Marine Fish Culture in Britain VI. The Effect of the Acclimatization of Adult Plaice to Pond Conditions on the Viability of Eggs and Larvae<br>By<br>A. B. Bowers<br>University of Liverpool<br>Marine Biological Station, Port Erin, Isle of Man

The value of acclimatization to fish-farm conditions of captive spawning stocks of marine flat fish was investigated by hatching and rearing to metamorphosis newly spawned eggs from plaice (Pleuronectes platessa L.) which had been kept in captivity for several months, and from plaice caught just before spawning. Survival was higher at all stages from egg to metamorphosis in the fish reared from eggs produced by the acclimatized stock, with an average of 206 metamorphosed fish per thousand eggs. The production from unacclimatized stock was 44 metamorphosed fish per thousand eggs. Biometric data are given for the fish reared.

A very cold winter inhibited spawning of a captive stock of plaice in 1963.

## Introduction

A series of experiments at Lowestoft (England) and Port Erin (Isle of Man) have indicated that mass culture of metamorphosed plaice in hatcheries is an attainable objective (Shelbourne, Riley and Thacker, 1963; Shelbourne, 1963, 1964; Riley and Thacker, 1963). Collection at sea of sufficient plaice eggs to stock a hatchery would be impracticable, and it is considered that very large numbers of eggs could be obtained only from a captive spawning stock of adult fish. It has been known for many years that plaice kept in marine ponds will produce viable eggs (Herdman, 1904) and there is a long tradition of production and hatching of plaice eggs at Port Erin. In 1960 Shelbourne (1963) successfully reared to metamorphosis larvae hatched from eggs produced by two stocks of captive plaice maintained by the Marine Biological Station. Shelbourne's 1963 experiment gave no clear indication of a relation between survival rates of eggs and treatment of spawning stock; he considered that the hazards of tank life under the technique then employed may have masked stock differences.

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Figure 1. Temperature of marine ponds at Port Erin in 1961-62 (solid line) and 1962-63 (broken line).

My attempts in 1961 to rear eggs from different stocks in Dannevig boxes and wooden incubators were unsuccessful, but in 1962 an experiment was carried out using glass incubators equipped to Shelbourne's design with results described below.

## Material and Methods

One hundred and twenty-one adult plaice were trawled in November, 1961, from coastal waters of the Isle of Man and carefully transported to the laboratory in keep tanks constantly refreshed with seawater. The fish were placed in a rectangular rock and concrete pond 15.7 m long by 9.4 m wide holding 3,270 hectolitres of clean seawater pumped from Port Erin Bay through cast iron pipes. The maximum depth of water in the pond was 2.6 m . The water was refreshed periodically by part draining the pond, so that the water level fell about 0.6 m , and pumping in clean seawater.

Twice a week the fish were fed on chopped raw herring flesh, boiled shelled mussels (Mytilus edulis) or worms (mainly Arenicola marina) dug from local beaches. They took food readily until the end of December when feeding virtually stopped; the pond temperature was then $2^{\circ} \mathrm{C}$ (Figure 1).

At the end of January 118 of these fish ( 50 male, 68 female) were transferred to a similar but smaller pond 11.7 m long by 9 m wide with a maximum depth of 2.6 m , holding 2,360 hectolitres to allow cleaning of the larger pond. The fish resumed feeding two weeks later; they started spawning on or about 10. February when eggs were first seen in the pond. This stock will be called the pond-wintered stock.

The larger pond was cleaned of all weed growth by scrubbing with fresh water and refilled with clean seawater. Ripe or near-ripe adult plaice ( 64 males and 75 females) trawled near the Isle of Man on various dates between 20. February and 6. March, 1962, were brought to the laboratory as before and put into the pond within six hours of capture. This stock will be called the sea-wintered stock. At least some of the fish started feeding within a few days of transfer to the pond. Sea temperatures in the trawling area were $6.3^{\circ} \mathrm{C}$ at
the beginning of the trawling period and $5 \cdot 2^{\circ} \mathrm{C}$ at the end. The pond temperatures over the period varied between $6^{\circ} \mathrm{C}$ and $2^{\circ} \mathrm{C}$; most of the fish were subjected to a rapid temperature change of 2 to $3^{\circ}$ (the difference between sea and pond temperatures). There was some shock-spawning of ripe and unripe eggs on the night after the fish were put in the pond; subsequent sampling of eggs showed that most of the shock-spawned eggs died. All newly spawned eggs sampled from the pond on and after 8. March (two days after the last addition of adult plaice to the pond) appeared to be healthy.

Eggs for the experiment were netted from either pond by skim-net and brought into the hatchery in a glass tank containing pond water. After about an hour, eggs damaged by the netting had settled to the bottom of the tank. Small quantities of the floating eggs were dipped out with a finger bowl from which the required stages were selected and pippetted out into a second bowl of clean seawater until 100 eggs had been counted; these were then poured gently into an incubator and the process repeated until the incubators were each stocked with 1,000 eggs.

The incubators were stocked as follows:-

| Row | Position <br> in Row | Date | Temperature <br> ${ }^{\circ} \mathrm{C}$ | Number <br> of eggs | Stage <br> of eggs | Parent <br> Stock |
| :---: | :---: | :---: | :---: | :---: | :--- | :--- |
| A | 1 | 15. March | $6 \cdot 2$ | 1,000 | early | sea-wintered |
| A | 2 | 29. March | $7 \cdot 8$ | 1,000 | mid | pond-wintered |
| A | 3 | 2. April | $7 \cdot 4$ | 1,000 | mid | sea-wintered |
| A | 4 | 15. March | $6 \cdot 2$ | 1,000 | early | pond-wintered |
| A | 5 | 15. March | $6 \cdot 2$ | 1,000 | early | sea-wintered |
| A | 6 | 30. March | $7 \cdot 8$ | 1,000 | mid | pond-wintered |
| B | 1 | 15. March | $6 \cdot 0$ | 1,000 | early | pond-wintered |
| B | 2 | 2. April | $7 \cdot 0$ | 1,000 | mid | sea-wintered |
| B | 3 | 30. March | $7 \cdot 7$ | 1,000 | mid | pond-wintered |
| B | 4 | 15. March | $6 \cdot 4$ | 1,000 | early | sea-wintered |
| B | 5 | 15. March | $6 \cdot 3$ | 1,000 | early | pond-wintered |
| B | 6 | 2. April | $7 \cdot 3$ | 1,000 | mid | sea-wintered |

Early eggs were stage 1 or 2 of APSTEIN's (1909) classification and mid-stage eggs were stage 7 to 9 .

Incubators were similar to those described by Shelbourne (1963). There was no direct control of temperature; a header tank supplied filtered seawater which could be cooled to a desired temperature but not warmed. Water flow was adjusted so that there was an exchange equal to the volume of each incubator every 24 hours; outflow water from the incubators ran to waste. Typical incubator water temperatures during the experiment are shown in Figure 2; the temperatures were similar in all incubators on the same day and none was consistently warmer or colder than others but differences in dates of stocking the incubators meant that eggs in experiments started late in the season passed through early stages of development at higher temperatures than those started early.

Top light was provided for 12 hours a day by fluorescent strip lights suspended well above the incubators. Midwater readings with an Eel underwater lightmeter showed approximately 700 lux in the centre of the incubators and 450 lux near the sides; there was a small variation from tank to tank.

The maintenance routine described by Shelbourne (1963) was followed. After the eggs hatched, the incubators served as rearing tanks; the larvae were


Figure 2. Seawater temperature in incubators 1962.
not moved to other tanks at any time during the experiment. Daily feeding with live nauplii of Artemia salina was started four days after hatching; feeding rates were adjusted to maintain a slight surplus of food in each tank as far as possible; there were short periods while food demand was changing rapidly when this was not achieved. Dead food material and dead plaice eggs and larvae were gently removed from the tanks by means of a pippette at least every other day.

Early stage eggs hatched in 15 days and were maintained for a further 75 days, by which time the larvae had metamorphosed. Mid-stage eggs hatched in 8 to 11 days and were maintained for a time calculated to give a product of ${ }^{\circ} \mathrm{C} \times$ days after hatching equivalent to that for the early eggs. At the end of the experiment the metamorphosed plaice were killed and preserved in $5 \%$ seawater-formalin and later counted, measured and examined.

Survival curves were plotted for all tanks, the number of eggs or fish surviving at any time being estimated by subtracting the cumulative number of known deaths from the initial stock. Final counts of fish in each tank showed that the method was not accurate, but it probably gave a reasonable approximation to the true survival curve. Inaccuracies probably arose as a result of rapid decay of very young larvae after death and consequent failure to see them on the tank floor, from scavenging by some fish which picked up and ate debris from the tank floor; and from cannibalism, which was occasionally observed in tanks with large size differences between fish. Counts of dead eggs were easy to make and were probably reliable. The greatest inaccuracies are thought to have occurred in the period 12 to 20 days after hatching; mortality was then high in larvae which had failed to feed; these fish were very thin, and difficult to see alive or dead.

## Results and Discussion

## Survival

Early eggs. Figure 3 shows the estimated number of eggs and larvae surviving at two day intervals throughout the experiment. There was a marked difference between stocks in early egg mortality; $45 \%$ to $50 \%$ of the seawintered eggs died before hatching compared with $6 \%$ to $13 \%$ of the pond-


Figure 3. Estimated number of surviving eggs and larvae from pond-wintered stock (solid circles) and sea-wintered stock (open circles) in incubators stocked with early eggs. Coincident points are plotted with one symbol only.
wintered eggs. Regular sampling of eggs left in the spawning ponds confirmed that there was heavy mortality among early eggs from the sea-wintered stock.

Hatching failure accounted for 8 to $9 \%$ of the surviving stock of sea-wintered eggs, but the failure rate in pond-wintered eggs varied in different tanks at $3 \%, 10 \%$ and $15 \%$. For 13 days after hatching there was steady mortality in both stocks; that in the tanks stocked with pond-wintered eggs showed more variation between tanks but on average the percentage loss was similar to that of the sea-wintered stock. There was heavy mortality in all tanks from the 13th to the 25th day after hatching; examination of the dead larvae showed that they had failed to feed, and had used up all the reserve of food available

Table 1
Plaice reared from eggs of different stocks; number hatched,
Egg stage
Stock
Tank number
Number of eggs
Days in tank
Degree-days from hatching
Number hatched
Estimated number feeding
Number surviving at end of experiment
\% of hatched larvae feeding
\% of hatched larvae surviving to end of experiment
\% of feeding larvae surviving to end of experiment

Early
Pond-wintered

| B 1 | A 4 | B 5 | Mean |
| :--- | ---: | ---: | ---: |
| 1,000 | 1,000 | 1,000 |  |
| 90 | 90 | 90 |  |
| 771 | 775 | 779 |  |
| 923 | 798 | 827 | 849 |
| 441 | 340 | 277 | 353 |
| 301 | 203 | 114 | 206 |
| 48 | 43 | 33 | 42 |
| 33 | 25 | 14 | 24 |
| 68 | 60 | 51 | 58 |

from the yolk sac. After the 25 th day mortality eased and there was a good survival of established feeders in the pond-wintered stock (Table 1); survival of feeding larvae of the sea-wintered stock was markedly poorer.

Mid-stage eggs. About $16 \%$ of the sea-wintered eggs died before hatching compared with $5 \%$ of the pond-wintered eggs. Mortality thereafter was similar on average in the two stocks but there was a fairly wide variation between individual tanks (Table 1): The greatest mortality occurred 11 to 17 days after hatching; deaths of non-feeding larvae occurred sooner after hatching than in the tanks stocked with early stage eggs because tank temperatures were higher.

The data in Table 1 and Figure 3 indicate that there were differences in viability between eggs from the two adult stocks; the differences showed principally in the egg and newly hatched stages. Despite large variations between tanks with the same stock and the same treatment it is clear that the pond-wintered eggs gave a higher survival from spawning to metamorphosis than did the sea-wintered eggs. The implication for large-scale hatchery practice is that long-term maintenance of a captive spawning stock or at least collection of adults well before spawning is advisable; this view was held by Dannevig (1895). The disadvantage of overwintering a spawning stock in outside ponds is that severe weather such as was experienced in 1947-48 and 1962-63 may inhibit spawning (Smith, 1948). In 1963 pond temperatures were below $2^{\circ} \mathrm{C}$ for many days in January and February (Figure 1) and a stock of 80 adult plaice overwintered in the ponds produced only a few thousand eggs. Very few females spawned at all, and none of these spawned completely. Dissection and examination of partly spent fish showed that there was a mass of eggs breaking down in the lumen of the ovary. Thirty-eight fish had died. In a large hatchery some control of water temperature would be necessary to ensure an adequate supply of viable eggs every year regardless of weather conditions; it would probably be sufficient to keep the temperature above $2^{\circ} \mathrm{C}$ at all times.

## Characteristics of reared plaice

The plaice reared to metamorphosis showed a wide variation in length of individual fish within any tank and in mean length of fish between tanks (Shelbourne, 1964). Abnormal pigmentation, characteristically taking the
number feeding and survival beyond metamorphosis

| Early Sea-wintered |  |  |  | Mid <br> Pond-wintered |  |  |  | Mid a-wintered |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| B 4 | A 5 | A 1 | Mean | A 2 | A 6 | B 3 | Mean | B 6 | B 2 | A 3 | Mean |
| 1,000 | 1,000 | 1,000 |  | 1,000 | 1,000 | 1,000 |  | 1,000 | 1,000 | 1,000 |  |
| 90 | 90 | 90 |  | 83 | 83 | 83 |  | 82 | 82 | 82 |  |
| 773 | 775 | 772 |  | 761 | 762 | 766 |  | 797 | 781 | 793 |  |
| 483 | 499 | 519 | 500 | 962 | 968 | 943 | 956 | 872 | 878 | 768 | 840 |
| 181 | 177 | 183 | 180 | 479 | 405 | 471 | 452 | 353 | 409 | 303 | 355 |
| 52 | 52 | 29 | 44 | 201 | 150 | 125 | 159 | 155 | 133 | 79 | 122 |
| 37 | 35 | 35 | 36 | 50 | 42 | 50 | 47 | 40 | 47 | 40 | 42 |
| 11 | 10 | 6 | 9 | 21 | 15 | 13 | 17 | 18 | 15 | 10 | 14 |
| 29 | 29 | 16 | 24 | 42 | 37 | 27 | 35 | 44 | 33 | 26 | 34 |


| Treatment of parent stock |  |
| :---: | :---: |
| Stage of eggs put into incuba |  |
|  |  |
| Number of fish reared |  |
| Mean length mm |  |
| Length range mm |  |
| Upper surface pigment: $\%$ of fish at each grade | Normal |
|  | Over 90- |
|  | 50-90\% |
|  | 10-50\% |
|  | less than $10-$ |
| Lower surface pigment: up to $50 \%$ |  |
| $\%$ of fish with pigment over |  |
|  |  |
| of fish with eyes on left side |  |


| Characteristics |  |  |  |
| :---: | :---: | :---: | :---: |
| Early |  |  |  |
| Pond-wintered |  |  |  |
| A $_{4}$ | B $_{5}$ | Mean |  |
| 203 | 114 | 206 |  |
| 13.0 | 13.6 | 13.0 |  |
| $9-18$ | $10-20$ |  |  |
| 32 | 56 | 34 |  |
| 6 | 6 | 6 |  |
| 13 | 10 | 10 |  |
| 21 | 6 | 19 |  |
| 28 | 21 | 31 |  |
| 35 | 38 | 37 |  |
| 7 | 9 | 8 |  |
| 44 | 10 | 34 |  |
| 4 | 1 | 2 |  |

form of one or two unpigmented areas of varying size on the upper surface, was very common and a considerable proportion of the fish showed some pigmentation of the under surface, which is normally white. Many fish had their tail fins bitten by other fish in the tank. Data on length, pigmentation and tail fin biting in the fish that survived to the end of the experiment are shown in Table 2. It is not clear from the data how far variation between tanks in mean length of fish, incidence of abnormal pigmentation and incidence of bitten tails was due to differences in egg stock and how far it was due to the density and size-distribution of surviving fish in each tank. There is some indication, for example, that the mean length of a tank population varies with population density and that pigment cover and tail biting are to some extent dependent on the size of each fish relative to that of all the other fish in the same tank. Further experiments have been carried out to investigate these points and the results will be published.

## Summary

1. Two stocks of adult plaice (Pleuronectes platessa L.) spawned in captivity in large seawater ponds constructed in rock and concrete.
2. One stock was acclimatized in a pond for a period of four months before spawning; the other stock was collected at sea at the beginning of the spawning season.
3. Eggs collected from each stock were hatched in incubators and reared to metamorphosis. Acclimatized stock produced eggs which gave a mean survival to metamorphosis of $20 \%$. Survival from unacclimatized stock was $4 \%$.
4. Differences in viability were most marked in early stages of development of eggs and larvae.
5. Biometric data on the plaice reared are briefly reported.
6. Severe winter conditions in 1962-63 inhibited spawning in a captive stock of adult plaice.
of fish reared

| Early Sea-wintered |  |  |  | Mid Pond-wintered |  |  |  | Mid Sea-wintered |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathrm{B}_{4}$ | $\mathrm{A}_{5}$ | $\mathrm{A}_{1}$ | Mean | $\mathrm{A}_{2}$ | $\mathrm{A}_{6}$ | $\mathrm{B}_{3}$ | Mean | B6 | $\mathrm{B}_{2}$ | $\mathrm{A}_{3}$ | Mean |
| 52 | 52 | 29 | 44 | 201 | 150 | 125 | 159 | 155 | 133 | 79 | 122 |
| 14.5 | 13.0 | 14.4 | 13.9 | $13 \cdot 8$ | $14 \cdot 3$ | $13 \cdot 9$ | 14.0 | 13.4 | $13 \cdot 8$ | $13 \cdot 5$ | 13.6 |
| 10-21 | 9-19 | 10-21 |  | 8-20 | 8-23 | 10-21 |  | 8-21 | 8-21 | 8-21 |  |
| 31 | 13 | 24 | 22 | 21 | 27 | 21 | 23 | 23 | 41 | 18 | 28 |
| 6 | 15 | 17 | 12 | 28 | 13 | 38 | 26 | 18 | 15 | 23 | 18 |
| 19 | 37 | 31 | 29 | 21 | 19 | 19 | 20 | 18 | 20 | 26 | 20 |
| 35 | 23 | 7 | 25 | 9 | 15 | 2 | 10 | 21 | 7 | 13 | 15 |
| 10 | 12 | 21 | 13 | 21 | 25 | 19 | 21 | 20 | 17 | 20 | 19 |
| 19 | 25 | 14 | 20 | 12 | 11 | 8 | 10 | 19 | 18 | 15 | 17 |
| 12 | 25 | 24 | 20 | 6 | 13 | 6 | 8 | 12 | 12 | 22 | 14 |
| 35 | 13 | 24 | 24 | 75 | 49 | 48 | 60 | 80 | 41 | 40 | 57 |
| 8 | 2 | 3 | 4 | 4 | 11 | 6 | 7 | 8 | 10 | 5 | 8 |

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