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# PAPER

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A comparison of growth rate of halibut larvae (<u>Hippoglossus</u> hippoglossus L.) fed wild zooplankton and enriched <u>Artemia</u>.

by

L.H. Skjolddal, T. Harboe, T. Næss, K.E. Naas, H. Rabben

Institute of Marine Reaserch Austevoll Aquaculture Research Station N-5392 Storebø NORWAY

# ABSTRACT

Halibut larvae at an age of 267 day degrees post hatching, were reared through first feeding, outdoors in 100 l plastic bags. There were three feeding regimes: wild zooplankton, <u>Artemia</u> enriched on the algae <u>Isochrysis galbana</u> and <u>Artemia</u> enriched with "Super Selco". The larval growth was very low the first three weeks probably due to low temperature and high larval age at onset of exogenous feeding. At Day 16 the mean myotome height and dry weight were significantly higher for the group fed wild zooplankton, than for the <u>Artemia</u> groups, and the larvae fed Super Selco enriched <u>Artemia</u> had a significant higher myotome height and dry weight than the larvae fed <u>Isochrysis</u> enriched <u>Artemia</u>. There were no significant differences in larval size at Day 23. The low survivals of the two groups fed <u>Artemia</u>, could have been caused by the incomplete digestion of <u>Artemia</u>.

## INTRODUCTION

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Cultivation experiments on halibut (<u>Hippoglossus hippoglossus</u> L.), have for the most part been concentrated on the early lifestages. The experiments have all in some way been related to the overall objective to develop a mass production method for halibut fry (Naas et al. 1987).

Start feeding of halibut has been done with increasing success the last few years. This can be attributed to several factors including improved handling of earlier stages with resulting increase in numbers of viable, functional larvae. In addition techniques and handling procedures are developed to a point where feeding trials can be performed in systems which give the halibut larvae a fair chance to feed and grow. This has made it possible to investigate first feeding/prey preference of halibut larvae both in an ecological as well as in a nutritional perspective.

The present study aims to compare somatic growth and survival of halibut larvae fed enriched <u>Artemia</u> and wild zooplankton.

# MATERIALS AND METHODS

# Eggs and larvae

Eggs were striped from one female and fertilized with sperm from one male of the broodstock at Austevoll Aquaculture Research Station. After 9 days in hatchery the eggs were transfered to 2.5 m<sup>3</sup> silos (Rabben et al. 1987), where they were kept during the yolk sack stage at about 7 °C. At an age of 253 day degrees after hatching, the larvae were transferred to outdoor startfeeding systems, at daytime. The larvae were 267 day degrees when prey organisms were offered.

## The rearing system

The experimental unit were 21 black plastic bags, submerged in an 280  $\text{m}^3$  outdoor tank, 12 m in diameter, were used. The tank was filled with sea water from 50 m depth, to a level of approximately 1 m one week before the transfer of the larvae. The water was stagnant. The plastic bags with a volum of 100 l, were filled with water from the tank immediately before the larvae were transferred.

The larvael groups containing 6 replicates were given different diets. The groups were fed <u>Artemia</u> enriched on <u>Isochrysis galbana</u> (Group 1), Super Selco enriched <u>Artemia</u> (Group 2) or wild zooplankton (Group 3), respectively. A starving group of 3 replicates were included. Each bag was supplied approximate 250. The water in the bags remained stagnant troughout the experiment.

### <u>Live feed</u>

Wild zooplankton, of the size fraction between 200 and 500  $\mu$ m, was collected from a pond by a wheel filter (Unik Filtersystems A/S, Norway) and size fractioned. <u>Artemia</u> (AF cysts from Artemia Systems, Belgium) was hatched from decapsulated cysts as described in Sorgeloos et al. (1986). The <u>Artemia</u> nauplii were enriched and administered to the halibut larvae 24 hours after hatching. Feeding regime was once a day. Zooplankton samples were collected twice a week with a 0.6 l tube sampler. in the middle of each bag. The samples were filtered through a 40  $\mu$ m net and fixed in acid Lugol's solution.

# <u>Larvae</u>

Once a week all the larvae in one bag from each group were sampled and conserved with 4 % formaldehyde for one month before examination of body length, myotome height, dry weight and presence of gut content. The numbers of larvae sampled for growth analyses are given in Table 1.

	moore of allow sumpled and examined in the three feeding groups.					
	Day 8	Day 17	Day 24	Day 30	Day 36	
Group 1	57	31	4			
Group 2	45	30	13	ς.		
Group 3	43	32	30	11	11	

Table 1. Numbers of larvae sampled and examined in the three feeding groups.

# Environmental measurement

Temperature was monitored once a day during the whole experiment. Samples of phytoplankton, chlorophyll a and ammonia were collected, and salinity and oxygen monitored 2 days a week until day 23. Temperature and salinity were measured with a calibrated WTW-salinometer, model L 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 in the middle of the bag with a 2 l Ruttner water sampler. Chlorophyll <u>a</u> were analyzed on a Perkin-Elmer LS-3B Fluorescence Spectrophotometer, and ammonia on а Shimadzu UV-160 spectrophotometer.

#### RESULTS

## Environmental parameters

The temperatures are shown in Figure 1. There was a steady increase of temperature (approximately 0.5 °C/day) from 7 °C at Day 1, interrupted by a lower platau between Day 16 and 20. The temperature increased to a level of approximately 16 °C at Day 22 to the end of the experiment.

The salinity at the bottom of the bag varied between 32.2 and 33.1 % , and at the surface between 33.4 and 15.5 % .

Oxygen levels varied between 12 and 15 ml/l during the experiment (Figure 2), and the ammonia concentration never exceeded 9.0  $\mu$ M (Figure 3). The concentration of chlorophyll <u>a</u> increased during the measured period, but was always below 0.35  $\mu$ g/l (Figure 4).

### Larvai survival

All the larvae in the starving group were dead at Day 12. At Day 23, all the remaining larvae in Group 1 (4 individuals) and Group 2 (13 individuals) were sampled. The survival at this time was about 1 % in Group 1, 3 % in Group 2 and 39 % in Group 3 (Figure 5). At Day 36 the larvae in Group 3 were almost metamorphosed and they were tansferred to an indoor flat-bottomed tank. The survival at this point was 33 %/bag.

## Larval growth

Based on myotome heights and dry weights, the larvae in all Groups had a long lag phase. until Day 23 (Figures 6, 7). After Day 23, the larvae in Group 3 had a substantial growth increase. The myotome heights and dry weights were significantly higher in Group 3 than in Group 2 at Day 8 (p<0.01 and p<0.001 respectively, t-test). Differences between Group 1, sampled at Day 8, and the other groups are excluded in the statistical analyses because the larvae in Group 1 were measured only after 2 days in the fixative. At Day 16 there were significant differences in both myotome heights and dry weights between all the groups (p<0.01, t-test), Group highest and Group 1 lowest. At Day 23 no significant differences were found. The specific growth rates (Houde and Schekter 19981) varied between 2.3 % and 7.6 %

for all the groups until Day 23. The specific growth rate for Group 3 increased to 22.9  $\tilde{c}$  from Day 23 to 30, and was 10.4 % between Day 30 to 36.

Based on this study and Boxaspen et al. (1990), the relationship between larval dry weight/length \* myotome height was fit to a polynomial function:  $1.0516*10^{-03}*X^2$  + 0.038139\*X + 0.249796. This equation gave a better fit (residual sum of squares = 58.31) than the exponential function:  $k1*X^{k2}$  (residual sum of squares = 75.08). The regression curve is shown in Figure 8.

Group 3 (wild zooplankton) had the highest frequency of larvae with food in the gut at Day 8 and 15. At Day 23 all the larvae, both in Group 1 and 3 had eaten (Table 2).

	Day 9	Dog 15	Day 22	Day 30	Dov 36
	Day 8	Day 15	Day 23	Day 30	Day 36
Group 1	53	42	100		
Group 2	69	23	62		
Group 3	70	63	100	91	100
Group 3	70	63	100	91	100

Table 2. Frequency of larvae with food in the gut, at different times.

#### DISCUSSION

The larvae had a low growth both in terms of myotome height and dry weight during the first three weeks after first feeding. Low temperature, 9 °C, causes a lower growth compared to 12 and 15 °C for halibut larvae (Rabben et al. 1990). However the mean dry weight exceeded 2 mg at approximately 8 days. The larvae in this experiment did not exceed 2 mg mean dry weight before after Day 23. Boxaspen et al. (1990) found that the mean dry weight of the halibut larvae had exceeded 2 mg at Day 14 after first feeding. However, Boxaspen et al. (1990) used higher temperatures than in the present experiment.

This experiment was started before the optimum first feeding age for the larvae in these systems was established. Harboe et al. (1990) have later suggested around 230

day degrees as the optimum first feeding age for the larvae. At 250 day degrees or later, unfed larvae use of their tissue bound energy, and therefore need time to build up the tissue again when fed. Periods of starvation result in slower development and increased mortality (Wright and Martin, 1985). The late onset of feeding could therefor explain the observed slow growth.

It seems that the halibut larvae have problems with digestion of <u>Artemia</u>. Boxaspen et al. (1990) never found empty <u>Artemia</u> shells in the larval gut, as they did with wild zooplankton.

Næss et al. (1990) stated that a high content of algae in the water seems to have a positiv effect on the survival and growth of halibut larvae. The algae content in our experiment was low (chlorophyll <u>a</u> lower than 0.35  $\mu$ g/l). This concentration approximates the chlorophyll <u>a</u> level in the "algae free" water in the experiment of Næss et al. (1990).

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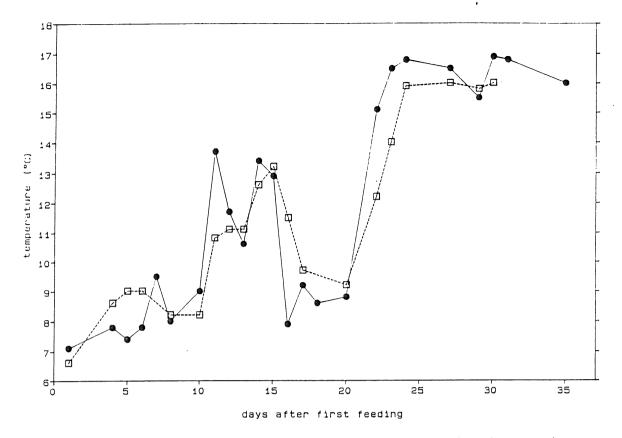


Figure 1. The development of temperature in the bag during the experiment.

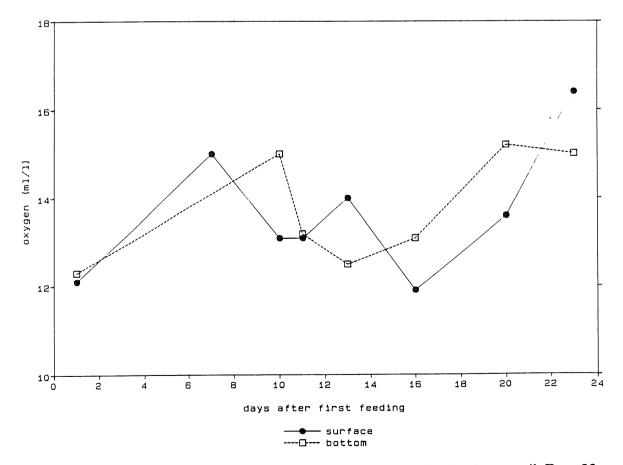


Figure 2. The development of oxygen concentration in the bag until Day 23.

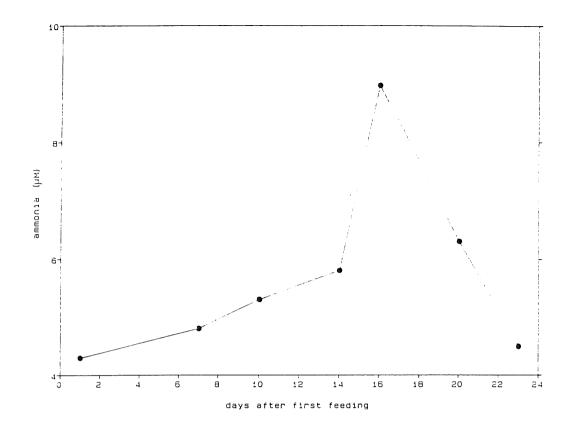


Figure 3. The development of ammonia concentration in the bag until Day 23.

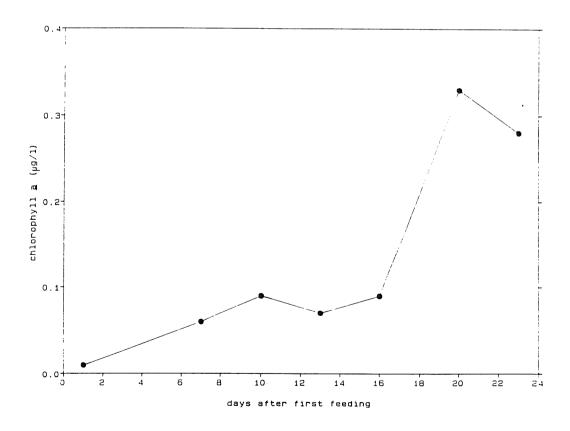


Figure 4. The chlorophyll <u>a</u> concentration in the bag until Day 23.

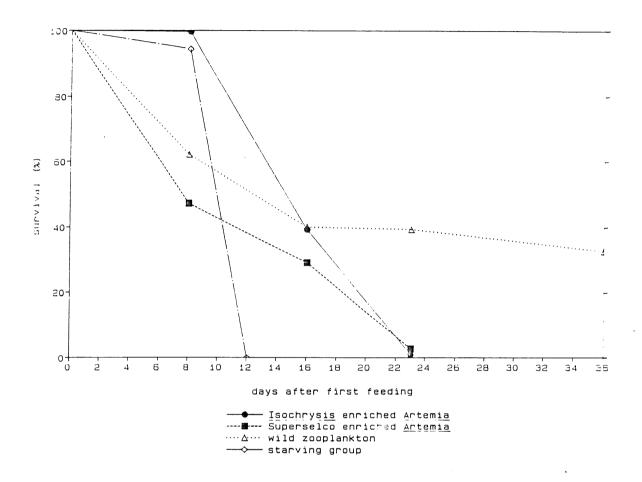


Figure 5. Percentage survival during the experiment.

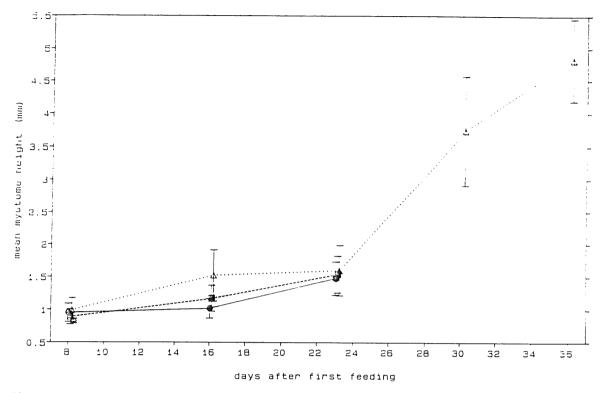


Figure 6. Mean myotome height of the halibut larvae in the experimental groups during the experiment.

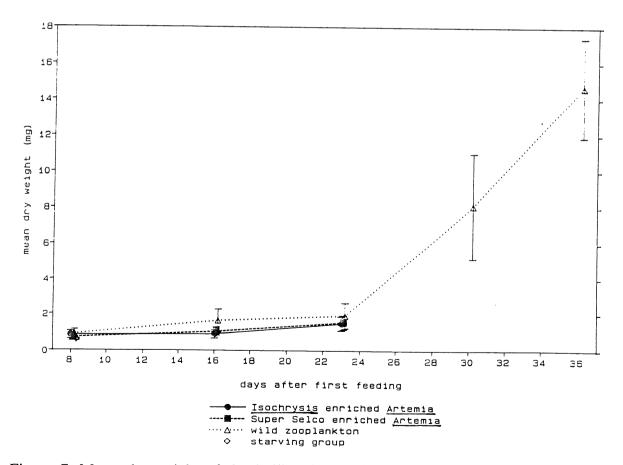


Figure 7. Mean dry weight of the halibut larvae in the experimental groups during the experiment.

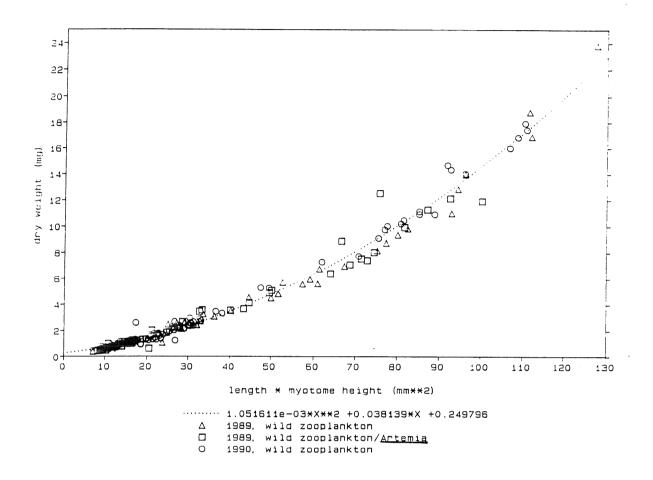


Figure 8. The regression curve between dry weight and length\*myotome height for halibut larvae fed wild zooplankton, from two different experiments.