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MORPHOMETRICAL, BEHAVIOURAL AND CHEMICAL CHANGES DURING GROWTH AND STARVATION OF HERRING AND PLAICE LARVAE

A thesis submitted for the degree of Doctor of Philosophy of the University of Stirling.

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Is ioma rud a tha 'n cuan a falach.

(Many's the thing the ocean hides.)

Gaelic proverb

The work presented in this thesis is the result of my own investigations and has neither been accepted nor is being submitted for any other degree.

Candidate

Supervisor

..16 Dec 1972.. Date

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MORPHOMETRICAL, BEHAVIOURAL AND CHEMICAL CHANGES DURING GROWTH AND STARVATION OF HERRING AND PLAICE LARVAE

A thesis submitted for the degree of Ph.D.

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ABSTRACT

Growth rates of herring (Clupea harengus) and plaice (Pleuronectes platessa) larvae were 0.22 and 0.16 mm/day respectively. The slope of the length-dry weight line for plaice larvae was 3.92. That for herring larvae was 4.57, which remained constant throughout starvation, although the intercepts decreased. If wet weights were used the slopes decreased. Relative condition factors were used to estimate nutritive condition over a wide size range. Condition factors based on length and weight were not very good for estimating nutritive condition, due to concurrent losses of length and weight. The ratio of eye to head height rapidly increased during starvation due to head shrinkage.

The sinking rate of herring larvae in sea water decreased from hatching to the end of the yolk sac stage but increased with further growth. Newly hatched plaice larvae were positively buoyant, but their sinking rate increased with development. In both species the rate decreased during starvation. This was suggested to be a

mechanism of energy conservation. Water content was inversely related to the sinking rate, but other body components also influenced it. Water provided the major upward vector, followed by fat; protein was responsible for the downward force. The decrease in sinking rate during starvation was partially due to the increasing percentage of water, but the largest proportion was from nitrogen catabolism.

The days of starvation to reach irreversible starvation increased during development; the rate of increase was greater in plaice. Over 50% of the life span of herring and plaice starved from the end of the yolk sac stage was beyond irreversible starvation. Ontogenetic changes in chemical composition were dependent upon larval size rather than age. Percent water decreased throughout development from the end of the yolk sac stage. In the period of initial post-hatching growth (up to 20 mm in herring and stage 2 in plaice) nitrogen and carbohydrate were laid down faster than triglyceride, suggesting that it was advantageous to the larvae to convert food largely into growth rather than simultaneously accumulating energy stores. During starvation percent water increased about 4% above the unstarved level; percent ash also increased. The percentage of triglyceride, carbohydrate, and carbon decreased in both species, as did nitrogen in plaice. In herring the percentage of nitrogen did not change throughout starvation, although

the actual amount decreased. The C/N ratio decreased in starved herring, but it did not show a consistent pattern in plaice. It was suggested that the use of nitrogen throughout starvation could be an adaptation by pelagic marine larvae to the planktonic environment, since nitrogen catabolism was responsible for their decreasing sinking rate.

Herring egg composition from different females was related to hatching success. The size of the larvae at the end of the yolk sac stage was compared to their chemical composition. Survival and chemical composition of 100 day-old herring and 50 day-old plaice larvae were altered after 20 days of feeding on diets of Artemia, rotifers, or plankton, but larval length was not affected. The size of the larval fat store was influenced by the amount of dietary fat. Herring larvae started feeding on rotifers 5 days post-hatching, 2 days earlier than on a mixture of barnacle and shrimp brine/nauplii and plankton. By 28 days the rotifer-feeders were significantly larger. 20 mm herring larvae ate 190 rotifers/larvae/day.

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INTRODUCTION

Considering the increasing world population and the millions of currently ill-fed people, it becomes evident that with even a daily protein requirement of 10 to 20 g (Hornig, 1966), the amount of food-protein available must be increased. Fishes, both marine and fresh water, have the potential of augmenting the world's much needed protein requirement. Although the amount of fish that can be taken from the sea is great, it is not unlimited as has been previously thought. Fecundity can be in millions (Blaxter, 1969); however, studies on some Scomberoids and Clupeoids indicate that the vast numbers of adults that inhabit the oceans represent only about 0.1 to 0.01% of eggs originally fertilized (Farris, 1960). If it were possible to increase this survival to 10 or even 1%, the amount of protein available from the sea could possibly be greatly increased. However, before this can be done factors affecting survival must first be defined and then their modes of operation understood. The greatest percentage of mortality occurs in larval stages (Beverton, 1962 and Gulland, 1965), and because of this larval survival is of particular interest to fishery biologists. Many factors influence survival of young fishes: among these are

predation, genetic defects, perturbations of the physical environment and starvation. The relative importance of each of these forms of mortality is not known, but it is thought that starvation can be a major source. It is this last form of mortality that this study examines.

A "critical period" in the life of larvae, associated with resorption of yolk, at which time mass mortalities could occur due to insufficient food or feeding difficulties was suggested by Hjort (1914, 1926). Whether or not this type of mass mortality does occur in the sea has been debated for some time. Marr (1956) reviewed the subject and showed a lack of substantial evidence for the presence of a "critical period" in the sea. Further workers such as Farris (1961) or Pearcy (1962) have not been able to show clearly that survival was markedly lower at the end of the yolk sac stage. However, laboratory studies have demonstrated that larvae of many species, deprived of food for a very short time after resorption of yolk (often only one to two days), reach a point of irreversible starvation. This condition was termed the point-of-no-return (Blaxter & Hempel, 1963). These larvae will not feed even if subsequently presented with food, resulting in complete mortalities (Blaxter & Hempel, 1963; Rosenthal, 1966; Lasker et al. 1970, and

O'Connel & Raymond, 1970). From this it is obvious that many marine fish larvae are greatly susceptible to starvation and possible mass mortalities.

The ability to determine the nutritional condition of sea-caught larvae could be of great help to fishery biologists in predicting larval survival and potential brood strength. Until now estimates of condition have been based on arbitrary indexes of emaciation, various morphological measurements such as

$$\frac{\text{weight (W)}}{\text{length (L)}^3}$$

or body height etc. (e.g. Shelbourne, 1957; Hempel & Blaxter, 1963; Blaxter, 1971); but these results have not been explicit. Chemical changes were thought to be a better way in which to delineate nutritive condition, while at the same time improving the knowledge of the physiology and biochemistry of marine fish larvae. Considerable chemical work has been done on both young and adult of fresh water species (e.g. Hayes, 1949; Smith, 1952; Phillips et al. 1964; Parker & Vanstone, 1966; Satomi, 1969, and Nagai & Ikeda, 1971). Much work has also been done on starvation of adult marine species (e.g. Milroy, 1908; Arevalo, 1948; Templeman & Andrews, 1956; Love, 1958; Kamra, 1966; Wilkins, 1967; Bromley, 1971, and Lavety & Love, 1971).

Limited chemical work has been done on marine fish eggs (e.g. Dakin & Dakin, 1925; Lasker, 1962, and Mengi, 1965): while chemical work on marine fish larvae is even more scarce. Apparently only three references, deal with chemical changes during starvation of marine fish larvae. Marshall, Nicholls & Orr (1937) determined fat in herring larvae. Lasker (1962) worked on sardine eggs and ovaries, but he also analysed changes up to the end of the yolk sac stage in larvae. May (1971) measured nitrogen, carbon, hydrogen and ash of grunion during 20 days of growth and starvation.

The present study on growth and starvation of marine fish larvae may be divided into a number of projects which were carried out on two species: herring (Clupea harengus L.) and plaice (Pleuronectes platessa L.). These species were chosen since rearing techniques were known and because the fishes represent systematically distinct teleosts. The main aim was to supply information on laboratory reared animals which would subsequently provide a basis for comparison of the same species caught in the sea. The main parts of the project may be listed as follows:

1. Morphometric - Physical changes in size and shape
2. Behavioural - Activity and buoyancy changes
3. Chemical - Changes in water, triglyceride, carbohydrate, total nitrogen, ash, total carbon and water.

Another aim of the starvation study was to identify clearly larvae which reached irreversible starvation or the point-of-no-return, or more generally to provide a method for accurately determining the nutritional status of larvae. It was also thought that although herring and plaice did not appear to have any great future potential in aquaculture, the techniques learned and used in this study could lend themselves to application in this field.

METHODS AND MATERIALS

Source and incubation of eggs

HERRING

To rear herring, gonads were taken from spawning fish captured by trammel net fishermen on the Ballantrae Bank, in the Firth of Clyde. Gonads were dissected out directly after capture, while still on board the fishing vessel, and individually placed in small glass jars. These were stored on a small amount of ice in a large vacuum flask while being transported to

Dunstaffnage. Adult herring, from which the gonads were taken, were also brought to Dunstaffnage for length and age determinations. Special care was taken upon dissection of gonads from parent fish to keep the ovaries intact and out of contact with water. Once eggs come in contact with sea water they become sticky. If this happens prior to dispersal on plates, achieving an even distribution becomes extremely difficult.

At Dunstaffnage eggs were fertilized in the following manner, based on the method of Blaxter (1968). Glass plates of about 20x50 cm were placed on the bottom of several shallow rectangular plastic containers. Sea water was added to a depth of about 10 cm. Groups of eggs were taken from one ovary, by means of a scalpel, and quickly shaken in the water for even dispersal. The scalpel was dried and this process continued until the plates were uniformly covered, but the eggs were not clustered. The plates were then transferred to a second container containing a milt suspension. Milt was from one or more males depending on whether or not parentage was needed to be known. After about 15 minutes in this solution the plates were taken out and rinsed to remove excess milt, by dipping them several times in clean sea water. Care was taken to remove excess milt,

because it fouls very quickly and would pollute the incubation tanks. The plates were then placed vertically back-to-back around the sides of a large plastic container with standing sea water. This same process was repeated on eggs from other females. Eggs from different fish were kept separately. The incubation tanks were covered with black plastic sheeting to keep out light. Plates with eggs on them were transferred daily to fresh sea water. Throughout development temperature was maintained at about 9.5°C., and eggs started to hatch after 13 days.

PLAICE

To rear plaice, fertilized eggs were collected from the spawning tanks of/White Fish Authority at Hunterston as well as from the Ministry of Agriculture, Fisheries and Food at Port Erin, Isle of Man and from the White Fish Authority at Ardtoe, Ardnamurchan and were transported to Dunstaffnage in sea water in plastic jars. These eggs, unlike those of herring, were from a group of various adults so that the parentage was not known. The jars were kept on a small amount of ice in vacuum flasks. Care was taken to use only minimal amounts of ice to avoid the temperature dropping to 4°C. or lower.

At the laboratory, eggs were allowed to equilibrate thermally with a tank of sea water of about 9.5°C. Water in the incubation tanks contained penicillin and streptomycin according to the method of Shelbourne (1964). Eggs were placed in this water where they remained until hatching. Time to hatching was dependent upon their developmental condition on arrival at Dunstaffnage.

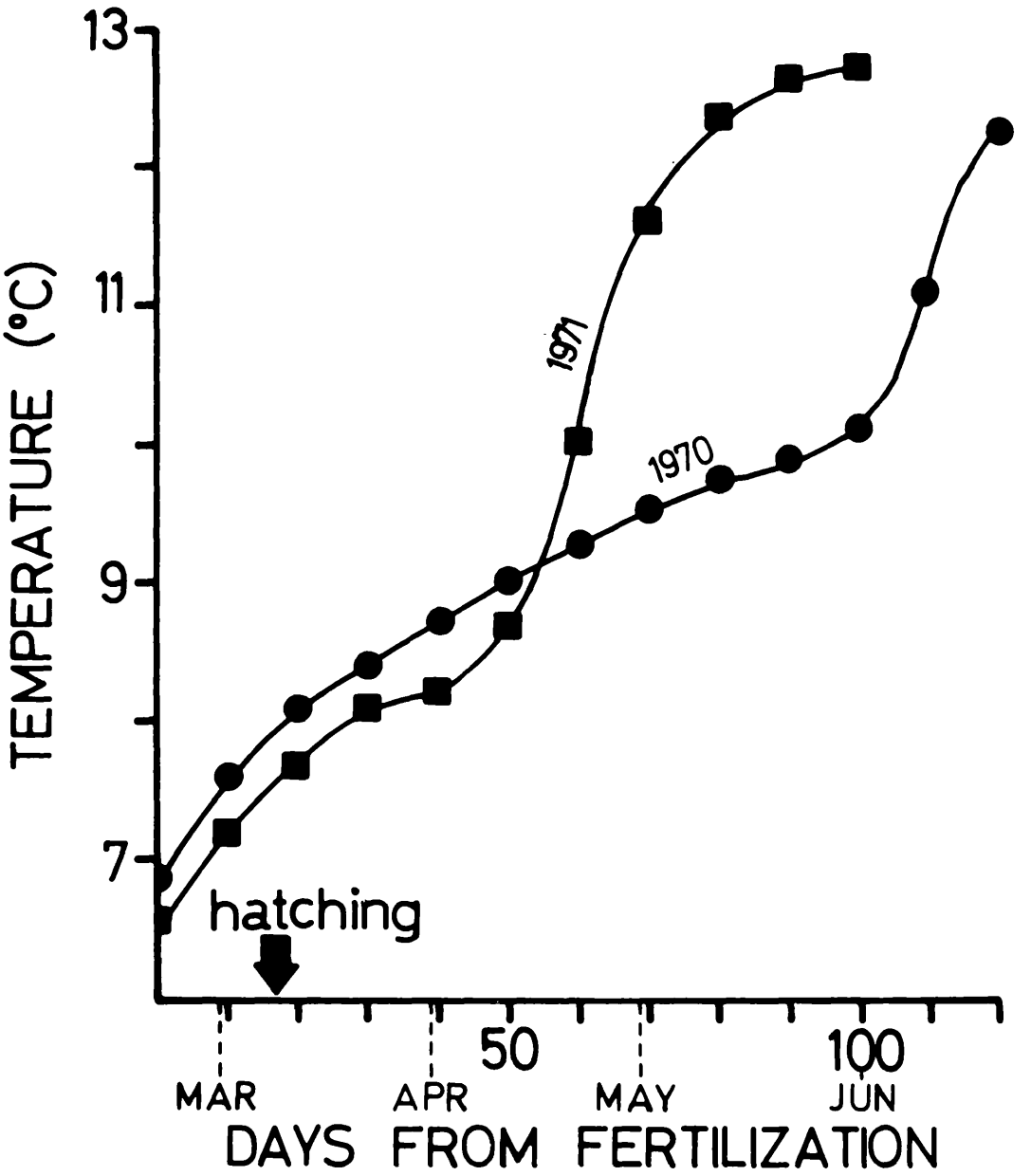
Rearing equipment

In this study the term "larvae" is used to refer to all stages of young fishes from hatching to metamorphosis. At hatching, larvae of both species were individually removed by means of a large pipette or in small groups with a glass beaker. Herring larvae were placed in round black plastic tubs with a matt finish, which varied in size from 20 to about 300 l. Two large rectangular tanks of about 200 l made of an ICI plastic, "Darvic", were also used in a 10°C. constant temperature room. Only the smallest tanks were used for plaice due to their lower spatial requirements. When herring approached metamorphosis they were transferred to a fibreglass tank about 1 m deep and 2 m in diameter, holding approximately 3000 l. This tank was translucent, so the outside was covered with black plastic sheeting to prevent

light entering through the sides. This helped to disperse larvae and their planktonic food from the edges of the tank. From hatching light was supplied by an 80 W fluorescent tube equipped with a diffuser and situated about 1.5 m above the tanks. A time clock was employed to turn lights on and off about one-half hour before sunrise and sunset respectively, although at the end of the yolk sac stage day length was sometimes increased to facilitate commencement of feeding. Up to 6000 newly hatched herring larvae were placed in the 300 l tanks. 800 to 1000 individuals could be placed in the smallest tanks if the larvae were not going to be fed; however, if food was to be added the number was reduced to a maximum of about 500. This was to reduce the occurrence of fish nipping at each other.

Water temperature in rearing tanks was recorded daily, and the pattern of changing temperature during 1970 and 1971 rearings is shown in Fig. 1. All of the rearing tanks except for the Darvic ones had a supply of running sea water. In those without running sea water 10% of the water was replaced each day. The smallest tanks had a flow of about 2 l/h, with faster flow in larger tanks. Water entered at the bottom and left at the surface. Larvae were prevented from being lost

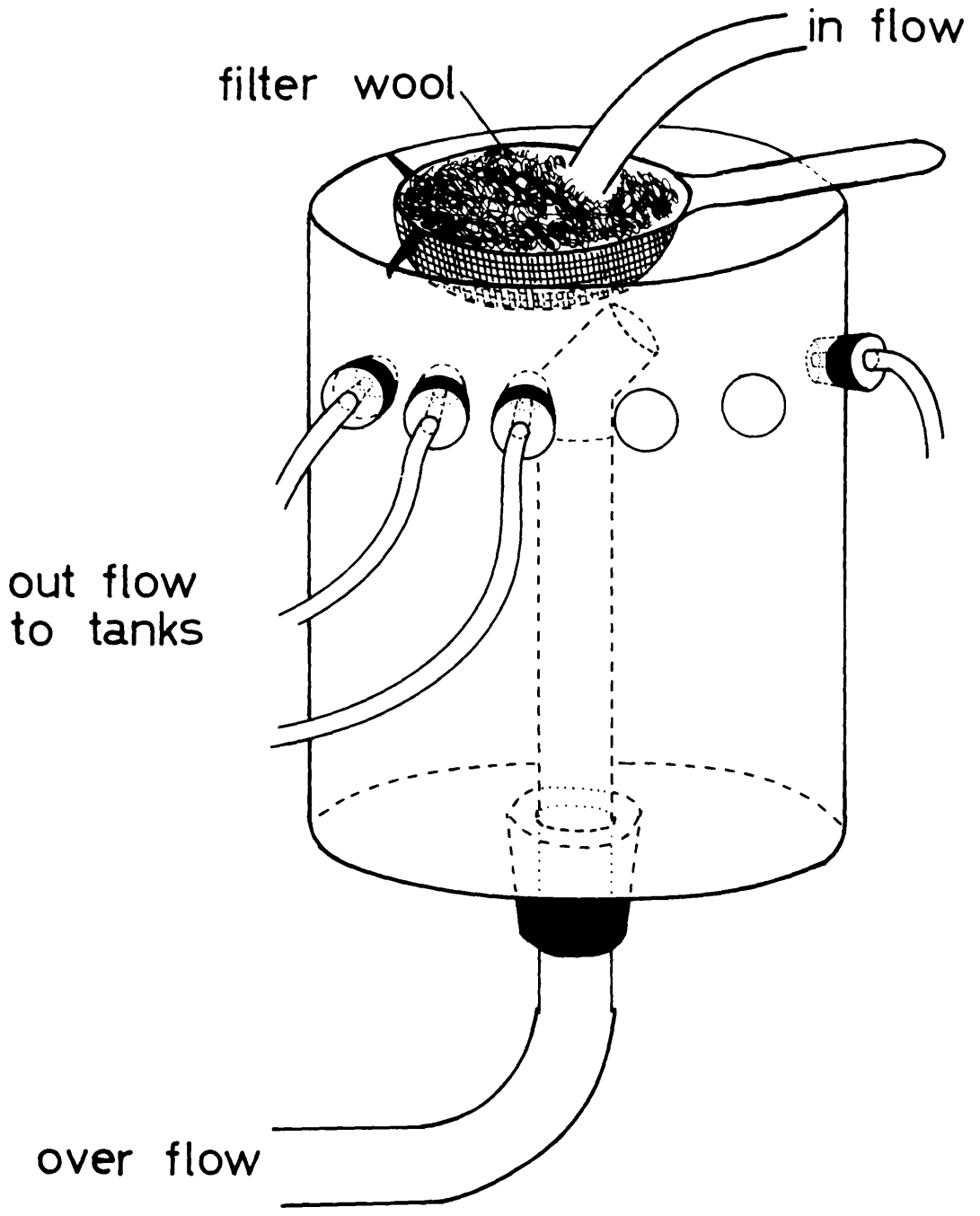
Fig. 1. Temperature changes during herring rearing. Smoothed curve shows changes during 1970 and 1971 rearings.


13
11
9
7
TEMPERATURE (°C)
1971
1970
hatching
50
100
MAR
APR
MAY
JUN
DAYS FROM FERTILIZATION

down the horizontal outflow pipe by means of a perspex ring of about 8 cm diameter and 5 cm high, which had a fine mesh glued to its lower side. The ring had a hole, the size of the outflow pipe, drilled in its side and was positioned over that pipe. The mesh was cleaned each day to prevent clogging.

Sea water was initially taken directly from the aquarium supply, but there was immediate trouble with small air bubbles forming on the sides of the tanks. Larvae would swallow these, then being unable to clear them from their gut would float at the surface until they died. This was unfortunate since it was the larvae most prone to take food which would eat the bubbles and die. To alleviate this problem a header tank of about 300 l was constructed above the rearing tanks. Water entering the header tank was passed over a tray with a series of baffles and then through a filter of synthetic fibre wool. Water in the tank was aerated by means of an air pump and heated with three 100 W heaters, after which it was fed into the rearing system. The water was then filtered a second time as it entered a 2 l constant level container (Fig. 2), one of which supplied up to six tanks with a uniform flow of water. During at least the first two months of the rearing the tanks were cleaned daily. This involved carefully siphoning debris and

Fig. 2. 2 l container used to maintain constant flow to rearing tanks. Details of water flow and filtration are shown, but the figure is not drawn to scale.


in flow
filter wool
out flow
to tanks
over flow

dead larvae, which were counted, from the bottom and skimming a bacterial film from the surface. This film trapped air bubbles and exacerbated the mortality due to swallowed bubbles.

Feeding

Herring larvae were initially fed Balanus nauplii and natural plankton sieved through a 400 μ screen. Balanus nauplii were collected by removing egg sacs from the adult barnacles and forcing the eggs through a screen into sea water. This caused the egg masses to break up and the eggs to hatch. Artemia nauplii were used as a supplement. All Artemia fed to larvae were from eggs from San Fransisco Bay. Those from the Great Salt Lake, Utah were not used due to reported contamination from DDT. Artemia eggs were incubated in 2 l beakers of sea water at 27°C., which was continuously and vigorously aerated. The nauplii were separated from egg cases and collected by attraction to light, the standard method described by the suppliers of the eggs. Rotifers (Brachionus plicatilis) were also used during the rearing. After metamorphosis both species of fishes were weaned onto chopped fish and squid. Plaice larvae were fed up to metamorphosis on Artemia nauplii and a supplement of sieved plankton.

Rotifers were cultured in a 35 l perspex tank. They were fed on the green algae Brachyamonas submarina, and with sufficient food a density of over 20 rotifers/ml was reached. Both the seed stocks of the algae and the rotifers were obtained from Mr. Martin Scott of the Dunstaffnage Marine Research Laboratory. Several hundred ml of bacteria-free algae were placed in a 1 l volumetric flask. An equal volume of half strength sea water was added. Sea water was first filtered through a "C" Millipore filter and then diluted with distilled water. Nutrient medium was then added at the rate of 1 ml/l, according to the directions of Mr. Scott and contained the following chemicals dissolved in 1 l of distilled water:

KNO_3 100 g

K_2HPO_4 10 g

E.D.T.A. 20 g

$FeSO_4 \cdot 7H_2O$ 2.5 g

$MnSO_4 \cdot 4H_2O$ 0.25 g

Thiamine 6 mg

Vitamin B_{12} 50 µg.

The flask of diluted algae was placed in a perspex water bath at 18°C. with two warm-white fluorescent lights supplying illumination from the side. The mixture was vigorously aerated and the top of

the flask was stoppered with synthetic fibre wool. When the algae had grown for several days to about its original concentration (dark green), it was transferred to a 2 l flask and diluted again with freshly filtered half-strength sea water. Nutrient medium was again added at the rate of 1 ml/l. This process was continued until two 20 l glass carboys of algae were available. These were maintained at 18°C. by means of 100 W immersion heaters. New algal cultures were started about every two weeks to avoid food shortages due to the possibility of the collapse of the cultures, which sometimes happened after 2 to 3 weeks of use. Each day 5 l of the algal solution were removed from each carboy and placed in the rotifer tank. Before rotifers were in sufficient numbers to be used for food an equal volume of water was removed from their tank prior to addition of algae. This water was filtered through a 50 μ screen to avoid loss of rotifers. When they were being taken for food about 10 l of water was removed from the rotifer tank and was filtered through the 50 μ screen to collect the rotifers. They were then transferred to a beaker of sea water and placed in the tanks with the larvae.

General sampling procedures

FIRST YEAR: GROUPED SAMPLES GROWTH AND STARVATION

The basic experimental sampling pattern was to rear larvae from hatching through metamorphosis and at various ages, starting at hatching to allow groups to starve. In this study "starvation" means deprivation of large particulate food such as plankton or Artemia. No attempt was made to control dissolved organic material or very fine particles, although all sea water was twice filtered through synthetic fibre wool. However, the extent of feeding on these small organic compounds as well as the benefit derived from them was assumed to be minimal. Feeding on these sorts of substances was described by Pütter (1909 a and b) and was reviewed by Morris (1955). Fish were sampled at the start of starvation and usually every three days thereafter until death, or until all larvae in a group being starved had been sampled.

In the first year the following sampling method was used: where possible groups of larvae in each condition(defined as active, inactive - that is at the point-of-no-return, and moribund) were captured by means of a pipette, rinsed in distilled water, grouped, quickly frozen, and then freeze-dried as soon as possible.

Some samples were subsequently stored in a desiccator in a deep freeze until needed for further work. Before weighing samples, the desiccator was removed from the freezer and left in the air to equilibrate with room temperature. A larva was then quickly removed and weighed to determine its dry weight. Individual and grouped larvae were then ground with a mortar and pestle, refrozen and then refreeze-dried. The increase in weight due to absorption of atmospheric water during the minute or so required to weigh the larva was less than 1% and considered insignificant. This procedure was used rather than allowing larvae to equilibrate with atmospheric vapor pressure, because variations in surface area of larvae led to differences in rate of absorption of water. However, after samples were ground, aliquots weighed for chemical analyses were allowed to equilibrate with atmospheric water content. In these samples surface area was comparable and weighing errors due to absorption of water amounted to about 3%. Some larvae at sampling were first anaesthetized in MS 222, at a concentration of 1:20,000, to see if this influenced the chemical results.

At hatching groups for chemical analyses contained about 20 herring larvae or about 30 plaice. These numbers were chosen to give samples of at least 2 mg. At each sampling some larvae

were also preserved in 4% neutral formalin. In the first year, starvations of herring were started at the end of the yolk sac stage and at 24, 41, 55, 74, and 88 days after hatching.

Plaice were grouped for sampling according to main stage classifications described by Ryland (1966) (see Table 1). In the first year plaice were starved from the end of the yolk sac stage, and at days 27 and 28 after hatching groups of larvae were selected at stages 3 and 4 respectively (see Ryland, 1966) and subsequently deprived of food, and at 41 days post-hatching a group at metamorphosis was allowed to starve. Larvae at stage 2 (16 days) as well as post-metamorphosis, 51 days post-hatching, were also sampled for growth changes.

SECOND YEAR: PARENTAL EFFECTS, INDIVIDUAL SAMPLES OF GROWTH AND STARVATION

In the second year it was decided to work, as much as practical, on individuals, to rule out the increasing variation in the size hierarchy which occurs with growth. In this year parental differences were also considered. Eggs from seven herring females were fertilized but only six yielded enough larvae for comparison during starvation at the end of the yolk sac stage. Unfertilized eggs were also compared for dry weight and chemical

Table 1. Plaice-larvae developmental stages from Ryland (1966).

Stage	length (mm)①	age (days)②	Description
1	7-7.5	0-15	Yolk sac present.
2	7.5-8.5	16-32	Notochord straight; hypural fin rudiment developing; yolk resorbed or remaining at first as a minute globule
3	8.5-10.0	33-49	Caudal extremity of notochord bent; marginal fin rays developing; eyes symmetrical.
4	10.0-11.5	40-67	Eyes symmetrical; flatfish shape develops.
5	> 11.5	> 67	Left eye on or beyond edge of head; the pupil visible from on top. When the eye reaches its final position metamorphosis is complete.

① Lengths approximated from Fig. 9 of Ryland (1966).

② Ages rounded off from Table 4 of Ryland (1966). The temperature during this period increased from approximately 7 to 12°C.

differences. Starvations of herring larvae were started from the end of the yolk sac stage and from 30 and 50 days post-hatching. In addition to the beginning of each starvation herring larvae were also sampled for growth at 38, 46, 53, 63, 80, and 94 days after hatching.

During starvation of 30 and 50 day-old herring larvae the percentage of 50 larvae capable of feeding on Artemia at each day of sampling was measured. When this reached 50% the population was considered at the point-of-no-return. This coincided with larvae hanging head down in the water, thus it was possible to pick out behaviourally larvae starved to the point-of-no-return. (In the text below this condition is referred to as PNR). Only very few plaice eggs were obtained in the second year; so the number of experiments had to be limited. The only starvation was of fish at the end of the yolk sac stage, but others were also sampled for growth changes at 12 days post-hatching. Plaice at the point-of-no-return were also observed to hang head down in the water. However, this was not the case for fish near metamorphosis, which swim on the bottom. In these fish the point-of-no-return was taken to be the time when they showed no interest in objects in their immediate vicinity nor a response to a weak jet of water from a pipette.

Dietary studies

At 73 days after hatching eight 20 l tanks each with 50 herring larvae were set up for comparison of growth and survival as well as subsequent chemical differences after feeding for 20 days on different diets. The diets were rotifers, brine shrimp, plankton sieved through 400 μ netting, or a mixed diet of the other foods. An additional two experiments were performed in the third year to test the benefit of rotifers as food for young herring. In the first of these, two groups of 500 newly hatched herring were placed in separate 20 l tanks and given food for 28 days. One group received rotifers and the other a mixed diet of *Balanus* nauplii, *Artemia* nauplii, and plankton sieved as above. Survival was followed over this period, and after 28 days 30 larvae were measured from each group for length comparisons. The second experiment measured feeding rate of 20 mm herring larvae on rotifers. Rotifers were added to 800 ml of sea water in a glass beaker, and their density was determined by counting numbers in two 5 ml aliquots. Twenty larvae, one day starved, were placed in this beaker, which was then floated in a large black tank. After 48 hours larvae were removed and counts of rotifers and empty loricas were made. From this the number of rotifers eaten per fish per day was calculated.

Plaice were also used in diet studies. Six 20 l tanks, each containing fifty 30 day-old plaice larvae, were set up in duplicate for comparison of three diets: rotifers, brine shrimp, and plankton sieved through 400 μ netting. The feeding regimes were maintained for 21 days before sampling. In diet studies on both herring and plaice samples of foods were taken for chemical analysis.

Buoyancy measurements

It was observed during the first year that the heads of herring larvae appeared to shrink during starvation, especially as compared to eye size, which remained constant. Mr. Andrew Packard, of the University of Edinburgh, who was working on brains of herring larvae, suggested that this might be due to utilization of diffuse fatty tissue found around the brain. In the second year, in addition to measuring the head height, sinking rates of anaesthetized larvae in sea water and various other salinities between 16 to 48‰ were also determined. Sinking rates were determined by the time required to fall through 10 cm in a 1 l cylinder. Stable temperature was maintained by placing the cylinders in a glass aquarium filled with tap-water, in a 10°C. constant temperature room. Larvae were rinsed with

water of the same salinity through which they would fall, before being placed in the cylinder. Temperature and salinity were recorded on each sampling day and all rates in 100% sea water are reported at 10°C. and 33‰. Solutions other than 100% sea water were varied with additions of distilled water or NaCl. By measuring the sinking rate at several salinities it was possible to find that equivalent to neutral buoyancy. At that point the density of the fish was determined, since the density of the water was known based on temperature and salinity.

Groups of 10 to 15 larvae were used for sinking rate determinations of herring up to 30 days post-hatching and of the youngest plaice. Further measurement of sinking rates of individual herring larvae was continued up to 63 days post-hatching. After this time most were too large for the cylinders, in that they tended not to fall quite vertically and quickly ran into the side of the cylinder invalidating any timing measurement. In older larvae, individuals were measured in different salinities before being used for morphological and other determinations. Larvae were measured for total length, eye and head height, and wet and dry weight.

Relative condition factor

In the starvation and dietary studies the morphometric condition of the larvae has been reported, as by Le Cren (1951), in terms of relative condition factor (RCF).

$$RCF = k\ 10^5 \text{ for herring;}$$

$$RCF = k\ 10^4 \text{ for plaice;}$$

where k is a constant from the equation

$$\text{weight} = k\ \text{length}^n.$$

The orders of magnitude 10^5 and 10^4 were arbitrarily chosen to make the RCF more easily handled (see the discussion for a theoretical treatment of condition factors).

Chemistry

Chemical analyses consisted of water, triglyceride, carbohydrate, total nitrogen and carbon, and ash. Where possible all analyses were run on each sample. This was done on all samples of pooled larvae, but when working with individuals the smallest gave only enough material for one determination. In this case other individuals of similar size were used for the other analyses.

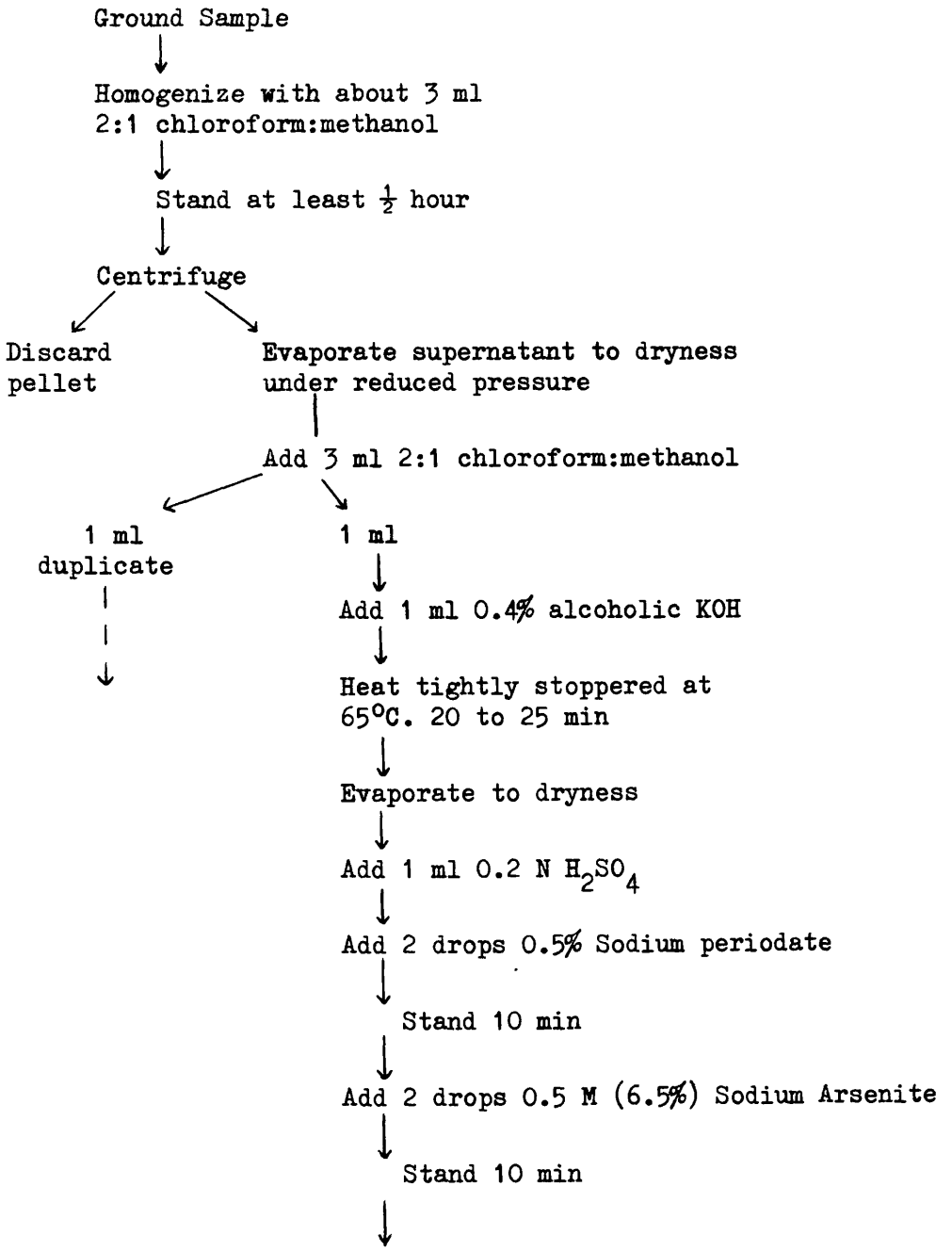
WATER

Wet weight was determined first by rinsing a larva, as quickly as possible, in distilled water to remove any salt water on its surface, which would affect the dry weight. It was then briefly dried on a filter paper and transferred to a small aluminium boat for weighing. Wet weight of each sample was recorded at 1, 2, and 3 min after placing the larva on the filter paper. Decrease in weight over this time was linear, so it was a simple matter to extrapolate to zero time. Weighings were done on a Beckman EMB 1 electrobalance, which could weigh samples to the nearest 0.5 µg. A larva was frozen as soon as its wet weight was determined. After freeze-drying the dry weight of larvae were determined, and the water content was calculated from this and the wet weight. The larvae were then stored for other chemical analyses.

TRIGLYCERIDE

Triglyceride was determined instead of total lipid, because it was thought to be a more sensitive indicator of nutritive condition. Furthermore, an adequate method for determination total lipid on micro-quantities was not available at the start of this study. The method used was that of van Handel (1961)

as modified by Barnes (personal communication). This method was usable on 150 µg, but about 500 µg were preferred. The method is shown in the flow chart below:



↓

Add 5 ml 0.2% (w/v) Chromotropic acid in 60% H_2SO_4 (v/v)

↓

Heat tightly stoppered in boiling water for 30 min

↓

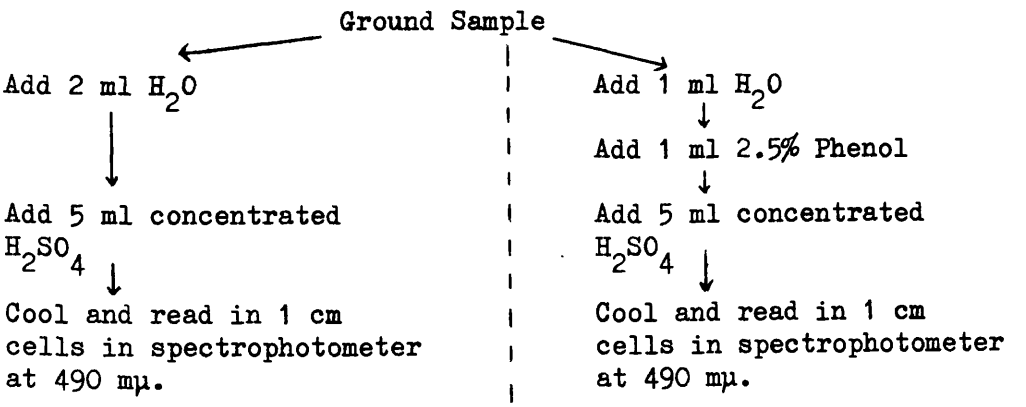
Cool and read in 1 cm cell in spectrophotometer at 570 mμ.

In this determination glycerol tristearate was the standard and was taken through the whole procedure. Blanks were run with each group of samples. The method was sensitive to 5 μg of triglyceride. The reactions are based on the extraction of lipids with chloroform:methanol, saponification of triglyceride fraction to glycerol, which in turn is converted to formaldehyde. This is then quantitatively determined in an oxidation mixture of chromotropic acid. Phospholipids were not removed before saponification as was done by van Handel (1961), because it was felt that any glycerol obtained from the saponification of these would be small compared to that from the triglycerides. Furthermore, the extraction with silicic acid and subsequent separation were steps that could increase the error. Since this reaction converts triglyceride to formaldehyde great care had to be taken to avoid contamination from external sources of formalin present in the laboratory. Its vapour also had to be avoided. This applied not only to the samples but also to the

reagents. Even with great care some samples were still contaminated, probably by vapour, and had to be discarded. Triglyceride determinations were initially performed on duplicate weighings of samples which were divided after extraction and run again in duplicate giving four replicates. After several runs of 25 samples the weighing duplicates were eliminated so that the sample number per run could be doubled. About 100 tubes plus standards were handled in each run.

CARBOHYDRATE

Carbohydrate was determined by the method of Dubois et al. (1956) with an additional blank without phenol as suggested by Barnes (personal communication). The method is shown in the flowchart below. The additional blank is shown to the left of the broken line.



The method is based on conversion of carbohydrate, in the presence of phenol and sulphuric acid, to furfural products. The acid has to be added onto the surface of the water within about 0.5 sec. This generates the heat required for the reaction, but long tubes must be used to avoid loss of the solution through splattering. Distilled water blanks and glucose standards were taken through the entire reaction. This method is sensitive to about 5 µg of carbohydrate. The original work of Dubois et al. (1956) was on fermentation solutions, where charring by the acid of substances other than carbohydrate should have been minimal. However, when using whole animal material, interference or masking of the colour reaction due to charring of non-carbohydrates is significant. It was for this reason that the blank without phenol was used. The colour developed from the interfered charring was measured as percent carbohydrate and subtracted from the sample with phenol to give the percent carbohydrate values reported. Although the reaction with the phenol present would work with 100 µg, the colour developed without the phenol was weaker and accurate estimates required about 300 µg.

TOTAL NITROGEN AND CARBON, ASH

Total nitrogen and carbon were determined on a Perkin Elmer Elemental analyzer. This machine combusts samples at 950°C.; ash values of samples combusted at this temperature were compared to those combusted for about 12 h at 520°C. in a muffle furnace. The elemental analyzer takes about 15 min to analyze one sample and satisfactorily works on about 150 to 200 µg of sample, although up to several mg could also be used. Samples were initially run in duplicate, but agreement soon showed this to be unnecessary. This method was especially suitable for use on individual larvae of very small size.

GLASSWARE CLEANING

All glassware used was initially washed in chromic acid, rinsed 10 times in distilled water, soaked overnight in distilled water, then rinsed again and dried. In the second year the procedure was changed to washing with the commercial detergent RBS 25, manufactured by Chemical Concentrates Ltd., London. Glassware in detergent was brought to near boiling then allowed to cool; after 10 rinsings it was dried. This method was much neater than working with chromic acid and gave satisfactory blanks.

Treatment of data

Percentages are relative values and so when one component increases another falls. Percentages thus show the relative size of a particular component or store in an individual but not the actual amount. Although changes in actual amount

(% x dry weight in mg x 10 = μg)

are useful during growth and especially starvation of a particular size group, they are difficult to use when comparing animals of different sizes. In order to compare changes during starvation of a particular constituent, independent of the other components, the amount of the constituent has also been shown as a percentage of the initial amount present at the start of the starvation. Thus results have been reported in three ways: percent of dry weight, actual amount (μg) and percent of initial amount at start of starvation. In this study, unless otherwise stated, all percentages refer to proportion of dry weight.

When rearing fish one finds an increasing size range during growth to metamorphosis. In this period of growth the fish are developing as well as increasing in size. Developmental condition is thought to increase with size more than with age. Accordingly most of the substances measured in herring showed a better correlation with size than with age of larvae (Figs 3 & 4), for this reason dependent variables have been initially plotted

Fig. 3. Herring percent water versus age. Representative points show poor correlation with age as compared to length (Fig. 4).

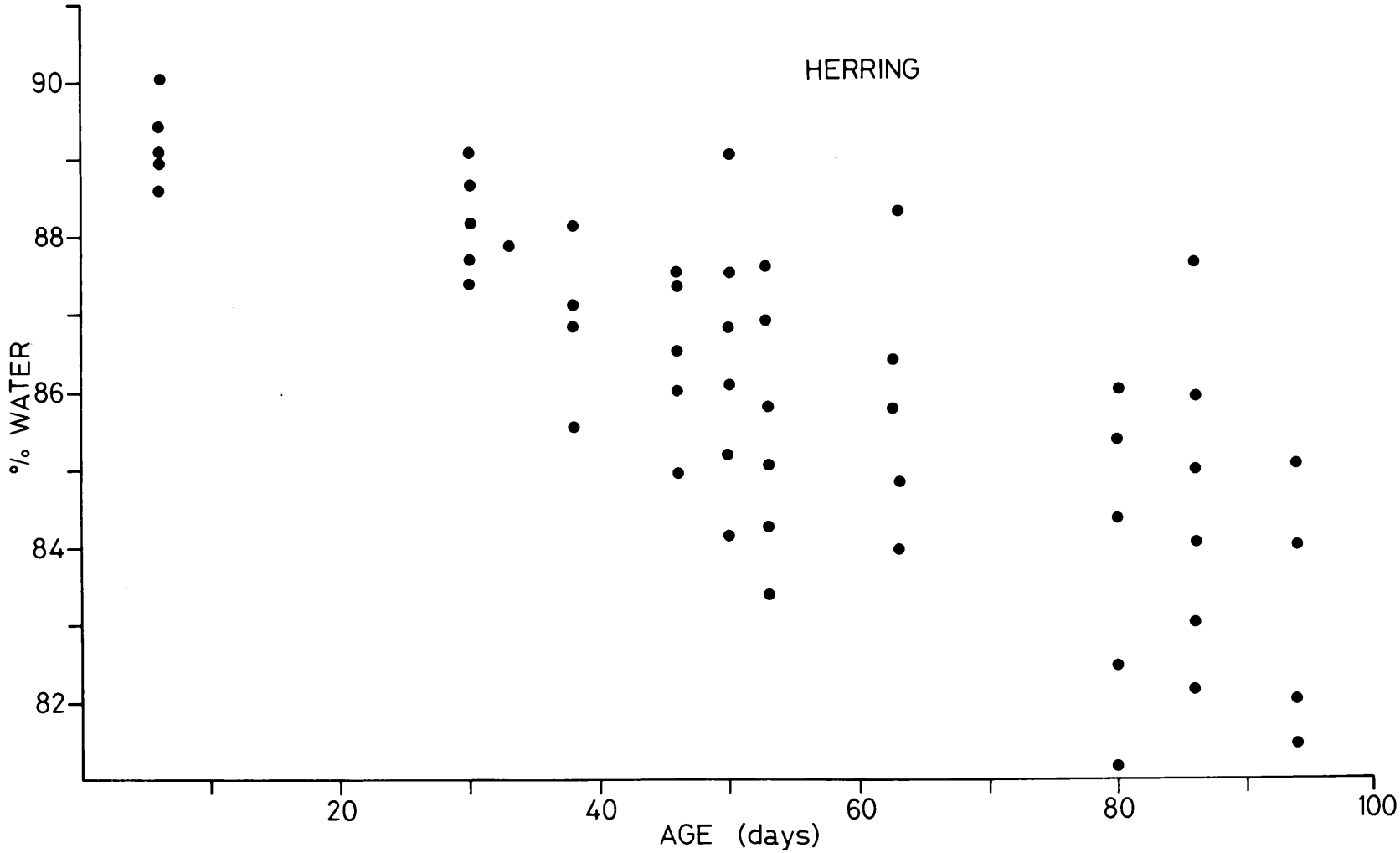
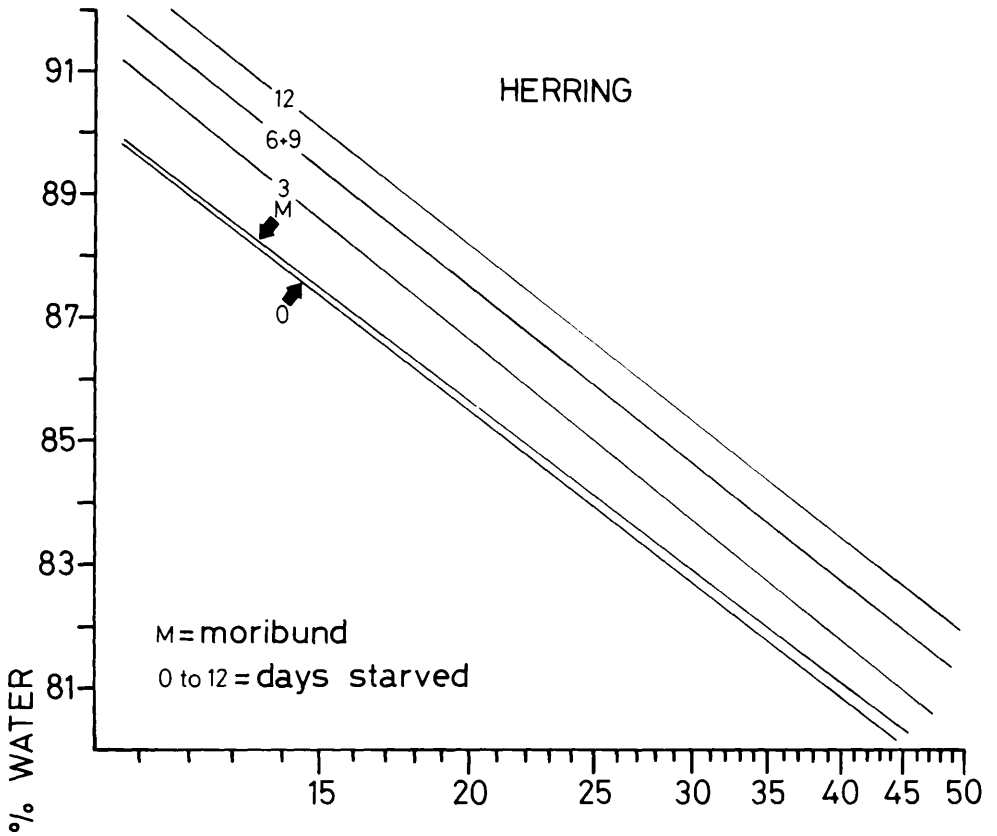
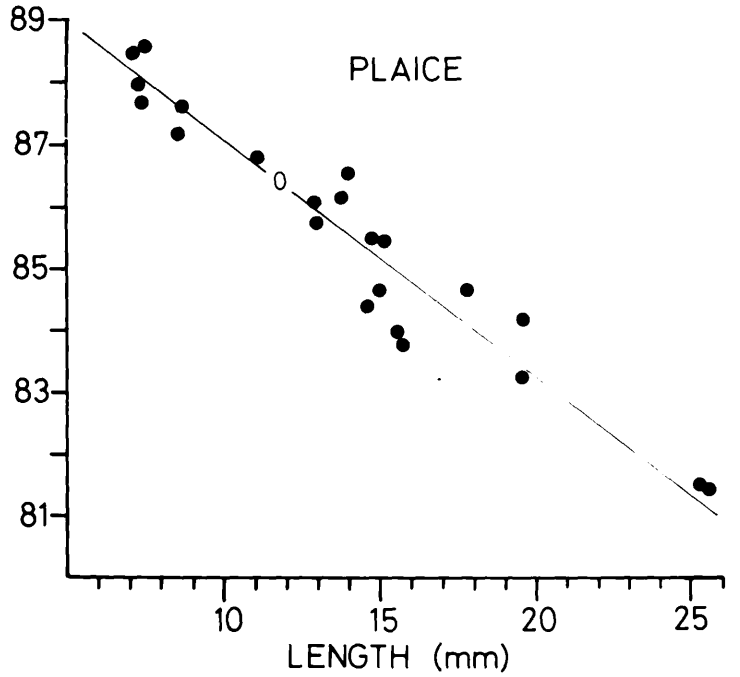

HERRING
90
88
86
84
82
% WATER
20
40
60
80
100
AGE (days)

Fig. 4. Percent water versus length.

A. Herring: Length is drawn to a logarithmic scale. Lines are shown for different degrees of starvation. Equations and other parameters of lines are given in Appendix 3.

B. Plaice: Length is drawn on an arithmetic scale. The line and some representative points, which show the spread, are drawn for unstarved fish. % water = - 0.38 length (mm) + 90.81; N = 33, $S_{y \cdot x}$ = 0.4908, SD_b = 0.0186, SE_c = 0.2395.


HERRING
12
6+9
3
M
0
M = moribund
0 to 12 = days starved
% WATER
91
89
87
85
83
81
15
20
25
30
35
40
45
50


PLAICE
0
89
87
85
83
81
10
15
20
25
LENGTH (mm)

against length of larvae. This was done for each day starved as well as unstarved larvae. Length was chosen instead of weight, because it is an easier reference and did not require a logarithmic scale for plotting the wider weight range. To compare changes during starvation of different sized herring larvae, six lengths were chosen as standard sizes. Unstarved lengths of 12, 15, 21, 25, 30 and 35 mm were chosen, because they best illustrated the differences between larvae of increasing size. The use of standard sized animals is based on the method of Barnes, Barnes and Finlayson (1963), but has been modified so that the length of animal at each day starved will vary according to changing length of larvae during starvation.

Computations and drawing of regression lines and calculations of other statistics were greatly aided by use of the Hewlett Packard desk computer 9100B. Statistical tests and references have been taken from Dixon and Massey (1957) as well as from Steele and Torrie (1960). When computing changes of some component against length, individual values were initially plotted for calculation of regression lines. High individual variability made composite drawings of successive regression lines during starvation unclear. For this reason the individual points have

not been drawn, but only the lines. In lieu of this, for each graph of the form $y = bx + c$, a table has been included to show pertinent parameters of lines. These tables contain the equation of line, the number of individuals in sample (N), the standard deviation of y for fixed x ($S_{y.x}$), the standard deviation of slope (SD_b), and the standard error of intercept (SE_c). The equations for these are from Steel & Torrie (1960).

RESULTS

Growth

The growth rate of herring larvae based on live lengths was 0.22 mm/day during 91 days post-hatching (Fig. 5A). The regression of length on age during this period gave the equation:

length (mm) = 0.22 Days + 7.74, (N = 155).

A later measurement of fish 212 days old showed the mean length was 92.2 mm, considerably higher than that predicted by the earlier growth rate (54.4 mm). Metamorphosis of herring larvae was observed to occur at about a length of 35 mm. The growth rate of plaice based on live length was 0.16 mm/day during 51 days post-hatching (Fig. 5B). The regression of length on age during this period gave the equation:

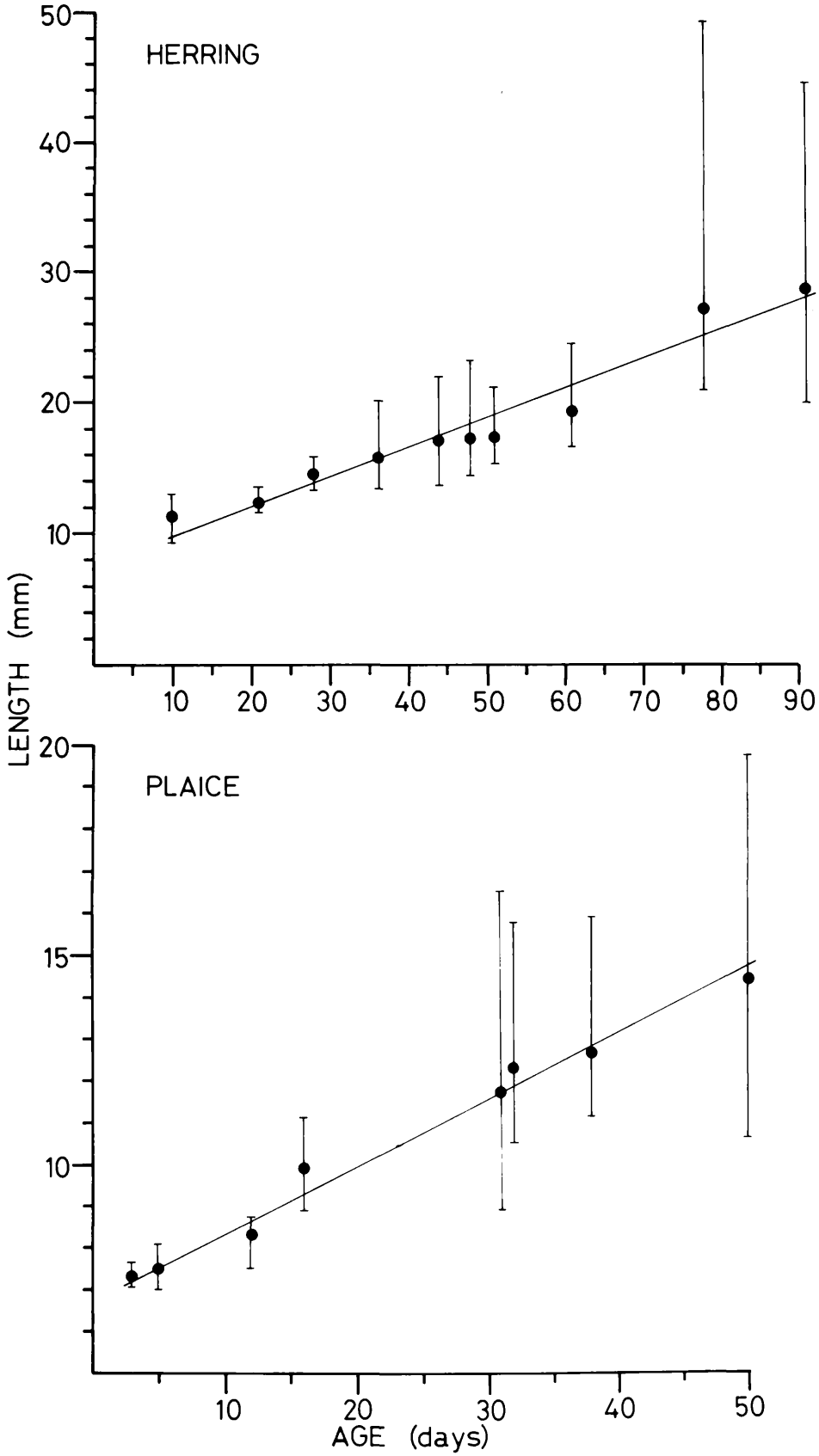
length (mm) = 0.16 Days + 6.67, (N = 127).

The mean length of the herring population, up to 21 mm, was found to noticeably change during starvation (Appendix 1A). Length increased during the first days of starvation but then decreased to less than the value at the start of starvation. Above 21 mm the large variation in larval size (size hierarchy effect - see Fig. 5) prevented these measurements from being made without sampling much larger numbers than were available.

Fig. 5. Larval growth rates. Means and ranges of lengths are shown for larvae reared during the first two years (1970 and 1971).

A. Herring: Length (mm) = 0.22 Days + 7.74; N = 155, $S_{y \cdot x} = 3.9146$, $SD_b = 0.0114$, $SE_c = 0.7328$.

B. Plaice: Length (mm) = 0.16 Days + 6.67; N = 127, $S_{y \cdot x} = 1.6959$, $SD_b = 0.0089$, $SE_c = 0.1576$.


HERRING
50
40
30
20
10
10
20
30
40
50
60
70
80
90
LENGTH (mm)
PLAICE
20
15
10
10
20
30
40
50
AGE (days)

Length-weight

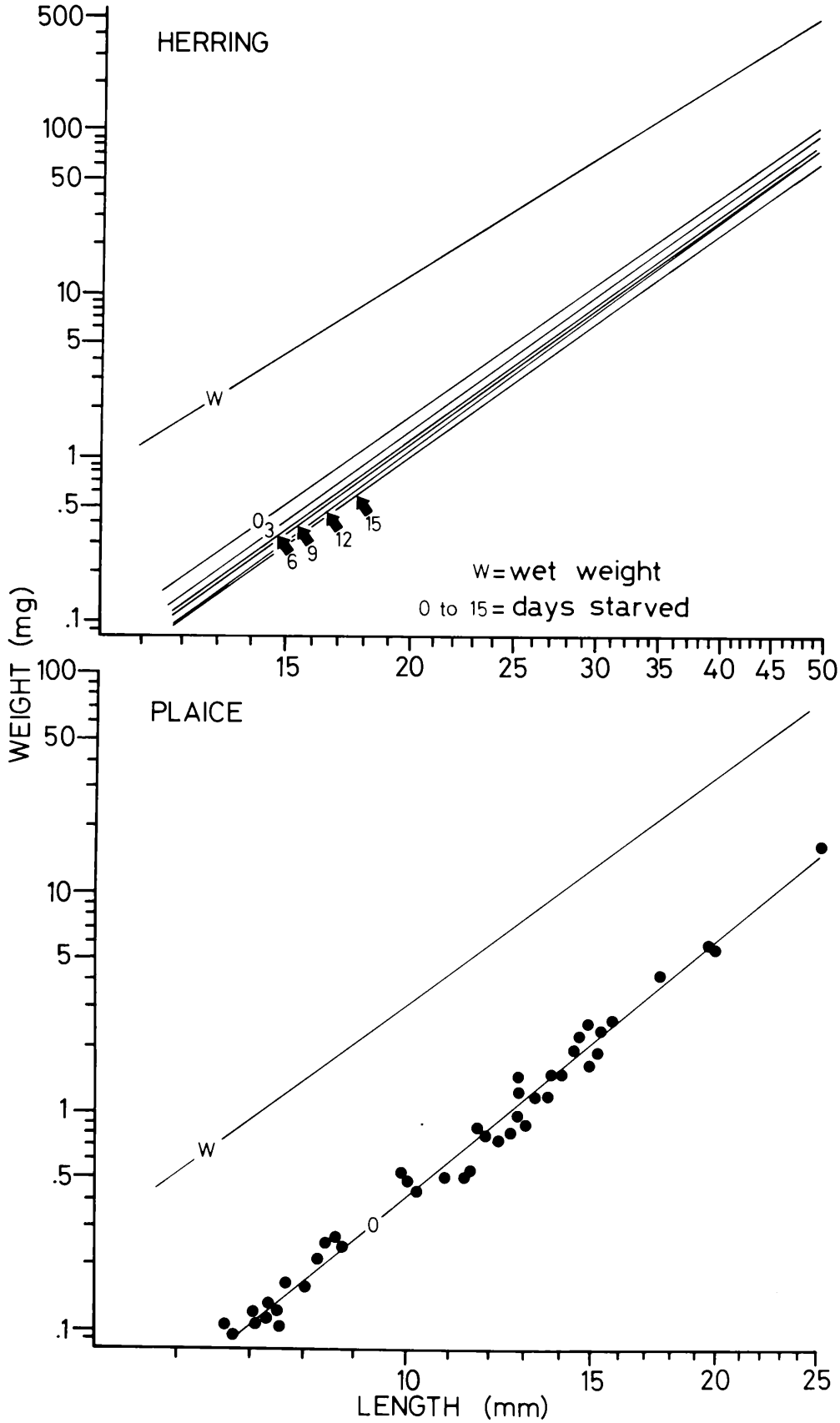
Length-weight relationships were based on live lengths and dry weights. Length-weight lines for herring were determined for unstarved larvae and for starved ones at each day of sampling (Fig. 6A). The lines were tested for homogeneity and were found to be different by a highly significant amount although no significant differences were found between the slopes (Appendix 2). The slopes were pooled to get the best estimate of the true slope and this was then used for determining the equations of the regression lines of weight on length for unstarved and starved larvae. It was these equations, based on the pooled slope, that were used for determining the weight of the standard length larvae at the different days starved. The original lines computed for each day starved were not used for this purpose. Changes in weight, both actual (mg) and relative (% of initial weight), during starvation of these larvae are shown in Appendix 1A. A length-weight line in plaice was determined only for unstarved larvae (Fig. 6B). The equation of this line was

$$\log \text{weight} = 3.9155 \log \text{length} - 4.3043.$$

Fig. 6. Length-weight relationships. Lines are shown using wet and dry weights of unstarved larvae. The wet weight line was calculated from water content of different sizes of larvae.

A. Herring: Lines are shown for different degrees of starvation. Equations and other parameters of the lines are given in Appendix 2.

B. Plaice: Lines are shown only for unstarved fish. Representative points show spread around line. Log dry weight = 3.92 log length - 4.3043; N = 71, $S_{y \cdot x}$ = 0.0658, SD_b = 0.0554, SE_c = 0.0587. Log wet weight = 3.56 log length - 3.0678.


HERRING
500
100
50
10
5
1
.5
.1
W
0
3
6
9
12
15
W = wet weight
0 to 15 = days starved
15
20
25
30
35
40
45
50
WEIGHT (mg)
PLAICE
100
50
10
5
1
.5
.1
W
0
10
15
20
25
LENGTH (mm)

There were insufficient numbers of larvae for determination of this relationship for each day starved.

Relative condition factor

Relative condition factor decreased during starvation of herring (Appendix 2 and Fig. 7A). These values are the antilogarithms of the intercept of the length-weight line for each day starved, times a factor to make the numbers more easily handled. For herring the best estimate of relative condition factor, when using live length and dry weight was

$$\frac{\text{weight}}{\text{length}^{4.571}} \times 10^5$$

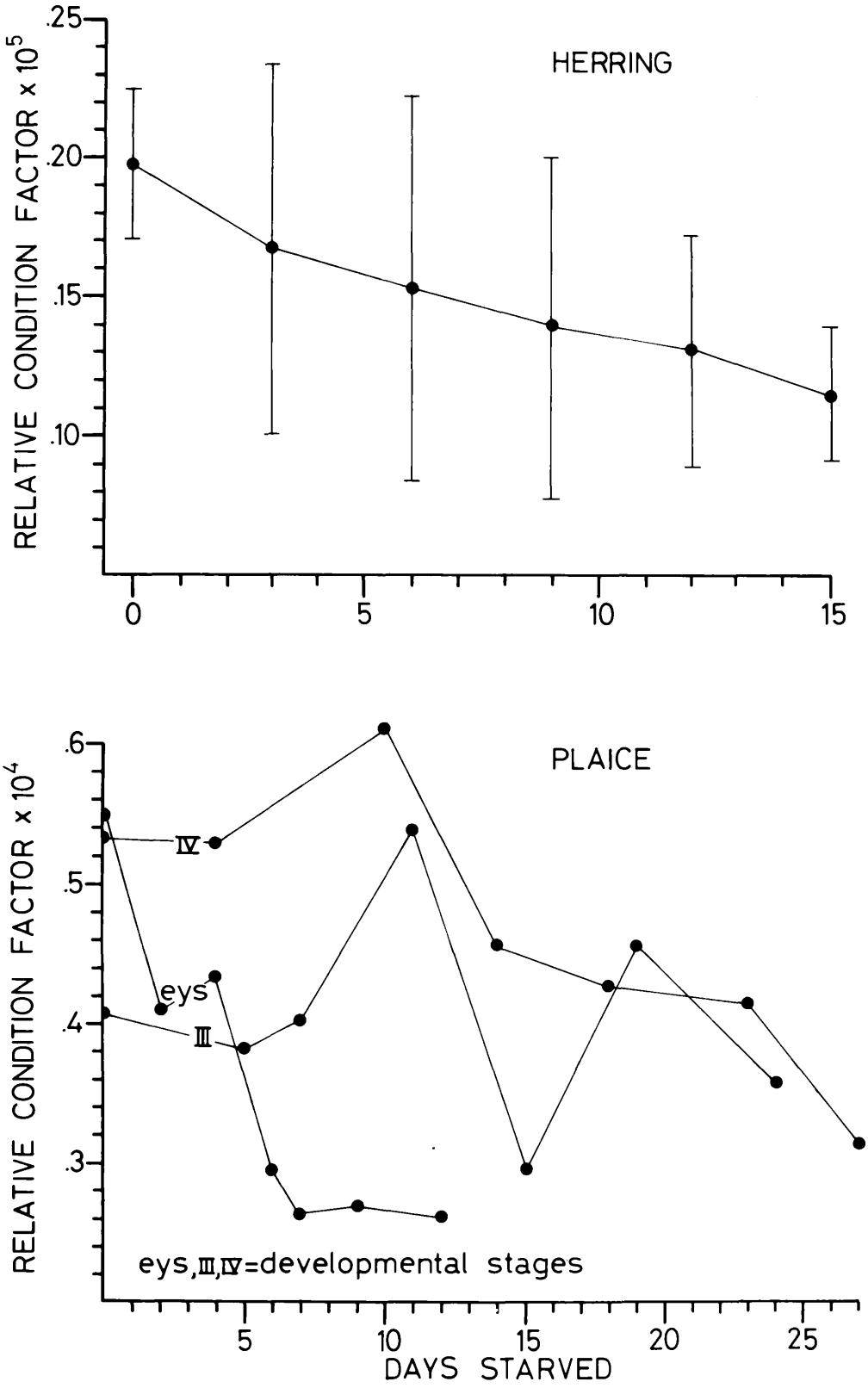
Using this relationship condition factor does not vary with size of larvae. For plaice relative condition factor changes were determined only during starvation from the end of the yolk sac stage. These values were calculated for individuals as

$$\frac{\text{weight}}{\text{length}^{3.9155}} \times 10^4 \quad \text{(Fig. 7B).}$$

Fig. 7. Relative condition factors during starvation.

A. Herring: $RCF = W/L^{4.57} \times 10^5$. Means and 95% confidence limits are shown.

B. Plaice: $RCF = W/L^{3.92} \times 10^4$. Changes are shown for different developmental stages.


HERRING
RELATIVE CONDITION FACTOR x 10⁵
.25
.20
.15
.10
0
5
10
15
PLAICE
RELATIVE CONDITION FACTOR x 10⁴
.6
.5
.4
.3
IV
eys
III
eys,III,IV=developmental stages
5
10
15
20
25
DAYS STARVED

Eye : head height ratio

During starvation of herring the height of the head decreased, and the top of it also changed in shape from convex to concave (Fig. 8). This was quantified by measuring eye height, which remained constant during starvation, as a percentage of head height. To be consistent with other analyses, this was initially plotted against length (Fig. 9A). However, the variation was extremely large and the data of eye : head height ratio during starvation was mainly limited to fish less than 25 mm, within which range it did not show good correlation with length. Therefore the eye : head ratio was plotted against age (Fig. 9B). The ratio in feeding larvae showed a slight tendency to increase with age, up to about 50 days followed by a small decrease. This could also be seen in the eye : head ratio when plotted against length. During starvation the ratio rapidly increased. The eye height as related to head height is probably of most use for assessing condition of the youngest larvae where the variation was least (Fig. 9B). Comparison of photos of fed and starved plaice showed that the eye : head ratio did not change (Fig. 10).

Fig. 8. Feeding and starved herring larvae. Both larvae are 12 days-old. The top one has been feeding, and the bottom one has been starved to the PNR. In the starved larva the top of the head as well as the gut region and the area around the pectoral girdle have shrunk.

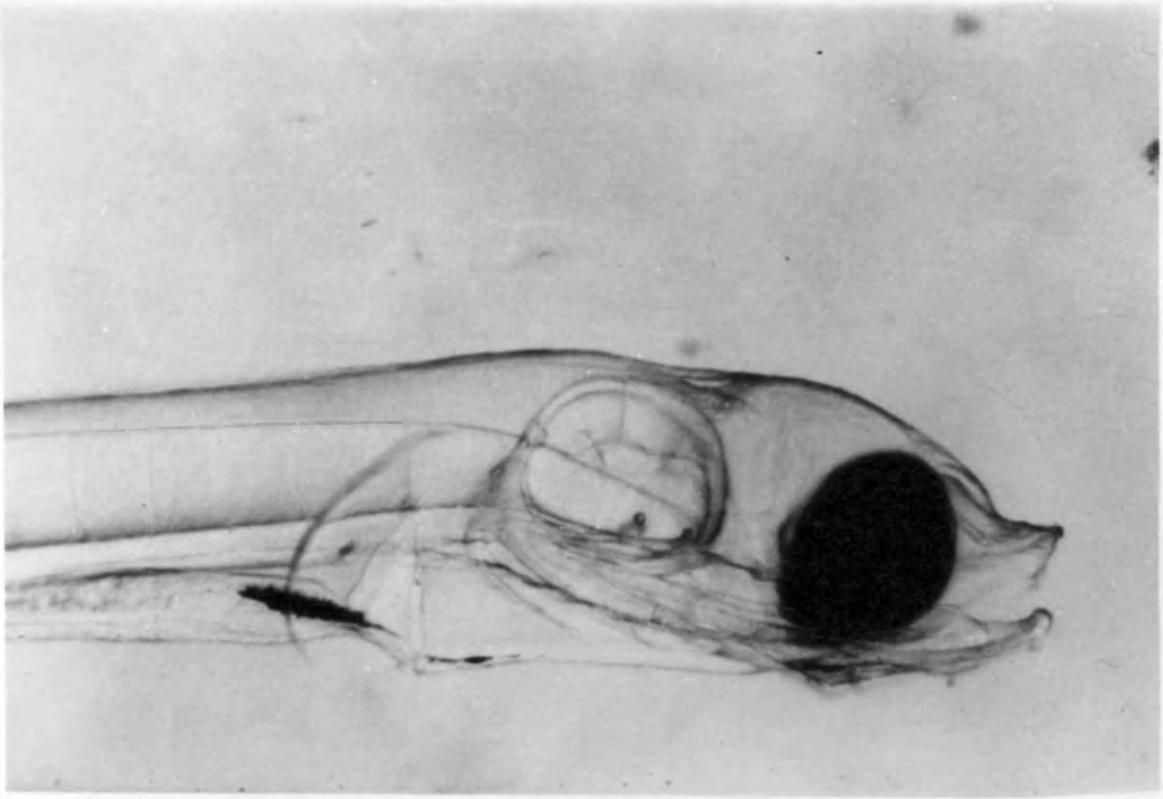


Fig. 9. Herring eye to head height ratios.

A. Eye : head height ratio versus length for feeding larvae. Representative points show the wide spread.

B. Eye : head height ratio versus age. Means and 95% confidence limits are shown for changes during growth and starvation of different age-groups of larvae.

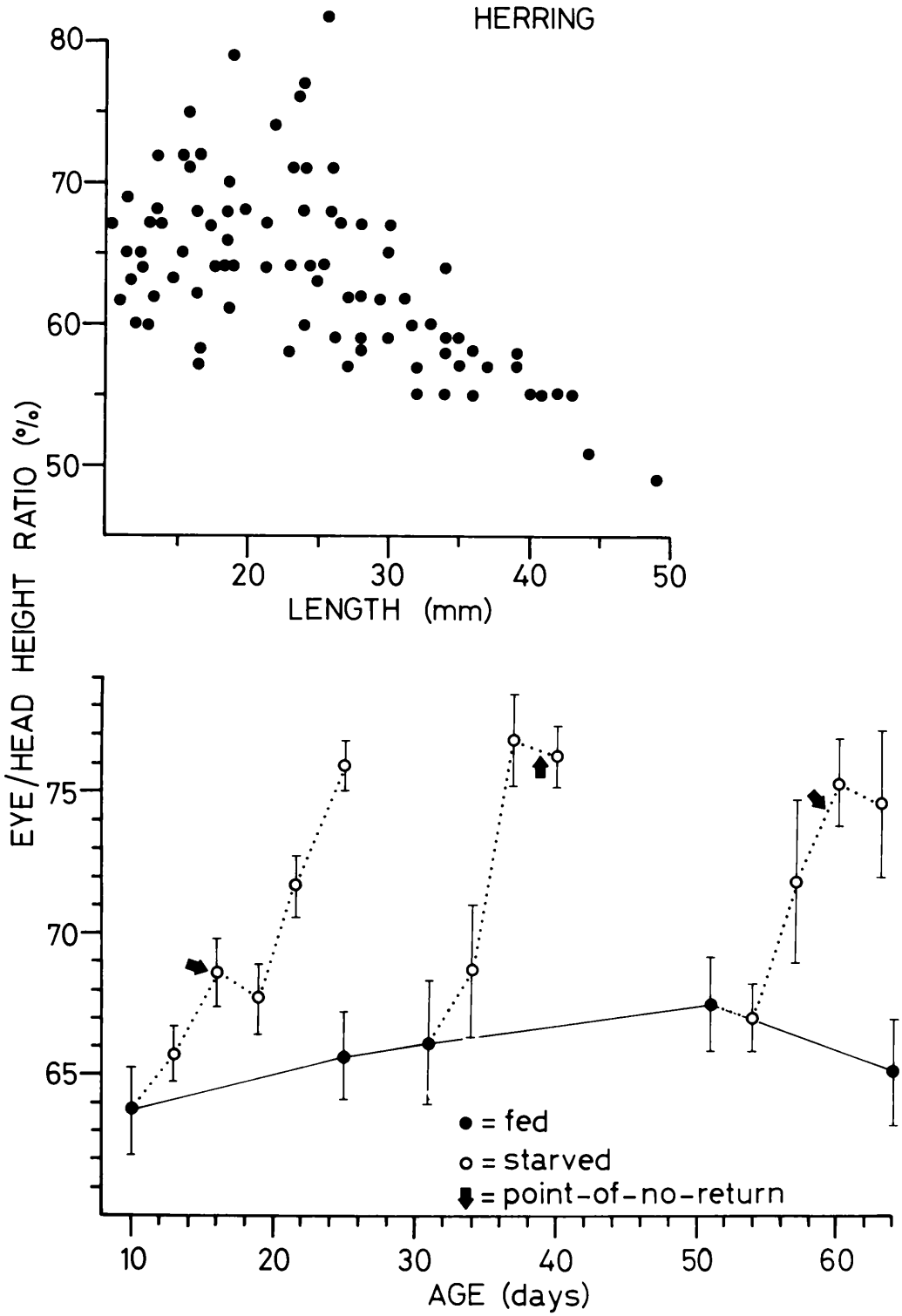
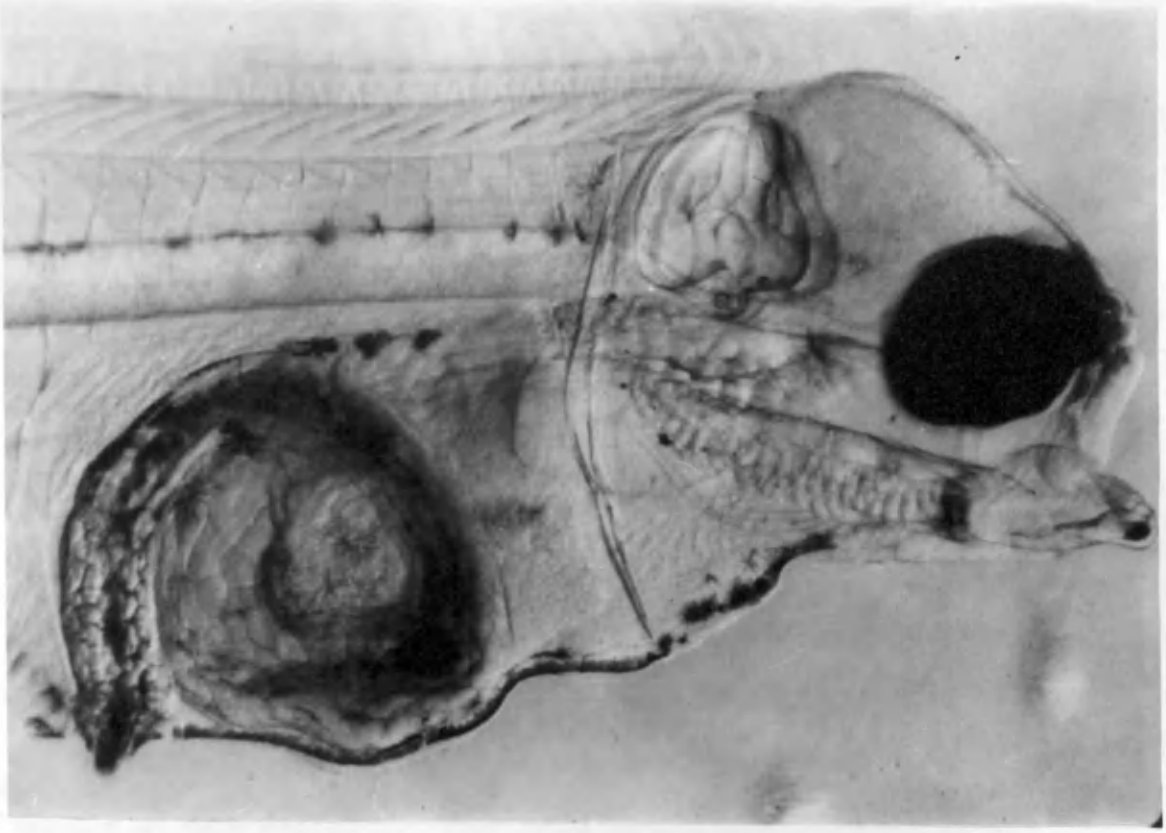
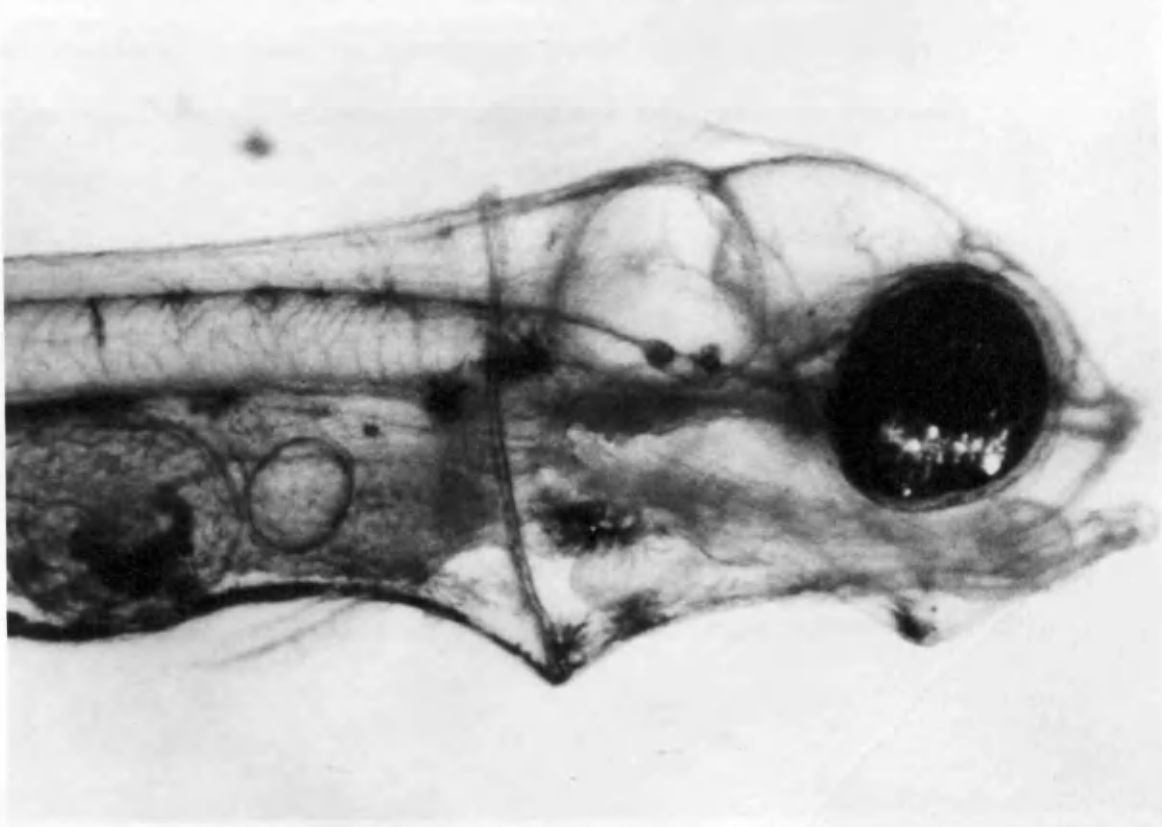

HERRING
80
70
60
50
EYE/HEAD HEIGHT RATIO (%)
20
30
40
50
LENGTH (mm)
75
70
65
● = fed
o = starved
= point-of-no-return
10
20
30
40
50
60
AGE (days)

Fig. 10. Feeding and starved plaice larvae. Both larvae are 12 days-old. The top one has been feeding, while the bottom one was starved to the PNR. In the starved one the gut and pectoral regions have shrunk, but the top of the head has not.





Sinking rate and water content

HERRING

The sinking rate of herring in sea water (33‰, 10°C.) at hatching was about 0.4 cm/sec. During the following days it decreased until by the end of the yolk sac stage it was slightly less than 0.24 cm/sec (Fig. 11A). If the herring larvae were not fed their sinking rate continued to decrease until they had a negative sinking rate, or rising rate, of 0.02 cm/sec, 12 days later. The rising rate after 12 days of starvation, of larvae in 33‰, was found by extrapolating the curve of sinking rate versus salinity, from sinking rates at 16, 20 and 27‰. The rate in 33‰ was not directly determined since the larvae were positively buoyant at this salinity. They could not be placed at the bottom of the water column, to determine their rising rate, without upsetting its stability. Sinking rates continued to decrease throughout starvation until the larvae became moribund, when they increased. Fish fed from the end of the yolk sac stage showed an increase in their sinking rate (Fig. 11A).

The salinity of the water also affected the sinking rate, for as the salinity increased the sinking rate decreased (Fig. 12A). Beyond the end of the yolk sac stage up to 25 mm changes in sinking rate of herring were plotted against length for 0, 3, 6 and 9 days of starvation as well as for moribund larvae (Fig. 13).

Fig. 11. Sinking rate and percent water versus age from hatching. Changes are shown from hatching to death from starvation. Feeding and moribund larvae, of the same age, are also shown. Each point represents a mean of at least 10 larvae.

A. Herring.

B. Plaice.

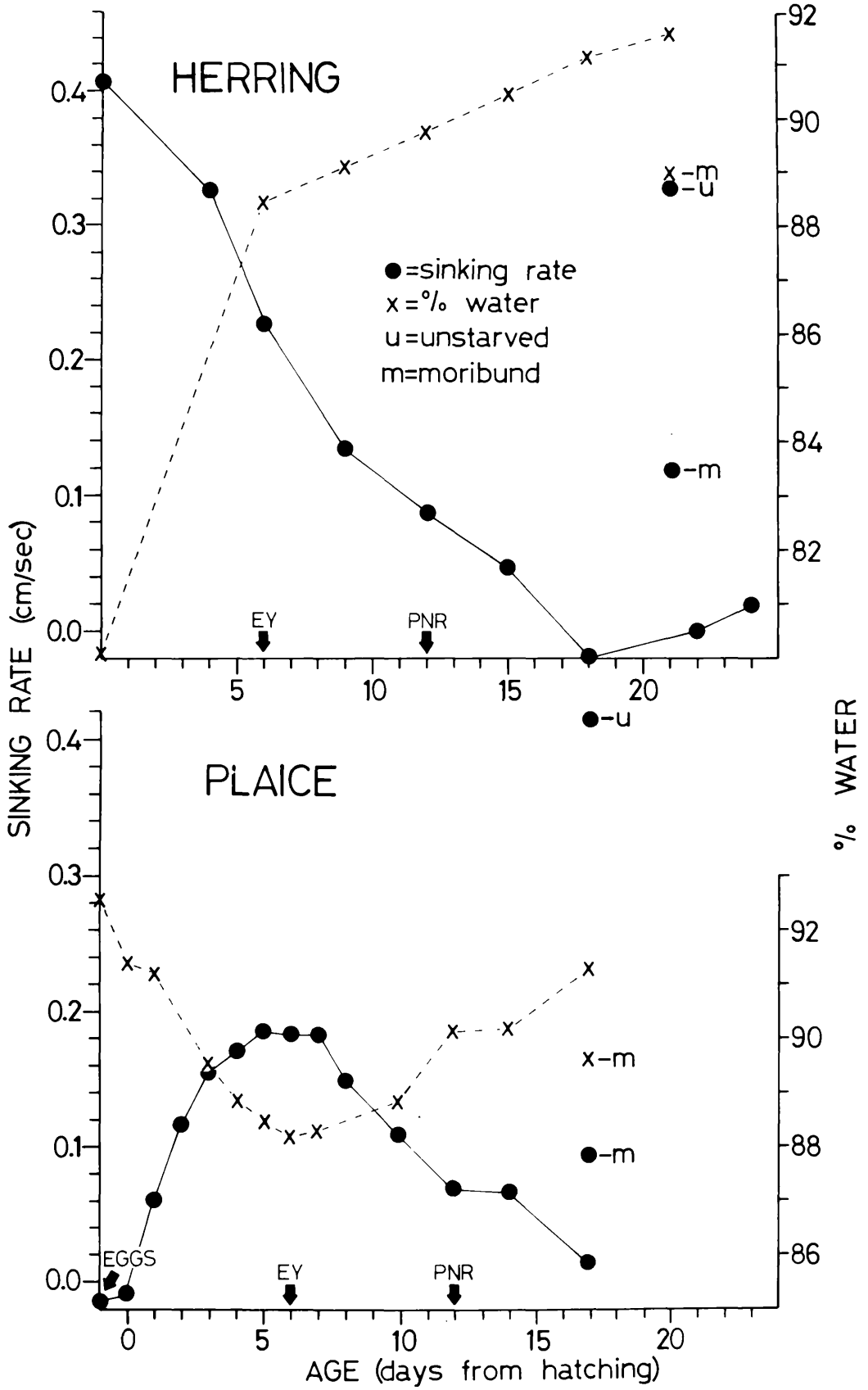

HERRING
●=sinking rate
x=% water
u=unstarved
m=moribund
x-m
●-u
●-m
●-u
EY
PNR
PLAICE
x-m
●-m
EGGS
EY
PNR
SINKING RATE (cm/sec)
% WATER
AGE (days from hatching)
0.0
0.1
0.2
0.3
0.4
82
84
86
88
90
92
0
5
10
15
20

Fig. 12. Sinking rate versus salinity. Lines are shown for larvae at hatching, the end of the yolk sac stage and the PNR. Each point represents a mean of at least 10 larvae.

A. Herring.

B. Plaice.

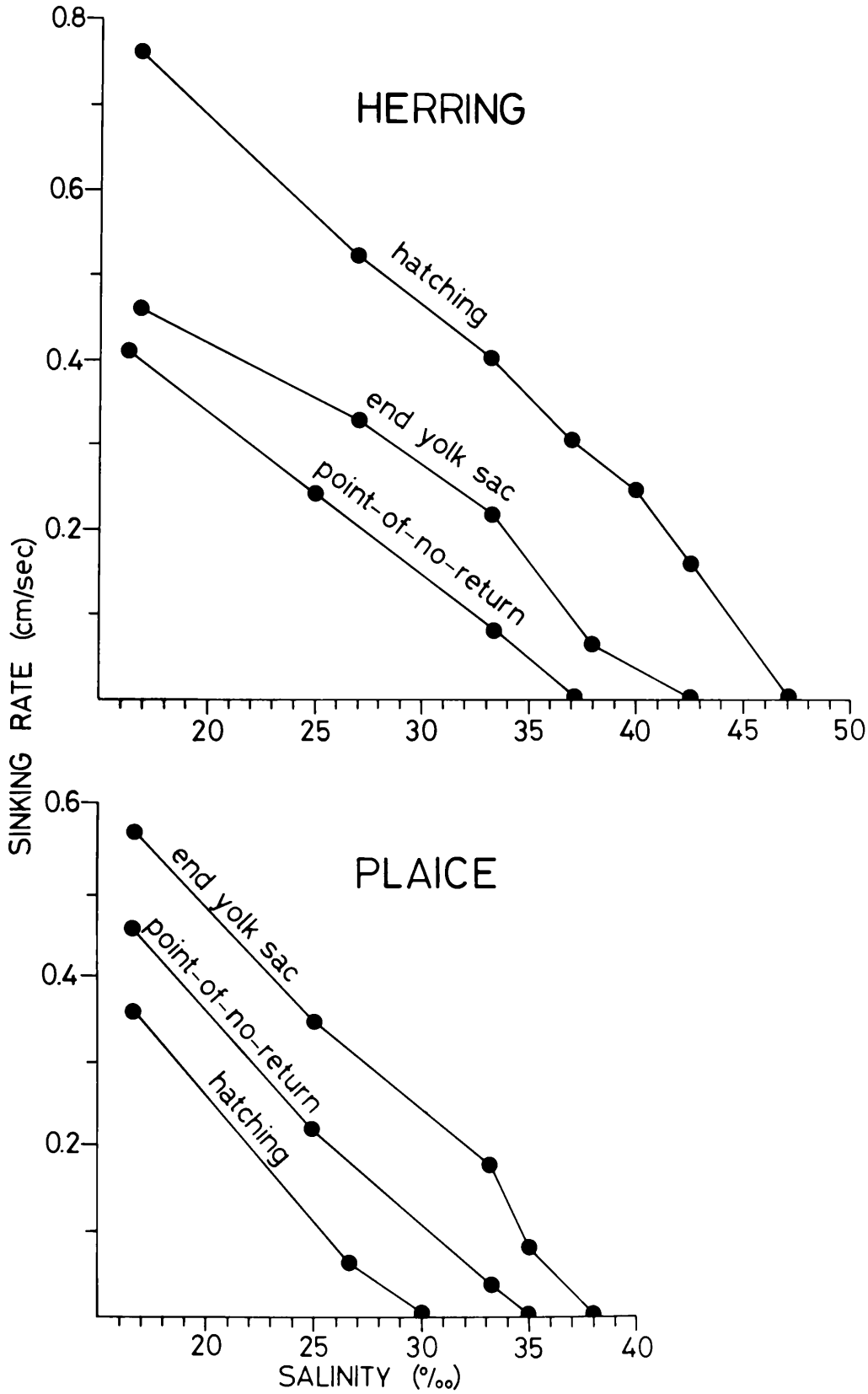
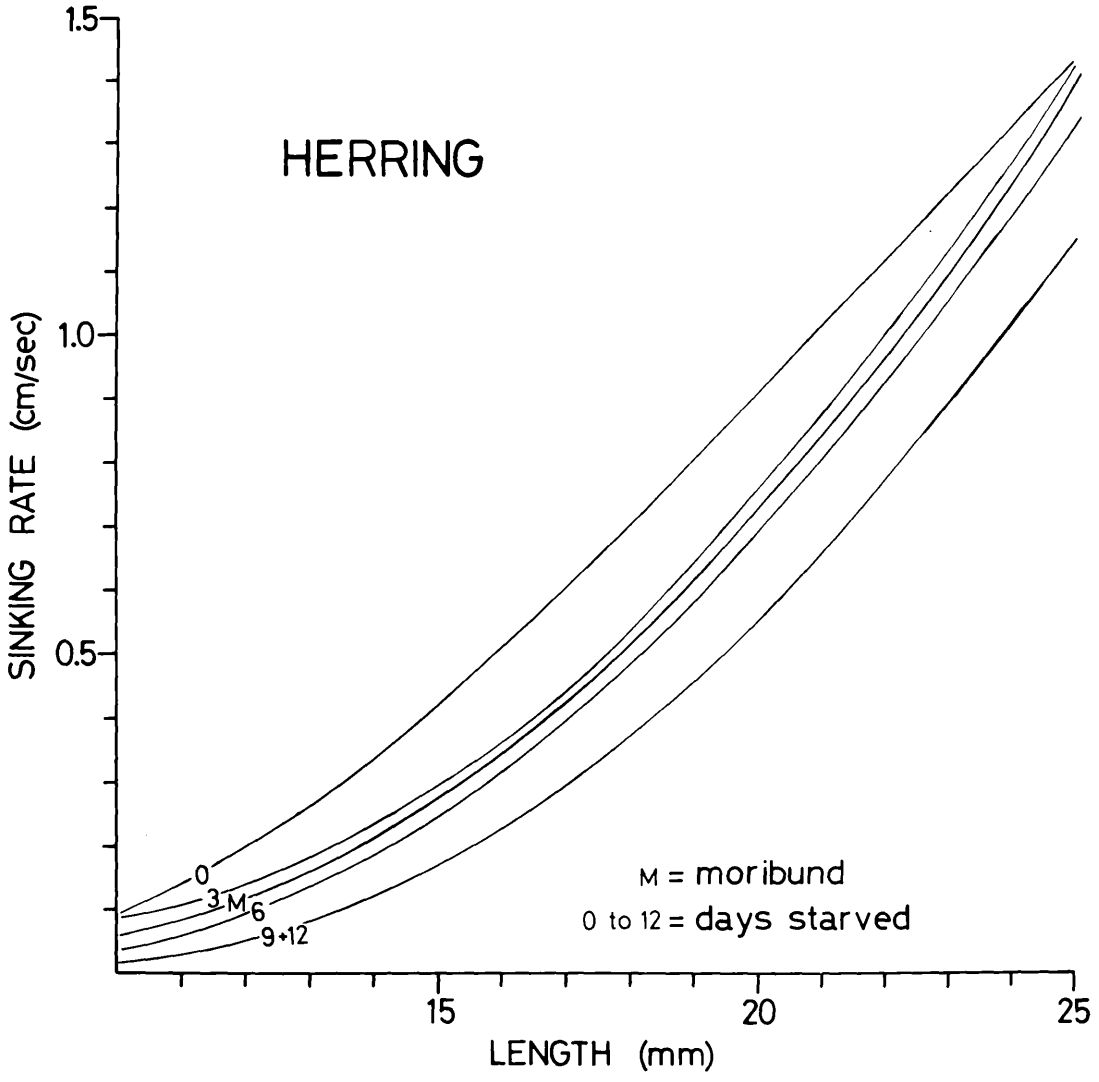

HERRING
hatching
end yolk sac
point-of-no-return
0.8
0.6
0.4
0.2
20
25
30
35
40
45
50
SINKING RATE (cm/sec)
PLAICE
end yolk sac
point-of-no-return
hatching
0.6
0.4
0.2
20
25
30
35
40
SALINITY (‰)

Fig. 13. Herring sinking rate versus length. Lines are shown for different degrees of starvation.

days starved	equation	N
0	$SR = -0.0125\ L + 0.0031\ L^2 - 0.0860$	76
3	$= -0.0848\ L + 0.0051\ L^2 - 0.4316$	27
6	$= -0.0686\ L + 0.0045\ L^2 - 0.2641$	29
9 + 12	$= -0.0798\ L + 0.0045\ L^2 - 0.3667$	38
moribund	$= -0.0753\ L + 0.0047\ L^2 - 0.3448$	38


HERRING
SINKING RATE (cm/sec)
1.5
1.0
0.5
0
3
M
6
9+12
M = moribund
0 to 12 = days starved
15
20
25
LENGTH (mm)

Water content of herring larvae increased, at least relatively, as their sinking rate declined (Table 2). Percent water increased more rapidly from hatching to the end of the yolk sac stage than during subsequent starvation. In the period prior to complete absorption of yolk there was an actual uptake of water (Table 2), while during starvation there was a loss of water, although the percentage increased. Within any one size group, during starvation, the sinking rate decreased inversely to percent water (Fig. 14A). This relationship approached linearity in the smallest herring larvae but became increasingly curvilinear in the larger ones. The relationship between sinking rate and percent water for larvae of all sizes was not constant during starvation but varied at each day sampled (Fig. 14A). The relationship was curvilinear, with the differences between days starved increasing with decreasing water content. Moribund larvae had a lower sinking rate for a given amount of water than any of the other larvae. This did not agree with the general pattern of increasing starvation, of increasing sinking rate for any given percentage of water.

Fig. 14. Sinking rate versus percent water.

A. Herring: Solid lines join different days of starvation or moribund condition for larvae of the same size. Broken lines join different sizes of larvae starved for the same number of days or moribund.

B. Plaice: Lines are shown for larvae from hatching to the end of the yolk sac stage and then during starvation.

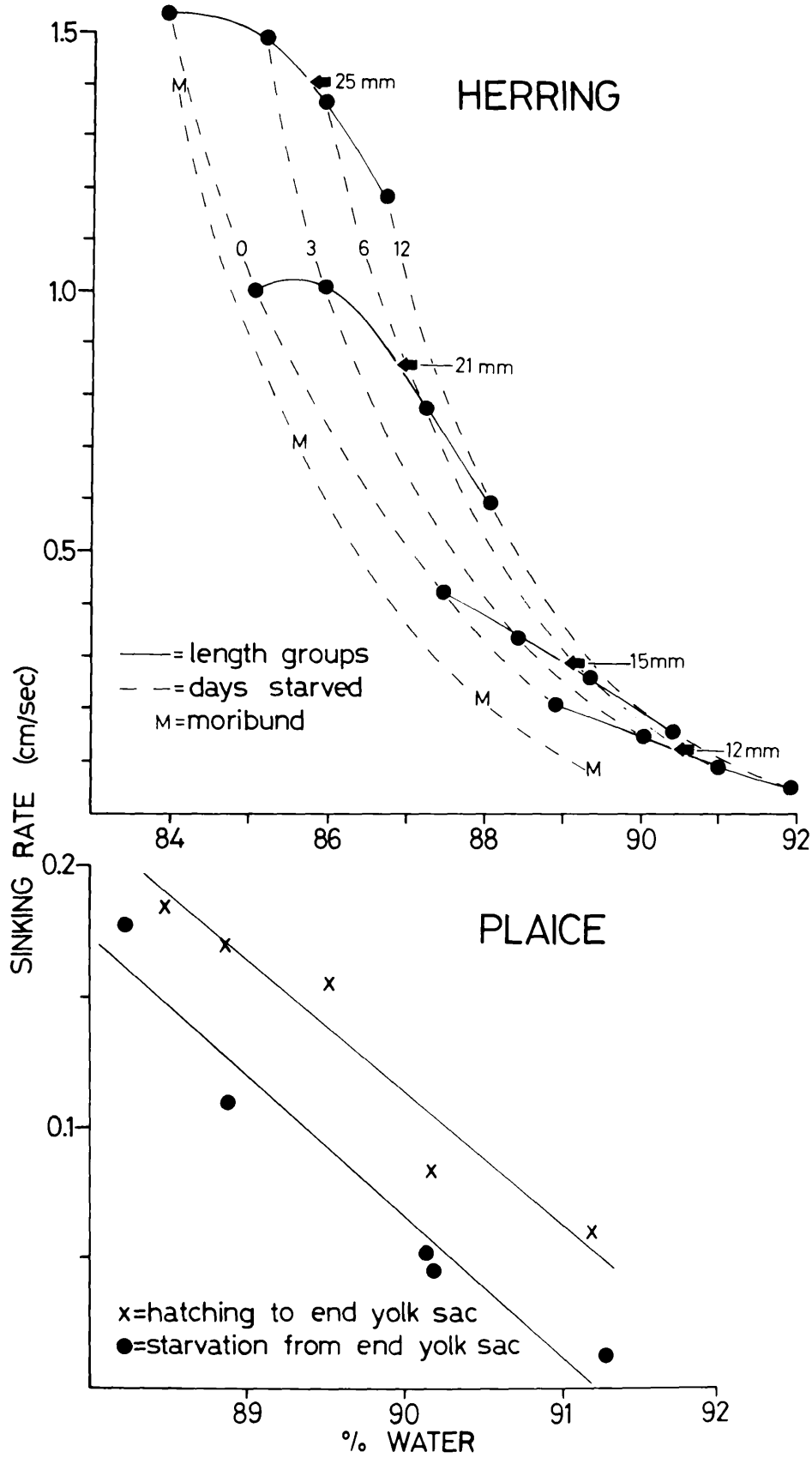

HERRING
1.5
1.0
0.5
25 mm
21 mm
15mm
12mm
M
0
3
6
12
= length groups
= days starved
M = moribund
84
86
88
90
92
SINKING RATE (cm/sec)
PLAICE
0.2
0.1
x=hatching to end yolk sac
●=starvation from end yolk sac
89
90
91
92
% WATER

Table 2. Herring and plaice water contents and sinking rates from hatching.

Age (Days)	Days starved	weight (mg) wet	dry	% water	mg water	Sinking rate (cm/sec)
	H E R R I N G					
Hatching	0	1.1394	0.2227	80.00	0.9116	0.407
6	0	1.3744	0.1586	88.46	1.2158	0.225
9	3	1.3501	0.1473	89.09	1.2028	0.132
12	6	1.2680	0.1301	89.74	1.1379	0.087
15	9	1.3110	0.1254	90.44	1.1863	0.047
18	12	1.1876	0.1051	91.15	1.0825	-0.020
21	15	1.1248	0.0946	91.59	1.0302	0.000
Moribund	15	0.8296	0.0920	88.81	0.7356	0.120
	P L A I C E					
Eggs	0	2.7139	0.2030	92.52	2.5109	-0.012
Hatching	0	1.7373	0.1508	91.32	1.3761	-0.008
1	0	1.6055	0.1408	91.23	1.4647	0.060
2	0	1.4898	0.1463	90.18	1.3435	0.117
3	0	1.3718	0.1439	89.51	1.2279	0.155
4	0	1.2203	0.1407	88.86	1.0796	0.170
5	0	1.1827	0.1318	88.47	1.0509	0.185
6	1	1.0761	0.1272	88.18	0.9489	0.182
7	2	1.0885	0.1279	88.25	0.9606	0.182
8	3					0.149
10	5	0.9666	0.1076	88.87	0.8590	0.109
12	7	0.9635	0.0950	90.14	0.8685	0.046
14	9	0.9342	0.0918	90.17	0.8424	0.045
17	12	0.9701	0.0845	91.29	0.8856	0.013
Moribund	12	0.7953	0.0826	89.61	0.7127	0.094
17	0	2.1593	0.2868	86.72	1.8725	0.419
31	0					0.949
38	0					1.120

PLAICE

Plaice eggs as well as the newly hatched larvae were positively buoyant. These had negative sinking rates or rising rates of 0.012 and 0.008 cm/sec respectively, which were extrapolated, as for the herring from sinking rates at 16, 20 and 27‰. The sinking rate increased from hatching to 0.182 cm/sec by the end of the yolk sac stage (Fig. 11B). Larvae subsequently deprived of food decreased in sinking rate, approaching neutral buoyancy 10 days later. Moribund larvae sank faster. Plaice larvae feeding from the end of the yolk sac stage showed a rapid increase in sinking rate with age (Fig. 11B). In plaice, as in herring, percent water was inversely related to sinking rate (Fig. 14B). The relative proportion of water in the larvae decreased from hatching through the yolk sac stage and with continued growth, but if larvae were deprived of food the percentage of water in them increased. The decrease in water up to the end of the yolk sac stage represented an actual loss of water, while the increase during starvation was only relative, since the actual amount of water decreased in this period (Table 2). The relationships between percent water and sinking rate of plaice before and after

depletion of yolk were quite different (Fig. 14B). Although the relationship from hatching did not appear to be linear, if in this instance linearity was assumed, the lines were found to be different by a highly significant amount ($F > 61$; 2, 6 df; $p < 0.01$).

Feeding behaviour

The number of days of starvation to the point when 50% of the population was too weak to feed is shown in Table 3. These values were based on visual estimates of the percentage of the population hanging head down in the water. Changes in the percentage of herring capable of feeding, based on feeding experiments, during starvation of 30 and 50 day-old larvae are shown in Fig. 15. There was excellent agreement between experimental and visual estimates. The time to reach the PNR increased with the size of the larvae for both species. Plaice were able to feed after longer periods of starvation than herring (Table 3).

Fig. 15. Percent of initial number of feeding larvae versus days starved. Lines are shown for two age groups. PNR is indicated by 50% feeding.

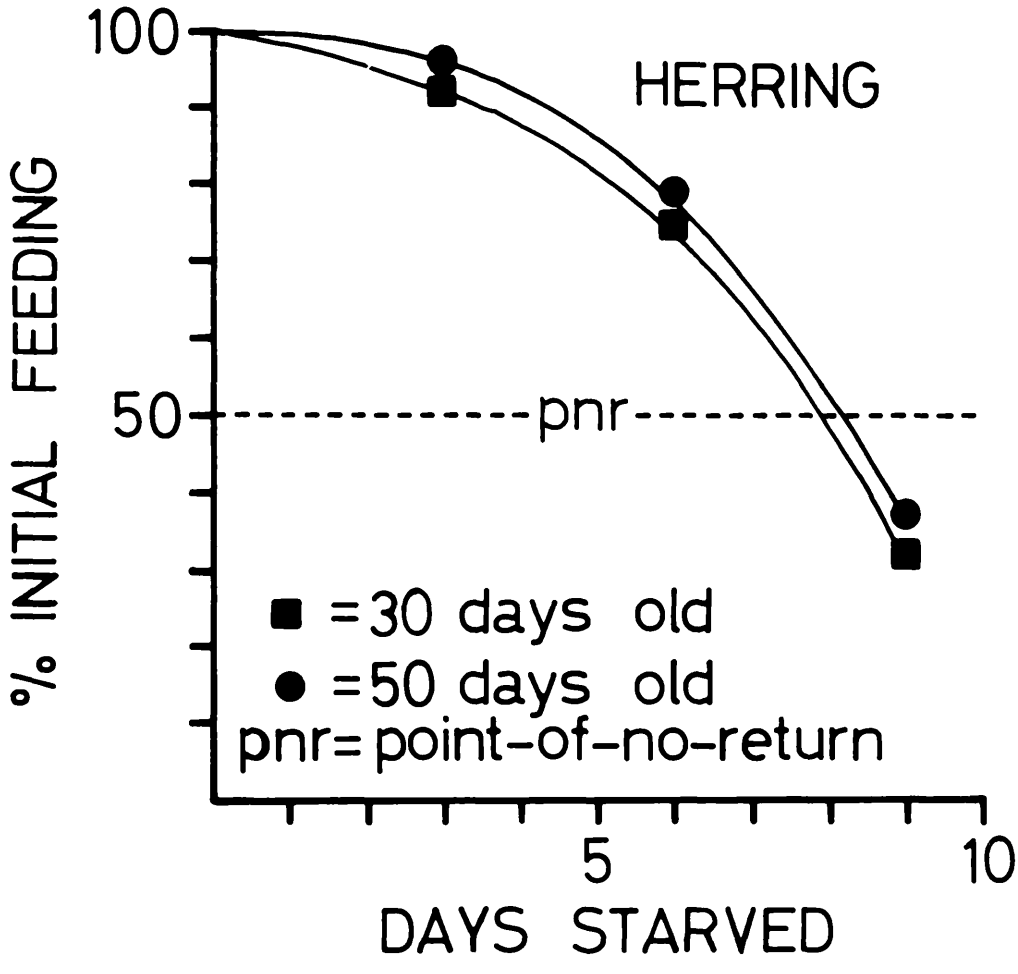

100
50
% INITIAL FEEDING
HERRING
pnr
■ =30 days old
● =50 days old
pnr= point-of-no-return
5
10
DAYS STARVED

Table 3. Days to point-of-no-return.

	Age (days) and/or developmental stage	Days to PNR
HERRING	6 end of yolk sac	6
	30	8
	50	8
	74	12
	88	15
PLAICE	End yolk sac	6
	stage 3	15
	stage 4	23

Chemistry

GENERAL NOTES

HERRING: The results of the chemical analyses on herring larvae have been treated in several ways. First of all, the percentage of the dry weight of each constituent was plotted against length for each day starved (Fig. 17). The equations and significance of the regression lines for water, triglyceride, carbohydrate, nitrogen, ash and carbon are shown in Appendices 3 to 8. Where the lines or slopes were found to be statistically indistinguishable, pooled estimates were made of the appropriate parameters to obtain the best estimates for the regression lines. These best estimates were then used to find the changes in the six standard-sized animals during starvation; the ages of the six standard sizes of herring larvae (12, 15, 21, 25, 30 and 35 mm) were taken from the growth rate data (Fig. 5A). The changes in percent composition of these different sized herring larvae during starvation as well as the changes in the percent of the unstarved amount were plotted against days (Figs 18 and 19, respectively). In addition to this the actual amounts of each constituent in the six standard sizes of herring larvae are listed in Appendix 1A.

Fig. 16. Herring percent water versus days starved for six standard sizes of larvae. The solid line shows ontogenetic changes while the broken ones delineate starvation. The PNR has been indicated when it occurred within the days of starvation shown in the figures. The age of the larvae at the start of each starvation is the age at the standard lengths taken from the growth rate (Fig. 5A).

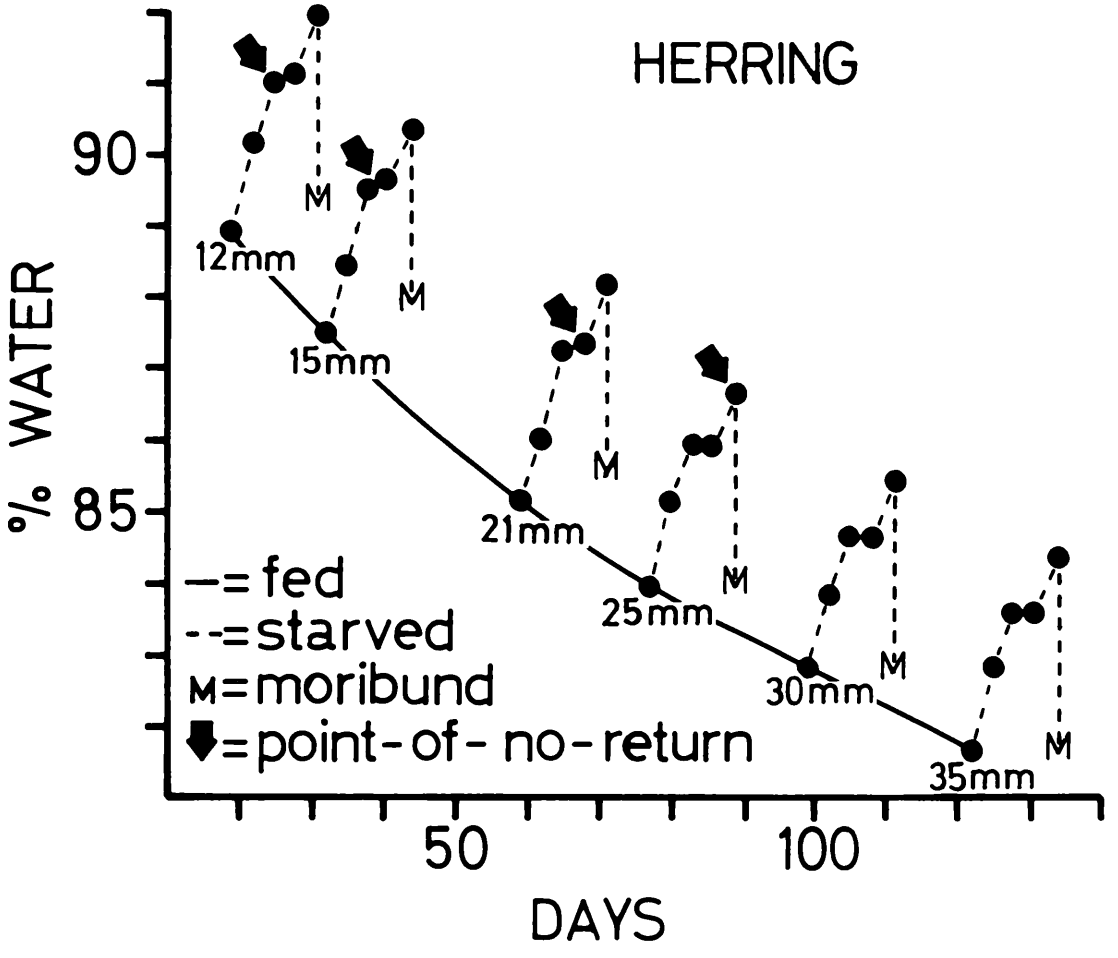

HERRING
% WATER
90
85
12mm
15mm
21mm
25mm
30mm
35mm
M
—= fed
--=starved
M=moribund
=point-of-no-return
50
100
DAYS

Fig. 17. Herring composition in percent of dry weight versus length. Composition is shown for fed and starved larvae. The numbers within each figure indicate the number of days of starvation.

A. Triglyceride: A concave quadratic relationship with length was found from 10 to 27 mm in length, but in larger larvae triglyceride decreased in a linear fashion. In both groups significant differences were detected during starvation. The equations and other parameters of the relationships are shown in Appendix 4.

B. Carbohydrate: In unstarved larvae three linear relationships were found with length: from 10 to 18 mm, carbohydrate increased; from 18 to 29 mm in length it decreased and then levelled off in the larger larvae. During starvation carbohydrate did not vary with larval size. Significant changes occurred during starvation. Equations and other parameters of the relationships are shown in Appendix 5.

C. Nitrogen: An increasing linear relationship was found for all larvae up to a length of 21 mm. In larger larvae percent nitrogen decreased as a logarithmic function of length. No significant changes were detected during starvation. Equations and other parameters of the relationships are shown in Appendix 6.

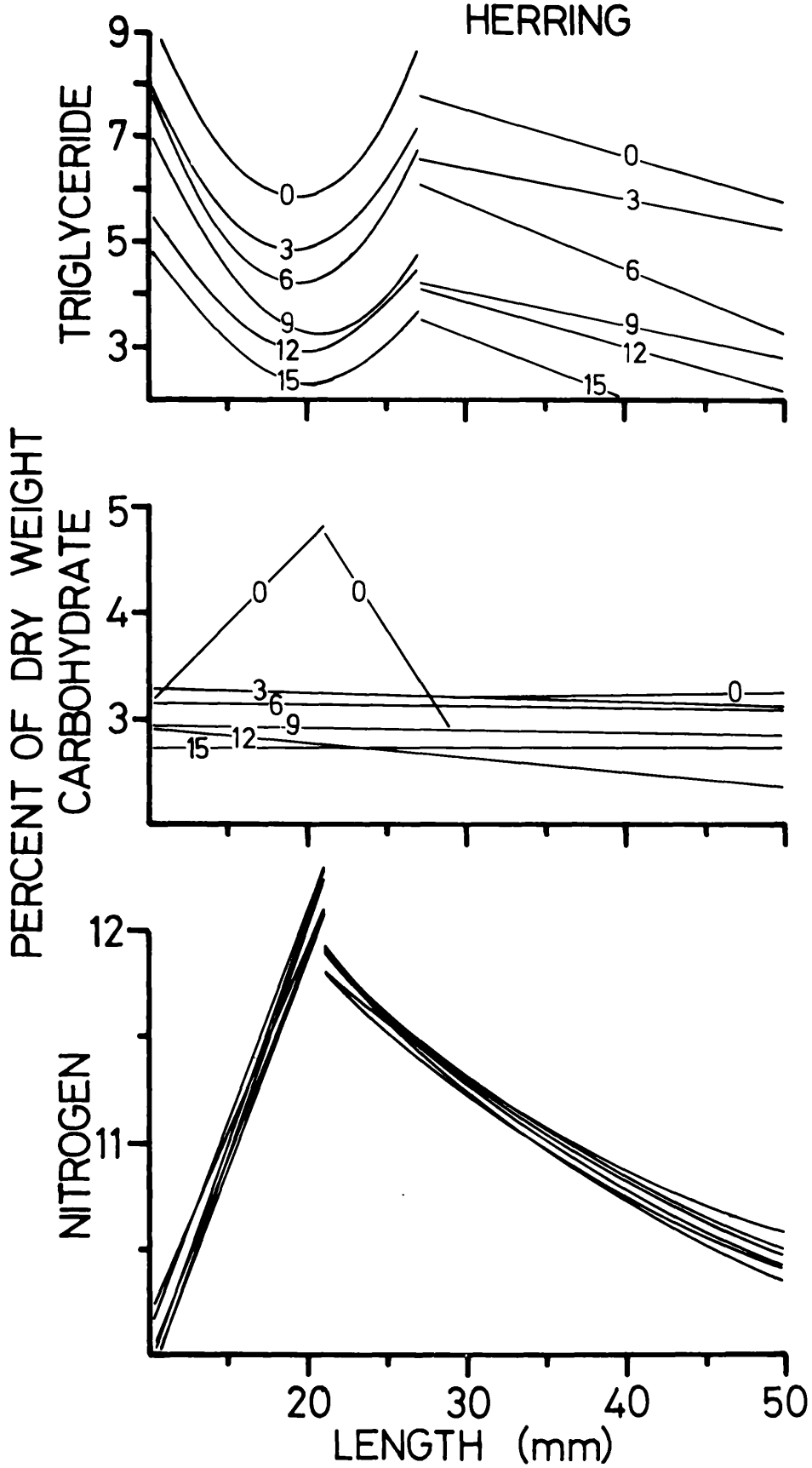

HERRING
TRIGLYCERIDE
9
7
5
3
0
3
6
9
12
15
0
3
6
9
12
15
PERCENT OF DRY WEIGHT
CARBOHYDRATE
5
4
3
0
0
3
6
9
12
15
0
NITROGEN
12
11
20
30
40
50
LENGTH (mm)

Fig. 17. (Continued).

D. Ash: From 10 to 25 mm in length percent ash did not change during growth, but it increased in larger larvae. Significant increases occurred during starvation. Equations and other parameters of the reltionships are given in Appendix 7.

E. Carbon: From 10 to 18 mm in length percent carbon increased with increasing larval length, but the relationship levelled off in larger larvae. Significant decreases occurred during starvation. Equations and other parameters of the relationships are given in Appendix 8.

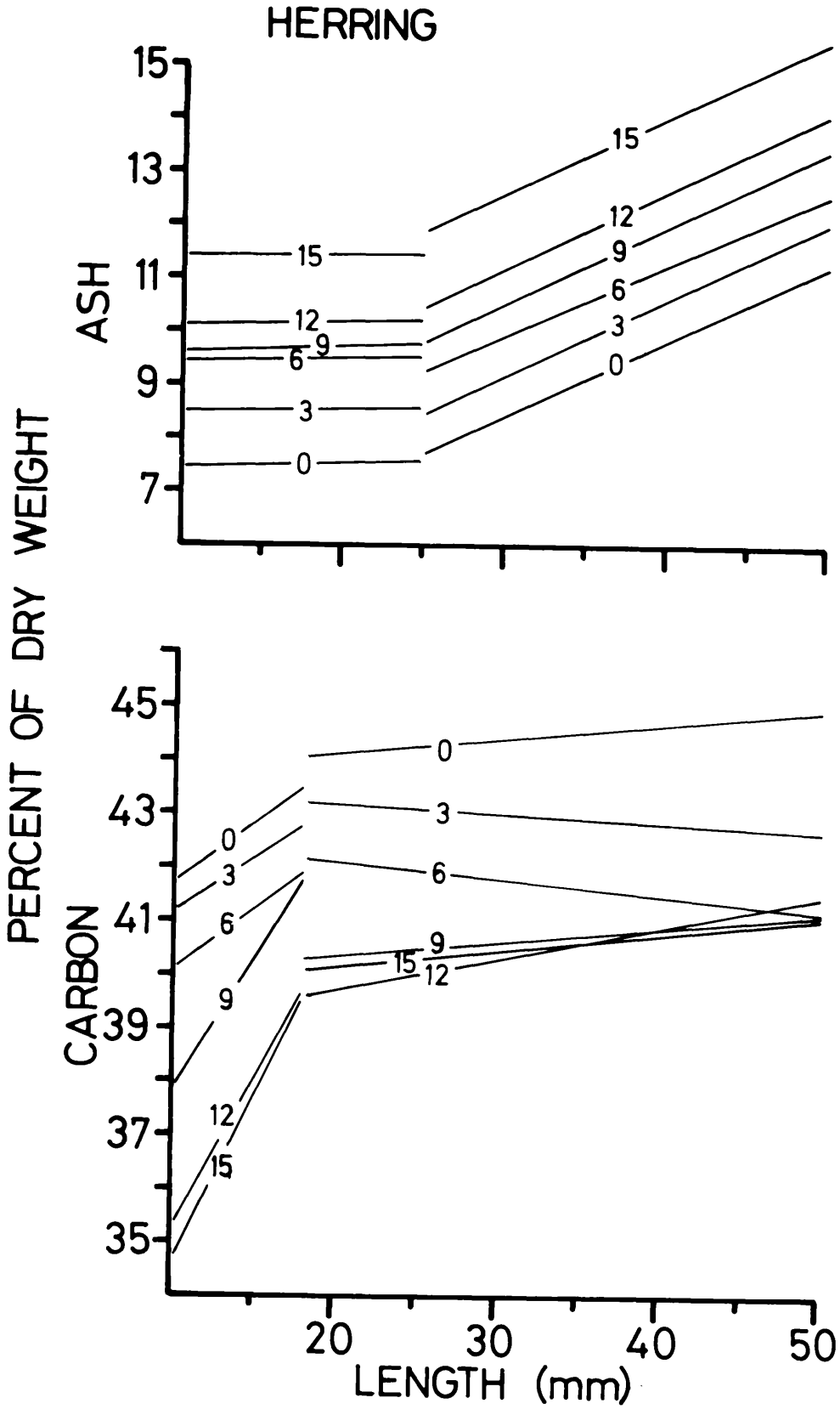

HERRING
PERCENT OF DRY WEIGHT
ASH
15
13
11
9
7
15
12
9
6
3
0
CARBON
45
43
41
39
37
35
0
3
6
9
12
15
20
30
40
50
LENGTH (mm)

Fig. 18. Composition of standard sized herring larvae during growth and starvation. Solid lines show ontogenetic changes; dotted ones delineate starvation. Open circles show composition after 3, 6, 9, 12 and 15 days of starvation. The PNR has been indicated when it occurred within the days of starvation shown in the figures. The age of the standard lengthed larvae, at the start of each starvation, was taken from the growth rate (Fig. 5A).

A. Triglyceride.

B. Carbohydrate.

C. Nitrogen.

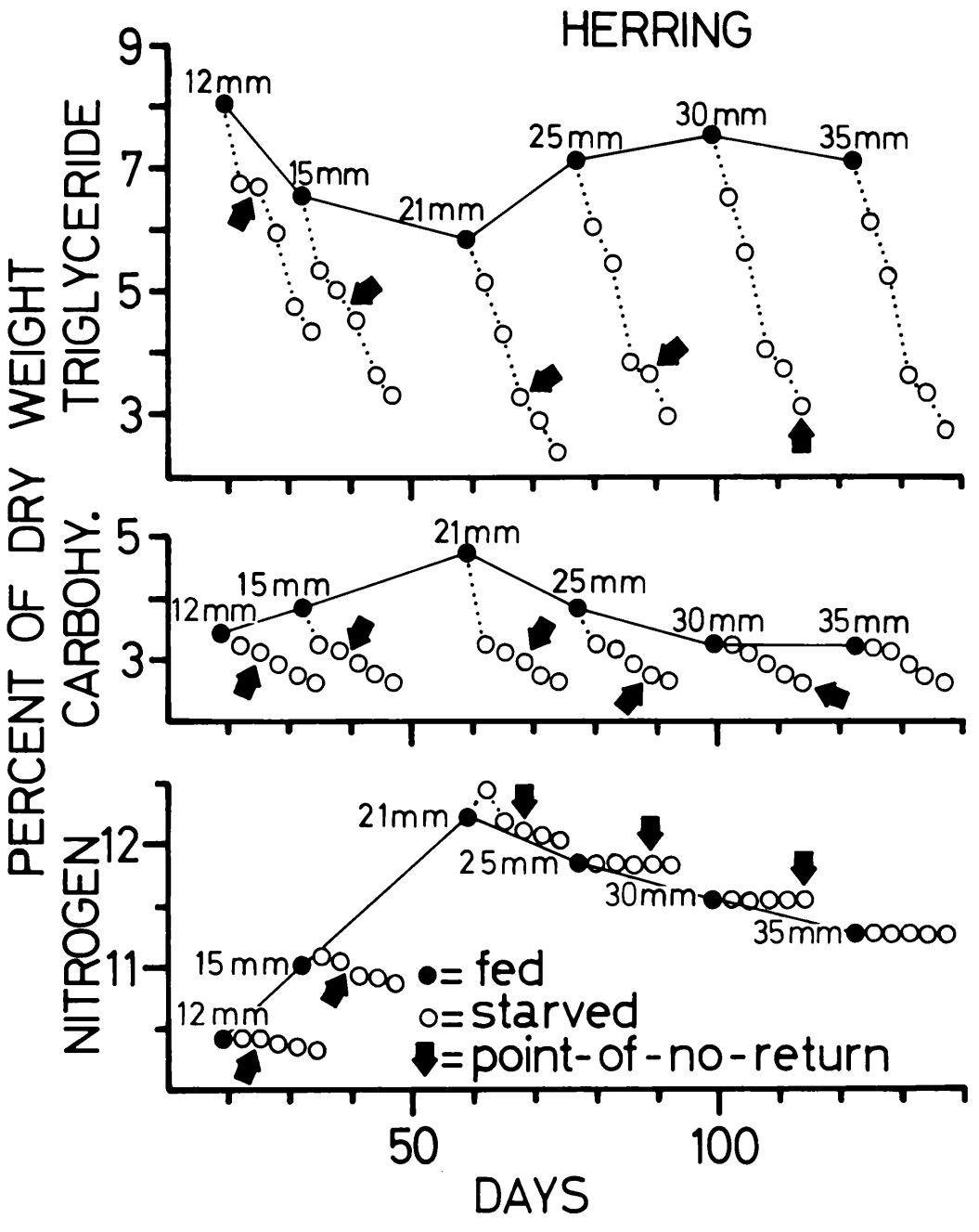

HERRING
PERCENT OF DRY WEIGHT
TRIGLYCERIDE
9
7
5
3
12mm
15mm
21mm
25mm
30mm
35mm
CARBOHY.
5
3
21mm
15mm
12mm
25mm
30mm
35mm
NITROGEN
12
11
21mm
25mm
30mm
35mm
15mm
12mm
●= fed
○= starved
⬇= point-of-no-return
50
100
DAYS

Fig. 18. (Continued).

D. Ash.

E. Carbon.

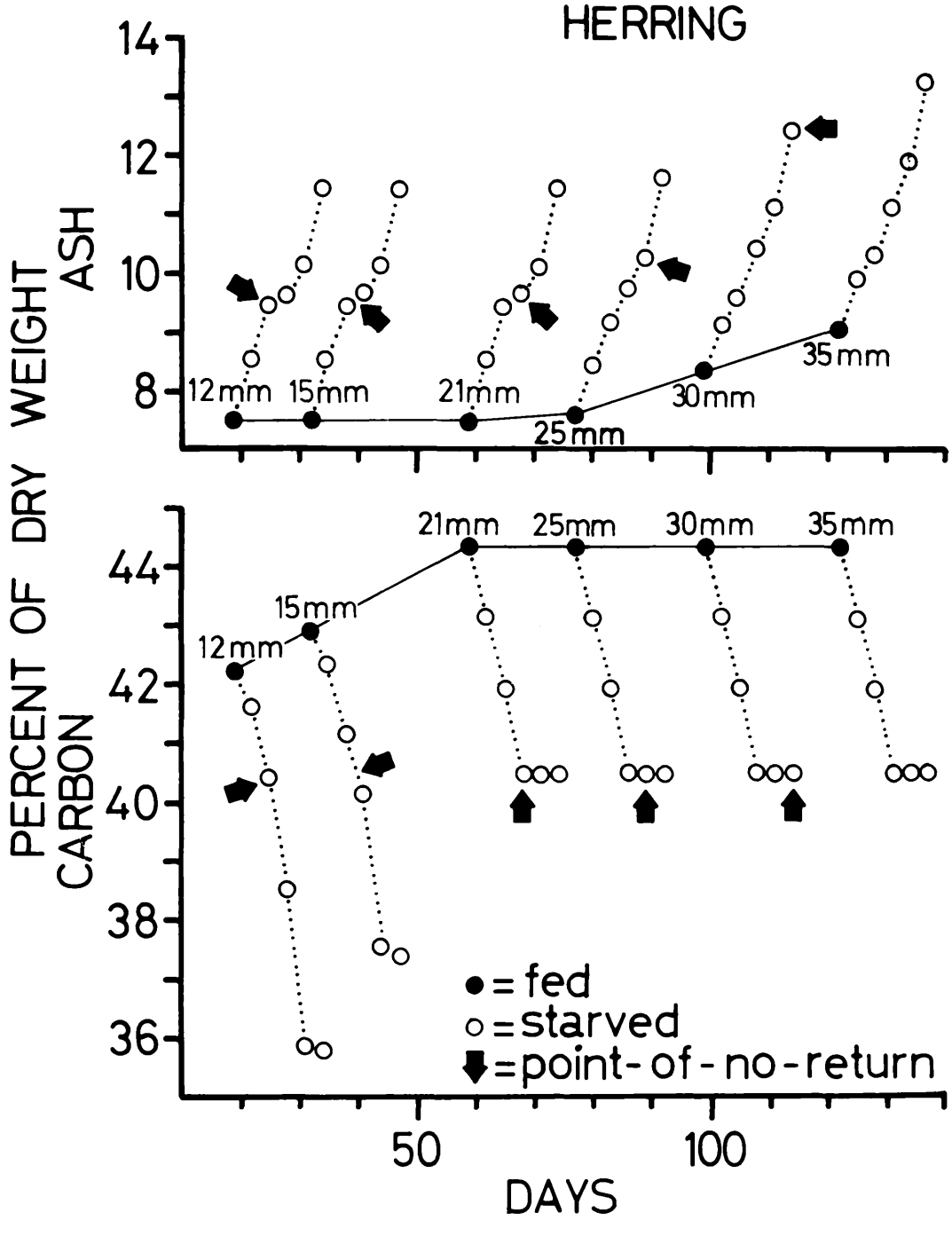

HERRING
PERCENT OF DRY WEIGHT
ASH
CARBON
14
12
10
8
44
42
40
38
36
12mm
15mm
21mm
25mm
30mm
35mm
● = fed
○ = starved
▼ = point-of-no-return
50
100
DAYS

Fig. 19. Percent of unstarved amount versus days starved for standard sizes of herring larvae. Composition is shown after 3, 6, 9, 12 and 15 days of starvation. Dotted lines connect each of these days between consecutive sizes of larvae to show changes in rates of depletion with growth. The PNR has been indicated when it occurred within the days of starvation shown in the figures. The age of the standard lengthed larvae, at the start of each starvation, was taken from the growth rate (Fig. 5A).

A. Triglyceride.

B. Carbohydrate.

C. Nitrogen.

D. Ash.

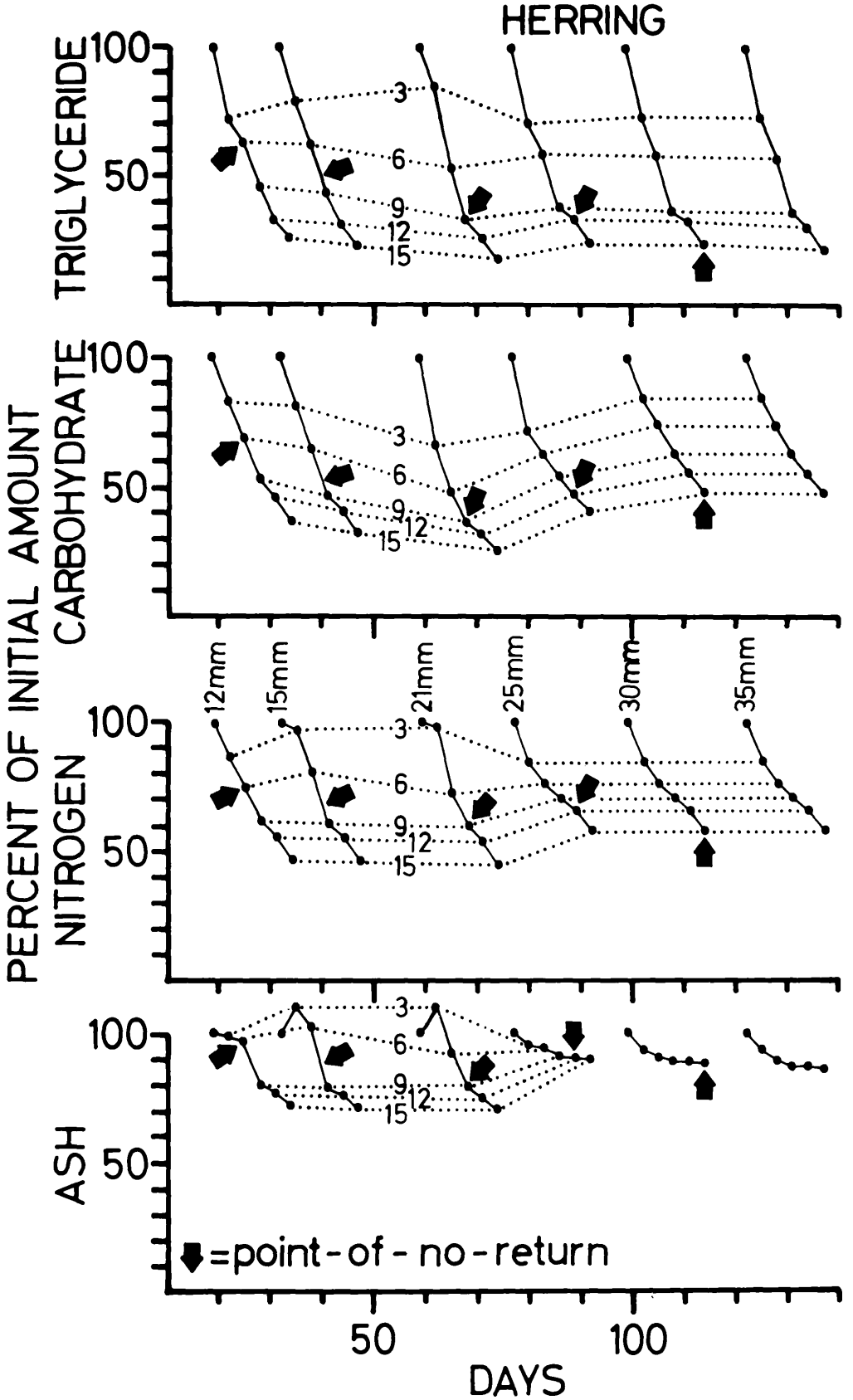

HERRING
TRIGLYCERIDE
CARBOHYDRATE
NITROGEN
ASH
PERCENT OF INITIAL AMOUNT
100
50
3
6
9
12
15
12mm
15mm
21mm
25mm
30mm
35mm
=point-of-no-return
50
100
DAYS

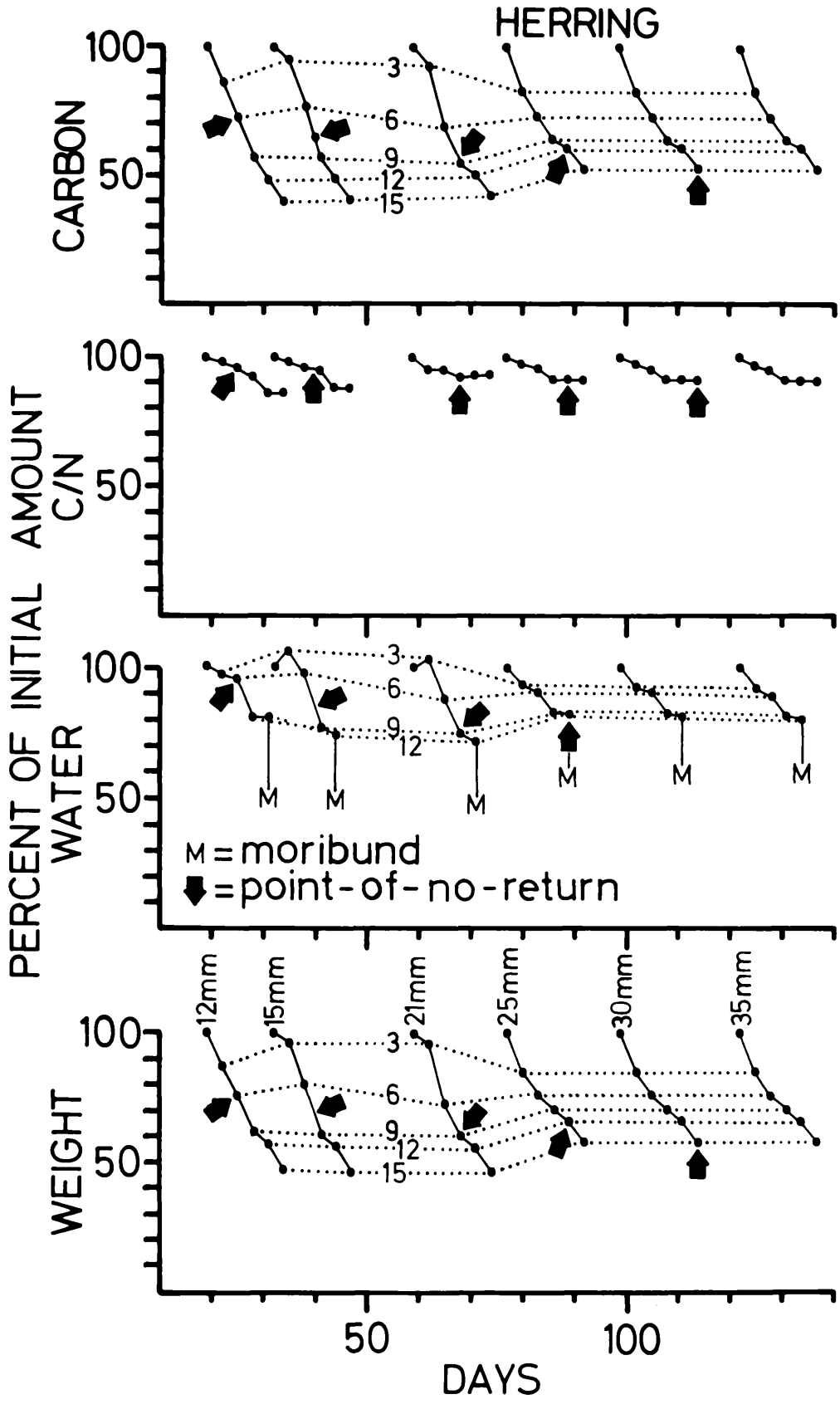
Fig. 19. (Continued).

E. Carbon.

F. Carbon/nitrogen ratio.

G. Water.

H. Weight.


HERRING
CARBON
100
50
3
6
9
12
15
C/N
WATER
M
M = moribund
= point-of-no-return
WEIGHT
12mm
15mm
21mm
25mm
30mm
35mm
PERCENT OF INITIAL AMOUNT
50
100
DAYS

PLAICE: Plaice were handled slightly differently. These larvae were initially sampled according to developmental stages so that it was not necessary to separate them further according to sizes, as was done for herring. The actual age of the plaice, at time of sampling, was used and not that taken from the growth rate as for herring. Their composition in percentage of dry weight and in the percentage of the unstarved amount, plotted against days, are shown in Figs 20 and 21, respectively. In each of these figures the composition changes during starvation are shown for larvae at the end of the yolk sac stage and stages 3, 4 and 5. In Fig. 20 the percent composition of unstarved larvae at stage 2 and just after metamorphosis are also included.

In all chemical analyses on both species of larvae the effect of MS 222 upon the results was undetectable.

HERRING COMPOSITION

WATER: The relationship between percent water and length of herring larvae was a negative logarithmic function [redacted] (Fig. 4A and Appendix 3). At any length, percent water increased during starvation over the first 12 days; no differences were distinguishable, however, from 6 to 9 days starved. When the

Fig. 20. Plaice composition as percent of dry weight during growth and starvation. Solid lines show changes during growth, connecting points (from left to right) for larvae at the end of the yolk sac stage, stages 2, 3, 4 and 5 and 10 days past metamorphosis. Broken and dotted lines show changes during starvation of larvae at the end of the yolk sac stage and stages 3, 4 and 5. The PNR has been indicated when it occurred within the days of starvation shown in the figures. Actual larval ages are shown.

A. Triglyceride.

B. Carbohydrate.

C. Nitrogen

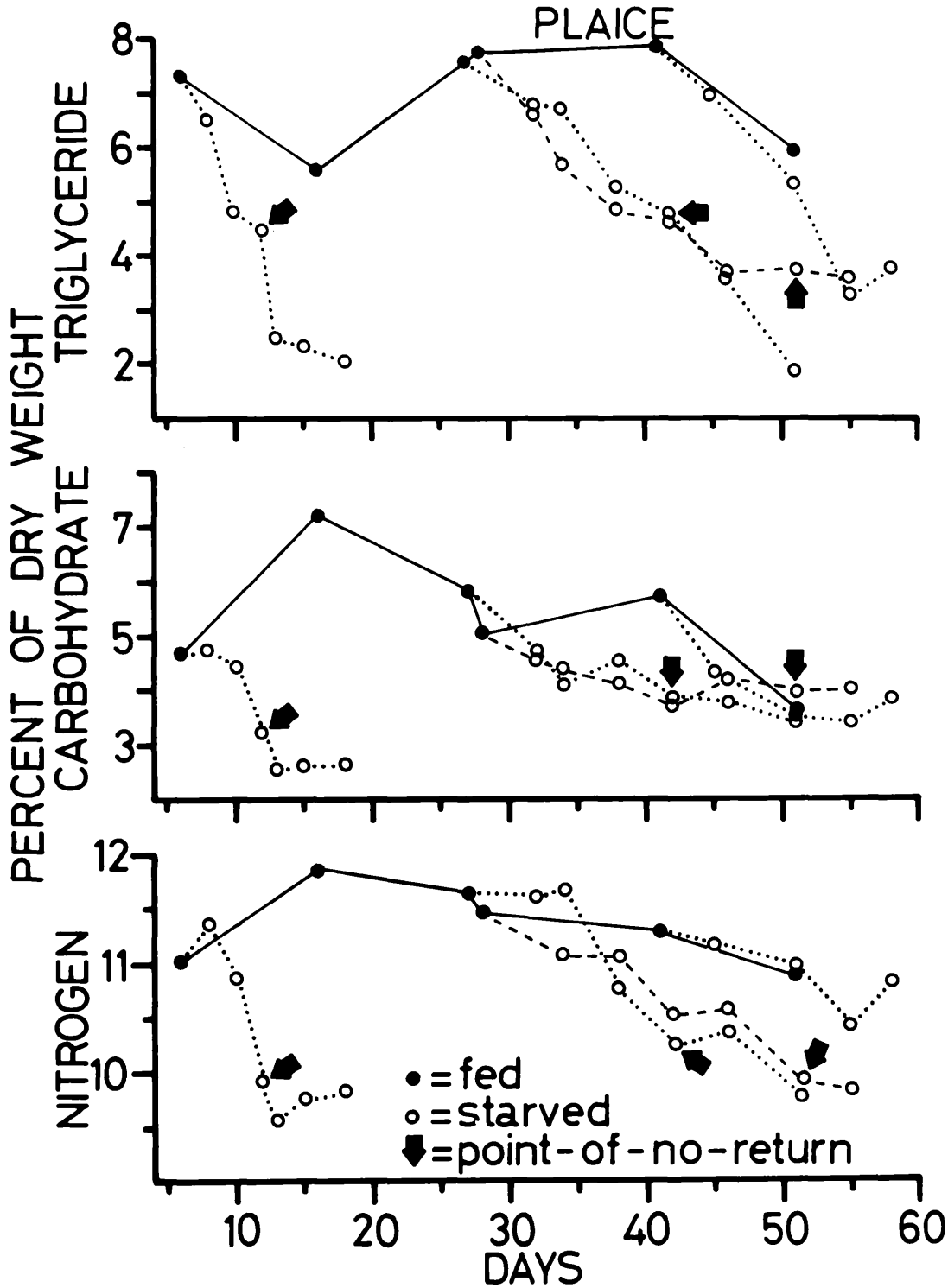

PLAICE
PERCENT OF DRY WEIGHT
TRIGLYCERIDE
CARBOHYDRATE
NITROGEN
8
6
4
2
7
5
3
12
11
10
● = fed
○ = starved
⬇ = point-of-no-return
10
20
30
40
50
60
DAYS

Fig. 20. (Continued).

D. Ash.

E. Carbon.

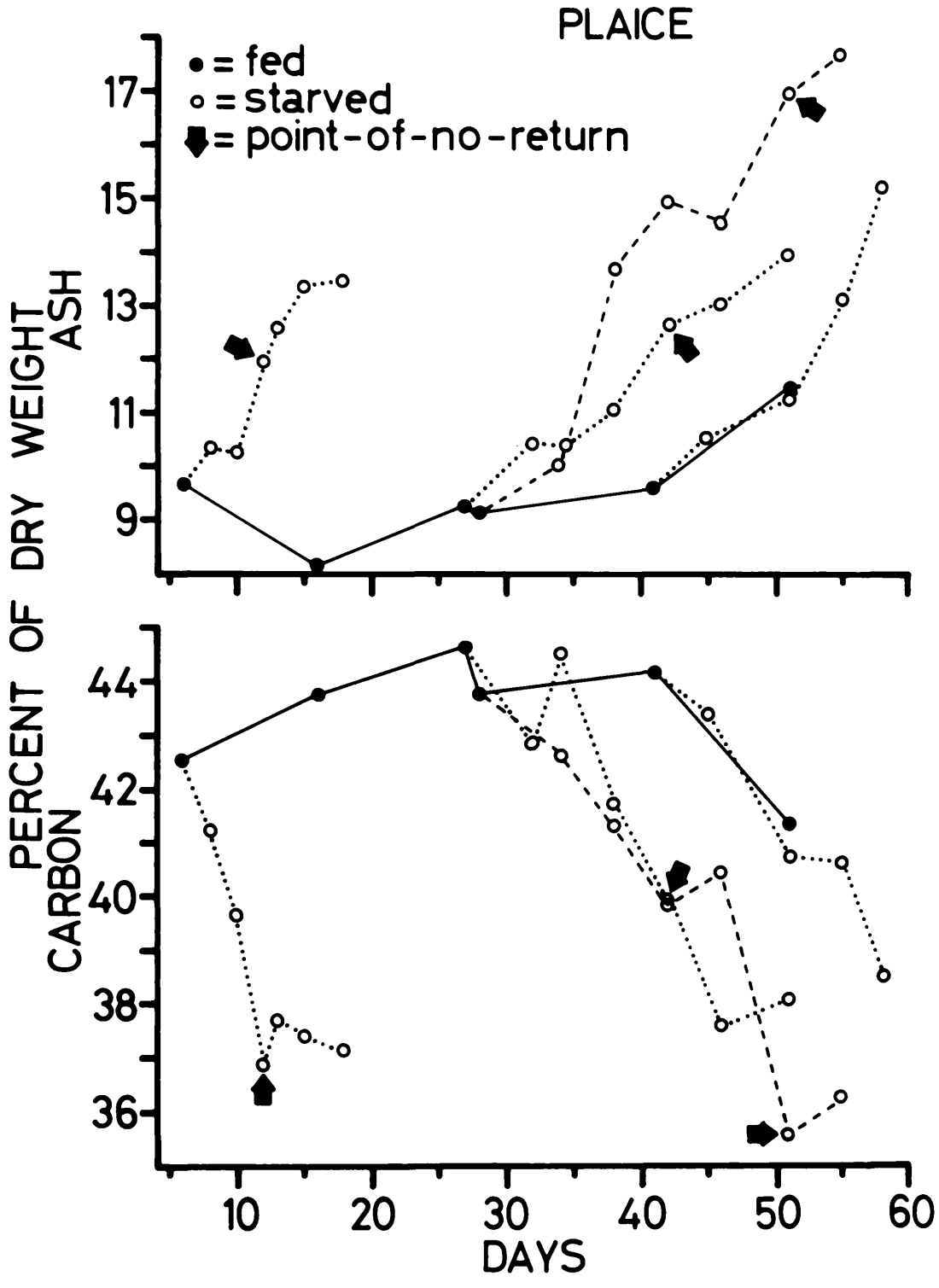

PLAICE
● = fed
○ = starved
= point-of-no-return
PERCENT OF DRY WEIGHT
ASH
17
15
13
11
9
CARBON
44
42
40
38
36
10
20
30
40
50
60
DAYS

Fig. 21. Plaice percent of unstarved amount versus days starved. Dotted lines connect similar days of starvation between larvae of consecutive developmental stages to show changes in rates of depletion with growth. The PNR has been indicated when it occurred within the days of starvation shown in the figures. Actual larval ages are shown.

A. Triglyceride.

B. Carbohydrate.

C. Nitrogen.

D. Carbon.

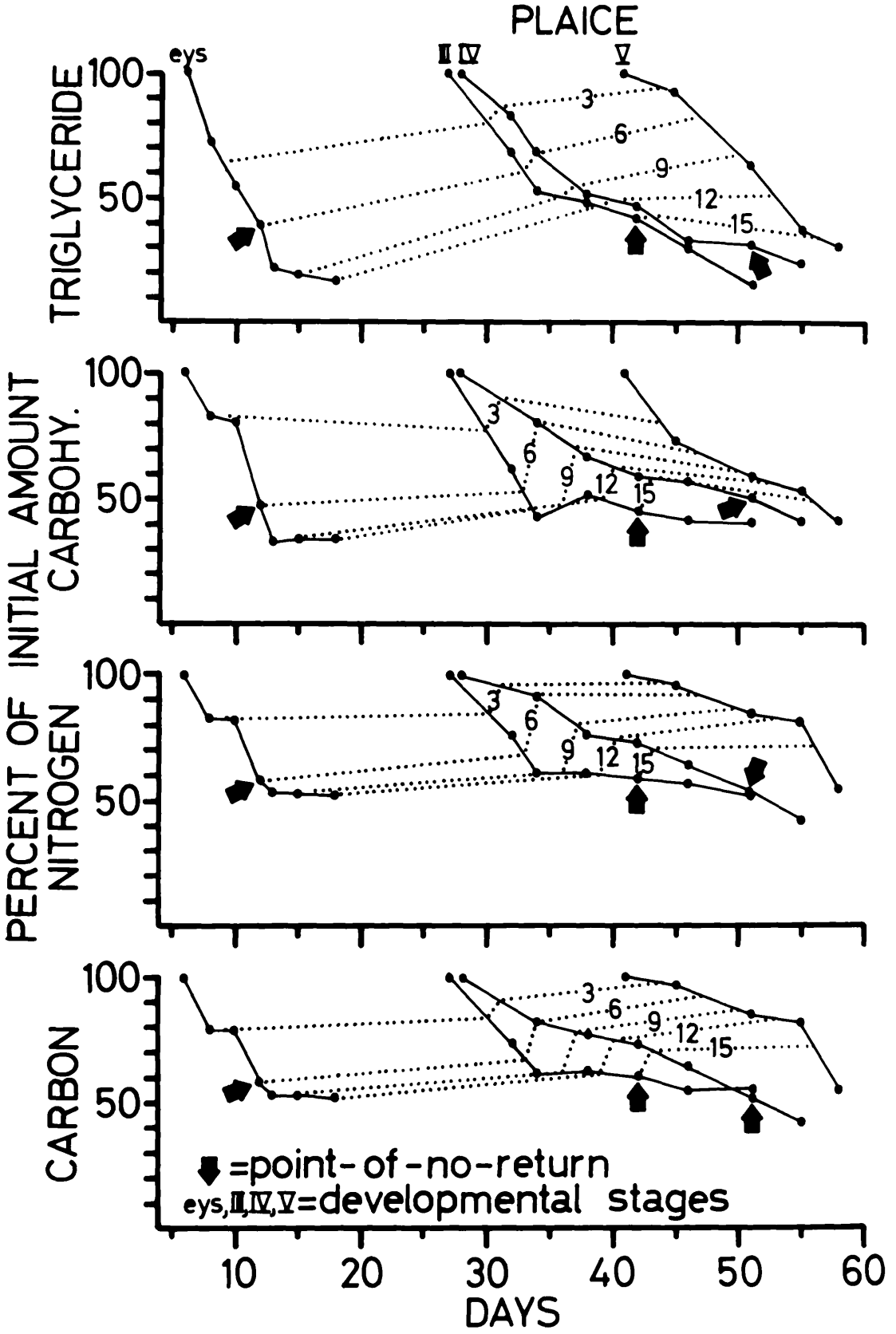
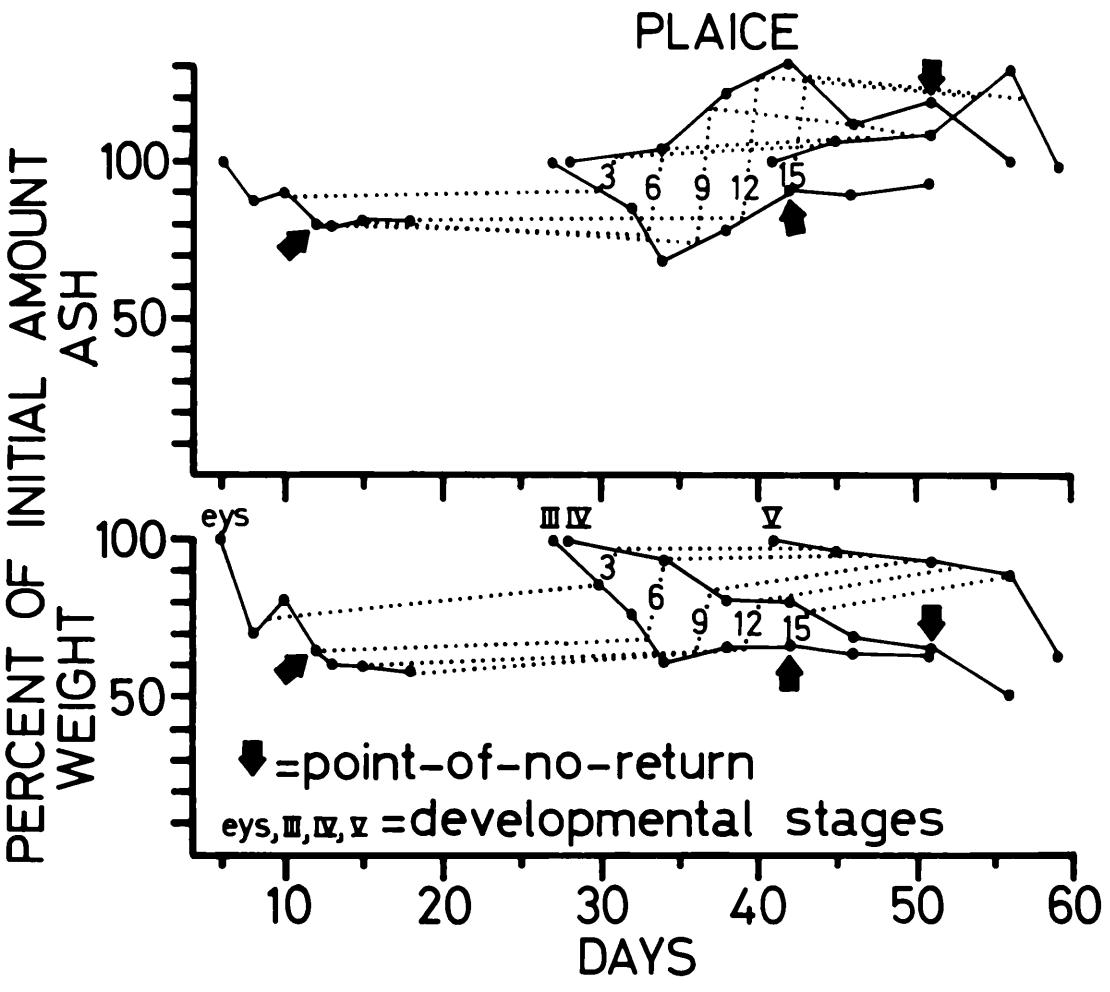

PLAICE
eys
II IV
V
TRIGLYCERIDE
100
50
3
6
9
12
15
PERCENT OF INITIAL AMOUNT
CARBOHY.
NITROGEN
CARBON
=point-of-no-return
eys, II, IV, V=developmental stages
10
20
30
40
50
60
DAYS

Fig. 21. (Continued).

E. Ash.

F. Weight.


PLAICE
PERCENT OF INITIAL AMOUNT
ASH
WEIGHT
100
50
3
6
9
12
15
eys
III IV
V
=point-of-no-return
eys, III, IV, V =developmental stages
10
20
30
40
50
60
DAYS

larvae became moribund the percentage of water in them decreased. The regression lines of percent water versus length for the various days starved were different by a highly significant amount, but the slopes were not significantly different (Appendix 3). The changes in percent water during the starvation of the six standard sizes of herring larvae are shown in Fig. 16. The increasing percentage of water during starvation was only a relative change, for there was an actual loss of water (Appendix 1A). The only exception to this was in 15 and 21 mm larvae where during the first 3 days of starvation there was an actual increase in water. The rate of loss of water during food deprivation to the PNR as a percentage of the initial amount present (Fig. 19G) tended to decrease as larvae increased in length, at least up to 21 mm. Above this length the rate of loss stayed about the same for the different sizes of larvae. In moribund larvae the loss of water as a percentage of the initial amount was almost twice that of larvae starved for the same number of days but not yet moribund (Fig. 19G).

TRIGLYCERIDE: In herring larvae this constituent showed a concave parabolic relationship with length up to 26 mm, decreasing from 8.08% in larvae at the end of the yolk sac stage to 5.72% in those just over 19 mm and then increasing to 7.84% by 26 mm. In larger larvae, triglyceride decreased in a linear fashion with increasing length to a value of 7.17% by 35 mm. The slopes of these curves were maintained throughout starvation (Fig. 19A and Appendix 4).

During 15 days of starvation triglyceride decreased approximately by 3.4 to 4.5% irrespective of the size of the larvae, but the percentage of the dry weight utilized to reach the PNR increased with increasing size from just over 1%, in the smallest herring larvae, to over 4% in those 30 mm long (Fig. 18A and Appendix 1A). The percentage of the unstarved store utilized to reach the PNR increased with the length of the larvae, from 37% in the smallest to 76% in those 30 mm in length (Fig. 19A and Appendix 1A). The decrease in the rate of triglyceride utilization to the PNR with increasing larval size was also shown in terms of the rate of depletion of the actual amount (Table 4).

Table 4. Rate of utilization to PNR (μg/mg unstarved dry wt/day)

	unstarved dry weight	days to PNR	Triglyceride	Carbohydrate	Nitrogen	Carbon	Ash
HERRING							
12 mm	0.1689	6	5.0	1.7	11.2	19.3	0.6
15 mm	0.4685	8	4.2	2.3	4.5	19.3	1.2
21 mm	2.1810	9	4.4	3.4	5.5	22.2	1.8
25 mm	4.8393	12	4.0	1.7	3.3	14.6	0.6
30 mm	11.1358	15	3.8	1.1	3.2	13.8	0.7
35 mm*	22.5286	15*	3.7	1.1	3.1	13.8	0.9
PLAICE							
End yolk sac	0.1271	6	7.4	4.2	7.6	38.9	3.3
Stage 3	1.055	15	2.9	2.1	3.2	11.9	0.5
Stage 4	1.786	23	2.3	1.1	2.2	9.1	+0.8
Stage 5*	2.242	17*	3.2	1.5	2.6	11.8	0.0

* Experiment terminated before fish reached PNR. Rate based on larvae at last day of experiment.

+ indicates increase of component.

CARBOHYDRATE: Percent carbohydrate during development showed three relationships with length: from the end of the yolk sac stage to 21 mm, from 21 mm to 29 mm and from 29 to 50 mm (Fig. 17B and Appendix 5). In the first phase percent carbohydrate increased with length from 3.41 to 4.78%. In the second, it decreased to 3.25% and then remained constant at this level in the third.

During starvation (days 3 to 15) the regressions of percent carbohydrate against length were highly significantly different, but the slopes were statistically indistinguishable and not different from zero (Appendix 5). The mean values of percent carbohydrate during starvation (days 3 to 15) are also shown in Appendix 5. In terms of percent changes per day of starvation the only differences between larvae of different lengths occurred during the first three days of food deprivation. In this period the percentage loss of carbohydrate was dependent on the size of the unstarved store (Fig. 18B). The percentage of the unstarved store utilized for any given number of days of starvation also increased with the level of carbohydrate in unstarved herring larvae (Fig. 19B). There was a trend for the rate of carbohydrate catabolism to the PNR to

decrease with increasing size, but this was in part masked by the varying size of the unstarved store (Fig. 19B and Table 4).

NITROGEN: The percentage of nitrogen in herring larvae displayed two relationships with length during growth: from the end of the yolk sac stage to 21 mm it linearly increased with length (10.42 to 12.23%); in larger larvae it decreased as a logarithmic function of length to 11.29% at 35 mm (Fig. 17C).

In both size groups no significant differences were detected during starvation for the regression lines of percent total nitrogen versus length (Fig. 18C and Appendix 6). However, when the actual amounts of nitrogen were considered obvious starvation decreases were observed (Fig. 19C and Appendix 1A). The percentage of the unstarved store used to the PNR tended to increase with increasing larval size although the rate of nitrogen catabolism to the PNR also varied with the size of the unstarved level of nitrogen (Table 4).

ASH: Percent ash showed two relationships with length: from the end of the yolk sac stage to 25 mm, feeding herring larvae contained 7.50% ash independent of their length, but in larger larvae the percentage of ash increased with length to 9.04% at 35 mm (Fig. 17D).

In all sizes of larvae percent ash significantly increased during starvation, but the rate of change of percent ash per unit length did not significantly vary (Appendix 7). Because of this the differences in the percentage of ash at the PNR were largely due to the differences in the number of days required to reach this condition (Fig. 18D and Appendix 1A). Although the percentage of ash increased up to the PNR the actual amount of ash, in all sizes of herring larvae, slightly decreased (Fig. 19D and Appendix 1A). There was no general pattern of the rate of utilization of ash to the PNR (Table 4).

CARBON: The percentage of carbon in herring larvae increased with development from the end of the yolk sac stage to 18 mm in length (42.22 to 44.34%). In larger fish percent carbon remained constant at the level reached by 18 mm (Fig. 17E).

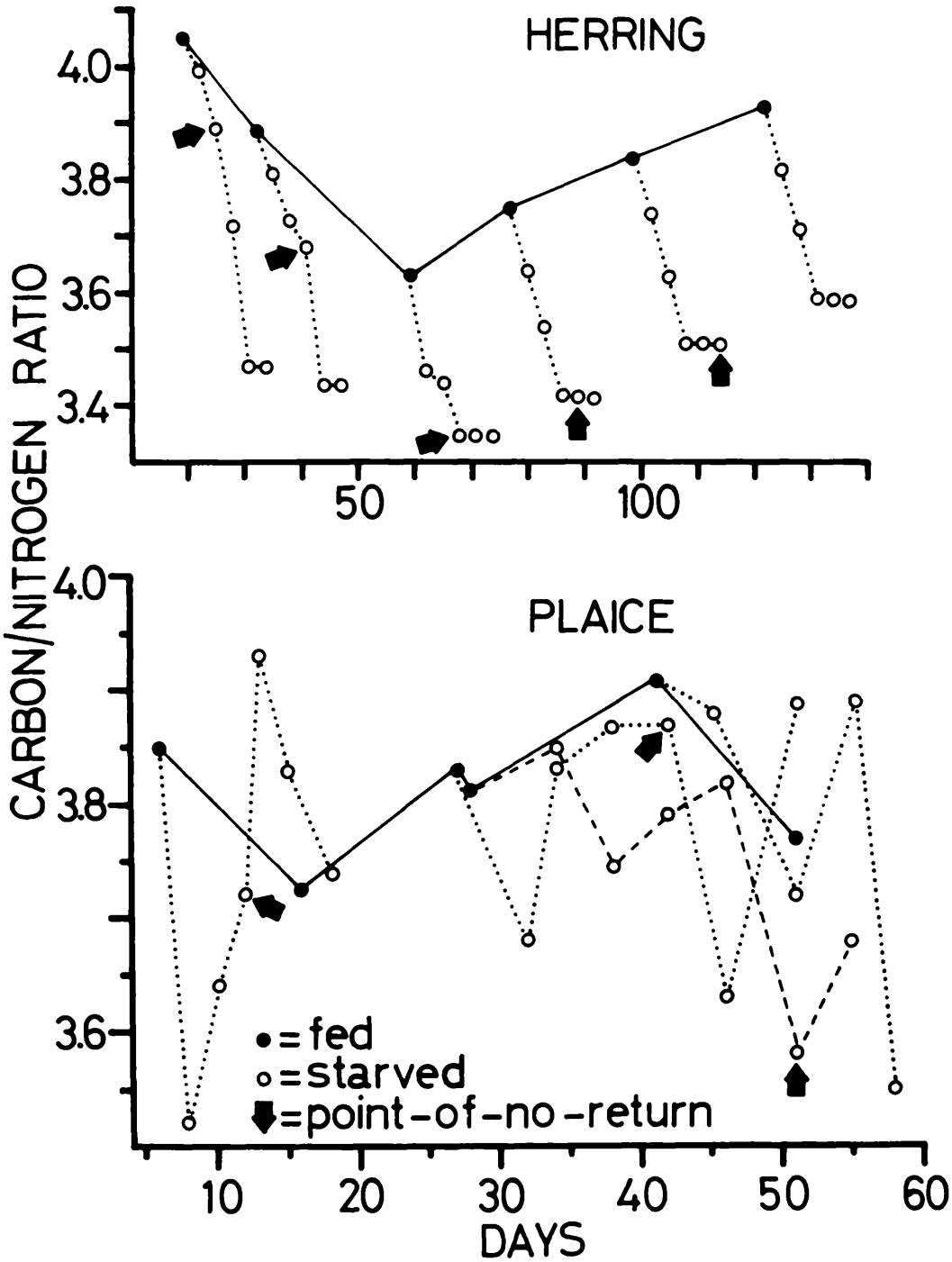
During the starvation of the smaller larvae the rate of increase of percent carbon with length changed between 6 and 9 days of food deprivation. However, within each group of days (0 to 6 and 9 to 15) the regression lines of percent carbon versus length were significantly different, but the slopes were not statistically distinguishable (Appendix 8). For larvae larger than 18 mm the regression lines of percent carbon versus length differed up to 9 days of starvation, but after this no further differences were detected. In all larvae from the end of the yolk sac stage to 30 mm percent carbon at the PNR was between 40.00 to 40.50% (Fig. 18E). However, the percentage of the unstarved store utilized to reach the PNR tended to increase in larger larvae (Fig. 19E), although the rate of carbon depletion to the PNR tended to decrease with increasing size (Table 4).

CARBON/NITROGEN RATIO: In feeding herring larvae the C/N ratio decreased with development from 4.05 at the end of the yolk sac stage to 3.63 at 21 mm in length. It then increased with further growth to 3.93 in 35 mm larvae (Fig. 22A).

Fig. 22. Carbon/nitrogen ratios during growth and starvation. Solid lines show changes during growth; dotted and broken ones delineate starvation. The PNR has been indicated when it occurred within the days of starvation shown in the figures.

A. Herring: Starvation changes are shown for the standard sizes of larvae (12, 15, 21, 25, 30 and 35 mm). The age of the standard lengthed larvae, at the start of each starvation, was taken from the growth rate (Fig. 5A).

B. Plaice: Growth changes are shown (from left to right) for larvae at the end of the yolk sac stage, stages 2, 3, 4 and 5 and 10 days post-metamorphosis. Actual larval ages are shown.


HERRING
PLAICE
CARBON/NITROGEN RATIO
4.0
3.8
3.6
3.4
50
100
10
20
30
40
50
60
DAYS
●=fed
○=starved
⬇=point-of-no-return

During starvation the C/N ratio decreased in all sizes of herring larve, but the size of the ratio at the PNR was dependent on the relative amounts of carbon and nitrogen at the start of starvation. Although the C/N ratio of all sizes of herring larvae decreased during starvation, the percentage of the decrease from the unstarved ratio was small as compared to other chemical components. In all larvae the difference in the C/N ratio between feeding larvae and those starved to the PNR did not exceed 10% (Fig. 19F).

PLAICE COMPOSITION

WATER: In feeding plaice larvae the percentage of water decreased during development inversely to larval length (Fig. 4B):

% water = - 0.38 length (mm) + 90.81.

The changes in percentage of water during starvation were followed only from the end of the yolk sac stage. In this period the water content increased from 88.18 to 90.14% by the PNR (6 days) and then to 91.29% by 11 days of starvation, but fell to 89.61% in moribund larvae (Fig. 11B). The increasing percentage of water in the plaice larvae was only a relative change, for there was an actual loss of water by the PNR (Table 2B).

However, the loss of water during starvation was not a continuous process, for an actual uptake of it occurred between 4 to 6 and 8 to 11 days of starvation. Although larvae at the PNR had lost only 8% of their initial amount of water, when they became moribund this loss increased to 25%.

TRIGLYCERIDE: At the end of the yolk sac stage plaice larvae contained 7.30% triglyceride. By stage two this decreased to 4.56%, but then it started to increase reaching 7.54% at stage 3, 7.79% at stage 4 and 7.85% by stage 5. Following the completion of metamorphosis the triglyceride store markedly decreased during the next 10 days to 5.95% (Fig. 20A).

During starvation the percentage of the unstarved amount of triglyceride catabolized during a given number of days of food deprivation generally decreased with increasing larval development (Fig. 21A). Furthermore, the rate of triglyceride utilization to the PNR decreased with increasing development (Table 4).

CARBOHYDRATE: In feeding plaice larvae the percentage of carbohydrate increased from 4.65% at the end of the yolk sac stage to 7.25% by stage 2. This store then started to decrease, reaching 5.06% at stage 4. Further development to stage 5 resulted in a small increase to 5.78%, but in the following 10 days carbohydrate content dropped to 3.65% in the newly metamorphosed plaice (Fig. 20B).

During starvation the level of carbohydrate was not depleted as quickly as triglyceride, and in all stages of plaice, that were starved, the loss of carbohydrate eventually levelled off (Fig. 20B). In larvae starved to the PNR, 50 to 55% of the unstarved carbohydrate was catabolized by the time this condition was reached (Fig. 21B). The rate of utilization of carbohydrate to the PNR decreased with increasing development (Table 4).

NITROGEN: Total nitrogen in feeding plaice larvae increased from 11.04% at the end of the yolk sac stage to 11.75% by stage 2. It then started to decrease and continued to do so through metamorphosis, reaching 10.90% 10 days after completion of metamorphosis (Fig. 20C).

In all of the starvations, where the PNR was reached, total nitrogen decreased to a level of between 9.90 to 10.30% (Fig. 20C).

Unlike herring larvae, in plaice the percentage of nitrogen, as well as the actual amount, decreased during starvation. The percentage of the initial unstarved amount of nitrogen depleted during any given number of days of starvation decreased with increasing development of the larvae (Fig. 21C). Furthermore, the rate of nitrogen utilization to the PNR decreased with increasing development (Table 4).

ASH: The percentage of ash in plaice larvae slightly decreased during development from 9.65% at the end of the yolk sac stage to 9.06% by stage 4. It then started to increase rapidly with further development reaching 9.62% by stage 5, and during the next 10 days newly metamorphosed plaice increased their ash content to 11.46% (Fig. 20D).

During starvation the percentage of ash increased in all developmental stages of plaice larvae that were starved (Fig. 20D). However, the actual amount of ash did not show a consistent pattern for all stages of development (Fig. 21E and Appendix 1B), although the rate of loss of ash to the PNR decreased with development and actually increased in actual amount over the unstarved value in stage 4 plaice larvae (Table 4).

CARBON: During development percent carbon increased from 42.53% at the end of the yolk sac stage to 44.62% at stage 3. The percentage then decreased to 43.79% at stage 4 followed by a slight increase to 44.20% at stage 5. Over the next 10 days the level of carbon in newly metamorphosed plaice fell to 41.35% (Fig. 20E).

In all of the developmental stages, which were starved, the percentage of carbon rapidly declined during starvation (Fig. 20E). Fig. 21D shows that the percentage of the unstarved amount of carbon used to any given number of days of starvation decreased with increasing development. The rate of utilization of carbon to the PNR also declined with increasing development (Table 4).

CARBON/NITROGEN RATIO: The C/N ratio decreased from 3.85 in plaice larvae at the end of the yolk sac stage to 3.72 by stage 4. It then increased to 3.91 by stage 5, and during the 10 days following the completion of metamorphosis the C/N ratio decreased to 3.79 (Fig. 22B and Appendix 1B).

In all developmental stages the C/N ratio did not show any consistent pattern during starvation (Fig. 22B).

Parental effects

In order to obtain some idea of the effects of genetic (parental) variability upon the differences between herring larvae, comparisons were made of eggs, and subsequently larvae, from eight female herring fertilized with a mixture of milt from two males. Various parameters of the parents, eggs and larvae are shown in Table 5. The lowest percentage of hatching occurred in eggs from female no. 4; in these hatching success was so low that not enough larvae were available for other comparisons. The most obvious difference between eggs of female no. 4 and the others was in the percentage of carbon which was almost 57%, compared with only 50 to 51% in all others (exceeding the 99% confidence limits of the other eggs Table 5). These eggs also had the highest amounts of carbohydrate and nitrogen, but these values were only slightly higher and within the 95% confidence limits. The only other peculiarity of female no. 4 was that it was slightly larger than the others. There appeared to be no relationship between hatching success and egg size. However, there was a highly significant correlation between egg dry weight and that of the larvae at the end of the yolk sac stage ($r = 0.9696$, $p < 0.01$) (Fig. 23).

Fig. 23. Larval dry weight versus egg dry weight. The relationship between the dry weights of herring eggs and larvae from different females is shown. The larvae were at the end of the yolk sac stage. Each point represents a mean of at least 10 eggs and larvae.

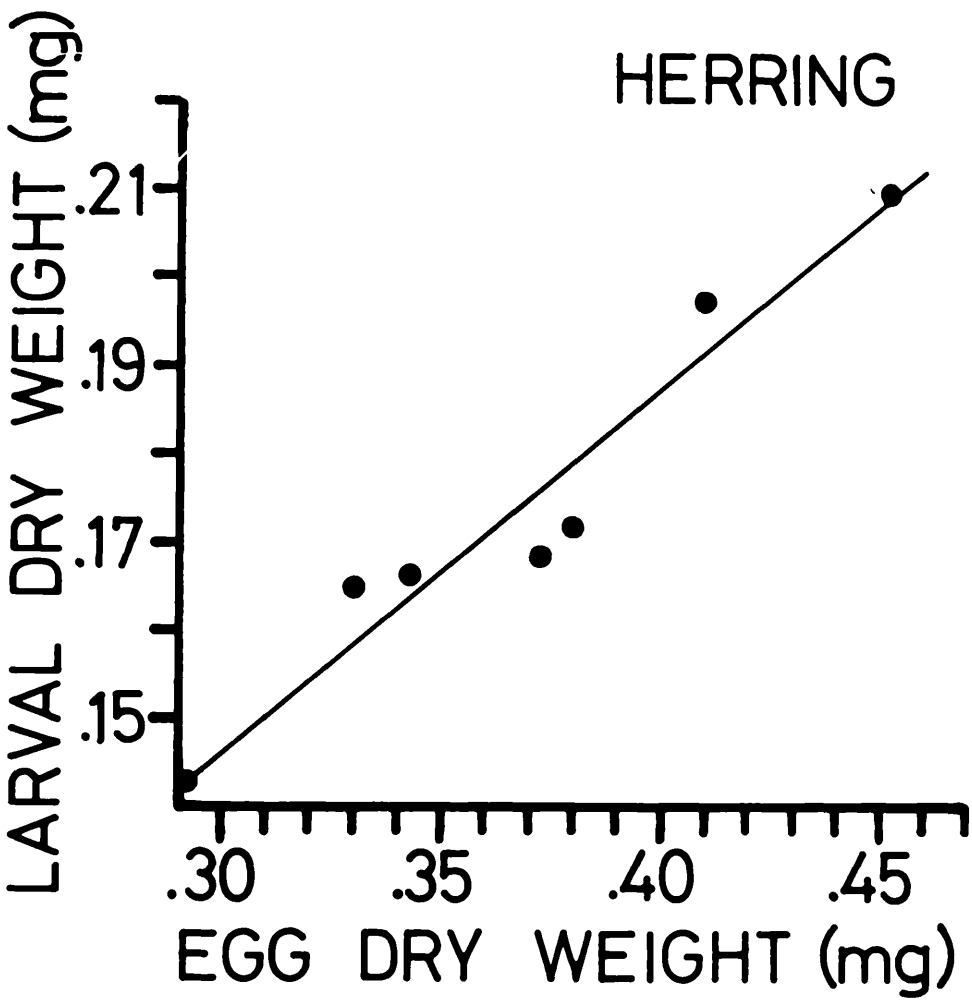

HERRING
LARVAL DRY WEIGHT (mg)
.21
.19
.17
.15
.30
.35
.40
.45
EGG DRY WEIGHT (mg)

Table 5. Herring parental comparisons.

	♀	Total length (cm)	Age	Mean egg dry wt. (µg)	Hatching success	End yolk sac larvae mean dry weight (mg)	Egg composition % dry weight: Triglyceride	Carbohydrate	Nitrogen	Ash	Carbon
♂ (2+3)x	1	33.0	3+	0.3732	>80	0.1681	7.93	4.55	11.71	4.47	50.24
	2	31.0	3+	0.3435	>80	0.1660	8.57	4.72	11.92	4.66	50.40
	3	31.5	3+	0.2907	30-50	0.1408	8.76	4.48	11.96	3.24	50.14
	4	34.0	3+	0.3783	< 0.5	-	7.22	4.98	12.05	4.43	56.98
	5	28.5	3+	0.4539	>80	0.2100	6.84	3.99	11.55	4.40	50.03
	6	32.5	4+	0.3315	>80	0.1646	6.50	3.71	11.19	3.51	50.70
	7	29.5	3+	0.3806	< 10	0.1715	7.87	4.38	11.50	4.04	50.08
	8	31.5	3+	0.4111	>80	0.1969	8.50	3.95	11.96	4.59	50.34
Mean		31.4		0.3704		0.1740	7.77	4.34	11.73	4.17	51.11
S.D. excl. ♀4								0.42	0.29		0.23

Total fat 8.35%* % protein 73.31*

♂	Total length (cm)	Age
1	34.5	4
2	33.0	3
3	31.0	3
4	28.0	-

(*) Total Fat = 100% - Σ(Protein + Carbohydrate + Ash) where protein = N x 6.25 (Lasker, 1962)

Comparisons of larvae from different females during starvation are shown in Appendix 9. The percent deviation of each chemical component of the larvae of each female from the mean percentage for each day starved is also shown. For each constituent deviations from the mean percentage for each day starved, were plotted against the dry weight of the larvae at the end of the yolk sac stage (Fig. 24).

No significant variation with larval size was found for triglyceride, nitrogen or water ($r = 0.174$, -0.213 and -0.208 respectively; 32 df in each and $p > 0.05$). Percent carbohydrate and ash decreased with increasing size ($r = -0.636$, 32 df and $r = -0.743$ respectively; in both relationships $p < 0.01$). However, these percentage decreases with increasing size were only relative changes, for when actual amounts of these components (Appendix 10) were considered significant positive correlations were obtained between carbohydrate and ash against the dry weight of the larvae at the end of the yolk sac stage ($r = 0.816$ and 0.920 respectively, in both $p < 0.01$) (Fig. 25). Percent carbon increased with increasing size as did the carbon/nitrogen ratio ($r = 0.608$ and 0.485 respectively; 33 df in each

Fig. 24. Deviation from mean percentage versus larval herring dry weight. At each day of starvation, past the end of the yolk sac stage, the mean percentage of a component was calculated between larvae from different females and the deviations from the mean were determined for each parental group. The mean deviations for each group, over all days starved, are plotted against the dry weight of the larvae from these parental groups. The mean of the deviations for each parental (size) group is shown. Individual values are given in Appendix 9.

A. Triglyceride.

B. Carbohydrate.

C. Nitrogen.

D. Carbon.

E. Ash.

F. Carbon/nitrogen ratio.

G. Water.

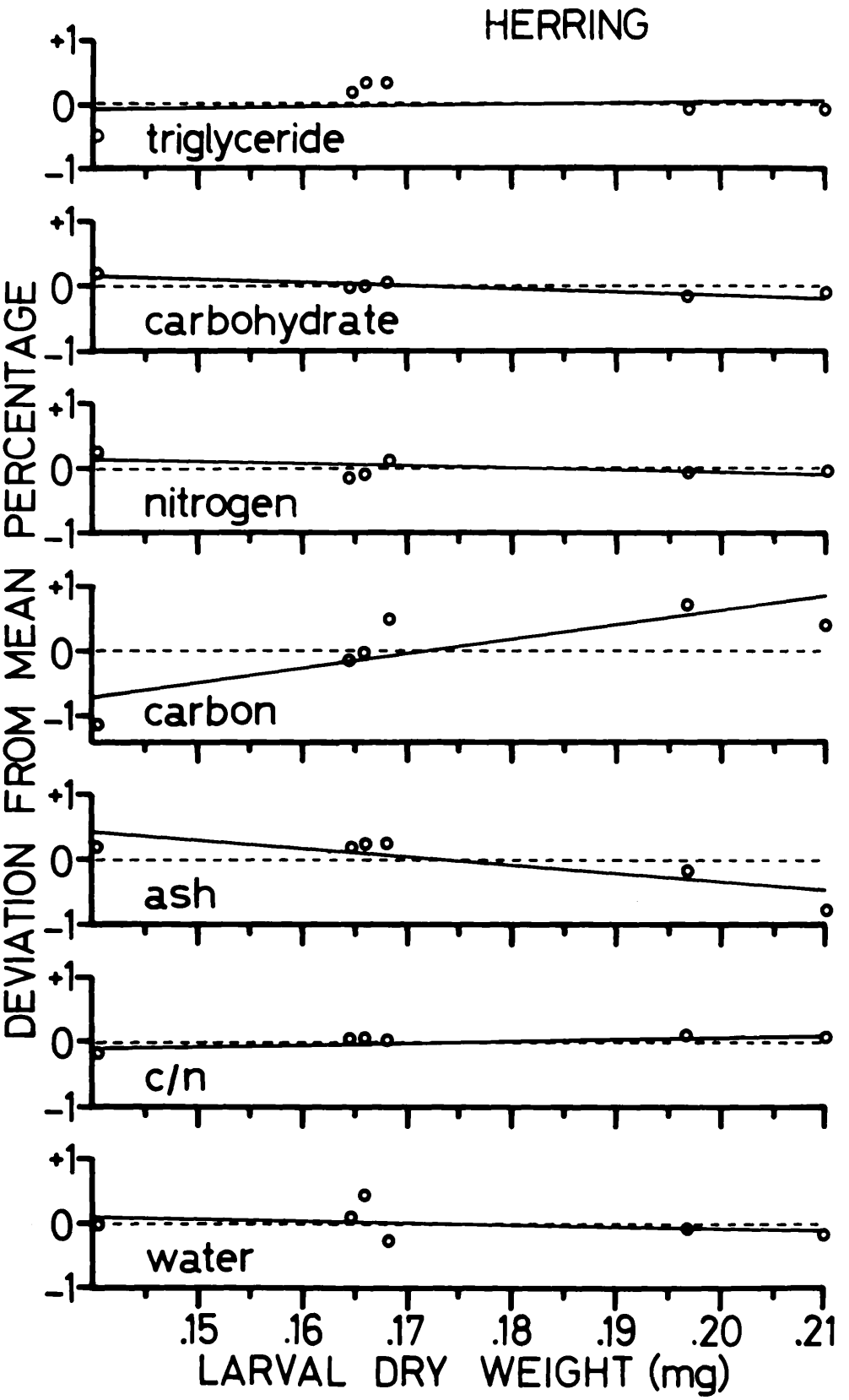
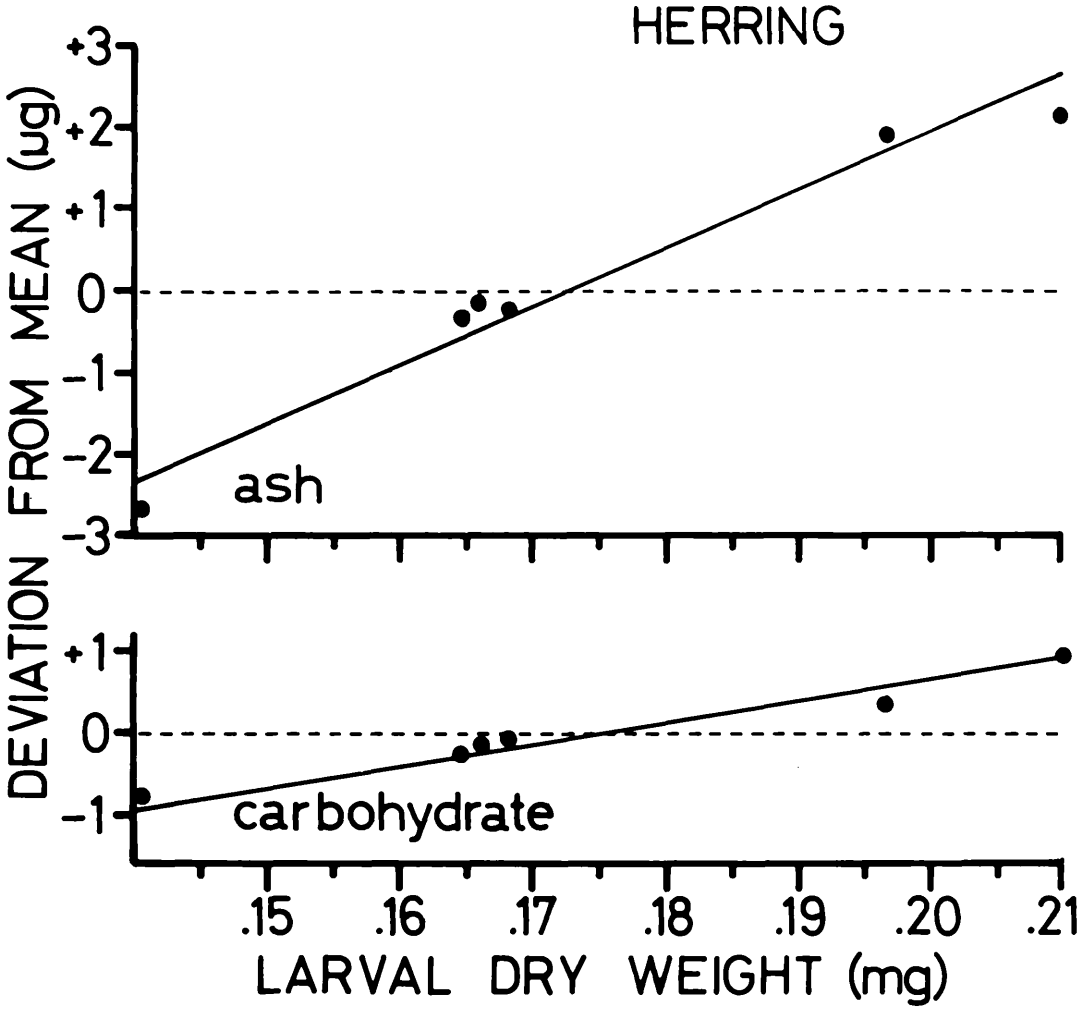

HERRING
+1
0
−1
triglyceride
carbohydrate
nitrogen
carbon
ash
c/n
water
DEVIATION FROM MEAN PERCENTAGE
.15
.16
.17
.18
.19
.20
.21
LARVAL DRY WEIGHT (mg)

Fig. 25. Deviation from mean amount (μg) versus larval herring dry weight. At each day of starvation, past the end of the yolk sac stage, the mean amounts of ash and carbohydrate were calculated between larvae from different females, and the deviations from the means were determined for each parental group. The mean deviations for each group, over all days starved, are plotted against the dry weight of the larvae from these parental groups. The mean of the deviations for each parental (size) group is shown. Individual values are given in Appendix 10.

A. Ash.

B. Carbohydrate.


HERRING
+3
+2
+1
0
-1
-2
-3
ash
+1
0
-1
carbohydrate
DEVIATION FROM MEAN (µg)
.15
.16
.17
.18
.19
.20
.21
LARVAL DRY WEIGHT (mg)

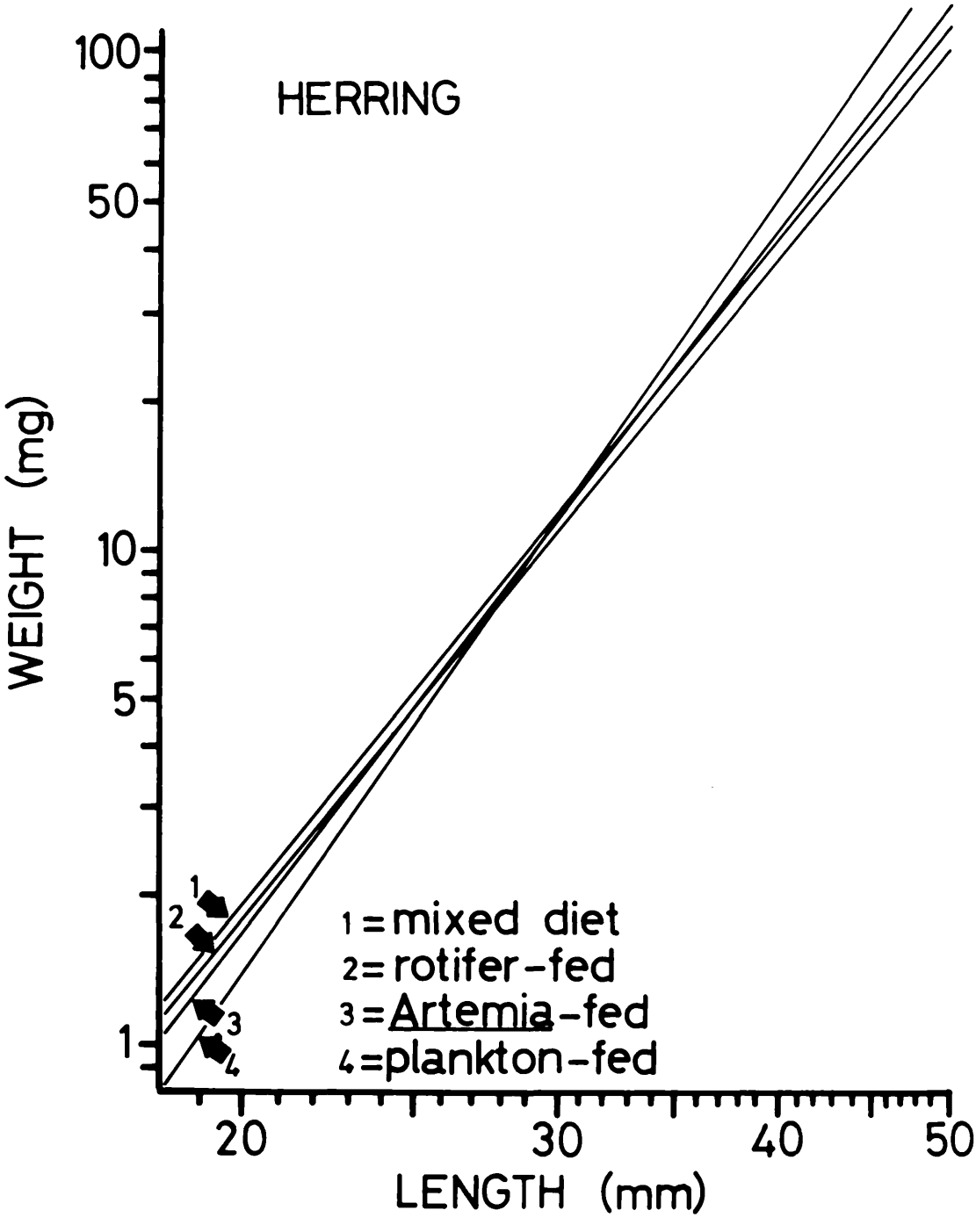
and in both $p < 0.01$) (Fig. 24). The positive correlation between carbon and the dry weight of the larvae resulted from an obvious increase in carbon. The increase in the C/N ratio against dry weight resulted from increases in carbon while nitrogen remained constant.

Dietary studies

HERRING

Dietary experiments were begun in 1971 on herring larvae 80 days post-hatching. The length-weight relationships of the herring larvae were compared after feeding for 20 days on diets of rotifers, natural plankton, Artemia, or mixed foods (Fig. 26), and the lines were found to be highly significantly different, although the slopes were not significantly different (Appendix 11). The pooled slope (4.6258) was used to obtain the best estimates of the individual lines and their relative condition factors. The lowest relative condition factor (0.1536) was found for larvae feeding on rotifers, followed by 0.1655 for Artemia-feeders, 0.1711 for plankton, and the highest (0.1764) was for those fed on a mixed diet. Comparison of lengths of larvae at the end of feeding revealed no significant differences between diets

Fig. 26. Herring length-weight relationships from different diets. Equations and other parameters of the relationships are shown in Appendix 11.


HERRING
100
50
10
5
1
WEIGHT (mg)
1
2
3
4
1 = mixed diet
2 = rotifer-fed
3 = Artemia-fed
4 = plankton-fed
20
30
40
50
LENGTH (mm)

($F = 0.07$; 3, 78 df; $0.9 < p < 0.95$). Survival during this experiment was highest for herring fed on rotifers (56%) followed by 52% for mixed diet, 48% for plankton-feeders, and the lowest was 44% for those fed Artemia (Fig. 27). No differences were found in the eye:head ratios between larvae on different diets ($F = 0.62$; 3, 56 df; $0.5 < p < 0.75$), although the highest value was found for rotifer feeders (Table 6A).

Chemical comparisons of larvae fed on different diets were based on percent values of triglyceride, carbohydrate, total nitrogen and carbon, ash and water (Table 6A). The only significant differences between larvae from different diets were found for triglyceride and carbon. Although there were significant differences of triglyceride in herring larvae feeding on different diets, Tukey's $w = 0.74$ (Steele and Torrie, 1960) showed that the only significant difference was that rotifer fed larvae had a smaller percentage of triglyceride. For carbon $w = 0.83$, showing that rotifers produced less carbon in larvae than plankton.

The major components of the natural plankton used as food and that found in the herring larval guts are shown in Table 7.

Fig. 27. Herring survival on different diets. Variation between duplicate tanks is shown.

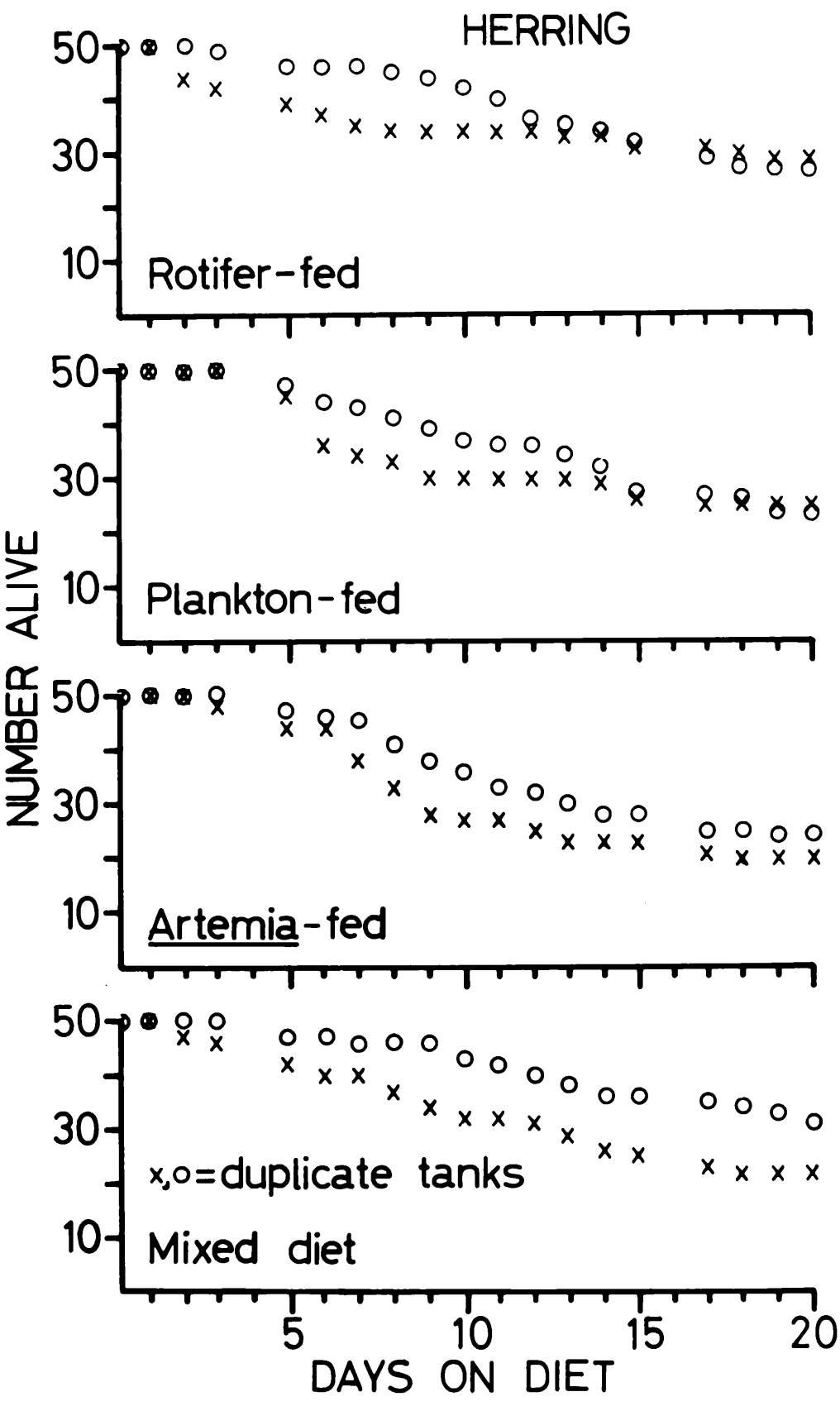

HERRING
50
30
10
Rotifer-fed
Plankton-fed
Artemia-fed
x,o=duplicate tanks
Mixed diet
NUMBER ALIVE
5
10
15
20
DAYS ON DIET

Table 6a. Larval comparisons from different diets.

H E R R I N G

		DIETS							
		Rotifers	Plankton	Artemia	Mixed	Cochrans(C)	ANOVA (F)=		Tukey's (w)
Triglyceride									
n=10 $\bar{x}$		6.23	7.26	7.88	7.50		13.39;3,36df;	p<0.01	0.74 ∴ Rot. diet => less tri. in larvae, but no other diff.
	S^2	0.3678	0.4306	0.4360	0.2562	0.2925, p>0.05			
Carbohydrate									
n=10 $\bar{x}$		3.81	3.47	3.65	3.74		0.76; 3,36df;	p>0.05	
	S^2	0.3802	0.1681	0.4611	0.1310	0.4043, p>0.05			
Nitrogen									
n=10 $\bar{x}$		11.57	11.47	11.64	11.64		0.38; 3,36df;	p>0.05	
	S^2	0.2676	0.1597	0.1663	0.1361	0.3667, p>0.05			
Carbon									
n=10 $\bar{x}$		43.59	44.69	44.20	44.27		4.35; 3,36df;	p<0.05	0.83 ∴ Rot. diet < Plank but no other diff.
	S^2	0.3858	0.2071	0.2388	1.0672	0.5620, p>0.05			
Ash									
n=10 $\bar{x}$		12.21	11.28	11.12	10.72		2.61; 3,36df;	p>0.05	
	S^2	1.4913	1.8161	1.9475	0.8391	0.3196, p>0.05			
Water									
n=15 $\bar{x}$		83.50	83.30	83.05	82.90		0.67; 3.56df;	p>0.05	
	S^2	1.3986	2.6254	1.7717	0.5237	0.4154, p>0.05			
Eye/head									
n=15 $\bar{x}$		65.53	63.93	62.40	63.40		0.62; 3,56df;	p>0.05	
	S^2	36.4095	35.6381	47.9714	46.4000	0.2883, p>0.05			

Table 6b. Larval comparisons from different diets.

P L A I C E

	DIETS					
	Rotifers	Plankton	Artemia	Cochrans(C)	ANOVA (F)=	
Triglyceride						
n=5 $\bar{x}$	5.90	5.71	6.13		0.16; 2,12df; p>0.05	
S^2	1.8859	1.3594	0.9569	0.4488, p>0.05		
Carbohydrate						
n=5 $\bar{x}$	4.90	4.23	4.65		1.25; 2,12df; p>0.05	
S^2	0.4007	0.3322	0.6988	0.4881, p>0.05		
Nitrogen						
n=5 $\bar{x}$	11.70	11.17	11.69			
S^2	0.2655	0.6155	1.0132	0.5349, p>0.05	0.77; 2,12df; p>0.05	
Carbon						
n=5 $\bar{x}$	40.14	41.30	40.87		0.77; 2,12df; p>0.05	
S^2	3.2667	0.5824	2.9428	0.4810, p>0.05		
Ash						
n=5 $\bar{x}$	11.74	11.07	9.86		1.40; 2,12df; p>0.05	
S^2	6.3341	1.9894	1.4164	0.6503, p>0.05		
Water						Tukey's (w)=
n=5 $\bar{x}$	82.71	84.59	85.63		5.67; 2,12df; p<0.05	2.34 ∴Rot. and Art. diets differ but no others.
S^2	1.1212	3.2564	1.3919	0.5644, p>0.05		

Table 7. Plankton contents.

Date	Copepod eggs	Copepods & Copepodites etc.	Cladocerans	Barnacle nauplii	Cyprids	bivalve larvae	decapod larvae	fish eggs
15 Mar.				****				
23		*		****				*
24		*		****				
27		*		****	*			
6 Apr.	*	*		***	**			
14	*	**		*	**			
15		***			**			
26	*	**			***			
27		***			**			
1 May		***			**			
4 May		****	*					
6								
13	*	***	**					
23		****	*		*			
24	*	***	**				*	
28		****				*		
29	*	***	**				*	
30	*	***	**				*	
31	*	***	**		*	*	*	
3 Jun.		***	**				*	
8		***	**			*	*	
10		***	**		*		*	

Plankton in larval herring stomachs 3 & 10 June
Copepods ***
Cladocerans **
Decapod larvae *
Cyprid *

**** 90%
*** 50-90%
** 10-50%
* < 10%

Herring larvae in the experiments appeared to take whatever was present.

Further experiments in 1972 on feeding rotifers compared with a mixed diet without rotifers to newly hatched larvae for 28 days showed rotifer-fed larvae reached a mean length of 13.95 mm while the control group reached only 12.68 mm. This difference was highly significant ($t = 7.82$; 58 df; $p < 0.01$). However, a difference was observed in age at commencement of feeding. Most rotifer-feeders started feeding 5 days and the others 7 days after hatching. If this is considered the growth rates for days fed were 0.61 mm/day for the rotifers and 0.60 mm/day for the mixed diet. Survival of both groups was 30% of the initial number feeding.

In the experiment on the feeding rate of 20 mm herring larvae on rotifers the initial concentration of rotifers was 23.2/ml, providing 18500 in 800 ml the capacity of the experimental tank. On day two the rotifer count was 13.7/ml, and the number of empty loricas was 11/ml. The sum of rotifers plus loricas was 24.7/ml, which closely agreed with the initial concentration of 23.2/ml. The total number of live rotifers

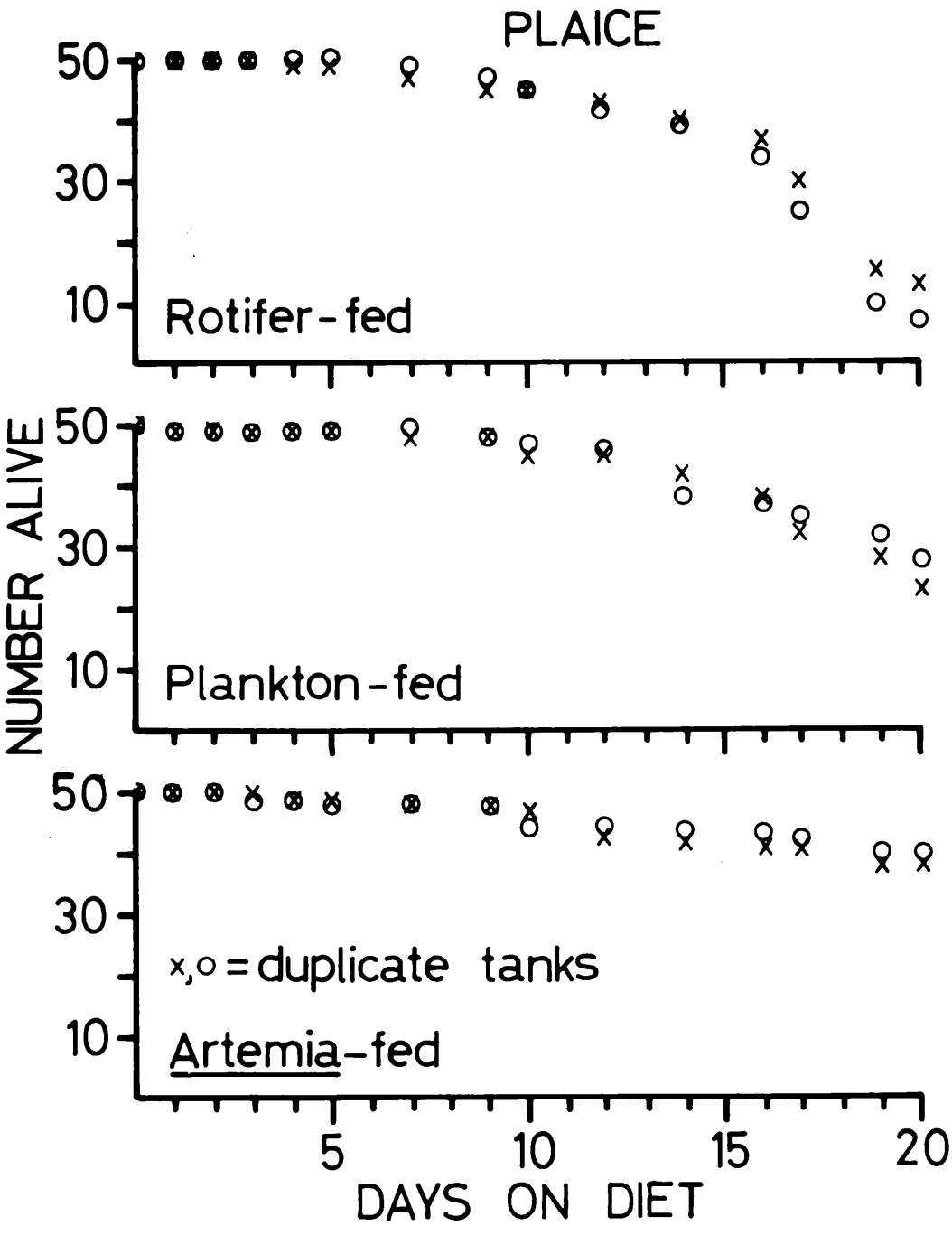
present in the 800 ml after two days of feeding by the herring was 10960. The number eaten in two days by 20 larvae was 7600 or 190 rotifers/larva/day.

PLAICE

Dietary experiments on plaice were carried out in 1971 on larvae 30 days after hatching. Plaice larvae fed for 20 days on diets of rotifers, plankton, or Artemia were compared for length differences. The variances of the length groups were tested by Bartlett's test and were found to be indistinguishable ($F = 1.57$; 2, 94 df; $0.1 < p < 0.25$). No significant differences were found between lengths of larvae on the different diets ($F = 3.31$; 2, 22 df; $0.05 < p < 0.1$). Survival during the dietary experiment was lowest for rotifer-feeders (14%) followed by 58% for plankton and 80% for plaice larvae fed on Artemia (Fig. 28).

The effect of diet on the chemical composition of plaice larvae is shown in Table 6B. The only significant differences were found in water content. Tukey's $w = 2.34$ showed the only significant difference was that rotifer feeders had a lower water content than those fed on Artemia.

Fig. 28. Plaice survival on different diets. Variation between duplicate tanks is shown.


PLAICE
50
30
10
Rotifer-fed
Plankton-fed
Artemia-fed
NUMBER ALIVE
x,o = duplicate tanks
5
10
15
20
DAYS ON DIET

Chemical comparison of foods (Table 8) showed Artemia had the highest triglyceride levels as well as high carbohydrate and carbon. Rotifers had low triglyceride, nitrogen and carbon, but carbohydrate was high. The plankton had low carbohydrate, but the other components were moderate.

Table 8. Chemical comparison of foods.

Food	Date	Composition (% dry weight) Triglyceride	Carbohydrate	Nitrogen	Ash	Carbon
Rotifers	15 March	4.41	9.11	8.73	7.31	36.64
	10 June	4.86	7.89	7.18	9.66	29.52
	Mean	4.64	8.50	7.96	8.49	33.08
Artemia	15 March	11.13	9.93	10.25	6.10	46.12
	7 April	11.18	15.19	8.79	5.61	45.88
	Mean	11.16	12.56	9.52	5.86	46.00
Plankton	15 March	8.02	6.27	9.41	15.10	38.65
	4 May	4.29	3.68	11.02	6.25	42.82
	6 May	6.78	5.03	9.60	10.30	39.90
	13 May	6.34	3.43	10.46	5.36	44.94
	24 May	5.66	3.71	10.46	13.15	43.50
	30 May	4.47	3.51	8.52	8.49	40.08
	31 May	4.12	3.25	10.04	6.20	41.06
	Mean	5.67	4.13	9.93	9.26	41.56
Balanus	15 March	5.72	5.65	11.67	7.32	42.80

DISCUSSION

Growth

Eighty day-old herring had a mean length between 25 to 26 mm, which was just about equal to that reported for reared larvae by Blaxter (1968) but considerably less than the mean length of 36.4 mm reported for wild larvae by Marshall et al. (1937). The differences between the reared and wild larvae could be due to actual differences in growth rate resulting from diet or other environmental or genetic differences. However, it was suspected that these size differences were at least partially due to variations in the survival patterns rather than to growth rate differences. Blaxter & Hempel (1963) showed that as the egg size increased so did the size of larvae at first feeding and their growth rate up to the end of the yolk sac stage. They also suggested that since larger larvae have larger jaws they can take a wider range of food and thus would be at an advantage over smaller individuals, especially when food is limiting. This is particularly true since larval herring show no food selection; what they take is limited by larval size (Marshall et al, 1937 and Blaxter, 1965). If smaller larvae are at a disadvantage one would expect that there would be a selective mortality of them in the sea, where food is not always abundant, whereas in the rearing situation they can survive since food is supplied in excess. Comparisons of the wild size range at 75 days

post-hatching from Marshall et al. (1937) and larvae reared in this present study for 78 days (Fig. 5A) showed that while the upper ranges corresponded at over 40 mm the lower limit of the natural population was 30 mm while that of the reared population was 22 mm. This suggests that the smaller ones are missing from the natural population, and their presence in the reared population lowered the mean size and growth rate. The mean length of reared juvenile herring after 212 days was 92.2 mm, which was higher than predicted by earlier growth. This may have been explained by the gradual change in diet to minced fish and squid after 90 days. If smaller larvae were not able to make the transition their death would have artificially increased the growth rate. The length at 212 days was close to that predicted for offshore, spring-spawning, herring (Marshall et al, 1939) and further suggests that the growth rate in the laboratory was comparable to that in the sea.

The larval plaice growth rate (0.16 mm/day) was considerably greater than that reported by Ryland (1966) (0.098 mm/day). Furthermore, the mean larval length after 50 days, near metamorphosis, was about 15 mm, which was longer than the 13 mm recorded by Riley (1966). He referred to his larvae as being smaller than wild larvae, which he suggested reached metamorphosis between 14 to 18 mm. Temperature differences may partially explain the differences in

growth rate of plaice in this study with that previously reported by Ryland (1966). In this current work larvae were maintained in a constant temperature room of 10°C., while Ryland's work was done at ambient temperatures which started off at 7 to 8°C. and did not reach 10°C. until about 30 days post-hatching.

Length-weight

The relationship between weight and length is described by the equation $W = k L^n$: where W and L are weight and length respectively, k is a constant and n an exponent (Ricker, 1958). The equation is most readily solved by conversion to logarithms i.e.

log weight = n log length + log k

and calculating the regression of log weight against log length. In this way n describes the slope of the line. When $n = 3$ the equation describes isometric growth, and when $n \neq 3$ allometric growth is represented. The value of n has been shown to change during ontogenesis, being highest (> 3) in the larval stage and decreasing towards isometric growth after metamorphosis (Marshall et al, 1937, 1939; Le Cren, 1951; Parker & Vanstone, 1966). That $n > 3$ in larval forms is not at all surprising considering the very elongated form of many larvae as compared to the adult

shape. Obviously height must increase faster than length during growth and development. Besides ontogeny many other factors can also affect the length-weight line, including environmental parameters as well as method of capture of fish (LeCren, 1951). He discussed sources of variability, especially in adult forms. The sources of variability for larvae are further complicated in that work is seldom done on fresh material. The slope of the line will also be affected by shrinkage if preserved larvae are used, as will the use of dry weight if percent water changes with length.

Condition factors

Condition factor is most often calculated as

$$\frac{W}{L^3} \times 1000$$

the factor 1000 being included to make a more easily handled number. This is based on the general assumption that weight increases as the cube of the length and thus gives a measure of the relative fatness or condition of the fish. However, this relationship describes isometric growth and is useful in comparing fish of different sizes only if growth is of this form. If there is allometric growth and $n > 3$, as in many larvae, then condition factor based on $\frac{W}{L^3}$ will increase with increasing length, independent

of any actual changes in nutritional condition. Because of this, meaningful comparisons of condition factor based on $\frac{W}{L^3}$ can only be made within very narrow length limits and ideally at one given length. This was pointed out by LeCren (1951) and utilized in practice by Hempel & Blaxter (1963), and Blaxter (1971). If it is desired to compare condition factor over a wider length range a different method must be used. It can be seen from the equation of the length-weight line that the proportionally constant (k) is also a function of the condition factor with isometric growth (n=3). In logarithmic form k is the intercept (i.e. when log L = 0, length = 1). Thus for isometric growth the condition factor will not change with length and will be equal to k x 1000. For allometric growth ($n \neq 3$), however, the intercept of the line can also be used as condition factor, giving a constant value over a range of constant slope (n). In this case condition factor = $W (p) \div L^n$ where n is the slope of the line and p is an arbitrary number used to make the condition factor more easily handled. This has been called relative condition factor (LeCren, 1951) and its value is obtainable directly from the equation of length-weight relationship. However, as pointed out by LeCren comparison of relative condition factors between groups of fish can only be made if the slopes of the length-weight lines between groups are equal.

In the current work the length-weight equation for feeding herring larvae from the end of the yolk sac stage to 49 mm (Fig. 6A) was log W = 4.57 log L - 5.7052 and was nearly identical to that reported for wild larvae by Marshall et al. (1937)(log W = 4.52 log L - 5.70). This shows that the rate of increase in weight with length, as well as the relative condition factor, were the same in the present rearing experiment as in the natural situation. Furthermore, Sameoto (1972) found the slope of the line to be 4.49 for herring larvae off the East coast of Canada. These equations are based on dry weight which yielded a higher slope than wet weight, since the larger larvae had a smaller percentage of water (see section on water content). Wet weight versus length gave

log W = 4.10 log L - 4.2421 (Fig. 6A).

In either case estimates of weight based on the cube of length were obviously inappropriate. Although the relative condition factor steadily decreased during starvation of herring larvae the largest drop occurred during the first three days (Fig. 7A). In this period, although the weight was decreasing, the length in many herring larvae was still increasing (Appendix 1) resulting in a more pronounced drop in relative condition factor. Weight changes in the six standard-length herring larvae, as a percentage

of the initial weight (Fig. 19H), showed that the rate of loss was closer to linear than asymptotic as suggested by Love (1970).

The length-weight relationship for plaice larvae was

$\log W = 3.92 \log L - 4.3043$

($N = 71$, $Sy.x = 0.0658$, $SD_b = 0.0554$, $SE_c = 0.0587$), which had a considerably steeper slope than the $n = 3$ reported by Ryland (1966). However, he did not compute an actual length-weight relationship but rather plotted the cube of length against weight and reported a high correlation coefficient. As in herring the slope was lower if wet weight was used instead of dry weight. For wet weight $\log W = 3.56 \log L - 3.0678$ (Fig. 6B). This slope was not as steep as for herring, but it was still greater than the $n = 3$ reported for adult plaice (Meek, 1903). Relative condition factor changes during starvation of plaice from the end of the yolk sac stage were based on $n = 3.92$. Like herring the steepest drop occurred during initial starvation. However, unlike herring the relative condition factor of plaice levelled off following the point-of-no-return, indicating proportionality between loss of length and weight.

Although the condition factor is of use in determining nutritive condition of adult or post-metamorphic fishes its use with larvae is limited (Fig. 7). The reason for this lies in the fact that both weight and length decrease during starvation of larvae due

to lack of bone, while in adult forms length does not significantly change with food deprivation. The pattern of weight loss during starvation of plaice larvae, at least up to stage three, levelled out after an initial loss (Fig. 21F). For both herring and plaice the percentage of weight lost was considerably larger in larvae than in starvation of various adults (Wilkins, 1967).

Shrinkage of length as well as weight during starvation may be a general phenomenon of larval teleosts due to lack of hard bony parts. Decreases of length during starvation of larvae have been previously reported by various workers (Farris, 1959; Blaxter & Hempel, 1963; Blaxter, 1969; Lasker, 1964; May, 1971).

Eye : head height ratio

Besides condition factor other morphological measurements such as body height have been used to assess the nutritive condition of larvae (Blaxter, 1971), but results were variable and not an explicit indicator of condition. The eye to head height ratio of herring larvae used in this experiment increased rapidly during starvation up to the point-of-no-return, showing that it was a reasonable parameter for estimating larval herring condition (Fig. 9B). Shrinkage of the head may have been due to utilization of diffuse fatty tissue located around the brain. This tissue was described by Mr. A. Packard of the University of Edinburgh during

dissection of brains of herring larvae (personal communication). Herring both larvae and adults readily use fat stores during starvation. Considering this it seemed reasonable that fat catabolism could have been the cause of the shrinkage of the head, especially since the brain and the surrounding fatty tissue are the only major components in the head region. Histological studies would help to answer the role of fat in this shrinkage. That plaice larvae did not show this shrinkage could be explained by a more ridged skull or a lack of metabolizable material in the head region.

Swimming behaviour

Larvae deprived of food to the point-of-no-return were observed to hang head down in the water, showing the anterior was the densest part of the body. It may have been that in feeding larvae fat deposits around the brain helped to reduce the higher specific gravity of this region, making it easier to maintain a horizontal position. Fat deposits in the head help to decrease density of the anterior end in other fishes (Bone, 1972). Maintaining a horizontal position, with a heavier anterior region, would have been most difficult when larvae were motionless as opposed to actively swimming. Herring larvae attempting to feed

take up an "S" position which is momentarily held while following the prey prior to springing forward in a capturing motion (Rosenthal, 1969). If during maintenance of the "S" position the anterior end of the larvae was gradually sinking it is obvious that more difficulty would be encountered in capturing food. This would be especially true for the youngest larvae since they are poorest hunters (Rosenthal, 1969; Blaxter & Staines, 1971).

Buoyancy

Sinking rates in sea water (10°C., 33‰) of anesthetized herring larvae decreased from hatching to the end of the yolk sac stage (Fig. 11A), while after this time they increased in feeding larvae. Rosenthal & Hempel (1971) described this same pattern, but they did not quantify it. Plaice larvae, however, sank at progressively faster rates from eggs to the end of the yolk sac stage, and this sinking rate continued to increase with larval growth. The similarity in sinking rate between herring and plaice larvae at the end of the yolk sac stage at just over 0.2 cm/sec may not have been purely coincidental, but rather a preferred value of physical advantage to marine larvae of this size and in this condition. In either case the sinking rate of herring decreased while that of plaice increased to reach this particular

rate by the end of the yolk sac stage. The differences between the species before and after this stage were probably due to differences in the habitats of eggs as well as of adults. Plaice eggs are positively buoyant in sea water and float; the same is true of newly hatched larvae. The increasing sinking rate would help them to keep off the surface and eventually aid them in settling. Herring on the other hand, at hatching, must rise to the planktonic zone from their demersal eggs. Although the decreasing sinking rate may not help them with this initial movement it could be of ecological significance during the yolk sac period by decreasing the energy expenditure required to remain in the planktonic zone.

During starvation the sinking rates of both herring and plaice larvae decreased until they became moribund, when the rates increased (Figs. 11 & 13). In these starving larvae the sinking rate decreased until the fishes were close to neutral buoyancy (Figs. 11A & 11B). It was thought that the change when they became moribund was a function of the breakdown in their osmoregulatory capability, resulting in a loss of water so making the larvae relatively more dense. Water content analyses have corroborated this hypothesis (Appendix 1; Table 3; Fig. 11). As larvae starved

and became weaker their decreasing sinking rate, or increasing positive buoyancy could have enabled them to spend less energy on maintaining their position in the water column. This would have conserved energy for use in attempting to capture food. Denton & Shaw (1961) suggested turbulence may distribute animals, close to neutral buoyancy, throughout the upper layers. This would apply to eggs with a small positive buoyancy and to larvae starved from the end of the yolk sac stage, but healthy larvae at the end of the yolk sac stage probably expend energy maintaining their position in the water column, since their sinking rate is much higher.

Water content was an obvious factor inversely related to sinking rate (Fig. 14). Shelbourne (1956) said that water was more important in regulation of buoyancy in marine fish eggs than fat or oil droplets. Table 9 shows the factors contributing to the buoyancy of plaice eggs. It can be seen that water provided about 9 times the lift of fat. Furthermore, it alone could account for the positive buoyancy of the egg, thus supporting the statement of Shelbourne. That water content should have been related to the sinking rate in such a manner is quite expected since the body fluids of herring and plaice larvae have a lower density than sea

Table 9. Plaice egg buoyancy.

Wet weight (mg) 4.9387 ρ Sea Water (10°C., 33‰) = 1.0255

Dry weight (mg) 0.3704

Component	ρ Egg component	$\rho_{sw}-\rho_E$	% dry wt.	mg	% wet wt.	volume (ml)	Dynes vol($\rho_{sw}-\rho_E$)981 cm/sec^2
Water	1.0094①	0.161		4.5683	92.50	0.0045258	+0.0715
Fat	0.926②	0.0995	20.82④	0.0771④	1.56	0.0000833	+0.0081
Protein + Carbohydrate	1.379③	-0.3535	75.00	0.2779	5.62	0.0002015	-0.0699
Ash			4.17	0.0155			⑤

① Egg fluid density based on salinity equivalent of 12‰ from Holliday & Jones (1967).

② Fat density from Brawn (1969).

③ Protein density from inverse of partial specific volume of protein from White, Handler & Smith (1964).

④ % Fat = 100 - (Protein + Carbohydrate + Ash).

⑤ Ash was not included in buoyancy forces since greatest part was already considered in density of body fluids.

water (Holliday & Blaxter, 1960; Holliday & Jones, 1967). However, Fig. 14 shows that the sinking rate was affected by more factors than just water, for if water was the sole controlling parameter of sinking rate one would have expected the relationship between these two factors to have been the same throughout starvation. That this was not the case for herring can be seen in Fig. 14A where the relationship between sinking rate and percent water obviously differed with days starved. Furthermore, for plaice the relationships between percent water and sinking rate before and after the end of the yolk stage were also different (Fig. 14B).

Factors other than water are also used in buoyancy regulation. In organisms which can secrete or collect gas this is usually the most significant component. Denton & Marshall (1958) discussed the factors contributing to the upward and downward forces on fishes without gas bladders. They also discussed the importance of oil in buoyancy of sharks. Iles & Wood (1965) mentioned the importance of water and oil in herring buoyancy. Pinder & Eales (1969) measured the density of gas-free fish and the fat content of salmon parr and smolt. Brawn (1969) looked at the effects of oil, gas, skeletal material, water and the remainder of the fish

on the buoyancy of Atlantic and Pacific herring. In order to estimate the effects of the various chemical components on the sinking rate of herring and plaice larvae between the end of the yolk sac stage and the point-of-no-return, the upward or downward forces, in dynes, were computed for each component (Table 10) in the following way.

The density of the body water was based on salinity equivalents at 10°C. for herring and plaice of 12.3 and 15.0‰ respectively (Holliday & Blaxter, 1960; Holliday & Jones, 1967). It was assumed for these calculations that the osmotic concentration of the body fluids remained constant up to when the fish became moribund, when there was a breakdown. Gray (1920) showed osmoregulatory ability in eggs was maintained until death. Thus, although percent water increased during starvation its density was assumed to be constant. However, there is some evidence that during starvation of cod, the blood becomes more hypotonic due to decreases in blood plasma proteins and glucose (Smallwood, 1916; Murachi, 1959, both cited by Love, 1970 and Kamra, 1966). If this was the case for larvae then the decreasing sinking rate would have been further aided by the decreasing density of body fluids which accounted for an increasing proportion of the larval body. Actual amounts of protein, carbohydrate and ash were converted to percent wet weight.

Table 10. Larval buoyancy components.

HERRING

				Weight (mg) Wet	Dry	% wet wt.	Rel %= % / 100-% Ash	Partial Density= Rel % x ρc / 100	Vol.(ml)= Wt.(g)/ρc	Net force (dynes)=⑥ (ρsw-ρc)x(vol)x(981cm/sec²)	Δdynes	dynes / wet wt.	Δ dynes / wet wt.
End of yolk sac (EY)				1.5327	0.1689								
Point-of-no-return (PNR)				1.4200	0.1278								
	ρ component	ρsw-ρc⑦		mg									
Water	1.0094[1a]	0.0161	EY	1.3638		88.98	89.72	0.9056	0.0013511	+0.0213		+0.0139	
			PNR	1.2922		91.00	91.78	0.9264	0.0012802	+0.0202	-0.0011	+0.0142	+0.0003
Fat	0.926[2]	0.0995	EY	0.0444		2.90[4]	2.92	0.0270	0.0000479	+0.0047		+0.0031	
			PNR	0.0317		2.23[4]	2.25	0.0208	0.0000342	+0.0033	-0.0014	+0.0023	-0.0008
Protein plus carbohydrate	1.379[3]	-0.3535	EY	0.1118		7.29	7.35	0.1014	0.0000811	-0.281		-0.0183	
			PNR	0.0841		5.92	5.97	0.0823	0.0000610	-0.0212	+0.0069	-0.0149	+0.0034
							Σ EY	1.0340					
							Σ PNR	1.0295					
Ash			EY	0.0127		0.83				⑤		Σ EY -0.0013	
			PNR	0.0121		0.85						Σ PNR +0.0016	
												Δ +0.0029	

PLAICE

				Weight (mg) Wet	Dry	% wet wt.	Rel %= % / 100-% Ash	Partial Density= Rel % x ρc / 100	Vol.(ml)= Wt.(g)/ρc	Net force (dynes)=⑥ (ρsw-ρc)x(vol)x(981cm/sec²)	Δdynes	dynes / wet wt.	Δ dynes / wet wt.
			EY	1.0761	0.1271								
			PNR	0.8337	0.0822								
Water	1.0114[1b]	0.0141	EY	0.9489		88.18	89.20	0.9021	0.0009382	+0.0130		+0.0121	
			PNR	0.7515		90.14	91.22	0.9226	0.0007430	+0.0103	-0.0027	+0.0124	+0.0003
Fat	0.926	0.0995	EY	0.0244		2.27[4]	2.30	0.0213	0.0000263	+0.0026		+0.0024	
			PNR	0.0206		2.47[4]	2.50	0.0231	0.0000222	+0.0022	-0.0004	+0.0026	+0.0002
Protein plus carbohydrate	1.379	-0.3535	EY	0.0905		8.41	8.51	0.1173	0.0000656	-0.0228	+0.0098	-0.0211	
			PNR	0.0518		6.21	6.28	0.0867	0.0000376	-0.0130	+0.0098	-0.0156	+0.0055
							Σ EY	1.0407					
							Σ PNR	1.0324					
												Σ EY -0.0066	
												Σ PNR -0.0006	
												Δ +0.0060	
Ash			EY	0.0123		1.14							
			PNR	0.098		1.18							

[1a] and [1b]. Fluid density of larval herring and plaice based on salinity equivalents from Holliday & Blaxter (1960) and Holliday & Jones (1967) respectively.
[2]. Fat density from Brawn (1969).
[3]. Protein density is inverse of partial specific volume (White, Handler & Smith 1964).
[4]. % Fat = 100 - Σ (protein + carbohydrate + ash).
[5]. Ash was not included in buoyancy forces since greatest part was already considered in density of body fluids.
[6]. + and - indicate upward and downward forces respectively.
[7]. ρ Sea Water (10°C., 33‰) = 1.0255.

Total lipid was taken as 100% minus the sum of these. Lipid density was taken from adult herring data of Brawn (1969), which she stated was typical of teleosts. Protein was calculated from total nitrogen times 6.025. This value was used instead of the more common value of 6.25, because it was found to be a better estimate for fish muscle (Bailey, 1937; Nottingham, 1952, both cited by Love, 1970). Protein plus carbohydrate density was taken as that for protein since this was the major portion. The value 1.359 g/ml is the mean density of protein as computed from the partial specific volume (White, Handler & Smith, 1964). Ash was not directly considered in contributions to the density, for in larvae there was no bone and by far the greatest proportion of ash was most likely from salts contributing to the osmotic equilibrium. The density of these salts was already taken into account in determination of the water density. That salts probably contributed the greatest amount of ash could be seen by multiplying the weight of water by 1.23% for herring and 1.50% for plaice to get the amount of salts needed to maintain observed the osmotic pressures. Computed in this manner the ash content exceeded the levels found by combustion of samples. However, this was easily explained since the osmotic pressures are expressed in salinity equivalents (Holliday & Blaxter, 1960), and not all osmotic pressure is derived from salts but also from organic compounds.

The theoretical densities of larvae at the end of the yolk sac stage and subsequent point-of-no-return were calculated from the proportion of chemical components and their densities. These computed densities for both species were higher than the actual values found for larvae from sinking rates at different salinities. However, the computed densities should only be considered approximations, because actual component densities were not known for these larvae and because total protein and fat were not in themselves chemically determined. A further complicating factor could have been changing densities of components during starvation which has been shown to occur in some lipid fractions (Mills, Chapman & McTaggart, 1972). The upward and downward forces (in dynes) from different chemical components contributing to density of larvae shown in Table 10 are only approximations for the reasons previously stated. The actual values are not as important as the relative amounts. Water and lipid were responsible for the upward forces on the larvae, while protein plus carbohydrate caused the downward forces. Water contributed the major upward force. For herring at the end of the yolk sac stage the lift per unit weight from water was almost five times that of lipid. The relative importance of water increased during starvation with depletion of lipid and increasing percentage of water. The high fat together

with water play a large role in the upward forces of buoyancy of the pelagic adult herring (Iles & Wood, 1965), but the major force is usually from the swim bladder (Brawn, 1969). Obviously in larvae without gas bladders the effects of water and lipid would have been much more important. Fat content of larval herring (Table 10) was much lower than of adults, which can be over 30% of the wet weight (Iles & Wood, 1965), but not much different from that reported for settled plaice, a non-fatty fish (Edwards, Finlayson & Steele, 1969). The lower fat in post-metamorphic plaice than herring probably reflected the lack of need for as well a developed buoyancy mechanism in demersal plaice. The lower fat in the larvae would have detracted from their upward forces, but this has been compensated for by water contents higher than in adults. Although the major lift was from water, which increased in relative importance during starvation, it was decreasing protein which was mainly responsible for the decreasing sinking rate during starvation.

The high density of their surrounding medium as compared to terrestrial organisms has enabled fishes to reduce structural components such as skeletal material (Lagler, Bardach & Miller, 1962). It has also led to modifications of the catabolic patterns during times of depletion. Fishes utilize proteins and phospholipids much

sooner in starvation than terrestrial forms, these variations being attributed to differences in support requirements between environments (Love, 1970). However, from the data presented here it appears that the ability to use protein is also especially advantageous for planktonic marine larvae. Newly hatched pelagic fish larvae live in an environment where food supply is thought to be limiting and where high mortalities may occur from its insufficiency (Hjort, 1914, 1926). It has been suggested that larger larvae, which can take a wider range of food, have an advantage over smaller ones especially if food is limiting (Blaxter & Hempel, 1963). From this it seems reasonable that larvae which can most quickly and efficiently convert captured food into increased growth, rather than just reserve material like fat and glycogen could have a definite survival advantage. This would seem to be especially true of proteins and phospholipids remain accessible and can be called on during food limitations.

It was earlier suggested that the decrease in their density during starvation with concurrent conservation of energy, otherwise expended in maintenance of their position in the water column, could be of definite selective advantage to larvae. If fat stores were accumulated for use in starvation, aside from the disadvantages mentioned above, their selective use during starvation would have

relatively increased the proportion of denser protein, resulting in a net increase in density. However, the ability to catabolize nitrogen resulted in a decrease in larval density. That this same pattern of buoyancy regulation and nitrogen catabolism occurred in larvae of herring and plaice, fishes in different orders, Isospondylii and Acanthopterygii respectively, suggests that it could be a general feature of pelagic larvae of marine teleosts. It thus appears that the ability to utilize protein from the start of food deprivation and the accompanying high water content to counterbalance low fat levels may be adaptations for buoyancy during planktonic life. Further evidence for this theory was found upon examination of the chemical changes during starvation of larvae from another family of the Acanthopterygii, the grunion (Leuresthes tenuis, Atherinidae). May (1971) followed changes in fat, protein and ash during starvation from the end of the yolk sac stage. He did not measure water content, so values comparable to herring were chosen. The density of sea water for grunion was taken from 18°C. and 33‰, the conditions May worked under. The density of the chemical components was computed as for the herring and plaice larvae. Appendix 12 shows that for the grunion as for herring and plaice the major upward force was from water, while the major downward force was from protein. However, more importantly it can be seen that the major change in forces during starvation resulting in the

decreased density was due to protein utilization. The similarities between all three species may reflect analogous adaptation to the planktonic environment. However, since teleosts phylogenetically [redacted] in relatively recent times (Young, 1962) the similarities between the species may reflect their common ancestry.

Point-of-no-return

The six days of starvation to reach the point-of-no-return for herring larvae at the end of the yolk sac stage agrees with those of Blaxter & Hempel (1963). The time to reach the PNR for plaice at the end of the yolk sac stage was the same as for herring, but as the plaice developed and approached metamorphosis the days of starvation to reach the PNR increased more rapidly than for herring (Table 3). Wyatt (1972) reported slightly longer periods to reach the point-of-no-return for plaice larvae. The differences between this study and his work may be due to the means of identification of the PNR. In this study the PNR in plaice was in large based on changes in swimming behaviour due to a shortage of animals for feeding experiments. However, some differences may also be explained by the small samples used by Wyatt (4 larvae).

The differences between herring and plaice larvae in the time required to reach the PNR were probably at least partially explained on activity differences, since plaice larvae even by

stage 3 have been observed to spend considerable time on the bottom of the rearing tanks. Blaxter & Staines (1971) showed that while herring activity increases during development that of plaice decreases as they approached metamorphosis. Ivlev (1961) and Love (1970) mentioned that the rate of depletion (weight or chemical) during starvation was partially dependent on the activity of the species, sluggish ones withstanding longer periods of starvation. The time to the point-of-no-return also increased with increasing size within a species; this also agrees with work of Ivlev (1961) who showed days to mortality during starvation increased with increasing size.

It has been shown that larvae deprived of food reach a point where they are no longer capable of feeding even if subsequently presented with food. These fish do not, however, immediately die but can live for considerable time even though they cannot recover. For instance, for herring deprived of food from hatching the amount of their life span beyond the point-of-no-return can exceed 50% (Fig. 11A). Furthermore, starved larvae become less active, hanging head down in the water column as well as becoming less dense so that they most likely do not sink out of the planktonic zone.

Considering this and that food levels in sea may be lower than required by young herring (Blaxter, 1966), it seems that starvation is a likely occurrence and that a large proportion of the starving larvae captured may be beyond the point-of-no-return. The chances of capturing larvae past the PNR should be increased by their decreased activity and subsequent lower ability to avoid nets. This could lead to an overestimation of potential future stock from larval censuses. Obviously a good method for distinguishing larvae in different nutritional conditions would help to reduce these problems.

Chemistry

GENERAL NOTES: LENGTH VERSUS AGE

The length of herring was found to be a better parameter than age against which to compare changes during growth up to metamorphosis. This has been shown for percent water (Figs. 3 & 4A) but was also true for the other components. This same situation has also been found for DNA content in the brains of herring larvae (A. Packard, personal communication). That this was the case is quite understandable if one considers the wide range of growth rates (Fig. 5A) obtained when the larvae were not only growing but also developing. It seems reasonable to assume that chemical changes during growth reflect developmental ones, and considering

the variation in growth rates length should be a better parameter than age against which to compare changes.

ONTOGENETIC CHANGES

Water content of plaice eggs prior to hatching was 92.5% which closely agrees with about 93% reported by Dakin & Dakin (1925). It is also close to that of other planktonic marine eggs e.g. Sardinops caerulea 91.2% (Lasker, 1962); Solea solea 90.8% (Flüchter & Pandian, 1968) and Gadus morhua 88.0% (Mengi, 1965). Water content of herring eggs was not measured in this study but has been reported to be 76% (Blaxter & Hempel, 1966). In herring, percent water increased from hatching to the end of the yolk sac stage, but it decreased in plaice (Figs. 11A & B). In this period there was an actual uptake of water in herring. This same phenomenon has also been reported for larvae of trout (Suyama & Ogino, 1958; Phillips, Podoliak, Dumas & Thoesen, 1958). The differences in water content between herring and plaice are probably related to buoyancy differences of the larvae as has been previously discussed. During growth from the end of the yolk sac stage percent water decreased with increasing length for both species (Fig. 4). This is probably a general phenomenon of growth during development.

Dickerson & Widdowson (1960) reported it for foetal humans and pigs, as well as citing previous examples of decreasing water with growth in mammals. In fish the phenomenon is quite well known (Kizevetter, 1948; Corti, 1950; Phillips, Livingston & Dumas, 1960; Parker & Vanstone, 1966; and Satomi, 1969). The probable cause of these changes in young fishes is that the dividing cells lead to high amounts of extracellular space and water content, both of which decrease with growth. Further evidence for this is shown in that cells increase in size with increasing length of fish (Love, 1958a).

In herring larvae the relative size of the triglyceride (neutral fat) store decreased from the end of the yolk sac stage to a length of 19 to 20 mm. It then increased between 20 and 26 mm, followed by a subsequent decrease (Fig. 17A). The initial decrease and subsequent increase coincided with increasing total nitrogen and carbohydrate to about 21 mm followed by decreases in these components (Figs 17B & C). Decreasing percent triglyceride in fish 26 mm and larger coincided with increasing percent ash from 25 mm (Fig. 17D). It should be noted that all of these changes were only relative ones as percent of the dry body weight, and that throughout this period of growth all constituents were increasing in actual amounts but at different rates (Appendix 1).

In the period from the end of the yolk sac stage to about 20 mm, nitrogen and carbohydrate were being laid down faster than neutral fat stores. Although triglyceride was not being laid down as fast as these other components, other fats such as phospholipids or cholesterol could have been expected to increase about as fast as protein, since they are associated with cell membranes and hence growth (Giese, 1962). That neutral fat stores were not laid down as rapidly as protein, supports the earlier suggestion that it is to the advantage of marine fish larvae from hatching to convert food into growth rather than into purely accumulated energy stores. The total nitrogen deposition representing protein would indicate growth. This increased growth would enable larvae to feed on a wider size range of food particles. After reaching about 20 mm the larvae started to accumulate neutral fat faster than protein. Larvae of this size probably reached a length enabling them to capture a sufficiently wide range of food to warrant a change in their metabolism toward increased deposition of fat. This accumulation of lipid would then increase the energy reserves of the larvae, since fat provides nearly twice the calories per unit weight of protein (9.5 Kcal/g as compared with 5.5 Kcal/g, Winberg, 1971). Triglyceride continued to increase as a percentage of the dry weight until the start of

increasing percentage of ash. Even after the percentage triglyceride started to decrease, fat stores were still being laid down faster than protein as indicated by the ratio of carbon to nitrogen (Fig. 22A). In organisms with low amounts of carbohydrate the carbon/nitrogen ratio indicates relative proportion of lipids to protein (Mullin & Brooks, 1970). The ash is probably an indicator of the start of ossification. The close correlation between the changing patterns of nitrogen and carbohydrate content may be explained by the requirement of a certain amount of carbohydrate for efficient utilization of protein as was shown to be the case for carp (Nagai, 1971).

The same general pattern was found during plaice ontogenesis (Fig. 20). However, the carbon/nitrogen ratio declined in newly metamorphosed plaice, while it continued to increase in herring (Fig. 22). Marshall et al. (1937) also found that larval herring increased the relative size of their fat store with growth towards the very high amounts retained by adults. Adult plaice, however, are not fatty fish and accordingly did not show continuously increasing fat reserves.

The similarities in the pattern of chemical changes during larval growth and development of these species suggest that the pattern may be generally applicable to marine fish larvae.

May (1971) showed that larval grunion have the same relationship between fat and protein. In grunion the fat decreased for 13 days after hatching, while protein increased then declined up to 25 days, when the experiment was terminated. Metamorphosis in this species occurs about 30 days after hatching at 18°C. (Ehrlich & Farris, 1971).

STARVATION CHANGES

WATER: During starvation, percent water increased in all sizes of both herring and plaice (Figs 11B & 16). This change during food deprivation, whether artificially starved in the laboratory or naturally in the sea, from lack of food or during use of reserves for gonad formation, has been reported for many species. Phillips et al. (1960) showed water increased in starved trout. Parker & Vanstone (1966) mentioned increases in water in salmon. Templeman & Andrews (1956) found about 4% increases in American plaice. Love (1960) reported 2 to 3% increases in starved cod. Iles & Wood (1965) reported an inverse relationship between fat and water in herring as well as reviewing previous work in this field. Wilkins (1967) showed water increased about 2% in starved herring as compared to unstarved fat free ones.

The same sort of range 2 to 4% was found for starved herring and plaice larvae and may be indicative of a general pattern in starvation of marine fishes, while fry of fresh water carp have shown increases of about 10% during starvation (Satomi, 1969). Love, Robertson & Strachan (1968) showed that during starvation of cod there was an increase in extracellular space and a decrease in cell size. The same phenomenon was also reported for American plaice (Templeman & Andrews, 1956); both of which would result in a relative increase in percent water. Extracellular space in the myotomes of herring larvae also appears to increase during starvation (R. Pemberton of Dunstaffnage Marine Research Laboratory, personal communication). The general situation was described by Love (1970):

> "Thus when fish are starved, the lipid reserves, wherever situated, decrease to a certain point beyond which the muscle protein is mobilized. As the protein decreases, the water increases, and this is mainly brought about by the shrinkage of the cells and a corresponding increase in the fluid between them."

From this it can be seen that increasing water content is a relative increase as has been found for herring and plaice larvae (Appendix 1 and Table 2).

TRIGLYCERIDE: Throughout starvation of all sizes of herring and plaice larvae, triglyceride continually decreased as a percentage of the dry weight (Figs. 18A and 20A). This component showed the greatest decrease in herring and was reduced to the smallest percentage of the original store in both species. Fat stores offer the larvae the greatest source of stored energy (Winberg, 1971), and considering this it is quite expected that neutral fat should be heavily drawn upon during starvation. That triglycerides were not totally depleted during initial starvation but rather used throughout suggests that a certain amount may be needed to sustain life or possibly for efficient utilization of other components, most likely protein. The percentage of the unstarved triglyceride store utilized to reach the point-of-no-return increased with increasing length of the larvae (Figs. 19 and 21A). This could be interpreted in two ways: first it could mean that larger larvae were capable of surviving greater depletion, or secondly that if triglyceride was used at about a constant rate then the smaller larvae could have used less of this store due to their shorter survival time. However, Table 4 shows for plaice and generally for herring that the rate of loss of triglyceride to the PNR tended to decrease with increasing development. Furthermore, for plaice it can be seen that

percentage of the unstarved store utilized to any particular number of days starved decreased as the larvae got larger (Fig. 21A). It thus seems that the efficiency of triglyceride catabolism increased during ontogenesis. Since smaller larvae were using neutral fat more rapidly the second explanation above can be ruled out. This supports contention that depletion resistance improved during development.

CARBOHYDRATE: The rate of carbohydrate utilization during starvation did not vary with the size of herring larvae, when it was about 3.25% of the dry weight or less (Fig. 18B). However, when more was present the rate of use was dependent on the store size. The rapid loss of carbohydrate to some minimal level is quite expected, since it is usually an energy store and expended during a short period of starvation (Love, 1970). Smith (1952) also showed carbohydrate of trout eggs was quickly utilized, and Barnes & Finlayson (1963) showed that carbohydrate present in starving barnacles was quickly depleted to some low value, after which protein and lipid were used.

The relatively constant rate of loss below 3.25% in herring larvae suggests that carbohydrate needs to be maintained at a minimal level. This situation could well be expected since,

as previously mentioned, carbohydrates may be needed for efficient protein utilization. Furthermore, Bailey (1952) claimed that carbohydrates must be present for complete oxidation of fats. The slight decrease in carbohydrate below 3.25% could be explained by decreases of these other components. The initial loss of carbohydrate probably represented depletion of the carbohydrate stores, while the carbohydrate present after this time may represent products formed from the conversion of fat or protein. It has been shown for salmon that glucose can be formed from lipid, masking glucose depletion during starvation (Robertson, Krupp, Favour, Hane & Thomas, 1961 cited by Love, 1970). The slight decline below 3.25% could also in part reflect differences in blood glucose. Kiermeier (1939, cited by Love, 1970) showed that blood glucose of active species slightly decreased during starvation while in less active fishes the level remained very constant. It should be noted that part of the minimal level of carbohydrate may consist of compounds such as glycoproteins, which are associated with cell membranes (Giese, 1962) and are not readily available as energy sources.

Plaice larvae showed a different pattern of carbohydrate depletion than herring. In these larvae there was no rapid initial decrease, but rather a more gradual loss (Fig. 20B).

Furthermore, the percentage at which the carbohydrate levelled off varied with developmental condition. In percentage terms, larvae starved from the end of the yolk sac stage reached a lower level by the point-of-no-return than larvae starved at stage 3 or more. This suggests that minimal carbohydrate requirements may increase during development. However, this increase may in part also be due to increases in glycoproteins or other bound substances.

NITROGEN: Changes in percent nitrogen during starvation of herring larvae were either very small or not at all detectable. However, the actual amount of nitrogen decreased from the beginning of starvation, showing that protein was being catabolized (Appendix 1). Plaice larvae not only showed decreasing amounts of nitrogen during starvation, but the percentage also declined showing it was being used faster than some other body stores. Breakdown of protein from the start of starvation was also found in grunion larvae (May, 1971). Although May reported percent protein increased during starvation, the actual amounts (percent times dry weight) decreased. The breakdown of protein from the start of starvation may appear to be somewhat surprising in that lipids are more commonly depleted before protein is

mobilized (Love, 1970), at least in adult fishes. This depletion in herring, plaice and grunion and the increasing water content of the first two species were probably related to the size of their fat stores. In these larvae at the end of the yolk sac stage percent fat, obtained by subtracting the difference of the other components from 100%, amounted to about 2% of the wet weight. The wet weight of the grunion was computed on the basis of 89% water, the same as for herring larvae. With such low fat levels larvae could not be considered fatty animals as compared with fish like adult herring. In fact this amount of fat was within the range of 0.8 to 3.1% reported for starved adult herring (Wilkins, 1967). He reported that

> "Once fat deposits are depleted or substantially reduced, then muscle proteins are metabolized for maintenance and the proportion of total nitrogen will tend to decrease".

Furthermore, Phillips et al. (1960) reported for trout that once fat was depleted to about 1.7%, protein was utilized. Since the fat stores of larvae were small, it seemed quite reasonable that protein should have been called upon from the start of starvation. This would also explain the increasing water content and be consistent with Love's (1970) report that in non-fatty fishes fat is depleted followed by a breakdown of protein and an increase in water.

The rate of nitrogen utilization per unit weight to the point-of-no-return decreased with increasing development as it did for triglyceride and carbohydrate (Table 4). However, herring showed the same pattern for nitrogen as for carbohydrate i.e. an increasing rate of use up to 21 mm length followed by subsequent decreases. The rate of use of carbohydrate and nitrogen during starvation may reflect the pattern of deposition of fat and these components as well as the relative proportion of them. It was shown that during ontogenesis the relative size of the triglyceride store decreased to a low value at 20 mm length followed by subsequent increases (Fig. 17A). Nitrogen and carbohydrate showed the opposite pattern. It then seems reasonable that during starvation a relatively larger drain would have to be made on these latter two components during the period of their increasing proportion. The reason plaice showed consistent decreases in their rate of use of chemical constituents was probably due to a lack of starvation samples in the period of increasing levels of nitrogen and carbohydrate and decreasing triglyceride (between days 5 and 16, Fig. 20). In terms of percent of the unstarved amount (Figs. 19 and 21) it could be seen that in both herring and plaice the proportion of nitrogen utilized to reach the PNR increased with development. This same general situation was found for both species

for triglyceride, carbohydrate and nitrogen and appears to suggest increasing depletion tolerances with growth and development. Furthermore, in plaice the percent of initial amount of nitrogen utilized for any given number of days starved decreased with growth as was found for triglyceride. Although this could mean increased efficiency, it could also reflect the increasing size of animal and greater total amounts of stores.

CARBON: Changes in carbon were probably in large part reflections of fat and carbohydrate, although it was also obviously dependent on protein. This can be seen in rates of loss to the point-of-no-return (Table 4). In any case carbon appeared to be a good index of condition (Figs. 18 and 20). In herring larvae, where percent nitrogen did not change much during starvation while triglyceride and carbohydrate decreased, the carbon/nitrogen ratio decreased. However, in plaice, where percent nitrogen, triglyceride and carbohydrate all declined, the carbon/nitrogen ratio gave no consistent pattern.

ASH: Ash content as a percentage of the dry weight markedly increased during starvation of both herring and plaice larvae (Figs. 18 and 20). However, when the changes were compared to the unstarved amount, it could be seen that these increases were largely relative, reflecting decreasing organic constituents. This was

especially true for herring. It also applied to plaice at the end of the yolk sac stage throughout starvation and to stage 3 plaice during the first 7 days of food deprivation. During subsequent starvation of these plaice and stages 4 and 5, actual ash increases were observed. The decreasing actual amounts of ash in herring larvae and early plaice larvae were probably due to losses needed for maintenance of osmotic equilibrium, since actual amounts of water decreased during starvation (Appendix 1). The increasing ash during starvation of older plaice may have been due to increasing skin thickness, since skin has a higher amount of ash than muscle (Young & Lorimer, 1960 cited by Love, 1970). Thickening of the skin has been shown to happen during depletion of adult herring (Hughes, 1963 cited by Love, 1970). Another explanation could have been the actual uptake of salts to help maintain osmotic balance due to the loss of nitrogenous compounds of small molecular weight.

Energy utilization

Energy expenditures in calories used to reach the PNR were estimated for both herring and plaice larvae by multiplying the quantities of chemicals used to the point-of-no-return by appropriate conversion factors for fat (9.5 cal/mg), carbohydrate (4.1 cal/mg) and protein (5.5 cal/mg) obtained from Winberg (1971).

These computed values were multiplied by 100 for clarity and are shown in Table 11. It can be seen that for any size of herring or plaice larvae maximum energy was derived from the catabolism of protein followed by triglyceride and then carbohydrate. There was roughly a three-fold difference between each group. This agrees with reports of energy sources for teleost eggs. Smith (1957) and Lasker (1962) showed the order of importance of food reserves was protein then lipid followed by carbohydrate. It can also be seen that the total energy expenditure to the point-of-no-return decreased with development of plaice, but that in herring it increased to a length of 21 mm followed by subsequent decreases. The reason for this is probably explained by the changes in utilization of protein and carbohydrate previously descussed.

An attempt was made to correlate losses of triglyceride, carbohydrate and protein with oxygen consumption of herring larvae at the end of the yolk sac stage. Holliday, Blaxter & Lasker (1964) reported the Q_{O_2} was about 2 for herring larvae during starvation, although there was some tendency for it to decrease in this period. This value was used in conjunction with oxygen combustion values for fat (2 ml/mg), carbohydrate (0.825 ml/mg) and protein (1.22 ml/mg) reported by Winberg (1971). With these values and the amounts of these chemical components utilized to the point-of-no-return (Appendix 1), the time to burn these components and reach the

Table 11. Calories* used to PNR (calories x100/mg unstarved dry weight/day).

	unstarved dry weight	Days to PNR	µg Protein N x 6.025	Calories x100 Triglyceride	Carbohydrate	Protein	cal x100
Herring							
12 mm	0.1689	6	25.3	4.8	0.7	13.9	19.4
15 mm	0.4685	8	27.1	4.0	0.9	14.9	14.8
21 mm	2.1810	9	33.2	4.2	1.4	18.2	23.8
25 mm	4.8393	12	19.9	3.8	0.7	10.9	15.4
30 mm	11.1358	15	19.3	3.6	0.5	10.6	14.7
35 mm**	22.5286	15**	18.7	3.5	0.5	10.3	14.3
Plaice							
End yolk sac	0.1272	6	45.8	7.0	1.7	25.2	33.9
Stage 3	1.055	15	19.3	2.8	0.9	10.6	14.3
Stage 4	1.786	23	13.3	2.2	0.5	7.7	10.0
Stage 5**	2.242	17**	15.7	3.0	0.6	8.6	12.2

** Experiment terminated before fish reached PNR. Rate based on larvae at last day of experiment.

* Calorific values for fat, carbohydrate and protein taken from Winberg (1971).

point-of-no-return was calculated (Table 12). The computed time (six days) agreed with the behavioural observations of the time to the PNR from yolk resorption (Table 3). These values can only be considered as approximate since total lipid was not known and because protein was not chemically determined. However, it is apparent that the time required to deplete the body components to the extent that the point-of-no-return is reached is very much dependent on oxygen consumption. Holliday et al. (1964) also showed that the Q_{O_2} was dependent upon activity being as much as 9 times the resting value in very active larvae. An increase to even $Q_{O_2} = 3$ would decrease the time to reach the PNR by 2 days or 33%. From this it is obvious that the observed decrease in activity during starvation is of great survival advantage (as long as activity is sufficient for searching), by extending the time over which larvae are capable of feeding. Blaxter (personal communication) recently has shown by activity meters that herring and plaice larvae decrease their activity during starvation. This same situation has also been shown for sole (Rosenthal, 1966). This agrees with the general concept of the metabolic rate decreasing during starvation due to a form of conservation metabolism (Phillips, 1969).

Table 12. Oxygen and time required to burn proximate compounds used to PNR, by herring larvae.

Components	Triglyceride	Carbohydrate	Protein
O_2 equivalent (ml/mg)①	2000	825	1220
Amount used to PNR (mg)②	0.0050	0.0018	0.0250
O_2 used to burn compounds (µl)	10	1.5	30.5

Total O_2 consumed = 42 µl

	Larval dry weight (mg)
EY	0.1689
PNR	0.1278
Mean weight	0.1483

Rate of larval O_2 consumption WT x Q_{O_2}③ = 0.1483 x 2 ≈ .3 µl/hr

Time required to use 42 ul O_2 or to reach PNR = $\frac{42}{.3}$ = 132.5 hrs = 6 days

If Q_{O_2} = 3 ⇒Rate O_2 consumption = .45 µl/hr ⇒days to PNR = 4

① O_2 equivalents from Winberg (1971)

② Amount used to PNR from Appendix 1A

③ Q_{O_2} = 2 from Holliday, Blaxter & Lasker (1964)

Identifying the PNR

One of the main aims of this work was to identify chemically the changes in herring and plaice larvae during starvation that led to the point-of-no-return. Percent changes are only relative and may not represent real changes in a component, but in terms of identifying the PNR they have been useful, sometimes more so than real changes. In herring and plaice larvae, increasing percent water was a very good nutritive indicator more so than the actual amount (Fig. 16 and Appendix 1). This was even more true for ash where the actual amount changed little, but the percentage rapidly increased. On the other hand in herring larvae percent changes in nitrogen were of no use in identifying condition, although they were of some use in plaice. In both species, however, actual amounts of nitrogen gave better indications of the condition of the larvae (Appendix 1). Percent changes of both triglyceride and carbohydrate gave good estimates of nutritional condition, but in both actual amounts showed greater differences between healthy and starved larvae. Changes in total carbon in percent or actual amount gave good condition estimates. However, ratios of carbon to nitrogen showed condition only for herring larvae. This ratio was not of much use for predicting condition of plaice larvae due to concurrent variation in both carbon and nitrogen.

In all chemical determinations it was imperative that only larvae of the same size or developmental state be included in one determination. Chemical comparisons between larvae of different sizes, regardless of age, could not yield meaningful results as to their nutritive condition.

Aside from chemical comparisons, and more easily determined, eye to head height ratios were useful for estimating nutritive condition. Length-weight relationships and relative condition factors could also be used, although the results are not very explicit due to concurrent decreases in length and weight. The sinking rates of anaesthetized larvae in water gave good correlation with condition. The best situation is to estimate larval condition by several methods and to pool the results.

Some parental effects on herring larvae

Aside from food and its availability other factors such as the size of larvae can affect their survival. Blaxter & Hempel (1963) showed that egg size is positively correlated with larval size and growth rate. Egg size varied between female herring, but did not necessarily depend on the maternal size (Table 5). In an attempt to relate egg size to larvae in chemical terms the composition of eggs was first examined in relation to egg size and hatching success (Table 5). Studies of chemical analyses on marine fish eggs have

been very limited. Mean values of percent composition were compared between herring eggs and published results on another clupeid (Sardinops caerulea) (Lasker, 1962). The nitrogen level in the herring eggs was nearly identical to that of the sardine (11.4%). However, triglyceride was nearly twice the level of 4% found in the sardine, while total lipid in herring eggs (total lipid = 100% - Σ(protein + carbohydrate + ash)) was less than the 13% of the sardine; thus fats such as phospholipid and/or cholesterol must have been considerably less in herring. Another major difference between the species was in carbohydrate levels: 4.17% in herring eggs and less than 1% in those of the sardine (Lasker, 1962). Ash content was also less in herring eggs than in those of sardines. These differences between species in amounts and composition of lipids, as well as of carbohydrate, probably reflect differences in developmental needs and energy sources of larvae of different sizes. At the end of the yolk sac stage herring larvae are 5 to 6 times the weight of sardines (sardine data from Lasker, 1962). It is generally known that metabolic rate per unit weight increases inversely to size (Schaeperclaus, 1933; Woynarovich, 1964, both cited by Phillips, 1969). Considering this and that sardines are smaller than herring, one would expect sardine larvae to have a higher energy requirement per unit weight at the end of the yolk sac stage. It can be seen from Table 5 and from the

work of Lasker (1962) that the sum of fat plus carbohydrate for both species was about equal, but fat was greater in the sardine. Since fat catabolism supplies more than twice the energy of carbohydrate, the differences between herring and sardines are quite understandable. However, the presence of more fat in the sardine egg could also be for buoyancy purposes, since sardine eggs are pelagic while those of herring are demersal. Although water provides the major lift to the pelagic plaice egg the role of fat should not be discounted since it has been shown to increase in relative importance during development (Dakin & Dakin, 1925).

Table 5 shows that there was no general pattern between hatching success and any of the measured parameters. The only obvious difference was in the percentage of carbon in female no. 4 where the value was over 6% higher than others and significantly different. The explanation for this abnormally high level of carbon was not obvious from the available data. It may, in part, have been due to the slightly higher level of carbohydrate, but fat must also have been involved, for the carbon to nitrogen ratio increased more than was explained by carbohydrate. A more detailed analysis of various lipid components would appear to be very beneficial. The low hatching success may have been due to factors more subtle than detected by proximate analyses. Hirao, Yamada & Kikuchi (1955) showed for rainbow trout eggs that neither water, total nitrogen nor

fat were related to hatching success, but iron content was. They also found a slight correlation between hatching success and vitamin B_2. The high level of carbon in all eggs (at least 50% as compared with that in larvae (40 to 44%) was probably due to glycoproteins and phospholipids found in chorion (Blaxter, 1969).

It was previously stated that Blaxter & Hempel (1963) showed that egg size influenced both size and growth rate of larvae. However, the influence of larval size upon chemical changes during starvation has not previously been looked at. An attempt was made to compare these factors (Figs. 24 & 25). The only significant correlations between percent composition and larval size were in carbohydrate and ash where negative correlations were found and carbon and the carbon/nitrogen ratio where a positive correlation was obtained. However, when carbohydrate and ash were compared as actual amounts versus larval size positive correlations were obtained. This means that both of these products decreased only in relative amounts, because some other factor was increasing more rapidly. Protein was ruled out with nitrogen as was triglyceride, but since the carbon/nitrogen ratio increased another fat component probably increased.

Dietary studies

The purpose of the diet experiments was to compare growth, survival and chemical composition of herring and plaice larvae fed for 20 days on foods commonly used in rearing. Artemia is a most commonly used food, which has been fed to many groups of fishes as has wild plankton (see May, 1970 for review of larval foods). The rotifer Brachionus plicatilis has been used less (May, 1970), but its use is becoming more widespread. Theilacker & McMaster (1971) used it for anchovy larvae, and it is also being used for various flatfishes (Howell, 1972). The effects of these foods was compared for herring and plaice with an additional mixture of these diets also fed to herring.

Comparison of the length-weight lines of herring larvae fed on different diets (Fig. 26), showed as in starvation (Fig. 6), that dietary differences or lack of food do not affect length-weight relationship of one size group more than another. That is the slopes of the lines were equal. However, the diets did alter the relative condition factor of larvae (Appendix 11). Lasker et al.(1970) found that larval anchovies fed on various natural diets had the same length-weight line. Farris (1956) showed that adult sardines fed on fish meal plus other additives had a length-weight relationship with the same slope as starved fish, but the fed fish had a higher

relative condition factor. He also found that if sardines were fed a high carbohydrate diet the slope was increased. The lack of differences in the lengths of larvae at the end of the dietary experiment (Appendix 11) may have been due to the wide size range, or it may reflect too short a period of diet differences. However, it seems more likely that all diets supplied adequate nutrition for growth over the experimental period, but that physical and/or chemical differences affected the chemical stores of the larvae and hence their condition. This same explanation should also apply to the lack of significant differences in the lengths of the plaice larvae.

The lower relative condition factor of herring larvae fed on rotifers was probably partially due to this food's significantly lower stores of neutral fat (Table 8). This component, however, could not by itself be entirely responsible for the differences in condition, for although Artemia-fed larvae had the highest amounts of triglyceride, they did not have the highest relative condition factor. The chemical stores of the larvae must interact with each other as well as with various physical parameters to produce the condition of larvae. In this particular case the relatively small size of the rotifers (about 100 μ) would have led to a net higher energy expenditure in the capture of food. This would also obviously

have a direct effect upon larval condition. The highest eye:head height ratio was also found in rotifer-fed herring, which agreed with the lowest relative condition factor for these larvae.

Although not significant at the 5% level in both larval species the highest level of triglycerides was in Artemia-feeders, while the lowest was in those fed on rotifers. This was also the pattern of triglyceride in the foods (Table 8). Bailey (1952) showed that fat deposits are usually laid down only when ingested fat exceeds that burnt for energy requirements. Considering this, it is quite expected that larvae fed on a diet with the most fat should have had the highest stores of it. The positive relationship between triglyceride content between larvae and their food did not appear in plaice larvae. In these larvae the lowest triglyceride was in plankton-feeders (Table 9). It may be that plaice larvae feeding on rotifers could make up for the low levels of neutral fat by conversion of the fairly high levels of carbohydrate found in rotifers, while when fed on plankton, also with fairly low levels of triglyceride, the conversion could not be made because of insufficient carbohydrate. The absence of this same pattern in herring may have been due to interspecific differences in efficiency of transformation of carbohydrate to fat. This suggests that the experimental foods did not have equal nutritive value for the two species of larvae. Further evidence for this was found in comparison

of survival rates during diet experiments (Figs. 27 & 28). The highest mortality of plaice larvae was on those fed rotifers, while for herring it was for Artemia-feeders. Since other diets had higher survival rates, it seems that rotifers and Artemia diets were not by themselves completely adequate for plaice and herring larvae respectively. The pattern of mortality for plaice suggests that some essential component may have been missing from the diet, or that there was an accumulation of something toxic.

The higher mortality of herring fed Artemia showed its incomplete adequacy and agreed with the findings of Blaxter (1968) that herring larvae fed on Artemia alone did not survive past 25 mm. A similar situation was found for cod reared on Artemia (Dannevig & Dannevig, 1950). The cause for this mortality may at least partially be found in the quantity and quality of Artemia lipids and those resulting in the fish larvae. Roberts (1970) showed that "0" group plaice continuously fed on high fat diets had abnormal areolar fat. It may be that accumulation of large lipid stores is detrimental to the larvae. However, it could also be the composition of the ingested lipids that is harmful. It has been shown that lipid stores usually closely resemble those ingested (Lovern, 1935). Phillips & Podoliak (1957) showed that too much low saturated fat in the diet of trout led to accumulation of it in the kidneys and

subsequent malfunctions. They also reported that an excess of highly saturated fat led to clogging of the intestinal tract and higher mortalities. Furthermore, they pointed out that fat can infiltrate the liver yielding swollen cells of impaired efficiency resulting in death. If the high fat content of Artemia is the cause of the poor survival of herring and cod larvae, then it might be possible by raising the rearing temperature to reduce fat accumulation and mortality. Phillips (1969) reported that fat requirements, prior to laying down of stores, increase with temperature.

Aside from high fat content, the concurrently high carbohydrate level of Artemia may have been partly responsible for the poor survival. Hess (1935), showed that trout cannot cope with high carbohydrate in the diet, because it is not a normal part of their food, and they have only a diffuse pancreas with few insulin producing Islets of Langerhans. Carbohydrate level in Artemia is over three times greater than in plankton, their normal food. Furthermore, Kitamikado, Morishita & Tochino (1965) found that high starch levels in the diet of rainbow trout decreased digestion of proteins resulting in decreased amounts available for metabolism. This sort of phenomenon may be especially critical to larvae, for as Phillips (1969) reported, small fast growing fish need more protein.

The similarity of growth rates per day of feeding of newly hatched herring on rotifers and mixed diets showed that in this sense one food had no clear advantage over the other. However, the small size of the rotifers enabled the larvae to start feeding two days earlier, which led to highly significant differences in length by 28 days. Thus, in terms of improving growth rates of larvae in the laboratory the rotifer Brachionus plicatilis was of significant value. This, however, did not improve survival, although survival might have been better in species with very small larvae and small mouths. It may be that rotifers used by themselves are not completely adequate for young herring, but if used in addition to other foods they may improve growth and survival. A similar situation was found in rearing anchovies on this rotifer (Theilacker & McMaster, 1971).

Conclusion

It is obvious from this discussion that this work only begins an understanding of the chemistry of herring and plaice larvae. Its purpose was to help identify and understand what happens to larvae of different sizes as they starve and reach the point-of-no-return and to show changes during growth and development of the larvae.

The parental and dietary studies were intended to examine how some aspects of these factors can influence the composition of the larvae as well as their survival. These purposes were reached, but in the process a multitude of other questions have been raised relating to mechanisms of changes and interactions between the main chemical components.

SUMMARY

1. Herring (Clupea harengus) and plaice (Pleuronectes platessa) were reared from eggs through metamorphosis during which time morphometrical, behavioural, and chemical changes were followed in relation to growth and starvation. The main aim was to supply information, based on laboratory reared animals, which would subsequently provide a basis for comparison with the same species caught in the sea. Another aim of the starvation study was to identify clearly larvae which reached irreversible starvation (the point-of-no-return).

2. Growth rates of herring and plaice larvae from the end of the yolk sac stage (6 days post-hatching) through metamorphosis were 0.22 and 0.16 mm/day respectively.

3. The slope of the length-dry weight line for plaice larvae was 3.92. That for herring larvae was 4.57, which remained constant throughout starvation, although the intercepts decreased. If wet weights were used the slopes decreased.

4. Relative condition factors (RCF) were used to estimate nutritive condition over a wide size range. RCF is a function of k from the equation $W = k\ L^n$, where W and L represent weight and length respectively. The use of RCF as opposed to the ordinary condition factor $\frac{W}{L^3}$ was discussed. Condition factors based on length and weight were not very good for estimating nutritive condition due to concurrent loss of both length and weight.

5. During starvation the ratio of eye to head height rapidly increased due to shrinkage of the head. This enabled this ratio to be used as a good indicator of nutritive condition.

6. The sinking rate of herring larvae in sea water (10^{o}C., 33‰) decreased from 0.407 cm/sec at hatching to 0.225 cm/sec at the end of the yolk sac stage. In feeding larvae the sinking rate then increased to 1.54 cm/sec by 25 mm in length. Newly hatched plaice larvae were positively buoyant, but their sinking rate increased to 0.185 cm/sec by the end of the yolk sac stage. The rate continued to increase during growth and development, reaching 1.120 by 38 days. In both species the sinking rate decreased during starvation. It was suggested that this is a mechanism of energy conservation. The changes in sinking rate from hatching between the species were related to habitat differences and behavioural changes during ontogenesis.

7. Water content was inversely related to the sinking rate, but other body components also influenced it. The forces contributing to the sinking rate from fat, carbohydrate, and protein, as well as water were computed during starvation, from the end of the yolk sac stage for both herring and plaice. In both species water provided the major upward vector, followed by fat, while protein was responsible for the downward force. The decrease in sinking rate during starvation was partially due to the increasing percentage

of water, although the major part of the decrease was due to nitrogen catabolism throughout starvation.

8. Herring and plaice at the end of the yolk sac stage required 6 days of starvation to reach the point-of-no-return (PNR). In both species the days of starvation to reach this condition increased during development, but the rate of increase was greater in plaice. Over 50% of the life span of herring and plaice starved from the end of the yolk sac was spent past the PNR, suggesting starved larvae may be caught in larval surveys.

9. Changes in water, triglyceride, carbohydrate, nitrogen, carbon, and ash were followed during ontogenesis from the end of the yolk sac stage through metamorphosis as well as during starvation of various size groups of herring and plaice larvae. Ontogenetic changes were dependent upon larval size rather than age. Throughout development from the end of the yolk sac stage to metamorphosis the percentage of water continuously decreased, from about 89 to about 82% in herring and from about 88 to about 84% in plaice. In the period of initial post-hatching growth (up to 20 mm in herring and stage 2 in plaice) nitrogen and carbohydrate were laid down faster than triglyceride. The pattern was then altered in larger larvae. It was suggested that the initial preferential deposition of protein to triglyceride was advantageous to the larvae in that it enabled them to put on growth more quickly than if they had been simultaneously

accumulating energy stores. This increased the size range of foods available to them and hence their chances of survival.

During starvation of herring and plaice larvae the percentage of water increased about 4% above the unstarved level. The percentage of triglyceride, carbohydrate, and carbon decreased in both herring and plaice as did nitrogen in plaice. However, in herring the percentage of nitrogen did not change throughout starvation, although the actual amount decreased. In both species percent ash rapidly increased during starvation. In herring the C/N ratio decreased in starved animals, but it did not show a consistent pattern in plaice due to concurrent changes in both carbon and nitrogen. The most striking feature of the starvation study was the continuous depletion of nitrogen throughout starvation in both species. It was suggested that since nitrogen catabolism was responsible for the decreasing sinking rate of the larvae during starvation, this unusually high rate of use could be an adaptation by pelagic marine larvae to the planktonic environment.

10. Herring egg composition from different females was related to hatching success. The eggs yielding the lowest hatching contained about 57% carbon, almost 7% more than the others and exceeding the 99% confidence limits of the others. Percent carbohydrate and ash showed significant decreases with increasing larval herring size at the end of the yolk sac stage. Percent carbon and the C/N ratio

increased with increasing larval size.

11. The size and chemical composition of 100 day-old herring larvae was compared after 20 days of feeding on diets of *Artemia*, rotifers, plankton, or a mixture of the others. The same was done for 50 day-old plaice, excluding the mixed diet. The diets did not alter the slopes of the length-weight line of herring larvae, but the intercepts were affected. The order of RCF for herring larvae fed on the different diets was (lowest first) rotifers, *Artemia*, plankton, and the mixed diet. The survival pattern of herring larvae on the different diets was rotifers 56%, mixed diet 52%, plankton 48% and *Artemia* 44%. There was a tendency for the size of the larval fat store to be related to the amount in the diet.

Newly hatched herring larvae started to feed on rotifers 5 days post-hatching, but those offered a mixture of *Balanus balanoides* nauplii, *Artemia*, and plankton did not start to feed until day seven. This led to a significant difference in length between the groups by 28 days. Herring larvae 20 mm in length were each able to eat 190 rotifers/day.

Survival of the plaice fed on the different diets was *Artemia*-fed 80%, plankton-fed 58%, and rotifer-fed 14%. There were no significant differences in larval size between the groups. The only significant difference in composition of the larvae fed on the different diets was in water content; the rotifer-feeders had the least. The relationship between larval and food composition was discussed.

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APPENDICES

Appendix 1A. Herring standard amounts.

Length mm	Age	Days starved	WATER wet wt. (mg)	WATER dry wt. (mg)	WATER % initial	WATER %	WATER µg	WATER % initial	TRIGLYCERIDE %	TRIGLYCERIDE µg	TRIGLYCERIDE % initial	CARBOHYDRATE %	CARBOHYDRATE µg	CARBOHYDRATE % initial	NITROGEN %	NITROGEN µg	NITROGEN % initial	ASH %	ASH µg	ASH % initial	CARBON %	CARBON µg	CARBON % initial	C/N	C/N % initial	Sinking rate cm/sec
12.00	19	0	1.5327	0.1689	100	88.98	1363.8	100	8.08	13.6	100	3.41	5.8	100	10.42	17.6	100	7.50	12.7	100	42.22	71.3	100	4.05	100	0.21
12.06		3	1.4843	0.1468	87	90.11	1337.5	98	6.72	9.9	72	3.26	4.8	83	10.44	15.3	87	8.53	12.5	99	41.62	61.1	86	3.99	98	0.15
11.94		6[a]	1.4200	0.1278	76	91.00	1292.2	95	6.70	8.6	63	3.12	4.0	69	10.41	13.3	76	9.48	12.1	96	40.47	51.7	72	3.89	96	0.09
11.68		9	1.1898	0.1053	62	91.15	1084.5	80	5.99	6.3	46	2.92	3.1	53	10.36	10.9	62	9.68	10.2	80	38.55	40.6	57	3.72	92	0.05
11.60		12	1.1854	0.0959	57	91.91	1089.5	80	4.75	4.6	33	2.77	2.7	46	10.34	9.9	56	10.17	9.8	77	35.92	34.4	48	3.47	86	0.05
11.46		15	0.7528[b]	0.0798	47	89.40[b]	673.03	49	4,36	3.5	26	2.69	2.2	37	10.32	8.2	47	11.43	9.1	72	35.80	28.6	40	3.47	86	0.10[b]
15.00	32	0	3.7330	0.4685	100	87.45	3264.6	100	6.54	30.6	100	3.88	18.2	100	11.02	51.6	100	7.50	35.1	100	42.89	200.9	100	3.89	100	0.42
15.42		3	3.9023	0.4515	96	88.43	3450.8	106	5.35	24.2	79	3.26	14.8	81	11.11	50.2	97	8.53	38.5	110	42.37	191.3	95	3.81	98	0.34
15.12		6	3.5424	0.3762	80	89.38	3166.2	97	5.08	19.1	62	3.12	11.7	65	11.05	41.6	81	9.48	35.7	102	41.17	154.9	77	3.73	96	0.26
		8[a]							(8)	14.9		(8)	9.5		(8)	34.7		(8)	30.4		(8)	128.4				
14.54		9	2.7681	0.2865	61	89.65	2481.6	76	4.50	12.9	42	2.92	8.4	46	10.93	31.3	61	9.68	27.7	79	40.19	115.1	57	3.68	95	0.16
14.47		12	2.7159	0.2610	56	90.39	2454.9	75	3.68	9.6	31	2.77	7.2	40	10.92	28.5	55	10.17	26.5	76	37.57	98.1	49	3.44	88	0.15
14.26		15	1.7932[b]	0.2168	46	87.91[b]	1576.4	48	3.30	7.2	23	2.69	5.8	32	10.88	23.6	46	11.43	24.8	71	37.40	81.9	40	3.44	88	0.23[b]
21.00	59	0	14.6869	2.1810	100	85.15	12505.9	100	5.85	127.7	100	4.78	104.2	100	12.23	266.7	100	7.50	163.6	100	44.34	967.1	100	3.63	100	1.02
22.13		3	14.9765	2.1027	96	85.96	12873.0	103	5.12	107.6	84	3.26	68.6	66	12.45	261.8	98	8.53	179.4	110	43.14	907.1	94	3.46	95	1.05
20.73		6	12.4554	1.5918	73	87.22	10863.6	87	4.28	68.2	53	3.12	49.7	48	12.17	193.7	73	9.48	150.9	92	41.93	667.4	69	3.44	95	0.78
20.29		9[a]	10.4062	1.3143	60	87.37	9091.9	73	3.20	42.1	33	2.92	38.4	37	12.08	158.8	60	9.68	127.2	78	40.50	532.3	55	3.35	92	0.60
20.16		12	10.0976	1.1996	55	88.12	8898.1	71	2.90	34.8	27	2.77	33.2	32	12.06	144.7	54	10.17	122.0	75	40.50	485.8	50	3.36	93	0.59
19.91		15	6.9360[b]	0.9961	46	85.63[b]	5939.3	47	2.37	23.6	18	2.69	26.8	26	12.07	119.7	45	11.43	113.9	70	40.50	403.7	42	3.37	93	0.71[b]
25	77	0	30.170	4.8393	100	83.96	25.3309	100	7.17	346.8	100	3.83	185.3	100	11.84	573.0	100	7.54	364.9	100	44.34	2145.8	100	3.74	100	1.54
		3	27.642	4.1104	85	85.13	23.5318	93	6.03	248.0	72	3.26	134.0	72	11.84	486.7	85	8.46	347.7	95	43.14	1773.2	83	3.64	97	1.50
		6	26.669	3.7470	77	85.95	29.9220	90	5.43	203.5	59	3.12	116.9	63	11.84	443.6	77	9.20	344.7	94	41.93	1571.1	73	3.54	95	1.36
		9	24.289	3.4126	71	85.95	20.8764	82	3.83	130.8	38	2.92	99.6	54	11.84	404.0	71	9.74	332.4	91	40.50	1382.1	64	3.42	91	1.18
		12[a]	24.027	3.2076	66	86.65	20.8194	82	3.62	116.1	34	2.77	88.8	48	11.84	379.8	66	10.30	330.4	90	40.50	1299.1	61	3.42	91	1.18
		15	17.712[b]	2.8215	58	84.07[b]	14.8904	59	2.92	82.4	24	2.69	75.9	41	11.84	334.1	58	11.63	328.1	90	40.50	1142.7	53	3.42	91	1.40[b]
30	99	0	64.406	11.1358	100	82.71	53.270	100	7.59	845.2	100	3.25	361.9	100	11.54	1285.1	100	8.31	925.4	100	44.34	4937.6	100	3.84	100	
		3	58.676	9.4585	85	83.88	49.217	92	6.55	619.5	73	3.26	308.4	85	11.54	1091.5	85	9.12	862.6	93	43.14	4080.4	83	3.74	97	
		6	56.318	8.6223	77	84.69	47.696	90	5.67	488.9	58	3.12	269.0	74	11.54	995.0	77	9.64	831.2	90	41.93	3615.3	73	3.63	95	
		9	51.291	7.8527	71	84.69	43.439	82	4.03	316.5	37	2.92	229.3	63	11.54	906.2	71	10.46	821.4	89	40.50	3180.3	64	3.51	91	
		12	50.590	7.3811	66	85.11	43.209	81	3.78	279.0	33	2.77	204.5	56	11.54	851.8	66	11.16	823.7	89	40.50	2989.4	61	3.51	91	
		15[a]	37.792[b]	6.4926	58	82.82[b]	31.299	59	3.13	203.2	24	2.69	174.6	48	11.54	749.2	58	12.56	815.5	88	40.50	2629.5	53	3.51	91	
35	122	0	122.839	22.5286	100	81.66	100.310	100	7.17	1615.3	100	3.25	732.2	100	11.29	2543.5	100	9.04	2036.6	100	44.34	9989.2	100	3.93	100	
		3	111.446	19.1353	85	82.83	92.311	92	6.14	1174.9	73	3.26	623.8	85	11.29	2160.4	85	9.86	1886.7	93	43.14	8255.0	83	3.82	97	
		6	106.624	17.4436	77	83.64	89.180	89	5.25	915.8	57	3.12	544.2	74	11.29	1969.4	77	10.38	1810.6	89	41.93	7314.1	73	3.71	94	
		9	97.107	15.8867	71	83.64	81.220	81	3.61	573.5	36	2.92	463.9	63	11.29	1793.6	71	11.19	1777.7	87	40.50	6434.1	64	3.59	91	
		12	95.416	14.9326	66	84.35	80.483	80	3.36	501.7	31	2.77	413.6	56	11.29	1685.9	66	11.90	1777.0	87	40.50	6047.7	61	3.59	91	
		15	72.052[b]	13.1351	58	81.77[b]	58.917	59	2.71	356.0	22	2.69	353.3	48	11.29	1483.0	58	13.30	1747.0	86	40.50	5319.7	53	3.59	91	

a Start of PNR

b Moribund

Appendix 1B. Plaice standard amounts.

Stage	Age	Days starved	Wet wt. (mg)	Dry wt. (mg)	% initial	TRIGLYCERIDE %	TRIGLYCERIDE µg	TRIGLYCERIDE % initial	CARBOHYDRATE %	CARBOHYDRATE µg	CARBOHYDRATE % initial	NITROGEN %	NITROGEN µg	NITROGEN % initial	ASH %	ASH µg	ASH % initial	CARBON %	CARBON µg	CARBON % initial	C/N	C/N % initial
EYS	6	0	1.0761	0.1272	100	7.30	9.3	100	4.65	5.9	100	11.07	14.0	100	9.65	12.3	100	42.53	54.1	100	3.85	100
		2	0.8766	0.1030	81	6.50	6.7	72	4.74	4.9	83	11.38	11.7	83	10.37	10.7	87	41.27	42.5	79	3.52	91
		4	0.9614	0.1070	84	4.80	5.1	55	4.42	4.7	80	10.88	11.6	83	10.29	11.0	90	39.63	42.5	79	3.64	95
		6[a]	0.8337	0.0822	65	4.42	3.6	39	3.28	2.7	46	9.91	8.2	58	11.54	9.8	80	36.92	30.4	56	3.72	97
		7	0.7823	0.0769	60	2.42	1.9	20	2.52	1.9	33	9.59	7.4	53	12.54	9.6	79	37.66	29.0	54	3.93	102
		9	-	0.0758	60	2.30	1.7	19	2.61	2.0	44	9.79	7.4	53	13.35	10.1	82	37.45	28.4	52	3.83	99
		12	0.8496	0.0740	58	2.08	1.5	17	2.69	2.0	44	9.93	7.4	53	13.48	10.0	81	37.12	27.5	51	3.74	97
2	16	0		.3940		4.56	18.0		7.25	28.6		11.75	46.3		9.48	37.4		43.73	172.2		3.72	
3	27	0		1.055	100	7.54	79.5	100	5.82	61.4	100	11.65	122.9	100	9.28	97.9	100	44.62	470.7	100	3.83	100
		5		0.801	76	6.78	54.3	68	4.78	38.3	62	11.64	93.2	76	10.44	83.6	85	42.87	343.4	73	3.68	96
		7		0.641	61	6.70	41.6	52	4.60	29.5	48	11.66	74.5	61	10.32	67.1	68	44.50	285.2	61	3.83	100
		11		0.696	66	5.29	38.1	48[a]	4.19	29.2	47	10.79	75.1	61	11.01	76.0	78	41.74	290.5	62	3.87	101
		15[a]		0.708	67	4.74	33.6	42	3.90	27.6	45	10.29	72.8	59	12.62	89.4	91	39.87	282.3	60	3.87	101
		19		0.670	64	3.53	23.6	30	3.80	25.5	41	10.37	69.5	57	13.05	87.4	89	37.66	251.9	54	3.63	95
		24		0.666	63	1.80	12.0	15	3.69	24.6	40	9.78	65.1	53	13.91	92.6	94	38.08	253.6	54	3.89	102
4	28	0		1.786	100	7.79	139.1	100	5.06	90.4	100	11.48	205.0	100	9.06	161.8	100	43.79	782.1	100	3.81	100
		4		1.736	97	6.68	116.0	83	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		6		1.687	94	5.61	94.6	68	4.35	73.4	81	11.06	186.6	91	10.02	169.0	104	42.63	719.2	92	3.85	101
		10		1.450	81	4.86	70.5	51	4.18	60.6	67	11.06	160.4	78	13.62	197.5	122	41.38	600.0	77	3.74	98
		14		1.424	80	4.62	65.8	47	3.75	53.4	59	10.53	150.0	73	13.97	198.9	123	39.87	567.8	73	3.79	99
		18		1.241	69	3.66	45.4	32	4.23	52.5	58	10.58	131.3	64	14.52	180.2	111	40.41	501.5	64	3.82	100
		23[a]		1.144	64	3.74	42.8	31	3.97	45.4	50	9.93	113.6	55	17.60	194.5	120	35.57	406.9	52	3.58	94
		27		0.909	51	3.60	32.7	24	4.08	37.1	41	9.84	89.4	44	17.76	161.4	100	36.23	329.3	42	3.68	97
5	41	0		2.242	100	7.85	176.0	100	5.78	129.6	100	11.29	253.1	100	9.62	215.7	100	44.20	991.0	100	3.91	100
		4		2.185	97	7.50	163.9	93	4.35	95.0	73	11.18	244.3	97	10.54	230.3	107	43.39	948.1	96	3.88	99
		10		2.075	93	5.36	110.7	63	3.68	76.5	59	10.96	227.7	90	11.32	234.7	109	40.76	846.0	85	3.72	95
		14		2.004	89	3.24	64.4	37	3.43	68.8	53	10.44	209.3	83	13.18	247.0	130	40.61	814.0	82	3.89	99
		17		1.407	63	3.73	52.1	30	3.83	53.6	41	10.84	152.4	60	15.28	213.9	99	38.51	541.4	55	3.55	91
Post meta.	51	0		2.578		5.95	153.4		3.65	94.1		10.90	281.0		11.46	295.4		41.35	1066.0		3.79	

a Start of PNR

Appendix 2. Herring length-weight relationships.

equations of form $y = bx + c$

Days starved	Equation log wt =	N	Stand. dev. of b	Stand. err. of c	Stand. dev. y for fixed x
0	4.5459 log L-5.6715	193	0.0268	0.0313	0.0539
3	4.6124 log L-5.8248	36	0.0868	0.0944	0.0388
6	4.5064 log L-5.7401	36	0.1011	0.1099	0.0425
9	4.5650 log L-5.8497	74	0.0993	0.1092	0.0604
12	4.6577 log L-5.9872	25	0.0234	0.0725	0.0505
15	4.6231 log L-6.0084	25	0.0395	0.0461	0.0411

1. Ho: Variances are homogeneous, $F = 2.73$; 5,64000 df; $.01 < p < 0.025$
2. Ho: one regression line can be used for all data
   $F = 80$; 10,377 df; $p < 0.01$
   $\therefore$ reject Ho
3. Ho: lines have equal slopes
   $F = 0.81$; 5,377 df; $0.5 < p < .75$
   $\therefore$ accept Ho
   pooled slope = 4.5710

Best estimates of individual regressions based on pooled slope

Days starved	log wt = 4.5710 log L -	relative c.f. $\times 10^5$
0	5.7052	0.1972
3	5.7761	0.1675
6	5.8163	0.1527
9	5.8569	0.1390
12	5.8838	0.1307
15	5.9395	0.1149

Appendix 3. Herring percent water versus length 10 to 45 mm.

equations of form $y = bx + c$

Days starved	Equation % water =	N	Stand. dev. of b	Stand. err. of c	Stand. dev. of y for fixed x
0	-15.4068 log L+105.5146	129	0.2588	0.3018	0.3644
3	-16.2791 log L+107.8236	71	0.2212	0.2523	0.2609
6, 9	-15.8030 log L+108.0266	37	0.2455	0.2784	0.1719
12	-15.8821 log L+108.8451	16	0.5737	0.6637	0.2611
III	-15.1611 log L+105.3484	21	0.7164	0.8044	0.2974

III = moribund

1. Ho: variances are homogeneous $F = 2.93$; 4,32000 df; $.01 < p$ 0.025
2. Ho: one regression line can be used for all data
   $F = 270$; 8,264 df; $p < 0.01$
   $\therefore$ reject Ho
3. Ho: all lines have equal slopes
   $F = 1.80$; 4,264 df; $0.1 < p < 0.25$
   $\therefore$ accept Ho
   pooled slope =-15.7516

Best estimates of individual regressions based on pooled slope

Days starved	% water = -15.7516 log L +
0	105.9787
3	107.1478
6, 9	107.9608
12	108.6722
15	106.0888

Appendix 4. Herring percent triglyceride versus length.

Days starved	10 - 27 mm Equation % triglyceride =	N	Days starved	27 - 45 mm equations of form y = bx + c Equation % triglyceride =	N	Stand. Dev. of b	Stand. Err. of c	Stand. dev. y for fixed x
0	$-1.7095\ L+0.0443\ L_2^2+22.22$	34	0	$-0.0885\ L+10.26$	29	0.0119	0.0860	0.2835
3	$-1.4202\ L+0.0369\ L_2^2+18.48$	25	3	$-0.0585\ L+8.17$	9	0.0206	0.1572	0.2963
6	$-1.6379\ L+0.0417\ L_2^2+20.32$	29	6	$-0.1185\ L+9.24$	9	0.0117	0.0757	0.1161
9	$-1.4199\ L+0.0343\ L_2^2+17.89$	30	9	$-0.0594\ L+5.77$	10	0.0064	0.0473	0.0968
12	$-1.0798\ L+0.0272\ L_2^2+13.61$	14	12	$-0.0764\ L+6.05$	5	0.0059	0.0383	0.0420
15	$-1.0324\ L+0.0254\ L^2+12.86$	13	15	$-0.1137\ L+6.65$	15	0.0138	0.0950	0.2012

Tests

1. Ho: variances are homogeneous, $F = 0.65$; 5,3000 df; $.25 < p < .50$ $\therefore$ accept Ho
2. Ho: one regression line can be used for all data
   $F = 477$; 10,65 df; $p < 0.01$
   $\therefore$ reject Ho
3. Ho: all lines have equal slopes
   $F = 1.89$; 5,65 df; $0.10 < p < 0.25$
   $\therefore$ accept Ho
   pooled slope $= -0.0835$
4. Ho: pooled slope is not significantly different from zero
   $t = 13.37$. 75 df; $p < 0.01$
   $\therefore$ reject Ho, $b \neq 0$

Best estimates of regressions

Days starved	Equation % triglyceride = −0.0835 L+
0	10.10
3	9.06
6	8.17
9	6.54
12	6.28
15	5.63

Appendix 5. Herring percent carbohydrate versus length.

equations of form $y = bx + c$

Days starved	Equations % carbohydrate =	N	Stand. dev. of b	Stand. err. of c	Stand. dev. of y for fixed x
0	10-21mm 0.1590 L+1.55	37	0.0128	0.0638	0.2242
	21-29mm -0.2260 L+9.48	26	0.0263	0.1472	0.3549
	29-45mm 0.0012 L+3.19	22	0.0104	0.0737	0.1830
3	-0.0034 L+3.32	27	0.0050	0.0399	0.1761
6	-0.0011 L+3.14	31	0.0039	0.0328	0.1585
9	-0.0030 L+2.97	44	0.0045	0.0385	0.2181
12	-0.0130 L+3.03	29	0.0042	0.0422	0.2036
15	-0.0008 L+2.71	29	0.0048	0.0491	0.2358

Tests 1. Ho: Variances are homogeneous days 3 to 15 $F = 1.472$; 4,28000 df;
$0.1 < p < 0.25$ $\therefore$ accept Ho

2. Ho: one regression line can be used for all data, days 3 to 15
$F = 18.82$; 8,150 df; $p < 0.01$
$\therefore$ reject Ho

3. Ho: all lines (days 3 to 15) have equal slopes
$F = 1.43$; 4,150 df; $0.1 < p < 0.25$
$\therefore$ accept Ho
pooled slope = -0.0046

4. Ho: pooled slope is not significantly different from zero.
$t = 1.68$, 158 df, $0.05 < p < 0.10$
$\therefore$ accept Ho, b=0

Best estimate of individual regressions days 3 to 15

Days starved	% carbohydrate = 0 L+
3	3.26
6	3.12
9	2.92
12	2.77
15	2.69

Appendix 6. Herring percent nitrogen versus length.

equations of form $y = bx + c$

10 - 21 mm

Days starved	Equation % nitrogen =	N	Stand. dev. of b	Stand. dev. of c	Stand. dev. y for fixed x
0	0.1700 L+8.54	31	0.0106	0.0548	0.1910
3	0.2060 L+8.04	21	0.0191	0.0920	0.2586
6	0.2139 L+7.82	28	0.0137	0.0638	0.1991
9	0.2212 L+7.66	21	0.0216	0.1034	0.2606
12	0.1964 L+8.00	13	0.0168	0.0937	0.2422
15	0.2205 L+7.66	14	0.0209	0.0996	0.2333

21 - 45 mm

Days starved	Equation log % nitrogen =	N	Stand. dev. of b	Stand. dev. of c	Stand. dev. y for fixed x
0	-0.1301 log L+1.2544	52	0.0094	0.0114	0.0063
3	-0.1610 log L+1.2990	7	0.0266	0.0322	0.0063
6	-0.1353 log L+1.2627	7	0.0388	0.0324	0.0063
9	-0.1335 log L+1.2625	10	0.0256	0.0312	0.0071
12	-0.1729 log L+1.3167	12	0.0276	0.0333	0.0084
15	-0.1395 log L+1.1815	16	0.0227	0.0278	0.0065

Tests

1. Ho: Variances are homogeneous, $F = 0.81$; 5,15000 df; $0.5 < p < 0.75$
   ∴ Accept Ho
2. Ho: one regression line can be used for all data
   $F = 1.75$; 10,116 df; $.25 < p < .5$
   ∴ Accept Ho
   pooled slope = 0.2001

1. Ho: Variances are homogeneous, $F = 0.44$; 5,4200 df; $.75 < p < .90$
   ∴ Accept Ho
2. Ho: one regression line can be used for all data
   $F = 1.49$; 10,92 df; $.1 < p < .25$
   ∴ Accept Ho
   pooled slope = -0.1400

Best estimates of individual regressions

Days starved	10 - 21 mm
0, 3, 6, 9, 12, 15	% N = 0.2001 L+8.02

Days starved	21 - 45 mm
0, 3, 6, 9, 12, 15	log % N = 1.2689 -0.1400 log L

Appendix 7. Herring percent ash versus length.

equations of form $y = bx + c$

10 - 25 mm

Days starved	Equation % Ash =	N	Stand. dev. of b	Stand. err. of c	Stand. dev. y for fixed x
0	0.0119 L+7.29	43	0.0160	0.0959	0.4507
3	0.0076 L+8.41	30	0.0170	0.0991	0.3952
6	0.0089 L+9.34	30	0.0141	0.0820	0.3305
9	0.0113 L+9.49	31	0.0203	0.1130	0.4287
12	0.0078 L+10.05	19	0.0214	0.1292	0.4224
15	0.0033 L+11.38	23	0.0214	0.1337	0.4933

25 - 45 mm

Days starved	Equation % Ash =	N	Stand. dev. of b	Stand. err. of c	Stand. dev. y for fixed x
0	0.1361 L+4.26	34	0.0142	0.1161	0.4782
3	0.1702 L+3.98	9	0.0378	0.2636	0.4702
6	0.1364 L+5.57	7	0.0331	0.2466	0.4227
9	0.1501 L+5.95	9	0.0408	0.3161	0.6523
12	0.1790 L+5.73	9	0.0214	0.1483	0.2592
15	0.1721 L+7.36	15	0.0320	0.2109	0.4223

Tests

1. Ho: variances are homogeneous $F = 0.197$; 5,3500 df; $.95 < p < .975$
   ∴ variances are homogeneous

2. Ho: one regression line can be used for all data
   $F=137$; 10,164 df; $p<0.01$
   ∴ reject Ho

3. Ho: all lines have equal slopes
   $F=0.03$; 5,164 df; $p>.99$
   ∴ accept Ho
   pooled slope = 0.0087

4. Ho: pooled slope is not significantly different from zero
   $t=1.13$; 174 df; $0.2<p<0.4$
   ∴ accept Ho, $b = 0$

1. Ho: variances are homogeneous $F = 3.52$; 5,5000 df; $.001 < p < .005$

2. Ho: one regression line can be used for all data
   $F=105$; 10,71 df; $p<0.01$
   ∴ reject Ho

3. Ho: all lines have equal slopes
   $F=0.49$; 5,71df; $.75<p<.9$
   ∴ accept Ho
   pooled slope = 0.1469

4. Ho: pooled slope is not significantly different from zero
   $t=14.84$; 73 df; $p<0.01$
   ∴ accept Ho, $b \neq 0$

Best estimates of individual regressions

Days starved	10 - 25 mm % Ash = 0L+
0	7.50
3	8.53
6	9.48
9	9.68
12	10.17
15	11.43

Days starved	25 - 45 mm % Ash = 0.1469 L+
0	3.90
3	4.71
6	5.24
9	6.05
12	6.76
15	8.16

Appendix 8. Herring percent carbon versus length.

equations of form $y = bx + c$

10 - 18 mm

Days starved	Equation % Carbon =	N	Stand. dev. of b	Stand. err. of c	Stand. dev. y for fixed x
0	0.2331 L+39.39	28	0.0423	0.1814	0.4182
3	0.1903 L+39.38	17	0.0448	0.1897	0.3834
6	0.2359 L+37.64	28	0.0538	0.2124	0.4495
9	0.5496 L+32.19	22	0.0858	0.3648	0.7870
12	0.5730 L+29.27	18	0.0718	0.3135	0.7025
15	0.6013 L+28.85	16	0.0938	0.4007	0.8077

18 - 45 mm

Days starved	Equation % Carbon =	N	Stand. dev. of b	Stand. dev. of c	Stand. dev. y for fixed x
0	0.0164 L+43.87	62	0.0123	0.1107	0.6985
3	-0.0237 L+43.79	11	0.0303	0.2411	0.6006
6	-0.0292 L+42.68	10	0.0295	0.2189	0.5054
9	0.0211 L+40.10	19	0.0295	0.2353	0.8078
12	0.0758 L+38.16	14	0.0372	0.3002	0.8603
15	0.0353 L+39.55	19	0.0408	0.3109	0.9454

Tests

1. Ho: variances are homogeneous a) days 0 to 6 $F = 0.24$; 2,9500 df; $0.75 < p < 0.90$
   ∴ accept Ho
   b) days 9 to 15 $F = 0.177$; 2,6000 df; $.75 < p < .90$
   ∴ accept Ho

2. Ho: one regression line can be used for all data
   a) days 0 - 6
   $F = 52$; 4,67 df; $p < 0.01$
   ∴ reject Ho
   b) days 9 - 15
   $F = 38$; 4,51 df; $p < 0.01$
   ∴ reject Ho

3. Ho: all lines have equal slopes
   a) days 0 - 6
   $F = 0.22$; 2,67 df; $0.75 < p < 0.90$
   ∴ accept Ho
   pooled slope = 0.2209
   b) days 9 - 15
   $F = 0.1$; 2,51 df; $0.90 < p < 0.95$
   ∴ accept Ho
   pooled slope = 0.5735

1. Ho: variances are homogeneous $F = 1.29$; 5,9500 df; $.25 < p < .5$
   ∴ accept Ho

2. Ho: one regression line can be used for all data
   a) all days starved
   $F = 42$; 10,123 df; $p < 0.01$
   ∴ reject Ho
   b) days 9 - 15
   $F = 0.69$; 6,46 df; $0.5 < p < 0.75$
   ∴ accept Ho

3. Ho: all lines have equal slopes
   a) all days starved
   $F = 1.16$; 5,123 df; $0.25 < p < 0.50$
   ∴ accept Ho
   pooled slope = 0.0192

4. Ho: pooled slope is not significantly different from zero
   $t = 1.93$; 133 df; $0.05 < p < 0.10$
   accept Ho, b=0

Best estimates of individual regressions

Days starved	10 - 18 mm	Days starved	18 - 45 mm
	% Carbon = 0.2209 L+		% Carbon = 0L+
0	39.57	0	44.34
3	38.96	3	43.13
6	37.83	6	41.93
	% Carbon = 0.5735 L+		
9	31.85	9	40.50
12	29.27	12	40.50
15	29.22	15	40.50

Appendix 9. Percent deviation from mean values of body component at each day starved for herring at the end of the yolk sac stage.

TRIGLYCERIDE

	Days starved												
	0		3		6		9		12		15		Mean
♀'s	%	Dev.	%	Dev.	%	Dev.	%	Dev.	%	Dev.	%	Dev.	Dev.
1	———		7.22	+0.08	6.83	+0.40	6.27	+0.57	5.38	+0.44	4.81	+0.29	+.36
2	8.96	+0.42	7.50	+0.36	6.84	+0.41	6.10	+0.40	5.26	+0.32	4.72	+0.20	+.35
3	8.18	-0.36	6.78	-0.36	5.42	-1.01	5.12	-0.58	4.58	-0.36	4.09	-0.43	-.52
5	———		6.79	-0.35	6.96	+0.53	5.46	-0.24	4.30	-0.64	4.78	+0.26	-.09
6	8.47	-0.07	7.76	+0.62	6.21	-0.22	5.82	+0.12	4.62	-0.32	4.32	-0.20	-.01
8	———		6.78	-0.36	6.30	-0.13	5.41	-0.29	5.50	+0.56	4.43	-0.09	-.06
Mean %	8.54		7.14		6.43		5.70		4.94		4.52		

CARBOHYDRATE

1	———		3.50	+0.16	3.16	+0.03	3.10	-0.04	2.78	-0.01	2.73	+0.10	+.05
2	3.24	-0.12	3.09	-0.25	3.11	-0.02	3.26	+0.12	2.96	+0.17	2.67	+0.04	-.01
3	3.49	+0.13	3.55	+0.21	3.50	+0.37	3.36	+0.22	3.10	+0.31	2.62	-0.01	+.20
5	———		3.50	+0.16	3.07	-0.06	2.86	-0.28	2.42	-0.37	2.50	-0.13	-.14
6	3.35	-0.01	3.11	-0.23	3.10	-0.03	3.11	-0.03	2.87	+0.08	2.72	+0.09	-.02
8	———		3.27	-0.07	2.83	-0.30	———		2.59	-0.20	2.56	-0.07	-.16
Mean %	3.36		3.34		3.13		3.14		2.79		2.63		

NITROGEN

1	———		9.56	-0.29	10.35	+0.09	9.75	+0.31	10.03	+0.37	9.98	+0.09	+.11
2	10.32	-0.13	9.81	-0.04	10.13	-0.13	9.37	-0.07	9.64	-0.02	9.43	-0.46	-.14
3	10.72	+0.27	10.14	+0.29	10.58	+0.32	9.55	+0.11	9.69	+0.07	10.30	+0.41	+.24
5	———		9.69	-0.16	10.60	+0.34	9.23	-0.21	9.80	+0.14	10.06	+0.17	-.06
6	10.32	-0.13	9.95	+0.10	9.64	-0.62	9.47	+0.03	9.30	-0.36	9.95	+0.06	-.15
8	———		9.95	+0.10	10.28	+0.02	9.26	-0.18	9.47	-0.19	9.64	-0.25	-.10
Mean %	10.45		9.85		10.26		9.44		9.66		9.89		

CARBON

1	———		41.55	-0.04	40.90	+0.44	39.35	+0.67	37.51	+1.60	35.56	-0.13	+.51
2	42.70	+0.41	41.20	-0.39	40.46	0.00	37.65	-1.03	36.22	+0.31	36.34	+0.65	-.01
3	41.89	-0.40	41.04	-0.55	39.12	-1.33	37.48	-1.20	34.11	-1.80	34.10	-1.59	-1.14
5	———		42.32	+0.73	40.43	-0.03	40.32	+1.64	35.20	-0.71	35.99	+0.30	+.39
6	42.29	0.00	41.41	-0.18	40.87	+0.41	38.19	-0.49	35.40	-0.51	35.65	-0.04	-.14
8	———		42.03	+0.44	40.95	+0.49	39.10	+0.42	37.00	+1.09	36.52	+0.83	+.65
Mean %	42.29		41.59		40.46		38.68		35.91		35.69		

ASH

	Days starved												
	0		3		6		9		12		15		Mean
♀'s	%	Dev.	%	Dev.	%	Dev.	%	Dev.	%	Dev.	%	Dev.	Dev.
1	———		8.34	-0.09	9.50	+0.13	10.20	+0.44	10.00	+0.23	11.01	+0.34	+.21
2	7.70	+0.35	8.43	0.00	9.48	+0.11	9.88	+0.12	10.04	+0.27	11.16	+0.49	+.22
3	7.04	-0.31	8.69	+0.26	10.01	+0.64	9.88	+0.12	10.09	+0.32	10.87	+0.20	+.20
5	———		7.89	-0.54	8.60	-0.77	8.84	-0.92	9.18	-0.59	9.56	-1.11	-.79
6	7.30	-0.05	8.62	+0.19	9.36	-0.01	10.15	+0.39	10.12	+0.35	10.96	+0.29	+.19
8	———		8.61	+0.18	9.25	-0.12	9.62	-0.14	9.18	-0.59	10.44	-0.23	-.18
Mean %	7.35		8.43		9.37		9.76		9.77		10.67		

C / N

1	———		4.35	+0.13	3.95	+0.01	4.04	-0.06	3.74	+0.02	3.56	-0.05	+.01
2	4.14	+0.09	4.20	-0.02	3.99	+0.05	4.02	-0.08	3.76	+0.04	3.85	+0.24	+.05
3	3.91	-0.14	4.05	-0.17	3.70	-0.24	3.92	-0.18	3.52	-0.20	3.31	-0.30	-.20
5	———		4.37	+0.15	3.81	-0.13	4.37	+0.27	3.59	-0.13	3.58	-0.03	+.03
6	4.10	+0.05	4.16	-0.06	4.24	+0.30	4.03	-0.07	3.81	+0.09	3.58	-0.03	+.05
8	———		4.22	0.00	3.98	+0.04	4.22	+0.12	3.91	+0.19	3.79	+0.18	+.11
Mean	4.05		4.22		3.94		4.10		3.72		3.61		

WATER

1	———		88.56	-0.62	89.07	-0.31	89.57	-0.66	91.16	-0.01	91.38	+0.21	-.28
2	89.03	+0.33	89.87	+0.69	89.62	+0.24	91.00	+0.77	91.37	+0.20	———		+.45
3	88.60	-0.10	89.50	+0.32	88.86	-0.52	90.40	+0.17	91.24	+0.07	91.01	-0.01	-.01
5	———		89.25	+0.07	89.53	+0.15	89.81	-0.42	90.83	-0.34	90.80	-0.37	-.18
6	88.46	-0.24	89.09	-0.09	89.74	+0.36	90.44	+0.21	91.15	-0.02	91.49	+0.32	+.09
8	———		88.80	-0.38	89.49	+0.11	90.14	-0.09	91.25	+0.08	91.18	+0.01	-.05
Mean %	88.70		89.18		89.38		90.23		91.17		91.17		

Appendix 10. Chemical deviation in µg from mean amount at each day starved for herring at the end of the yolk sac stage.

C A R B O H Y D R A T E

	Days starved 0		3		6		9		12		15		
♀	µg	Dev.	µg	Dev.	µg	Dev.	µg	Dev.	µg	Dev.	µg	Dev.	Mean Dev.
1			5.37	-0.25	4.69	+0.10	3.85	-0.12	2.74	-0.26	2.54	-0.04	-0.11
2	5.61	+0.47	4.50	-1.12	4.33	-0.26	3.93	-0.04	3.05	+0.05	2.42	-0.16	-0.18
3	4.50	-0.64	4.26	-1.36	3.92	-0.67	3.23	-0.74	2.38	-0.62	1.99	-0.59	-0.77
5			6.82	+1.20	5.78	+1.19	4.93	+0.96	3.41	+0.41	3.10	+0.52	+0.86
6	5.31	+0.17	4.58	-1.04	4.03	-0.56	3.90	-0.07	3.02	+0.02	2.57	-0.01	-0.25
8			6.05	+0.43	4.78	+0.19			3.43	+0.43	2.85	+0.27	+0.33
Mean	5.14		5.62		4.59		3.97		3.00				

A S H

1			12.78	-0.47	14.11	+0.36	12.68	-0.17	9.85	-0.77	10.23	-0.18	-0.25
2	13.34	+2.01	12.27	-0.98	13.20	-0.55	11.91	-0.94	10.36	-0.26	10.13	-0.28	-0.17
3	9.07	-2.26	10.42	-2.83	11.22	-2.53	9.50	-3.35	7.76	-2.86	8.27	-2.14	-2.66
5			15.38	+2.13	16.19	+2.44	15.24	+2.39	12.93	+2.31	11.84	+1.43	+2.14
6	11.58	+0.25	12.70	-0.55	12.18	-1.57	12.73	-0.12	10.64	+0.02	10.37	-0.04	-0.34
8			15.94	+2.69	15.62	+1.87	15.05	+2.20	12.15	+1.53	11.61	+1.20	+1.90
Mean	11.33		13.25		13.75		12.85		10.62		10.41		

Appendix 11. Herring length-weight lines for different diets.

equations of form $y = bx + c$

Diet	Equation log wt =	N	Stand. dev. of b	Stand. err. of c	Stand. dev. y for fixed x
Rotifers	4.4767 log L-5.5982	23	0.0940	0.1134	0.0464
Artemia	4.7472 log L-5.9571	20	0.1098	0.1236	0.0451
plankton	4.7758 log L-5.9844	17	0.0904	0.0939	0.0348
mixed	4.5288 log L-5.6138	22	0.1866	0.2192	0.0462

Tests

1. Ho: all lines have equal variances
   $F = 0.46$; 3,10000 df; $0.5 < p < 0.75$
   ∴ lines have homogeneous variances.
2. Ho: one regression line can be used for all diets
   $F = 4.18$; 6,74 df; $p < 0.01$
   ∴ lines are not equal
3. Ho: all lines have equal slopes
   $F = 1.86$; 3,74 df; $0.1 < p < 0.25$
   ∴ lines have common slope
   pooled slope = 4.6258

	Best estimates of individual regressions	Relative condition factors $x10^5$
Rotifers	log wt = 4.6258 log L-5.8136	0.1536
Artemia	-5.7811	0.1655
plankton	-5.7535	0.1764
mixed	-5.7666	0.1711

Appendix 12. Larval grunion[1] buoyancy.

Days from hatching	Dry weight (mg)	% Water[2]	Wet weight[2]
7	0.409	89	3.718
13	0.311	91	3.456

Component	ρ Larval component[3]	ρ_{sw} / ρ_L[4]	Weight (mg)	Vol(ml)= $\frac{wt(g)}{\rho_L}$	Force (Dynes)= ($\rho_{sw}-\rho_L$)x(vol)x981cm/sec^2[5]	Dynes / wet wt.	Δ Dynes
Water	1.0094	0.0144	Day 7/3.309	0.00328	+0.0463	+0.0125	
			Day 13/3.145	0.00312	+0.0441	+0.0128	+0.0003
Protein	1.379	0.3535	Day 7/0.271	0.0001965	-0.0681	-0.0183	
			Day 13/0.218	0.0001580	-0.0548	-0.0159	+0.0024
Fat	0.926	0.0995	Day 7/0.106	0.000145	+0.0112	+0.0030	
			Day 13/0.065	0.0000702	+0.0069	+0.0010	-0.0020

Δdynes to Day 13 = +0.0007

① Chemical composition and weights from May (1971)
② % water taken as same for herring larvae (Appendix 1). Wet weights were computed from dry weight and % water.
③ Component densities taken as for herring (Table 10).
④ Sea water density (1.0238) taken for water at 18°C. and 33‰, which May (1971) used.
⑤ + and - signs indicate upward and downward forces respectively.