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GROWTH AND SURVIVAL STUDIES OF HALIBUT (HIPPOGLOSSUS HIPPOGLOSSUS L.) FROM HATCHING TO BEYOND METAMORPHOSIS CARRIED OUT IN MESOCOSMS.

by

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ABSTRACT

To each of five floating plastic bags 1500 halibut larvae were transferred from the hatchery one day after hatching. The plastic bags had a volume of 11.5 m^3, a total depth of 5.5 m and they were moored to floating collars. The bags were made of polyethylene coated with black PVC. Each bag was covered with a roof so the larvae were in a totally dark water column the first month. The general salinity was that of Atlantic water. Supplied water had a salinity of 30-32^o/oo. The halibut larvae reached the functional stage 40 days after hatching and natural zooplankton was added. At this time opening gradually was made in the roof of three of the bags while in two bags light was supplied by underwater lamps. Survival to the first feeding stage was about 45%. Almost all functional larvae started to feed, and they reached a size of 20 mm at metamorphosis and bag termination 45 days after first feeding was observed. The dry weight had increased from 600 to 3500 ug in the period of active feeding. The mean overall survival was 3.3%.

INTRODUCTION

The first described hatching experiment with halibut was carried out by Rollefsen (1935) at Trondheim Biological Station, Norway. He sicceded to keep the larvae alive for 10 days.

In 1972 successfull hatching was carried out with the Pacific halibut (Forrester and Alderdice 1973) and they kept the larvae for 6 days. In both experiments eggs were obtained from halibut in captivity.

The development of Pacific halibut larvae in the open sea has been described (Thompsen and van Cleve 1936), but from Atlantic halibut collected material has been fragmented although a large number of eggs have been collected the last four years in Northern Norway (Haug et al. 1984).

In Norway hatching and rearing experiments were initiated in 1974 (Solemdal et al. 1974) and they have continued ever since although reports have been rather few. Until 1982 all eggs were obtained from females captured with gill nets at the spawning grounds. In 1980 two larvae survived to a size of 30 mm in a mesocosm experiment (Blaxter et al. 1983).

A brood stock was established in the Bergen area during 1982/83 and the first eggs were stripped from them in February 1983. Since than two other brood stocks have been established in Norway and a coordinated halibut research program launched in Norway with five participating institutes. This report describes the first successfull large scale rearing of juvenile halibut within this program.

MATERIALS AND METHODES

The experiment was carried out in the Hyltropond at the Aquaculture Station Austevoll. The eggs were stripped from a 43 kg large female 2 March and fertilized by sperm from one 23 kg large male. The eggs were incubated in an incubator (Rabben et al. 1986) for 14 days at a mean temperature of 6 ^{o}C. One day after hatching the larvae were transferred in black 30 l plastic bags to the pond and placed in large floating plastic bags the day after.

In each of five bags about 1500 larvae were supplied. The bags had a volume of 11.5 m^3, a diameter of 2 m, a sylindrical part of 4m and a conical part of 1.5 m (total water column about 4.5 m). A bottom layer of sea water with increased salinity (38 o/oo) was supplied to the bags by a tube connected to the bottom (Fig. 1). Bottom water could be drained through the same tube. The bags were filled with sea water filtered through a sand filter. Salt (NaCl) was added to increase the overall salinity to about 35 o/oo. At the top there was a layer with salinity about 25 o/oo.

There was no exchange of water during most of yolk sac stage. Then there was an exchange of about 5%/day increasing to about 25%/day at the end of the experiment. Water was removed by an overflow system which had a sieve (mesh size 200 um) at the transition from sylindrical to the conical part of the bag. The bottom water was exchanged every week except the first two weeks. The filtrated detrites was put on formaline (4%) for later inspection. Both dead and live larvae in the bottom water were preserved. Oxygen and also salinity and temperature were measured in every meter of water column before exchanging the bottom water.

Samples were taken for rough estimation of zooplankton supplied. In addition bags zooplankton sampling was carried out by a tube sampling a 3.5 m water column (8 litre). Occasionally halibut larvae were in the tube sampled water and were preserved with the filtrate of zooplankton (mesh size 40 um). Larvae other than bottom larvae, called water column larvae, were still sampled mostly from the upper layer of the water column.

The plastic bags were made of polyethylene and were coated with a black film of PVC. The bags were moored to floating collar and were covered with a roof in which there was an inspection triangel. This could be closed by a special type of burseal.

When the larvae reached the time near functional stage (day 32) the triangel was opened permanently in three bags. In the other two light was supplied by immersing an underwater lamp giving a conic light beam in the bag. The bags with opening had about one quarter of the roof removed at the time of zooplankton adding (day 40). A small triangel opening was made in the lamp bags

on day 58. On day 66 the roof was completely removed from all bags except one, which were about half covered rest of the time.

At termination (day 88 for three bags, day 96 for two) all surviving juveniles were transferred alive to laboratory tanks.

During the experiment some water column larvae were sampled (mostly from near the surface) for gut content inspection. In addition a large number of live larvae were preserved in the filtrate from the bottom water. All larvae were examined for stage of development, occurance of deformations and gut content, and were counted together with dead larvae. Further the live larval standard length and myotome hight was measured and the dry weight determined after 24 h at 60 ^{o}C.

A study of the neuromast organ went on parallell to this experiment and has been described by Økland et al. (1986).

RESULTS

Hydrografi

The temperature increased from 3 ^{o}C at day 1 (one day afterhatching) (Fig. 2) to near 9 ^{o}C at day 30 (15 April) followed by a rapid increase in temperature and in early May it was about 13 ^{o}C in the whole water column (day 55). Then there was a variable temperature in the rest of the experiment with a rapid temperature increase before termination of the last two bags (after day 90). The salinity was over 35 o/oo the first two weeks declining to about 32.5 o/oo. From day 70 the salinity had declined to below 30 o/oo, except in the bottom water, as a result of the adding of more bracish water.

The oxygen saturation was between 75 and 90% in the water column except in the high salinity bottomlayer (below 4 m depth) where it varied from 30 to 80%. After light was introduced the saturation was about 90%, but it did not increase substansially as a result of phytoplankton blooms.

Larval survival

The large volume and low density of larvae made sampling difficult and only the initial number (about 1500) and the number of surviving halibut (a mean of about 50) is known, which is a mean overall survival of 3.3%. Only a small fraction of the dead larvae was drained during water exchange. Assuming however that the number of dead larvae detected in the bottom water reflected the general mortality pattern, the main mortality took place in two periods (fig. 3), from about day 20 to day 35, and after time of first feeding from day 50 to day 60. The indicated survival of 45% at time of first feeding is much the same as the survival at that time estimated by visual observation. After day 60 very few dead (and live) larvae were found in bottom water. At this time the relative curve shows an overall survival of about 3% which is near the true estimate of 3.3%

As seen by inspection the larvae reached the functional stage between an age of 37-43 days. Few larvae with deformations were detected. The main distribution was difficult to observe, but a deaper distribution with time was observed the first 20 days. At day 30 larvae again were observed nearer the surface and the more shallow distribution was maintained until larvae reached the functional stage. Later on the larvae at least parts of the day were observed near the surface special when zooplankton was supplied. At termination nearly all the larvae at some times were observable in the water column.

First feeding was observed by gut inspection on day 43, but might have started somewhat earlier. Metamorphosis took place gradually, and in late May (about day 75) most of the larvae observed were in an early phase of metamorphosis. At termination (about day 90) on transferance to tanks in laboratory, most of them could dwell at the bottom (90%) and only few were still typical larvae.

Larval food

Before introduction of zooplankton the zooplankton density was very low as nearly no organisms were found in tube samples from one of the bag. The food supply samples are not yet beeing counted, except for samples at time of first feeding (table 1). After

that time the total density of zooplankton was increased to near 21 organisms pr. litre with 70% Calanus (mostly copepodites 1-3) and other calanoid copepods (mainly Pseudocalanus and Temora (day 51 in table 1). At that time the number of copepod nauplii was higher in Pb3 compared to a bag without larvae (PbZ). In a period in PbZ there was a steady increase towards bigger organisms of Calanus and number of cal. copepods while the number of nauplii at the same time decrease. The nauplii was small organisms always less than 400 um never including bigger Calanus nauplii. Copepod eggs were found in fairly high quantities in PbZ while there were few eggs in Pb3 decreasing to 0 org./litre at day 73. The results indicate high densities of Halosphaera minor at time of first feeding until day 51 at least. At day 51 the number of organisms were much the same or higher in Pb3 compared to PbZ, with a great reduction in total number of cal. copepods at day 66 and later on and of Calanus at day 73 and 82. In Pb3 there was a marked decrease in total number of org./litre from 20.9 at day 51 to 7.7 at day 82, for cal. copepods and Calanus the reduction was from 15 to 1.7 and 7.8 to 0.4 org./litre respectivily. A brief check of food supply samples revealed that Calanus was supplied to the bags during the whole experiment in accordance with the concentration in PbZ (table 1), together with small but constant amounts of cladocerans althogh not found in PbZ.

Gut inspections of halibut larvae revealed that they included a number of organism groups in their diet from first feeding and onward (table 2, figure 4). Organisms less than 400 um were still never found and gut content include cop. nauplii only once. Calanus copepodites 5 never was found until day 68 (23 May) in spite of fairly high concentration in Pb3 the time before that. Mostly organisms with length of 400-1600 um was preyed upon including clagocerans, except in the period with high densities of H minor. Among the group 2 (400-800 um) and group 3 (800-1600 um) organisms cal. copepodites 1-3, cal. cop. 4-5 and Calanus cop. 1-2 most heavily were preyed upon.

In the two last period the gut inspection only was on bottom larvae (se figure).

Larval growth

At day 6 the total mean dry weight was near 1000 ug with the yolk sac making up 85% of the weight (table 3, figure 5). At day 37 total mean dry weight was about 580 ug (yolk sac 43%). At time when first feeding was observed (day 43) there was varying degree of yolk sac resorption. The mean dry weight of larvae without yolk sac was 710 ug (table 3). The result indicate an increase in larval dry weight from day 47-51 followed by a seemingly stagnant period of growth (day 51-59). From that on dry weight increased rapidly to about 3500 ug at day 81 and 13.5 mg at day 100 a few days after termination and transferance to the laboratorium.

Specific growth rate from day 6 to time of first feeding (day 44, larvae with yolk sac) was 3.7%. The specific growth rate was 4.5% and 5.3% from day 44-81 and day 44-100 respectively. From day 81-100 the specific growth rate was 6.9%.

Larval mean standard length increased from 7.5 mm at day 6 to 12.5 mm at first feeding. On day 81 they had a length of 16 mm increasing to about 20 mm at day 100. The corresponding daily length increment in the periods was 0.13, 0.10 and 0.21 mm respectively. In one bag with lamp the measured mean standard length at termination was about 21 mm compared to about 18 mm in a bag with only natural light, indicating a possible difference with regard to light regimes.

Myotome width was 0.45, 0.86 ($\pm$0.07) and about 2.5 mm on day 6, 44 and 81 respectively.

DISCUSSION

The halibut larvae has in many respect a deviating development from other flatfish in the North Atlantic: They are usually spawned at large depths, even down to 1200 meter in Norwegian fjords:, they have a high neutral bouancy both as eggs and also as yolk sac larvae, at least for a period:, they have a very long resorption time for the yolk sac (over 40 days for full resorption):, and they need 30-40 days to develope their organ systems for first feeding. They have a very well developed

neuromast organ system with long protruding cupulae (Økland et al. 1986). The rather large egg (3.2 mm) and first feeding larvae (12 mm) is also unusual. The special biology adress special precaution to be taken. To establish a rearing system that takes care of for all the demand, seems to be a must as very few larvae have been brought through to do comented first feeding although a number of conditions have been tested since 1974 (Solemdal et al. 1974).

The first successfull rearing was carried out in 1980 when two larvae reached a size of about 30 mm (Blaxter et al. 1983). That experiment was carried out in black plastic bags with a volume of 2 m^3, conical bottom, increased overall salinity and with increased bottom salinity. An inoculum of zooplankton supplied the larvae with food. This years experiment was a further elaboration of that study and methodical a further elaboration of the study last year when feeding and growth of three larvae was observed. The black plastic bag is assumed to give the larvae an illusion of being at large depth and the large water volume will reduce the frequency of contact with the bag wall and with other larvae. As the sea water was filtered, the number of other organisms was very low during the yolk sac stage. As a result the neuromast organ was intact at first feeding while in small rearing system they will easily be destroyed (Økland et al. 1986).

A black wall in rearing experiments has been considered an advantage since Blaxter carried out his experiment on herring (Blaxter 1968) and recent experiment has supported this. One of the advantage for the larvae seems to be improved feeding success due to better contrast of the prey organism. In addition the halibut larvae had a pelagic distribution in contrast to in light-colered bags which were tested in 1985 (Berg et al. 1985). Altogether black plastic bags gave far higher survival than in parallell experiment in light-colered bags (Rabben et al 1986).

In most halibut experiments a large fraction of larvae have different types of deformation. Although the reason for these are not fully understood, the rearing conditions during the yolk sac stage might cause a number of them. The scarcety of deformations in this experiment should indicate that the egg group

had a high quality and also that the rearing conditions in the bags during yolk resorbtion was appropriate. The neutral boyancy was not measured, but from earlier observations we know that it change during larval development. The vertical distribution variance in this experiment may well have been caused by a change in neutral bouancy although we do not know the effect of decreased salinity after two weeks. Larvae floating in the surface layer in a salinity of 25 o/oo has earlier been observed (Senstad 1984). Seemingly the change in neutral boyancy in this experiment was within a rather narrow range of 30-35 o/oo as nearly no larvae were observed at the surface during time of yolk resorbtion.

During most of yolk sac resorbtion time yolk sac dry weight was higher compared to Blaxter et al. (1983), which are probably due to slower development rate at the low temperature in March.

The increased mortality observed by the increased number of dead larvae in bottom water on day 50, might either be due to high temperature at that time or due to larvae beyond point of no return which eventually died. The apparently stagnant growth at the same period (day 51-59) and fairly low daily length increment from day 44-88 indicate PNR-larvae. Furthermore little differences in food concentration in PbZ and Pb3 at day 51, and highest concentration of cal. copepods in Pb3, indicate low grazing at that time, in spite of still 40% larval survival. The reason for this has to be studied in more detail, with special regard to suitable prey size and concentration and time of introduction.

Although table 1 shows little about size of larval diet (mostly because of a natural zooplankton succession in PbZ), it indicates the ability of at least elder larvae of grazing down zooplankton to very low level in spite of fairly low concentration of suitable prey items from day 66. This also include bigger organisms (as mentioned there was still addition of fairly high amounts of Calanus during the whole experiment). This is important because figure 3 indicates grazing mostly on smaller organisms (less than 1600 um), which probably is an effect of bottom larvae gut inspection.

The seemingly lack of cladocerans in PbZ in spite of continous

supply, which agree with larval gut content, is somewhat mysterious but may well be an effect of the tube sampling methode knowing that cladocerans usually have a shallow distribution. In addition it is usual observing cladocerans floating at the water surface as an effect of being filtrated through a filter with a certain mesh size. That cladocerans mostly was found in water column larvae sampled from near the water is a further statement of this. The result was probably a rapid grazing down of this organisms after zooplankton adding.

This in turn agree with the observance of larval ability to detect addition of zooplankton, which should indicate that they had brought the density of organisms to such a low level that they would go actively for the added watermass which they after a few weeks learning "knew" contained high densities of prey organisms. This conditioned behaviour should be studied in more detail under other circumstances.

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Table 1. Food supply as number/litre on day 44 and 47, organisms (number/litre) found in plastic bag 3 and in one bag without larvae at various larval ages.(PbZ). Eggs of copepods were not counted.

	LARVAL AGE IN DAYS									
	44	47	51		66		73		82	
	Food supply		PbZ	Pb3	PbZ	Pb3	PbZ	Pb3	PbZ	Pb3
Calanus sp. cop. 1	1.0	0.1	0.6	0.1	0.0	0.0	0.0	0.0	0.0	0.0
cop. 2	1.0	0.1	2.4	2.0	0.0	0.0	0.0	0.0	0.0	0.0
cop. 3	0.3	0.0	3.5	2.9	0.5	0.5	0.1	0.0	0.0	0.0
cop. 4	0.0	0.0	0.4	2.0	3.5	3.5	2.8	0.3	1.8	0.0
cop. 5	0.0	0.0	0.5	0.8	5.6	0.9	5.9	0.0	5.4	0.4
adults	0.0	0.0	0.0	0.0	1.3	0.0	0.3	0.0	0.3	0.0
Cal. cop. 1-3	0.3	0.2	1.5	2.1	3.4	0.9	6.4	0.4	8.5	0.6
4-5	0.4	0.3	0.9	3.4	1.0	0.3	0.3	0.0	3.0	0.1
adults	0.1	0.1	0.8	1.4	2.4	0.1	2.4	0.0	1.8	0.3
Acartia juv.	0.2	0.1	0.3	0.3	0.9	0.8	0.5	0.4	0.5	0.3
adults	0.1	0.0	0.0	0.0	0.0	0.4	0.5	0.1	0.5	0.0
Paracalanus sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Copepod nauplii	0.5	0.2	2.9	1.4	24.4	2.6	18.6	3.4	5.5	3.6
Cyclopoids	0.1	0.1	0.1	0.4	0.3	0.5	0.4	1.4	0.3	0.5
Euphausiid larvae calyptopis	0.7	0.0	0.4	0.6	0.1	0.0	0.0	0.0	0.0	0.0
Cladocerans	2.3	0.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Cypris	1.6	0.0	1.3	0.4	0.1	0.8	0.3	0.0	0.1	0.3
Bivalves	3.2	0.0	1.4	0.3	2.5	5.9	4.0	4.1	1.0	0.3
Gastropods	0.4	0.0	0.4	0.0	0.1	0.3	0.4	0.3	1.3	0.9
Polychaets	0.2	0.0	0.4	0.1	0.0	0.0	0.0	0.0	0.0	0.4
Rotifers	2.6	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Halosphaera minor	3.1	0.3	0.9	2.6	0.0	0.0	0.1	0.0	0.0	0.0
Copepod eggs	*	*	**	*	**	*	**	0.0	**	0.0

* rather few eggs were found

** rather many eggs were found

Table:2: Number of organisms of various size groups found by gut inspection. w.c are water column larvae, b are larvae found near or at bottom.

DAYS:	44-47		50-54		59-68	72-81
	w.c	b	w.c	b	b	b
GROUP 1, <400um:						
Copepod nauplii	0	0	0	0	0	1
Bivalves	3	0	1	3	2	0
Gastropods	1	0	1	0	0	0
H. minor	1	0	58	3	0	0
GROUP 2,400-800um:						
Cal. cop. 1-3	5	0	1	5	0	8
Acartia juv.	0	0	0	0	1	0
Cyclopoids						
Cladocerans	5	8	16	0	10	15
Calyptopis	2	0	0	1	0	0
Harpacticids	0	0	2	3	2	4
GROUP 3,800-1600um:						
Calanus cop.1-2	17	1	2	1	0	0
Copepods undef.	0	0	7	1	3	2
Cal. cop.4-5 and ad.	0	0	4	13	16	4
Cypris	0	0	2	0	0	1
GROUP 4, 1600-2400um:						
Calanus cop.3-4	1	0	0	1	5	0
GROUP 5, >2400um:						
Calanus cop.5 and ad.	0	0	0	0	2	3

Table 3. Larval and yolk sac mean dry weight with 95% confidence limits (p=0.05, student's t). b are larvae preserved from bottom water, w.c are larvae preserved from water column. In Pb1 there were 5 yolk sac larvae of 9 inspected on day 44.

AGE	Larval Yolk sac d.w.	n	Plastic bag number	Type of larvae
6	0.1431 ±0.0076 0.8436 ±0.0184	10		Control group
18	0.2220 ±0.0151 0.7539 ±0.0285	8	Pb1	b
25	0.2832 ±0.0077 0.6444 ±0.0194	10	Pb3	b
31	0.3778 ±0.0126 0.5899 ±0.0293	10	Pb3	b
31	0.3381 ±0.0243 0.5746 ±0.0240	8	Pb2/3/4	w.c
37	0.5051 ±0.0228 0.3760 ±0.0191	10	Pb5	b
44	0.5918 ±0.0831 0.3582 ±0.0370	9 5	Pb1	b
44	0.7107 ±0.1006	3	Pb4	w.c
47	0.6153 ±0.0581	4	Pb1	W.c
51	0.8987 ±0.0602	28	Pb4/5	w.c
54	0.8735 ±0.1100	6	Pb4	b
59	0.9310 ±0.6595	4	Pb2/4	b
68	1.5110 ±0.3721	4	Pb2/3/4	b
72	2.3345 ±0.7046	3	Pb2	w.c
81	3.6210	1	P2	w.c
100	13.4627 ±8.1519	3		lab.

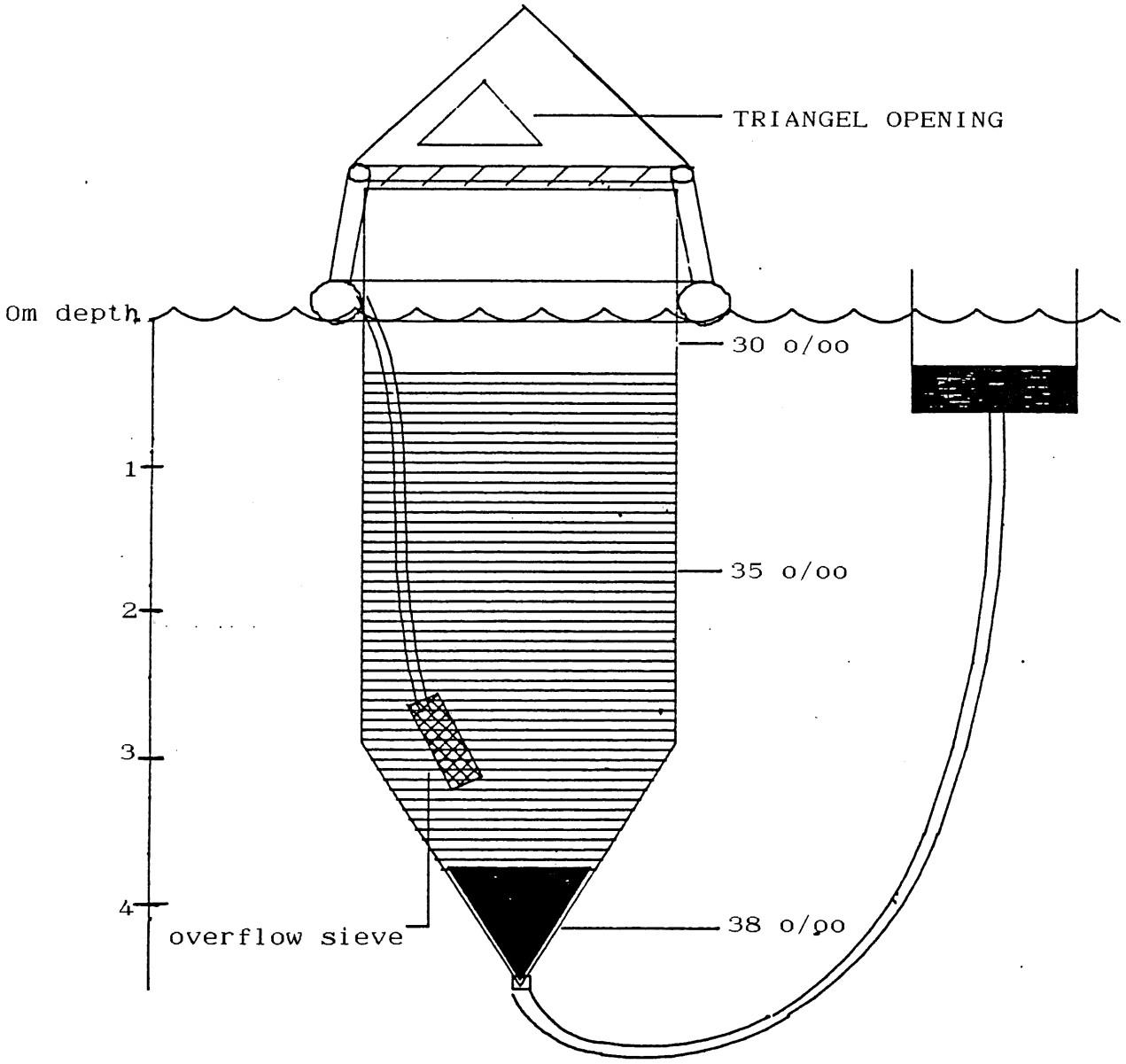

Figure 1. Plastic bag system for rearing of halibut fry.

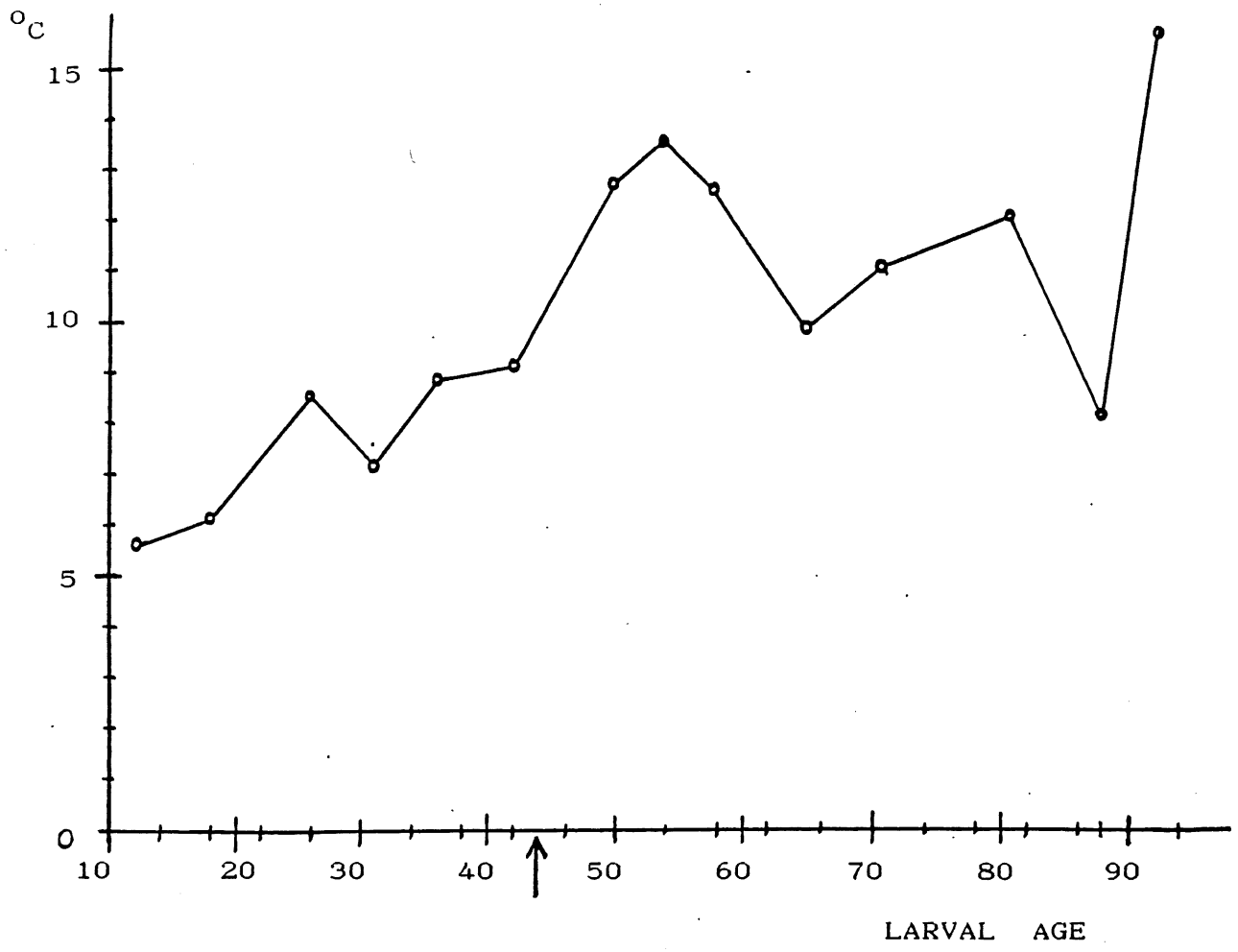

Figure 2. Temperature in the bags (measured in Pb3) during the experiment at 2m depth. In the first 10 days of the experiment there was a steady increase in temperature from 3 to 5 °C (as measured in the pond).
Arrow indicate when gut content first time was found.

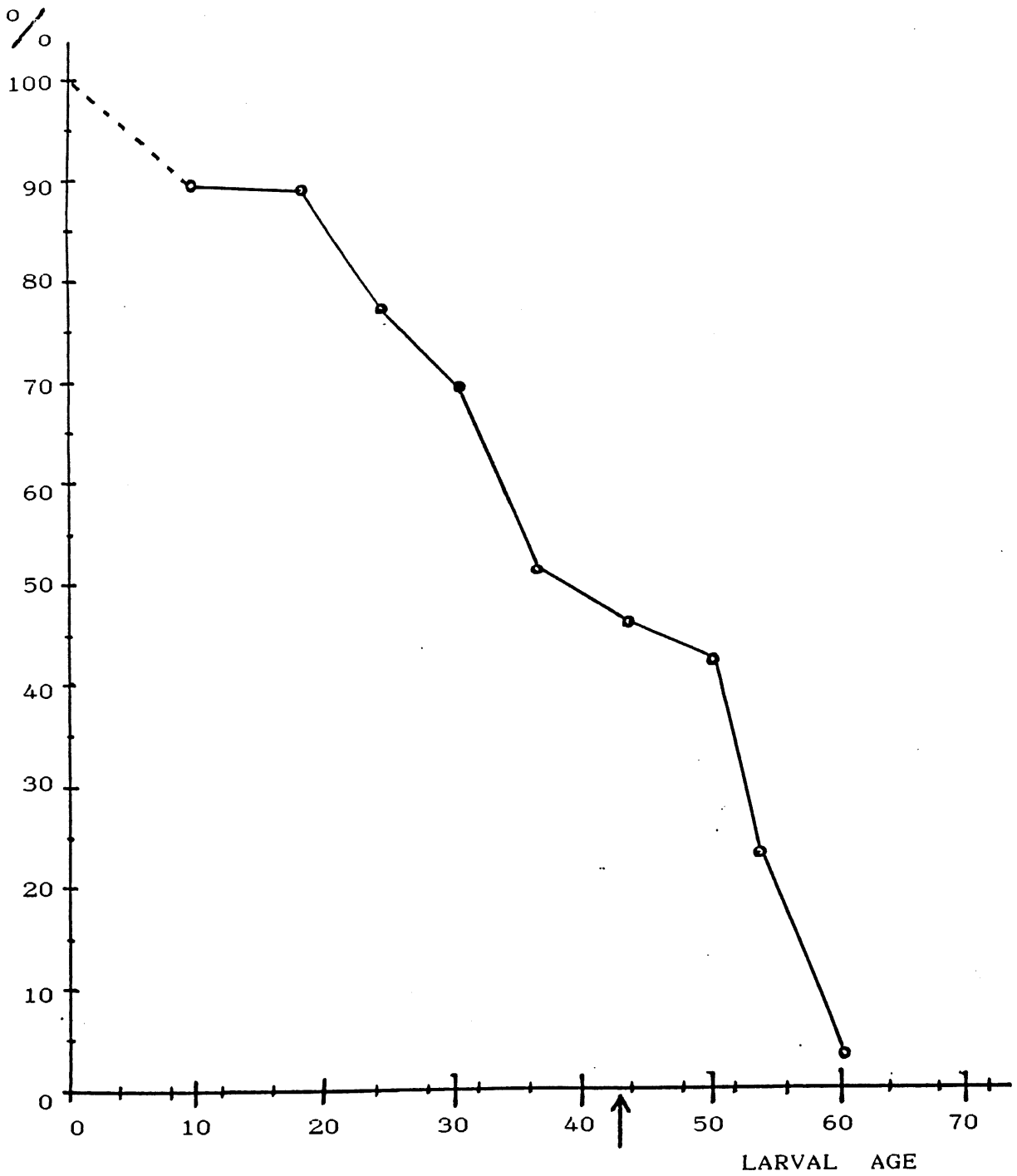

Figure 3. Relative survival of larvae, based on number of dead larvae found in bottom water. Arrow indicate when gut content first time was found.

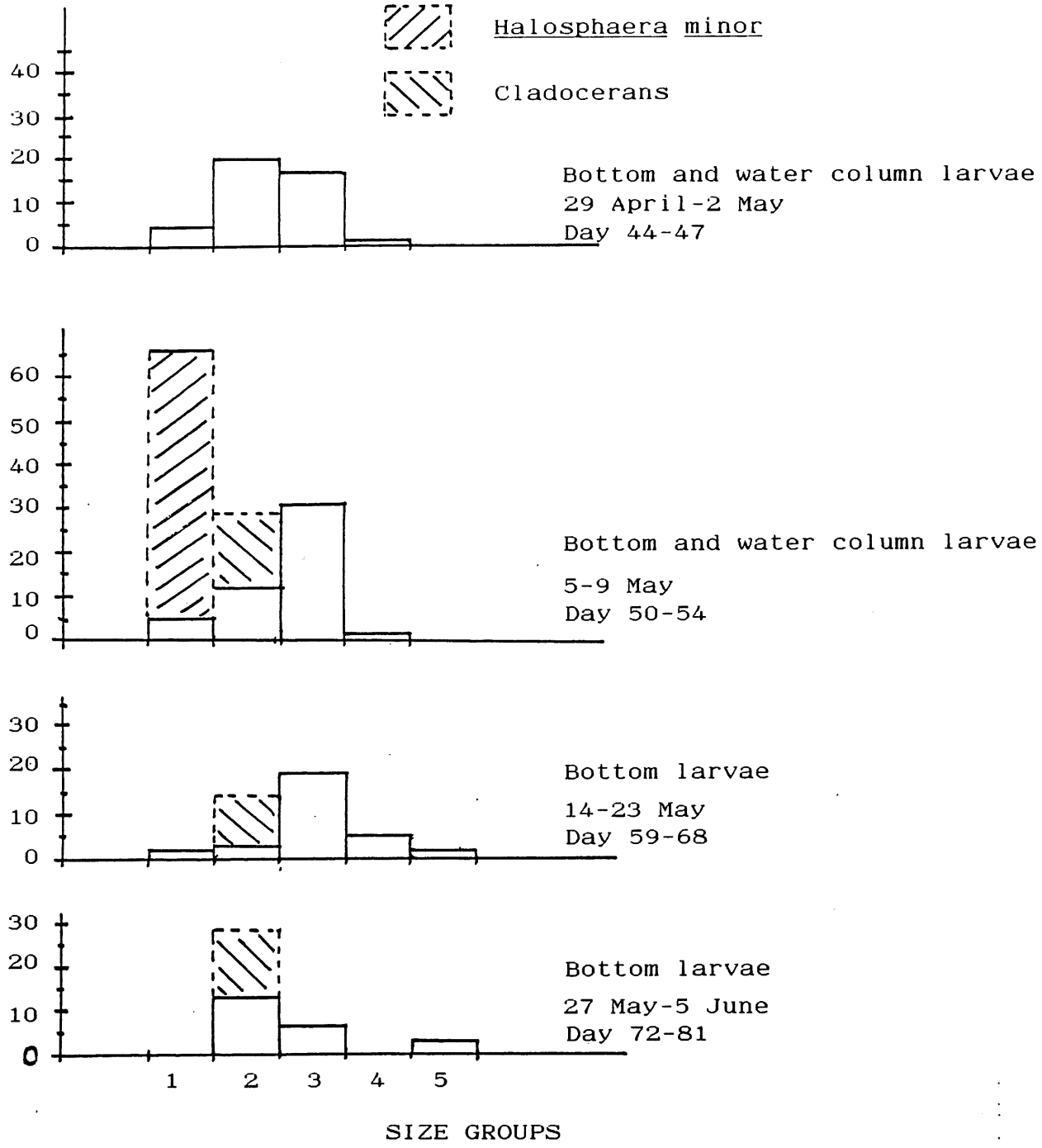

Figure 4. Rough size distributions of larval gut content
Various size groups are defined in table 2.

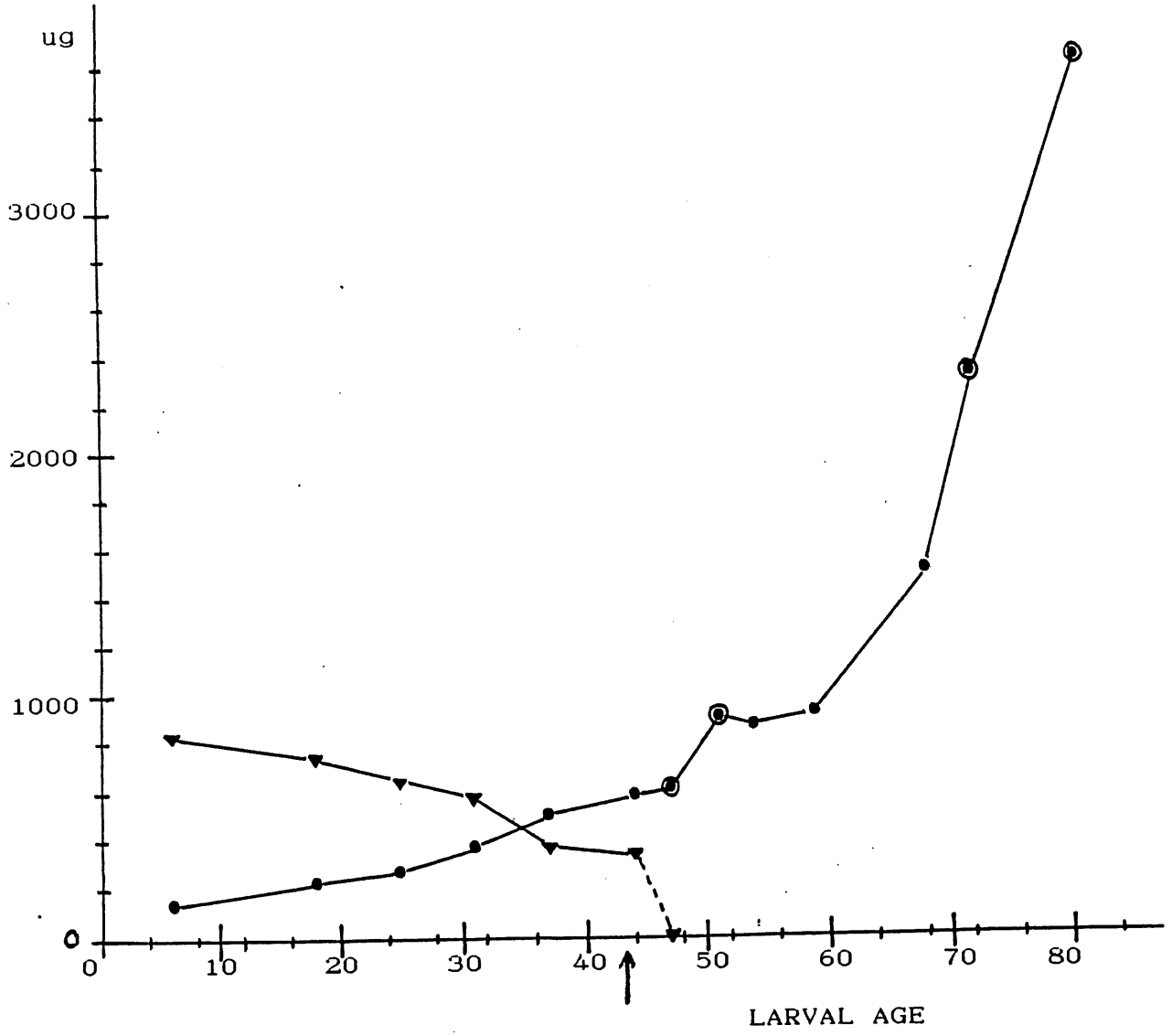

Figure 5. Average dry weight of larvae sampled from bottom water (●), larvae sampled from water column (◉) and yolk sac of larvae sampled from bottom water (▼). Arrow indicate when gut content first time was found.