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GROWTH AND OTOLITH RING DEPOSITION IN TELEOST LARVAE

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CONTENTS

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ABSTRACT

Growth and otolith ring deposition were examined in the larvae of turbot, plaice, herring, and in salmon embryos. Larvae were reared under various light, temperature, and feeding regimes in order to measure the response of ring deposition to different sets of environmental cues. Ring deposition in herring larvae was measured under a wide range of rearing conditions to provide the basis for ageing wild larvae collected in the Clyde and the Minch.

In turbot, salmon, and herring, both growth and ring deposition rates were altered under different rearing conditions. Ring deposition rates were higher under conditions which produced faster growth. Ring deposition in these species was not directly affected by the environmental conditions, instead, the differences observed were the result of the effect that the rearing conditions had on growth and activity. Neither the growth nor the ring deposition rates of plaice larvae were affected by any of the experimental conditions tested.

Length was significantly more important than age in determining ring number in individual turbot and herring larvae. At the population level there was a strong positive correlation between growth rate and ring deposition rate in turbot and herring. Age was the most important factor in determining ring number in the plaice larvae examined.

Otolith ageing techniques could not be used successfully to estimate growth rates for the wild herring larval populations sampled.

INTRODUCTION

Ageing and growth studies

Age and growth studies in fish populations form the basis of most work in fisheries biology and in experimental studies. Such studies provide a measure of the interaction between the organism and the environment since the age structure and growth rates of a population are responsive to environmental conditions. Measured changes in these characteristics often result from changes in food availability, fishing pressure, pollution, or climatic changes (Beckman, 1943b; McLeay & Brown, 1974; Postuma, 1971).

There are several methods of measuring fish growth directly. In the laboratory, experimental animals can be weighed, measured or analysed periodically. Growth is measured directly as a change in time, since individual age is usually known. In the field, measured fish are tagged, released, and measured again on recapture, to obtain the increment in growth in the intervening time period (Everhart *et al.*, 1975). An estimate of the population growth rate can be obtained by calculating the differences between length-frequency modes identified in large samples of a population (Lager, 1952; MacDonald & Pitcher, 1979). This method assumes that there is a normal distribution of individual lengths about distinct mean values which correspond to separate age classes (Petersen, 1891). It is most accurately applied in species where spawning is restricted to isolated periods which are known, and in species which take several years to reach the asymtotic length.

Measures of individual growth can be obtained if individual age is determined. In many species, growth may be a continuous process, but it does not proceed at a constant rate. The periods of faster

or slower growth are distinguished by distinct layers in body tissues which grow by accretion. The apparent relationship between variation in growth rates and patterns in permanent tissue has led to the established methods of ageing and interpreting the past growth history of individual organisms.

Growth bands are the regular characteristics used for the ageing and growth calculations in trees (Henderson, 1946), marine mammals (Bryden, 1972), large game mammals (Mitchell, 1967), fish (Bagenal & Tesch, 1968), polychaetes (Olive, 1977), stromatolite formations (Jones, 1981), corals (Hudson, 1981), barnacles (Bourget & Crisp, 1975b), and molluscs (Weymouth & McMillan, 1931; Kenny, 1977). The interpretation of the banding patterns gives not only the overall growth of the individual, but also the response of that individual to short-term environmental changes (Pannella & MacClintock, 1968).

The main structures used in the ageing of fish are the vertebrae, scales, and otoliths. The techniques and problems of ageing studies are reviewed in most fisheries biology texts (e.g. Lagler, 1952; Royce, 1972; Everhart *et al.*, 1975; Ricker, 1977; Bagenal & Tesch, 1968). Fin rays (Boyko, 1946), opercular bones (Bardach, 1955; Fagade, 1974) and cleithra (Casselman, 1974) are also used to age some species. Ageing of cartilaginous fish has been accomplished by examination of growth bands on dorsal spines and vertebrae (Holden & Meadows, 1962). There is a good deal of variation among different species as to which structures are most suitable for ageing (Bilton & Jenkinson, 1968; Meunier *et al.*, 1979). Calcified structures have been used since the late 19th century as internal time markers for ageing individuals. Several more specific and more sophisticated techniques for the examination and interpretation of these structures have been introduced in the past 10-15 years. These include

examination by electron microscopy (Tanaka *et al.*, 1981), scanning electron microscopy (Dunkelberger *et al.*, 1980) and stereoscan (Blacker, 1977), fluorescent tags (Weber & Ridgeway, 1962; Holden & Vince, 1973; Bingel, 1981a) and studies of the uptake of radioactive labelled compounds (Mugiya, 1974; Ottaway and Simkiss, 1977a).

Scales are used to age many temperate species. The information they provide is used to describe individual growth and to define the age structure of populations. A pattern of annual rings can be identified in most species without difficulty. Under normal conditions, scales can be removed from live fish without ill effects allowing subsequent samples to be taken from the same individuals at a later date. To measure individual growth rates, it is assumed that changes in scale size are directly related to changes in body size. 'Backcalculation' of fish size at any previous age was developed by Dahl (1910) and Lea (1910) and utilises the relation: $l_1 r_2 = l_2 r_1$, where l refers to the length of the fish, r to the radius of the scale, at the subscripts *1* and 2 to points in time previous to and at capture. Where the relationship between the body length and the scale radius does pass through the origin, the Fraser-Lee modification is used for backcalculation (Bagenal & Tesch, 1968). This technique is reviewed by Lagler (1952) and Bagenal & Tesch (1968). Ottaway and Simkiss (1977b) refined a technique of incubating scales and measuring the ^{14}C-glycerine uptake to obtain instantaneous growth rates. Although the rates of scale growth are not yet directly comparable with changes in length, they are of value in comparing the relative growth rates of several populations under varying environmental conditions. Changes identifiable in instantaneous growth rates are the result of the condition of the individual and its response to environmental change.

Vertebrae have been used to age fish, especially skates and rays, in those cases where bands laid down on the centrum appear to occur in annual cycles. In tag and return studies, fluorescent tags have been used to mark the vertebrae of individuals in order to measure the rates of ring deposition (Holden & Vince, 1973).

Otoliths are widely used for ageing, especially demersal species (Williams & Bedford, 1974). As with scales, they are used to define the age structure and growth rates of populations (Liew, 1974; Hunt, 1979). Otoliths can be used for stock identification and racial investigations, especially in herring stocks as the nucleus appears to be of a different structure in different populations (Einarsson, 1951; Parrish, 1958; Messieh, 1972; McKern & Horton, 1974; Wood, 1979). This may be due to differences in temperature or feeding conditions experienced during the first year of life.

Biological basis for the ageing of fish

The process by which fish growth is translated into patterns of calcification has been studied in detail by Kobayashi *et al.* (1964) and Mugiya (1964). Hickling (1931), Timola (1977), Dunkelberger *et al.* (1980) and Bingel (1981b) have shown that the formation of the otolith involves the deposition of calcium (aragonite) crystals on a protein matrix. During periods of rapid growth in the individual, wide bands (incremental zones) are formed with bundles of calcium crystals arranged radially. When fish growth is slowed, a thinner band of predominantly protein is visible (Dannevig, 1956; Watabe *et al.*, 1982). The chemical composition of these layers differs in the proportion of protein and calcium and these differences can be related to seasonal changes in the constituents of the endolymph, the fluid bathing the otoliths in the labyrinth (Mugiya, 1964; Love, 1980).

The control of calcification, from the theories developed in studies of mammals and molluscs, has been suggested to lie with the action of alkaline phosphatases present on the protein matrix of calcifying tissues (Fleisch & Neuman, 1961; Alcock & Shils, 1969; Ali *et al.*, 1970). The action of these enzymes may change the ionic balance locally, favouring the initiation of calcification. They may act to hydrolyse ATP in order to provide energy for the uptake of calcium (Ali *et al.*, 1970). Their action may be to hydrolyze and inactivate pyrophosphate, which is an inhibitor of calcification (Fleisch & Neuman, 1961).

Radioactive labels have been used to measure the contributing sources of calcium for deposition on fish scales by Mugiya (1980) and on fish otoliths by Irie *et al.* (1967). Calcium ions may be transported across the gills from the surrounding water, and through the gut walls from dietary intake to provide calcium for deposition. Food was the predominant source of calcium for otolith formation (Irie *et al.*, 1967). Scale formation was supplied with calcium taken up from the surrounding water (Mugiya, 1980).

The rates and periodicity of calcification have been determined for otoliths of rainbow trout, *Salmo gairdneri*, by Mugiya *et al.* (1979) and goldfish, *Carassius auratus*, by Mugiya *et al.* (1981).

The periodicity of the calcium deposition process which has been detected on a seasonal (Dannevig, 1956; Mugiya, 1964) and daily (Mugiya *et al.*, 1981) level seems to reflect the physical patterns of deposition visible on the otoliths of many adult species. Pannella (1971) first published evidence of rings deposited with greater frequency than yearly rings on the otoliths of silver hake, *Merluccius bilinearis*; cod, *Gadus morhua*; and squirrel hake,

Urophycis chuss. Based on the examination of 25 tropical and four temperate fish species, he went on to claim that patterns of ring deposition matched daily and lunar growth cycles (Pannella, 1974). On this basis, the width of individual growth bands could be correlated with the fish's daily growth increment (Gjøsaeter and Øiestad, 1981), much the same as annual growth bands have been matched to the fish's yearly growth increment.

Use of otoliths in studies of fish larvae

The existence of otolith ring deposition cycles of daily or fortnightly period has far-reaching effects on larval growth studies (Brothers *et al.*, 1976). Before the development of larval otolith techniques, work on larval growth and survival utilised age determinations based on length and morphological development. At least one of the three pairs of otoliths (the sagittæ, in the sacculus) is present from the time of hatching. If calcification proceeds in the same manner as in adult fish, the deposition patterns visible in larval otoliths can be related to the growth history of the individual throughout the larval and juvenile stages.

One of the most promising applications of otolith ageing techniques is the determination of survival rates of wild larvae. The question of survival in the larval stages is important in fisheries biology as many believe that the brood or year class strength (later abundance or success of each year's reproductive contribution) is determined during this period (Hjort, 1926; Dragesund & Nakken, 1971; Shepard & Cushing, 1980). Hjort (1914, 1926) formulated possible mechanisms explaining mortality in the earliest larval stages. From his hypothesis, it has generally been

accepted that most of larval mortality is due to starvation (Ehrlich, 1974; May, 1974; Buckley, 1979).

The phrase 'critical period' has been attached to the transition from yolk-sac nutrition to the initiation of exogenous feeding, when most starvation mortality is assumed to occur. It has also come to be used in a broader sense to describe either specific developmental stages when larval mortality is high (in rearing experiments) or an undefined phase when the strength of a year-class is determined. Hjort's hypothesis and the evidence for critical periods in studies of larval survival have been reviewed by Marr (1955) and May (1974). Their analysis of the survival curves of several species provided no conclusive evidence either for large mortalities at the end of the yolk sac stage, or for any sudden decrease in abundance which could be identified as a critical period for determining year class strength. The abundance of Atlantic mackerel, *Scomber scombrus*, larvae sampled in the plankton declined steadily with time throughout the season. Survival curves of Pacific sardine, *Sardinops caerulea*, or jack mackerel, *Trachurus symmetricus*, larvae did show some rapid changes in abundance, but these could be explained by gear selectivity leading to undersampling of certain larval stages.

High mortalities at the end of the yolk sac stage have been observed in many species reared in the laboratory (Marr, 1955; Blaxter, 1968). Large morphological changes are associated with development in most species, such as the development of organ systems, the transition from cutaneous to branchial respiration, or the eye migration and shift in body orientation in flatfish. The vulnerability of these larvae may be increased at such times. None of these

events have proved useful in the field as critical periods for predicting year class strength.

If the factors that influence year class strength do have their effect in the larval stage, there is likely to be some variation between species as to the action of these factors. It is evident that different species vary in their resistance to starvation or poor feeding conditions in the larval stages (Ehrlich, 1972; Houde, 1974; Houde & Schechter, 1978). There is some evidence that predation plays a large role in determining larval survival (Johannessen, 1980).

The existing literature contains many descriptions and explanations of larval mortality. Theoretically, the observed patterns of larval mortality could be formed in several ways. Age- or stage-dependent mortality may occur when crucial developmental events are not passed successfully by all larvae due to genetic variation inherent in the population. This could be identified by monitoring the abundances of larvae as they grow older or pass through consecutive stages. The magnitude of this kind of mortality could vary from year to year as a result of both the size of the gene pool and the selective pressure of the environment. Alternatively, variations in individual growth rate, whether due to genetic variability or environmental conditions, may define the pattern of larval mortality. This supposes that some sizes or stages are not inherently 'difficult', but are more vulnerable than others to starvation, predation, or other environmental stress. Those larvae spending more than the optimal time in such stages face a lower probability of survival.

The ability to age individual larvae and to measure individual growth rate would aid in resolving the many questions of larval

mortality simply by identifying which larvae (young, old, slow- or
fast-growing, yolk-sac, cutaneous respiring, metamorphosing, etc.)
disappear from the population.

Growth studies of fish larvae have been restricted by the
inability to age individuals. Previous studies of wild larvae and
0-group fish involved periodic sampling of large numbers of indivi-
duals. The shift in each length or stage mode within the population
in consecutive samples provided a measure of population growth rate
(Tibbo & Henri Lagaré, 1960; Baker, 1972; Das, 1972; Graham, 1972;
Boyer *et al.*, 1973; Ware & Henriksen, 1978). The use of probability
paper described for biological applications by Harding (1949) and
Cassie (1954) was often employed to help separate the various age
groups by their length frequency modes (Hourston, 1958; Townsend &
Graham, 1981). These groups could be identified as separate spawnings
and estimates made of larval drift. The relative abundances of the
different size groups indicated patterns of larval mortality.

Laboratory studies with larvae of known age provided data on
growth and developmental rates of larvae under different conditions.
Using this information, large samples of larval populations of mixed
ages could be separated on the basis of size or developmental stage
into different age groups (Ellersten *et al.*, 1980; Nichols, pers.
comm.).

With the verification of daily otolith ring deposition (one
ring/day) in the larvae of several species, the use of otolith age
determination in larval population studies has become wide-spread.
To date, daily otolith rings have been reported for larvae of
Northern anchovy, *Engraulis mordax*, and Californian grunion,
Leuresthes tenuis, by Brothers *et al.* (1976). Taubert & Coble (1977)

found daily rings in pumpkinseed, *Lepomis gibbosus*; green sunfish, *L. cyanellus*; bluegill, *L. macrochirus*; and *Tilapia mossambica*. Daily otolith rings in larvae of Atlantic silverside, *Menidia menidia*, were reported by Barkman (1978), in Atlantic herring, *Clupea harengus L.*, by Lough *et al.*(in press) in redfish, *Sebastes spp.*, by Radtke (1980), and in cod by Radtke and Waiwood (1980). Studies of otoliths in the fry of nehu, *Stolephorus purpureus*, by Struhsaker and Uchiyama (1976), and fry of sockeye salmon, *Oncorhynchus nerka*, by Wilson and Larkin (1980) confirmed the presence of daily otolith rings.

Otoliths have been used for age determinations and estimations of growth in Northern anchovy larvae by Methot & Kramer (1979), in French grunt larvae, *Haemulon flavolineatum*, by Brothers & McFarland (1981), in largemouth bass larvae, *Micropterus salmoides*, by Schmidt & Fabrizio (1980), in herring larvae by Townsend & Graham (1981), in pescada larvae, *Plagioscion monti*, by Worthmann (1980), in cod larvae by Gjøsæter and Tilseth (1981), in redfish larvae by Penney & Anderson (1981), and in walleye pollack, *Theragra chalcogramma*, by Walline (1980).

A comparison of the estimates of larval growth based on otolith age determination and those measured by shifts in length frequency modes in Atlantic herring found good agreement between the two methods (Townsend & Graham, 1981).

The key problem in assessing the accuracy of ageing larvae from otolith rings is that of relating the cycle of ring deposition to the growth and activity pattern of the larva. Growth of larvae is affected by temperature (Houde, 1974; Kramer and Zweifel, 1970; Laurence, 1978; Bengtson and Barkman, 1981), food availability (O'Connell & Raymond, 1970; Ellersten *et al.*, 1975; Werner & Blaxter,

1980; Dowd & Houde, 1980), larval density (Doyle, 1975), and by photoperiod (Barahona-Fernandes, 1979; Biete & Green, 1980; Dowd & Houde, 1980).

In adults, growth and reproductive activity influence the nutritional condition of the individual (Le Cren, 1951), resulting in the observed annual otolith ring pattern (Mugiya, 1964). If larval rings are deposited by the same processes, diurnal variations in food availability, feeding activity, temperatures, etc. may cause acute changes in the nutritional condition of the larvae resulting in the observed pattern of daily ring deposition. Alternatively, environmental conditions may affect larval condition in such a way as to establish an internal rhythm of ring deposition which is not diurnal (Brothers *et al.*, 1976). If ring deposition is controlled directly or through feeding by cyclic environmental factors, then the deposition pattern should be subject to alteration by environmental manipulation. If the pattern of ring deposition is controlled by larval condition, then the observed rings should reflect larval growth, as opposed to diurnal fluctuations.

There are few published accounts of attempts to manipulate ring deposition by altering environmental conditions. These studies have concentrated on the effects of starvation (Wood, 1979) or unseasonal temperatures (Taubert & Coble, 1977). Photoperiod, feeding frequency, and temperature have been examined for their effect on ring deposition in starry flounder, *Platichthys stellatus*, and in chinook salmon, *Oncorhynchus tshawytscha*, (Neilson, J. pers. comm.).

The present work

The present work describes the pattern of otolith ring deposition in four species of teleosts differing greatly in larval life history: herring; plaice, *Pleuronectes platessa*; turbot, *Scophthalamus maximus*; and Atlantic salmon, *Salmo salar*. In order to measure the importance of environmental cues (exogenous rhythms) and larval condition (endogenous rhythms) as factors influencing otolith ring deposition, the differences in the otoliths of larvae reared under different environmental cycles were examined. Ring deposition was examined under various regimes of temperature, photoperiod and food ration. Several photoperiods were tested to produce different patterns of feeding activity. Temperature was used to alter growth rate, producing larvae of the same age but different lengths. Food rations were controlled to produce larvae in varying nutritional conditions.

In order to investigate the biological basis for otolith ageing techniques, larval growth and development were compared with the observed otolith ring deposition patterns. In particular, herring larvae of known age were examined from a wide variety of rearing conditions. These included larvae reared in laboratory tanks of different sizes, and in large enclosures exposed to natural photo-period. The large enclosures differed in these conditions as one was situated in the northwest of Scotland and the second on the south coast of Norway. Samples were taken from the Firth of Clyde and the Minch waters and used to assess the suitability of otolith ageing techniques for growth studies of wild herring larvae. Age determina-tions were made for these samples of herring larvae based on deposi-tion rates measured in larvae of known age. Larval growth rates for the wild populations were predicted in three ways, 1) from the ages

obtained from ring deposition rates of reared larvae; 2) backcalcula-
tion; and 3) assuming that ring number was equivalent to larval age.
These methods, which were dependent on otolith analysis, were compared
with growth rates obtained for the same wild larvae by direct measure-
ments of larval length and the increase in size since the estimated
time of hatching.

The theoretical and technical problems of otolith ageing methods
are discussed in detail.

MATERIALS AND METHODS

Larval   rearing

Herring

Ripe spring spawning herring were collected in the Firth of
Clyde (West Coast, Scotland) at the end of February or early March.
The gonads were removed on board ship, stored on ice and returned to
the Dunstaffnage Marine Research Laboratory (Oban) where the eggs were
plated out and fertilized according to the methods of Blaxter (1968).

The eggs were incubated in 120 ℓ circular black plastic tanks
in circulating sea water. The plates were moved to clean tanks every
other day during the two-week incubation period. The glass plates
were then distributed evenly among the rearing tanks just before
hatching. Hatching lasted over five days in 1980 with peak hatching
occurring between 30th and 31st March. In 1981, the hatching lasted
three days and peak hatching occurred between 27th and 28th March.
The larvae for otolith studies came from one tank containing larvae
hatched over one 24 h period only, during the hatching peak.

In 1980, larvae were reared in 120 ℓ circular black plastic
tanks as described by Ehrlich (1972). In 1981, two tanks served as
stock populations for otolith work. In addition to the routine 120 ℓ
tank, one 500 ℓ light-coloured fibreglass tank was stocked with
cultured algae and rotifers two days before the introduction of yolk
sac larvae five days after hatching (day 5). A slow circulation was
maintained only during the daylight hours until feeding was establi-
shed. After the larvae ceased feeding on rotifers at day 24, a con-
tinuous water flow was introduced. The larvae were often attracted
to the walls of the tank, especially in the first days of feeding.
In order to prevent this, black paper was attached around the wall

area to the outside of the tank, and an opaque annulus placed around
the rim of the tank to shade the walls.

In 1980 the larvae were fed from day 6 on newly-hatched *Artemia*
nauplii (source of eggs: California, U.S.A.). The diet was supple-
mented with live, natural plankton after day 19 and periodically with
copepods from splash pools (*Tigriopus sp.*) later in the season. In
1981, food was first presented on day 4 and consisted of rotifers
and *Tisbe sp.* nauplii. *Artemia* nauplii (source of eggs: Eilat,
Israel; Macau, Brazil) were introduced beginning on day 6 and natural
plankton on day 7. *Tigriopus* was collected and cultured so as to
provide a regular food supply. Larvae in the 500 ℓ tank were weaned
onto dry food by day 90 and weaning of the larvae in the 120 ℓ tanks
began on day 120.

Temperatures in the rearing tanks were not controlled and rose
gradually from 8°-14°C during the rearing season (for example, see
Fig. 1).

Light for the black rearing tanks was provided by 80W fluores-
cent strips giving 100 lx at the water surface. A single 150W bulb
provided lighting for the 500 ℓ tank, giving a surface illumination
of 130 lx at the tank centre. A photoperiod regime of 18 h of light
and 6 h of darkness (18L/6D), controlled by time clocks, was main-
tained from hatching.

Samples of herring larvae were made available from two separate
rearing experiments using large enclosure systems under conditions
of natural photoperiod. Larvae reared in large plastic bags at
Loch Ewe (west coast of Scotland) were supplied by J. Gamble
(Department of Agriculture and Fisheries for Scotland, Aberdeen) who
describes the rearing conditions in detail (Gamble, 1981). Samples

Fig. 1: Water temperatures in the herring larval stock tanks during the 1981 season. Individual points represent daily morning temperatures. ● 120 tank. O—O 500 tank.
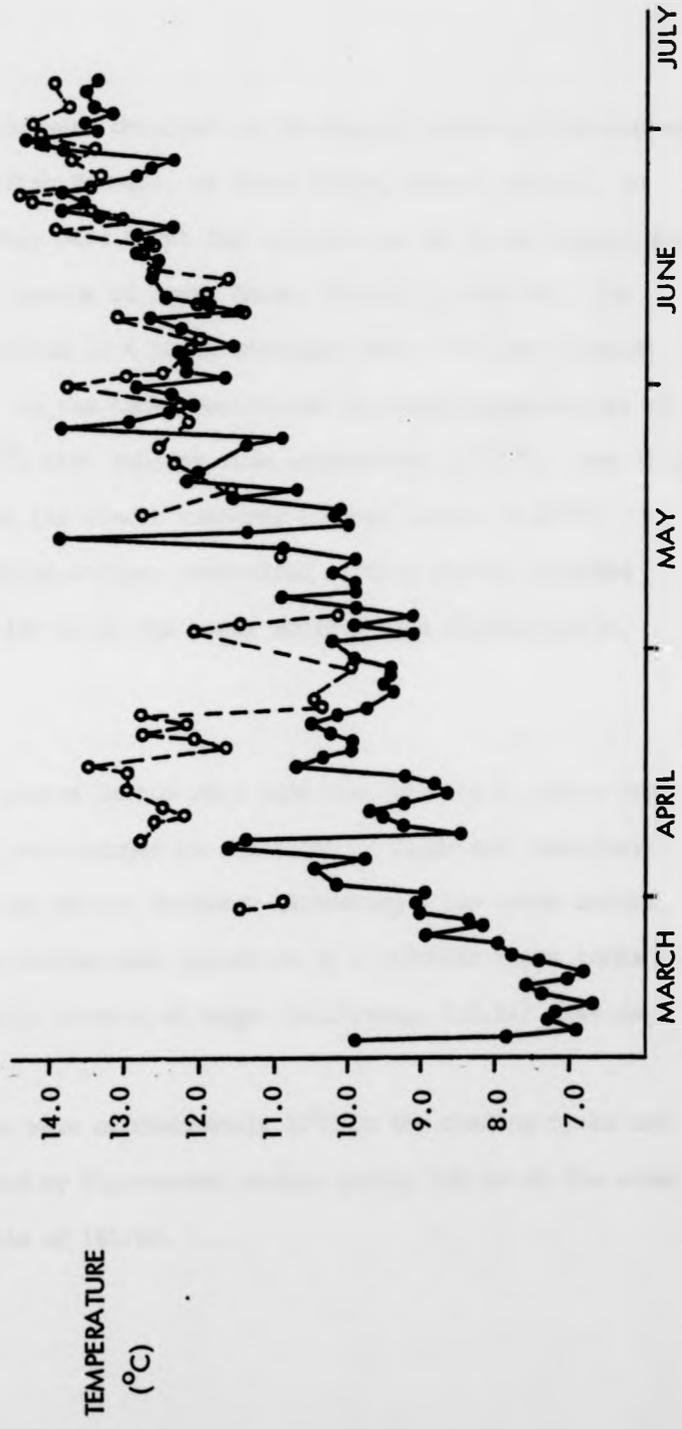
of herring larvae reared in a large concrete basin (pond) at
Flødevigen, Norway were obtained from V. Øiestad (Aquaculture
Department, Institute of Marine Research, Bergen, Norway). The
rearing conditions and results of the pond experiments are described
by Ellersten *et al.* (1975).

Turbot

Turbot larvae were obtained on the day of hatching from Scottish
Sea Farms Ltd., Fish Farmers, at South Shian, Connel, Argyll, in
August, 1980. They were first fed rotifers on day 2 and weaned onto
Artemia nauplii (source of eggs: Macau, Brazil) by day 10. The
larvae were reared in 30 ℓ black circular tanks. A slow circulating
flow (< 2 ℓ/min) in the tanks maintained the water temperatures at
approximately 20°C with ambient room temperature of 25°C. One 120 ℓ
tank was used for its slower turnover to rear larvae at 24°C.
Fluorescent lighting strips, controlled by time clocks provided
illumination of 130 lx at the water surface on a 12L/12D cycle.

Plaice

Batches of plaice larvae were made available by D. Neave from
broodstock which were caught in the Firth of Clyde and transferred
to the Dunstaffnage Marine Research Laboratory a few weeks before
spawning. These larvae were reared in 30 ℓ circular black tanks and
fed *Artemia* nauplii (source of eggs: California, U.S.A.) five days
after hatching.

Temperatures were approximately 10°C in the rearing tanks and
light was provided by fluorescent strips giving 130 lx at the water
surface on a cycle of 18L/6D.

Salmon

Throughout November and December 1980 migrating Atlantic salmon were stripped and the eggs fertilized at the Awe District Fishery Hatchery at Inverawe, Argyll. The eggs were incubated in the dark in hatchery trays under running spring water. Temperatures during incubation at the hatchery rose from 2-6°C during the course of the season. Just before eyeing (Lagler, 1952), groups of eggs were transferred to the Dunstaffnage Laboratory and acclimated over 24 h to 8°C. There they were held in 15 cm diameter glass dishes in spring water brought from the hatchery. The eggs were kept in the dark in an 8°C constant temperature room. The age of salmon embryos was recorded in number of days post-fertilization (dpf) and in degree days (degdays). The age in degree days was calculated as

(degdays) = dpf x $^{\circ}$C (incubation temperature)

so that embryos developing at different temperatures could be compared on the same time scale.

Manipulation experiments

Throughout the rearing seasons several sets of experiments were run to determine the response of otolith ring patterns to environmental conditions (e.g. light, temperature, and feeding). Larvae were held for periods of five days to one month under controlled conditions and the changes in otolith ring number and larval growth were analysed by repeated sampling of the population in the tank.

Herring

The manipulation experiments using herring larvae in 1980 and 1981 are listed in Table 1. These experiments were carried out on

TABLE 1

MANIPULATION EXPERIMENTS USING HERRING LARVAE

1980 and 1981

TANK	AGE (days from hatching)	TREATMENT (L = hours of light, D = hours of darkness)
1980		
1	4-23	18L/6D, starved*
32	4-23	18L/6D
33	4-23	18L/6D, feeding delayed to day 17
34	43-50	24L
36	43-50	6L/6D
37	43-50	18L/6D
38	43-50	18L/6D, starved
39	43-50	24D
1981		
51	6-10	18L/6D, feeding delayed to day 7
52	6-10	18L/6D, feeding delayed to day 10
53	6-10	18L/6D, starved
71	30-34, 45-52	18L/6D, 8°C
72	30-34, 45-52	18L/6D, 10°C
73	30-34, 45-52	18L/6D, 15°C
74	45-52	24L
75	45-52	6L/6D
76	45-52	18L/6D, low food ration
77	45-52	18L/6D

*larvae were fed as normal unless otherwise specified.

larvae transferred from the stock tanks to 30 ℓ black circular tanks
in a temperature controlled air-conditioned room. Usually, 30-50
larvae were stocked in each tank. Larvae in the experimental tanks
were fed only with *Artemia* nauplii to ensure uniform feeding condi-
tions between the tanks. Room temperature remained fairly constant
at 15°C and the water circulating in the tanks, unless specially
heated, was approximately 8°C.

To compare larvae of different growth rates at the same age,
three separate rearing temperatures were chosen: 8°, 10° and 15°C.
The water temperatures of individual tanks were altered by placing
a single 100W heater (Interpet, Super Maxamatic) in the bottom of
each tank. These aquarium heaters were controlled by internal
thermostats and accurately maintained the preset temperatures
throughout the period of the experiments. The experimental food
regimes tested were: starvation, delayed first feeding and low food
rations. The light regimes chosen for manipulations were intended
to represent the extremes in possible photoperiods: 24 L, 12L/12D,
6L/6D and 24 D. For photoperiod experiments individual 30 ℓ tanks
were placed in black boxes, each fitted with one 80W fluorescent
tube, controlled by a time clock.

Turbot

The turbot larvae were reared under the experimental conditions
listed in Table 2. Each 30 ℓ tank was stocked with 500 yolk sac
larvae. Tanks subjected to 24L and 6L/6D were placed in the photo-
period boxes (see herring experiments). The water temperature was
raised in one treatment by using a larger volume tank where the
retention time allowed the circulating water to heat up to 24°C.

TABLE 2

MANIPULATION EXPERIMENTS USING TURBOT LARVAE

August 1980

TANK	AGE (days after hatching)	TREATMENT (L = hours of light, D = hours of darkness)
1	0–23	24L, 20°C*
3	0–14	6L/6D, 20°C
4	0–17	12L/12D, 20°C
5	0–6	12L/12D, starved, 20°C
6	0–23	12L/12D, 24°C

*larvae were fed as normal unless otherwise specified.

Plaice

Thirty to fifty plaice larvae were transferred to 30 l experimental tanks at various stages during development (Table 3). Newly hatched plaice were used to test the effect of delayed feeding and starvation on deposition of the first ring. Larvae were held at 10°C under a 18L/6D cycle. One group was fed on *Artemia* daily from day 5, one group starved, and in the third group feeding was delayed until day 8. Day 8 was taken as the limit for survival before first feeding, as determined by Ehrlich (1972).

In order to assess the influence on ring deposition of separate light or temperature cues, a second batch of plaice were taken at day 42, before settling (Ryland, 1965) and subjected to either light or temperature cycles. One group was held at 10°C under a 12L/12D cycle. A 100W aquarium heater, powered by a time clock, was placed in a second tank and larvae were held under 24L with a daily cycle of increasing and decreasing temperature which ranged from 10–15°C. A third tank was maintained at 10° under 24L. All larvae were fed daily with *Artemia* nauplii.

Salmon

Salmon embryos were ideally suited for manipulation experiments as yolk sac nutrition removed any variation in feeding behaviour between treatments or individuals. Two experiments were run with salmon to compare the effects of light regimes and temperature on the growth of the embryo and the deposition of otolith rings (Table 4). In the first experiment, eggs were kept in duplicated groups of 20 eggs in 15 cm diameter glass dishes at 8, 10 and 15°C. The experiment began at 46 days post-fertilization (dpf) when the otoliths

TABLE 3

MANIPULATION EXPERIMENTS USING PLAICE LARVAE

TANK	AGE (days after hatching)	TREATMENT (L = hours of light, D = hours of darkness)
1	0-13	18L/6D,*
2	0-13	18L/6D, feeding delayed to day 8
3	0-13	18L/6D, starved
4	42-50	12L/12D
5	42-50	24L, temperature cycle
6	42-50	24L

*larvae were fed as normal unless otherwise specified.

TABLE 4

MANIPULATION EXPERIMENTS USING SALMON EMBRYOS

TANK	AGE dpf	degday	TREATMENT (L = hours of light, D = hours of darkness)
Experiment 1			
1a,b	48-82	120-376	24D, 8°
2a,b	48-82	120-444	24D, 10°
3a,b	48-82	120-614	24D, 15°
Experiment 2			
1	70-78	140-204	24D, 8°
2	70-78	140-220	24D, 10°
3	70-78	140-260	24D, 15°
4	70-78	140-204	12L/12D, 8°
5	70-78	140-220	12L/12D, 10°
6	70-78	140-260	12L/12D, 15°
7	70-78	140-204	6L/6D, 8°
8	70-78	140-220	6L/6D, 10°
9	70-78	140-260	6L/6D, 15°

TABLE 4

MANIPULATION EXPERIMENTS USING SALMON EMBRYOS

TANK	AGE dpf	degday	TREATMENT (L = hours of light, D = hours of darkness)
Experiment 1			
1a,b	48-82	120-376	24D, 8°
2a,b	48-82	120-444	24D, 10° ·
3a,b	48-82	120-614	24D, 15°
Experiment 2			
1	70-78	140-204	24D, 8°
2	70-78	140-220	24D, 10°
3	70-78	140-260	24D, 15°
4	70-78	140-204	12L/12D, 8°
5	70-78	140-220	12L/12D, 10°
6	70-78	140-260	12L/12D, 15°
7	70-78	140-204	6L/6D, 8°
8	70-78	140-220	6L/6D, 10°
9	70-78	140-260	6L/6D, 15°

were just visible as points of calcification. Experiment 1 was
terminated after 36 days (82 dpf), when all the embryos in the
various treatments were hatched. Experiment 2 compared combined
effects of temperature and light regimes. Three glass dishes (15 cm
diameter), containing groups of 20-30 eggs at 70 dpf, were placed in
water baths at 8, 10, and 15°C. The dishes in each water bath were
separated by black plastic sheeting so that they could be exposed
separately to 6L/6D, 24D, and 12L/12D light regimes (see Fig. 2).

Because the eggs were hardy and easy to handle, it was possible
to measure the oxygen consumption of individual eggs to compare the
effect of light cycles on metabolism. Every 4 h, over a 24 h period,
three eggs were removed from each of the three light regimes at 8°C
and placed in individual flasks for 90 min periods in a Gilson
Differential Respirometer (Gilson Medical Electronics, France). The
respiration rates (μl $O_2/\mu g$ larvae/h) for each sample were calculated
and the coincidence between metabolic cycles and otolith rings
examined.

Field studies

Sampling

The technique of ageing larvae from counts of otolith rings was
applied to herring larvae captured off the west coast of Scotland in
1980 and 1981 (Fig. 3).

Herring larvae were captured in the Firth of Clyde on 21st May
1980, between 27th April and 1st May 1981, and on 28th May 1981.
The sites sampled were between the Isles of Arran and Bute in 1980,
and between the Isle of Sanda (off the Mull of Kintyre) and Pladda
(off the Isle of Arran) in 1981. The samples were obtained by
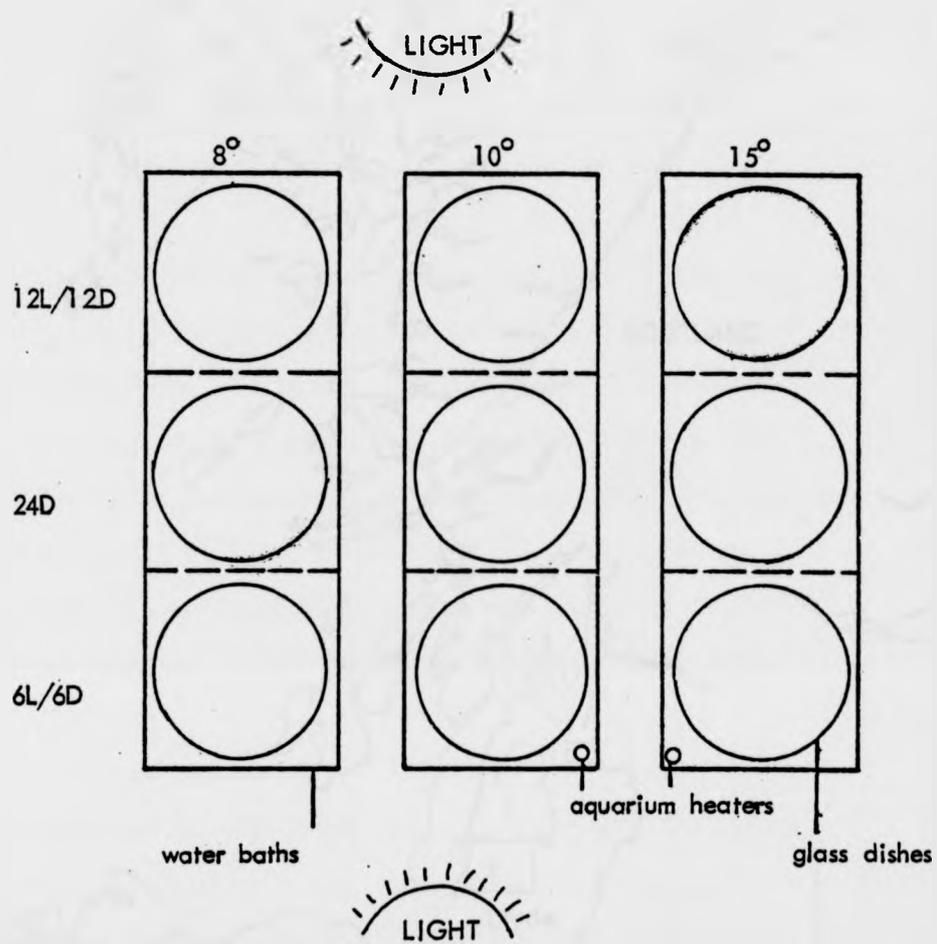
Fig. 2 : Design of the salmon embryo manipulation
experiment, Experiment 2.

Fig. 3 : Sampling areas for wild herring larvae.
a) Clyde, May 1980. b) Minch, October 1980.
c) Clyde, April and May 1981.

oblique hauls with a 2 m ring net (young fish trawl, Southward, 1970) from the RV Calanus (SMBA). Most of the hauls were done at night (2200-0500 h), although daylight sampling in late April 1981 was also successful in producing sufficient numbers of larvae for analysis. The herring larvae were immediately removed from the sample and preserved in 70% alcohol. In order to prevent decalcification of the otoliths (Radtke, 1980) the alcohol was changed after 24 h and replaced with absolute alcohol on return to the laboratory.

Samples were obtained between 1st and 20th October 1980 from the west coasts of Scotland and N. Ireland during the 1980 ICES Herring Larval Survey (FRV Scotia, DAFS, Aberdeen). Larvae were sampled with a modified Dutch Gulf III towed obliquely from the maximum depth which was dependent on bottom topography. The samples were washed into 4% buffered formalin. Those larvae taken for otolith analysis were immediately removed and frozen. Later they were thawed, measured, and stored dry in individual glass tubes.

Shrinkage of preserved larvae

The shrinkage in body length due to handling and alcohol preservation was calculated in order to convert all field measurements to fresh lengths. Three separate studies were made on herring larvae of various sizes.

A group of yolk sac larvae was removed from the rearing tanks and one sample measured immediately after anaesthetising with MS-222 (Sandoz). The remainder of the group was taken to sea in thermos flasks and subjected to net capture and tows from RV Seol Mara (SMBA). Following methods described by Hay (1981),

the larvae were poured over the stern into a 1.0 m diameter stramin net and towed at 1-2 knots for 15 min. Subsamples from each tow were preserved in 70% alcohol immediately after the tow and after delays of 5, 10, and 15 min. The larvae were transferred after 24 h to absolute alcohol and measured after one week.

After feeding was established in the rearing tanks a second group of larvae was removed, a sample anaesthetized and measured, and the remainder subjected to a simulated netting and towing experience. Groups of larvae were placed in a hand net and held in a drain with a constant seawater flow for 15 min. They were preserved in 70% alcohol after delays of 5 and 15 min. They were transferred to absolute alcohol after 24 h and measured after one week.

Any larvae which were brought on board alive during the field sampling were measured immediately using mm grid paper, preserved in separate vials, and treated as described for a calculation of shrinkage.

The correction for shrinkage in frozen and formalin preserved larvae was taken from Townsend & Graham (1981).

Otolith techniques

Sampling procedures

The larvae sampled from rearing experiments were anaethetized with Benzocaine (Ethyl para-aminobenzoate, Aldrich Chemicals, Dorset), the standard (notochord) length was measured, feeding condition noted, and in the case of herring, the larvae were staged according to Doyle (1977). The larvae were stored frozen on labelled glass slides for later otolith preparation.

Each frozen larva was thawed by placing a drop of water on the storage slide. After thawing, it was moved to a glass slide marked with a circle on the underside with alcohol-based marker. The slides were labelled by code as to species, source (laboratory-reared or wild), and numbered consecutively to avoid any bias in reading the otoliths. Against each code number the length, date of sample, source and feeding state were recorded. The otoliths were removed by teasing them out of the head with fine glass needles, working at 250-500 x under a binocular microscope (Wild M5). The otoliths were placed within the marked circle and set aside to dry for at least 30 min. Several mounting media were used on the early samples. Euparal (GBI labs) and DPX (Searle Scientific Service) were least acceptable because of the thickness of the mount and the drying time (24 h minimum) necessary before examination. DPX was also used without a coverslip to reduce the thickness, but the preparation did not always remain stable after cleaning the slide following examination. The most satisfactory procedure was to place a drop of immersion oil over the otolith, cover it with a coverslip and secure that in place by painting the edges with nail varnish. This gave the greatest resolution for examining and photography. The preparations could be viewed within one h after dissection.

Difficulties in mounting some otoliths often gave rise to problems in reading the rings. In cases of very small otoliths (<50 μm diameter), especially of turbot, it was difficult to manipulate the otolith into a position on the slide which showed the clearest rings. The otoliths are plano-convex in shape and show the clearest rings when the plano-side or flat-side is face up from the slide. The rings in inverted otoliths were very faint and often the

rings at the edge could not be seen at all. Instead of the normal mounting procedure these small otoliths were mounted on coverslips 50 mm x 22 mm, in oil, and covered with small circular coverslips 10 mm diameter. These preparations could then be viewed from either side, regardless of how the otolith was mounted.

In otoliths from older larvae the amount of deposited material sometimes occluded the earlier rings. In these cases the otoliths were fixed with epoxy cement or nail varnish to glass slides and ground carefully by hand using 600 grit wet carborundum paper or ground glass plates checking the progress of the grinding at frequent intervals under a dissecting microscope. When the plane of the nucleus was reached, the preparation was polished with commercial metal polish, washed, and then coated with DPX. This treatment was usually successful in presenting all the rings clearly for counting.

Counting rings

In keeping with the literature, the individual otolith rings or bands were identified as one complete set of light and dark deposition patterns. One ring (band, or increment) was the area from the beginning of one dark band (transmitted light) to the beginning of the next dark band (Fig. 4). For all the species, the rings were counted along one radius, adjusting the focus along the otolith to make sure that each ring was sharply focused when counted.

Three types of rings could usually be identified on the otolith: 1) Yolk sac rings. These rings were sometimes laid down during the period before feeding. They did not seem to represent any regular periodicity, and were usually fainter than the normal rings and ranged in number from 2-5.

Fig. 4: Diagramatic representation of typical larval otolith explaining terminology and axis of measurement.
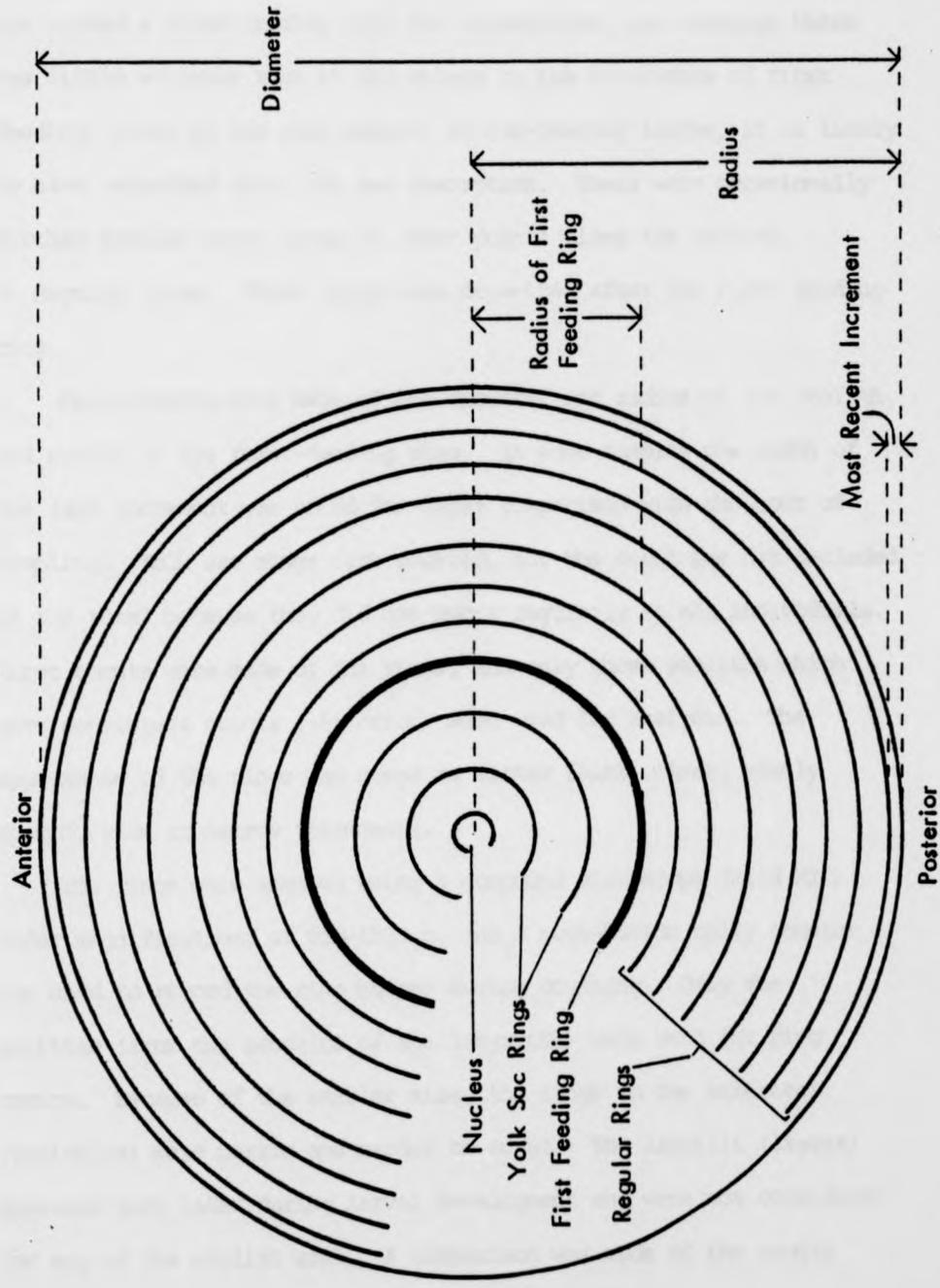
2) First feeding ring. This ring was found on almost all otoliths examined. It occurred between 8-13 μm from the nucleus and was considerably darker and thicker than the normal rings. In many cases the following light band was wider than in normal rings. This ring was termed a first-feeding ring for convenience, and although there was little evidence that it did relate to the occurrence of first feeding, since it was also present in non-feeding larvae, it is likely to have coincided with yolk sac absorption. There were occasionally further similar heavy rings at later points along the otolith.

3) Regular rings. These rings were deposited after the first feeding ring.

Measurements were made of the diameter and radius of the otolith, and radius of the first-feeding ring. In some samples the width of the last increment was noted for later comparison with the hour of sampling. Yolk sac rings were counted, but the count was not included in the total because they did not occur regularly in all individuals. Three counts were made of the rings, and only those otoliths which gave consistent counts (<5% error) were used for analysis. The appearance of the rings was noted as either faint, clear, evenly spaced, wide or narrow increments.

The rings were counted using a compound microscope (Wild M20) under magnifications of 600-1500 x, and a push-button tally counter was used to record the ring number during counting. Only the sagittae (from the sacculus of the labyrinth) were used for ring counts. Because of the smaller size, the rings on the asterisci (utriculus) were narrow and harder to count. The lappilli (lagena) appeared much later during larval development and were not considered for any of the otolith work. A comparison was made of the counts

obtained on each sagitta. The difference between the counts was not significant (t = 0.226; d.f. 77; P <0.001) so little attempt was made to remove or to count the rings on both otoliths.

A selection of 10 otolith preparations of plaice, turbot, and herring from different sources was read separately by a group of volunteers. The resulting counts were compared following the methods of Beamish & Fournier (1981) for an estimate of reader error.

Periodicity of ring deposition

A series of hourly samples taken over a 30 h period was examined in order to monitor the growth of the most recent otolith increment. The state of completion of the last band was used to identify periods of active ring deposition and to describe the synchronisation of ring deposition between individuals. Three herring larvae at day 115 were taken for each sample. The width of the most recent band in these samples and in most of the wild larvae captured was assessed qualit-atively as either new, half-formed, or completed. Whenever possible the width was measured using an occular micrometer.

In herring larvae, the width of the otolith rings was examined in relation to short-term changes in growth rate during larval development. The width of each ring was measured in a series of herring otoliths of different ages and sources. The image of the otolith was projected directly onto a Hewlett Packard 9111A Graphics Tablet (digitizer) using a drawing tube attachment mounted on a Wild M20 microscope. The position of each growth ring along the longest radius from the nucleus to the posterior edge of the otolith (see Fig. 4) was entered via the digitizer to an HP9825A desk-top computer and the width of each band calculated. The pattern of ring widths

was compared between individuals, between areas of the otolith in one individual, and between various rearing condition.

Treatment of data

Data recording

For each individual 12 parameters were recorded, although not all were used in the major portion of the analysis. The main parameters used were: age, number of days since first feeding, body length, source or treatment, otolith radius, and number of rings. The date sampled, otolith diameter and radius of the first-feeding ring, the number of yolk sac rings, and the standard deviation of the ring counts were also recorded.

The data were stored on the 2980 IBM at the Edinburgh Regional Computing Centre (ERCC). The statistical programme package used for some of the analysis of the data was SPSS (Nie *et al.*, 1975) and was available directly as part of ERCC facilities.

Statistical analysis

The major part of the analysis concentrated on the relationships between ring number, age, length, and growth condition. This was done using analysis of variance, analysis of covariance, and multiple regression techniques.

Multiple regression techniques were the most useful for separating out the influences of endogenous and exogenous factors on ring deposition. Step-wise regressions were performed beginning with larval length as a predictor of ring number and adding into the regression calculations larval age. By comparing the increase in the multiple correlation coefficient that each incremental parameter

incurred, it was possible to assess the importance of that parameter
in explaining the number of otolith rings observed (Nie *et al.*, 1975;
Quinn, 1980).

Predictive growth curves for wild herring larvae

The main aims of studying the rings on otoliths are to investi-
gate the growth and survival rates of populations of wild larvae.
Because the deposition of rings in reared herring larvae did not
prove to be straightforward, it was necessary to use several methods
to estimate the growth and survival of wild larvae. The curves
obtained by each method were compared with each other and with the
range of growth curves of larvae of known age.

In the first estimation method, body length was plotted against
the ring number observed for each individual. Assuming that ring
number was equivalent to larval age, the regression equation fitted
to these plots represented an estimation of growth rate for each
wild population sampled (Lough *et al.*, in press; Methot & Kramer, 1979).

Five groups of larvae of known age were also used to produce
ageing calibrations for the wild larvae. These stock populations of
reared larvae came from the 1980 and 1981 Dunstaffnage rearing tanks
(120 ℓ and 500 ℓ), the Loch Ewe plastic bags, and the Norway pond.
Wild herring larvae were aged individually, based on the ring depo-
sition rates observed in each known-age group, producing a second
series of estimated growth curves.

The third estimation method, the last involving otoliths, was
backcalculation. The dependence of otolith radius on larval length
must be shown before backcalculating growth rates. This is usually
demonstrated by a high correlation coefficient for the regression

on radius (Y axis) on length (X axis). The Geometric Mean (GM) functional regression is suggested for the prediction of x-values from y-values (Ricker, 1975) and was used in this analysis since larval length is predicted from otolith radius for backcalculation. Larval length at yolk sac absorption was estimated by calculating the length at first ring formation. Individual growth rates were calculated using the Fraser-Lee modified equation for backcalculation (Bagenal and Tesch, 1968):

$$r_1(l_2-c) = r_2(l_1-c) \qquad \text{in the form:}$$

$$\frac{l_2 - \dfrac{(c + (l_2-c)r_1}{r_2}}{\text{no. of rings}} = \text{growth rate}$$

where l = larval length, r = otolith radius, the subscripts 1 and 2 refer to the values at first ring formation and capture, respectively and c = the constant of the GM functional regression of length on radius.

In addition to these ageing methods, the growth of the wild larval populations sampled was estimated using direct measurements of larval length, assuming an average hatching length, and calculating the growth during the time estimated to have elapsed since hatching.

The estimations of growth obtained from all these methods were compared and the problems involved will be discussed in detail.

RESULTS

Turbot

Larval rearing

Turbot larvae were reared to metamorphosis in 50 days in 30 ℓ black circular tanks, fed on *Artemia* nauplii. They were weaned onto dry food beginning on day 45. Growth and ring deposition were analysed for larvae from hatching to day 23 under varying conditions. Larvae were reared at 20°C under photoperiods of 24L, 12L/12D and 6L/6D, and at 24°C under 12L/12D. Starved larvae were maintained at 20°C under 12L/12D. Larvae reared under 6L/6D survived to day 14, when 10 larvae were recovered from the tank. The 12L/12D treatment was terminated on day 17 with six survivors. A total of 12 larvae survived the experiment in the 24L and 24°C treatments. Two larvae survived the starvation conditions to day 6.

Growth

The final mean length ($\bar{x} \pm 1$ SD) of the larvae recorded at the termination of each experimental treatment was 3.76 ± 0.00 mm for starved larvae, 4.91 ± 0.4 mm for 6L/6D, 5.77 ± 1.11 mm for 12L/12D, 9.43 ± 1.75 mm for 24L, and 9.44 ± 1.5 mm for 24°C. The maximum length observed for all the treatments was 11.36 mm at day 23.

The growth model fitted for all the treatments combined was:

$$y = 1.16 + 0.21 \, e^x \qquad r = 0.92 \qquad n = 124$$

where x = age in days and y = length in mm (Fig. 5).

A comparison of the growth rates in the individual treatments (Table 5) showed that turbot larvae grew at a similar rate under conditions of 24L and 24°C and significantly faster than under
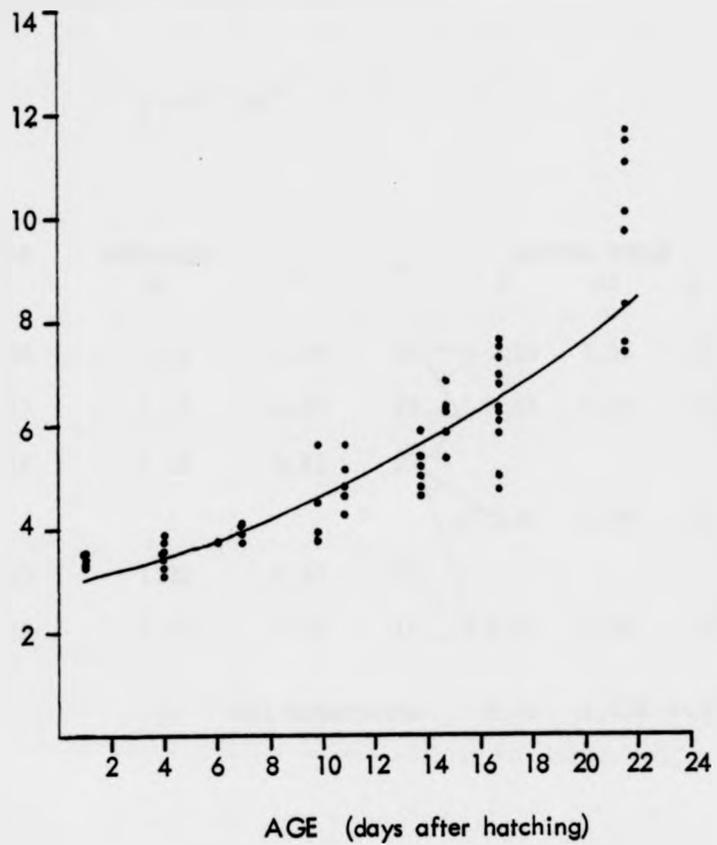
Fig. 5: Growth of turbot larvae, all treatments combined. Points represent individual larvae.

TABLE 5

COMPARISON OF THE GROWTH OF TURBOT LARVAE
REARED UNDER EXPERIMENTAL CONDITIONS

$$y = b + ae^x$$

TREATMENT	SLOPE (a)	INTERCEPT (b)	r	n	ANCOVA TABLE F	df	p
24L	0.26	1.14	0.84	28	0.28	1,53	>.50
24°C	0.27	1.14	0.87	28	3.98	2,81	<.025
12L/12D	0.16	1.18	0.83	29			
					0.93	1,49	>.50
6L/6D	0.13	1.22	0.95	23			
starved	0.19	1.12	0.35	16	6.05	2,64	<.005
All treatments					6.75	4,118	<<.001

conditions of 12L/12D. During the 6 days that they survived, starved larvae grew better than those under 12L/12D and 6L/6D. The growth rates of larvae reared under 12L/12D and 6L/6D were similar.

The coefficient of variation represents individual variation as a proportion of the mean so that the variability at any mean value can be directly compared both within and among populations. The coefficient of variation (CV) is calculated:

$$CV = \frac{sd}{\bar{x}} \times 100$$

where $\bar{x}$ = the mean, and sd = the standard deviation measured for each group.

The coefficients of variation in length were similar in each treatment, except under starvation conditions. The variation in each population gradually increased with age (Fig. 6).

Development of the otolith

A total of 77 larvae, aged 1-23 days post hatching, were used for otolith preparations. Of these otoliths, 11 were discarded as broken or unreadable. The otoliths were relatively large, ranging in size from 9 µm radius in a 3.5 mm larva to 90 µm in a 10 mm larva. The first feeding ring usually occurred between 9 and 11 µm from the nucleus and was first observed in larvae between day 1 and 7. The regular rings were very clear and evenly spaced (Plate 1). There were 2-3 yolk sac rings present in a few larvae, but most larvae had none.

Ring deposition

At day 4, three out of ten feeding larvae had deposited one
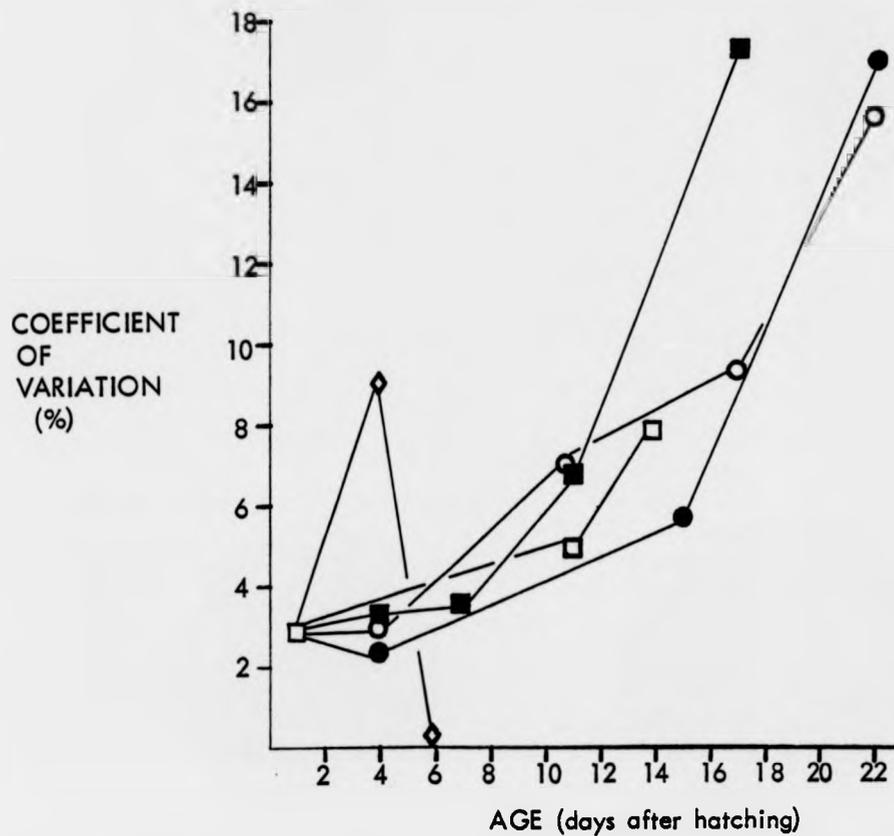
Fig. 6 : The effect of experimental treatment on individual
variation in length for turbot larvae, measured by
the coefficient of variation for each treatment.
Points represent the coefficient of variation of the
mean length of each sample.

● 24 L
■ 12L/12D
□ 6L/6D
○ 24°C
◇ starved

Plate 1.

Otolith development in turbot larvae

a: 1 day old, 3.40mm, 1 ring
b: 4 days old, 3.36mm, 2 rings
c: 8 days old, 4.84mm, 5 rings
d: 10 days old, 4.96mm, 7 rings
e: 23 days old, 9.44mm, 22 rings

scale bar = 20μm
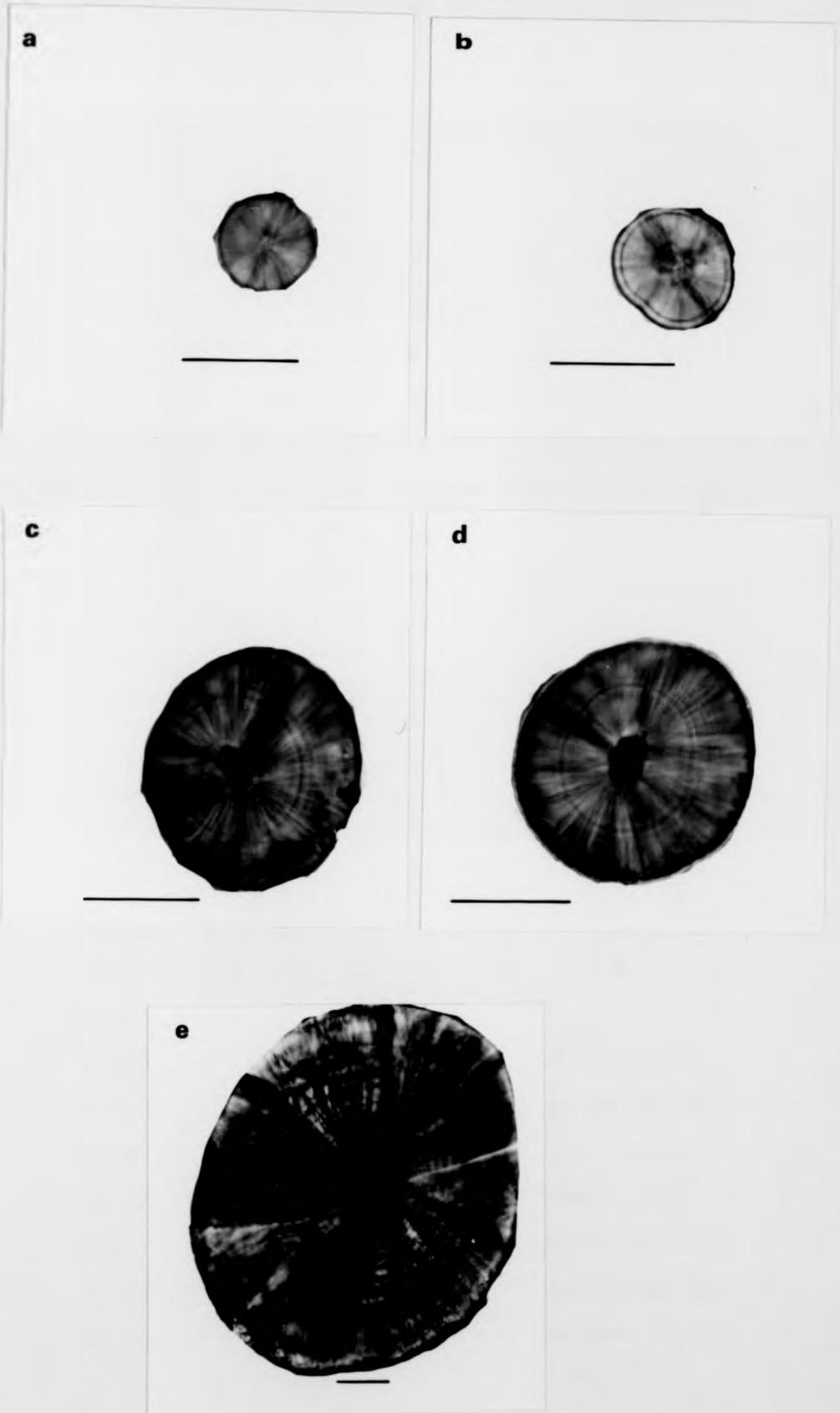
otolith ring. Starvation to this point (two days) did not affect
the ring deposition, as two of the four starved larvae sampled had
also deposited one ring. The starved larvae survived until day 6,
but laid down no further rings (Fig. 7). The second ring was laid
down in feeding larvae (the remaining four treatments) beginning at
day 7, and by day 11 most feeding larvae had more than two rings.
Ring numbers observed after day 18 were approximately equal to
larval age. Larvae surviving to day 6 under starvation conditions
had 1.0 ± 0.50 rings. At day 11, larvae sampled from the 6L/6D
treatment had 4.0 ± 0.99 rings, but the larvae which survived to day
14 in this tank had only 1.5 ± 1.38 rings. At day 17, larvae reared
under 12L/12D had 12.7 ± 2.77 rings. Larvae in the 24L treatment
had 17.8 ± 6.73 rings and those in the $24^{\circ}C$ treatment had $21.48 \pm$
0.83 rings at day 23. The average ring deposition rate for each
treatment during the 23 days of the experiment could be fitted to a
linear model:

|			
24L	$y = 1.00x - 2.80$	$r = 0.91$	$n = 14$
$24^{\circ}C$	$y = 1.00x - 3.05$	$r = 0.96$	$n = 17$
12L/12D	$y = 0.75x - 2.06$	$r = 0.88$	$n = 22$
6L/6D	$y = 0.38x - 0.18$	$r = 0.95$	$n = 8$
starved	$y = 0.07x + 0.15$	$r = 0.29$	$n = 11$

where x = age in days and y = number of rings.

Ring deposition was similar under conditions of 24L and $24^{\circ}C$
($F = 0.002$ df 1,28 p >0.25), and significantly higher than in the
other tanks ($F = 3.792$, df 4,66, p <0.01).

In all the tanks ring deposition varied significantly with
larval length among five length groupings tested (Fig. 8) ($F = 7.41$,
df 4,15, p <0.005), and there was a strong correlation between the
growth rate in each treatment and the ring deposition rate (Fig. 9):
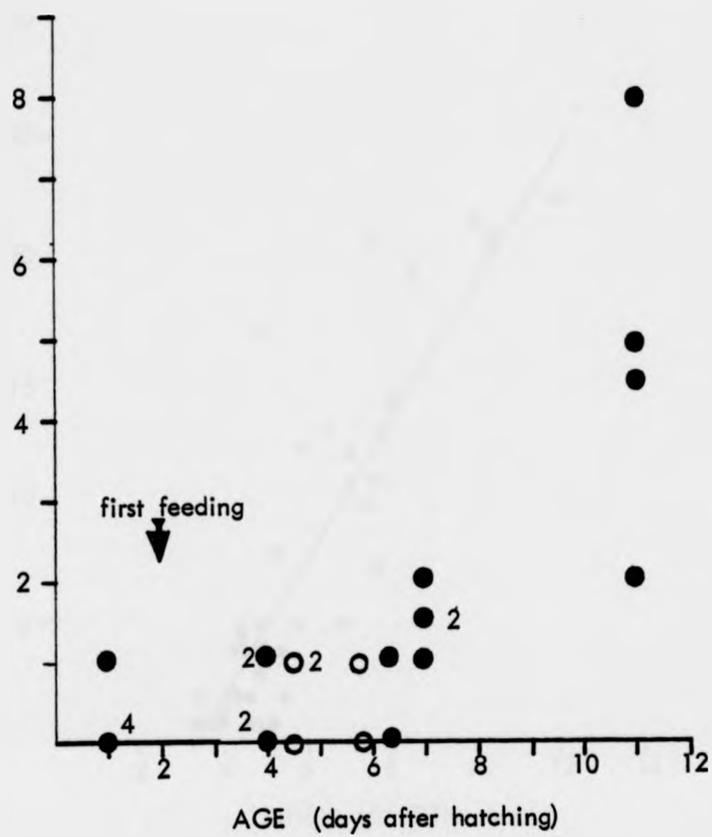
Fig. 7: The effect of starvation on early ring deposition in turbot larvae. Points represent individual larvae. The number of larvae with the same number of rings is indicated by the values beside each point.

○ starved
● feeding

NUMBER
OF
RINGS
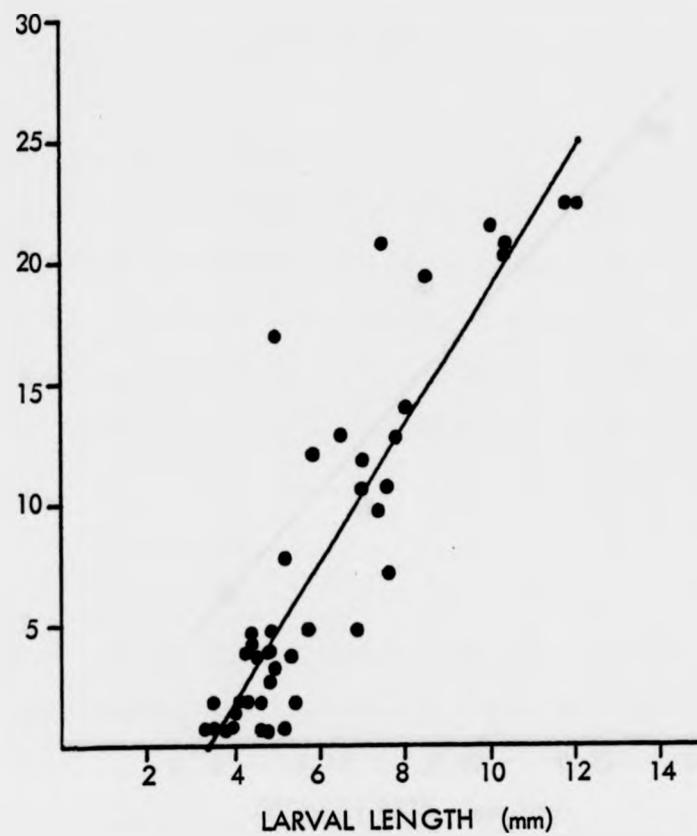
LARVAL LENGTH (mm)

Fig. 8 :   The relationship between larval length and number of
rings in turbot larvae, all treatments combined.
Points represent individual larvae.
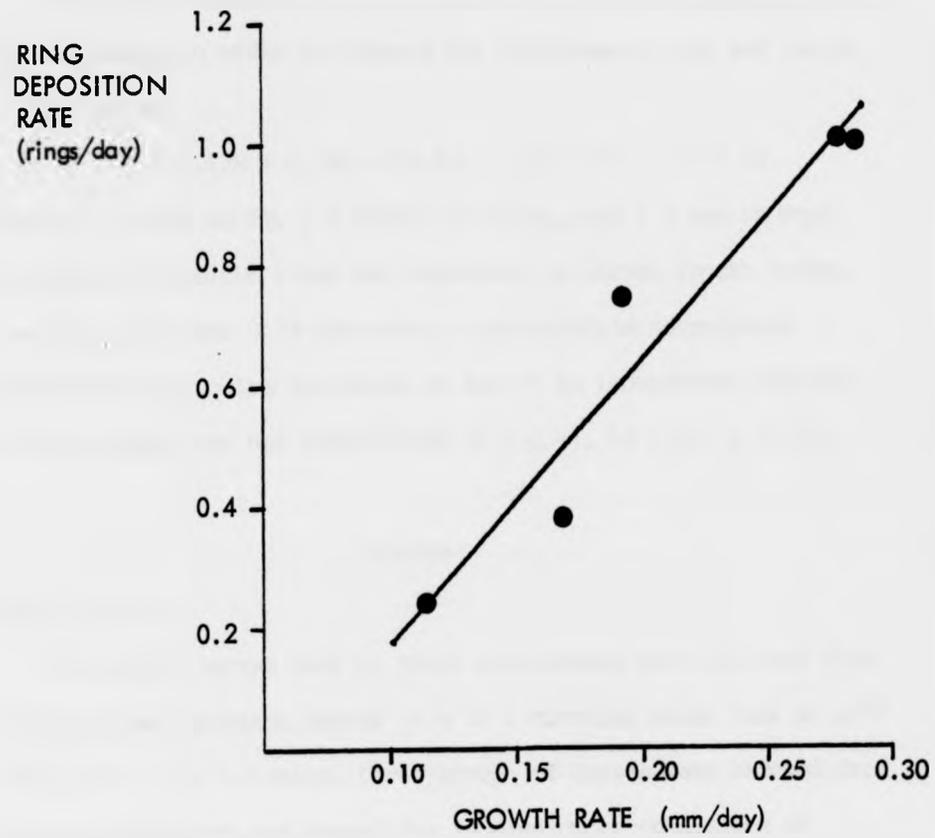
Fig. 9 : The relationship between growth rate and ring deposition rate in turbot larvae. Points represent the growth and ring deposition rate for each treatment.

$$y = 3.787 \, x - 0.299 \qquad r = 0.97 \qquad n = 5$$

where x = growth rate (mm/day), and y = ring deposition rate (rings/day).

A multiple regression equation was fitted to the data from all the treatments in order to compare the influence of age and length on ring number:

$$y = 2.83x + 0.18z - 10.83 \qquad r = 0.91 \qquad n = 64$$

where x = length in mm, y = number of rings, and z = age in days. The number of otolith rings was dependent on larval length rather than age, since the 0.7% increase in the multiple correlation coefficient due to the inclusion of age as an independent variable for ring number was not significant (F = 0.44, df 1,61, p <0.25).

Plaice

Larval rearing

The plaice larvae used in these experiments were obtained from a single spawning batch reared in a 30 ℓ circular black tank at 10°C under 18L/6D. At hatching, three groups of larvae were removed from the main population and reared for 13 days under conditions of starvation, normal feeding, and delayed first feeding. Survival to day 13 was good (10-15%). At day 42, three groups of larvae were removed from the main population and exposed to conditions of 12L/12D, 24L, and 24L with a diurnal temperature cycle. The treatments continued until day 50 and there were no mortalities in the tanks during this time.

Growth

The mean length of newly hatched plaice larvae in all the treatments was 6.39 ± 0.29 mm and increased to 6.78 ± 0.58 mm at day 13.

The older larvae tested grew on average from 8.13 ± 0.51 mm at day
42 to 9.35 ± 0.58 mm at day 50 (all treatments combined). At day 50
the largest larva was 10.04 mm and came from the tank with the
imposed temperature cycle. The lengths of the plaice larvae in all
six treatments was fitted to a linear growth model (Fig. 10):

$$y = 0.06x + 6.39 \qquad r = 0.91 \qquad n = 46$$

where x = age in days and y = length in mm.

There was no difference in growth between the three tanks in
the feeding experiment (F= 0.82, df 2,9, p >0.50). The light and
temperature regimes tested on older larvae also had no affect on
growth (F = 0.62, df 2,8, p >0.50).

In the absence of food, the coefficient of variation for length
was less constant and reached higher levels than for feeding larvae
(Fig. 11). In older larvae the coefficient of variation for length
ranged from 5.2-7.7%.

Development of the otolith

The otoliths of 58 plaice larvae were examined from these
experiments. The larvae ranged in age from 0 (day of hatching) to
50 days. Five otoliths were discarded from the analysis because
they were broken or unreadable.

Two pairs of otoliths were present in plaice embryos about four
days before hatching. The third pair of otoliths was observed after
day 8. On hatching the otoliths were 10 μm radius and usually showed
two rings formed during the embryonic stage. Otoliths were 15 μm in
radius at day 13 and increased to 50-90 μm at day 50 (Plate 2).
From day 4 onwards, 3-6 yolk sac rings were visible in all larvae.
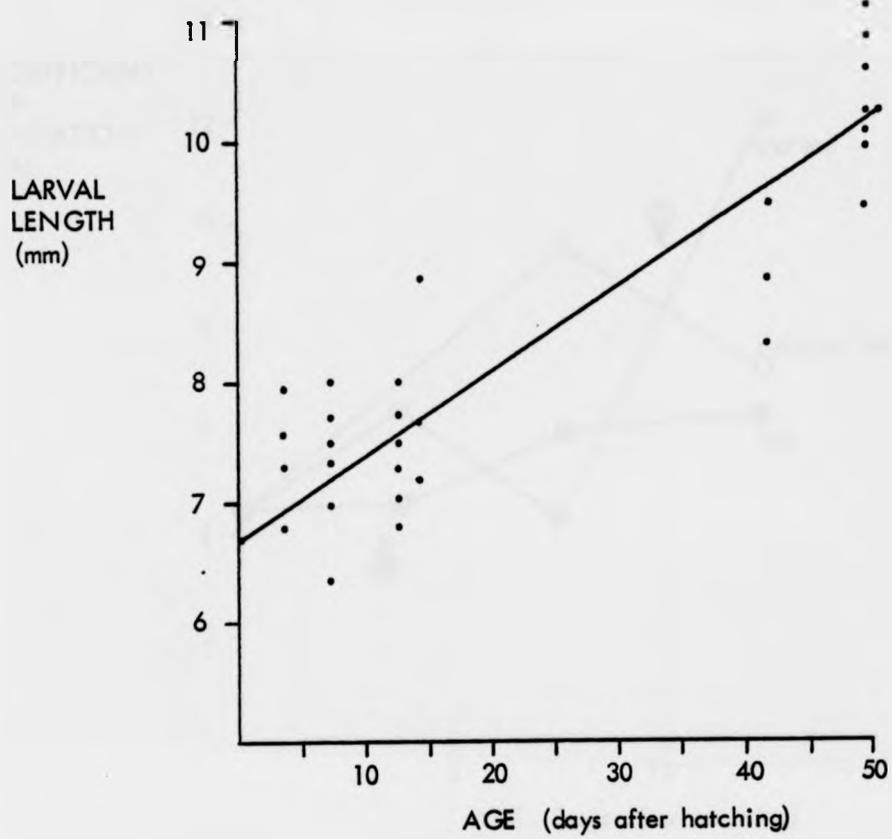
Fig. 10 : Growth of plaice larvae, all treatments combined. Points represent individual larvae.
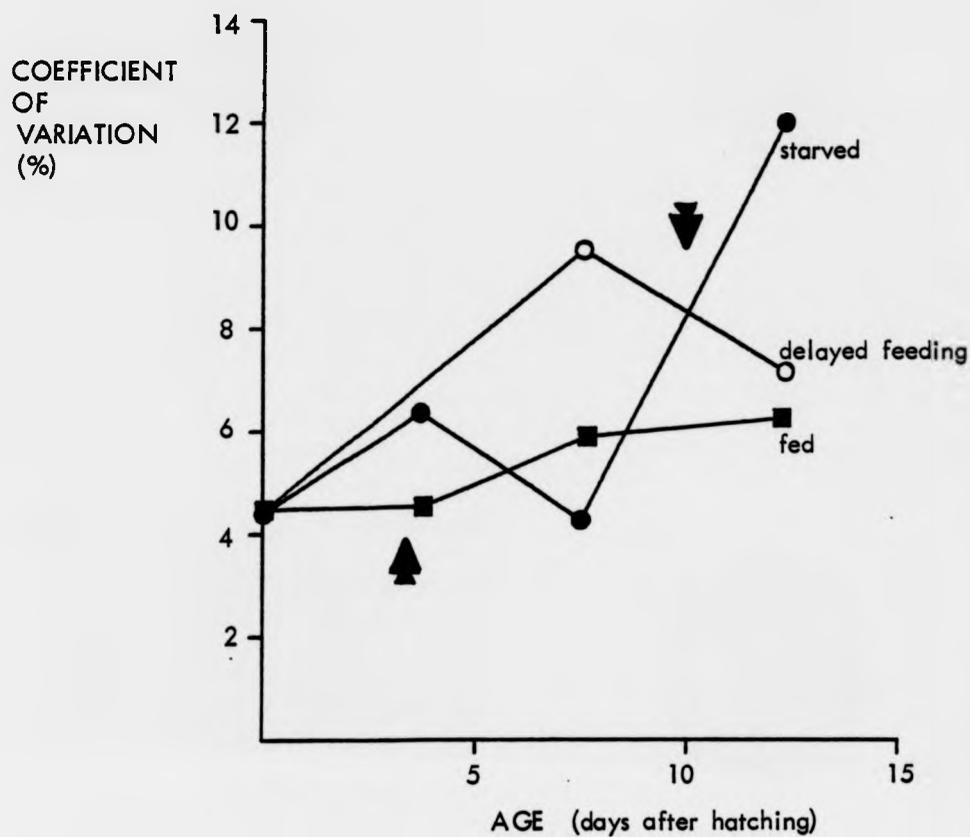
Fig. 11 : The effect of starvation and delayed feeding on individual variation in length for plaice larvae, measured by the coefficient of variation for each treatment. Points represent the coefficient of variation of the mean length for each sample. Arrows indicate the time of first feeding in the fed and delayed feeding treatments.

Plate 2.

Otolith development in plaice larvae

a: 0 days old,   6.47mm,   0 rings
b: 4 days old,   6.31mm,   4 rings
c: 8 days old,   6.97mm, 11 rings
d: 15 days old,   6.89mm, 11 rings
e: 42 days old,   8.79mm, 29 rings
f: 50 days old,   8.88mm, 49 rings

scale bar = 20μm
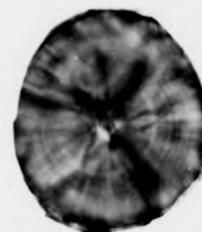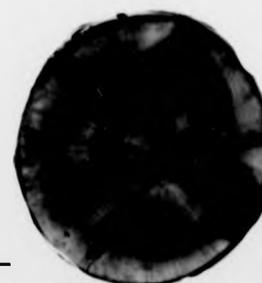
Ring deposition

The deposition of otolith rings began within the first four
days after hatching. Neither starvation nor the delay of first
feeding had any effect on ring deposition (F = 1.70, df 2,7, p >0.50).
The deposition rates of the older larvae were also unaffected by the
different light and temperature regimes (F = 1.60, df 2,10, p <0.25).
The ring deposition rate calculated for all six treatments combined
(day 0-50) was linear (Fig. 12):

$$y = 0.74x + 0.46 \qquad r = 0.97 \qquad n = 50$$

where x = age in days and y = number of regular rings.

Larvae at day 13 had an average among all of the treatments of
10.31 ± 0.91 rings. The maximum number of rings observed at this
time was 11.8 rings under starvation conditions. Continuous light
with a temperature cycle produced the individual at day 50 with the
highest number of rings (48). The average ring number for 50 day
old larvae in all of the treatments was 37.80 ± 4.31 rings.

The interaction of larval length and age in determining otolith
ring number was expressed by the multiple regression equation:

$$y = 0.40x + 0.74z - 4.77 \qquad r = 0.92 \qquad n = 53$$

where x = length in mm, y = number of rings, and z = age in days.
Larval age was significantly more important than length in determin-
ing ring number in plaice larvae (F = 23.38, df 1,50, p <0.001).
There was an 18% increase in the multiple correlation coefficient
after including age as an independent variable for determining ring
number.

NUMBER
OF
RINGS

AGE (days after hatching)
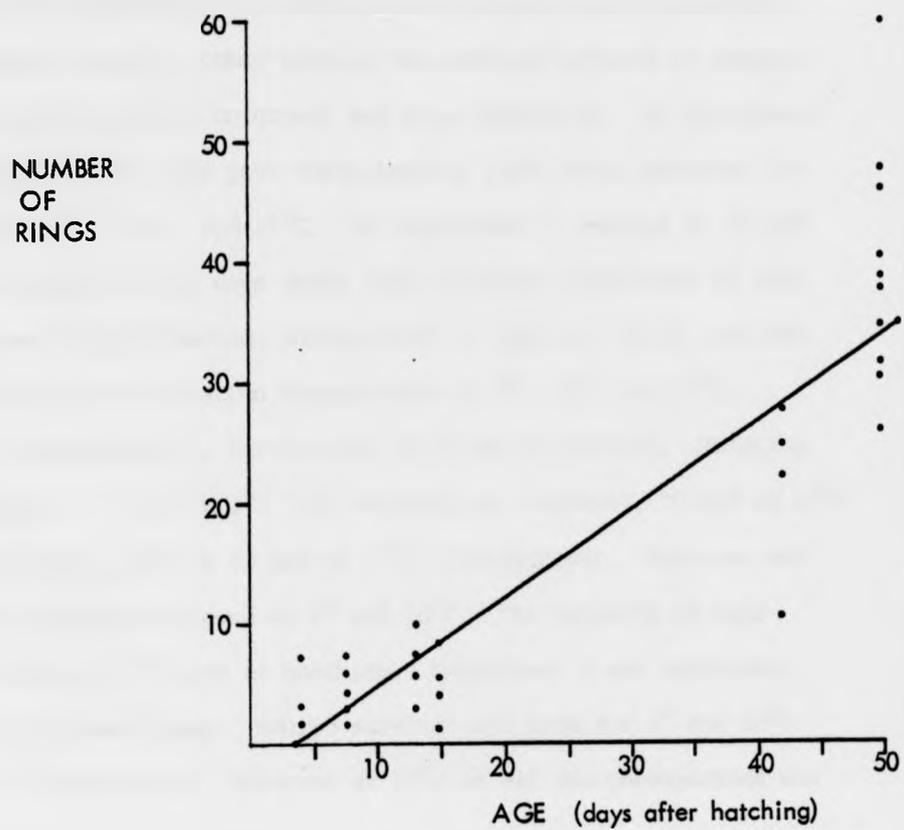
Fig. 12 : Ring deposition in plaice larvae, all treatments
combined. Points represent individual larvae.

Salmon

Incubation and rearing

Salmon embryos were incubated in small glass dishes in two separate experiments, one testing for the effects of incubation temperature and the other testing the combined effects of temperature and photoperiod on growth and ring deposition. In Experiment 1, embryos at 48 days post-fertilisation (dpf) were incubated for 36 days at 8°, 10°, and $15^\circ C$. In Experiment 2, embryos at 70 dpf were incubated for 8 days under nine different conditions of temperature and photoperiod: photoperiods of 12L/12D, 6L/6D, and 24D each tested at incubation temperatures of 8°, 10°, and $15^\circ C$.

In Experiment 1, larvae were 16-17 mm at hatching. Hatching occurred at 80 dpf at $8^\circ C$ (376 degree-days, degdays), 70 dpf at $10^\circ C$ (340 degdays), and at 60 dpf at $15^\circ C$ (300 degdays). Survival and hatching rates were good at 8° and $10^\circ C$. The majority of eggs incubated at $15^\circ C$ died at hatching. Experiment 2 was terminated before hatching began. Embryo survival was good for 8° and $10^\circ C$ in all photoperiods. Survival at $15^\circ C$ in all the photoperiods was about 3%.

Growth

Salmon embryos in Experiment 1 grew from 8.75 mm ± 0.06 at 48 dpf (120 degdays), to 15.62 mm ± 0.45 at 8°, 20.00 mm at 10°, and 23.00 mm at 15° by 81 dpf (376, 444, 614 degdays respectively, as shown in Fig. 13). Embryos in Experiment 2 were on average 10.8 mm ± 0.11 in length at 70 dpf (140 degdays). They increased in length to 13.97 ± 0.33 at 8° (204 degdays), 14.48 ± 0.28 at 10° (220 degdays), and 17.04 ± 0.69 at 15° (260 degdays).

Fig. 13 : Growth of salmon embryos in Experiment 1.
Points represent the mean lengths ± 1 sd
for each sample. Samples which contained only
one individual are represented by single points.
a) age in days post-fertilisation. b) age in
degree-days.

■ -- 15°C
○ -- 10°C
● -- 8°C
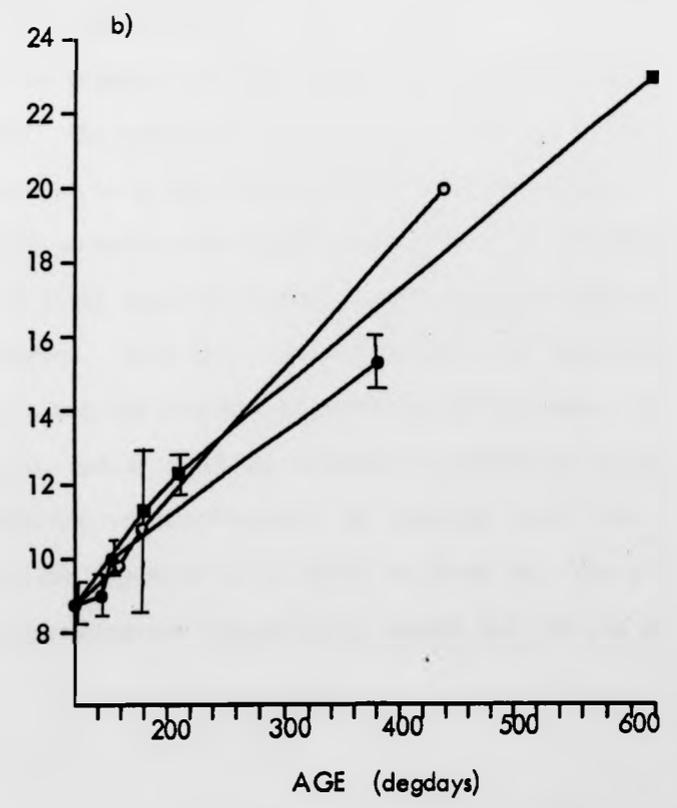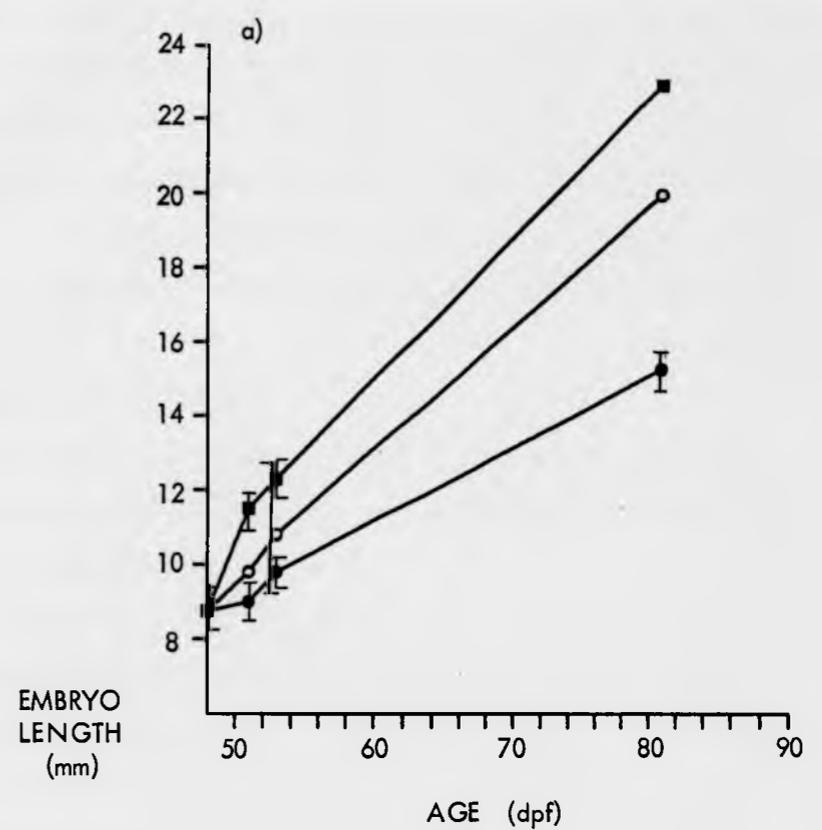
The growth rates in Experiment 1 were significantly affected by the incubation temperature. The effect of temperature on length could be compensated for by comparing the treatments on the basis of degree days (Table 6). The lengths of embryos in Experiment 2 were significantly different at the different temperatures (F = 105.78, df 2,22; p <0.001). However, the different light regimes of 6L/6D, 12L/12D or 24D had no effect on growth (F = 0.66, df 2,22, p >0.50).

Development of the otolith

From these experiments, 115 embryos age 48 to 81 dpf were sampled for otolith examination. From these 35 otoliths were discarded as unreadable.

Otoliths were first observed in salmon embryos at 45 dpf. The embryos were about 7.6 mm in length, with developed otic capsules and body movements visible within the egg. In the early formation of the otolith a chain of 2-5 individual nucleii grew into contact with one another (Plate 3). Each nucleus had 1-5 individual rings, but after making contact, continuous rings were laid down around the edges of all the nucleii, bonding the whole structure together. The otolith at this stage was between 70-90 μm in length and 40-60 μm in width. The radius of the individual nucleii was 4.5-9.0 μm before the deposition of the first continuous ring. The radius of this first ring was 10.8-13 μm and occurred after 50 dpf in 9-11 mm embryos. This ring corresponded with the vascularisation of the gills and the complete pigmentation of the eyes. A distinct heavy band, radius 22-29 μm, occurred in embryos of 15 mm at the time of yolk-sac vascularisation. At hatching (16-17 mm) a similar heavy band was deposited at a radius of 50-60 μm. The size of the otolith at hatching was 200-220 μm in length and 130-150 μm in width.

TABLE 6

COMPARISON OF THE GROWTH OF SALMON EMBRYOS

INCUBATED AT THREE TEMPERATURES

EXPERIMENT 1

TREATMENT	GROWTH MODEL	N	r	F	df	p
8°	y = 0.22x - 2.07	17	0.99			
10°	y = 0.32x - 6.32	10	0.99	11.32	2,30	<0.005
15°	y = 0.40x - 9.06	12	0.99			

x = age in dpf, y = length in mm.

8°	y = 0.03x + 5.22	17	0.99			
10°	y = 0.03x + 5.05	10	0.99	3.14	2,30	<0.10
15°	y = 0.03x + 6.37	12	0.99			

x = age in degree days, y = length in mm.

Plate 3.

Otolith development in salmon embryos

a: 48 dpf (120 degdays),   8.71mm,   0 rings
b: 51 dpf (150 degdays),   9.60mm,   1 ring
c: 53 dpf (195 degdays), 12.32mm,   4 rings
d: 73 dpf (170 degdays), 11.50mm,   9 rings
e: 81 dpf (368 degdays), 15.20mm, 20 rings
f: 81 dpf (434 degdays), 20.00mm, 30 rings

scale bar = 20μm

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f: 81 dpf (434 degdays), 20.00mm, 30 rings

scale bar = 20μm
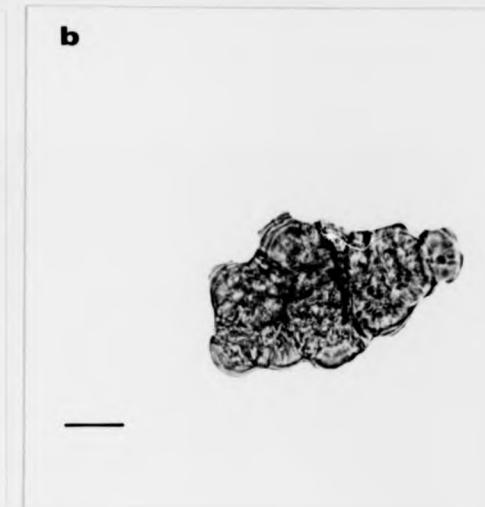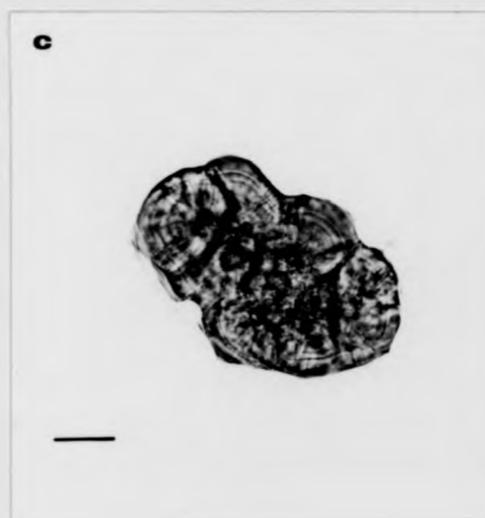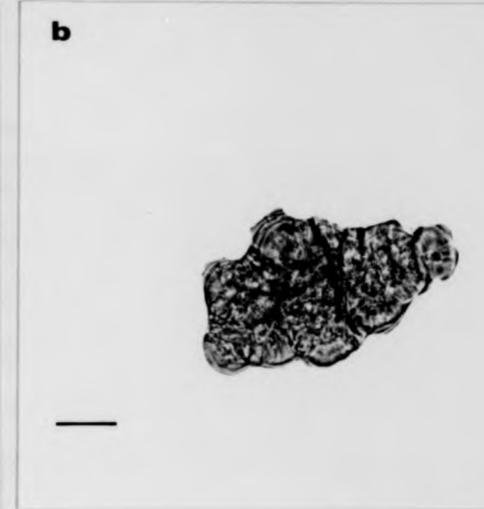
Ring deposition

The ring deposition rates of salmon embryos between 48 and 81 dpf ranged from 0.49 to 0.92 rings/day, depending on incubation temperature. Ring deposition varied significantly between embryos incubated at 8° and 10^OC in Experiment 1 (see Table 7). The otoliths of the few larvae hatched at 15^OC were well developed, but the rings were occluded and difficult to read and the results could not be included in the analysis. In the first five days of the experiment, ring deposition at 15° was similar to that at 8^OC. The rate of ring deposition was not directly related to temperature since a comparison of ring deposition by degday still showed differences between the treatments, although the significance of the difference was slightly lower using degdays (ring/degday) (Table 7).

The combined effects of light cycle and temperature accounted for differences in ring number in the different treatments in Experiment 2. Embryos in the experiment deposited between 1 and 2.3 rings/day. The effect of light regime was greater than that of temperature (see Table 8). The rate of ring deposition in embryos incubated under 6L/6D was 0.2-1.7 times greater than those held under 12L/12D or 24D at 8° and 10°. At 15^OC, light regime had the least affect on ring deposition (Table 8, Fig. 14).

Ring deposition was not completely controlled by light cycle. In only one case (10^OC) the double light cycle (6L/6D) came close to doubling the number of rings deposited. Fitting the data from all the treatments to a linear regression model showed that ring number was better correlated with embryo age ($r = 0.97$) than with length ($r = 0.88$). Calculating ring deposition rates for all the embryos in all treatments on the basis of degday rather than dpf gave only a slightly better fit ($r = 0.98$).

TABLE 7

COMPARISON OF RING DEPOSITION RATES OF SALMON EMBRYOS

INCUBATED AT THREE TEMPERATURES

EXPERIMENT 1

TREATMENT	DEPOSITION MODEL (for ages > 50 dpf)	r	F	df	p
8°	$y = 0.64x - 32.47$	0.99			
10°	$y = 0.92x - 46.07$	0.99	9.37	2,30	<0.001
15°	$y = 0.50x - 23.98$	0.69			

x = age in days pf, y = no. of rings.

TREATMENT	DEPOSITION MODEL	r	F	df	p
8°	$y = 0.08x - 11.66$	0.99			
10°	$y = 0.09x - 13.69$	0.99	6.42	2,30	<0.005
15°	$y = 0.03x - 4.53$	0.69			

x = age in degdays, y = no. of rings.

TABLE 8

COMPARISON OF RING DEPOSITION RATES OF SALMON EMBRYOS
INCUBATED AT THREE TEMPERATURES AND THREE LIGHT REGIMES
EXPERIMENT 2

LIGHT/TEMP	8°	10°	15°	
24D	$y = 1.72x - 117.42$	$y = 1.8x - 128.25$	$y = 2.23x - 152.29$	
	$r = 0.99$	$r = 0.98$	$r = 0.98$	
12L/12D	$y = 1.80x - 122.46$	$y = 1.17x - 77.53$	$y = 2.28x - 154.50$	x = age in dpf
	$r = 0.97$	$r = 0.88$	$r = 0.89$	y = no. of rings
6L/6D	$y = 2.3329x - 161.96$	$y = 2.04x - 139.62$	$y = 2.18x - 147.46$	
	$r = 0.98$	$r = 0.98$	$r = 0.93$	

Effects	F	df	p<
Temperature	9.31	2,18	0.005
Light	12.58	2,18	0.001
Interactions	3.74	4,18	0.01

Fig. 14 : Ring deposition in salmon embryos in Experiment 2.
Points represent the mean ring number ± 1 sd for
each sample. Samples which contained only one
individual are represented by single points.

■━━━━━■ 24 D
▲━━━━━▲ 12L/12D
●━━━━━● 6L/6D

NUMBER
OF
RINGS
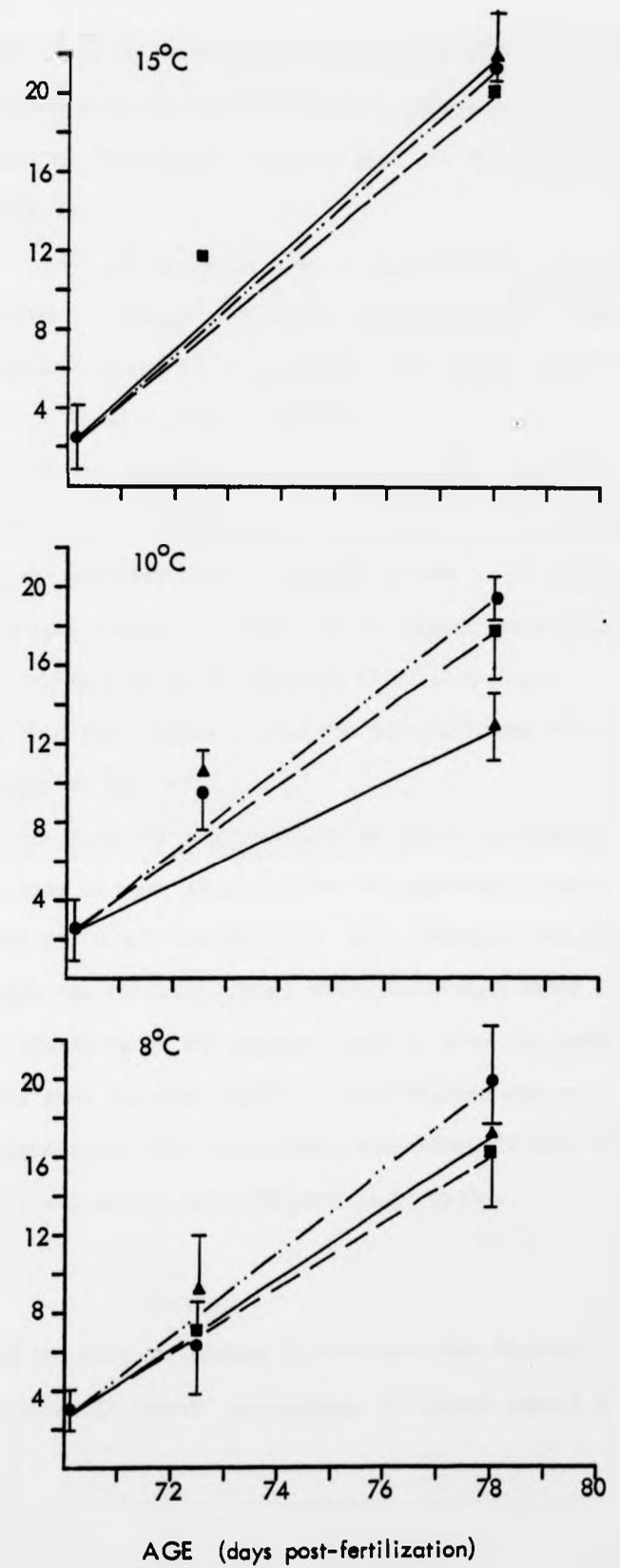
AGE (days post-fertilization)

Respiration studies

The respiration rates of salmon eggs incubated at $8^{\circ}C$ under three different light regimes were compared in order to relate periods of high oxygen consumption, reflecting growth or physical activity, with ring deposition patterns.

Eggs incubated under 24D showed a peak in oxygen consumption of 180 $\mu\ell$ $O_2/\mu g/h$ at 1200 h. Oxygen consumption dropped by 1600 h and remained fairly constant around 50 $\mu\ell$ $O_2/\mu g/hr$. The lowest level of oxygen consumption was 15 $\mu\ell$ $O_2/\mu g/h$ at 0400 h.

Under 12L/12D, oxygen consumption was at its highest level at 0600 h, 3 hours before the lights came on. At lights on the consumption level dropped and remained fairly constant around 40 $\mu\ell$ $O_2/\mu g/h$.

The level of oxygen consumption under 6L/6D dropped quickly at 0800h (one hour before lights on) to the minimum of 20 $\mu\ell$ $O_2/\mu g/h$. Oxygen consumption then rose steadily over the next 20 hours to a peak of 105 $\mu\ell$ $O_2/\mu g/h$ at 0600 h.

The effect of the cyclical light regimes on oxygen consumption was to shift the timing of peak activity from the midmorning under 24D to predawn under 6L/6D and 12L/12D (Fig. 15). Overall, the level of oxygen consumption was slightly higher under 6L/6D than under 24D or 12L/12D. The differences in the average level of activity between the three treatments show the same trend as their differences in patterns of ring deposition, both ring number and metabolic rate were increased in embryos exposed to more frequent light cycles.

Herring

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Fig. 15 : Oxygen consumption of salmon embryos incubated at 8°C under different light regimes, measured over a 24h period. Arrows indicate the beginning of each light (open) and dark (filled) period.

Dunstaffnage Laboratory in 1980 and 1981 (two 120 ℓ tanks and one 500 ℓ tank), large plastic bags in Loch Ewe, and large ponds in Norway; 2) manipulation experiments on larvae from the laboratory stock tanks; 3) wild populations sampled from the Firth of Clyde in 1980 and 1981 and from the Minch in 1980; 4) ageing and predictions of growth rates of wild larvae; 5) periodicity in ring deposition and the relationship between ring width and larval growth.

These results were based on the examination of otoliths from 172 laboratory reared larvae from the stock tanks, 165 larvae from manipulation experiments, 24 larvae from the Loch Ewe bags, 39 larvae from the Norway pond, and 399 wild larvae from the Clyde and the Minch. Out of these otoliths, nine were discarded as unreadable from the laboratory stock tanks, two from the manipulation experiments, eight from the Loch Ewe bags, 12 from the Norway ponds, and 11 from the wild larvae.

Survival of reared herring larvae from hatching to metamorphosis was estimated at 1% in the Dunstaffnage laboratory tanks, except in 1980 where a rapid increase in mortality after day 50 led to the termination of the larval season by day 78, 14% in the Loch Ewe bags (J. Gamble, 1981), and 10-11% on the Norway pond (Ellersten *et al.*, 1975).

The early development of the otolith was examined only in larvae from the Dunstaffnage laboratory tanks, but the pattern is likely to be similar for all the herring larvae examined. Two pairs of otoliths were present in herring embryos from 10 days pre-hatching. At hatching the otoliths were spherical, with a radius of 9-10.8 μm (Plate 4). The first feeding ring was prominent in most larvae (reared and wild) at a radius of 10.8-11.7 μm and coincided with

Plate 4.

Otolith development in herring larvae

a: 10 days old, 14.20mm, 2 rings
b: 16 days old, 13.62mm, 4 rings
c: 33 days old, 21.31mm, 15 rings
d: 52 days old, 29.64mm, 43 rings
e: 95 days old, 35.00mm, 87 rings
f: 99 days old, 45.00mm, 90 rings
    scale bar = 20μm
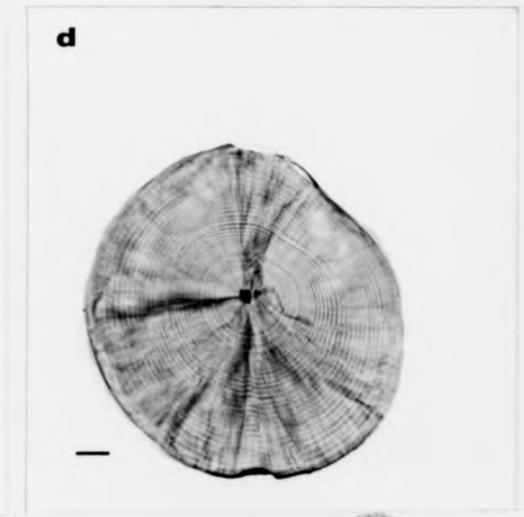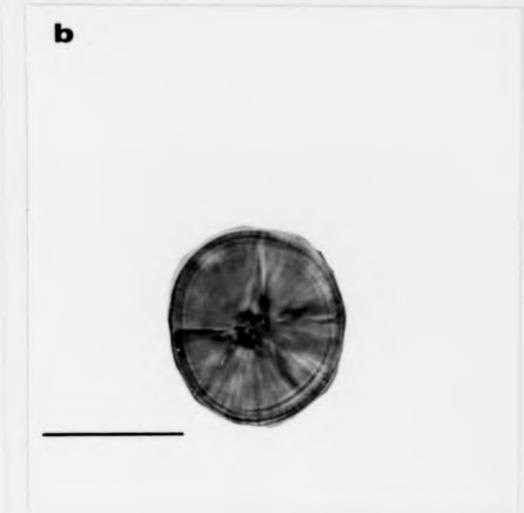
Plate 4.

Otolith development in herring larvae

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e: 95 days old, 35.00mm, 87 rings
f: 99 days old, 45.00mm, 90 rings
      scale bar = 20μm
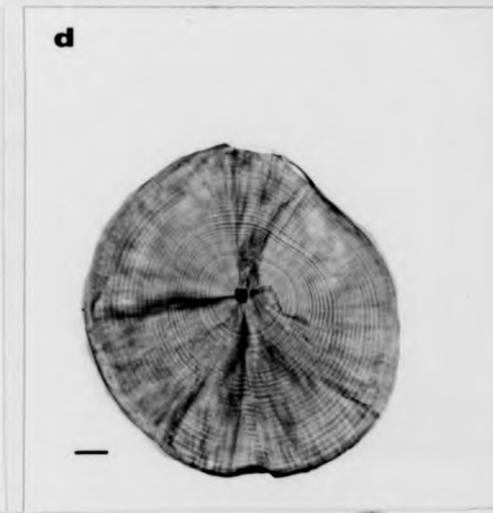
yolk sac absorption. The otoliths became hemispherical with a radius of 25-45 μm when the larvae reached 15-20 mm in length. In larvae >25 mm the otoliths began to elongate into an oval shape until they resembled the adult otoliths in metamorphosing larvae >30 mm. The radius from the nucleus to the posterior edge of these otoliths ranged from 140-240 μm.

Stock populations

Growth: Herring larvae reared in the spring of 1980 in a 120 ℓ tank were on average 8.92 ± 1.00 mm in length at hatching and grew to 17.53 ± 1.75 mm by day 58. In spring 1981 larvae hatched at an average length of 8.86 ± 0.09 mm. Larvae reared in the 120 ℓ tank reached 21.55 ± 4.08 mm by day 95, while those reared in the 500 ℓ tank were 30.28 ± 5.90 mm at the same age. The larvae obtained from the Loch Ewe bags were 9.24 ± 0.71 mm at hatching and 26.37 ± 1.06 mm at day 52. The samples made available from the large ponds at Flødevigen, Norway, contained 10 day old larvae of 13.13 ± 0.71 mm and 99 day old larvae of 48.10 ± 3.83 mm.

Larval growth in the five stock populations was fitted to linear models (Fig. 16):

1980	120 ℓ tank	$y = 0.12x + 10.26$	$r = 0.84$	$n = 95$
1981	120 ℓ tank	$y = 0.12x + 10.07$	$r = 0.89$	$n = 89$
1981	500 ℓ tank	$y = 0.22x + 9.11$	$r = 0.91$	$n = 104$
Loch Ewe bags		$y = 0.29x + 9.44$	$r = 0.84$	$n = 24$
Norway ponds		$y = 0.42x + 9.48$	$r = 0.96$	$n = 31$

where x = age in days, and y = length in mm.

An exponential growth model gave a slightly better fit for the data from the Loch Ewe bags ($y = a + be^x$, $r = 0.88$), but the linear model was used for comparisons with the other populations.

Fig. 16 : Growth of herring larvae in the five stock populations. Points represent individual larvae.
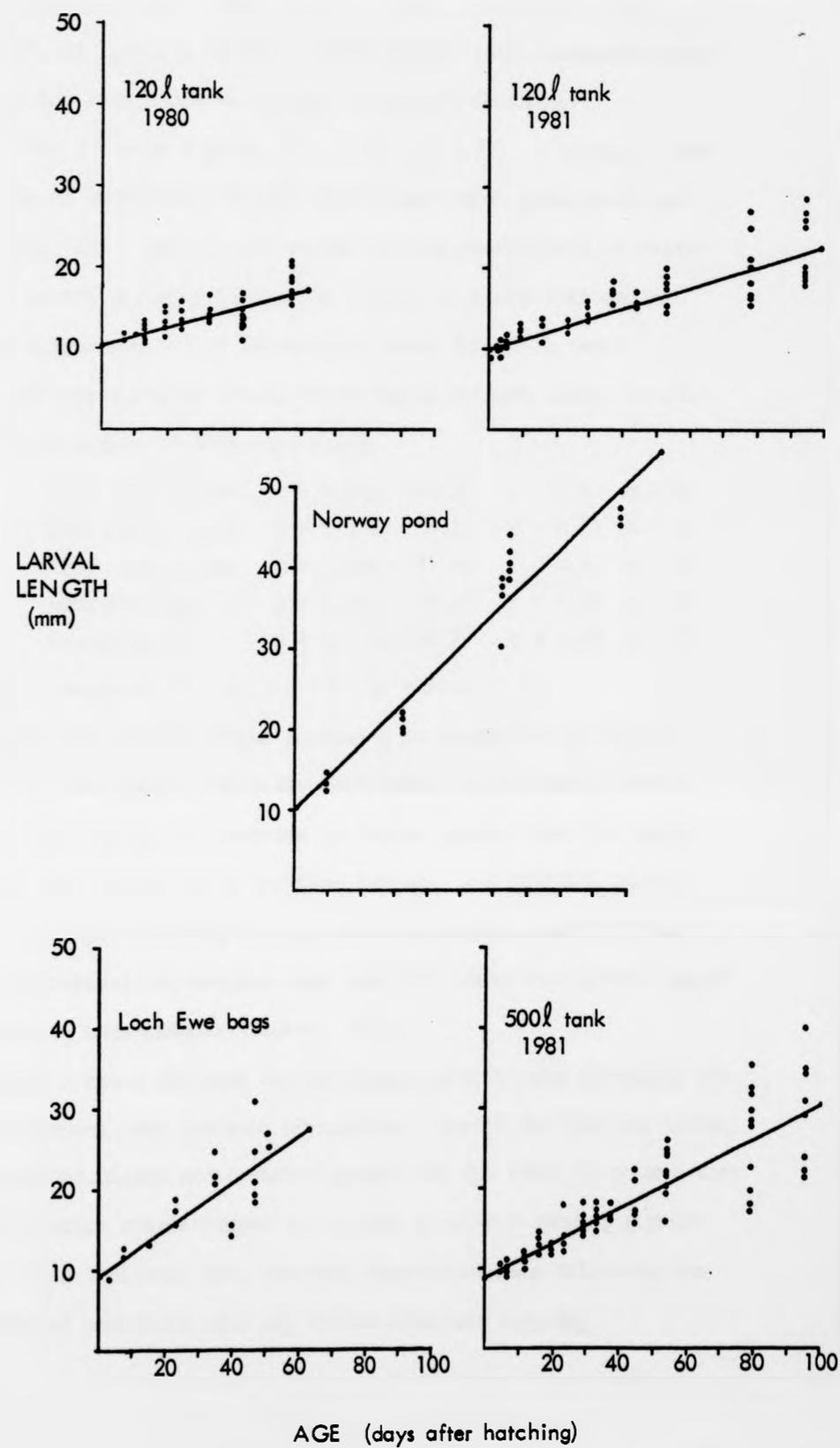
There was little difference in the growth rates of the slowest growing larvae, those reared in 120 ℓ tanks in 1980 and 1981 (F = 0.63, df 1,182, p <0.50). Larval growth rate increased significantly for each increase in size of rearing container: 120 ℓ ⟶ 500 ℓ ⟶ bags ⟶ ponds (F = 43.98, df 4,339, p <0.001). The variation in individual lengths within each stock population was large (Fig. 17). The highest values for the coefficient of variation in length occurred in the 500 ℓ tank, although this may be because larger samples of larvae were taken from this tank.

There was a strong linear relationship between larval length and otolith radius in each population:

1980	120 ℓ tank	y = 5.95x - 63.25	r = 0.83	n = 41
1981	120 ℓ tank	y = 5.51x - 54.11	r = 0.93	n = 49
1981	500 ℓ tank	y = 7.19x - 81.50	r = 0.96	n = 82
Loch Ewe bags		y = 6.35x - 66.93	r = 0.96	n = 24
Norway ponds		y = 11.99x-174.29	r = 0.97	n = 31

where x = length in mm, and y = otolith radius in μm.

Since the otolith radius increased in proportion to larval length, it was possible to obtain estimates of individual growth rate by calculating the increase in larval length from the length at first ring formation to the time sampled, and dividing by the number of rings, assuming a ring deposition rate of one ring/day. The GM functional regressions were used for predicting larval length from otolith measurements (Ricker, 1975).

Table 9 shows the mean backcalculated growth rate alongside the observed growth rate for each population. Except for the bag larvae, where backcalculated and observed growth was the same, it seemed that backcalculation overestimated the growth in slower growing populations. This indicates that the ring deposition rate following the formation of the first ring may differ from one ring/day.
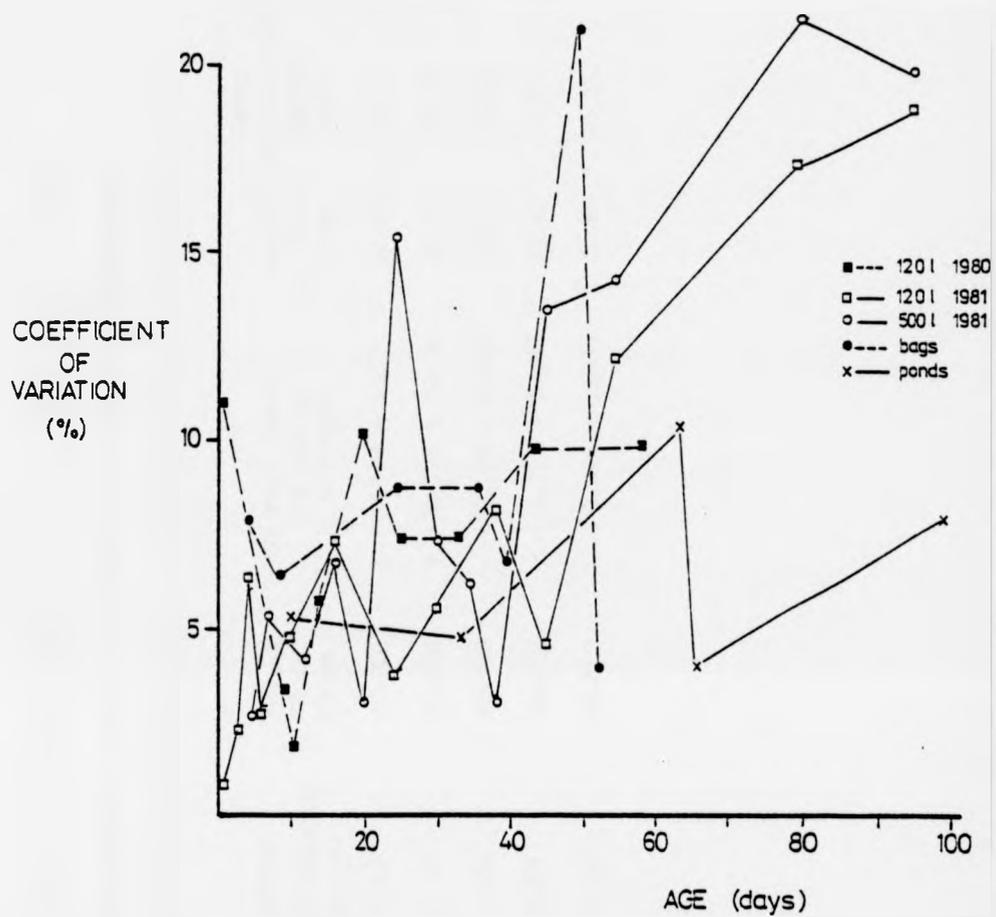
Fig. 17 : Individual variation in length of herring larvae in each
stock population, measured by the coefficient of variation.
Points represent the coefficient of variation of the mean
length for each sample.

TABLE 9

COMPARISON OF THE MEASURED AND BACKCALCULATED GROWTH RATES

IN THE HERRING STOCK POPULATIONS, GIVING THE GM REGRESSION

FUNCTION FOR BACKCALCULATING LENGTH AT FIRST RING FORMATION.

	GROWTH measured directly (mm/day)	90% CF for slope	GM reg. funct. x = radius y = length	length at first ring	MEAN BACKCALCULATED GROWTH RATE	1 sd
1980 120 ℓ tank	0.12	±0.001	y = 0.14x + 11.33	12.83	0.35	0.013
1981 120 ℓ tank	0.12	±0.001	y = 0.17x + 10.30	12.11	0.29	0.012
1981 500 ℓ tank	0.22	±0.001	y = 0.13x + 11.60	13.05	0.25	0.008
Loch Ewe bags	0.29	±0.004	y = 0.15x + 10.86	12.49	0.29	0.012
Norway ponds	0.42	±0.001	y = 0.07x + 16.91	17.69	0.35	0.013

Ring deposition: Herring larvae did not deposit any otolith rings
before hatching. Yolk sac rings were rare in all larvae except those
reared in the 500 l tank where 2-5 yolk sac rings were common.

Ring deposition proceeded differently in each stock population:
1) 120 l tank, 1980. There were 2-4 regular rings deposited by day 8
on the otoliths of larvae reared in 1980. Larvae with >10 rings were
not observed until after day 30. The maximum number of rings obser-
ved was 62 in the largest larva surviving to day 78.
2) 120 l tank, 1981. The first otolith rings were observed in 1 and
3 day old larvae in the 120 l tank, but it is likely that these were
actually very heavy yolk sac rings. The second regular ring was
deposited by day 16 and a maximum of 61 rings was observed at day 95
in these larvae.
3) 500 l tank, 1981. On day 5, a single larva from the 500 l tank
had deposited 1 regular ring. Half of the larvae sampled on day 7,
one day after the observed onset of feeding, had 1 regular ring and
further rings were deposited by day 12. At 95 days a maximum of 91
rings was observed.
4) Loch Ewe bags. The first otolith ring was laid down by day 4 in
larvae reared in the Loch Ewe bags, and 3 rings had been deposited
by day 8. A maximum of 46 rings was observed at day 48.
5) Norway pond. The 10 day old larvae reared in the Norway ponds
had 2-3 rings and by day 99 had deposited a maximum of 85 rings.

The individual variation in ring number for larvae of the same
age was very large, but the data could be best fitted to a linear
regression model (Fig. 18):

Fig. 18 : Ring deposition in each of the stock populations
of herring larvae. Points represent individual
larvae.
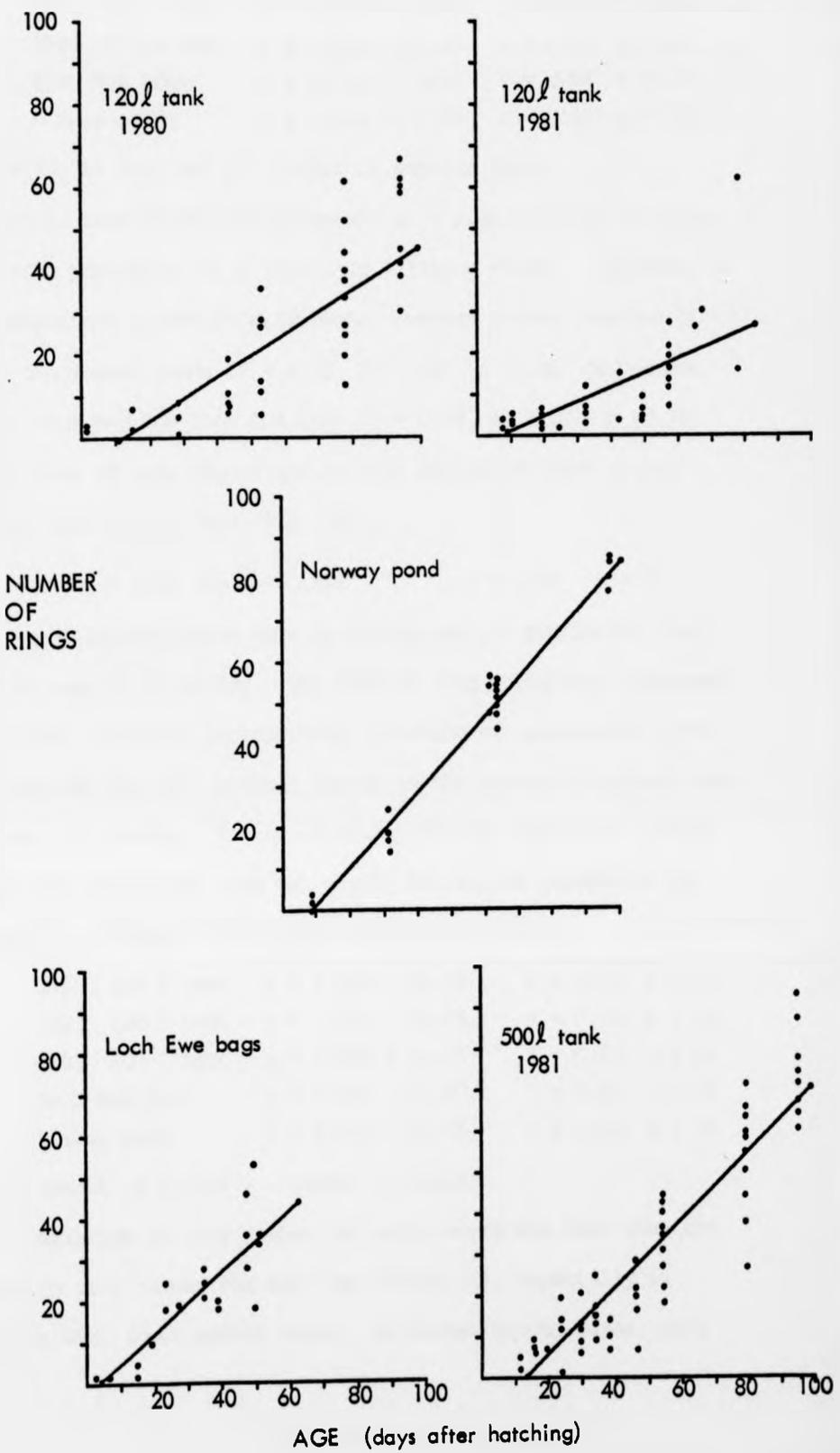
1980	120 ℓ tank	$y = 0.34x - 2.59$	$r = 0.72$	$n = 41$
1981	120 ℓ tank	$y = 0.53x - 5.74$	$r = 0.86$	$n = 49$
1981	500 ℓ tank	$y = 0.83x - 10.54$	$r = 0.94$	$n = 82$
Loch Ewe bags		$y = 0.74x - 2.03$	$r = 0.94$	$n = 24$
Norway ponds		$y = 0.92x - 7.84$	$r = 0.99$	$n = 31$

where x = age in days and y = number of regular rings.

Overall, ring deposition proceeded at a significantly different rate in each population (F = 17.93, df 4,221; p <0.001). However, a closer comparison showed no differences between larvae from the 500 ℓ tank and the Norway ponds (F = 2.41, df 1,110, p <0.25) or between the 500 ℓ tank and the Loch Ewe bags (F = 0.96, df 1,103, p <0.50).

The rates of ring deposition in each population were highly correlated with growth rate (Fig. 19):

$$\log y = 0.64 \log x + 0.238 \qquad r = 0.88 \quad n = 5$$

where x = population growth rate in mm/day and y = population ring deposition rate in rings/day. The rate of ring deposition increased steadily with increased growth rates, reaching an asymptotic level of one ring/day for fast growing larvae as the population growth rate approached 0.40 mm/day. The relationship between population growth rate and ring deposition rate was partly due to the dependence of individual ring number on individual length (Fig. 20):

1980	120 ℓ tank	$y = 2.33x - 25.32$	$r = 0.93$	$n = 41$
1981	120 ℓ tank	$y = 3.79x - 41.55$	$r = 0.94$	$n = 49$
1981	500 ℓ tank	$y = 3.26x - 36.07$	$r = 0.93$	$n = 82$
Loch Ewe bags		$y = 2.09x - 17.87$	$r = 0.92$	$n = 24$
Norway pond		$y = 2.05x - 24.15$	$r = 0.96$	$n = 31$

where x = length in mm and y = number of rings.

The variation in ring number for each length was less than the variation in ring number for each age (Table 10), especially in populations with lower growth rates. At higher growth rates, ring
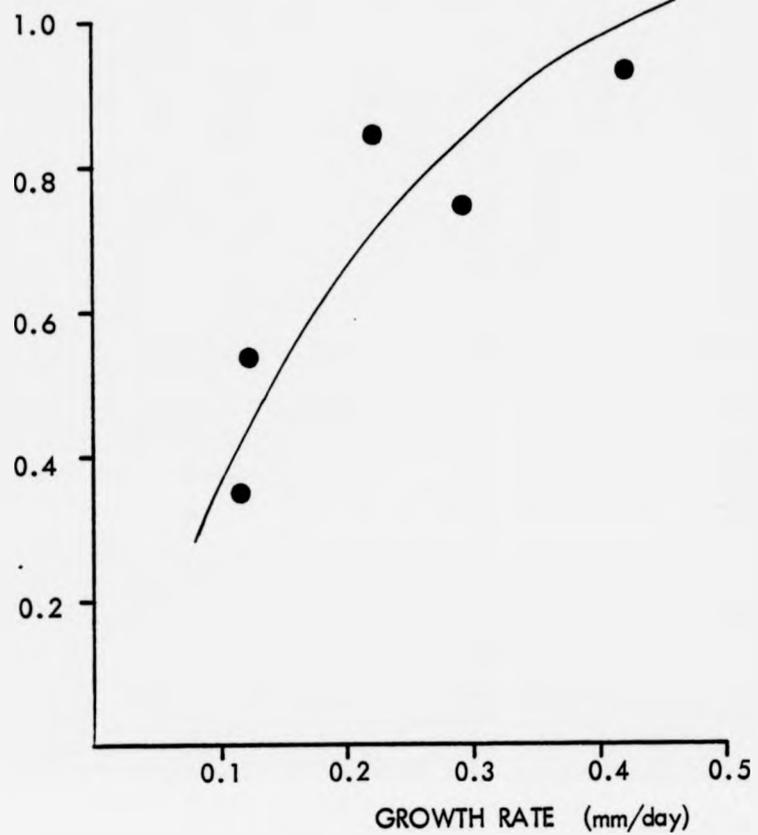
Fig. 19: The relationship between growth rate and ring deposition rate in herring larvae. Points represent the growth and ring deposition rates for each stock population.

Fig. 20 : The relationship between larval length and number
of rings in herring larvae for each stock population.
Points represent individual larvae.
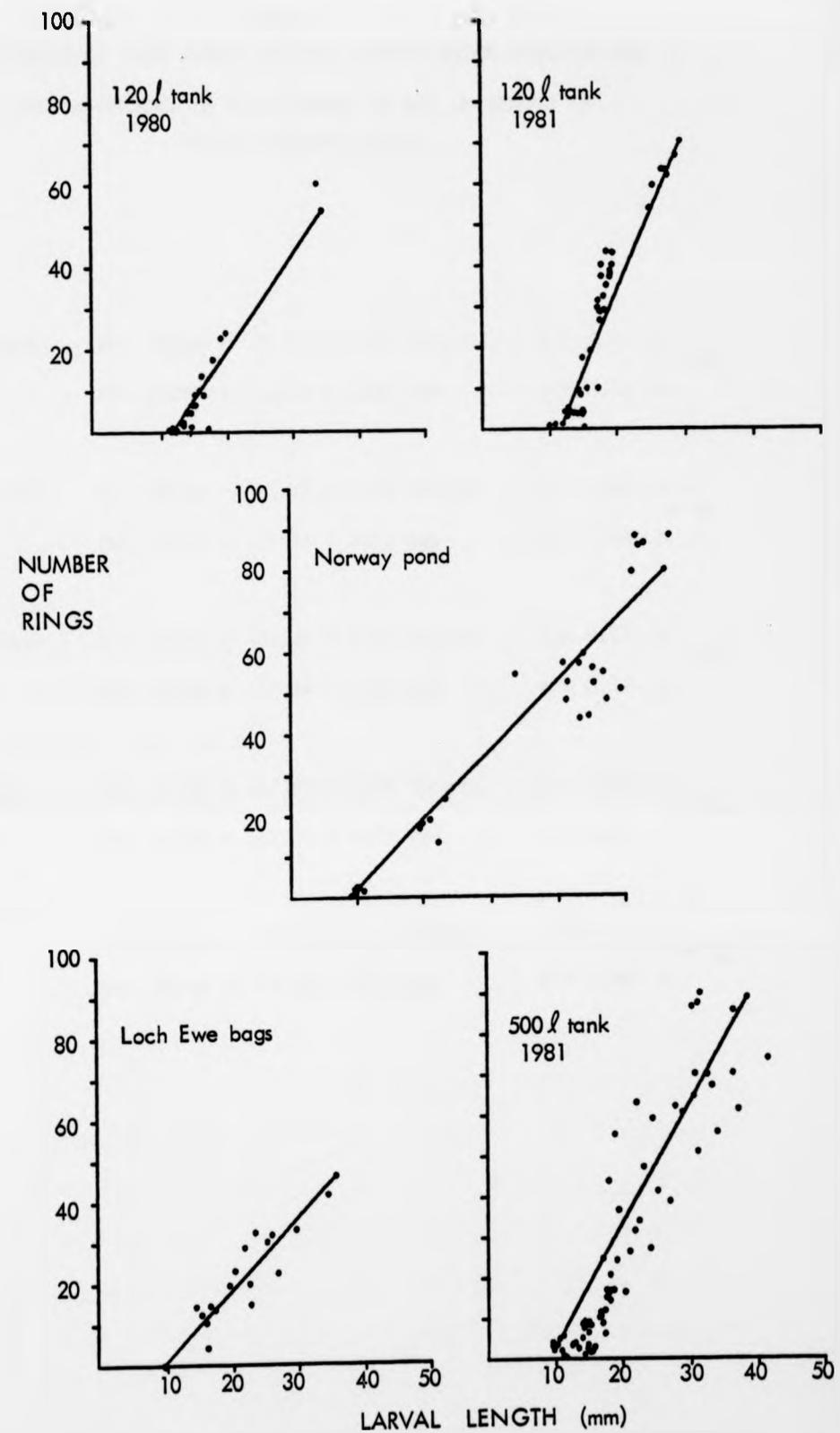
TABLE 10

COMPARISON OF THE STRENGTH OF AGE OR LENGTH AS A
PREDICTOR OF RING NUMBER IN THE HERRING STOCK POPULATIONS.

THE DEPENDENCE OF RING NUMBER ON AGE OR LENGTH IN
REARED HERRING LARVAE.

120 ℓ tank	no. rings = -25.32 + 2.33 length	r = 0.93	n = 41
1980	no. rings = -2.59 + 0.34 age	r = 0.72	n
120 ℓ tank	no. rings = -41.55 + 3.79 length	r = 0.94	n = 49
1981	no. rings = -5.74 + 0.53 age	r = 0.86	n
500 ℓ tank	no. rings = -36.07 + 3.27 length	r = 0.93	n = 82
1981	no. rings = -10.54 + 0.83 age	r = 0.94	n
Loch Ewe	no. rings = -17.87 + 2.09 length	r = 0.92	n = 24
bags	no. rings = -2.03 + 0.74 age	r = 0.94	n
Norway	no. rings = -24.15 + 2.05 length	r = 0.96	n = 31
basin	no. rings = -7.84 + 0.92 age	r = 0.99	n

deposition approached one ring/day and ring number was slightly better correlated with age than with length.

In order to quantify the influences of age and length on otolith ring number, multiple regression equations were fitted to the herring larvae data:

1980	120 ℓ tank	$y = 2.27x - 0.12z - 19.16$	$r=0.88$ $n=203$
1981	120 ℓ tank	$\} \; y = 3.11x - 0.01z - 33.58$	$r=0.91$ $n=201$
1981	500 ℓ tank		
Loch Ewe bags		$y = 0.99x + 0.44z - 11.33$	$r=0.98$ $n= 30$
Norway pond		$y = 0.95x + 0.96z - 19.15$	$r=0.95$ $n= 40$

where x = length in mm, y = number of rings, and z = age in days.

Larval age was more important in determining ring number in the bag and pond populations (both of which had high growth rates and high ring deposition rates), than in laboratory reared larvae. The increase in the multiple correlation coefficient after the inclusion of age as an independent variable for determining ring number was 3% in the 1980 laboratory tank, 0.6% in the 1981 laboratory tanks, 9.1% in the Loch Ewe bags, and 6% in the Norway ponds. The increase was not significant in any of the populations (1980: $F = 6.12$, df 1,200, $p < 0.25$; 1981: $F = 1.198$, df 1,198, $p < 0.25$; bags: $F = 2.46$, df 1,27, $p < 0.25$; pond: $F = 2.30$, df 1,37, $p < 0.25$).

Manipulation experiments

In 1980, herring larvae were removed from the 120 ℓ stock tank at day 4 and reared until day 23 under conditions of starvation, normal feeding, and delayed feeding. A second group of larvae was taken from the stock tank at day 43 and held for seven days under conditions of 24L, 6L/6D, 24D, and at 18L/6D with and without food. The larvae exposed to 24D were provided with food, but it is unlikely that they could feed effectively in the dark. There was no evidence

of feeding when these larvae were examined at the termination of the experiment. In 1981, prefeeding larvae were removed from the 120 ℓ stock tank at day 6 and reared until day 10 under starvation and delayed feeding conditions. Larvae at day 30 were taken from the 120 ℓ tank and reared at 8°, 10°, and $15^O C$ until day 34. A second group of larvae from the 120 ℓ stock tank were reared at 8°, 10°, and $15^O C$ from day 45 to day 52. Larvae at day 45 were taken from the 500 ℓ stock tank and reared for seven days under conditions of 24L, 6L/6D, and under 18L/6D at normal and low food levels (approximately half the level of food organisms presented to the normal feeding larvae). In general, survival and growth of herring larvae in the six manipulation experiments was poor.

Growth: Starvation and the delay of first feeding did not affect larval length significantly in either 1980 or 1981, although starved larvae were generally smaller (Table 11a). Larval length at the termination of each experiment was also unaffected by photoperiod or food ration in either year (Table 11b). The effect of rearing temperature, tested in two experiments in 1981, on larval growth was unclear. There was an increase in the final mean length of larvae reared at higher temperatures in the first experiment, but the difference was not significant. The final mean lengths of larvae in the second experiment were significantly different in each treatment, but the differences showed no trend in relation to different rearing temperatures (Table 11c).

The length-frequency distributions of the stock population sampled at the initiation of each manipulation experiment and the final population surviving in each treatment is shown in Fig. 21.

TABLE 11

COMPARISON OF THE FINAL LENGTHS OF HERRING LARVAE IN MANIPULATION EXPERIMENTS

YEAR	FINAL AGE	INITIAL LENGTH	TREATMENT	FINAL LENGTH	N	F	df	p
a) Effects of Starvation and Delayed Feeding								
1980	23	7.67± 0.29	starved	11.04 ± 0.58	3			
			fed	12.86 ± 1.68	5	2.47	2,9	<0.25
			fed on day 17	11.60 ± 0.59	4			
1981	10	10.07± 0.24	starved	10.67 ± 0.09	3			
			fed on day 7	11.31 ± 0.18	3	3.50	2,6	<0.10
			fed on day 10	12.16 ± 0.32	3			
b) Effects of Photoperiod and Food Ration								
1980	50	14.22± 1.41	18L/6D, starved	14.75 ± 1.12	4			
			18L/6D, fed	15.22 ± 0.98	4			
			6L/6D, fed	15.50 ± 1.29	3	0.07	5,15	>0.50
			24L, fed	15.12 ± 1.23	4			
			24D, fed	14.19 ± 1.79	5			
1981	52	19.07± 2.58	18L/6D, low food	21.12 ± 3.51	5			
			18L/6D, fed	18.88 ± 1.22	10	1.30	3,30	<0.50
			6L/6D, fed	18.89 ± 2.51	11			
			24L, fed	19.42 ± 2.04	8			

TABLE 11, cont.

c) Effects of Temperature

YEAR	FINAL AGE	INITIAL LENGTH	TREATMENT	FINAL LENGTH	N	F	df	p
1981	34	14.51± 1.87	8°	13.49 ± 2.57	3			
			10°	14.24 ± 2.31	3	0.76	2,4	>0.50
			15°	15.41 ± 2.03	4			
1981	52	15.78± 0.73	8°	16.58 ± 0.95	8			
			10°	14.85 ± 1.04	6	4.44	2,19	<0.05
			15°	16.00 ± 1.89	8			

Fig. 21: The distribution of larval lengths for each treatment at the start and end of each herring larvae manipulation experiment. a) Effects of starvation and delayed feeding. b) Effects of photoperiod and food regime. c) Effects of temperature.

N.B. This figure shows the lengths of all surviving larvae and includes some individuals that were not used for otolith analysis
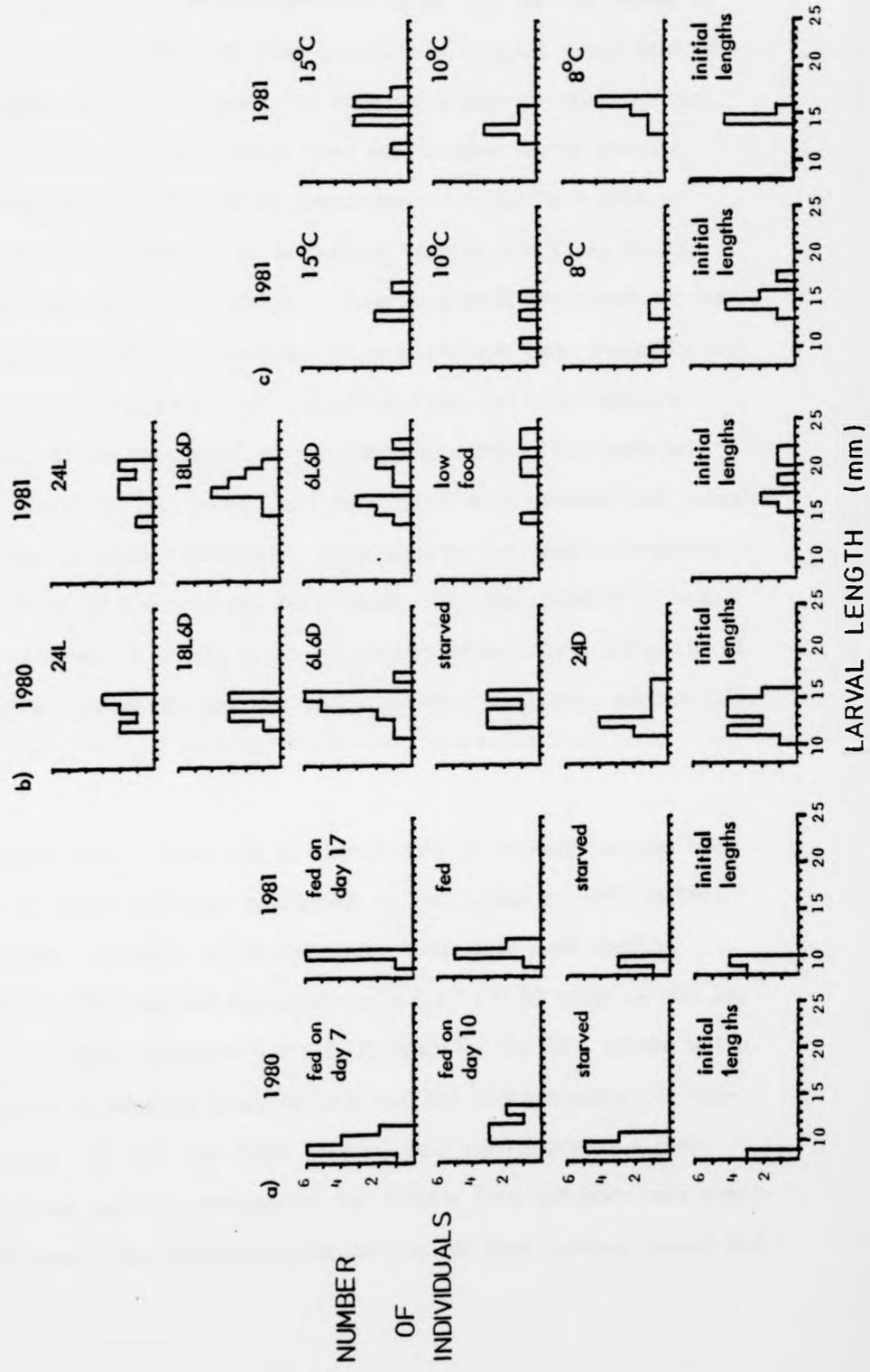
The range of lengths in each treatment observed at the termination
of each experiment was usually similar to the range of lengths of
the original group. The existence of larger and smaller larvae in
the final samples indicates that the initial samples taken from the
stock tanks did not represent the wide variation in length within
the population. It is unlikely that any changes in the length-
frequency distributions of the treatments were due to growth or
shrinkage of the larvae. It is unclear whether the final lengths
observed were due to the effect on growth of each treatment, or due
to selective mortality, which may or may not have been caused by the
experimental conditions. All individuals may not have responded
similarly to the conditions within each treatment. For example,
larvae reared in 1981 under low food supply had a greater mean length
than those in other treatments. Reference to the length-frequency
distribution of the original group shows that the increase in mean
length observed is likely to be the result of selective mortality of
the smaller larvae who may not have been able to compete successfully
for a limited supply of food.

Ring deposition: There was no significant difference between the
number of rings deposited by starved or fed larvae in 1980 or 1981
(Table 12a). Starved larvae had fewer rings than most feeding
larvae, in 1980 they had deposited only 0.67 ± 0.58 rings by day 23,
but in 1981 they deposited 3.0 ± 2.00 rings by day 10. Larvae which
experienced a delay in first feeding had the highest number of oto-
lith rings. In 1980 the first feeding ring was observed in these
larvae after yolk sac absorption, but before food had been made avail-
able to them. The different photoperiods and food rations tested did

TABLE 12

COMPARISON OF THE NUMBER OF RINGS AT TERMINATION OF EACH HERRING LARVAE MANIPULATION EXPERIMENT.

YEAR	FINAL AGE	INITIAL RING No.			TREATMENT	FINAL RING No.			N	F	df	p

a) Effects of starvation and delayed feeding

YEAR	FINAL AGE	INITIAL RING No.			TREATMENT	FINAL RING No.			N	F	df	p
1980	23	0.00	±	0.00	starved	0.67	±	0.58	3			
					fed	2.86	±	3.08	5	2.03	2,9	<0.25
					fed on day 17	3.87	±	0.63	4			
1981	10	1.25	±	1.50	starved	3.00	±	2.00	3			
					fed on day 7	2.00	±	1.73	3	0.46	2,6	>0.50
					fed on day 10	3.33	±	1.53	3			

b) Effects of photoperiod and food ration

| YEAR	FINAL AGE	INITIAL RING No.			TREATMENT	FINAL RING No.			N	F	df	p
1980	50	4.00	±	0.00	18L/6D starved	10.72	±	6.89	4			
					18L/6D fed	8.05	±	3.02	4			
					6L/6D fed	9.30	±	8.74	3	0.33	5,15	>0.50
					24L fed	5.50	±	1.29	4			
					24D fed	9.40	±	7.29	5			
1981	52	19.28	±	8.74	18L/6D low food	30.54	±	15.60	5			
					18L/6D fed	23.66	±	4.68	10			
					6L/6D fed	22.45	±	10.05	11	1.79	3,30	<0.25
					24L fed	25.37	±	6.23	8			

Table 12, cont.

c) Effects of temperature

YEAR	FINAL AGE	INITIAL RING No.	TREATMENT	FINAL RING No.	N	F	df	p
1981	34	13.00 ± 0.30	8°	7.00 ± 1.41	2			
			10°	9.50 ± 8.49	3	1.01	2,4	<0.50
			15°	15.10 ± 5.46	3			
	52	9.94 ± 5.11	8°	8.05 ± 6.52	8			
			10°	5.72 ± 2.76	6	3.93	2,19	<0.05
			15°	16.07 ± 10.08	8			

not affect the number of rings deposited in older larvae in either 1980 or 1981 (Table 12b). Larvae experiencing starvation or low food supply had the highest number of rings. Larvae reared under extreme conditions of 6L/6D and 24D had more rings than larvae reared under long photoperiods with a plentiful food supply. Larvae reared at 15°C had more rings than larvae reared at 10°C or 8°C, though the difference was significant only in the second temperature experiment (Table 12c).

The relationship between ring number and larval length was not altered by any of the manipulation treatments, which suggests that ring number cannot be manipulated independently of length in herring larvae.

Starvation and delayed feeding	1980	$F = 1.55$, df 2,29, $p < 0.25$
	1981	$F = 0.04$, df 2,20, $p > 0.50$
Photoperiod and Food Ration	1980	$F = 1.28$, df 4,19, $p < 0.50$
	1981	$F = 0.16$, df 3,49, $p > 0.50$
First temperature experiment		$F = 0.55$, df 2,20, $p > 0.50$
Second temperature experiment		$F = 0.55$, df 2,33, $p > 0.50$

The average number of rings observed at the termination of each experiment was a reflection of the lengths of the surviving larvae rather than a direct result of manipulation of ring deposition by experimental conditions.

Wild populations

Shrinkage of preserved larvae: In herring larvae, the amount of shrinkage in body length due to handling and preservation was greater in smaller larvae than in larger larvae. The preserved length was altered somewhat by delay in preservation after capture, but the trend was not consistent or significant (Table 13). The shrinkage

TABLE 13

SHRINKAGE IN LENGTH OF HERRING LARVAE DUE TO HANDLING AND PRESERVATION

FRESH LENGTH (mm) x̄ ± SD(N)	TREATMENT	DELAY BEFORE PRESERVATION (min)	PRESERVED LENGTH (mm) x̄ ± SD(N)	% SHRINKAGE	S I G N I F I C A N C E		
					t	df	p
9.20 ± 0.50 (21)	Net recapture (1.0 m Stramin)	0	7.94 ± 1.12 (8)	13.7	effect of delay in preservation:		
		5	7.73 ± 0.91 (15)	15.9	0.49	21	>0.50
		10	7.24 ± 0.68 (7)	21.3	1.49	20	<0.50
		15	9.01 ± 0.36 (6)	2.0	10.33	11	<0.001
					effect of preservation:		
					3.20	27	<0.001
11.94 ± 0.77 (20)	Laboratory simulated net capture	0	10.49 ± 0.57 (9)	12.1	effect of delay in preservation:		
		5	10.69 ± 0.79 (13)	10.5	0.92	10	<0.50
		15	10.99 ± 0.47 (10)	7.9	0.20	21	>0.50
					effect of preservation:		
					7.56	27	<0.001
30.83 ± 0.76 (3)	Field sampling	-	29.33 ± 1.15 (3)	4.9	effect of preservation:		
					2.25	4	<0.10

in yolk sac larvae (9.20 ± 0.50 mm) towed in a 1.0 m stramin net
increased from 14% when preserved immediately after capture, to 21%
when preservation was delayed for 10 min. The amount of shrinkage
after a 15 min delay between capture and preservation dropped to 2%.
In contrast, larvae at 11.94 ± 0.77 mm subjected to simulated net
handling in the laboratory showed a steady decline in the amount of
shrinkage as preservation was delayed longer. Shrinkage in these
larvae was 12% when preserved immediately after 'capture' and drop-
ped to 8% when preservation was delayed for 15 min. The few larger
larvae which were captured alive during field sampling were preserved
within 5 min of capture. The amount of shrinkage in these larvae
was 5%.

Based on these results, the larvae used for growth and otolith
studies were preserved within 5 min of capture. Larval length was
always measured at least one week after preservation (see Methods).
The lengths of alcohol preserved larvae were converted to fresh
lengths as follows:

preserved length (mm)	x	shrinkage factor	=	fresh length (mm)
<9		1.13		<10
9-20		1.10		10-22
20-28		1.06		22-30
>28		1.04		>30

Growth: Herring larvae sampled in the Clyde on 24th May, 1980
ranged from 15 to 25 mm in length (fresh length, calculated from
preserved length and corrected for shrinkage). Assuming that these
larvae hatched during the first week of April, they could be 44-51
days in age at the time sampled. If growth was linear during this
time, these larvae (hatching at 9 mm) grew at a rate of 0.23 mm/day,

taking the mean value of the range in lengths and ages. Autumn-
spawned larvae sampled in the Minch between 2-17 October, 1980 were
between 9 and 20 mm in length. There was a general increase in
length of 6 mm over the 15 days of the cruise, resulting in an
estimated growth rate of 0.40 mm/day. This estimate is only an
approximation since the larvae were sampled over a wide geographical
area and most probably did not belong to the same spawning batch.
The larvae captured during the first of the 1981 sampling trips
(25th April - 1st May, 1981) in the Clyde, ranged from 13 to 22 mm
in length. Reports of spent herring in the Clyde indicated that
hatching could have occurred between the 3rd week in March and the
2nd week in April (C. Dempsey, P. McLaughlin, pers. comm.). These
larvae could have ranged in age from 17 to 42 days by the 1st of May,
at the end of the first sampling trip. Assuming an average hatching
length of 9 mm, growth rate of these larvae could be 0.23-0.31 mm/
day. The Clyde herring larvae sampled on 28th May, 1981 were 22.72-
32.45 mm in length. If these larvae were part of the same population
previously sampled, then they grew an average of 10 mm in the 30 day
period between samples at a rate of 0.33 mm/day.

Ring deposition: In general, the otolith rings of wild larvae were
more distinct and more evenly spaced than in reared larvae. The
number of rings found on the otoliths of larvae collected in 1980
in the Clyde ranged from 5 rings in a 19.00 mm larva to 22 rings in
a 24.00 mm larva. Herring larvae of 8.78 mm and 19.55 mm in the
Minch in 1980 had 1 and 24 otolith rings, respectively. The minimum
ring number observed in 1981 Clyde larvae was 0 in a larva of 15.63 mm,
and a maximum of 55 rings in a 31.20 mm larva.

The larvae sampled in the Clyde in 1981 showed a strong correlation between larval length and the number of otolith rings (Fig. 22). This correlation was not as strong in the Minch 1980 samples and was absent in the Clyde 1980 samples, probably because the range in lengths was much smaller. The data was fitted to a linear regression model:

1980	Clyde	y = 0.22x + 16.49	r = 0.50	n = 84
1980	Minch	y = 0.45x + 10.76	r = 0.78	n = 24
1981	Clyde	y = 0.32x + 16.27	r = 0.94	n =174

where x = number of regular rings and y = length in mm.

The relationship between length and ring number differed significantly in the three populations of wild larvae (F = 4.95, df 2,278, $p < 0.05$).

Ageing and predicted growth rates for wild larvae

The ages of individual wild larvae were calculated from the observed number of otolith rings based on the ring deposition rates measured in the stock populations. Estimated growth curves were constructed using the measured length at the calculated age for each individual (see Fig. 23). These estimated population growth rates for each group of wild larvae varied, as would be predicted from the variation in ring deposition rates among the stock populations. The estimated growth rates for wild herring larvae ranged from 0.07–0.20 mm/day, for larvae caught in 1980 in the Clyde; 0.15–0.41 mm/day for Minch larvae in 1980; and 0.11–0.30 mm/day for Clyde larvae in 1981.

Estimated growth curves were also produced by plotting larval length against number of rings, assuming that the rate of ring deposition was constant at one ring/day throughout the larvae stage

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$$1980 \quad \text{Clyde} \quad y = 0.22x + 16.49 \quad r = 0.50 \quad n = 84$$
$$1980 \quad \text{Minch} \quad y = 0.45x + 10.76 \quad r = 0.78 \quad n = 24$$
$$1981 \quad \text{Clyde} \quad y = 0.32x + 16.27 \quad r = 0.94 \quad n = 174$$

where x = number of regular rings and y = length in mm.

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Fig. 22 : The relationship between larval length and
number of rings in wild herring larvae.
Points represent individual larvae.
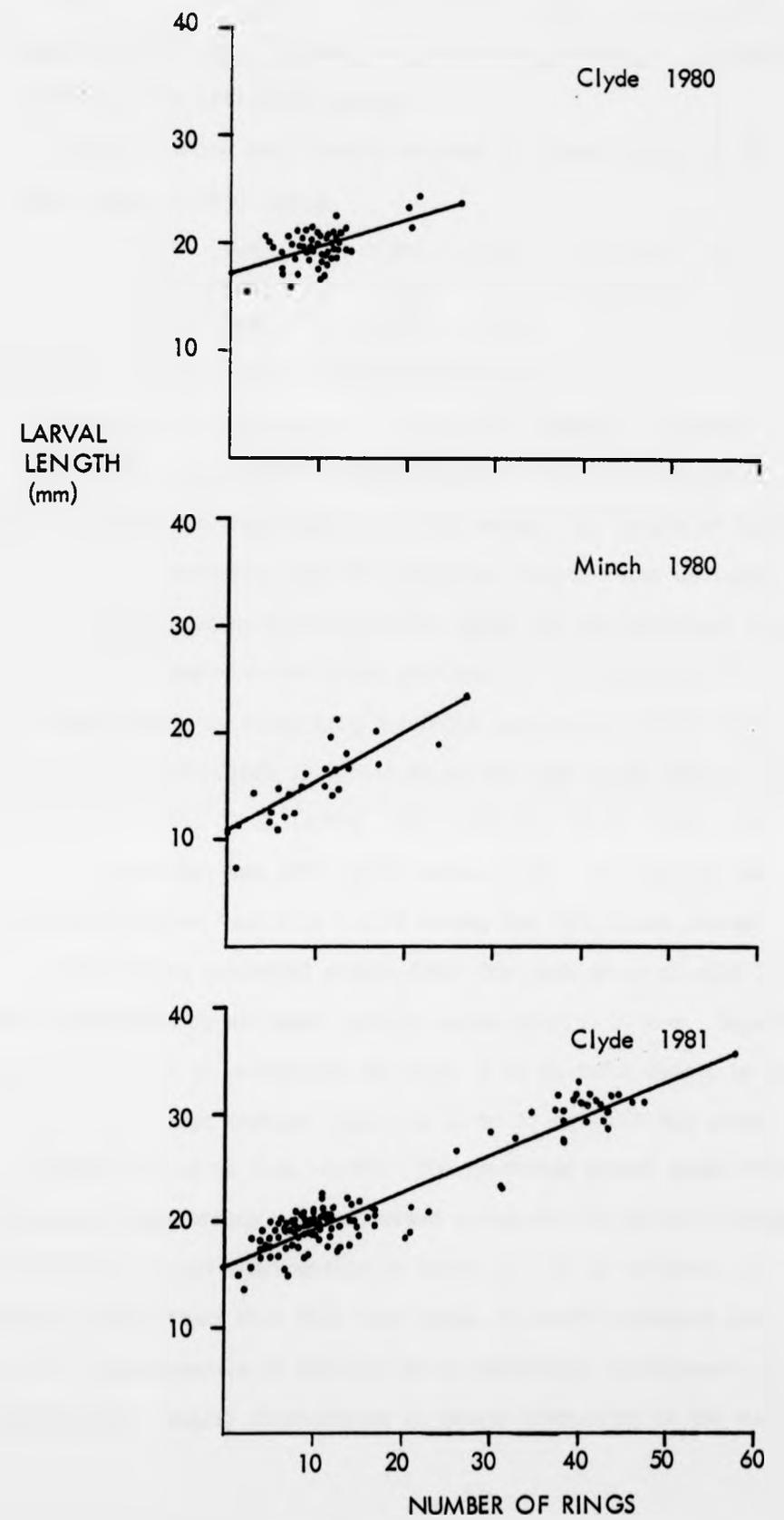
(see Fig. 23). The growth rates estimated in this way were 0.22 mm/day for 1980 Clyde larvae; 0.45 mm/day for 1980 Minch larvae, and 0.32 mm/day for 1981 Clyde larvae.

Otolith radius was linearly related to larval length in the three groups of wild larvae.

1980	Clyde	$y = 2.25x - 15.02$	$r = 0.54$	$n = 84$
1980	Minch	$y = 1.69x - 2.56$	$r = 0.74$	$n = 24$
1981	Clyde	$y = 4.08x - 58.69$	$r = 0.93$	$n = 174$

where x = larval length in mm and y = otolith radius in μm.

The poor correlation in the Clyde 1980 samples is probably a result of the small range in sizes sampled. Because the otolith size increased in proportion to larval length, the length of larvae at first ring formation and the individual growth rates of larvae could be estimated by backcalculation using the GM functional regression, as was done with the stock populations (see Appendix III). The mean lengths at first ring formation estimated in this way were 14.76 mm for 1980 Clyde larvae; 9.85 mm for 1980 Minch larvae; and 17.33 mm for 1981 Clyde larvae. The estimated growth rates were 0.41 ± 0.03 mm/day for 1980 Clyde larvae; 0.64 ± 0.14 mm/day for 1980 Minch larvae, and 0.26 ± 0.01 mm/day for 1981 Clyde larvae.

The various estimated growth rates for each group of wild larvae are represented by estimated growth curves in Fig. 23 a–c. Depending on which method of estimation was used, a 20 mm larva caught in the Clyde in 1981, for example, could be 12 to 35 days old and growing at a rate of 0.11 to 0.64 mm/day. The predicted growth rates for the three wild populations are summarised alongside the growth estimates from direct length measurements in Table 14. It is difficult to quantify the effect that this wide range of growth estimates would have for measurements of herring larval mortality, recruitment, or monitoring of yearly fluctuations in growth conditions in the wild.

Fig. 23 : Predicted growth curves for wild herring larvae.
Growth models were constructed based on the
ring deposition rates of the stock populations
a) 120ℓ tank, 1980. b)120ℓ tank, 1981.
c) 500ℓ tank, 1981. d) Loch Ewe bags.
e)Norway ponds; on the assumption that
f) larval age = number of rings; and by
g) backcalculation.
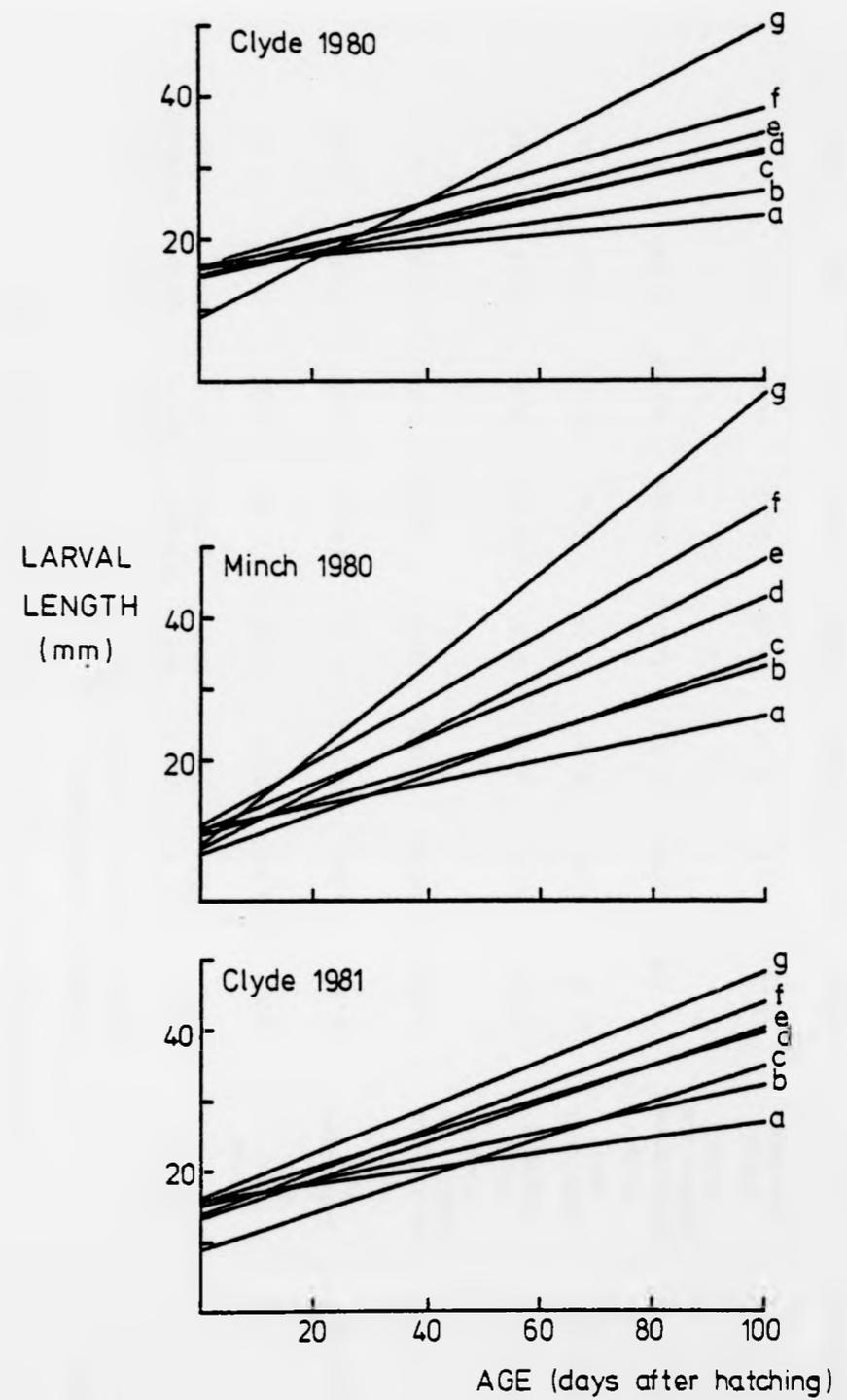
TABLE 14

GROWTH RATES PREDICTED BY SEVERAL METHODS FOR WILD HERRING LARVAE

SOURCE	METHOD OF PREDICTING	Based on Ring Deposition in:					Assuming age = No.of rings	Back-cal-cula-tion	Direct length measure-ment
		120 ℓ 1980	120 ℓ 1981	500 ℓ 1981	Loch Ewe bags	Norway pond			
Clyde 1980	Slope (mm/day)	0.07	0.11	0.18	0.16	0.20	0.22	0.41	0.23
	Intercept (mm)	16.30	15.84	14.59	16.17	14.94	16.49	9.00	9.00
	*90% confidence limits for slope	0.002	0.005	0.009	0.007	0.010	0.012	0.350	-
	Slope (mm/day)	0.16	0.24	0.38	0.33	0.41	0.45	0.64	0.44
Minch 1980	Intercept (mm)	10.35	9.39	6.80	10.08	7.52	10.75	8.00	8.00
	*90% confidence limits for slope	0.004	0.007	0.013	0.011	0.016	0.018	0.260	-
	Slope (mm/day)	0.11	0.17	0.27	0.24	0.30	0.32	0.26	0.29
Clyde 1981	Intercept (mm)	15.98	15.25	13.42	15.78	13.98	16.27	9.00	9.00
	*90% confidence limits for slope	0.001	0.001	0.001	0.001	0.001	0.001	0.40	-

*values for backcalculated predictions are
1 SD of the mean of individual growth rates

Although the growth rates estimated by some of these methods were similar, certain characteristics of larval development could not be predicted correctly with the assumption of daily ring deposition (ring deposition rate of one ring/day). The magnitude of the error can be demonstrated by examining the timing of first ring formation (indicating the age at yolk sac absorption) predicted by these various methods (Table 15, Appendix III).

The assumption that ring number is equal to larval age greatly overestimated the age at yolk sac absorption in the Clyde larvae in 1980 and 1981. These results support the observations in laboratory reared larvae that ring deposition does not begin at hatching in herring larvae.

The age at yolk sac absorption for the Clyde 1981 larvae was consistently overestimated when otolith radius was used to predict length. If the relationship between larval length and otolith radius was not described correctly, or if the larvae sampled were not from the same population, then errors would be introduced into the prediction of length at first ring formation. Overestimates of growth could be the result of such errors. However, if larval length is predicted correctly, an underestimate of growth rate, resulting from ring deposition rates greater than one ring/day, will also lead to errors in calculating the age at yolk sac absorption.

Backcalculated growth rates assume that once ring deposition begins with the first feeding ring, it proceeds at a constant rate of one ring/day. However, the backcalculated growth rates seem to be overestimates of growth in the Clyde 1980 and Minch 1980 populations, and underestimates of growth in the Clyde 1981 larvae. It is reasonable to conclude from this that ring deposition in wild herring

TABLE 15

GROWTH RATE AND AGE AT FIRST RING FORMATION (YOLK SAC ABSORPTION)
PREDICTED BY SEVERAL METHODS FOR WILD HERRING LARVAE

METHOD	Clyde 1980		Minch 1980		Clyde 1981	
	GROWTH RATE	Age at yolk absorption	GROWTH mm/day	yolk sac absorption Age (days)	GROWTH mm/day	yolk sac absorption Age (days)
1. ring = age	0.22	35	0.45	7	0.32	24
2. backcalculation	0.41	14	0.64	3	0.26	32
3. direct length measurement	0.23	11	0.40	5	0.29	28
4. ring deposition rate as determined by growth rate	at 0.66 rings/day	7	at 1.00 rings/day	5	at 0.74 rings/day	10

can differ from one ring/day between yolk sac absorption and the time samples (see Table 15).

It is possible to predict the ring deposition rates of wild larvae from the relationship between population growth rate and ring deposition rate established for the stock populations. If the direct measurements of growth in the wild populations are correct, the predicted ring deposition rates would be 0.66 rings/day in 1980 Clyde larvae, 1.00 ring/day in 1980 Minch larvae, and 0.74 rings/day in 1981 Clyde larvae. In contrast to the previous estimates of age at yolk sac absorption, the growth curves produced using these deposition rates suggest that first ring formation occurred at 7.2 days in Clyde 1980 larvae, at 5.0 days in Minch 1980 larvae, and at 10.5 days in Clyde 1981 larvae.   These figures seem to be more reasonable estimates of the age at yolk sac absorption. Reared herring larvae complete the yolk sac stage 6-10 days after hatching (Ehrlich, 1972; Doyle, 1975). Although temperatures in the rearing tanks are often slightly higher than sea temperatures, the estimates of age at first ring formation seem to be reasonable if based on ring deposition rates of less than one ring/day.

Periodicity in ring deposition and widths of individual increments

Deposition of the most recent otolith increment was not synchronised between individual larvae and was observed to be at various stages of completion (fully-formed, half-formed, or newly-initiated) in individual larvae sampled at any time during the day or night, from laboratory tanks or from the wild (Fig. 24). Although there was no evidence of the action of any environmental cues to initiate the deposition of each band, this does not exclude the possibility that feeding bursts could initiate ring deposition.
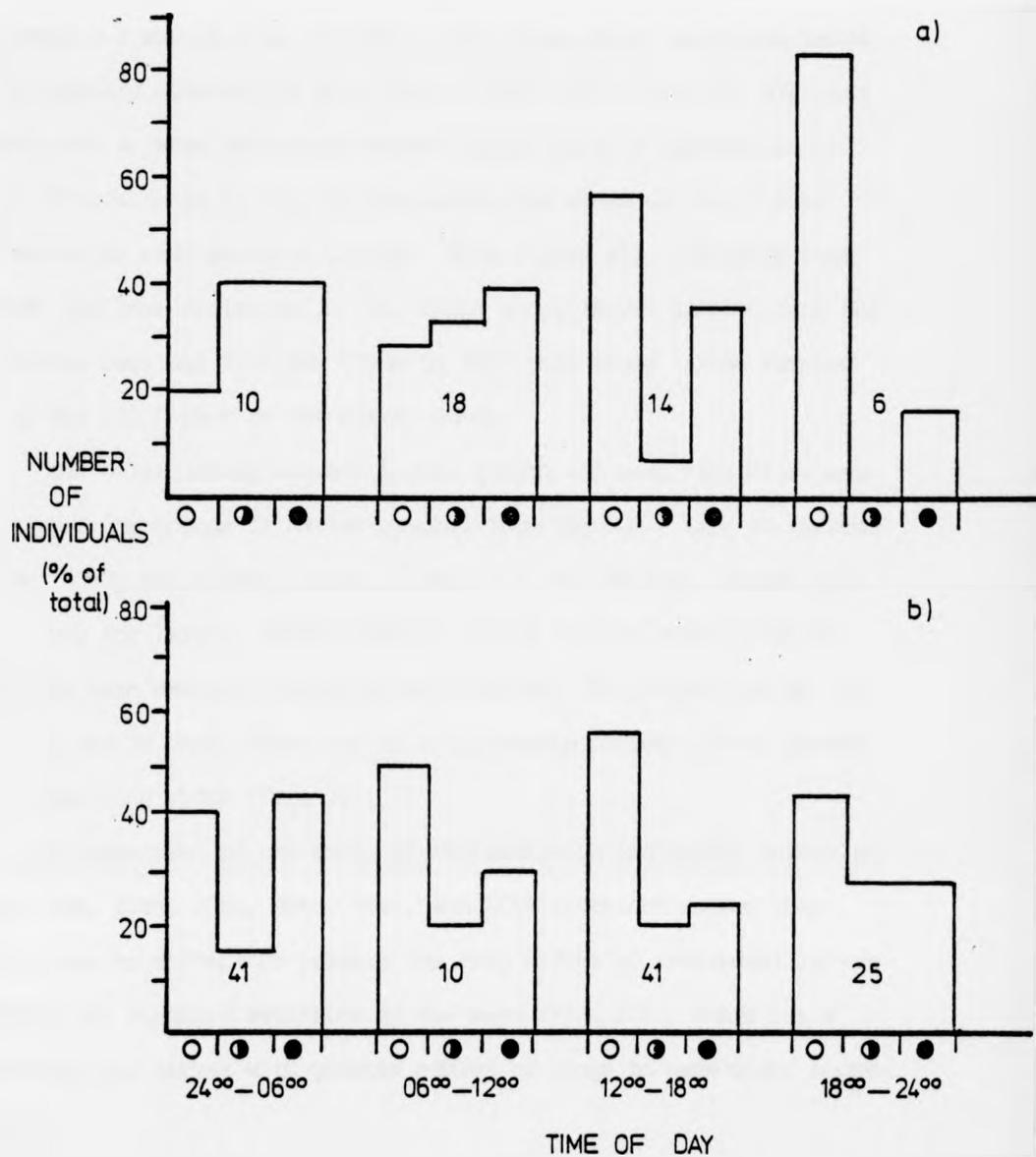
Fig. 24 : Synchronisation of ring deposition in herring larvae.
Distribution of the state of completion of the most recent
increment in individuals sampled over 24h periods.
a) laboratory reared larvae (500ℓ tank, 1981). b) wild larvae
(Clyde 1981). Numbers within each block indicate sample
size. Symbols represent ○ newly-formed, ◑ half-formed,
or ● completed increments.

The individual bands measured on herring larval otoliths were between 0.2 and 14.0 μm in width. The rings of an individual larva were usually constant in size (C.V. = 14.5, SD = 3.24, N = 97), but there was a large variation between individuals as represented by the vertical bars in Fig. 25 indicating the range of ring widths observed in each group of larvae. This figure also indicates that there was less variation in ring width among larvae sampled from the Loch Ewe bags and from the Clyde in 1981 than among larvae sampled from the 500 ℓ tank or the Norway ponds.

The relationship between larval length and mean ring width was examined for groups of larvae sampled from the 500 ℓ tank at various ages. Only the oldest larvae, those at 54 and 79 days, showed any tendency for larger, faster-growing larvae to have wider rings on average than smaller, slower-growing larvae. In younger larvae, at 12, 34 and 38 days, there was no relationship between larval growth and mean ring width (Fig. 26).

A comparison of the width of increments in individual larvae at the 10th, 20th, 30th, 40th, 50th, and 60th increment showed that there was no difference between the ring widths of individual larvae within one standard deviation of the mean (Fig. 27). There was a tendency for larvae with greater number of rings to have wider increments.

Reader error

The counts of larval otolith rings are subject to errors caused by the subjective interpretation of different readers. The main factors which can reduce the variability between readers are experience, discussions with other readers, and standard definitions of rings (Appendix II).

Fig. 25: Individual ring width in typical herring larvae
from four of the stock populations. Vertical
bars indicate the range in the width of selected
increments observed within each population.
a) Loch Ewe bags. b) wild larvae. c) 500ℓ tank, 1981.
d) Norway pond.
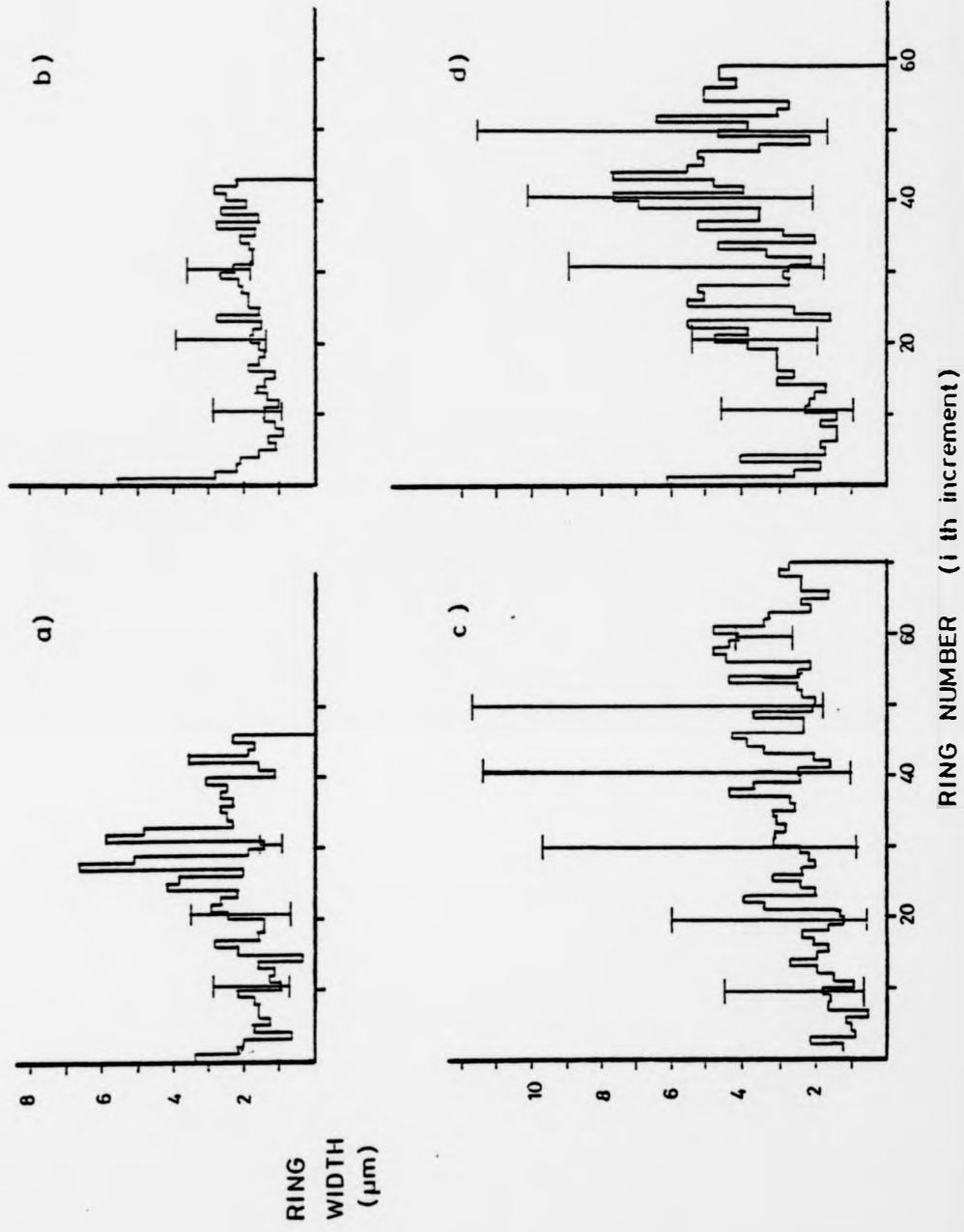
Fig. 26 : The relationship between larval length and mean ring
width in herring larvae of different ages, 500ℓ tank, 1981.

a)   □ -- day 12
      ● -- day 34
      △-- day 38

b)   ▲ -- day 54
      o -- day 79
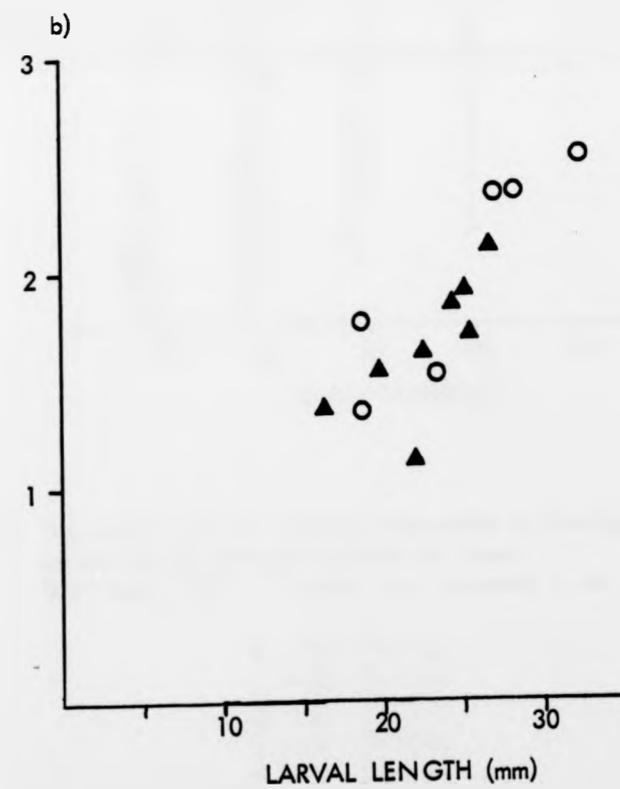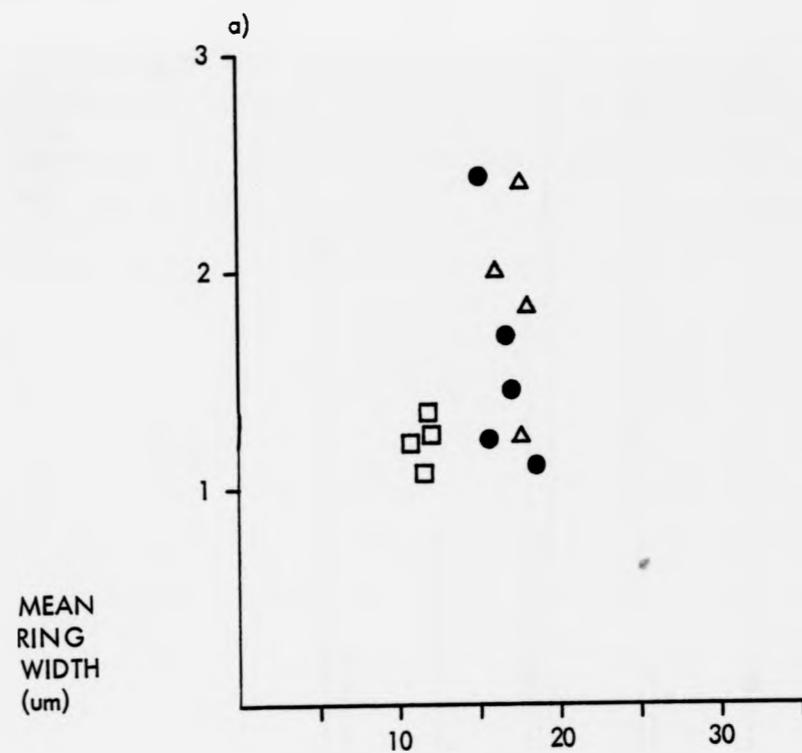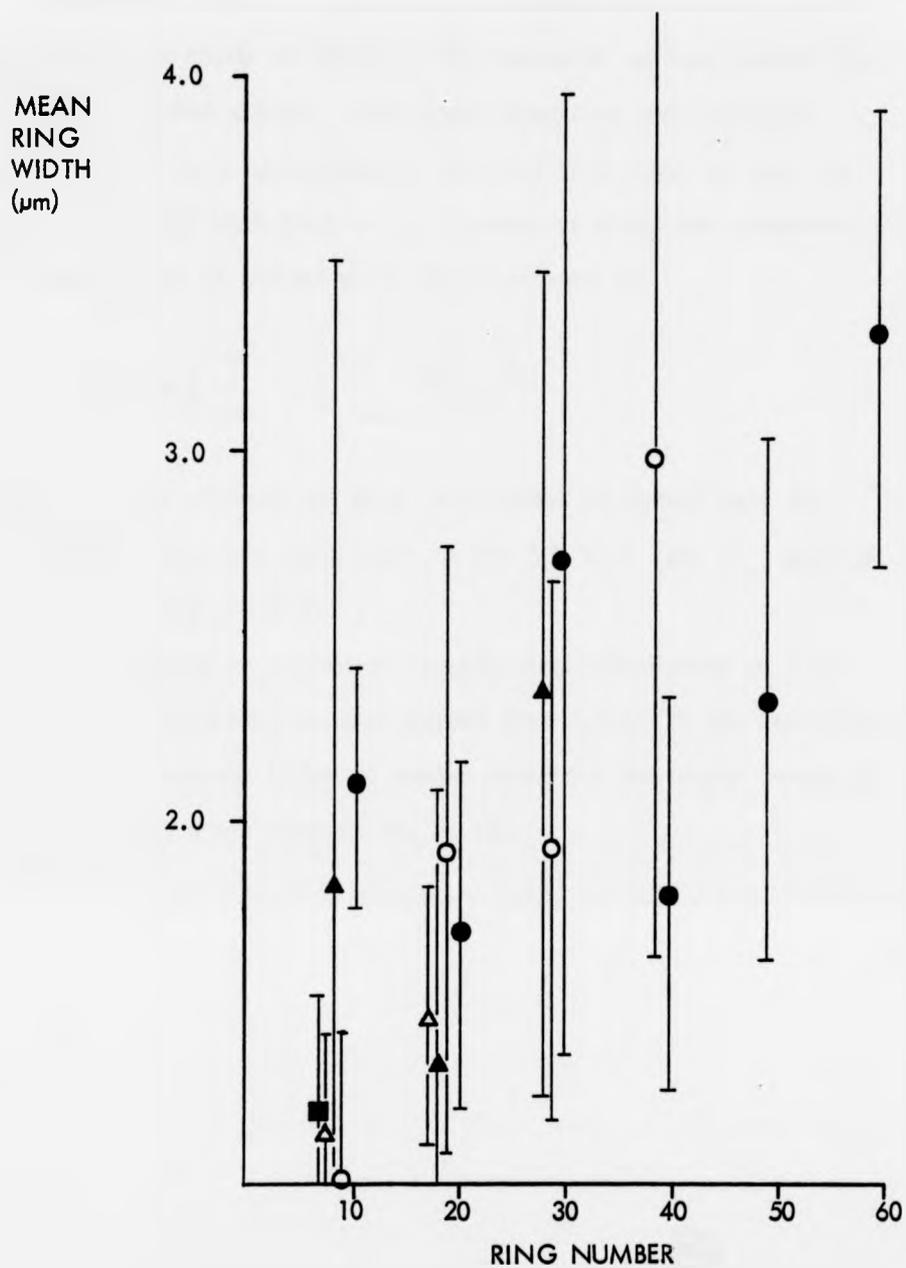
Fig. 27: The mean width of selected increments in herring
larvae having different numbers of rings.
500ℓ tank, 1981. Vertical bars represent 1 sd.

■ --10 - 20 rings
△ --20 - 30 rings
▲ --30 - 40 rings
○ --40 - 50 rings
● --60 - 70 rings

The average index of reader error (Beamish and Fournier, 1981) was used in this study to quantify the variation in ring counts due to interpretational errors. This index describes the variation between readers as a percentage of the mean ring count so that the error for otoliths with high or low numbers of rings are comparable. The average index of reader error is calculated as:

$$100 \times \frac{1}{N} \sum_{j=1}^{N} \quad \frac{1}{R} \sum_{i=1}^{N} \frac{|X_{ij} - X_j|}{X_j}$$

where N = number of otoliths read, R = number of counts made for each otolith, X_{ij} = ith ring count of the jth fish, and X_j = average ring count for the jth fish.

The variations in counts of otolith ring number made in this study by three different readers ranged from 7.0-51.7% for individual otoliths. The average index of reader error for the whole sample of 10 otoliths from three species was 22.6%.

DISCUSSION

Growth

The changes in larval growth rate due to manipulation of the
rearing environment have been amply recorded in the literature.
The effect of rearing temperature on growth and activity of fish
larvae was investigated in order to determine temperature tolerance
limits and optimal temperatures for growth of Atlantic silversides
by Bengston and Barkman (1981) and of yellowtail flounder *Limanda
ferruginea*, by Howell (1980). Measurement of larval growth and
development rates were made at different temperatures for herring
by Hempel and Blaxter (1963), and for northern anchovy by Kramer
and Zweifel (1970) in order to provide baseline data for ageing
wild larvae. Studies such as those of Laurence (1973) on tautog,
Tautoga onitis, and Houde (1974) on bay anchovy, *Anchoa mitchilli*;
sea bream, *Archosargus rhomboidalis*; and lined sole, *Achirus lineatus*,
used different rearing temperatures to produce larvae of different
growth and metabolic rates in order to measure efficiency of energy
utilization. In general, higher rearing temperatures resulted in
smaller larvae at hatching and at yolk sac absorption due to increased
inefficiency of yolk utilization, but produced higher growth rates in
feeding larvae. Comparative studies of the growth of cod and haddock,
Melanogrammus aeglefinus, showed considerable variation between
species in temperature lethal limits (Laurence, 1978).

Food density and composition of food organisms has also been
shown to affect larval growth. Riley (1966) and O'Connell and
Raymond (1970) found that both growth and survival were regulated by
feeding levels in plaice and anchovy larvae. Werner and Blaxter
(1980) reported that growth of herring larvae was significantly

lower at low feeding densities but that increased food above an
optimum level did not alter growth or survival. The effect that
variations in feeding conditions in the wild could have on larval
growth was described by Shelbourne (1957) for plaice, Ellersten *et
al.* (1975) for herring, and Houde & Schekter (1978) for anchovy and
sea bream, stating that larvae showed better growth when feeding in
areas or periods of high prey densities. A greater range of prey
organisms and sizes of prey also led to increased growth and survi-
val in herring larvae (Blaxter, 1968; Ellersten, 1975). Complete
starvation and the delay of initial feeding have been shown to
affect growth rate and survival, but there is a wide variation in
the responses of different species. Larvae of the grunion seemed
to be resistant to starvation and began to grow at the same rate
whenever feeding was initiated (May, 1971). Ehrlich and Blaxter
(1976) found that plaice larvae were more resistant to starvation
than herring larvae, and Houde (1974) reported that feeding could
be delayed without deleterious effects for 12-24 h longer in lined
soles than in sea bream or bay anchovy.

The growth and survival of reared organisms also respond to
rhythmicity in the environment. Dowd and Houde (1980) found that
sea bream larvae grew better with long photoperiods (19L/5D) at
reduced prey densities, but at normal prey densities growth was
greater under 13L/11D. Long photoperiods (18L/6D) produced better
growth in sea bass, *Dicentrarchus labrax*, than continuous light or
12L/12D in experiments reported by Baharona-Fernades (1979). A
cyclic temperature regime resulted in higher growth rates in under-
yearling sockeye salmon and Biette and Geen (1980) claimed that these
results support the theory that diel migrations are energetically
advantageous.

Other factors may affect larval growth in artificial environments. Theilacker (1980) reported that jack mackerel larvae grew faster when reared in larger rearing containers. Crowding has been found to retard larval growth especially in species where social behaviour leads to a dominance hierarchy and when food supply is reduced (Brown, 1946; Magnuson, 1962; Nagoshi, 1967a; Eaton and Farley, 1974). However, Doyle (1975) reported no effects of crowding on growth of plaice larvae at densities up to 64 larvae/ℓ but survival was lower at the higher densities.

Sensory deprivation can also lead to poor growth and survival in reared larvae (Blaxter, 1970, 1975).

The means by which these various environmental conditions affect larval growth is not completely understood. Increased temperature, the onset of a light period, or the presence of food probably stimulates larval activity either behaviourally with increased swimming and feeding activity or physiologically by increasing metabolic rate (Kramer and Zweifel, 1970; Blaxter and Ehrlich, 1974; Houde and Schekter, 1980; R. Batty, pers. comm.). Larval growth and survival is generally better under longer photoperiods because larvae have longer to feed and are therefore less likely to suffer starvation (Dowd & Houde, 1980). Laurence (1972) showed that swimming activity and sustained swimming speed decreased in starved largemouth bass larvae, making them less able to feed, which could lead to slower growth and reduced chances of survival. In general, light, temperature and food availability seem to affect growth by controlling feeding behaviour. However, the response of different species to these conditions may vary and some species may grow well in conditions that limit others (Houde and Schekter, 1980).

In the present study continuous light, higher temperatures, temperature cycles, and larger rearing containers seemed to stimulate faster growth. In contrast to other studies, continuous light produced the highest growth rates in turbot larvae. In all cases (except salmon embryos where yolk sac nutrition seemed adequate to provide for growth at higher metabolic rates) photoperiods of 6 h or less of light were disadvantageous for growth and survival. Even though larvae reared under 6L/6D experience a total of 12 h light within each 24 h, the disruption of feeding activity after 6 h was apparently detrimental to both growth and survival.

May (1971) and Houde and Schekter (1980) described how different species respond differently to environmental changes. Some species respond with the ability to alter feeding behaviour and growth rate when conditions change, while other species tend to maintain stability in growth over a wide range of conditions. These growth characteristics represent life history strategies which would confer an adaptive advantage on certain species, depending on environmental conditions. Theoretically, larvae with stable growth rates could be more resistant to low food conditions whereas larvae with flexible growth rates can take immediate, opportunistic advantage of high food concentrations. The diversity of life history strategies in the larval stage has been described, for the first time in the wild, for an entire community of first feeding larvae in a small lake by Keast (1980).

Each species tested in this study differed in response to environmental manipulation. Growth in turbot larvae seemed to depend on the level of feeding activity, which increased with continuous light and at a higher temperature. Plaice larvae were static in

response over the range of conditions tested since their growth rate was not affected by either light or temperature cycles, starvation or delayed initial feeding. Higher rearing temperatures and an increased number of light periods probably stimulated the metabolism of salmon embryos. An increase in physiological activity could only have resulted in the higher growth rates observed in this study if yolk nutrition was sufficient to supply the required energy. It is unclear from this study to what degree the growth of herring larvae can be altered under different rearing conditions. Herring larvae grew faster in larger rearing containers, and it is probable that larvae reared in the Norway ponds actually grew faster than wild larvae, due to higher temperatures and more constant food supplies. Growth and survival in 30 l tanks was so poor that any effect of environmental manipulation was masked by mortality patterns in the tanks. Herring larvae grow at different rates depending on feeding level, until an optimal level of feeding is reached (Werner and Blaxter, 1979). They do not seem able to alter prey consumption and growth to match continued increased prey density.

It is difficult to state conclusively whether a hardy species is one whose growth response is more, or less plastic. The survival of larvae of a less responsive species is less affected by a range of environmental conditions but a more responsive species may make better use of advantageous changes. In the context of the species studied, the growth rate of turbot and herring larvae were the most plastic, salmon seemed to respond to some environmental parameters more than others, and plaice larvae were very resilient in that growth rate was similar under different conditions.

Ring deposition

There was a wide variation in the pattern of ring deposition
observed in the four species studied. Using multiple regression
techniques it was shown that ring number was more dependent on length
in some species than in others. In turbot and herring larvae ring
deposition rate varied in accordance with growth rate, and in these
two species growth could be manipulated by altering the rearing con-
ditions. Growth was probably influenced by the control that each
treatment had on feeding activity. It is unlikely that this activity
in itself initiated the deposition of otolith rings. This was evi-
dent from the large individual variation both in ring number and in
the state of completion of the last band observed in herring larvae
of the same age, experiencing the same conditions. The number of
rings was also not related to the number of light or temperature
cycles experienced either in standard or experimental conditions.
In fact, turbot and herring larvae experiencing twice as many light
cycles (6L/6D) had fewer rings than larvae reared at 12L/12D, 18L/6D,
24L. Otolith ring deposition was not directly controlled by environ-
mental cues since it did not continue in the absence of larval growth,
even though a cycle of environmental cues was present. Salmon
embryos were an exception in that an increased number of light
cycles did increase ring deposition, but respiration studies showed
that the overall level of physiological activity was also increased
by light. In general, the results of this study indicate that when-
ever increased activity (due to rearing conditions) resulted in
higher growth rates, a greater number of otolith rings was deposited.

Otolith growth and ring deposition may proceed differently in
different species, as a comparison of these results with the

literature would suggest. Mugiya *et al.* (1981) found a diurnal rhythm of calcium deposition on the otoliths of adult goldfish. Tanaka *et al.* (1981) found a similar cycle for juvenile *Tilapia nilotica*, and showed that ring deposition activity ceased at lights-on and resumed 2-3 h later corresponding to the formation of a new increment. It is likely that diurnal cues reinforce the diurnal physiological rhythms which are stronger in adults and juveniles than in young larvae.

Daily ring deposition has been established for several species of fish larvae under laboratory conditions. In other species, a diurnal pattern of ring deposition cannot be demonstrated conclusively with the existing evidence. Radtke and Waiwood (1980) observed four increments in cod larvae four days after hatching, but these were deposited during the yolk sac stage and the experiment was terminated at day 4, before feeding began. Nevertheless, they recommend the use of otolith rings to measure growth of wild cod larvae, and this technique has been used by Gjøsaeter and Tilseth (1981) although they reported a coefficient of only 0.55 for the correlation between age estimated from otolith rings and age estimated from staging and temperature data. Lough *et al.* (in press) report that the third regular increment was first observed in reared herring larvae beginning at day 16. Although the rearing experiments were terminated at day 18, they estimated a mean age of 22 days for a larvae with three increments and assumed that ring deposition occurred on a daily basis after the third increment. It is notable that of the eight studies which have applied otolith ageing techniques to wild larve, the ring deposition rates of only four of the species had been measured under controlled conditions (see p. 10) and two of these confirming experiments covered only a small period of the larval stage.

Both Taubert and Coble (1977) and Tanaka *et al.* (1981) proposed that the rhythmicity of otolith ring deposition was controlled by an internal clock, and entrained to a diurnal rhythm by environmental cues. The initial rhymicity (before entrainment) of the internal clock could vary among individuals in different species resulting in a wide range of deposition rates in individuals experiencing the same diurnal environmental conditions. The initiation of a diurnal rhythm of behaviour and activity which could result in daily ring deposition, or at least ring deposition at a constant rate, may develop at different ages in larvae of different species. Behaviour patterns of larvae do change with age and development as described by Noakes (1978).

The process of ring deposition itself is probably controlled enzymatically. The production or activation of a reservoir of such enzymes could be controlled by different means in different species. Light or temperature cycles could provide the cues in some species, in others the enzymatic control could be linked to diurnal behaviour patterns. In some species the enzyme reservoir could be completely controlled by the amount of energy available for growth, and therefore dependent on individual growth rate.

The biochemical process of ring deposition is probably the same in all species, however, the control of the process may not be. In herring and turbot, larval length was the most important factor in predicting the number of rings. This may be interpreted as meaning that ring deposition was controlled by fish growth. The dependence was not as strong in plaice or salmon, and in these species ring deposition may be controlled by the level of physiological activity alone. The presence of more rings in longer larvae than in shorter

larvae of the same age can be explained by cycles or bursts of growth or physiological activity, each cycle producing a single ring. These cycles could be either less than, equal to, or greater than one day, if their duration was determined by the requirement of a certain amount of energy for continued growth.

Although Taubert and Coble (1977) reported that ring deposition ceases completely under certain conditions, it has generally been assumed that larvae will always deposit one ring per day but that band widths will be altered corresponding to individual growth rate, since otolith size must remain the same in relation to larval size in order to be functional. In the majority of herring larvae examined in this study there was no significant correlation between individual growth rate and the width of the otolith rings. The slow growing larvae in this study deposited rings that were similar in appearance to those of fast growing larvae, but took a longer time to complete each increment. There is some support, among those working with primary growth increments of adult fish, for concluding that during periods of slow growth the deposition of one ring may take more than one day.

The interspecific differences in ring deposition observed in this study may be explained by the different growth characteristics of each species. As observed in herring and turbot larvae, the rate of ring deposition increased with increased growth rate. This may be a general case so that larvae with higher relative growth rates, or larval stages of shorter duration, have higher ring deposition rates, or at least deposition rates averaging at one ring/day. It is notable that the species that have been shown to deposit one ring/ day have larval stages of <60 days, whereas species which metamorphose

after a longer period have ring deposition rates which vary with growth rate (Table 16).

Predictions of growth in wild herring larvae

The growth rates measured in the stock populations of herring larvae agreed well with previous reports for herring reared in similar conditions (Table 17). Doyle (1975) measured growth rates of 0.12 mm/day and Ehrlich (1972) measured growth rates of 0.22 mm/day for herring larvae reared in 120 l tanks at the Dunstaffnage Laboratory. A growth rate of 0.30 mm/day was reported by Gamble (1981) for autumn-spawned Minch larvae reared in the Loch Ewe bags. Ellersten *et al.* (1975) reported growth rates of 0.32-0.51 mm/day for spring-spawned Atlanto-Scandian herring reared in large ponds in Norway.

There are few published studies of the growth of herring larvae in either the Clyde or the West Coast of Scotland. The major work on the growth of wild herring larvae was done by Marshall *et al.* (1937, 1939) who estimated growth rates of 0.49 mm/day in autumn-spawned Clyde larvae and 0.45 mm/day in spring-spawned Clyde larvae. These early estimates are considerably higher than the growth rates measured in the past 10 years. Recent growth studies of herring larvae have dealt only with autumn-spawned larvae. In the north-eastern Atlantic, Wood and Burd (1976) reported growth rates of 0.25 mm/day for the Longstone herring and 0.35 mm/day for the Whitby/Dowsing stock and Hempel and Schnack (1976) measured growth of 0.25 mm/day in Banks and Downs larvae. The growth of autumn-spawned herring larvae in the northwestern Atlantic has been studied since the early 1960's. The published estimates are summarised by Townsend

TABLE 15

RELATIONSHIP BETWEEN THE DURATION OF THE LARVAL
STAGE, THE AVERAGE RATE OF RING DEPOSITION, AND
THE RESPONSE OF RING DEPOSITION RATE TO
ALTERATIONS IN GROWTH RATE FOR SEVERAL FISH SPECIES

Larvae	Duration of Larval Stage (d)	$\bar{x}$ Ring Deposition Rate (ring/day)	rings/day altered at different growth rates	Source
Atlantic silversides	30–35	0.97	no	Barkman (1978
Green sunfish	30–40	1.0	only at completed cessation of growth	Taubert & Coble (1977)
Plaice	50–60	0.74	no	This study
Turbot	60–70	1.02	yes	This study
Northern anchovy	70–75	1.02	yes	Methot & Kramer (1979)
Herring	90–120	0.67	yes	This study

TABLE 16

SUMMARY OF HERRING LARVAL GROWTH RATES
UNDER REARED AND WILD CONDITIONS

SOURCE (of eggs)	REARING CONDITIONS	GROWTH RATE (mm/day)	REFERENCE
A. Stock Populations, This study			
Clyde, Spring	120 ℓ tank	0.12	
	500 ℓ tank	0.22	
Minch, Spring	plastic bag	0.29	
Atlanto-Scandian, Spring	ponds	0.42	
B. Stock Populations, Previous studies			
Clyde. Spring	120 ℓ tank	0.12	Doyle, 1975
		0.22	Ehrlich, 1972
Minch, Autumn	plastic bag	0.30	Gamble, 1981
Atlanto-Scandian, Spring	ponds	0.32-0.51	Ellersten, 1975
C. Wild Larvae, This study			
Clyde, Spring 1980	--	0.23	
Clyde, Spring 1981	--	0.23-0.33	
Minch, Autumn 1980	--	0.40	
D. Wild Larvae, Previous studies			
Clyde, Autumn	--	0.49	Marshall *et al.*, 1937
Clyde, Spring	--	0.45	Marshall *et al.*, 1937
Longstone, Aut.	--	0.25	Wood and Burd, 1976
Whitby/Dowsing, Autumn	--	0.35	Wood and Burd, 1976
Banks, Downs, Autumn	--	0.25	Hempel and Schnack, 1971
Gulf of Maine, Autumn	--	<0.14-0.34	summarised by Townsend and Graham, 1981.

and Graham (1981) and range from <0.14 to 0.34 mm/day. It is inter-
esting to note that among the estimates summarised, those growth
estimates based on shifts in modal or mean length frequencies range
from <0.14 to 0.28 mm/day while growth estimated from otolith rings
was 0.34 mm/day.

The calculation of growth rates based on an assumed daily ring
deposition in this study also tended to overestimate growth. If the
rings are assumed to represent a constant time scale, individual
growth rates estimated by backcalculation would greatly overestimate
the growth of slow growing larvae and underestimate the growth of
fast growing larvae. It was clear that the assumption of daily ring
deposition was incorrect for these herring larvae. The number of
rings deposited during the increase from one length to the next was
shown to be constant, because of the dependence of ring number on
length. In herring larvae it seems that each ring represents a
different length of time in slower or faster growing larvae.

With the exception of backcalculations, the estimates of growth
rate for the Clyde and Minch herring larvae accomplished by direct
length measurements and otolith ageing fit within the range of pub-
lished growth rates. The direct measurements of growth make many
assumptions about hatching times, dispersion, and mixing of age
groups, but they seem to be reasonable first estimates of growth in
terms of the published literature. The ageing of these wild larvae
using the ring deposition rate of the slowest growing reared larvae
provided the lowest estimate of growth, in most cases unreasonably
low (0.07-0.15 mm/day), indicating that ring deposition rates in
rearing experiments can be different from those in the wild.
Estimates of growth rate based on the assumption that the number of

rings is directly related to the number of days since hatching, as well as estimates based on the ring deposition rate in the Norway ponds (one ring/day) were similar and surprisingly, these estimates were very close to the directly measured rates of growth. The similarity between these two estimates of larval growth, that from direct measurements of length and that from the comparison of length at consecutive otolith increments which are assumed to represent age, would at first glance lend sufficient support for the use of otolith increments for ageing herring larvae. However, it should be remembered that it is the value of the slope of the regression equation of ring number and larvae length (mm/ring) and the slope of an estimated increase in length over time (mm/day) which are similar. This is not the same as if two values for an identical relationship were found to be the same, especially if ring deposition varies with growth rate. The real growth rate of wild herring larvae cannot be estimated correctly by assuming that ring number is equal to age, because ring deposition does not begin at hatching. Using the ring deposition rates measured in fast-growing reared larvae will also lead to incorrect predictions of growth in the wild. If the wild larvae are growing slower than some reared larvae (slower than 0.4 mm/day) then it is likely that ring deposition in the wild will be lower than one ring/day. The difference in ring deposition will tend to underestimate the age of wild larvae. The ages of older larvae will be progressively more underestimated, which will affect predictions of growth rate. Unfortunately, if ring deposition in herring larvae is dependent on growth rate, as suggested (see Fig. 19), then the ageing of wild herring larvae cannot be accomplished if the growth rate is unknown.

Assessment of otolith techniques for

studies of fish larvae

Several disadvantages of otolith ageing were identified in this study. The verification and calibration of ring deposition rates must be carried out for each species using larvae of known age. However, laboratory reared larvae are not always suitable for making predictions about wild populations of larvae since their growth characteristics are often different. Individual variation in reared larvae can be very high, and it is generally considered that it is only a small proportion of fast growing reared larvae which corres- pond to the wild population. In order to relate the otolith data of reared larvae to those in the wild, larvae should be reared under conditions which resemble the wild as closely as possible. The amount of variation within the population must be examined, especially in species where individual variation in growth rate could result in variations in ring deposition.

The use of larval otoliths, like the ageing methods for adult fish, involves the collection of information about individuals for predictions of population growth. Due to variability in the popula- tion, and to the possibility of selective mortality, individual growth rates may vary markedly from the population growth curves (Ursin, 1963; Ricker, 1975). Although in some cases herring growth rates estimated on the basis of daily ring deposition were similar to measured and published reports of herring growth, experimental data showed that individual larvae could vary as much as 100% in ring number at the same age. The justifications for using methods which can produce acceptable results on the population level, but are incor- rect on the individual level, are not discussed in the literature.

There are many technical problems associated with otolith ageing methods, especially in larval fish (see Appendix II). The preservation of wild larvae is critical in order to prevent dissolution or damage to the otoliths (Radtke, 1980; Kramer, Nichols, pers. comm.). The dissection and preparation of larval otoliths is often tedious and time consuming, and always requires a great deal of concentration. Care must be taken for the successful grinding and polishing of the otoliths of larger larvae. In addition to the preparation of larval otoliths, many of the problems of counting the increments have not yet been resolved. Although the increments have been defined, described, and illustrated in the literature, the rings on many otoliths (especially in larvae approaching metamorphosis) are still subject to errors in interpretation. Differences in the interpretation of these rings can lead to variations of up to 100% in the counts made by different readers, even those with experience of larval otoliths (Gjøsaeter, pers. comm.). Much of the reader error can be reduced by careful definition and instruction as described in Appendix II. The variation in ring number resulting from reader error was estimated in this study to be 22.7%. These errors, combined with the variability in the ring deposition rates of some species and the effort involved in the analysis, make the use of otoliths for the ageing of the larvae of certain species somewhat questionable. In certain cases the analysis of length-frequency modes, or developmental staging, combined with the knowledge of spawning and incubation times may be adequate to provide accurate information for larval growth studies. For other species, notably anchovy and menhaden, the ageing of larvae using otolith rings is a standard procedure. In species such as these, where the deposition rate is presumably constant at

one ring/day over a wide range of conditions, the ability to age larvae is a valuable asset to larval work.

The knowledge of an individual's age increases the accuracy of growth estimates and allows detailed studies of mortality patterns and individual life histories. Before this technique can provide the answers relevant to fisheries biology in more species the biological significance (or the controling stimulus) of the rings should be studied.

It is clear from these results and discussions that more critical investigations of larval growth and otolith ring deposition are necessary before accepting this technique as a universal method. The manipulation of otolith ring deposition was demonstrated in this study in different species under varying conditions, but the environmental factors controling ring deposition were not clear. A more complete understanding of ring deposition and the physiological control of this process may give a clearer explanation for the response of otolith rings to internal or external stimulii.

In order to determine successfully the periodicity of ring deposition, especially in response to environmental cues, the process of ring deposition must be studied at the microscopic or biochemical level. Following the techniques of Mugiya (1964), the endolymph fluid of larvae sampled over 24 h periods could be analysed for protein content. The rhythm of changes in protein content would indicate the periodicity of ring deposition.

The timing of calcium deposition on the otolith could be monitored histochemically using a method developed for shell deposition of oyster larvae by R. Millar and M. Scott (pers. comm.). Frozen sections of larvae sampled over 24 h periods could be stained for

alkaline phosphatase activity indicating the existence and sites of active calcification.

Even after the deposition process has been explained, and the deposition rates for each species measured, the problem still exists of how to relate the results from experimental work to the wild population. This is not a critical problem with species in which the ring deposition rates are stable over a wide range of conditions, but for larvae with variable deposition rates (such as herring and turbot in this study) it is necessary to know what the growth characteristics of the wild population are in order to select the appropriate range of results from the experimental data. Unfortunately, these growth characteristics are the same parameters that are meant to be estimated by the method in question. It will require several years of comparative studies of laboratory reared and wild larval growth and ring deposition for each population before ring numbers could be used generally in studies of fish larvae.

SUMMARY

1. The ageing of individual adult fish based on differential pat-
    terns of calcification in bony structures is an important tool
    for fisheries biology. The discovery of daily rhythms of ring
    deposition on the otoliths has extended this ageing technique
    for use in larval fish studies.

2. Salmon embryos from fertilisation through hatching, and turbot,
    plaice and herring larvae from hatching to metamorphosis were
    kept under various light, temperature and feeding regimes in
    order to study the effects of environmental conditions on growth
    and ring deposition. Ring deposition rates in turbot, salmon,
    and herring were increased significantly under conditions lead-
    ing to faster growth, such as long photoperiods, larger rearing
    containers, and higher temperatures. Neither growth nor ring
    deposition rates of plaice larvae were altered by any of the
    different rearing conditions tested. The growth rates observed
    under the conditions tested ranged from 0.13 to 0.27 mm/day in
    turbot, from 0.03 to 0.06 mm/day in plaice, from 0.22 to 0.40
    mm/day in salmon embryos, and from 0.12 to 0.42 mm/day in herring
    larvae. The ring deposition rates measured under these condi-
    tions ranged from 0.10 to 0.93 rings/day in turbot, from 0.84
    to 1.12 rings/day in plaice, from 0.50 to 2.33 rings/day in
    salmon, and from 0.34 to 0.92 rings/day in herring larvae. In
    turbot and herring larvae the number of otolith rings observed
    was dependent on the length of the individual larva. This rela-
    tionship held at the population level as well, such that higher
    ring deposition rates were observed in faster growing populations.
    The relationship was linear for turbot larvae for the range of

growth rates observed. In herring larvae, the relationship
between growth rate and ring deposition rate seemed to be
logrithmic, reaching an assymptote of one ring/day at growth
rates approaching 0.40 mm/day, for the range of growth rates
observed.

3. The successful manipulation of otolith ring deposition seemed
    to result from the effects that the rearing conditions had on
    growth and activity, rather than from the direct triggering
    effects of environmental cues. In all the larvae examined,
    significant differences in ring deposition were observed only
    where there were significant differences in growth rate. There
    was no synchronisation of ring deposition with diurnal environ-
    mental cues in either reared or wild herring larvae. Larval
    length was significantly more important than age in determining
    ring number in turbot and herring larvae. Age was the most
    important factor in determining ring number in plaice larvae.

4. Wild herring larvae were sampled from the Firth of Clyde in
    1980 and 1981 and from the Minch in 1980 in order to assess the
    suitability of otolith ageing methods for herring larval studies.
    Because of the large individual variation in the ring deposition
    rates of reared larvae, the accurate ageing of wild larvae was
    difficult. Four methods were used to estimate the growth rates
    of wild herring larvae: i) assuming that ring number was equal
    to larval age; ii) ageing each wild larva on the basis of the
    average ring deposition rates of reared larvae; iii) backcalcula-
    tion, assuming a ring deposition rate of one ring/day; and
    iv) measuring the increase in length from an estimated hatching
    length and date of hatching. These methods produced a wide

range of growth estimates for each wild population. The assumption that ring number was equal to age led to incorrect predictions for length at yolk sac absorption, and backcalculations led to overestimated growth rates, indicating that ring deposition does not begin at hatching and may proceed at a rate different from one ring/day in wild herring larvae.

5. The controlling mechanisms of ring deposition and the deposition rates of known-aged larvae under conditions resembling those in the wild should be thoroughly investigated before otolith ageing techniques are adopted universally for larval studies.

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APPENDIX I

Selected Examples of Larval Otoliths

Appendix 1.

A comparison of the otoliths of similar
aged herring larvae from different sources.

a: 120 ℓ tank 1980,   58 days old,   18.40mm, 19 rings
b: 120 ℓ tank 1981,   45 days old,   15.84mm, 10 rings
c: Loch Ewe bags,   48 days old,   18.95mm, 28 rings
d: Clyde 1981,   ? 59 days old,   29.12mm, 43 rings
e: 500 ℓ tank 1981,   45 days old,   22.72mm, 30 rings
f: Norway pond,   63 days old,   37.44mm, 48 rings

     scale bar = 20μm
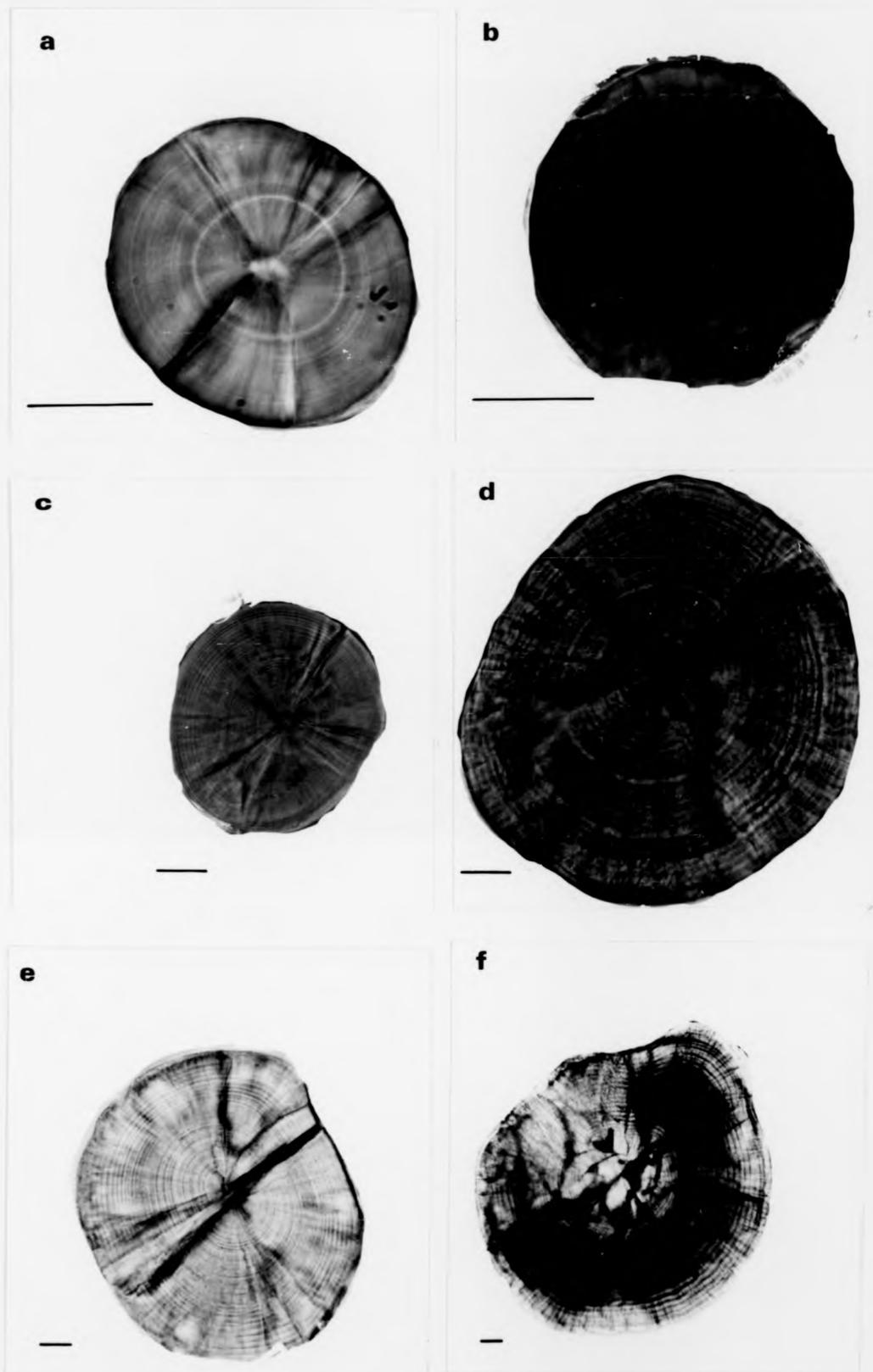
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e: 500 l tank 1981,   45 days old,   22.72mm, 30 rings
f: Norway pond,   63 days old,   37.44mm, 48 rings

scale bar = 20μm

APPENDIX II

Proceeding of Workshop on Larval Otoliths,

Bergen, Norway, January, 1982.

This report was written jointly by
O. Bergstad, H. Gjøsaeter, P. Munk,
and A. Geffen. It was not included
in assessing the thesis for the
Ph.D. degree, but is bound in for
the reader's interest.

Report on an

OTOLITH WORKSHOP

in

Bergen, January 18-29, 1982

Department of Fisheries Biology
University of Bergen
and
Institute of Marine Research
Bergen
1982

PREFACE

Since the publication of Pannella's paper on daily growth rings
in fish otoliths in 1971, there has been a growing interest in
the use of these rings for ageing. For many species, this
method has been verified and is used routinely. For other
species there is evidence that considering the primary growth
rings as daily marks is too simplistic, and that growth also
is an important regulator of the ring formation. Therefore,
it seems necessary to verify the reliability of the method
for each new species and probably also for different environ-
ments.

To discuss the problems involved in ageing fish by means of
daily growth rings and to try to verify the method for some
selected species, a workshop was arranged. As the participants
were mainly interested in larval and juvenile cod and herring,
and in adult sardinella, the work was concentrated on these
species.

The following questions were put forward for discussion:

1. Can primary growth rings be used as a practical tool
   for ageing the species in question?

2. How should the otoliths be prepared and read to
   give optimal results?

3. What kind of research should be undertaken to
   improve the methods?

The workshop held in Bergen on January 18-29, 1982, was organized
by the Department of Fisheries Biology, University of Bergen, in
cooperation with the Institute of Marine Research and with help
from the Zoological Laboratory, University of Bergen. The
Norwegian Fisheries Research Council gave financial support
to one of the participants, and one participant holds a NORAD
fellowship.

Bergen, February 26, 1982

Jakob Gjøsæter

INTRODUCTION

It was discovered, in the early seventies, that the otoliths
of some tropical and temperate fishes contained primary growth
increments which seemed to be formed daily. (Pannella 1971,
1974). Since then, several research workers have studied the
potential use of counting these increments as a routine ageing
method. As far as temperate species are concerned, the work
has concentrated on larvae and juveniles. The number of species
found to have primary otolith increments has steadily increased.
Since the list includes species from very different environ-
ments and geographical regions, it seems reasonable to assume
that an incremental growth is a universal phenomenon in fish
otoliths.

Before being accepted for routine ageing, the method should be
verified for each new species under consideration, especially
if this species seems to differ from earlier studied species
in terms of behaviour, habitat, growth etc.
Ring deposition of one ring/day has been established for some
species by reading otoliths from fishes of known age. However,
it is not known whether the results of these laboratory studies
are applicable to field studies. The mechanisms involved in
increment formation are as yet unknown. Therefore it is diffi-
cult to assess which factors (environmental or internal) in-
fluence the deposition of zones. Light, temperature and feed-
ing cycles have been proposed as possible rhythmic stimuli that
could function as trigger factors for the zone deposition.
So far, no experiments undertaken to study the influence of
these environmental stimuli have given conclusive support for
one or the other. The influence of these factors on ring depo-
sition could differ among species and in different environments.

A difficulty with starting to use the method for ageing wild
fish, even where a daily deposition of increments has been con-
firmed on "known-age" material, is the possibility that fluc-
tuations in growth rate could affect the deposition rate, slow-
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ing it down or even stopping it.

In addition to these theoretical problems, the participants
in this workshop had previously encountered various practical
problems in preparing and reading otoliths. Even though the
counts of each person seem quite consistent, comparison of dif-
ferent counts of otoliths prior to this workshop has shown
that there can be a substantial variation between readers.
Thus there appeared to be an obvious need for reaching a point
where all counters agree on a certain definition of an increment.

It was therefore decided to organize a workshop, where in addi-
tion to an intercalibration of counters, the more basic problems
concerning this ageing method and the literature could be dis-
cussed.

It was decided to concentrate the practical work on larval
material of the two temperate species cod (Gadus morhua) and
herring (Clupea harengus), in addition to adults of a tropical
Sardinella species (Clupeoid).

SHORT COMMENTS ON PREVIOUS WORK ON OTOLITHS OF COD, HERRING
AND SARDINELLA.

Cod.

The amount of work done prior to this workshop is rather preli-
minary if the motivation is to establish a practical ageing
method for larval and juvenile cod. Radtke and Waiwood (1980)
presented results of a study on otoliths from lab-reared larvae
up to an age of four days using SEM. They found that one zone
was formed daily from the day after hatching and onwards.
T. Dale (pers. comm.) came to the same conclusion after TEM -
studies of laboratory reared yolk sac larvae. He could detect
faint zones prior to hatching in some larvae, but a prominent
hatch-line was formed within 24 hous after hatching. The dis-
tinctness of the zones formed daily after hatching was variable.
H. Gjøsæter (1981), using a light microscope, found a good cor-
relation between the number of increments and age up to an age
of about 40 days. He concluded that one zone was formed per
day from 4-5 days after hatching and onwards.

These reports support the assumption that zone formation is
rhythmic during periods of larval life, but the conclusions
which can be drawn are limited by several factors e.g. the
limited age range examined, the small sample sizes and the un-
usual rearing conditions. In addition there have been problems
with finding a standardized way of reading cod otoliths.
O.A. Bergstad (pers. comm.) has undertaken a test where diffe-
rent readers counted the same otoliths. He detected a consid-
erable variation between readers, especially for the older
larvae.

Herring.

Lough et al. (1980) report a study of otoliths from herring
larvae. They kept the larvae for 18 days in the laboratory,
and by then the larvae had deposited only three increments in
their otoliths. The first increment formed soon after yolk sac
absorbtion. Then there was a delay of about one week before

the second increment appeared, and when the rest of the larvae died after 18 days they had just deposited a third increment. H. Gjøsæter (1981) found that the increment deposition was daily for herring kept in a large out-door basin starting at an age of 3-4 days when the larvae were released in the basin counting to the end of the experiment at a larval age of 135 days.

A. Geffen (pers. comm.) has studied the otoliths of herring larval groups with different growth rates. Her findings indicated that the ring deposition rate is dependent on the growth rate. Larger larvae had more increments than smaller ones of the same age, this applied both for within rearing groups and between rearing groups. Only the fastest growing larvae, with a mean growth rate of 0.4 mm/day had a ring deposition rate of nearly one ring/day. These results indicate that primary ring counting is probably not applicable for ageing herring larvae in the field.

There have also been some problems with defining the rings in herring in a standard way to get indentical counts for different readers, but the problem is not as severe as in cod.

Sardinella.

H. Gjøsæter (1981) found primary growth increments in the otoliths of adult Sardinella longiceps caught in the Gulf of Aden which were relatively easy to count. Assuming a daily deposition rate, the age corresponded more or less to what was previously known about the growth of this species and the spawning season in the area.

P. Dayaratne (unpubl. mat.), working with adult S. sirm from Sri Lankan waters, has found increments, the number of which corresponds well with the age deduced from length frequency analysis and from the known spawning time of the species. Even though the otoliths of these species are more easy to read than the temperate ones, there has been a variation between different counters in test runs. There is also a tendency for the otoliths to become more difficult to read when the preparations get older, which could be caused by the preparation technique in use.

RESULTS OF THE WORKSHOP

Definitions of terms.

As a result of discussion during the workshop several defi-
nitions were formulated as a quide for the interpretation of
zones to be counted for the purpose of ageing. On the basis
of the work with cod, herring and Sardinella the following
counting procedure was designed for light microscope examina-
tions.
One ring (zone, or increment) was defined as the distance from
one discountinuity layer to the next. This is visible as the
distance from the beginning of one dark band to the beginning
of the next dark band. The dark bands were used for counting
rather than the light bands.

It was sometimes necessary to alter the focus while counting
the rings, but care must be taken in interpreting the "move-
ment" of the rings when the focus is changed and of the opti-
cal phenomena that this produces at the edge of the otolith.
The fact that a ring was not visibly countinous around the
entire otolith was not a criteria for excluding these rings
from the counts.

In herring and cod the first ring was usually a very prominent
band. This ring was chosen as the first ring for counting
purposes. Rings could sometimes be seen inside the prominent
ring but since their presence and number was not consistent
these rings were not included in the ring counts. They should
be excluded until their significance is investigated more clo-
sely. If no prominent ring is visible on the otolith, coun-
ting should begin at a speciefied distance from the nucleus
which is approximately equal to the size of the otolith at·
hatching (herring: 10.8 μm: cod: 6 μm). In juveniles where it
is often difficult to grind the otolith to see the innermost
rings, a measured distance could be taken from the nucleus once
this distance has been calibrated to equal a constant number
of rings.

The edge of the otolith was included in the ring counts if the beginning of the next increment was visible at the edge. Optical phenomena produced at the otolith edge often made the interpretation of the last band difficult.

The last rings or the group of rings closest to the edge of the otoliths of Sardinella and juvenile cod were very narrow and it was important to make the transition between counting wide rings and narrow rings correctly. This could be done by carefully following the gradual reduction in zone width approaching the edge.

The otolith of the oldest herring and cod larvae, and of the juvenile cod and the Sardinella were best counted at the lowest magnification possible. In these otoliths many of the rings that were visible at 1000x could not be included in the ring counts for the purposes of ageing. The interpretation of these rings and the selection of "daily" rings was accomplished more successfully, with better agreement between readers and with age, when done under lower magnification (400x) (see Table 1.). Before going to lower magnification as a standard method a thorough calibration must be complete for these counts on known-age material.

In the herring larvae examined differences in the ring structure of reared and wild larvae were observed. The first prominent ring described for reared cod and herring larvae was not as common in the wild material, although ring deposition started from the same distance from the nucleus. The rings of wild larvae were generally more distinct (clearer) and more evenly spaced than in reared larvae.

Standard Methodology.

A standard method for the collection, preparation and examination of otolith material was designed. This standardization is a necessary part of future larval studies utilizing otoliths. These methods expand on the manual for otolith work produced at La Jolla, California (1977).

TABLE 1.

RESULTS OF INTERCALIBRATION FOR 40 D. OLD COD LARVAE

	Counts obtained by counter:								
	1	2	3	4	5	6	$\overline{X}$	SD	C.V.
	50.3	68	59.6	45.7	-	-	55.9	9.9	17.7
Group 1.	38.0	47	45.4	45.0	-	-	43.6	4.0	9.1
	54.3	65	58.6	44.7	-	-	55.6	8.5	15.3
	27	18.5	37.0	29	-	-	27.9	7.6	27.2
	-	40.7	48	33	-	36.2	39.5	6.5	16.4
Group 2.	-	38	44	36.7	-	37.8	39.1	3.3	8.4
	-	39	41.5	36	-	-	38.8	2.7	6.9
	-	27	36	28	-	-	30.3	4.9	16.2
	-	38.3	47.5	34.7	-	40.2	40.2	5.4	13.4

1. Group: Counts were made without giving any instructions to the counters.

2. Group: All counters used 400x magnification and counted the zones which appeared most prominent.

I. Collecting specimens

Field samples should be preserved in buffered 95% alcohol.
Buffered formalin may be acceptable for periods of 1-2 months.
Freezing or drying the larvae are also possible preservation
methods. Any other preservation methods should be tested first
for their effects on otoliths.

A measure of the shrinkage in length, weight or other altera-
tions in the morphology of the larva caused by the preservation
should be made.

Relevant information should be recorded for each haul, such
as depth, time of day, haul duration, location, water tempera-
ture, light levels. It is also valuable to record an estimate
of abundance of food organisms if possible.

Lab samples. All calibration work to establish the deposition
rate and time of first ring formation must use larvae hatched
within one 24 h period, preferably during the peak of hatching.
There should be intensive sampling around the stages of hatching,
yolk aborption and first feeding. Later samples should be
frequent (every 2-7 days) and cover the entire larval period,
but this depends both on the hypothesis being tested and the time
and resouces available. The number of larvae required for a
good sample should be determined by first using at least 10
larvae of each age to check the amount of variation in ring
number, for each size group at each age. This will determine
how many larvae are needed to give a representative sample in
the future. If there is little variation in ring number among
size groups, or little variation in size among individuals of
the same age, then three larvae at each age could be sufficient
for a representative sample the entire size range of larvae.
Lab. samples should be frozen or dried (if the dry weight is to
be taken). Larvae preserved in this way can be stored on mic-
roscope slides until dissection. Measurements made on lab.
samples should be taken from fresh material whenever possible
to give an additional measure of shrinkage or alterations due
to preservation. A detailed measure of feeding condition is
more of a possibilty when working with lab. samples.

II. Measurements for the larvae

The measurements to be made on each larvae should consist of
1) the standard (notochord) length (taking care consistently
measure the upper jaw), 2) some measure of larval condition
such as dry weight, myotome height, eye height, distance bet-
ween the eyes, feeding incidence, and 3) the developmental stage.
The staging should be done using a standard, published method
whenever possible. The staging system used should always be
reported. 4) The time of day sampled.

III. Removing the otoliths

Larval otoliths should be removed working under binocular mic-
roscope at about 50x magnification with fine needles (insect
needles or fine glass needles). The larvae should be soaked
in distilled water to make them flexible for easier dissection.
All the otoliths should be removed from each larva until it
has been determined which pair is the sagittae. Only the sagit-
tae should be used for ageing. Both sagittae should be used
as a safeguard in case one of the pair is not clear. It is
possible to try mounting one of the pair convex-side up and the
other convex-side down on the slide.
A circle should be marked on the underside of the slide and the
otoliths placed within it. All markings should be done with
a xylene-proof marker. The otolith should be dried for approx-
imately ½ hour before mounting.

The otoliths of juveniles need to be ground in order to see
the inner rings. The otoliths should be scraped clear of all
the adhering tissue (working under a binocular microscope) either
in water or very dilute (0.19%) acid. After drying thoroughly
(½h) the otoliths can be mounted with superglue or mounting
medium onto a slide. Grinding should be done using very fine
carborundum (wet-dry) paper placed in water in a petri-dish.
A dentists drill can also be used for grinding. The grinding
process should be done at the dissecting microscope. If the
grinding paper is fine enough, no further polishing is neces-
sary to clear the surface.

Commercial metal polish is suitable for polishing if needed. The otoliths can be mounted with medium under coverslips, or viewed under a drop of water or immersion oil.

IV. Clearing and staining techniques

There are no staining methods in common use for small otoliths. Some techniques were tried at the workshop, but without improvement in ring clarity. These methods could not be investigated adequately in the time available, and it is possible that clearing, staining, or other methods of microscopy could increase the clearness and visibility of the rings.

V. Mounting the otoliths

Of the different mounting media discussed, Pro-texx, Microkitt, or immersion oil were considered to produce the best results for permanent otolith preparations. Canada-balsam is not recommended since it disrupts the ring structure after a period of one month. This may be due to a reaction between the medium and the otolith itself or because the otoliths were not properly dried before mounting. Canada balsam is acidic and probably does cause some dissolution of the otolith. Neutralized Canada balsam is available, but its use as a permanent mountant has not been tested. In most cases the coverslips should be sealed with nail varnish to prevent evaporation of the medium. Evaporation can cause the coverslip to be sucked down onto the otoliths and crush them, or it can pull them apart as the medium shrinks. Nail varnish also prevents the coverslips from sliding when using immersion oil.

VI. Examining the otoliths

Several measurements should be made when examining each otolith. The diameter and radius should be measured using the longest diameter and radius in larval otoliths and a radius from the nucleus to the posterior edge in juvenile otoliths. The exact radius used for juveniles should be that which gives the most simple form to the relationship between otolith size and fish size.

The measurements made on each otolith should include the dia-
meter, radius, radius of the first prominent ring and subse-
quent prominent rings, individual zone width if possible, and
information on the stage of completion of the last increment.

VII. Counting rings

The counts of ring number should be made at least three times,
recording the mean and standard deviation of the counts. These
counts should be separated in time so that the decisions made
in one counting do not influence the results of the next.
The limit of acceptable error in counts should be set before
the examinations are begun. Ring counts should be checked inde-
pendently by at least one other experienced reader. Each reader
should count with a tally-counter for ring numbers higher than
20. This makes the counting easier and avoids biasing one count
with the results of another.

A Counting procedure was developed for cod, herring and sardi-
nella otoliths as a guide for interpreting rings.

1. If a prominent first ring is present in all larvae this
    seems to be a good place to start counting, making a
    separate record of the number of rings visible inside
    this band. There is evidence for earlier rings from EM
    work but these are not consistently visible in the light
    microscope. There is also the possibility of using the
    radius of the otolith at first feeding or hatching (if this
    is constant) and beginning to count the first ring visible
    outside that distance.

2. In small otoliths it is important to count all the rings,
    focusing several times to verify the counts. The edge
    is counted as a ring if it appears as a dark band.

3. In larger otoliths it is likely that counting every ring
    gives counts much higher than the age. If only the hea-
    viest bands are counted there is better agreement with

age and also between readers. This is best accomplished by focusing so that the outer bands are thick and fuzzy and not divided into smaller rings.

4. The width of the outer rings in older larvae + juveniles tends to decrease towards the edge and all of these narrow rings should be counted as separate rings.

VIII. Suggested equipment for otolith work

Freezer for storing samples

Dissecting microscope (with x50 mag.)
Compound microscope (with x1000 mag.)
Photographic equipment

Tally counter
Fine needles
Grinding papers
Mounting media
Nail varnish

Extra equipment helpful in otolith work

Microscope attachments:

   video vamera + monitor
   viewing screen
   polaroid camera
   large-format negative camera
   discussion tube
   drawing tube

Dental drill and grinding discs
Digitizing table
Desk-top computer

EVALUATION OF TECHNIQUE FOR AGEING OF ADULT, JUVENILE AND
LARVAL FISHES.

The use of observed number of primary growth increments for
ageing fishes will depend on several factors. There seems
to be no basis for the general assumption that increment
formation is a daily event in most fish species unaffected
by other factors, either genetic or external. At this point
it seems impossible to draw conclusions from results obtained
for one species to validate the use of increment counts as
an ageing method for another species.

Adult tropical fishes.

For adult tropical fishes the available data support the
assumption of daily increment formation. The age determina-
tions made by increment counts show good correspondence with
age as obtained by other methods i.e. length frequency modal
progression and/or knowledge of spawning season. In some
cases the use of otoliths appears as the only useful and rea-
sonably precise method. When prepared in a proper manner
the primary increments of tropical fishes (see Sardinella sp.)
appear more distinct and easier to count than is the case
for most temperate and boreal fishes. It is, however, also
for these species a definite requirement that the assumption
of daily increment formation is verified.

Juveniles of cold-temperate fishes.

Counting of primary increments of otoliths from juveniles of
cod have been attempted, at present only from field-caught
specimens. The conclusions drawn from this material rest on
the same assumptions which are necessary for larval cod.
In addition, a verification of a regular pattern of zone
formation during the juvenile period is needed. If these
requirements can be met, and if the counting pattern is stan-
dardized, the method seems promising for ageing juvenile cod.

Larvae of temperate and boreal species.

For larval fishes it has been shown that the rate of increment formation may vary considerably within the same species. If a relationship between age and number of increments is established for a stock or a species with due consideration of the possible deviations in slope and time of first zone formation, this can be used for ageing of larvae from this particular unit. Calibration of counts between counters is necessary to prove the validity and usefulness of this relationship. It is also important to base the establishment of this relationship on larvae of known age from a wide spectrum of age-groups (preferably from hatching to metamorphosis in as many steps as possible).

It should be emphasized that knowledge of the mechanisms involved in trigging or entraining zone formation must be the basis for using the established regression equation for ageing larvae from other stocks. These may live under very different conditions compared to the population for which the regression was established. Knowledge of the underlying mechanism and/or of factors which are affecting the regularity of zone formation will be a necessary guideline for what to expect when examining larvae from other conditions.

The total effort required for all steps in the routine use of the method should be considered when comparing with other available ageing methods (i.e. stageing by anatomical features, length frequency modal progression etc.). If the increase in precision of an age determination by increment counts is small, the time needed for special preservation of samples, dissection, mounting and several determination of increment number for each otolith, may cost too much to allow it for routine use. For species where the increments are rather indistinct, a fact which will always reduce the precision of the counts, this comparison is especially relevant. For other species where the counting is easy and also reasonably constant from one counter to another, ageing by some relation between the age and the number of increments still seems to by very promising.

REQUIREMENTS FOR ANSWERING BASIC QUESTIONS CONSERNING
OTOLITH STUDIES -   SOME RECOMMENDATIONS .

A short list of questions which at this stage seem relevant
will then be:

- When does the first prominent ring seen in the light
  microscope form?
- Is the zone formation regular throughout the larval period?

- If so, which factors are involved in entraining the mech-
  anism of zone formation?
- Is the rate of zone formation independent of growth rate?

- Is the step from a rearing experiment to the wild immedi-
  ately acceptable?

- If a straightforward relationship exists between age and
  the number of zones, how precise will the age determinati-
  ons by this method really be?

- Which limits are set for the amount of error acceptable
  for routine use? (this is likely to be governed by for
  what purpose the ageing is done i.e. growth studies, sur-
  vival studies, studies of anatomical development etc.)

- If the method of increment counting proves unsuitable for
  routine ageing, can otoliths be used to gain other infor-
  mation about early life history?

It seems clear that the use of primary increment formation
may be useful as a practical ageing method if the following
requirements are met. (the list will most probably prove
incomplete, but may serve as a useful starting point).

1. One species has to be considered at a time, possibly also
   each stock or population if growth rate or other factors
   are assumed to be variable between them. Special consider-
   ation should be directed to the effects of cyclic environ-
   mental stimuli.

2. The mechanism of zone formation and/or factors which influence deposition rate and the synchronization of ring deposition should be investigated.

3. The time for the formation of the first increment should be determined.

4. For each unit considered (species, stock or population) a wide spectrum of age-classes of which the exact age is known from another independent ageing method should be used.

5. The regression of number of increments vs. age must be based on results of 3 and 4.

6. The precision of the regression obtained and of age determinations made from it should be measured.

7. The precision of an age determination by this method should be tested against other available methods (if any exist) by a cost-benefit analysis (i.e. is enough precision gained by using this method to pay the costs of time and effort in preparation).

8. If the relationship between age and number of increments proves unsuitable for making age determinations the otolith may possibly provide other useful information about early life history events. The study of otoliths may prove valuable for getting information of condition, of which major stages which have been passed etc.

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Proceedings of Otolith Workshop held at NMFS La Jolla 1977
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APPENDIX III

Models for Predicting Growth Rate and Age at Yolk Sac Absorption for
Wild Herring Larvae

Prediction Models for Wild Herring Larvae

A.   GROWTH

Model (1) rings = age: plot ring no. with length as a dependent
variable.

estimated growth curve

Clyde 1980 $y = 0.22x + 16.49$

Minch 1980 $y = 0.45x + 10.79$

Clyde 1981 $y = 0.32x + 16.27$

x = age in days, y = length in mm.

Model (2) backcalculation:

$$L_2 - \frac{C + (L_2 - C)R_1}{R_2} = \text{growth rate}$$
$$\text{no. rings}$$

L_2 = larval length at capture

R_1 = otolith radius at first ring formation

R_2 = otolith radius at capture

C = y intercept of GM functional regression.

GM functional regression equations:

Clyde 1980 $y = 0.24x + 12.16$

Minch 1980 $y = 0.44x + 5.14$

Clyde 1981 $y = 0.23x + 14.87$

x = radius in μm, y = length in mm.

Estimated growth curve

Clyde 1980 $y = 0.41x + 9.00$

Minch 1980 $y = 0.64x + 8.00$

Clyde 1981 $y = 0.26x + 9.00$

x = age in days, y = length in mm.

Model (3) direct measurement:

$$\frac{\text{(length at capture)} - \text{(length at hatching)}}{\text{estimated time since hatching}} = \text{growth rate}$$

estimated growth curve

Clyde 1980 $y = 0.23x + 9.00$

Minch 1980 $y = 0.40x + 8.00$
                     *
Clyde 1981 $y = 0.29x + 9.00$

*mean of three measurements 0.23, 0.31, 0.33 mm/day (see p. 63).

$\bar{x} = 0.29 \pm 0.002$.

B. ESTIMATED AGE AT YOLK-SAC ABSORPTION USING DIFFERENT GROWTH MODELS

Assuming a) ring deposition begins at yolk sac absorption

at otolith radius 10.8 μm

b) hatching length for spring-spawned larvae = 9 mm

for autumn-spawned larvae   = 8 mm

c) ring deposition rate = 1 ring/day

$$\frac{\text{(length at first ring formation)} - \text{(length at hatching)}}{\text{growth rate}}$$

= age at yolk sac absorption.

For Clyde 1980

by Model 1): first ring formed at 16.71 mm

$$\frac{16.71 \text{ mm} - 9.00 \text{ mm}}{0.22 \text{ mm/day}} = 35.0 \text{ days}$$

by Model 2): first ring formed at 14.76 mm

$$\frac{14.75 \text{ mm} - 9.00}{0.41 \text{ mm/day}} = 14.0 \text{ days}$$

by Model 3): first ring formed at 11.47 mm

$$\frac{11.47 \text{ mm} - 9.00 \text{ mm}}{0.23 \text{ mm/day}} = 10.74 \text{ days}$$

For Minch 1980

by Model 1): first ring formed at 11.21 mm

$$\frac{11.21 \text{ mm} - 8 \text{ mm}}{0.45 \text{ mm/day}} = 7.1 \text{ days}$$

by Model 2): first ring formed at 9.85 mm

$$\frac{9.85 \text{ mm} - 8 \text{ mm}}{0.64 \text{ mm/day}} = 2.9 \text{ days}$$

by Model 3): first ring formed at 9.85 mm

$$\frac{9.85 \text{ mm} - 8 \text{ mm}}{0.40 \text{ mm/day}} = 4.6 \text{ days.}$$

For Clyde 1981

by Model 1): first ring formed at 16.59 mm

$$\frac{16.59 \text{ mm} - 9 \text{ mm}}{0.32 \text{ mm/day}} = 23.7 \text{ days}$$

by Model 2): first ring formed at 17.33 mm

$$\frac{17.33 \text{ mm} - 9 \text{ mm}}{0.26 \text{ mm/day}} = 32.0 \text{ days}$$

by Model 3): first ring formed at 17.03 mm

$$\frac{17.03 \text{ mm} - 9 \text{ mm}}{0.29 \text{ mm/day}} = 26.7 \text{ days.}$$

IV

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