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LABORATORY EXPERIMENTS ON SOME FACTORS AFFECTING THE SURVIVAL OF MARINE TELEOST LARVAE*

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Introduction

There has been remarkably little precise experimental work upon the conditions necessary for the successful rearing of marine larval fish. The experiments described here were therefore undertaken as part of a study of the biology of the common shore fish *Blennius pholis* L. and *Centronotus gunnellus* (L.) (Qasim, 1956 *a*, *b*, 1957 *a*, *b*). A preliminary report of the initial experiments (Qasim, 1955) has already revealed the necessity of providing suitable conditions, not only of food, but of diurnal changes in illumination. The present account describes another series of experiments subsequently undertaken to investigate the influence of food, light, water agitation and temperature on the survival of larvae of the two species. In addition to these factors, there are observations on the 'gas disease', noticed in various species by many authors and attributed by some to supersaturation of water with gases (Soleim, 1940 ; Dannevig & Dannevig, 1950 ; Henly, 1952).

Material and Methods

Large number of egg masses of *B. pholis* and *C. gunnellus*, generally with advanced stages of embryonic development were collected from between the tide marks by turning over stones and transferred to glass tanks in the laboratory. Here, they were kept well aerated and provided with continuously running sea water. On hatching, fairly equal numbers of larvae were distributed between several similar jars, each containing about 25 litres of sea water. This water was well circulated by streams of air bubbles. Parallel experiments were then set up in which batches of larvae were exposed to different conditions, the influence of which is discussed below. In each series of experiments, conditions other than those under investigation were kept identical in all jars, as far as possible. For example, in experiments dealing with food, changes of water were carried out similarly in all the jars, and in experiments with light, all jars were provided with running sea water. To obtain significant data from each experiment, at least three replicate jars were used for each condition investigated.

Moderate quantities of food were added to the experimental jars, generally twice a day. At the same time the larvae which had died were removed

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and their numbers from each jar were recorded. Most of the experiments were carried out in an underground aquarium room, where the fluctuations in daily temperature were not more than 1°C (usually much less) and the seasonal changes in temperature were only slightly greater than those in the Menai Straits.

Nutritional Value of Some Phytoplankton Cultures

Probably the most important factor in rearing larvae is the provision of suitable food. Until the present work was undertaken there was little evidence available to show to what extent fish larvae can meet their needs for normal growth directly from the phytoplankton. Detailed studies of gut contents of many species of young fish taken in tow-nettings revealed the presence of diatoms and green plant remains which, together with copepods and other Entomostracea, appear to form a considerable proportion of their food (Lebour, 1918-22). Gross (1937) fed young herring on phytoplankton cultures, but the findings of other authors based on observations in nature as well as in aquaria have indicated that phytoplankton is of poor food value to young fishes (Soleim, 1942 ; Dannevig, 1947). Other authors, however, have held that even dissolved organic matter may provide them with some nutrition (Morris, 1955).

In previous experiments (Qasim, 1955) marine fish larvae were observed to ingest large numbers of diatoms and flagellates as a result of the regular swallowing of water which forms part of the mechanism of osmoregulation (Smith, 1932 ; Keys, 1933). The survival rate of *Blennius pholis* larvae, however, in jars containing *Chlamydomonas* sp. and *Isochrysis galbana* Parke, two flagellates, generally considered to be valuable food organisms to invertebrate larvae, was only slightly greater than those kept in jars without food (Qasim, 1955).

To investigate further the relative importance of these food organisms, this experiment was first repeated by offering the same two flagellates (*Chlamydomonas* and *Isochrysis*) in a mixed state to 50 larvae of another shore fish, *C. gunnellus*. The same number of larvae was kept in each of three similar jars without food. To ensure, as far as possible, that the larvae did not get any extraneous food, the sea water used in all six jars was previously passed through sintered glass filters. The survival rate of the total number of larvae under these two conditions is shown by the curves A and B in Fig. 1. It can be seen that the larvae fed on flagellates showed slightly better survival than those kept without food. The difference seemed significant, for there was good agreement between replicates of both experimental and control jars. When in a later group of experiments each of the two species of flagellates were offered separately, the survival rate was again better with flagellates, the difference being slightly more marked in jars containing *Isochrysis* (Fig. 2, A, B and C). *Isochrysis* has previously been shown to be a very good food for oyster larvae (Port Erin *Flagellate* I in Bruce, Knight & Parke, 1940).

A comparison of the survival of the larvae kept without food, as controls in the two experiments, will indicate that those of the later experiment survived slightly longer than those of the earlier one. This is probably because the eggs of *C. gunnellus*, when collected with advanced embryos late in the breeding season, begin to hatch immediately. Since this hatching is probably due to some mechanical stimulus, such as shaking, as in *B. pholis*, (Qasim, 1956b), it is probable that the larvae used

in the latter experiment had greater supplies of yolk left, which enabled them to survive longer.

Further investigations on the food value of flagellates with larvae of *B. pholis* gave similar results (Fig. 3, A, B and C), the larvae showing slightly better

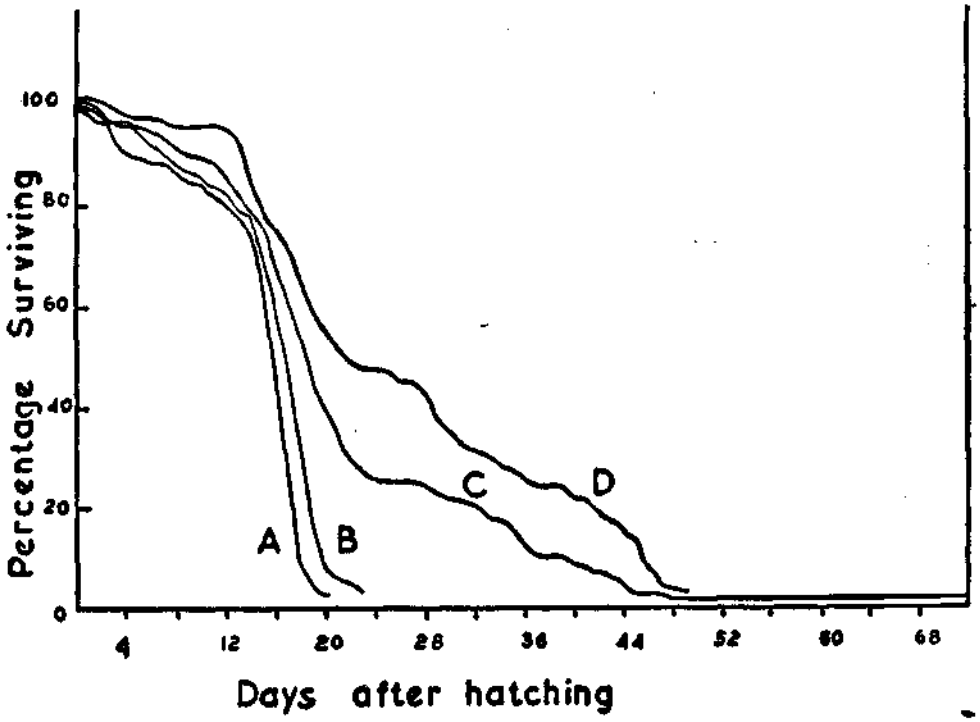

FIG. 1. Percentage of larvae of *C. gunnellus* surviving at the end of each day, when kept under different conditions of food. All larvae were given continuous illumination. A, without food; B, with flagellates; C, with *Artemia* nauplii; D, with *Artemia* nauplii and flagellates.

survival when fed on *Isochrysis*. The consistency in the results of all these experiments clearly suggests that the flagellates have some slight value as food, but the effect is not conducive to much greater longevity.

Larvae fed on nauplii of *Artemia* or *Balanus* survived much longer. They grew fairly rapidly and a small percentage continued to survive until metamorphosis. Each species, when young, showed its own characteristic behaviour of catching the nauplii. Larvae of *B. pholis*, which have no external yolk sac, generally began feeding on nauplii within 24 hours after hatching, whereas larvae of *C. gunnellus*, which have a small external yolk sac, did not start feeding until about 2-3 days old.

The question then arose, as to whether the survival rate would be improved by the initial ingestion of flagellates, as a result of osmoregulation, before feeding

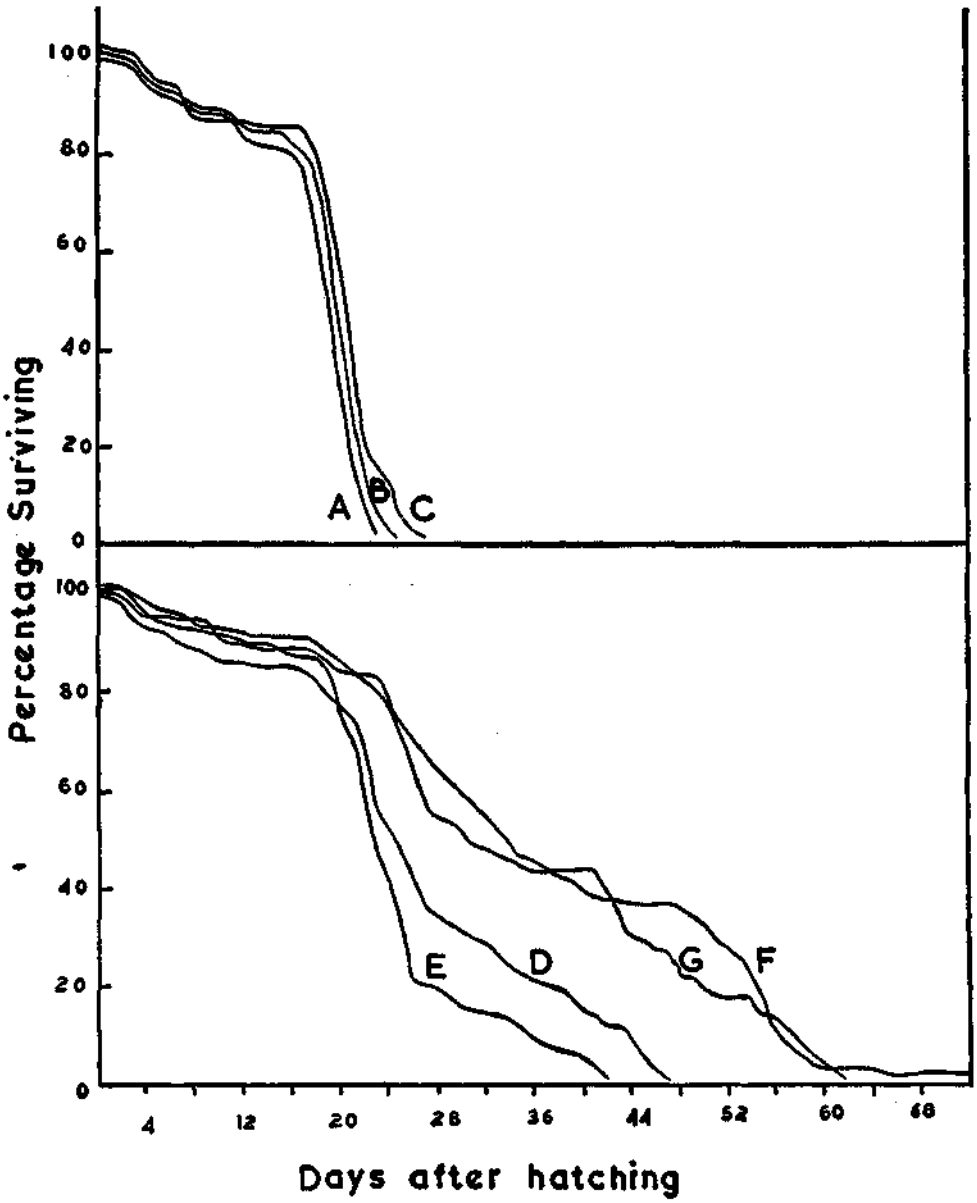

FIG. 2. Percentage of larvae of *C. gunnellus* surviving at the end of each day, when kept under different conditions of food and illumination. A, without food, continuous light; B, with *Chlamydomonas*, continuous light; C, with *Isochrysis*, continuous light; D, with *Artemia* nauplii, continuous light; E, with *Artemia* nauplii and flagellates, continuous light; F, with *Artemia* nauplii, alternate light and darkness; G, with *Artemia* nauplii and flagellates, alternate light and darkness.

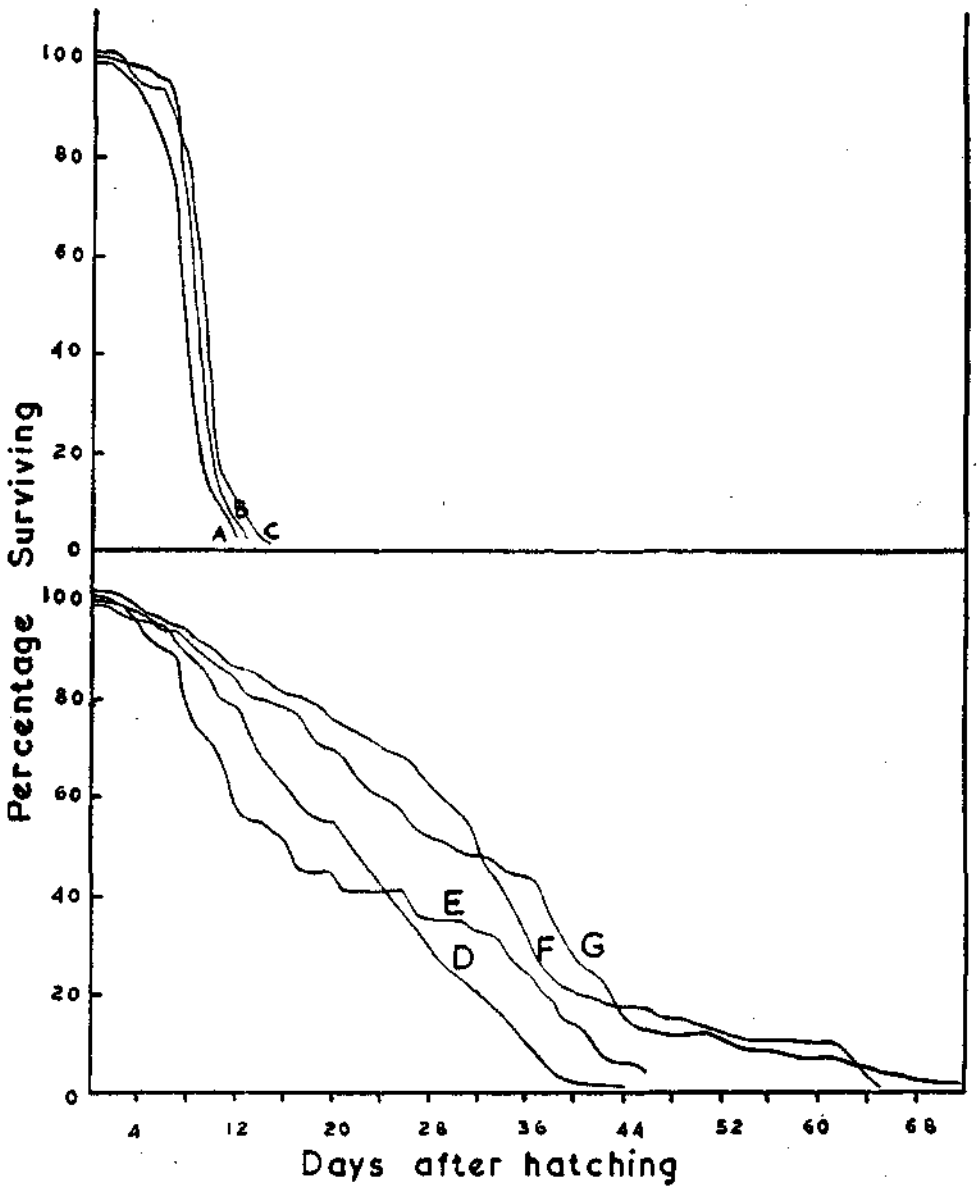

FIG. 3. Percentage of larvae of *B. pholis* surviving at the end of each day, when kept under different conditions of food and illumination. A, without food, continuous light; B, with *Chlamydomonas*, continuous light; C, with *Isochrysis*, continuous light; D, with *Artemia* nauplii, continuous light; E, with *Artemia* nauplii and flagellates, continuous light; F, with *Artemia* nauplii, alternate light and darkness; G, with *Artemia* nauplii and flagellates, alternate light and darkness.

on nauplii began. This was investigated by feeding the larvae, from hatching onwards, with a mixture of *Artemia* nauplii and flagellates. Controls were fed with *Artemia* nauplii alone. In the first series of experiments, larvae of *C. gunnellus* fed on such a mixed diet showed somewhat better survival than those fed on *Artemia* nauplii alone (Fig. 1, C and D). But later more detailed experiments failed to confirm this (compare survival curve E with D, and G with F, in Fig. 2). Similarly, with larvae of *B. pholis*, mixing flagellates with nauplii used as food produced no significantly better survival (Fig. 3, E and D ; G and F). Evidently flagellates are of very little direct food value to these fishes.

Diurnal Changes in Illumination

Other factors including light are of importance in rearing. The influence of these factors upon the survival of cod larvae has been investigated fairly extensively by Dannevig (1932) and by Dannevig & Sivertsen (1933), while Shelbourne (1953) has shown that young plaice take food only in the light.

It soon became evident from early experiments that *B. pholis* behaved similarly ; in complete darkness it stopped feeding, became inactive and eventually died, presumably from starvation. If, after being kept in the dark for a while, the larvae were again exposed to light, they became very active, and showed more intensive feeding on nauplii than did larvae which had been kept continuously under illumination. By giving alternate periods of light and darkness, it was possible to obtain better survival rates throughout the experiment than by giving continuous illumination (Qasim, 1955). Further experiments on the influence of light on the survival of *B. pholis* abundantly confirmed this. Several jars containing larvae of *B. pholis* were exposed to 7-8 hours of light during the day and were covered with thick black cloth during the night. The same number of jars were kept under continuous light. During illumination, the sources of light were fluorescent tubes in both cases. The survival rate of the total number of larvae contained in all jars under each of these two conditions is shown in Fig. 3. The two curves D and E in Fig. 3 represent two different kinds of food, *Artemia* nauplii and *Artemia* nauplii plus flagellates respectively, kept under continuous illumination, whereas F and G represent similar food conditions, kept under alternating periods of light and darkness. On comparing the survival curves, it will become evident that, under both food conditions, the larvae given alternate periods of light and darkness survived better than those kept under continuous illumination. Similar results under these conditions of food and light were obtained with larvae of *C. gunnellus* (Fig. 2, D, E, F and G).

Water Agitation

In early experiments glass plunger plates were used to stir the water, as advocated by Garstang (1900) and Lebour (1923), but heavy mortalities followed and it seemed that larvae were being injured by sharp edges of the plates. It was not thought advisable to continue plunger-jar experiments with smoother plates, since observations on the behaviour of the larvae suggested that they were unfavourably disturbed whenever the plungers were set in motion. The air bubbles, used for stirring thereafter, seemed to distrub the larvae far less. The steady circulation of water set up allowed the nauplii to congregate in certain places, generally nearest the light, where

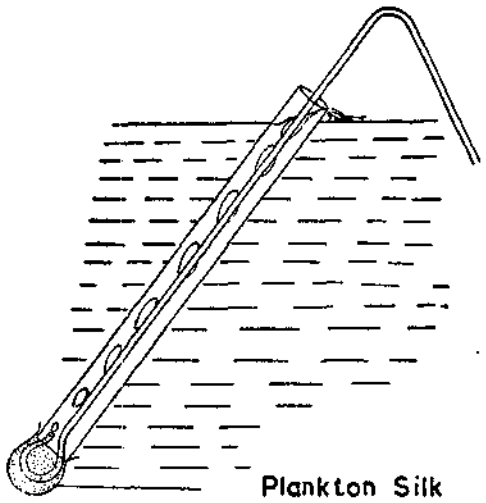

FIG. 4. A closed bubbler which consists of a narrow glass tube enclosed by a wider tube resting on the curved lower end of the former. The lower end is covered with plankton silk.

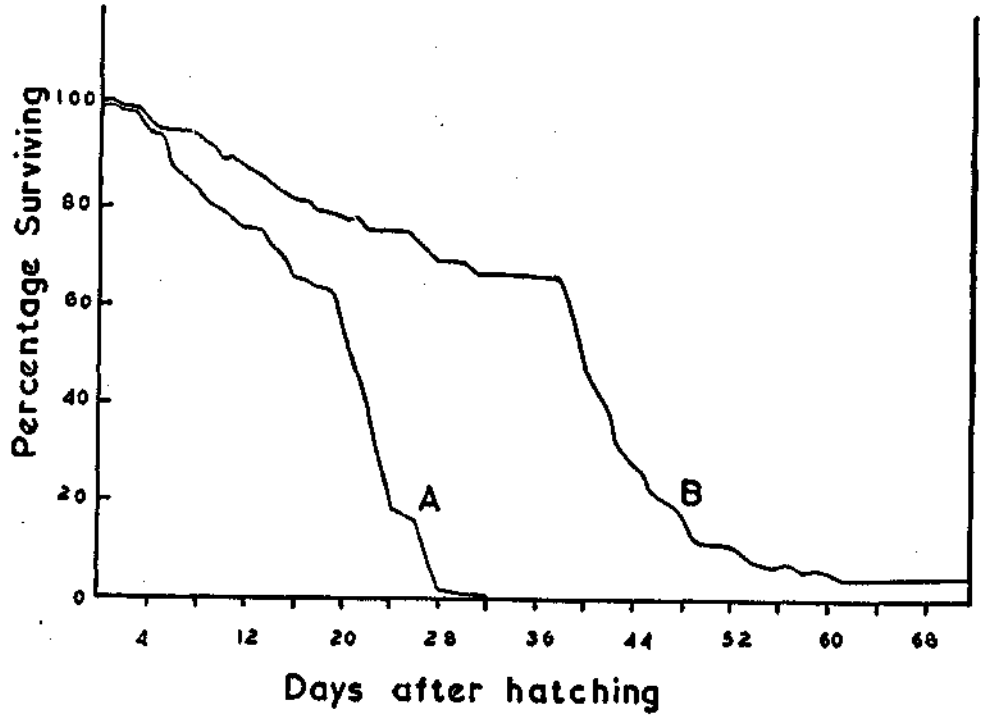

FIG. 5. Percentage of larvae surviving at the end of each day, when kept in jars provided with two different methods of air bubbling. A, closed bubblers; B, open bubblers.

they were easily caught by the fish larvae. Occasionally, however, larvae would be caught in the streams of air bubbles and carried upwards rather violently.

To test the possibility that such occasional disturbance by the bubbles might be unfavourable, the following experiment was carried out with larvae of *B. pholis*. A bubbling method was devised, which caused very little disturbance but gave apparently adequate circulation of water. Compressed air was blown into the water down a narrow glass tube with a recurved lower end. This tube was totally enclosed in a wider tube, which had its lower end covered with plankton silk to prevent entry of larvae. The open upper end of the wide tube projected about one inch above the water level in the jar. In operation, water was drawn from the bottom of the jar into the lower end of the wide tube, where aeration took place, and the aerated water trickled steadily from the upper end under the propulsion of the rising bubbles of air (Fig. 4). These special aerators were called 'closed bubblers' to distinguish them from the ordinary 'open bubbler' which allowed the larvae direct access to the rising stream of air bubbles.

Five jars with about 30 larvae in each were provided with such closed bubblers and the same number were kept bubbled rather hard with open bubblers. Alternate day and night illumination was given to all jars and *Artemia* nauplii were provided as food. The survival rate of the total number of larvae under each of these two conditions is shown in Fig. 5 (A and B). There was good agreement between the replicates.

It can be seen from Fig. 5 that in the jars provided with open bubblers, the larvae survived much longer than those bubbled by the closed method. They also grew much more quickly. Ordinary bubbling would appear to be not only the simplest method of aeration, but also the best.

Temperature Tolerances of Larvae and Embryos

Previous authors have shown that the ability of adult fish to resist extreme ranges of temperature varies greatly from species to species (Britton, 1924 ; Hathaway, 1927; Doudoroff, 1942). Such specific differences in the temperature tolerances of fish and of other poikilothermal animals may explain differences in their geographical distributions and breeding ranges (Orton, 1920 ; Runnstrom, 1927). There have not been many precise experiments, however, on the tolerances of eggs and larvae. These are likely to be more sensitive to extreme conditions than the adults are. Conclusions of great ecological significance are likely to emerge from experiments dealing with the temperature tolerances of fish. It is also of importance to have the knowledge of the optimum conditions of temperature, if a species is to be kept safely in the laboratory.

The experiments on which the following conclusions are based dealt mostly with the survival time of the larvae kept at constant temperatures. Equal numbers of larvae were kept in jars, each of which was provided with a thermostatically controlled heating arrangement. For maintaining constant temperatures below 10°C, some such jars were kept in a culture cabinet, running at 0-1°C, which had a large glass window. All jars had similar natural illumination. The jars were supplied with *Artemia* nauplii and kept well aerated by bubbling compressed air. Before

the larvae were transferred into various jars, each jar was filled with fresh sea water at the exact temperature at which the larvae were originally kept. After the transfer of the larvae, the refrigeration and heating in all the jars were switched on. The larvae in this way were subjected to changes in temperature which were only gradual and which took between 12 to 16 hours to reach the levels desired for the experiment. When these levels had been reached, the experiment was considered to have begun. The larvae which died were removed twice a day and their total numbers from each jar were counted after every 24 hours.

The percentages of larvae of *C. gunnellus* surviving at various temperatures during the first seven days of an experiment are given in Table I. On the eighth day practically all the fish died, probably because of a cut in electric power and refrigeration failure, so this experiment had to be discontinued. Differences in survival rates between the various batches had already become clear however. Both 15° and 20°C eventually proved lethal to this species. The optimum temperature seemed to be about 5°C. At lower temperatures it is not certain that the larvae were very adversely affected. Even at 0-1°C practically all remained healthy for the first few days, but later some began to show signs of inactivity and considerable numbers died. This mortality, however, may well have been due to starvation, as these low temperatures were beyond the tolerance limit of the *Artemia* nauplii which were given as food.

TABLE I

Percentage of larvae of *C. gunnellus* surviving at the end of each day, when kept at various constant temperatures. (The experiment was unfortunately terminated after 7 days.)

Number of days.	Temperatures					
	0-1° C	3° C	5° C	10° C	15° C	20° C
1	98	96	100	100	92	90
2	96	94	96	94	82	50
3	80	88	96	92	40	8
4	78	84	94	88	10	0
5	74	80	92	88	5	
6	72	76	90	86	0	
7	70	76	86	84		

A similar experiment with larvae of *B. Pholis* was carried through to completion without accident, but pressure of other work did not allow a complete record of the daily mortality at each temperature to be maintained. The results of this experiment are therefore shown in Table II as the maximum survival times at the various temperatures. It will be seen that all low temperatures up to 5°C were rapidly lethal (Table II). The larvae showed signs of distress as soon as or even before the required temperatures were reached and all larvae kept at 0°, 2° and 5°C died within 48 to 72 hours. At the lethal high temperature (30°C), there was no sign of shock at first. Some larvae began feeding at this temperature and continued to do so until they started dying in multitudes, their maximum survival time being 4 days.

TABLE II

Maximum survival time of the larvae of *B. pholis* at each constant temperature.

Temp.	0-1° C	2° C	2° C	5° C	23-25° C	30° C
Survival in days.	2	2	3	36	25	4

Similar experiments were made on embryos of *C. gunnellus*. Egg masses, with embryos clearly visible to the naked eye, were collected early in the breeding season. Each mass was then carefully broken into several portions and the portions from each mass were distributed between various constant-temperature jars. Within a few days after the commencement of the experiment, it became evident that the eggs kept at 15° and 20°C were turning opaque, and after 10-15 days most of the eggs at these temperatures were dead. On examining under a microscope, the dead eggs were found to be attacked by fungus. At temperatures 0-1°, 3°, 5° and 10°C, the eggs remained healthy for about 20 days, but eventually all died because of fungal infection. Under such conditions, it is difficult to say whether temperature alone was responsible for causing an early mortality at 15° and 20°C. It is of course possible that growth of fungus was encouraged more at higher temperatures than at low temperatures. Embryos of *C. gunnellus* proved to be able to withstand freezing, for an egg mass was kept in a deep freeze cabinet until it was frozen solid. On thawing, the embryos were found to be healthy and showing their characteristic movement inside the egg capsule. Other authors have shown that adult fish of some species are capable of surviving, after being frozen solid for a long time (Borodin, 1934).

These studies, therefore, show that the larvae and possibly the embryos of *C. gunnellus* are extremely intolerant of higher temperatures, but are resistant to low temperatures. Larvae of *B. pholis*, on the other hand, are intolerant of low temperatures, but can withstand comparatively high temperatures. These differences are associated with differences in the geographical distribution and breeding habits of the two species. *C. gunnellus* is an Arctic species which in European waters reaches its southern limits in the English Channel. Since its eggs and larvae require low temperatures, the fish breeds during the coldest time of the year (January-March). This short breeding season, occurring at the same time throughout the geographical range, is probably an adaptation which enables the larvae to take advantage of the short Arctic season of plankton production (Qasim, 1956a, 1957b). *B. pholis*, on the other hand, being a southern species (Mediterranean-boreal), breeds over a prolonged period during spring and summer (Qasim, 1956b, 1957a). Its larvae are not exposed, therefore, to the low temperatures which they cannot tolerate.

GAS DISEASE

It was often noticed that some of the larvae of *B. pholis* developed ' gas disease ' and died. The larvae showed symptoms similar to those described earlier in the young cod (Henly, 1952). They became buoyant, and though they made repeated attempts to swim down, they generally remained for the most part floating near the surface until they died, generally a day or two after the onset of the condition. On examination, their swim bladders were found to be unusually enlarged, apparently as a result of excessive gas secretion. Cases of gas disease were not very frequent, but occurred in most of the jars, and generally started when the fish were almost

one month old. The majority of larvae which died showed no sign of gas disease, but gradually turned pale.

The larvae of *C. gunnellus*, which lack swim bladders, showed no sign of gas disease. Larvae which died developed opaque spots, generally appearing first in the posterior region of the body, and soon spreading all over it. Such larvae, when examined, showed no gas bubbles either externally or internally.

It seemed likely that the gas disease of *B. pholis* was due to malfunctioning of the gas gland associated with the swim bladder, but it is uncertain whether the freedom from gas disease in *C. gunnellus* was due to the absence of these organs or whether it was due to the lower temperatures prevailing in the season during which the larvae were reared. The warmer weather later in the summer, when *B. pholis* were being reared, may well have caused the water, drawn from the cooler sea, to be supersaturated with gases.

Summary

The survival rates of larvae of *Blennius pholis* and *Centronotus gunnellus* have been studied in the laboratory, under different experimental conditions. Survival of larvae in jars containing *Chlamydomonas* and *Isochrysis* was slightly better than in jars without any food. The flagellates, however, seemed of very little food value, prolonging life only for two or three days. Larvae fed on flagellates together with *Artemia* nauplii survived no better than larvae fed on nauplii alone.

Larvae under alternate periods of light and darkness became more active, grew more quickly and survived longer than those kept under continuous illumination. The moderate agitation of the water caused by an unconfined stream of air bubbles was shown to have a beneficial effect on growth and survival and was found to be preferable to the use of plungers. A small proportion of the larvae of both species were reared until fully metamorphosed.

When kept at constant low or high temperatures, larvae of *C. gunnellus* (an arctic-boreal species) withstood 0° to 10°C, but soon died at 15° and 20°C. Larvae of *B. pholis* (a mediterranean-boreal species) were killed at 0° to 5°C, survived at higher temperatures of up to 25°, but soon died at 30°C.

Some larvae of *B. pholis* died of gas disease, their swim bladders being greatly distended. No cases of gas disease were seen in *C. gunnellus*, a species which lacks a swim bladder.

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