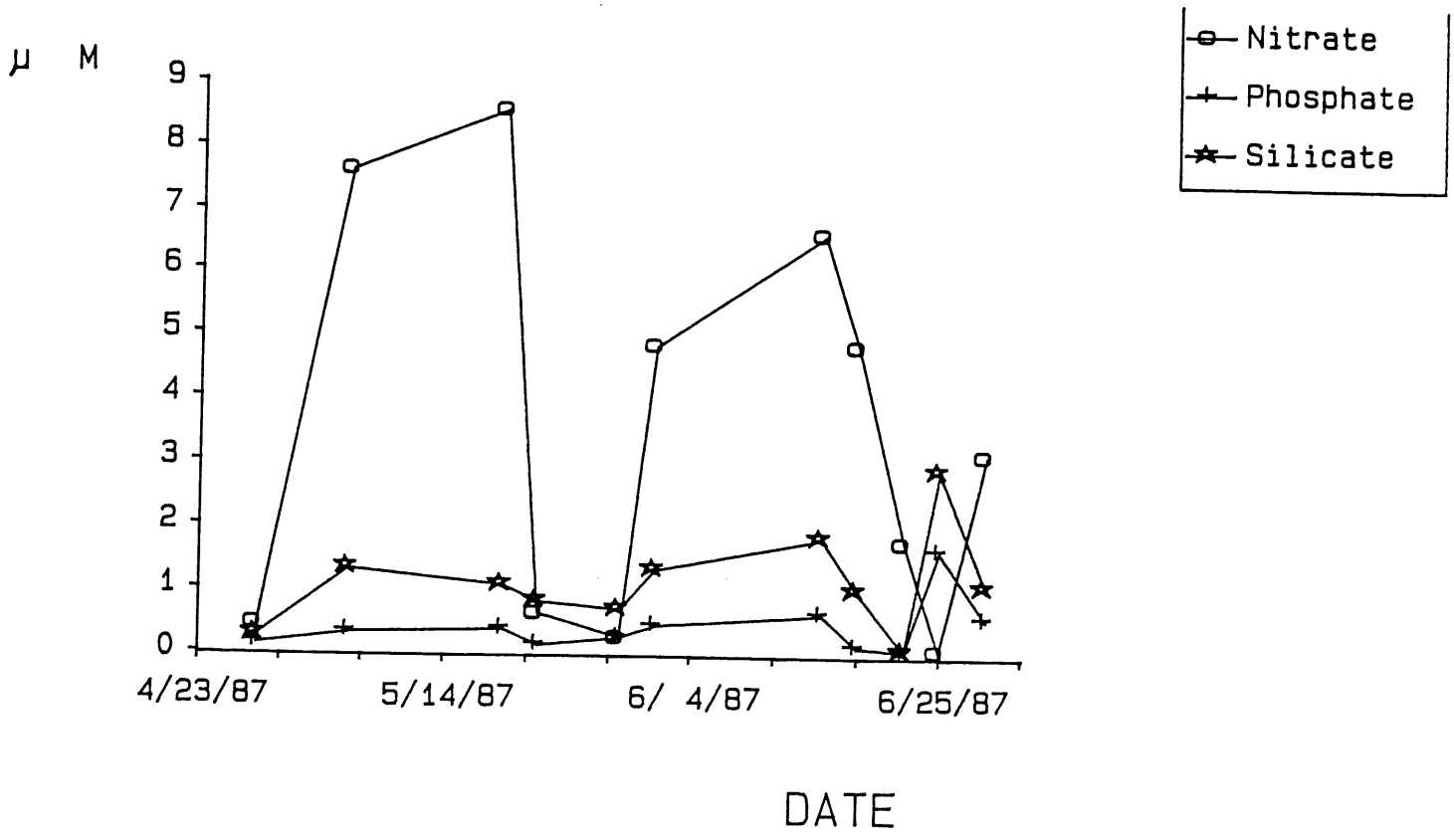

Figure 3. The concentrations of nutrients in bag (Ph 4) during the experiment.

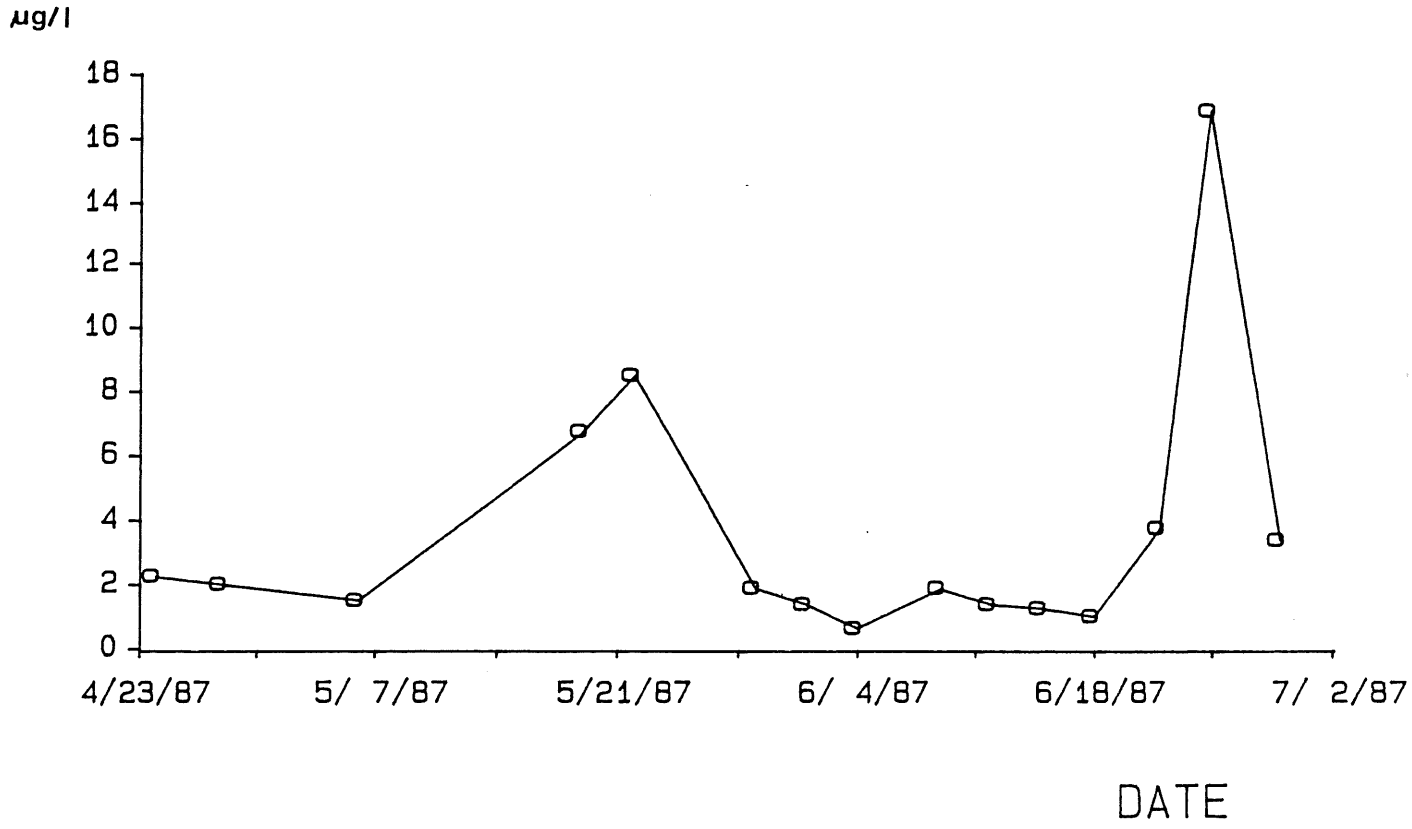

Figure 4. The concentration of chlorophyll a in bag (Ph 4) during the experiment.

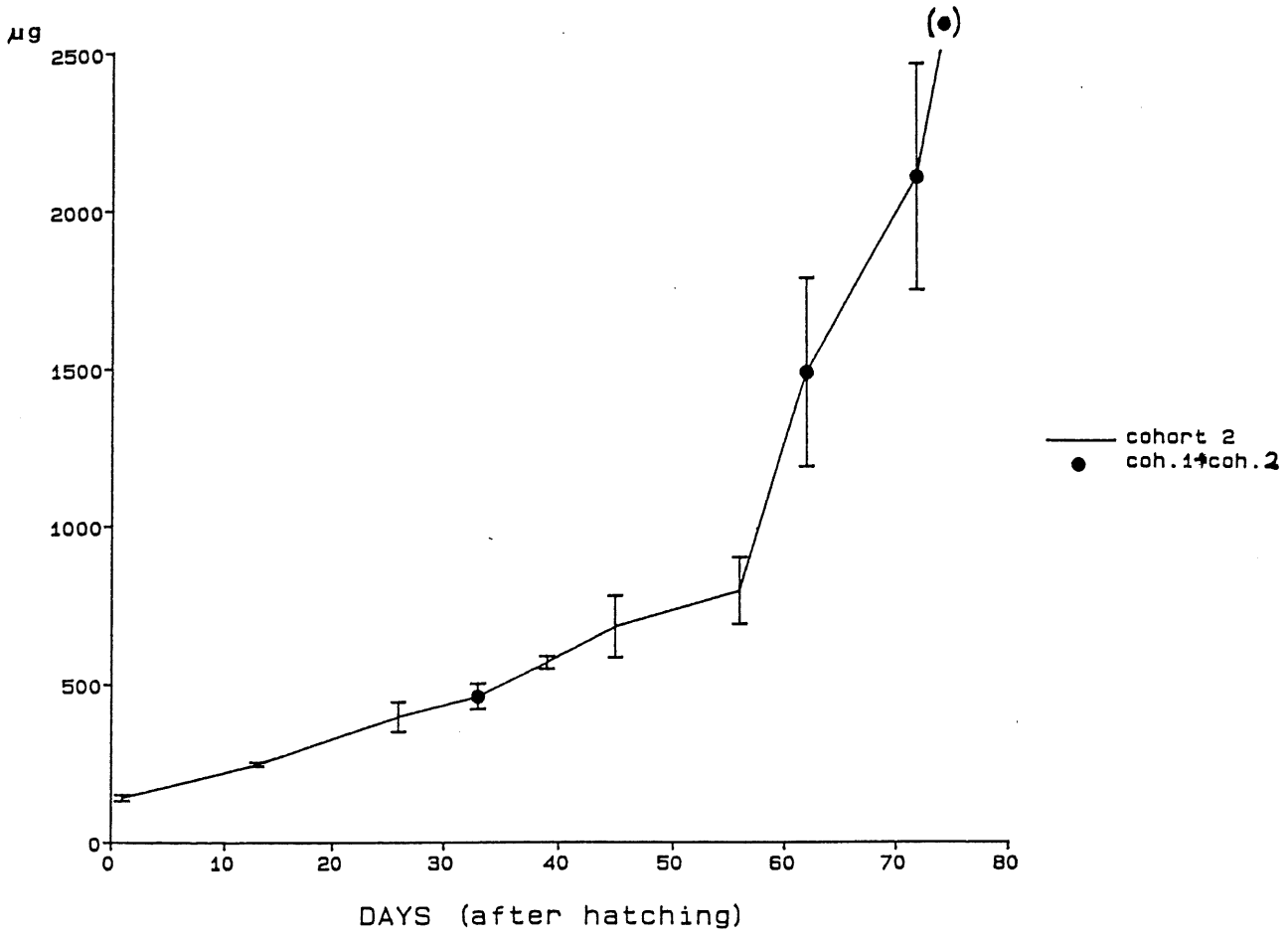

Figure 5. Average dry weight of larvae sampled from both cohorts. 95 % confidence limits are given (n= 4-10). (●) represents 4094 µg ± 2472 (n=5) on D83.

Table 1. Phytoplankton development in Bag Ph 4 in numbers per millilitre. Cell volumes of dominant species were estimated.

DATE	28.4.	6.5.	19.5.	22.5.	29.5.	1.6.	15.6	18.6.	22.6.	25.6.	29.6.
DIATOMS											
Cerataulina pelagica	7	14		14		28			168	707	1 764
Chaetoceros sp.	1 512	14	21	21	21	42					21
Diatoma elongatum						14	189	1 638	2 772	1 638	882
Leptocylindricus danicus	140	210	252	7		7			63	84	192
Nitzschia closterium	3 402	756	378	504	378	1 386	6 300	1 134	504	252	126
" sp.	14	28	7				14			14	
Odontella sp.							7				
Skeletonema costatum	1 512	7									
Thalassionema nitzscoides	14										
Thalassiosira sp.	7										
Unid. penn. diatoms	308	2 772	4 158	15 498	11 466	23 436	2 646	3 402	1 764	756	252
Unid. sentr. diatoms	21		14								
DINOFLAGELLATES											
Unid. dinofl.	28	28	70		35	70	504	35	7		
OTHER PHYTOPLANKTON											
Emiliania huxleyi							7				
Unid. flagellates	1 386	2 268	1 386	1 134	1 008	2 520	4 536	1 512	21 294	34 524	49 392
MICROZOOPLANKTON											
Lohmaniella oviformis									7	6	7
Strombidium sp.	21	7	63	14	7	28	28	77	28		3
TOTAL CELL NUMBER	8 351	6 097	6 286	17 178	12 908	27 503	14 203	7 721	26 572	37 975	52 629
% DIATOMS (OF CELL VOLUME)	93.3	96.4	97.7	99.7	99.3	99.1	92.2	92.1	82.9	72.2	73.1

Table 2. Zooplankton development in bags Z2 and Z3 in numbers per litre. Sampling device was a 10 l tubesampler.

DATE	29.4	6.5	12.5	20.5	29.5	5.6 [1]	10.6	23.6	29.6
Calanus finmarchicus St.1	0.3	0	0	0	0	0	0	0	0
St.2	1.8	0.3	0	0	0	0	0	0	0
St.3	2.4	7.2	0.1	0	0	0	0	0	0
St.4	0.2	2.9	2.5	3.1	1.1	0.1	0.1	0	0
St.5	0	0.3	0.1	2.1	1.8	1.7	2.9	1.5	0.8
adults (female)	0	0	0	0	0.4	1.0	1.3	0.6	1.2
(male)	0	0	0	0	0	0.2	0.1	0	0
Calanoid nauplii	11.0	10.2	16.2 [2]	22.9	23.4	24.2	17.8	29.0	17.1
Pseudocalanus juv.	1.1	0.8	1.2	3.4	15.0	35.7	29.3	10.3	5.0
ad.	1.4	2.2	1.4	0.5	1.6	4.0	4.4	3.8	2.2
Acartia juv.	0.2	0.4	0.8	0.5	0.7	0.3	0.4	0	0.1
ad.	0.6	1.3	0.2	0.6	0.7	0.8	0.9	0.6	0.3
Temora juv.	0	0.1	0.1	0.2	0.8	1.5	1.7	0.3	0.3
ad.	0	0	0	0.1	0.1	0	1.5	0.9	1.5
Centropages juv.	0.1	0.6	0.8	3.5	1.6	0.3	0.6	0	0
ad.	0.1	0.2	0	0	0.6	0.3	0.4	0.1	0.1
Cladocerans	0.2	2.0	0.2	0.3	0.0	0.8	0.2	0	0
Other spp.	0.2.	0.3	0.1	0.0	0.2	0.5	0.8	0.2	0.1

1) Lower concentrations in surface samples
2) Mostly Pseudocalanus

Table 3. Zooplankton development in Bag P2 (cohort 2) in numbers per litre. Sampling devices were (CS); water coloumn sampler (10l tube) and (SS); water surface sampler (10 l bucket).

DATE	5.5.	12.5.	20.5.	25.5.	29.5.		5.6.	10.6.	
AGE (days Cohort 2)	46	53	61	66	70		77	82	
SAMPLING DEVICE	CS	CS	CS	CS	CS	SS	CS	CS	SS
Calanus finmarchicus St.1	0	0	0	0	0	0	0	0	0.1
St.2	0	0.2	0	0.1	0.1	0	0	0.1	0
St.3	0	0.3	0.2	0	0	0	0	0	0
St.4	0	0.3	2.0	0.3	0	0	0	0.6	0
St.5	0.2	0	0.9	0.9	0.1	0.4	0.5	0.2	0.7
Ad (♀)	0	0	0.3	0	0.2	0	0.3	0.1	1.6
Calanoid cop. (Ad.+juv)	0.4	0.8	1.0	0.7	0.6	3.7 [1]	2.2	1.4	1.4
Cladocerans	4.3	0.9	2.0	0.3	0.1	0.3	14.9	2.4	1.9
Calanoid nauplii	1.9	0.3	0.8	3.6	7.8	11.8	17.7	16.9	11.3
Other spp.	0	0	0	0	0	0.2	0	0.1	0.4

1) Mostly Centropages

Table 4. Zooplankton development in bags P3 and P5 in numbers per litre. Sampling devices were (CS); water coloumn sampler (10 l tube) and (SS); water surface sampler (10 l bucket).

DATE		23.4	28.4	12.5	25.5	29.5	2.6		5.6		10.6	
AGE	Cohort 1	47	52	66	79	83	87		90		95	
	Cohort 2	34	39	53	66	70	74		77		82	
SAMPLING DEVICE		CS	CS	CS	CS	CS	CS	SS	CS	SS	CS	SS
Calanus finmarchicus	St.1	25.4	3.5	0	0	0	0	0.1	0	0	0.2	0.8
	St.2	21.1	22.1	0.2	0	0	0.1	0	0.1	0.1	0.1	0.7
	St.3	3.2	13.8	3.2	0.5	0.1	0.1	0	0	0	0.1	0.6
	St.4	0	2.7	6.3	9.2	3.7	3.6	0.5	1.5	0.4	0.8	3.2
	St.5	0	0.1	0	1.6	3.7	4.3	0.2	1.9	1.4	4.9	11.5
Calanoid cop. (Ad. + juv.)		1.2	4.7	5.2	3.5	5.5	5.5	3.1	8.6	5.1	6.7	6.8
Cladocerans		0.4	1.2	0.1	0	0	0.3	8.3	13.4	18.2	17.3	63.7
Calanoid nauplii		1.0	1.5	5.9	3.5	3.1	8.7	7.1	12.8	9.7	8.2	3.8
Other spp.		0.4	0.4	0.7	0.1	0.5	0.2	0.9	0.7	0.8	0.8	2.9

Table 5. Composition of gut content in both cohorts of larvae.

					1)							
AGE (days)		46	51	56	56	62	62	66	70	75	83	TOTAL	%
DATE		6.5	27.4	15.5	15.5	8.5	21.5	12.5	29.5	21.5	29.5		
COHORT No:		2	1	2	2	1	2	1	2	1	1		
NUMBER OF GUTS EXAMINED (n =)		3	2	8	2	8	3	2	7	3	5		
Calanus finmarchicus	St.1	0	4	1	0	8	0	1	0	1	0	15	5
	St.2	3	5	2	0	23	0	6	0	0	2	38	12
	St.3	1	0	17	0	19	3	2	1	3	7	53	16
	St.4	1	0	1	1	1	1	0	8	2	9	24	7
	St.5	0	0	0	0	0	0	0	1	0	0	1	
Calanoid copepods (incl. copepodites)		0	0	0	10	0	0	2	9	3	19	43	13
Cladocerans		1	0	0	1	0	2	2	65	15	51	137	42
Calanoid nauplii		0	0	0	4	0	0	0	0	0	0	4	1
Other spp.		0	0	1	0	0	0	0	1	0	6	8	3
TOTAL		6	9	22	16	51	6	10	85	24	94	323	
FOOD ORGANISMS/LARVA		2	5	3	8	6	2	5	12	8	19		

1) BAG P 2 (Fed enriched zooplankton)

Table 6. Estimated number of successfully startfed larvae, and percent growing larvae, including larvae which died of known causes after first feeding (D65).

	AGE (days)	BAG No: 1) 1	2) 2	3	4	5	6	7	8	9	10	11	12	TOTAL
Estimated number prior to observed first feeding	D40	400	400	900	800	200	200	200	200	200	200	200	200	4100
Larvae for various analysis	>D65	0	7	24	7	13	6	1	1	19	1	1	2	82
Vibriosis														
Coh.1;	D94-97				3)									
Coh.2;	D82-84	0	0	36	18	31	34	21	30	22	19	9	37	257
Number of metamorphosed larvae	>D100	4	9	8	23	4	5	2	3	4	1	1	4	4) 50
Total number of growing larvae	>D65	4	16	68	30	48	45	24	34	45	21	11	43	389
% growing larvae		1	4	8	4	24	23	12	17	23	11	6	22	10

1) This bag was terminated D65
2) This bag was restarted with cohort 2 larvae D47 and fed enriched zooplankton
3) Died in tank after ended experiment
4) When 3) is not counted.

Table 7. Fatty acid composition of total lipid in halibut larvae before startfeeding (Bag P11), and startfed with natural zooplankton (Bag P 9) and cultivated zooplankton (Bag P2).

Fatty acid	Bag P11 (D32)	Bag P9 (D43)	Bag P2 (D51)
14:0	2.4 ± 0.06	2.3 ± 0.06	3.2 ± 0.40
15:0	0.5 ± 0.00	0.6 ± 0.06	0.4 ± 0.00
16:0	18.5 ± 0.26	18.5 ± 0.57	18.7 ± 0.51
16:1(n-9)	1.2 ± 0.06	0.7 ± 0.06	0.7 ± 0.00
16:1(n-7)	1.7 ± 0.06	3.1 ± 0.32	3.7 ± 0.79
16:2(n-4)		0.1 ± 0.00	0.2 ± 0.06
17:0	0.4 ± 0.00	0.5 ± 0.06	0.4 ± 0.06
16:4(n-3)	0.2 ± 0.06	0.3 ± 0.06	0.1 ± 0.06
18:0	6.7 ± 0.15	6.0 ± 0.76	5.1 ± 0.47
18:1(n-9)	5.2 ± 0.12	6.0 ± 0.00	4.1 ± 0.25
18:1(n-7)	2.1 ± 0.10	3.0 ± 0.29	3.4 ± 0.12
18:1(n-5)	0.4 ± 0.06	0.9 ± 0.25	0.4 ± 0.06
18:2(n-6)	1.1 ± 0.06	1.2 ± 0.10	1.3 ± 0.06
18:3(n-6)	0.1 ± 0.00	0.1 ± 0.00	0.5 ± 0.12
18:3(n-3)	0.3 ± 0.06	0.6 ± 0.10	1.2 ± 0.12
18:4(n-3)	0.7 ± 0.00	1.2 ± 0.10	2.5 ± 0.12
20:0	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00
20:1(n-9)	3.0 ± 0.29	0.6 ± 0.06	0.4 ± 0.06
20:1(n-7)	0.3 ± 0.06	0.1 ± 0.06	0.2 ± 0.00
20:4(n-6)	2.3 ± 0.17	0.8 ± 0.23	0.9 ± 0.10
20:4(n-3)	0.4 ± 0.00	0.7 ± 0.12	1.0 ± 0.06
20:5(n-3)	10.9 ± 0.00	17.1 ± 0.80	13.9 ± 0.25
22:0			
22:1(n-11)	1.0 ± 0.00	0.2 ± 0.00	0.1 ± 0.00
22:1(n-9)	0.2 ± 0.00	0.1 ± 0.00	0.1 ± 0.00
22:5(n-3)	1.2 ± 0.06	1.3 ± 0.30	1.6 ± 0.06
22:6(n-3)	31.8 ± 0.06	28.8 ± 0.40	29.9 ± 1.60
24:0			0.1 ± 0.00
24:1(n-9)	2.5 ± 0.12	1.9 ± 0.32	1.5 ± 0.06
% saturates	28.6 ± 0.2	28.0 ± 1.4	28.1 ± 0.6
% monosaturates	17.6 ± 0.2	16.5 ± 0.6	14.5 ± 0.9
% (n-3) PUFA	45.4 ± 0.2	50.0 ± 1.3	50.2 ± 1.4
% (n-6) PUFA	3.5 ± 0.2	2.1 ± 0.2	2.7 ± 0.1
(n-3)/(n-6)	12.9 ± 0.9	23.6 ± 2.3	18.6 ± 0.9
% unknown	5.0 ± 0.1	3.3 ± 0.2	4.4 ± 0.2