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PRODUCTION OF JUVENILE FLATFISH SPECIES IN DIFFERENT SIZED MESOCOSMS

by

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ABSTRACT

Mesocosm studies with larvae of halibut (Hippoglossus hippoglossus), sole (Solea solea) and turbot (Scophthalmus maximus) was carried out at Austevoll Aquaculture Station and at Flødevigen Biological Station in 1985. Very low survival was observed for halibut beyond first feeding in plastic bags. Sole larvae had very high survival in plastic bags, but fairly low in basin studies. Turbot had survival below 10% in plastic bag studies, no survival in one of the basin studies and about 10% in another basin study. Altogether 5-10 000 sole and turbot survived beyond metamorphosis.

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INTRODUCTION

Successful production in mesocosms of juveniles of marine organisms has been carried out for a number of species such as oyster (Ostrea edulis), Atlantic cod (Gadus morhua) (Øiestad et al. 1985, Folkvord et al. 1985) and a number of flatfish species (Haugen 1982, Kuhlman et al. 1981, Rollefsen 1946, Øiestad et al. 1976). However, commersial large scale production is only carried out with oyster. An appropriate technology for commersial production of juveniles of Atlantic cod has been developed recently (Folkvord et al. 1985). In Norway a similar program to that on cod has started for a number of flatfish species to evolve a commersial feasible method for large scale production of juvenile flatfish by use of outdoor systems like plastic bags, ponds and basins. In 1985 these studies have included four flatfish species: halibut (Hippoglossus hippoglossus), plaice (Pleuronectes platessa) which will not be included in this report, turbot (Scophthalmus maximus) and sole (Solea solea). The studies have been carried out at Austevoll Aquaculture Station and Flødevigen Biological Station, two institutions belonging to Institute of Marine Research in Bergen.

MATERIALS AND METHODS

HALIBUT

An experiment with halibut larvae was carried out in ten large cylindrical plastic bags with conical bottoms. The bags were filled with seawater of about 30 o/oo. A salinity gradient was established by adding a solution of NaCl. Water depths in the bags were 2.5m in the shallow bags (called Pb.1-Pb.5) with a volume of 7.5 m^3 and 4m in the deep bags (Pb.6-Pb.10) with a volume of 11.5m^3.

Halibut larvae were transferred to the bags 1-2 days after hatching (Table 1). Every second week one of the plastic bags was terminated to examine larval development and survival. After refilling with seawater and establishing the salinity gradient new larvae were transferred to the bag from the same cohort kept in the laboratory. The quantity of larvae transferred was reduced from 1000 to 500 or less at second and third start due to mortality.

Temperature, salinity and oxygen was measured frequently in every 1m depths in the water column and at the bottom. Monitoring of phytoplankton composition, nutrient salts and chlorophyll a in 1m depth was also carried out. Zooplankton was sampled in the whole water column with a tube which isolated a volume of water from the surface to near the bottom of the plastic bag. By filtering the enclosed water through 40 µ mesh size zooplankton was collected for preservation and later examination.

On 11 and 21 April and on 12 May the plastic bags were enriched with nutrient salts (nitrate, phosphate and silicate), principally to increase primary production and thereby preventing oxygen deficiency . In addition bottom water was frequently removed from the bags with a pump to prevent oxygen depletion. The number of dead larvae in the removed bottom water was counted.

From 19 April natural zooplankton was sampled (mesh size 120 µ) and organisms larger than 350 µ (the fraction was gradually increased towards bigger organisms) were added to the bags as potential prey items. Abundance and composition of zooplankton was monitored before and after adding.

After final termination of the experiment (56 days) the larvae which had survived were collected in one of the shallow bags for further monitoring.

TURBOT AND SOLE

During the summer 1985 experiments with turbot and sole were carried out in plastic bags, net cages and two artificial basins filled with seawater from the fjord nearby. Larvae of turbot were either bought from Scotland, obtained by stripping newly caught fish in Denmark or by stripping a broodstock at Flødevigen Biological Station. Larvae of sole were all obtained from natural spawning of a newly established broodstock at Flødevigen Biological Station.

One of the experimental basins, Svartatjønn, was located near the Austevoll Aquaculture Station, and it had a volume of about 23000 m^3 and a maximum depth of 4 m (Øiestad et al. 1984). Surface seawater was pumped into the basin and a propeller moored to a float in the centre ensured homogeneity of watermasses during the experiment.

To increase the primary production and control the oxygen saturation, the basin was fertilized with nitrate, phosphate and silicate at amounts calculated to give initial concentrations of 10, 2 and 10 µM respectively.

To monitor the basin system abiotic and biotic data were sampled and analysed twice a week at the depths of 0, 1, 2 and 3m. These included temperature, salinity, oxygen saturation, pH, nutrient salts, chlorophyll *a*, phytoplankton and zooplankton samples.

The plastic bags were identical to the bags described for halibut and they were floating in the basin as did the net cages. Preparation of a salinity gradient in the bags was performed for all turbot groups, the salinity ranging from 29 o/oo at the surface to 37 o/oo at the bottom. The transfer of different groups of larvae is summerized in Table 2. Zooplankton for feeding fish larvae in plastic bags was collected in the Hyltro-pond from mid-June to mid-July as nauplii of *Centropages* sp. and *Acartia* sp. occurred at densities between 20 to 100/l. From mid-July zooplankton to the bags were obtained by filtering seawater from Svartatjønn in which there had been a sharp increase in density of cladocerans (from mid-July) and nauplii of calanoid copepods (from

late July). The water was mainly left stagnant for the first 3 weeks to ensure the added zooplankton to remain in the bags. Temperature, salinity and oxygen were monitored twice a week followed by tube samples of larvae and zooplankton. The same sampling procedure was followed for the net cages. None experiments from net cages will be reported.

At the Flødevigen Biological Station the experiment was carried out in a 4400 m3 basin with a maximum depth of about 4m (Øiestad et al. 1976). The basin was filled in early June with nutrient-rich water from 75m depth with a salinity of about 34.5 o/oo. From 9 July a steady supply was added (1900 l/h) to the deepest part of the basin to avoid anaerobic conditions. Temperature, salinity and oxygen were monitored once a week. The experiments started 8 June and planned termination is in late September.

Sampling prosedure of zooplankton and fish larvae applied in the Flødevigen basin has earlier been described by Kvenseth and Øiestad (1984).

About 10 000 newly hatched turbot larvae were released in the basin on 8 June (Danish group). Another release was made 11 July with 40 000 larvae (Scotish group). From 31 July to 13 August 13 000 larvae were released in the basin (Flødevigen group). From 20 June to 4 July 67 800 sole larvae were released in the basin and from 18 July to 31 July 41 500 sole larvae were released. A limited number of larvae were gutted and the content divided into categories.

Mature oysters were transferred to a basket in the basin with about 15 days intervals (27 June: 6 ind., 17 July: 5 ind., 31 July: 5 ind.). Once a week a panel of black plastic settlers were introduced into the basin. These 20 x 20 cm squares were situated vertically at the following depths: 0, 0.5, 1.5, and 2.5m.

RESULTS

HALIBUT

From 20 March there was an increase in temperature from 6.5 ^{o} C to 13.5 ^{o} C at the end of the experiment. The steep salinity gradient established near the bottom with an increase in salinity from slighty above 30 o/oo to near or above 40 o/oo, was temporary degraded about 10 April with a marked decrease of salinity in all depths of the plastic bags. At the same time oxygen depletion occured in most of the bags. Repeated addition of NaCl and removing of bottom water with a pump reestablished the salinity gradients and a fairly well oxygenated water was maintained until the end of the experiment. There was a marked increase in concentration of nutrient salts after fertilization followed by an increase in chlorophyll.

Rotifers was numerically the most dominant zooplankton organism during most of the experiment occuring at high densities (maximum 400/l) about one month after start of the experiment (Fig. 1). Appendicularians and tintinnids were also among the main constituents of zooplankton at certain times.

The main constituents of natural zooplankton added from 19 April was copepod nauplii with a steady increase in abundance of copepodites. Maximum values observed were 60 nauplii/litre and 6 copepodites/litre.

Removed bottom water was often loaded with dead larvae during the experiment, but seemingly the larvae experienced an extra high mortality about 10 April when the salinity gradient was disintegrated. Live larvae were caught in bottom water samples as late as 30 April when a larva with grey-greenish stomach content, probably detritus, was observed. Larvae were seldom observed directly in the water column except in P6 where as mush as 50 larvae were observed in the surface layer. The surface-dwelling larvae seemed to be in bad condition and late in the experiment some had air bubbles in the gut.

At first bag termination 77% of the larvae in cohort 1 had survived (Table 1) while for cohort 2 nearly all larvae died. At second termination (day 28) 8% survived from first cohort and 12% from second while none and 0.1% respectivly survived to third termination (day 42). At final termination of repeat-started bags three larvae survived altogether. A considerable increase in length and bodyheight and their red-brownish gut content indicated that these larvae were beyond first feeding. All were transferred alive to the same bag and one of these larvae was regularly observed until metamorphosis took place in early June (about 80 days post-hatching) when the length of the larva was estimated to 25-30 mm. When terminating this bag 27 June, one dead larva being about 20 mm in length, still not metamorphosed, was found while the metamorphosed halibut observed in early June was not found.

A very high fraction of the halibut larvae alive at second bag termination (day 28) was functional (80 and 100%).

TURBOT AND SOLE

The temperature in the basin at Austevoll increased rapidly from 12°C in mid-June to 16 C in the end of June. During the rest of the experiment the temperature fluctuated between 15°C and 20°C.

During a period of heavy rainfall in late July the salinity decreased from the initial 30 o/oo to 28 o/oo. At the same time pH-values decreased to below 7 for almost 2 weeks, probably due to reduced primary production and soil drainage. The oxygen saturation also had its minimum value in the same period, being as low as 76%, but for the rest of the experiment it was above 100%.

In June and July the phytoplankton was dominated by small (<5 µM) flagellates, periodically reaching more than 15 million cells pr. litre. A crack in this population was observed in late July. Later on the phytoplankton biomass mainly consisted of diatomes and coccolithophorids.

Temperature in the bags followed that of the basin. The homogeneity of the basin water ensured almost equal temperatures within all depths. The salinity gradient in the bottom water of the bags was maintained several weeks after start of the experiments. No oxygen depletion was observed in any of the bags.

The main zooplankton organism in the basin in late June and early July was rotifers which was replaced gradually by cladocerans in mid-July, represented by Podon sp. and Evadne nordmanni. During July there was a marked increase in numbers of copepod nauplii (Table 3).

At Flødevigen the temperature in the basin was about 14°C when the first turbot larvae were released 8 June. 19 June it increased to 17°C. The temperature fluctuated between 19-22°C to mid-August. Due to the constant inflow of water near the bottom the temperature from mid-July in this layer was between 13-15°C. Near the bottom the oxygen declined to 1.7 ml/l on 9 July. From that date the constant renewal of water in the deepest part kept the oxygen saturation at a high level also in the bottom water.

Calanoid copepod was the dominating organism group in the Flødevigen basin (Table 5). First feeding organisms for the sole and turbot larvae increased in density to about 50/litre. The decline in nauplii coincide with an increase in density of copepodites (from 1 to 10/litre).

Population estimates and survival

At Austevoll survival of the first turbot group which hatched 6 June was about 7% on day 38 when calculated from day 2 after hatching. At a mean length of 16.2 mm they were transferred to a tank at the station. Weaning of these larvae to an artificial diet was successful, and only 1% died during the next 10 days.

The second group of turbot larvae experienced heavy mortality 10 days after hatching and of the original 115.000 only about 1000 survived (about 1%) to day 40 when these were transferred to the station at a mean length of 15.9 mm.

Survival of sole larvae varied between 1-45% to beyond metamorphosis which took place between day 15-20. The metamorphosed sole spent most of the time at the walls of the plastic bags occasionally performing pelagic swimming. Ultimate survival values of sole and turbot larvae in the basin will be available in October.

During late July about 3 400 juvenile turbot were caught alive in the Flødevigen basin and transferred to the laboratory and given a dry pellet diet. The survival of this cohort was at least 10% to metamorphosis as a quantity probably went to the bottom of the basin.

Distribution of turbot and sole larvae and juveniles

In the plastic bags the turbot larvae remained in deeper parts until day 10-15. Later on they where mainly distributed in the upper 1/2 m where they might form dense schools. A similar behaviour was observed for the sole larvae. After metamorphosis the juveniles still spent most of their time in the upper half meter, the turbot even in the upper cm.Sole might in periods stay deeper in the bags either pelagic or settled onto the wall. They might in periods cover most of the upper 5 cm of the wall close to the surface.

In the Flødevigen basin the vertical distribution of turbot larvae at an age of 4, 6 and 9 days in daylight and 9 days old in the dark is shown in Table 4. In daylight it is clearly shown that there is a trend to move to the upper layer with increasing age. The trend is even more pronounced at night. Apart from the 4 days old larvae rather few larvae appeared to be below 1m depth.

Sole larvae had their main distribution below 1m during daytime sampling in both periods (Table 5). Very few sole larvae were observed in night samples. A change in distribution with age or size can not be considered until they have been further examined.

The juvenile turbot were distributed in the upper 1/2 m from 19 July where they easily could be collected. The sole larvae were not observed after metamorphosis. On the black plastic sheets settling of oyster larvae has been observed.

Diet of sole and turbol larvae

Different stages of calanoid copepods were the dominant diet for sole larvae from first feeding to a size of 5mm (Fig. 2). Larger larvae (5-7mm) preyed on polychaet larvae, oyster larvae and cladocerans. The turbot larvae preyed only on nauplii (standard length of larvae 2.6-7.1 mm ; Fig. 3).

DISCUSSION

HALIBUT

The experiment was ment to give an answer on the rigth larval age for transfer from laboratory tanks to plastic bags. The study was severly reduced due to lack of older larvae (4 weeks and 6 weeks). The experiment was also disturbed by collaps in water quality so the conclution might be rather tentative. However, high survival of newly hatched larvae for two weeks (Pb.1) and 4 weeks (Pb.2 and Pb.7) seems to indicate that this procedure is promising. Besides, those surviving 4 weeks had a very high fraction of functionality, compared to parallel groups in the laboratory. As reported by Blaxter et al. (1983) some few halibut larvae survived to beyond first feeding when functional larvae were transferred to plastic bags. However, the observed survival was far below that observed by Blaxter et al. (1983) who reports 4% beyond metamorphosis.

In future experiments with halibut larvae in mesocosms or enclosed systems one has to be able to control effectively some crucial environmental conditions. The salinity gradient and the oxygen saturation are factors of great importance because of the tendency of halibut larvae to sink until they are neutral boyant with the seawater. In this experiment a lack of salinity gradient together with low oxygen content near bottom in a period, may have caused serious damage to the larvae resulting in severe mortality. In addition to the oxygen depletion in the bottom water, the removal of this water and the adding of NaCl solution may have caused stress to the larvae. Consequently it is of great importance to find a system which provide high oxygen saturation and a reliable salinity gradient, and in cases of oxygen depletion make it possible to remove the bad water without disturbing the larvae.

In spite of high mortality this experiment has shown the possibility of rearing halibut larvae past first feeding in an enclosure system. Improved environmental conditions in the bags will probably have significant effects on the results of such experiments.

TURBOT AND SOLE

Survival

Sole larvae had a high survival rate beyond metamorphosis in plastic bags although the larval density was as high as 1/2 per litre. At higher initial densities the survival rate was reduced to a low level. The survival rates in the basins are still not known, but sampling indicates fairly low survival through the pelagic stage. It might be caused by either low food levels (Flødevigen) or bad water quality (pH < 7 in Austevoll). By keeping the sole to a more advanced stage in plastic bags this problem might be of lesser importance, but this has still not been demonstrated.

Turbot larvae have generally lower survival rate to metamorphosis than sole and this was demonstrated in the plastic bag experiments. However, in the basin at Flødevigen the turbot seems to have higher survival rates than sole. The very low survival of turbot in the basin at Austevoll in July and August, was probably due to either the low pH-value or general unfavourable water quality.

Diet

The sole larvae were able to ingest far larger food organisms than the turbot larvae during first feeding. They had also a far more varied diet as illustrated in Fig. 2 and Fig. 3. Seemingly the sole larvae are able to select food organisms occuring at low densities from the zooplankton (Table 6). These conditions might partly explain why they are more successful in rearing experiment than turbot larvae. The vertical distribution of both sole and turbot larvae in the plastic bags coincide with the observations from the Flødevigen basin. Advanced sole larvae were not sampled from the basin so the upward migration observed in the bags of sole larvae was not verified.

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TABLE 1. Plastic bag experiment with two cohorts of halibut. Number of larvae transferred to each bag and number of larvae alive at bag termination (underlined) has been given. (term: age at termination, restart: age of larvae when transferred from laboratory)

	COHORT 1 a)					COHORT 2 b)				
AGE	Pb.1	Pb.2	Pb.3	Pb.4	Pb.5	Pb.6	Pb.7	Pb.8	Pb.9	Pb.10
0	1000	1000	1000	1000	control	1000	1000	1000	1000	control
14 term.	767					3				
15 restart	1000					1000				
28 term.		78					119			
29 restart		442					95			
42 term.			0					1 c)		
56 term.	0	2 c)		0		1 c)	0		0	

a) start of experiment 20 March, terminated 15 May.

b) start of experiment 28 March, terminated 23 May.

c) transferred alive to a shallow bag, which was terminated 27 June.

TABLE 2. Release of different groups of Turbot and Sole larvae in plastic bags, net cages and the pond.

System:	Volume: (m3)	Larvae group:	Numbers released:	Age from hatching: (days)	Date of release:
Bag 1-4	11.5	Turbot PV-1	8700 x 4	1	7.6
Bag 5	7.5	Sole TU-1	5000	3	8.7
		Turbot PV-3	18800	3	11.8
Bag 6	7.5	Sole TU-3	6900	3	15.7
Bag 7	7.5	Sole TU-2	4100	1	11.7
Bag 8	11.5	Sole TU-5	20000	2	11.7
Bag 9	7.5	Turbot PV-2	25000	3	17.7
Bag 10-11	11.5	Turbot PV-2	25000 x 2	3	17.7
		Turbot PV-3	18800 x 2	3	11.8
Bag 12	11.5	Sole TU-4	19200	2	23.7
		Sole TU-4	17000	0	23.7
Bag 13	11.5	Turbot PV-3	33700	3	11.8
Bag 14	7.5	Sole TU-6	14800	2	29.7
Bag 15	11.5	Turbot PV-4	20800	3	12.8
Bag 16	7.5	Turbot PV-4	20800	3	12.8
Cage 1	8.0	Turbot PV-2	12000	3	17.7
Cage 2	8.0	Turbot PV-4	10400	3	12.8
Basin	23000.0	Sole TU-1	14000	3	8.7
		Sole TU-1	12000	2	9.7
		Sole TU-2	10800	2	11.7
		Sole TU-2	24000	4	15.7
		Sole TU-3	15100	3	15.7
		Sole TU-3	18000	3	17.7
		Sole TU-3	28000	2	15.7
		Turbot PV-2	12000	3	17.7
		Turbot PV-4	86500	3	12.8

TABLE 4. Vertical distribution of turbot larvae 14 - 19 July

AGE in days	4	6	9	9
Depth in m	10 a.m % (n)	10 a.m % (n)	10 a.m % (n)	24 pm % (n)
0,0	- -	- -	8,6 (3)	39,8 (33)
0,5	5,7 (7)	46,1 (41)	20,0 (7)	14,5 (12)
1,0	28,5 (35)	25,8 (23)	45,7 (16)	26,5 (22)
2,0	45,5 (56)	21,3 (19)	22,9 (8)	12,1 (10)
2,5	20,3 (25)	6,7 (6)	2,9 (1)	7,2 (6)

TABLE 3. Zooplankton composition and abundance (numbers pr. litre) in the basin Svartatjønn 2-23 July 1985. Meshsize 40 um pumped at 2 m depth.

	2.	5.	8.	9.	10.	12.	15.	16.	19.	23.
ROTIFERS	32.0	219.6	11.8	23.3	8.9	80.0	91.6	92.4	0.0"	0.0"
NAUPLII	1.6	0.3	0.8	0.4	0.6	0.7	0.7	0.9	2.2"	18.9"
CAL.COPEPODS	0.0	0.0	0.7	0.2	0.3	0.5	0.6	0.5	0.4"	0.6"
CLADOCERANS	0.0	2.7	9.1	15.1	13.1	9.7	31.9	37.3	11.6"	3.7"

" estimated from 1 m depth

TABLE 5. Vertical distribution of sole larvae in the Flødevigen basin early in the two periods of larval release.

	3 - 5 July		19 - 24 July	
Depth (m)	%	(n)	%	(n)
0.0	5,4	(3)	1,5	(1)
0,5	5,4	(3)	1,5	(1)
1,0	19,6	(11)	22,1	(15)
2,0	35,7	(20)	22.1	(15)
2,5	33,9	(19)	52,9	(36)

Table 6

Zooplankton organisms in the Flødevigen expressed as number/ litre and %.

Date:	JUNE 25		JULY 3		JULY 9		JULY 16	
	n/l	%	n/l	%	n/l	%	n/l	%
Copepoda								
nauplii	13,86	90,5	3,77	62,7	2,33	16,7	48,0	83,4
juvenils	1,13	7,4	1,53	25,6	9,70	69,6	4,73	8,2
adults	,05	,3	,10	1,7	,90	6,5	1,83	3,2
Cladocers	,18	1,2	,13	2,2	-	-	-	-
Gastropods	,06	,4	,43	7,2	,73	5,3	,07	,10
Bivalves	,04	,2	-	-	,27	1,9	-	-
Polychaets	,01	,0	,03	,6	-	-	2,93	5,10

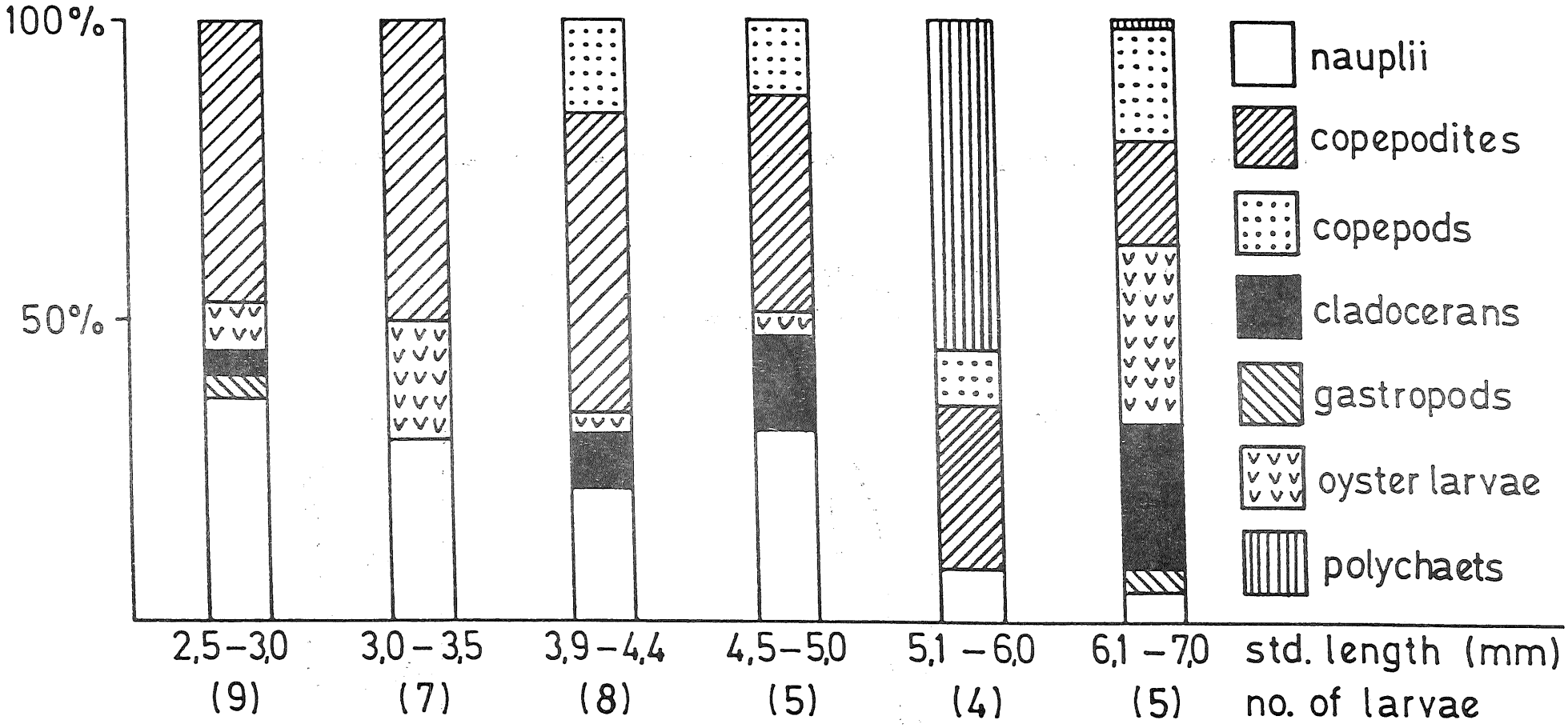

FIGURE 2. Composition of gut content of sole expressed as percent in the Flødevigen basin.

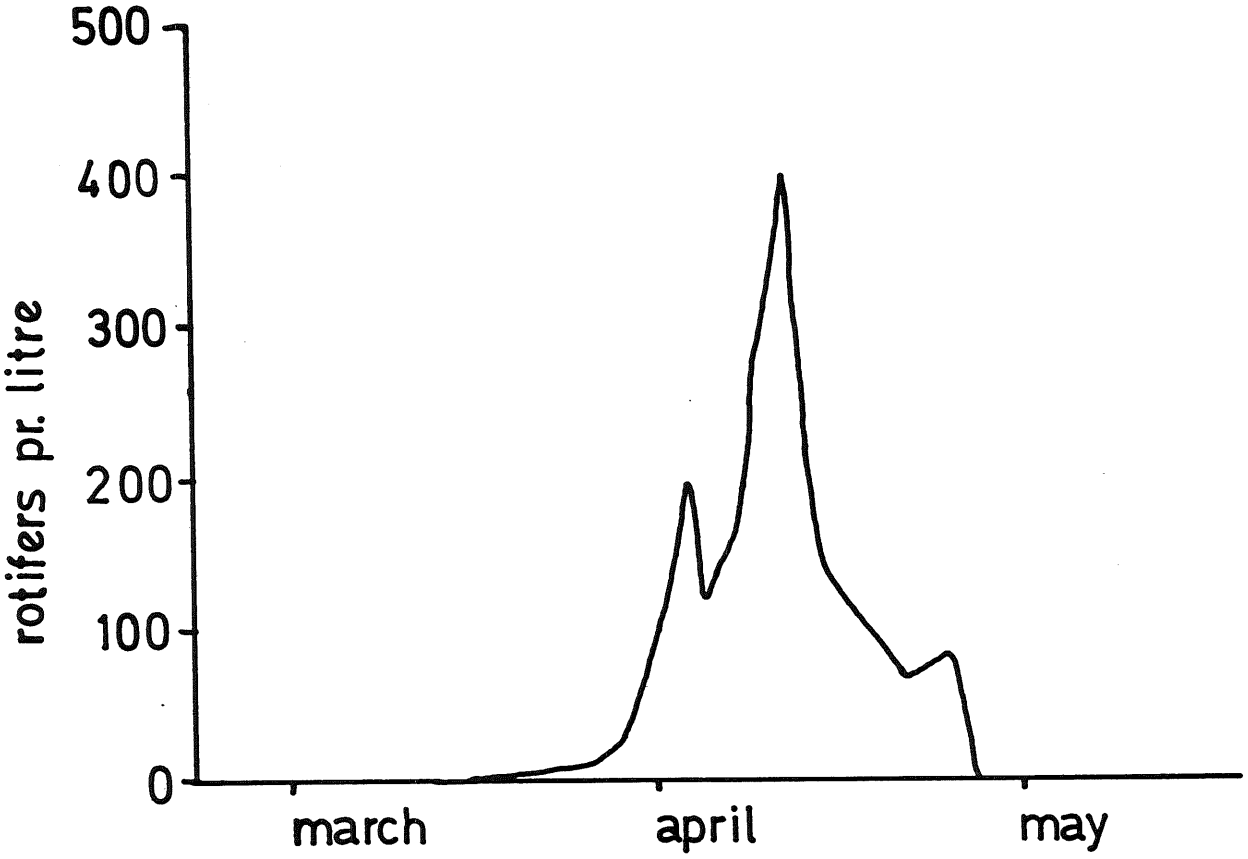

FIGURE 1. The abundance of rotifers in bag 2 during the experiment.

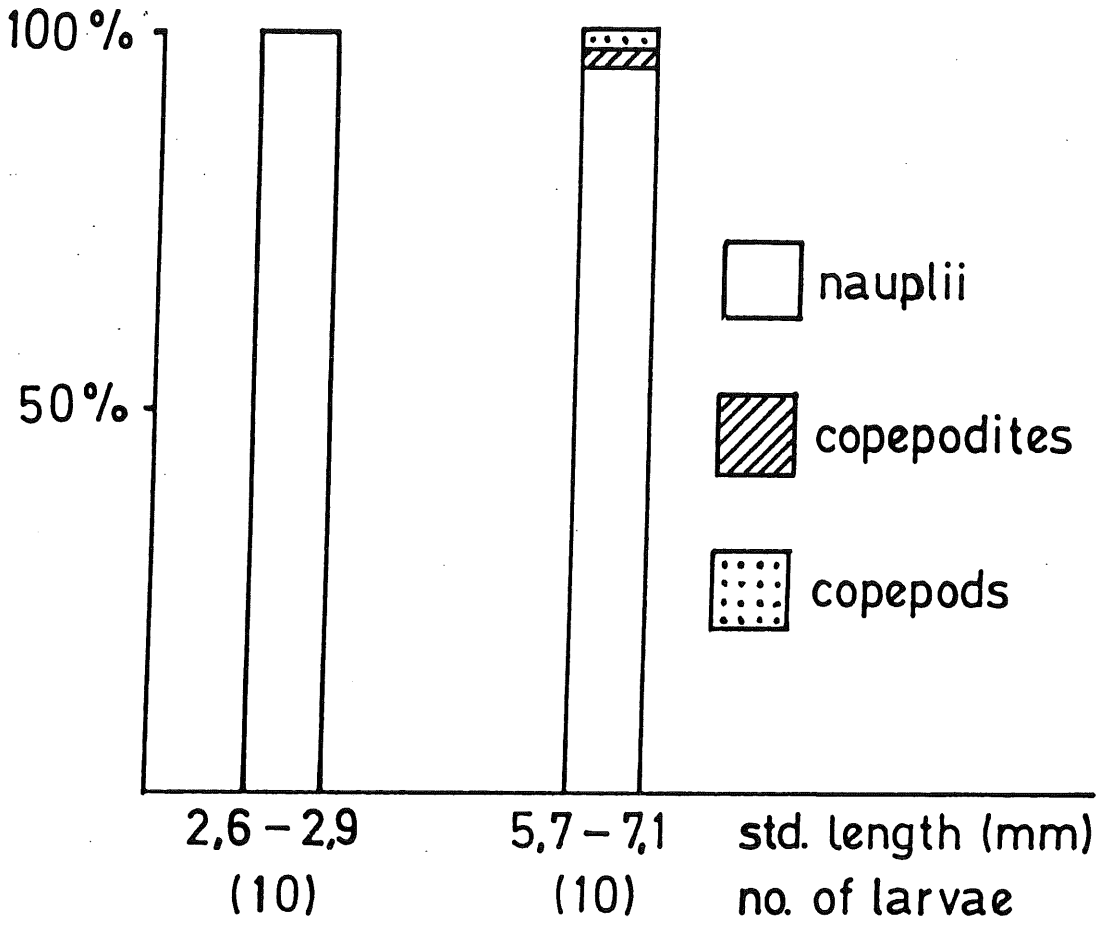

FIGURE 3. Composition of gut content of turbot larvae expressed as percent in the Flødevigen basin.