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GREEN WATER IN LARVICULTURE - An experiment with natural phytoplankton in tanks for first feeding of halibut larvae (Hippoglossus hippoglossus L.)

by

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Abstract

At 232 day degrees, halibut larvae were transferred from indoor tanks to 1.7 m^3 outdoor tanks for first feeding. The number in each tank was approximately 750. Three tanks were continuously given algal suspension ("green water") and supplied nonenriched Artemia instar II. Six tanks were given filtered deep water ("clear water"). Three of the six were supplied nonenriched Artemia, and three were supplied Artemia prefed in green water.

Feeding incidence at day 3 was 47 % in green water and 0 % in clear water. Larval growth was significantly higher in green water compared to clear water, while no significant difference was found between the clear water groups given prefed and nonenriched Artemia. The mean myotome heights for all groups were 0.75 - 0.78 mm at day 7. At day 14 and 21, the mean heights were 1.49 and 1.86 mm in the green water group and 0.84 and 1.05 mm in the clear water groups. The survival rates were also much higher in green water. Out of a total of approximately 2250 halibut larvae in the green water tanks, 684 larvae were found alive at the end of the experiment. Corresponding numbers for the clear water tanks were 57 out of 4500. Preliminary results indicate no nutritional effect of the algae.

INTRODUCTION

In the 1980'ies several studies were conducted in order to evaluate the use of different enclosures or mesocosms as part of production lines for marine fish larvae (Kvenseth and Øiestad al. 1984, Øiestad et al. 1985, Naas et al. 1987, Paulsen 1988). In terms of survival and growth, an extensive approach, combined with the use of wild zooplankton has in most cases proven to be superior to an intensive strategy with the use of cultured prey organisms (Witt et al. 1984, Paulsen 1988, Skjolddal et al. 1990). Although most of the research has focused on the nutritional aspects of wild zooplankton compared to Artemia or rotifers, there are several other characteristic aspects of the mesocosm approach that probably have an influence on the success. Among these are; large volumes combined with relatively low larval and prey concentrations; green water systems; outdoor systems with high light intensities and natural photoperiods.

Both in small scale intensive laboratory experiments and in large scale intensive production lines, the green water technique, with the use of an algal suspension in the rearing tanks, is favored by many culturists (see Jones et al. 1981). Without further comments to why, the green water is often referred to as a part of the standard procedure. Attempts to analyze the effects of the algal cells has been reported by Houde (1975, 1978), Moffat (1981) and Meeren (1989). Houde and his coworkers addressed the function of algae as water quality stabilizers in static rearing systems, while Moffat (op cit.) and Meeren (op cit.) points to the possible function of the algae as direct nutrition for the larvae.

The present study was a part of a comprehensive program on the various aspects of using mesocosms for the first feeding of halibut larvae, and the aim was to identify the main effects of algae in rearing tanks in relation to survival and growth of the fish larvae.

MATERIALS & METHODS

The experiment was conducted on Atlantic Halibut larvae (Hippoglossus hippoglossus L.) reared through the yolk sac stage at Austevoll Aquaculture Research Station. Methods are described by Rabben et al. (1987). The temperature was 7° C through the yolk sac stage. At an age of 232 day degrees after hatching, which is close to optimal time for first feeding in these systems (Harboe et al. 1990), the larvae were transferred to 9 outdoor tanks. These were 1.5m in diameter and 1m high, and were supplied with two types of water, "green water" and "clear water".

Green water:

The "green water", an algal suspension consisting of natural phytoplankton, was produced in a 280 m^3 outdoor basin supplied with unfiltered deep water (from 55 m). Twice a week the water was added N-P-K complex fertilizer (21-4-10) to a theoretical available nitrogen content of 30 μM. Water circulation was maintained by air bubbling, and the water flow through the basin corresponded to less than 20 % of the water volume per day. This algal culture was established two weeks before the experiment began.

Clear water:

Deep water (from 55 m), filtered through sand (10 μm) and cartridge (5 μm), was used as "clear water". To maintain the same temperature as the green water, the clear water was adjusted through a coil inside the green water basin.

Experiment:

Three tanks (replicates) were supplied with green water, and 2x3 tanks with clear water. The water flow was 1-2 l/min. Approximately 750 halibut larvae were carefully released in each tank at night time. All tanks were covered with black polyethylene netting reducing sunradiation with 70 %.

In the clear water tanks, one group (Group 1) were fed unenriched Artemia instar II (3 tanks) and one group (Group 2) Artemia instar II prefed for 2 hours in green water (3 tanks). The halibut larvae in the green water tanks (Group 3) were given unenriched Artemia instar II. An amount corresponding to 200 items/l were administered daily to each tank.

The experiment lasted 21 days. The proportion of functional larvae (without physical deformations) were estimated at the start of the experiment. Net samples of fish larvae from each tank were taken at day 3, 7, 14 and 21 for analyses of growth (myotome height) and feeding incidence. At the end of the experiment the number of remaining larvae in each tank was counted. The samples were fixed in 4 % formaldehyde and stored for one month before further analyses in a dissecting microscope. A light microscope was used to investigate possible algal material in

the guts.

Temperature was monitored daily. Twice a week samples of phytoplankton, Artemia densities, chlorophyll a and ammonia were collected and oxygen content and salinity were measured. Viable counts of bacteria in the water from one tank of each group were measured weekly on two different media, TCBS (Tryptone citrate Bile Salt, Oxoid) and MBA (Marine broth Agar, Difco). The petri dishes were incubated at 10 °C for at least 10 days.

Temperature and salinity were measured with a calibrated WTW-salinometer, model LF 191, and oxygen with an YSI- oximeter, model 57. These parameters were monitored both at the surface and bottom. Samples for phytoplankton, chlorophyll a and ammonia were taken at 0.5 m with a 2 litre Ruttner water sampler. Phytoplankton samples were fixed in acid Lugol's solution in addition to 4 % formaldehyde. Identification and counting were done in an inverted face contrast microscope. Chlorophyll a were analyzed on a Perkin-Elmer LS-3B Fluorescence Spectrophotometer, and ammonia on a Shimadzu UV-160 spectrophotometer.

Zooplankton samples were collected with a 5 litre plexiglas Schindler trap (Schindler 1969) at 0.5 m in the middle of the tanks. These were filtered through 40 μm and immediately fixed in acid Lugol's solution. Zooplankton samples were taken immediately before the daily supply of Artemia in order to evaluate the survival of the food organisms and the predation pressure from the fish larvae. Further analyses were done in a dissecting microscope.

In this report deformed larvae with yolk sac oedema or abnormal mouth opening ("gapers") are registered, but excluded from the presented data.

RESULTS

Hydrography

The water temperature through the experiment varied between 6 and 14°C (Fig. 1a). However, the temperature differences between the tanks with clear water and green water were small. pH was always higher in the green water due to the higher primary production (Fig. 1b), but no extreme values was measured. Salinity was very similar in all tanks and varied between 32.3 and 34.0 ppt during the experiment. The concentrations of ammonia were always low (Fig. 1c), highest values (8.3 μM) was recorded in the green water the first sampling date. Oxygen levels varied between 9 and 11 ml/l in the clear water tanks and between 12 and 15 ml/l in the green water tanks (Fig. 1d)

Bacteria

Viable counts of bacteria are listed in Table 1. MBA counts were higher in Group 2 at day 7, compared to the other groups. Groups 1 and 3 were virtually indifferent at day 7, whereas at days 14 and 21, MBA counts in Group 1 were lower than in the two other groups. TCBS counts, which is a measurement of Vibrio spp. (Bolinches and Egidius 1987), were similar in all groups at day 14, whereas at day 7, Group 3 showed higher counts compared to the others. At day 21, no counts were registered on this medium in the samples from Group 3, and

only a very limited number in Group 1.

Table 1. Viable counts of bacteria in the rearing water of each group.

Day	Medium	Group 1 (Clear unenr.)	Group 2 (Clear pref.)	Group 3 (Green w.)
Day 7	MBA	$3.7*10^4$	$2.0*10^5$	$4.2*10^4$
	TCBS	$3*10^2$	$3*10^2$	$9*10^2$
Day 14	MBA	$5.2*10^4$	$1.9*10^5$	$1.7*10^5$
	TCBS	$4*10^3$	$3*10^3$	$2*10^3$
Day 21	MBA	$4.3*10^3$		$7.7*10^4$
	TCBS	$4*10^1$		ND

ND = None detected

Algae

The concentration of algae in the green water was between one and two orders of magnitude higher than in the clear water (Fig. 2a,b). The first ten days of the experiment the green water consisted of small flagellates and very high numbers were recorded (90 million cells/l). After the flagellate bloom had elapsed the algal composition changed and by the end of the experiment diatoms were dominating.

The succession in the phytoplankton population from flagellates to diatoms was also visible in the total cell volume (Fig. 3a) and chlorophyll a (Fig. 3b)

recordings. However, although the cell numbers were much lower at the end of the experiment, both the total cell volumes and chlorophyll a were high due to the larger sized diatom cells.

Food (Artemia density)

Each tank received a daily food supply of 350 000 Artemia corresponding to a concentration of 200 items/l. Already at day 8, significant differences in the food densities among the groups were observed (Fig. 4). Small differences were found within the clear water tanks where the food densities most of the time varied between 200 and 600 Artemia/l. Increasing densities in the first half of the experiment, indicated minor predation from the halibut larvae. The opposite tendency was observed in the green water tanks, where the Artemia was more or less grazed down to a minimum before new Artemia was added. Except for the first sampling date (day 5), the food densities were always much lower in the green water than in the clear water. The concentrations also decreased through the experiment, and only 5.5 Artemia per litre were recorded at day 19.

Feeding incidence

Large differences in feeding incidence between the groups were observed in the first week of the experiment (Fig. 6). At day 3, no specimens with food in the gut were observed in groups 1 and 2. At the same time 46 % of larvae in Group 3 had Artemia remains in the gut. At day 7 the proportions with food in gut were 26.6, 5.0 and 41.2 respectively for groups 1,2,3. At day 14, which is considered beyond time of death in starvation groups, very high proportions were found with filled gut in all groups. No remains of phytoplankton were identified in any larvae

at day 3.

Growth of halibut larvae

At the start of the experiment all the larvae were uniform in size with an average myotome height of 878 μm (Fig. 6). A significant decrease ($p < 0.0001$, t-test) to approximately 780 μm was observed in all groups during the first three days. No significant differences in myotome heights were found from day 3 to 7 between the groups.

From day 7 to day 14 the larvae in Group 3 showed a considerable growth and almost doubled their myotome heights (to 1486 μm). This corresponded to a daily increase of the myotome height of 9.5 %. The larvae in Groups 1 and 2 increased from 760 μm to 840 μm on average in the same time period, corresponding to 1.5 % per day. Although this increase was significant ($p < 0.001$, t-test), the myotome heights were significant less than in Group 3 at day 14 ($p < 0.0001$, t-test). No significant differences in myotome heights were found between the groups 1 and 2 at day 14.

From day 14 to 21 the myotome heights of the Group 3 larvae increased significantly from 1486 to 1861 μm ($p < 0.001$, t-test), corresponding to 3.2 % per day. Group 1 larvae had grown significantly from 842 to 1058 μm ($p < 0.001$, t-test) which corresponded to 3.3 % per day. In Group 2 no such estimation could be made because only two larvae survived to day 21.

Survival

The survival were also much higher in Group 3 than in Groups 1 and 2. Out of a total of approximately 2250 halibut larvae in the three green water tanks, 684 were found alive at the end of the experiment (≈30 %). Corresponding number for Group 1 was 55 (≈2.4 %) and for Group 2, 2 (≈0.01 %). In addition, 106 living larvae in the green water and 160 in the clear water had been sampled during the experiment.

DISCUSSION

The results show that the algal suspension has a remarkable effect on the onset of feeding, growth and survival of halibut larvae. The most striking difference is the higher feeding incidence observed during the first days in green water compared to the clear water groups. Another point to be noted is the minimal growth of the larvae which actually start to take food in clear water. Obviously some factors of the green water induce a more successful seeking, capturing and utilizing of prey organisms. In this discussion both abiotic and biotic factors must be considered.

Algal effects on biotic parameters

A possible nutritional effect of the algae is directly as first food. In cod, Meeren (1989) observed a filter-feeding mechanism which retained phytoplankton cells larger than 10 μm. No free algal matter was found in the guts during the first days, but the cell wall of the small flagellates, dominating the green water at this time, are very easily destroyed through fixation and probably also by digestion.

Thus, the absence of identified algal cells in the guts of fixed larvae does not exclude ingestion of phytoplankton in this experiment. Through drinking activity, which is shown to be present in marine fish larvae (cod, Mangor-Jensen & Adoff (1987); halibut, Tytler & Blaxter (1988); Fundulus, Guggino (1980), it is likely to assume a passive transport of algae to the gut. The energy supply of algae through the drinking activity is probably of minor importance, and can not sustain growth. However, effects as micronutrient can not be discounted. Moffatt (1981) stated that it is unrealistic that algal cells present in the gut do not provide at least minimal nutrient value for fish larvae. Some nutritional effect of algae as first food can therefore not be rejected for halibut larvae, but can not explain the pronounced difference in onset of feeding among the groups tested.

An indirect nutritional effect of the algae through the gut content of the Artemia, seems likely to be rejected. The larvae in the tanks given prefed Artemia did not perform any improved growth and survival compared to the larvae given unfed Artemia. Artemia prefed in green water are also supposed to have gut content which would increase their visibility for the fish larvae. However, no differences which could correspond to such effects (i.e. feeding incidence), were found between the two clear water groups (Group 1 and 2).

Another possible indirect effect of the algae is through a "microbial loop" (Azam et. 1983). The algae show a kind of symbiotic relationship to bacteria, with elements of both competition and commensalism (Bratbak & Thingstad 1985). Thereby, the algae radically changes the microbial environment in the tanks, possibly making way for bacteria acting antagonistic towards opportunistic

pathogens, which in other systems may dominate the bacterial population (Bolinches & Egidius 1987, Hansen & Bergh unpublished data). A nutritional effect of the bacterial additives has been shown to be beneficial to other fish larvae (Gatesoupe 1989, Gatesoupe et al. 1989). The high viable counts in Group 2 correlates with the early mortality in this group. As the concentration of algae in this group was very low, it is likely that the bacterial population was caused by large amounts of available organic matter, originating from dead larvae and, possibly, Artemia.

Algal effects on abiotic parameters

The differences in performance of the halibut larvae in the two water types, are probably not caused by differences in the water quality parameters such as, pH, NH_4 and O_2 because of the water exchanges in the tanks. Introduction of algae is, otherwise, known to have positive effects on the water quality by reducing the metabolic by-products in static rearing systems (Houde 1975, 1978). The early drop in temperature have probably reduced the growth in the first part of the experiment, but the drop was similar or even higher in the green water tanks.

A very important effect of the algae is by increasing the turbidity or clarity of the medium. This will increase the light attenuation coefficient of the water and furthermore lead to a decrease in the visual range of the halibut larvae (cf. Aksnes & Giske 1990). For fish larvae with a strong anti-predator behaviour the clear water can have a stressing effect and lead to higher energy consumption by unnecessary swimming activities. A reasonable hypothesis is that the presence of algae will lower the stress of the rearing environment, reduce the anti-predator

behaviour and thereby induce a more "normal" and effective seeking and capturing behavior of the larvae. Furthermore, higher attenuation coefficient due to the algal suspension will also result in a sharp light intensity gradient in the water column. Consequently the larvae will have an increased option of positioning according to optimal light intensity (see Mangor-Jensen & Naas 1990). A conductive consideration of this aspect can not be drawn from the present data and must be followed by further investigations.

In addition to be a visual feeder, the halibut larva is supposed to use chemotaxis in food seeking and has developed an olfactory organ before time for first feeding (Knutsen 1989). Chemical stimuli from the algae on the halibut larvae can therefore be of importance.

Food concentration

The increasing Artemia concentrations in the clear water tanks indicated a high survival of the food organisms. The growth to larger stages, which easily could be observed in the clear water tanks, made the older Artemia too big for the halibut larvae. Consequently, the food densities in this tanks after some days did not correspond to the real densities of suitable prey items. Studies have shown good survival and growth of fish larvae at low zooplankton densities (25 - 300 items/l) when reared in green water (see f. ex. Houde 1975, 1978, Moffatt 1981), while higher food densities seem to be necessary in "cleaner" water types (Lasker et al. 1970, Hunter & Thomas 1974). In our experiment, the main differences in feeding incidence occurred while the food concentrations were around 200 items/l (Fig. 4). The delayed onset of feeding in clear water may have been avoided by an

increased food concentration at the start of the experiment. On the other hand, too high food concentrations may have induced a confusion effect (Miller 1922). This effect lead to lower capture rates and more irrelevant behaviour (Miliski 1979). The presence of algal cells may have reduced the visible range and thereby reduced the confusion effect.

The analyses from this study is still in a preliminary phase. A more thoroughly preparation of the data is in progress. Additional analyses of the microbial environment in the tanks, larval bacterial intestinal flora and fatty acid content of both algae and the different Artemia groups will be included.

CONCLUSION

By using green water the halibut larvae show substantial survival and growth. The role of algae is still not fully understood. However, a nutritional effect of algae seems to be of minor importance compared to effects on the light regime.

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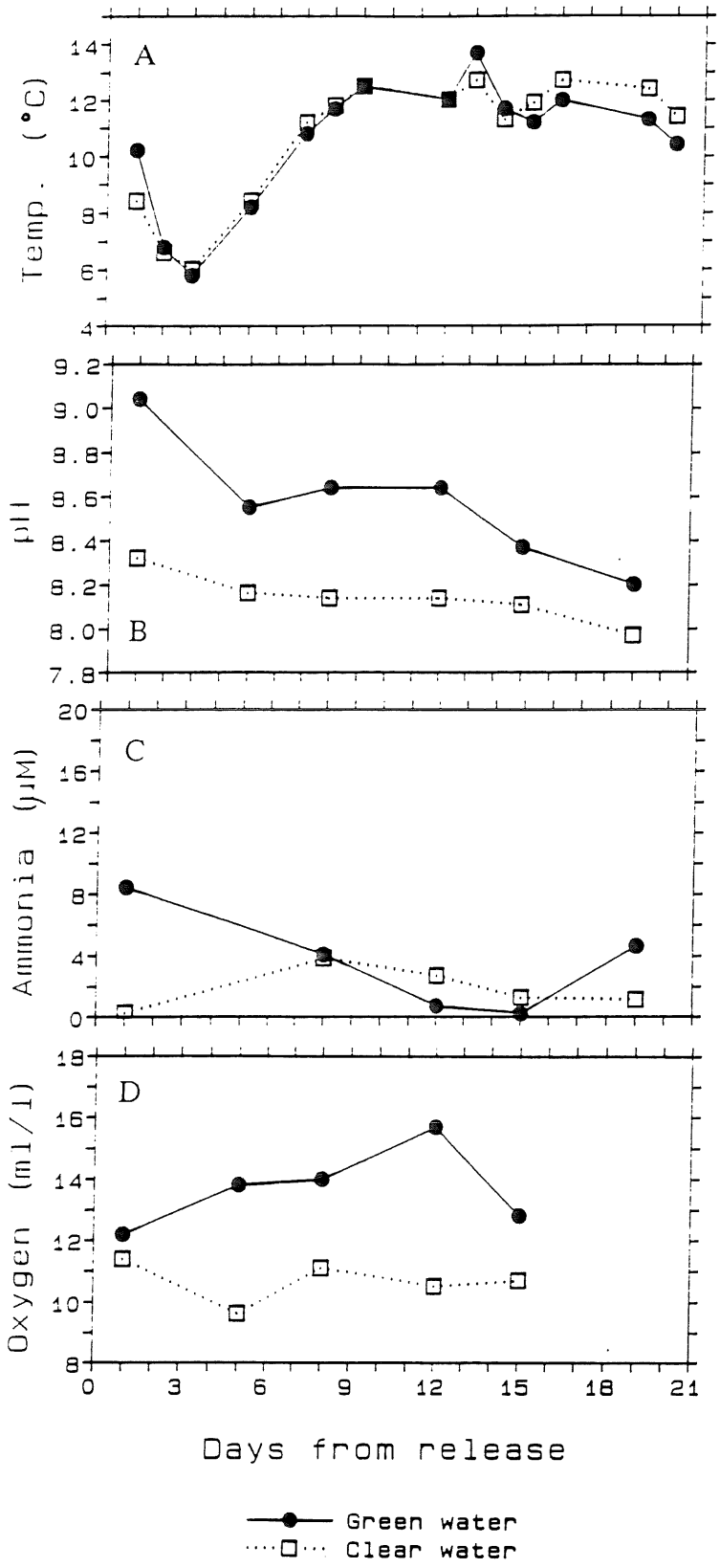

Fig. 1. The development of a) temperature; b) pH; c) ammonia concentration; and d) oxygen; in the green water and clear water tanks during the experiment.

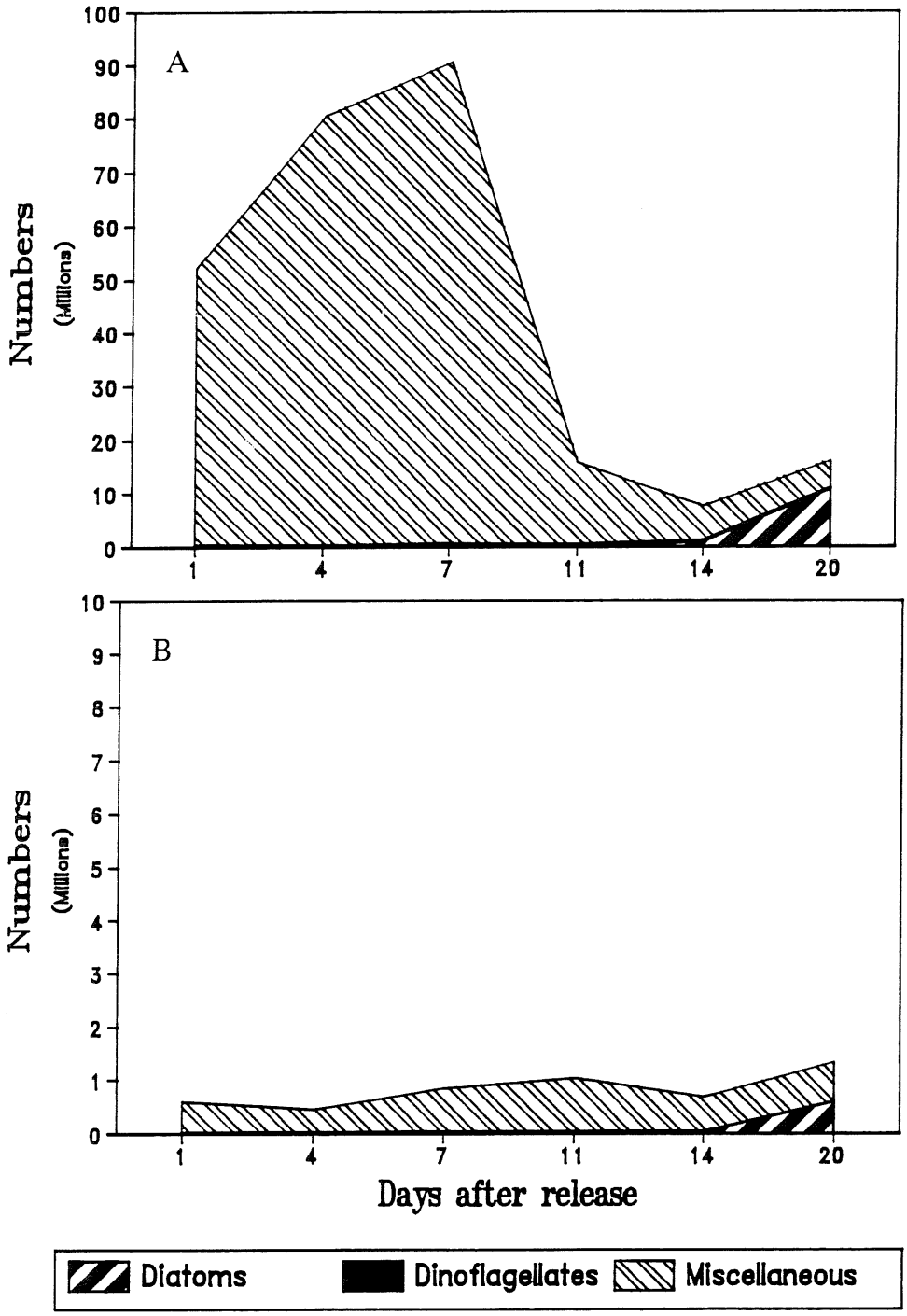

Fig. 2. Concentrations of the different phytoplankton groups in: a) the green water tanks and b) the clear water tanks. The group miscellaneous was dominated by small undetermined flagellates (<5 μm).

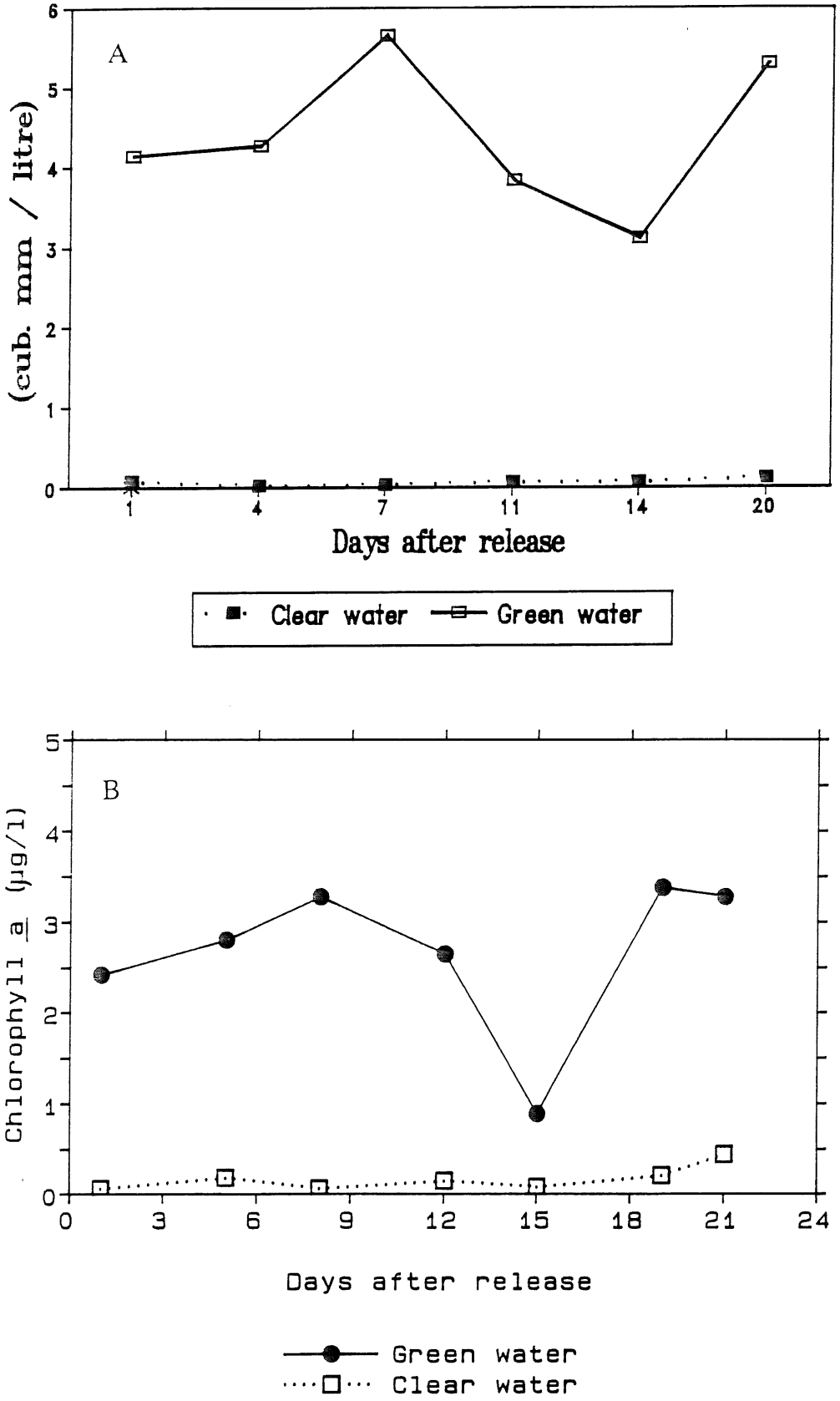

Fig. 3. a) Cell volume of phytoplankton and b) concentrations of chlorophyll a in the green and clear water tanks during the experiment.

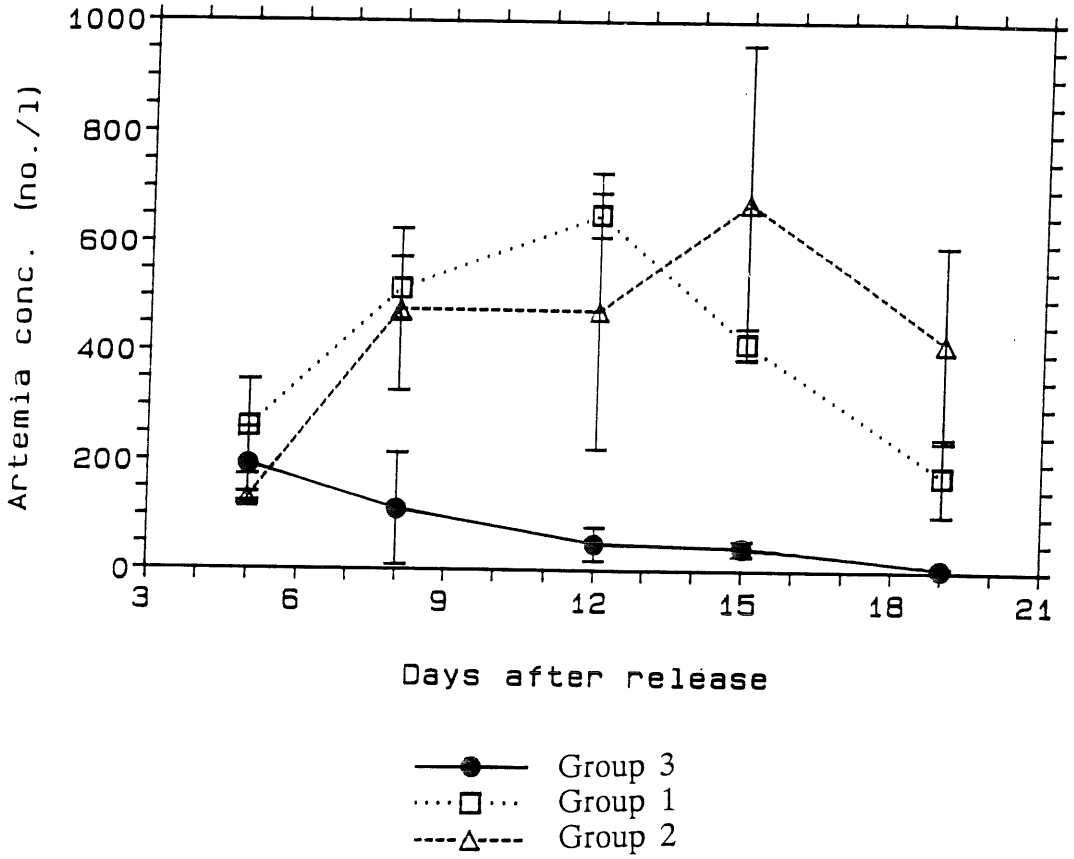

Fig. 4. Concentrations of Artemia in the three experimental groups during the experiment. Samples are taken immediately before new food supply.

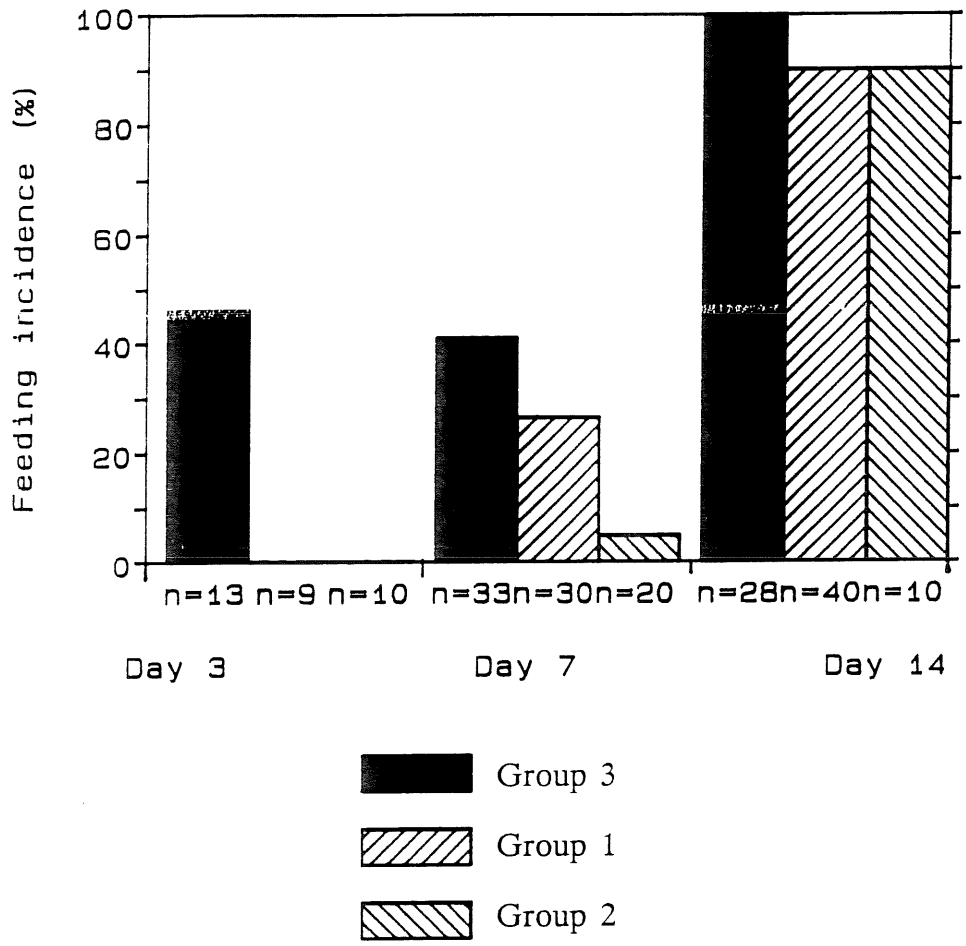

Fig. 5. Feeding incidence of halibut larvae in the three experimental groups during the experiment.

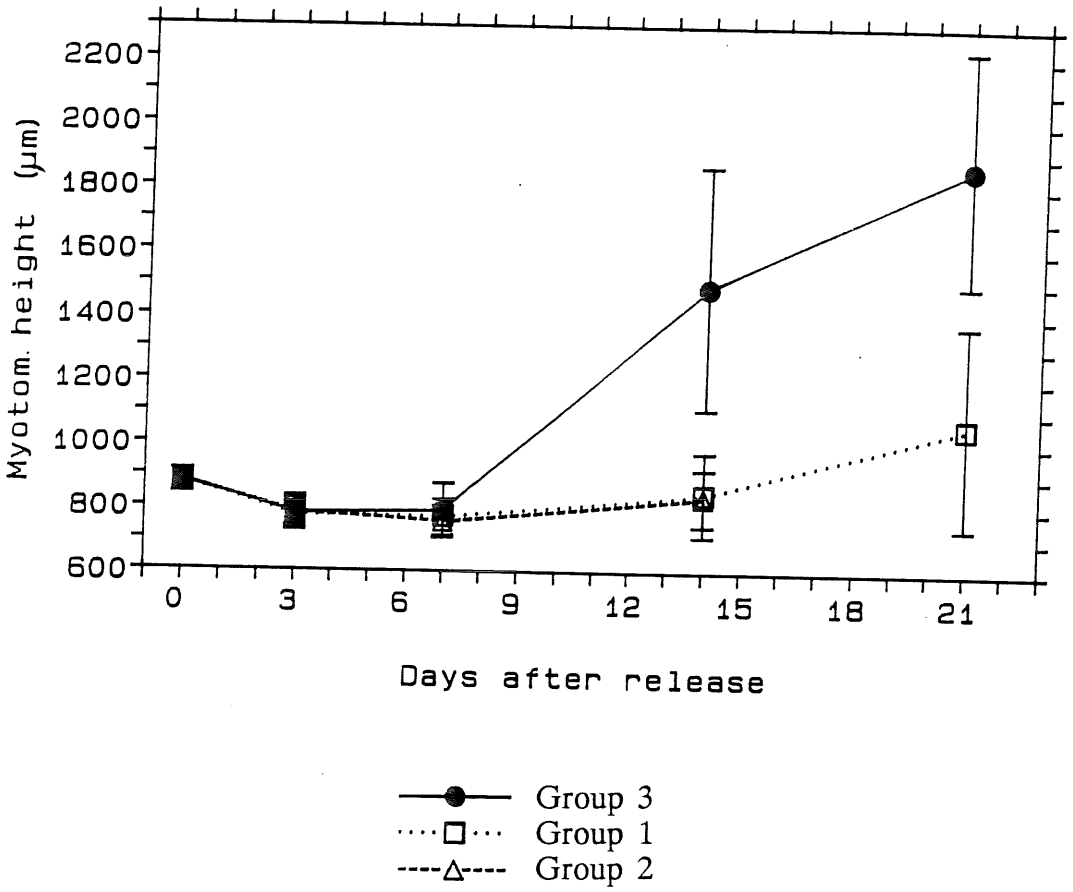

Fig. 6. Growth of halibut larvae in the experimental groups during the experiment.