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# Lack of substratum effect on the growth and metamorphosis of larval plaice *Pleuronectes platessa*

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ABSTRACT: Larval plaice *Pleuronectes platessa* hatched from artificially fertilised eggs were reared in tanks containing sand of 3 grain sizes (62 to 125 µm, fine; 125 to 250 µm, medium; 1 to 2 mm, coarse), and in the absence of sand. Although there were significant differences in growth, development and mortality between 3 replicate experiments, the differences were not related to substratum type. It is concluded that substratum type per se is unlikely to affect growth or the timing of metamorphosis. However, in the sea, substratum characteristics, because they control the distribution of suitable food organisms, may influence growth and metamorphosis indirectly.

## INTRODUCTION

Plaice *Pleuronectes platessa* releases its eggs offshore on discrete spawning grounds (Harding et al. 1978) and the metamorphosing larvae settle inshore on sandy beaches in depths of a few metres or less (Lockwood 1974, Van der Veer & Bergman 1986). In most cases the spawning grounds are situated so that currents deposit the larvae in areas where the whole coastline is suitable for settlement, on the sandy coasts of the eastern North Sea, for example. There are certain regions, however, as on the west coast of Britain, where much of the coastline is rocky. In such environments metamorphosing larvae may find themselves in areas that are unsuitable as nursery grounds. Under these conditions a delay in metamorphosis, and hence an increase in the possibility of locating a suitable settlement site, would clearly be of advantage. This paper describes the results of laboratory experiments designed to determine whether substratum type, per se, affects growth and time to metamorphosis. We hypothesised that given equal and adequate food rations, settling larvae would grow faster and metamorphose when they were younger on fine sand (i.e. $< 0.5$ mm), but that growth would be slower and metamorphosis delayed on coarser sand or in the absence of sediment.

## MATERIALS AND METHODS

All experiments were done in a temperature-controlled room in a 12:12 h LD schedule with 286 photopic lux (= 143 µW $cm^{-2}$) during the light phase. Sets of 4 black circular, polythene tanks, 42 cm in diameter and with a 20 cm depth of flowing, aerated seawater were used throughout. Water temperatures varied from means of 11.2 (± 1.4 SD) to 12.9 (± 0.3 SD) °C between experiments. One of each set of 4 tanks had no sediment. To the other three were added 600 ml of sand with grain size 62 to 125 µm (fine), 125 to 250 µm (medium) or 1 to 2 mm (coarse). This volume of sand covered the bottom of the tanks to a depth of about 4 mm. The 2 finer sands were initially sterilised with bleach and washed with hot fresh water. All 3 grades were thoroughly washed with seawater before adding them to the experimental tanks. This treatment was to ensure that no food organisms present in the sediment confounded the results.

The larvae were obtained by artificially fertilising eggs and rearing them in the laboratory. Eggs and sperm were stripped from ripe adult plaice caught previously by trawling in the Firth of Clyde, Scotland. Approximately 4 to 5 wk after hatching, random samples of about 30 larvae were carefully removed from the rearing tanks. To prevent damage while being



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handled and measured the larvae were anaesthetised in 1:10 000 MS222 in a small Perspex dish, placed on photographic paper in a darkroom and exposed by flash to give a silhouette (Neave & Batty 1982). They were then revived in fresh seawater and gently added to the experimental tanks, ca 30 per tank. The photographs were later used to assess the initial stages of the larvae (Ryland 1966) and to measure their total lengths under a binocular microscope using camera lucida and a digitising tablet connected to a microcomputer. The great majority of larvae at the beginning of the experiment were between Stage 3a' and 3b' of Ryland (1966), the point in development when they start to spend an increasing amount of time on the bottom.

Approximately 1000 *Artemia* sp. nauplii (San Francisco Bay) were added daily to each experimental tank. At the end of the day the fine mesh filter over the tank outlet was replaced with one of a coarser mesh so that remaining nauplii were flushed out overnight. After 11 to 13 d, depending on when the first metamorphosed fish was observed in the experimental tanks, all larvae were removed, counted, staged and measured. Three experiments, each with 3 replicates of the 4 substrata (treatments), were carried out from early May to early June. At the end of each experiment, all sand was removed from the tanks, washed and replaced.

Because the growth of individuals could not be followed, growth rate in length (GL) for each individual sampled at the end of the experiment in each tank was calculated as the change in length (mm degree-day$^{-1}$) over the course of the experiment and expressed as a proportion of the initial mean length ($L_1$):

$$GL = \frac{(L_2 - L_1)}{D \cdot L_1}$$

where $L_2$ = individual length at the end of experiment; and D = number of degree-days. Because biological zero is taken as −1 °C, degree-days for each tank was calculated as the sum of (degree-days +1) over the experiment. Changes in developmental stage were calculated in the same way, i.e. $GS = (S_2 - S_1)/D \cdot S_1$ where $S_1$ and $S_2$ are the stage equivalents of $L_1$ and $L_2$. For ease of data handling, prior to calculation the stages were coded on an arbitrary scale of 1 to 11. On this scale a value of 4 represents Ryland's (1966) Stage 3a' and 11 represents Stage 5 (the fully metamorphosed larva).

Prior to analysis the data were checked for normality and for homogeneity of variances. Subsequently all 3 experiments were combined and examined by analysis of variance using a nested model with substratum and experiment as the 2 main effects. Tank or replicate nested within substratum was used as an error term. Differences in mortality between substrata were also examined by comparing within each experiment (Chi square test) the numbers surviving on each substratum. All statistical tests were done using the SAS statistical package.

## RESULTS

Results for the change in length (GL) and stage (GS) on the various substrata are shown in Fig. 1 and Table 1. All sets of data for both GL and GS were normally distributed (Kolmogorov D statistic, $p > 0.9$) but their variances were markedly heterogeneous (Fmax test, $p < 0.005$) and neither square root nor logarithmic transformation corrected the problem. The latter finding, strictly speaking, violates one of the basic assumptions of the analysis of variance but heterogeneity of variances will not affect conclusions based on nonsignificant results and will only affect those based on significant results if they are close to the chosen level of significance (Underwood 1981). In the present case the results of the tests were either very significant or clearly nonsignificant and therefore the marked heterogeneity does not affect the conclusions drawn from them. The analyses of variance indicated that, although there was a highly significant difference between experiments ($p < 0.0001$), there was no difference between substrata either for GL or GS ($p > 0.97$ for GL and $p > 0.89$ for GS). There was no interaction between substratum and experiment ($p > 0.83$ for GL and $p = 1$ for GS).

There were significant differences in mortality between substrata in the 3 experiments (Table 2; $p = 0.02$ or less). There was little consistency in the results,

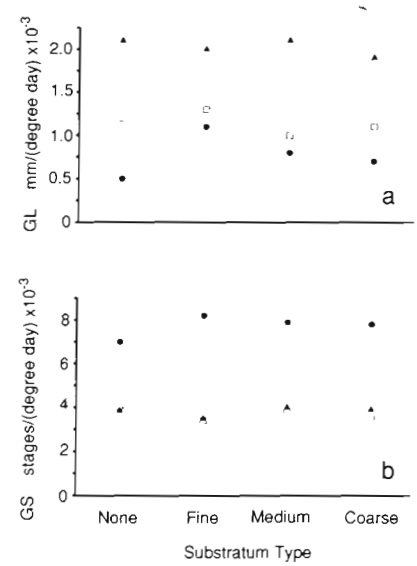


Fig. 1 *Pleuronectes platessa*. Growth of larvae on different substrata: (a) in length (mm degree-day$^{-1}$, GL) and (b) in stage (coded as in text). (●) Expt 1; (▲) Expt 2; (□) Expt 3

Table 1. *Pleuronectes platessa*. Mean lengths (mm) and stages (coded as in text) of larvae at the start and end of the experiments. Ages of larvae (days from fertilisation) at the start and end of the experiments are also given

| Expt no. | Substratum | Length | | Stage | | Age | |
|---|---|---|---|---|---|---|---|
| | | Start | End | Start | End | Start | End |
| 1 | None | 9.87 | 10.67 | 4.47 | 9.14 | 47 | 60 |
| | Fine | 9.89 | 11.43 | 4.24 | 9.25 | | |
| | Medium | 9.84 | 11.00 | 4.29 | 9.29 | | |
| | Coarse | 9.82 | 10.87 | 4.36 | 9.37 | | |
| 2 | None | 9.87 | 12.93 | 6.11 | 9.81 | 48 | 61 |
| | Fine | 9.98 | 12.94 | 6.57 | 9.94 | | |
| | Medium | 9.74 | 12.73 | 5.94 | 9.46 | | |
| | Coarse | 9.69 | 12.48 | 6.02 | 9.54 | | |
| 3 | None | 9.78 | 11.33 | 6.10 | 9.24 | 50 | 61 |
| | Fine | 10.0 | 11.74 | 6.66 | 9.45 | | |
| | Medium | 9.95 | 11.31 | 6.35 | 9.52 | | |
| | Coarse | 10.31 | 11.79 | 6.94 | 10.10 | | |

however, except that (1) the 2 highest mortalities were both recorded on coarse substrata (Expts 1 and 3) and (2) there was no significant difference in mortality among experiments on the 2 most 'suitable' (fine and medium).

## DISCUSSION

The dispersal of planktonic fish eggs is generally regarded as a passive process and is dependent upon hydrographic and meteorological factors, principally currents, tides and wind. After the eggs have hatched and the larvae have developed swimming abilities, active behavioural mechanisms may come into play. These mechanisms ensure that the larvae arrive at or near their juvenile habitat (Boehlert & Mundy 1988, Miller 1988). Adverse physical factors, such as offshore winds affecting species with inshore or estuarine habitats, may act to prolong the dispersal phase and even in some cases affect year class strength (see e.g. review by Doherty & Williams 1988). Under such conditions it may be advantageous for larvae of benthic species to delay their settlement and metamorphosis until they arrive in suitable habitats. Although the ability to delay metamorphosis is common among invertebrates (Doyle 1975, Jackson & Strathmann 1981, Butman 1987) even circumstantial evidence of its presence in fishes is scarce (Pearcy et al. 1977, Moser 1981) and in only one, the reef fish *Thalassoma bifasciatum*, has the phenomenon been substantiated (Victor 1986).

The results of the experiments described here showed convincingly that, at least under the experimental conditions used, substratum type alone does not affect the growth of young plaice, nor does it have any effect on the timing of metamorphosis. The significant inter-experiment effect observed was presumably due to the variation between batches of larvae caused by different parentage.

Although growth and metamorphosis must be closely linked, the few studies that have examined the relationship between length and age at metamorphosis (Chambers & Leggett 1987) indicate that length at metamorphosis is less variable than age. This finding suggests that fish larvae tend to metamorphose within a critical size range rather than after a specific length of

Table 2. *Pleuronectes platessa*. Mortalities on different substrata for each experiment. N: number of individuals at start of experiment; %M: percent mortality at end of experiment

| Substratum | Expt 1 | | Expt 2 | | Expt 3 | | Within-substratum chi square | p |
|---|---|---|---|---|---|---|---|---|
| | N | %M | N | %M | N | %M | | |
| None | 86 | 5.8 | 98 | 2.0 | 95 | 13.7 | 10.2 | 0.006 |
| Fine | 90 | 5.6 | 96 | 8.3 | 91 | 1.8 | 0.6 | 0.75 |
| Medium | 90 | 8.9 | 99 | 14.1 | 95 | 11.6 | 1.3 | 0.53 |
| Coarse | 90 | 22.2 | 100 | 6.9 | 99 | 40.4 | 31.9 | <0.001 |
| Among-substratum | | | | | | | | |
| Chi square | 17.5 | | 10.2 | | 42.7 | | | |
| p | <0.001 | | 0.017 | | <0.001 | | | |

time. In the plaice, however, the situation may be somewhat different because Riley (1966) has suggested that the onset of metamorphosis in this species is controlled more by a mechanism dependent on temperature and time (age) than by the attainment of a critical length.

If the substratum had inhibited metamorphosis, either by reducing overall growth rate or slowing growth above a certain critical stage, then one of 2 effects might have been expected. In the first case there would have been lower overall growth and a lower average larval stage at the end of the experiment on unsuitable substrata. In the second, initial growth rate might have been faster so that the larvae reached the critical stage earlier and then stopped growing. In the latter case a lower GS variance might have been expected. The experimental technique did not allow the 2 putative growth patterns to be distinguished but there was no evidence of differences in GS variances between the substrata.

It could also be postulated that the lack of substratum effect observed could have been caused by selective mortality of different sized fish. If growth rate had been high on suitable substrata but the fast-growing and hence larger fish were subject to higher mortality, then the measured growth rate would have been reduced. Conversely, if growth rate had been lower on unsuitable substrata but selective mortality occurred among the slowest-growing (smallest) fish then the measured growth rate would have been increased. In either case, however, selective mortality would have acted to reduce variance. There was no correlation between variance and mortality and so a strong effect of selective mortality seems unlikely.

Overall, the experiments suggest that substratum type per se is not important in affecting growth or the timing of metamorphosis and that some other factor(s) must be in operation. The most important of these is probably the presence of suitable food at the time of settlement. Creutzberg et al. (1978) showed that well-fed fish are less active and proposed that areas with abundant suitable food act as 'traps' for settling larvae. Furthermore, it has been suggested that in the Wadden Sea there is a direct relationship between the abundance of settled fish and macrobenthic biomass (Zijlstra et al. 1982, Bergman et al. 1987). Whether the latter situation is caused by a concentrating effect of the behavioural response to food is not known, but if such an effect exists it could also apply to other areas that are markedly heterogeneous in substratum characteristics. If initial settlement takes place on rock, weed or gravel substrata, for example, the lack of suitable food would induce movement to other areas until an appropriate settlement site was found. This selection process could continue for some time because settling plaice can survive for many days without feeding (Wyatt 1972) and the absence of food is likely to reduce growth and the ability to metamorphose. In this sense, then, substratum type could have an effect, albeit indirect, on the timing of metamorphosis.

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