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An Intensive Approach to Atlantic Halibut Fry Production

IVAR HOLMEFJORD,[1] JON GULBRANDSEN, INGRID LEIN AND TERJE REFSTIE
AKVAFORSK, Institute of Aquaculture Research, N-6600 Sunndalsøra, Norway

PHILIPPE LÉGER
Artemia Systems SA, Oeverstraat 7, B-9200 Baasrode, Belgium

TORSTEIN HARBOE AND INGVAR HUSE
Austevoll Aquaculture Research Station, Institute of Marine Research, N-5392 Storebø, Norway

PATRICK SORGELOOS
Laboratory of Aquaculture & Artemia Reference Center, University of Ghent, Rozier 44, B-9000 Ghent, Belgium

SYLVIE BOLLA, YNGVAR OLSEN, KJELL INGE REITAN,
OLAV VADSTEIN AND GUNVOR ØIE
SINTEF, Center of Aquaculture, N-7034 Trondheim, Norway

ANNE DANIELSBERG
SINTEF, NHL, N-7034 Trondheim, Norway

Abstract

The larval stage is regarded to be the main bottleneck of halibut production. Halibut eggs were obtained from captive broodstock both by stripping and by natural spawning. Artificial photoperiods were used to increase the total spawning season. Yolk sac larvae are presently produced either in small stagnant units or in large flow through systems. A major consideration is to avoid stress of the larvae, caused by mechanical disturbances of the larvae and by high bacterial load or high ammonia levels in the water.

The experiments showed that halibut larvae began to ingest algae earlier than rotifers *Brachionus* sp. Supplementation of algae to first feeding tanks resulted in enhanced survival and growth rate of the larvae. Both rotifers and *Artemia* can be enriched with very high levels of highly unsaturated n-3 fatty acids (n-3 HUFA). Enriched live feed, containing high levels of n-3 HUFA and total lipids, enhanced both survival and growth of the halibut larvae. The highest growth rates were obtained with wild zooplankton and addition of algae, but enriched cultivated feed combined with algae resulted in growth of the same magnitude as with wild zooplankton.

Atlantic halibut *Hippoglossus hippoglossus* L. is regarded as a promising aquaculture species for cold waters, due to its high market price and relatively high growth rate at low temperatures. It is, therefore, of great interest to develop cultivation methods for this species.

This paper reviews results of a three-year research project, "Intensive production of halibut fry" (1988–1990), funded by The Royal Norwegian Council for Scientific and Industrial Research (NTNF) and FINA Exploration Norway. The main goal of the project was to develop industrial processes for intensive cultivation of halibut fry.

The general strategy of the work has been that the development of technical equipment and production methods must be based on knowledge of the requirements of the larvae. Environmental, physical and microbial conditions of the yolk sac larvae, as

[1] Corresponding author.

well as the nutritional requirements through the phases of first feeding and weaning, are all believed to be important.

At the time the project was initiated, it was realized that the development of an intensive methodology for halibut fry production would need time. This was based on the assumption that all intensive production systems require a high level of basic knowledge on the components of the system. However, an intensive approach was regarded as critical in order to establish profitable production methods.

The commercial production of halibut fry must include different activities such as broodstock management, hatching of eggs, incubation and mass rearing of yolk sac larvae, live feed production, first feeding, and weaning to formulated diets.

Broodstock and Egg Production

When the experimental activity with Atlantic halibut in Norway was increased during the early 1980s, eggs were obtained from fish caught from the spawning grounds (Haug 1990). Because of the unsatisfactory egg quality and unstable supply, several broodstocks of Atlantic halibut were established. At AKVAFORSK, Sunndalsøra, the first broodstock fish were introduced in 1984.

Two groups of wild-caught broodstock were held in circular concrete tanks (10 m diameter, 1 m water depth). The fish were held under ambient temperature conditions (7–13 C), and were fed to satiation on a moist pelleted diet two or three times a week, except during the spawning season when feeding was stopped. Sporadic outbreaks of the ectoparasite *Entobdella hippoglossi* were effectively treated with formaldehyde (Holmefjord et al., in preparation).

The natural spawning season of Atlantic halibut normally lasts 2–3 mo. Commercial production of halibut, however, may imply the availability of eggs at different times of the year. As a consequence an important part of the broodstock work was to examine the possibility of extending the spawning season by photoperiod manipulation as applied for other marine fish (Devauchelle et al. 1988; Carillo et al. 1989).

One group of spawners was held under natural photoperiod conditions; in the second group the annual cycle was shifted two months ahead of the normal cycle. The shift in light cycle was introduced gradually during the autumn prior to spawning. In the first season after initiation of the experiment, the average time of the first spawned batch of each female was one month earlier in the group receiving the shifted light regime compared to normal light cycle. The difference increased to approximately two months in the second and third year (Holmefjord et al., in preparation).

The results showed that the halibut responded to a shift in photoperiod by a corresponding shift in spawning time. This suggests that halibut eggs could be produced at different times of the year by use of appropriate artificial photoperiods. Further work is needed to elucidate possible interaction effects between photoperiod, temperature and salinity on the spawning time and egg quality. Such interactions have been reported for turbot (Devauchelle et al. 1988).

As has been shown for turbot, halibut is a multiple spawner with individual ovulatory rhythms (McEvoy 1984; Howell and Scott 1989; Bromage et al. 1991; Holmefjord 1991; Norberg et al. 1991). Hand-stripping is the dominant method for egg collection, and accurate estimates of the individual spawning rhythms is necessary to obtain high hatching rates (75–85%) (Bromage et al. 1991; Holmefjord 1991; Norberg et al. 1991). Both a better knowledge of ovulatory rhythms and improved stripping procedures will be important in the future for improving the quantity and quality of stripped eggs.

Until 1989, hand-stripping was the only method for egg production. From 1989 onwards, naturally fertilized eggs were observed in one out of two broodstock tanks (Holmefjord et al., in preparation). Both the fertilization rate and the hatching rates were highly variable, but most of the females in

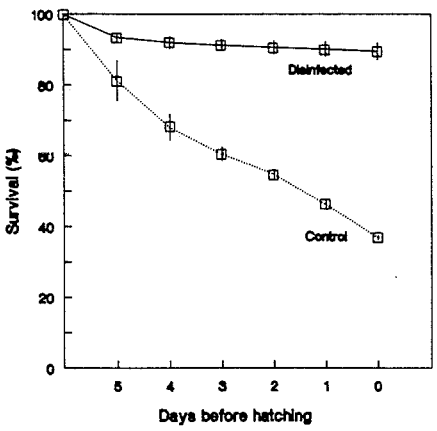

FIGURE 1. *Survival of disinfected (400 ppm glutaric dialdehyde/10 min) and untreated eggs incubated in upstream incubators supplied with membrane filtered water. Bars indicate SE.*

this tank produced one or more batches with high fertilization rates. Natural fertilization has also been observed in one other halibut broodstock in Norway. The factors inducing natural fertilization are still unknown.

Both spawned and stripped egg batches exhibited highly variable hatching rates. Naturally spawned eggs often became heavily loaded with bacteria during the last 4–5 d prior to hatching. Effort was therefore made to develop efficient egg disinfection procedures. Three chemicals were tested: Glutaric dialdehyde, Chloramine-T and Hypochlorite. Each of the chemicals was tested at different concentrations and exposure periods up to 10 min. Experimental methods are described by Salvesen et al. (1991). The results revealed high surface disinfection effects and increased survival of eggs and larvae for many of the applied conditions. Negative effects on larvae were, however, observed for most treatments with hypochlorite and for some with Chloramine-T. Based on these results a treatment of 10 min in 400 ppm glutaric dialdehyde solution was adopted for a large scale experiment. This treatment improved both survival of eggs (Fig. 1) and viability of larvae. The effects of the disinfection are, however, variable and partly depend on the initial bacterial load on the eggs (Salvesen et al. 1991).

Further research to optimize the disinfection conditions and to evaluate potential sub-lethal and long-term effects on the larval development is in progress. The described experiments were carried out with eggs older than 50 d° (d° = day–degrees = number of days × temperature (C)). More information is needed on the effect of disinfection at different developmental stages of the eggs.

Egg Incubation

Stripped eggs were kept cool (4 C) in ovarian fluid until fertilization. Eggs and milt were mixed with UV-treated and 0.2 μm filtered sea water (wet fertilization). Naturally spawned eggs were collected from a mesh screen at the outlet of the broodstock tank. After removal of dead eggs, the living eggs were gently transferred to the incubator systems.

Conical upstream incubators (volume 25–250 L) were used for incubation of eggs. The outflow was at the top of the incubator through a fine mesh screen. The incubators were kept in a temperature controlled room, and the water temperature was adjusted to 5 ± 0.5 C. Ambient seawater (29–32 ppt) was used in the incubators. Daily introduction of seawater of 34–35 ppt through the bottom inlet was used to separate floating, living eggs from sinking dead eggs; the latter were removed through a bottom outlet. The same procedure was used at hatching. The water flow was stopped after adding the salt water, and the newly hatched larvae floated to the top, and were gently transferred to the larval incubation systems.

Hatching occurred after approximately 16–17 d at 5 C (85 d°), and the blastopore closed at about 9–10 d (45–50 d°). After blastopore closure, the eggs are more resistant to mechanical stress, and successful transport can be carried out with eggs of 50–80 d° (Holmefjord and Bolla 1988).

Yolk Sac Larvae

The Atlantic halibut larvae hatch at a very early stage of development, and compared to most other marine fish, have a long yolk sac period (Blaxter et al. 1983; Pittman et al. 1990). At a temperature of 4.5 C, the larvae will depend on their yolk sac resources for 45–50 d before they start to feed (Lein and Holmefjord 1992).

It is very important to give the larvae adequate environmental conditions, since even moderate stress factors may influence the viability of the larvae. Effects of different environmental factors have been studied in small scale experiments, using 3 L glass jars as test chambers for incubation of the yolk sac larvae. Oxytetracycline at 25 ppm was supplemented to this stagnant system. Large silos (volume 3,000–14,000 L) with a slow upstreaming waterflow were also tested as larval incubation units (Rabben et al. 1987).

Newly-hatched halibut larvae are very sensitive to mechanical stress (Opstad and Raae 1986). When an exchange rate of 30% per day was started at different larval ages (0, 6, 12, or 24 d after hatching), highest survival was obtained when the water exchange was initiated 24 d after hatching. In this regime the frequency of deformed larvae and the mortality were low and not significantly different from the stagnant control, while earlier initiation of flow reduced survival and percentage of normal larvae.

In small stagnant units the concentration of unionized ammonia in water increased linearly during the first 30 d of the yolk sac stage (Fig. 2). After day 30, the concentration of ammonia increased rapidly, and could eventually reach potentially harmful concentrations for the larvae during the last part of the yolk sac period. This accumulation of ammonia could be reduced by water exchange during the yolk sac period. When a water exchange of 30% per day was introduced from day 26, the accumulation of ammonia was reduced by 85%. The bacterial concentration was also reduced to about 20% of that found in the stagnant

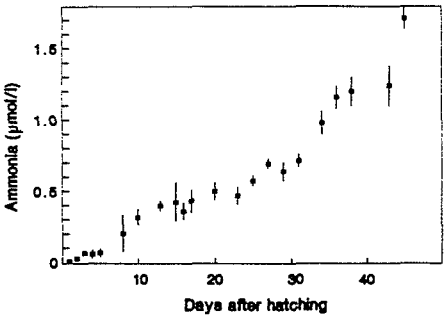

FIGURE 2. *Accumulation of ammonia in stagnant glass jars (volume 3 L, 5 C) during the yolk sac period (60 larvae/L). Values are means of 2 replicates and bars indicate SEM.*

control, but there was no difference in the survival of larvae.

Yolk sac larvae were kept at low temperature (4 C) almost up to first feeding, whereas first feeding larvae were kept at ambient seawater temperatures (7–12 C). A few days before transfer into the first feeding tanks, the water temperature in the small stagnant systems was raised to ambient seawater temperature, in order to avoid a temperature shock. The temperature increase, however, causes an increased fraction of unionized ammonia (Bower and Bidwell 1978).

The risk of potentially toxic NH3-levels in small, stagnant systems is a disadvantage in comparison with the flow-through systems. Nonetheless, the mortality rates and frequencies of abnormal larvae have been low in small, stagnant systems as compared to the results obtained in the different flow-through systems that have been tested. The survival during the first month of larval incubation has been very high (>95%) in 3 L glass jars, while in larger flow-through systems, a peak in mortality usually occurred about 14 d after hatching. The mean survival up to first feeding in large flow-through systems was 63–70% in 1988–1990. From the results obtained it appears that the halibut yolk sac larvae should be held in stagnant systems or very strictly controlled flow-through systems during the first 3–4 wk.

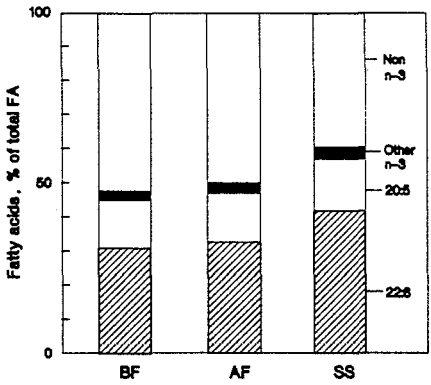

FIGURE 3. *Fatty acid distribution in rotifers fed on bakers yeast and an emulsified lipid source, before feeding (BF) and 4 h after feeding of daily ration (AF). The fatty acid distribution of the lipid source (Super Selco, Artemia Systems, Belgium) is shown in the right bar (SS). Values are means of 5 replicates.*

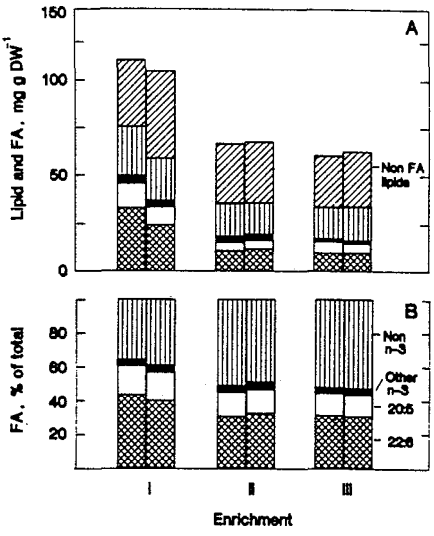

FIGURE 4. *Lipid and fatty acid content in enriched rotifers. The rotifers used were cultivated on yeast and Super Selco (see Figure 3). Values are means of 5 replicates, sampling for series I and III was performed 24 h after application of enrichment. A: Total content of fatty acids and n-3 fatty acids (mg FA/g dry matter). B: Relative fatty acid distribution (% of total FA). I: Short-term enriched with SS. II: No short-term enrichment. III: Short-term enriched with squid meal.*

Live Feed Production

The dietary level of highly unsaturated n-3 fatty acids (n-3 HUFA) in the food is critical for marine fish larvae (Watanabe et al. 1983; Izquierdo et al. 1989). Juvenile stages of coldwater-copepods generally contain high levels of n-3 HUFA, and these copepods probably constitute an important fraction of the natural prey organisms for halibut larvae. In order to be able to rely on the commonly used live feed organisms *Brachionus* and *Artemia*, special culture and enrichment procedures were developed to incorporate comparable levels of (n-3) HUFA as in copepods.

Rotifers were cultivated to levels of 3–400 ind./ml under weak aeration in 200 L cultures with daily doses of 20–30 g yeast and 2–3 g Super Selco® (SS), Artemia Systems NW/SA, Belgium. Lipid analyses according to Rodriguez Rainuzzo et al. (1989) on the produced rotifers, revealed that the sum of 22:6 n-3 and 20:5 n-3, constituted almost 50% of their total fatty acids (Fig. 3).

The Super Selco diet yielded an efficient long-term enrichment of the rotifers. The relative distribution of fatty acids in the rotifers was close to that of their dietary lipids. An appropriate fatty acid composition in rotifers may thus be achieved during their cultivation by choosing a dietary lipid source with appropriate fatty acid composition.

The lipid content of rotifers which are cultivated on yeast and SS will normally be in the range of 13–15% (DW basis). Aiming to further increase their level, short term enrichment procedures were tested with 50 ppm SS or 100 ppm squid meal added to the culture tank in the morning and the afternoon. The SS-enrichment resulted in enhanced total levels of lipids, but the relative content of n-3 HUFA was only slightly enhanced (Fig. 4). The squid meal enrichment gave no further change either in quantitative or in relative fatty acid content (Fig. 4). This is probably due to the high initial n-3 HUFA-level in the rotifers fed SS and to

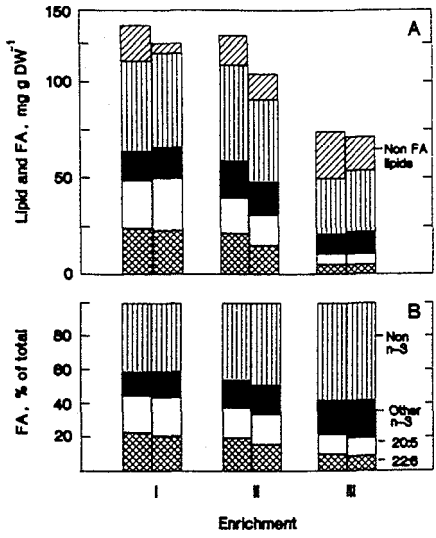

FIGURE 5. *Lipid and fatty acid content in enriched* Artemia. *A: Total content of fatty acids and n-3 fatty acids (mg FA/g dry matter). B: Relative fatty acid distribution (% of total FA). I:* Artemia *fed 300 ppm of SS 6 h, 24 h and 36 h after hatching. II:* Artemia *fed 300 ppm of SS 6 h and 12–18 h after hatching. III:* Artemia *fed 0.3 g of squid meal per liter culture medium 6 h and 12–18 h after hatching.*

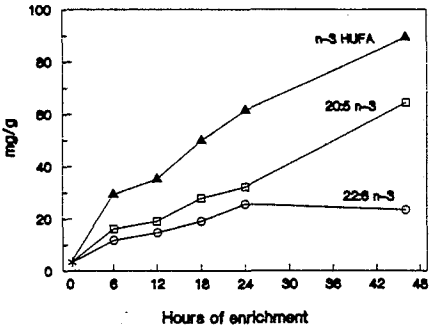

FIGURE 6. *Increase of n-3 HUFA with time during enrichment (0–46 hours) of* Artemia *using Super Selco. Values are means of three replicates.*

the rather low lipid content of the squid meal (Rodriguez Rainuzzo et al. 1989).

Artemia was decapsulated and hatched at 28 C according to standard procedures (Sorgeloos et al. 1986).

The fatty acid composition of the *Artemia* nauplii was modified through short-term enrichment (Léger et al. 1986, 1987) with either SS (300 ppm doses applied 6 h after hatching and again after 12–18 h, or after 6 h, 24 h and 36 h) or squid meal (300 ppm doses 6 h and 12–18 h after hatching).

The highest level of n-3 HUFA and lipids was obtained with the longest enrichment with SS (Fig. 5). In another experiment where *Artemia* was enriched with SS, the content of 20:5 n-3 and total n-3 fatty acids increased during 46 h of enrichment, whereas the increase of 22:6 n-3 levelled off after 24 h (Fig. 6). The content of n-3 HUFA was high compared to that found in naturally occurring zooplankton. Relatively high mortality is noticed when *Artemia* is short-term enriched at high densities (200–300 animals/ml) for more than 24–36 h with emulsified diets. Enriching at lower densities solves this problem. It should also be noticed that the mortality rate of the *Artemia* was enhanced after their transfer to cold water, in particular in the SS-enrichment series.

The characterization of fatty acid and lipid composition of wild zooplankton was undertaken in order to establish a reference for the enrichment of rotifers and *Artemia*. The zooplankton was sampled from a commercial research hatchery at the western coast of Norway from April to end of June 1990. The species analyzed involved some of the most common zooplankton used as feed for marine larvae, i.e., *Acartia* sp. and *Calanus finnmarchicus*. Both the total and relative level of n-3 HUFA found in the zooplankton in this study showed some variation from April to June, but was throughout within the range which may be obtained by enrichment of rotifers and *Artemia*.

The frequency of malpigmentation, however, is still higher among flatfish juveniles grown on rotifers and *Artemia* compared to juveniles grown on natural zooplankton. A recent study by Kanazawa (1991) showed that the levels of 22:6 n-3, phospholipids and vitamin A are all of great importance

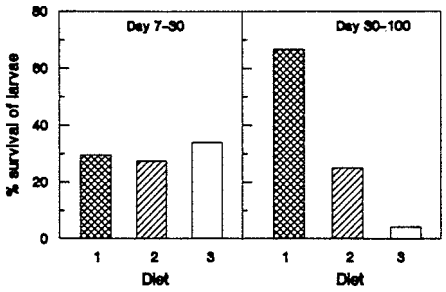

FIGURE 7. *Effect of enrichment period and enrichment source on survival of halibut larvae fed 3 different diets containing rotifers (cultivated on bakers yeast and Super Selco) and* Artemia. *Diet 1: Rotifers enriched with SS for 24 h and* Artemia *enriched with SS for 48 h. Diet 2: Rotifers cultivated on yeast and SS and* Artemia *enriched with SS for 24 h. Diet 3: Rotifers and* Artemia *enriched with squid meal for 24 h.*

for the development of the larval retina and further for the development of pigmentation. The information on phospholipid and vitamin A levels in rotifers and *Artemia* is scarce, and the importance of several factors must be considered in the future improvements of enrichment procedures for cultivated live feed.

First Feeding

Morphological studies on halibut larvae have shown that a functional mouth and intestine are developed at approximately 150 d° after hatching (Blaxter et al. 1983; Pittman et al. 1990). Studies on cod (van der Meeren 1991) and anchovy (Moffat 1981) showed uptake of algae >6–12 μm during the first days of feed intake, suggesting that larvae at that stage behave as active filter feeders.

An experiment was carried out in 3 L glass chambers for investigating if halibut larvae would ingest and assimilate algae when offered at an early stage. The ingestion rate of *Tetraselmis* sp. in halibut larvae increased up to a peak at day 43 (220 d°) (Reitan et al. 1991). The assimilation efficiency of ingested algae was <1% throughout the yolk sac period. The clearance rate of algae was much higher than the drinking rate, indicating an active filter feeding process (Reitan et al. 1991). These results were confirmed in a first feeding experiment where the larvae showed an active intake of algae in the early stage of first feeding. The highest frequency of larvae with algae in their guts was found 200–245 d° after hatching (at mean temperature 4.5 C), and a peak in the rotifer ingestion was observed between 245 and 325 d° (Lein and Holmefjord 1992). Harboe et al. (1990) reported an optimal age for first feeding of halibut larvae at around 230 d° (at 7 C).

The initial experiments with rotifers and *Artemia* as first food for halibut larvae showed similar survival, but lower growth rates as compared to wild zooplankton (Bolla 1989; Holmefjord et al. 1989). The n-3 HUFA of collected plankton was higher than that of rotifers and *Artemia*. The lower n-3 HUFA content in the cultivated food was reflected in the n-3 HUFA content of the larvae fed these organisms (Bolla 1989).

Culture tests with extra-enriched rotifers (Fig. 4) and *Artemia* (Fig. 5) were carried out in 250 L first feeding tanks. Light was provided from above, rotifers were added, and a slight waterflow (300 ml/min) was started before transfer of the larvae. After 14 d of feeding, the light regime was changed to submerged illumination. The larvae were fed rotifers for 3 wk, then both rotifers and *Artemia* for 1 wk, and finally after 4 wk only *Artemia*. Diets 1, 2 and 3 included rotifers and *Artemia* enriched according to treatments 1, 2 and 3, respectively (Figs. 4, 5). Each diet was tested in three replicates.

During the first 7 d after transfer, a high larval mortality was observed in all groups. This early mortality was probably due to transfer stress, larval quality and technical problems with the rearing system. The survival from day 7–30 and from day 30–100 is shown in Fig. 7.

The experiment was terminated after 100 d. Larvae fed rotifers and *Artemia* enriched according to treatment 1 (Figs. 4, 5) showed

the highest survival from day 7–100 (26.9%). The corresponding survival of halibut larvae given enrichment treatments 2 and 3 was 6.7% and 1.7%, respectively. Larvae fed diet 1 displayed both higher growth and survival from day 30 to day 100, but a high frequency of malpigmentation (42%) was observed, whereas the larvae fed the other diets had a normal pigmentation. Both the total lipid content and the proportion of n-3 HUFA was highest in diet 1, and both factors may thus be important for the larval viability.

Both wild zooplankton and cultured rotifers and *Artemia* have been used in first feeding experiments with halibut larvae. Growth obtained in different first feeding experiments is shown in Fig. 8. A complete comparison of the growth rates is not possible due to few weight determinations available for comparable stages, and also temperature differences (approximately 10–12 C for cultured food and 10–16 C for zooplankton). Nevertheless, Fig. 8 shows that cultured food with low levels of n-3 HUFA and no supplement of algae yielded a very poor larval growth. Both n-3 HUFA enrichment and algal addition to the water during first feeding had positive effects on larval growth (Fig. 8). The highest growth rates were obtained with collected zooplankton. Cultivated enriched live feed yielded nearly the same growth, even at lower temperatures.

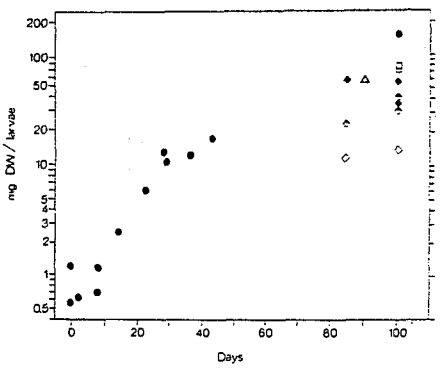

FIGURE 8. *Biomass increase in larvae offered the following feeding regimes: Filled circle: Wild zooplankton, algae. Filled diamond: High n-3 HUFA rotifers and* Artemia, *no algae. Diamond, top filled: Medium n-3 HUFA rotifers and* Artemia, *no algae. Open diamond: Low n-3 HUFA rotifers and* Artemia, *no algae. Open square: Medium n-3 HUFA rotifers and* Artemia, *algae. Open triangle: Rotifers,* Artemia, *zooplankton, algae.*

Discussion and Conclusions

The results of the experiment on photoperiod manipulation showed that the halibut broodstock responded to a shift in photoperiod by a corresponding shift in spawning time. This suggests that halibut eggs could be produced at different times of the year by use of appropriate artificial photoperiods.

Yolk sac larvae are presently produced in small stagnant units or in larger incubators with a slow upstreaming waterflow. The highest survival and the lowest frequencies of abnormal larvae have so far been obtained in small, stagnant systems with low concentrations of antibiotics. The advantages of the smaller and larger systems ought to be combined in the future work, to develop an efficient production system for this stage.

Several feeding experiments on Atlantic halibut have been carried out with collected wild zooplankton as the first feed (Blaxter et al. 1983; Berg and Øiestad 1986; Skjoldal et al. 1990). Relatively high growth rates were obtained in these experiments, but the availability of the appropriate sizes and species of zooplankton is often unpredictable and limited to a restricted season.

Cultivation of the live feed organisms *Brachionus* and *Artemia* provides a stable year-round supply of food. Improved enrichment methods for rotifers and *Artemia* resulted in larval growth of the same magnitude as with wild zooplankton. By combining the photoperiod manipulation of broodstock and the use of enriched *Brachionus* and *Artemia,* halibut fry is possible to produce at any time of the year.

Acknowledgments

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