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DECREASED DISCRIMINATION DURING SETTING AFTER PROLONGED PLANKTONIC LIFE IN LARVAE OF *SPIRORBIS BOREALIS* (SERPULIDAE)

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(Text-figs. 1 and 2)

Many larvae of benthic invertebrates search actively for a place to settle, as the end of the planktonic stage approaches. During this process they show considerable powers of discrimination and they can postpone metamorphosis until their search is successful (Thorson, 1950; Wilson, 1952). Since their swimming is feeble and usually undirected, apart from a negative reaction to light in many species, their random explorations must often become very prolonged. Such prolongation will generally be dangerous because planktonic larvae are particularly vulnerable to predators, and because development cannot be long delayed without seriously weakening larvae such as those of barnacles and *Spirorbis*, which do not feed during the searching phase. It therefore seemed possible that searching larvae would become more prone to settle, and consequently less discriminating in their choice of environment, if the planktonic stage were prolonged. Wilson (1952, 1953) has suggested that *Ophelia* larvae behave in this way. Such behaviour would be of adaptive value, in avoiding the dangers of an excessively long planktonic stage. It would also agree with Lorenz's widely accepted idea of instinctive behaviour, as the expression of a conative urge towards a goal, which grows gradually stronger if attainment of the goal is delayed (Thorpe, 1948).

The gregarious reaction of *Spirorbis* larvae (Knight-Jones, 1951) offered a good opportunity for investigating this possibility. These larvae pass successively through a surface-swimming stage, searching stage, setting action pattern and cataclysmic metamorphosis. They were readily obtained by collecting adult *Spirorbis borealis*, epizoic on *Fucus serratus*, during the neap tide periods of larval liberation, and placing them without delay in troughs of sea water. As soon as larvae were liberated in sufficient numbers, they were distributed equally between the various series of experiments in progress, to ensure homogeneity of material. Possible effects, due to diurnal variations in activity, were guarded against by bright illumination at night and by collecting adults and starting experiments at intervals throughout the day and night. Parent *Spirorbis* did not readily liberate larvae at night, but bright illumination and changes of sea water induced some to do so.

Lowered Level of Selection after Prolonged Swimming

Larvae were removed immediately after liberation, and distributed, about twelve to each bowl, between four batches of glass bowls, each of which had been wiped with cotton-wool just previously, and filled with 250 ml. of sea water. *Spirorbis* larvae rarely attach themselves to freshly wiped glass surfaces, and these were kept for various periods without metamorphosis, until offered the choice of substrata described below.

The four batches of bowls were used in four series of experiments, one series involving freshly liberated larvae and the other three series involving larvae which had been kept swimming for 3, 6 and 12 hr. respectively. In each experiment the larvae were offered a choice between two similar pieces of freshly collected *Fucus serratus*, placed about 6 cm. apart. One piece of each pair, termed the 'previously colonized' piece, had already been exposed to larvae, which had attached themselves to it in numbers varying between 12 and 150, while the other was bare, having been kept under similar conditions, but without larvae. Variation in the numbers of larvae settled on the previously colonized pieces was difficult to control, but in this type of experiment variation within wide limits has been found to have no significant effect on the results, as indeed can be seen from close inspection of the figures in Table I.

A fifth series of similar experiments was carried out using larvae which had been kept from metamorphosis for 24 hr. The majority of such larvae were incapable of locomotion, so those used in this series were obtained by keeping large numbers of larvae in beakers for 24 hr., and then selecting those which still swam.

The numbers of larvae setting subsequently on the previously colonized pieces and on the bare control pieces in the course of each experiment are shown in Table I, together with the angular transformations of the fractions $\frac{\text{no. settled on control}}{\text{total no. settled}} = \sin^2\theta$ (Fisher & Yates, 1948). This transformation was used to render the variance independent of the mean, and so promote homogeneity in the statistical treatment. Analysis of variance of θ throughout the five series indicates a difference between series, which is significant at the probability level 0·05. Further analysis indicates a highly significant difference ($P = 0{\cdot}01$) between the results from freshly liberated larvae and those from all other larvae. There are no significant differences between the four series of experiments in which these other larvae were used, resulting from the increase in the period of enforced delay from 3 to 24 hr.

In deriving a mean value of θ for each series, it was found as a result of a χ^2 test that the individual larvae could not justifiably be treated as units, perhaps because all those in certain batches had been affected by small inequalities of lighting. Instead it was considered that the most efficient treat-

TABLE I. NUMBERS OF PREVIOUSLY SETTLED *SPIRORBIS* ON PREVIOUSLY COLONIZED/BARE SURFACES, PLUS NUMBERS OF LARVAE SETTING IN EACH EXPERIMENT

Angular transformations $\left(\frac{\text{no. setting on bare surface}}{\text{no. setting on both surfaces}} = \sin^2\theta\right)$ are given in brackets.

Larvae were kept without surfaces suitable for setting for				
0 hr.	3 hr.	6 hr.	12 hr.	24 hr.
69/0+ 8/0 (0)	32/0+ 3/1 (30·0)	32/0+ 1/4 (63·4)	97/0+6/1 (22·2)	42/0+0/0 (—)
28/0+ 6/1 (22·2)	12/0+ 3/0 (0)	83/0+ 2/2 (45·0)	57/0+3/3 (45·0)	132/0+0/0 (—)
25/0+ 7/1 (20·7)	25/0+ 5/2 (32·3)	21/0+ 2/1 (35·3)	53/0+3/3 (45·0)	15/0+2/0 (0)
52/0+ 5/5 (45·0)	57/0+ 5/3 (37·8)	39/0+ 2/0 (0)	25/0+8/2 (26·6)	83/0+1/0 (0)
41/0+12/2 (22·2)	49/0+ 2/0 (0)	78/0+ 2/0 (0)	51/0+2/2 (45·0)	45/0+1/0 (0)
19/0+14/6 (33·2)	30/0+12/1 (16·1)	30/0+10/2 (24·1)	57/0+4/5 (48·2)	53/0+1/1 (45·0)
30/0+11/0 (0)	12/0+14/3 (24·8)	39/0+10/2 (24·1)	56/0+1/8 (70·5)	67/0+7/2 (28·1)
32/0+11/2 (23·1)	40/0+10/8 (41·8)	26/0+ 5/6 (47·6)	77/0+6/6 (45·0)	83/0+4/3 (40·9)
74/0+12/0 (0)	35/0+ 7/6 (42·8)	61/0+ 7/1 (20·7)	62/0+2/7 (61·9)	39/0+0/1 (90·0)
130/0+ 9/4 (33·7)	87/0+ 5/5 (45·0)	154/0+ 7/5 (40·2)	89/0+9/2 (25·2)	93/0+2/1 (35·3)
51/0+13/2 (21·4)	75/0+ 6/9 (50·8)	31/0+ 1/5 (65·9)	43/0+6/4 (39·2)	27/0+1/1 (45·0)
91/0+ 7/8 (46·9)	72/0+10/6 (37·8)	80/0+ 4/5 (48·2)	57/0+5/3 (37·8)	87/0+1/1 (45·0)
81/0+ 7/3 (33·2)	37/0+ 8/6 (40·9)	49/0+10/9 (43·5)	54/0+8/2 (26·6)	56/0+2/0 (0)
52/0+13/0 (0)	51/0+13/3 (25·7)	44/0+ 6/2 (30·0)	65/0+2/1 (35·3)	38/0+2/3 (50·8)
92/0+ 6/7 (47·2)	49/0+ 8/3 (31·5)	43/0+ 5/3 (37·8)	85/0+1/1 (45·0)	30/0+2/1 (35·3)
45/0+13/1 (15·5)	52/0+ 4/8 (54·7)	54/0+ 8/3 (31·5)	80/0+3/4 (49·1)	59/0+3/0 (0)
33/0+10/3 (28·7)	44/0+ 2/6 (60·0)	52/0+ 2/3 (50·8)	30/0+0/0 (—)	44/0+0/0 (—)
24/0+11/3 (27·6)	34/0+ 3/7 (56·8)	44/0+ 2/2 (45·0)	32/0+1/0 (0)	35/0+0/0 (—)
24/0+ 9/3 (30·0)	70/0+ 3/9 (60·0)	39/0+ 0/6 (90·0)	30/0+1/1 (45·0)	34/0+0/0 (—)
40/0+ 8/5 (38·3)	50/0+ 5/5 (45·0)	27/0+ 1/0 (0)	21/0+0/1 (90·0)	44/0+1/1 (45·0)
Totals 192/56	128/91	87/61	71/56	30/15
248	219	148	127	45
Weighted mean θ with S.E. $\pm\Delta\theta$ (25·36 ± 3·35)	(39·31 ± 3·10)	(39·67 ± 3·90)	(41·45 ± 3·43)	(32·33 ± 4·42)
P from $\frac{45-\theta}{\Delta\theta}$ <0·001	>0·1	0·2	>0·2	0·05

ment was to take each experiment as a unit, but to weight the result according to the number of larvae involved, so as to minimize the effect of those batches which had large errors due to small numbers of larvae setting. The weighted mean for each series, with its standard error, is given in Table I. To ensure that this treatment was valid, a more rigorous treatment was also employed, in which each experiment was treated as a unit without weighting: the results were not significantly different from those given in Table I. It is therefore clear that freshly liberated larvae showed well-marked selection of the previously colonized pieces of *Fucus*, whilst larvae which had been kept swimming for 3 hr. or longer showed little evidence of discrimination. Indeed, in some of the series involving prolongation of the planktonic life, the evidence is consistent with random setting. The somewhat anomalous results, obtained from the 24 hr. series, may perhaps have been due to the fact that the larvae of this series had been selected for their vigour, whereas all the other larvae were unselected.

The decrease in numbers setting on the *Fucus*, in experiments which involved delayed metamorphosis (Table I), was due partly to prior setting on the glass of the dishes, but mainly to weakness of the remaining larvae. Their swimming became slower and was occasionally interrupted, until eventually they became entirely immobile. A few showed some of the changes of metamorphosis without becoming attached, but many retained their larval form for several days, remaining motionless and clearly doomed to die unmetamorphosed.

Percentages of Larvae Settled, in the Presence of Previously Settled Individuals and in Isolation, after various Periods of Swimming

Choice of substrata which bear previously settled individuals is probably associated with the fact that setting is delayed in isolation (Knight-Jones, 1951). It seemed that the lesser degree of choice exercised after prolongation of the planktonic life might be due to more hasty setting, in gregarious and in isolated larvae alike.

Two parallel series of experiments were set up, using equal numbers of freshly liberated larvae in small beakers, each of which had been wiped just previously and filled with about 50 ml. of sea water. The larvae in one series were distributed so that each beaker contained five, which may be termed 'associated' larvae. Those in the other series were isolated, each beaker containing a single larva. The associated larvae were offered small pieces of *Fucus*, which bore previously settled *Spirorbis*, whilst the larvae of the other series were offered similar pieces of bare *Fucus*. Each of the two series was made up of four similar batches, A, B, C and D. In A the larvae were offered *Fucus* immediately after liberation, but in B, C and D they were kept without *Fucus* until 3, 6 and 12 hr. respectively after liberation. After adding *Fucus*,

numbers of larvae metamorphosed were counted at hourly intervals. The experiments were repeated during the liberation peaks of several months, until 800 larvae had been used, comprising a hundred associated and a hundred isolated larvae in each of the four batches.

Fig. 1 shows the curves, representing percentages metamorphosed against time, which resulted from these experiments. In each batch the curve relating

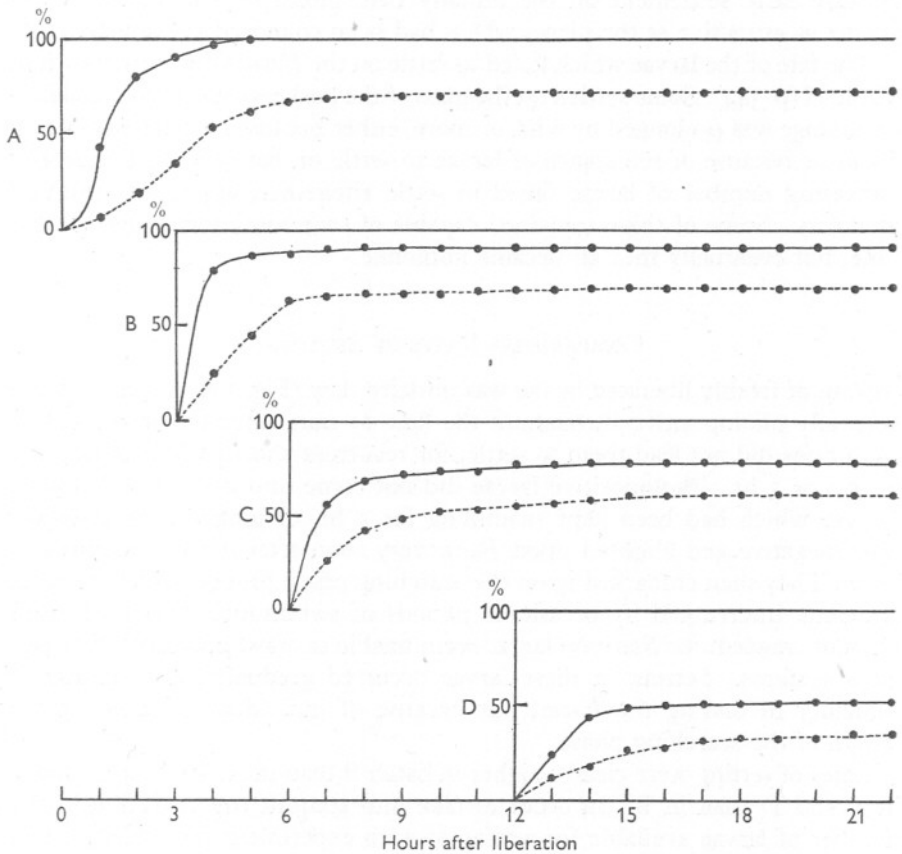

Fig. 1. Percentages metamorphosed, recorded at hourly intervals, in four similar groups of larvae. One group (A) was presented with *Fucus* without delay, whilst the others (B, C and D) were kept without substrata suitable for setting for periods of 3, 6 and 12 hr. respectively. Of the two curves drawn from each group, the continuous line relates to associated larvae, the interrupted line to isolated larvae.

to associated larvae is much steeper than that relating to isolated larvae. The difference between the two curves gives some indication of the degree of choice to be expected from each batch in gregariousness tests of the type described above. In batch A, for instance, eighty of the associated larvae settled within 2 hr., whereas only a quarter of that number of isolated larvae

settled within the same time. This agrees very well with the ratio between the numbers of larvae, which settled on previously colonized and bare *Fucus*, in gregariousness tests using freshly liberated larvae (Table I). In the remaining batches the curves are closer together, so the proportion setting in a given time on the bare *Fucus* is considerably higher. In gregariousness tests with similar batches of larvae (Table I) the ratio approached equality, probably because early settlement on the initially bare pieces of *Fucus* made these almost as attractive as the pieces which had been colonised previously.

The fate of the larvae which failed to settle on the *Fucus* (Fig. 1) is shown in Table II (p. 344). Some settled on the glass of the beakers, but when the planktonic stage was prolonged by 6 hr. or more, either because of delay in offering *Fucus* or because of reluctance of larvae to settle on bare *Fucus*, a gradually increasing number of larvae failed to settle altogether, apparently through weakness. Some of these remained capable of swimming for a considerable time, but eventually they all became immobile.

Comparative Rates of Setting

Setting in freshly liberated larvae was initially slow (Fig. 1 A) for larvae were generally photopositive throughout the first 15 min. after liberation and, if conditions did not lead them to settle, for recurrent short periods during the next 2 or 3 hr. Photopositive larvae did not come into contact with *Fucus*. Larvae which had been kept swimming for 3 hr. or longer were generally photonegative and alighted upon *Fucus* very soon after it was presented to them. They then embarked upon the searching phase proper, which involves crawling, interrupted by occasional periods of swimming. Previously they had not crawled, for *Spirorbis* larvae seem unable to crawl upon freshly wiped glass surfaces. Setting in these larvae occurred gradually, not because of difficulty in finding the *Fucus*, but because of individual variation in the length of the searching phase.

Rates of setting were clearly higher in batch B than in A, but seemed lower in C and D than in B. In order to take into account the reduction in the number of larvae available for setting as each experiment proceeded, a plot of the function $\frac{\log \frac{x}{x-N}}{t}$ was made, where x is the total number of larvae which finally settled on the *Fucus* and N is the number that had settled at time t (Fig. 2). The slope of this curve, $\frac{d \log \frac{x}{x-N}}{dt}$, gives an individual estimate of the rate of setting, which is independent of the number of larvae available. Considering the associated larvae first, the plot for batch A is a straight line,

which intercepts the base-line at about 15 min. after liberation (i.e. at the end of the entirely photopositive phase), and thereafter indicates a constant rate of setting of 0·44 of the population per hour. The plot for batch B is not a straight line, probably because the population is not homogeneous, for setting

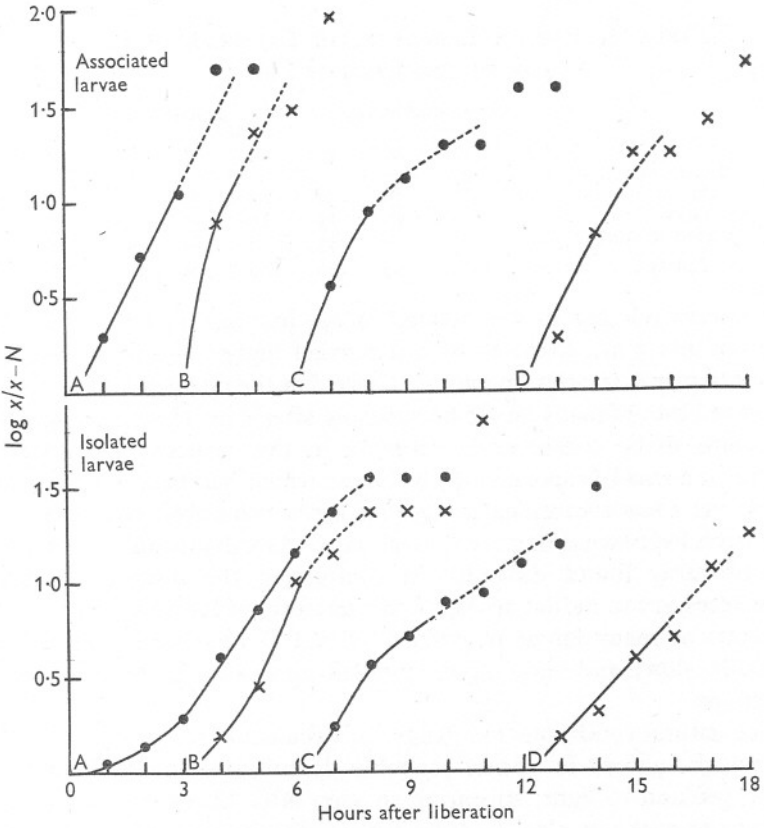

Fig. 2. The results recorded in Fig. 1, treated so as to obtain measurements of the rates of setting which are independent of the numbers of larvae available. A, B, C and D mark the curves relating to each batch of larvae. x = total number of larvae which settled on *Fucus* in each batch. N = number of these which had settled in a given time. The points where N approaches x are inaccurate, being based on small numbers of larvae, so these parts of the curves are dotted.

was delayed in some but not in others. The great majority of these larvae would have settled earlier, if they had had the opportunity, and it seems likely that these settled first, at about double the rate of batch A. The remainder corresponded to those individuals of batch A which were still swimming 3 or 4 hr. after liberation. Their setting was not delayed by the experiment, so it went on at the same rate as in batch A. In batches C and D the rate of

setting was secondarily lowered, probably because exhaustion, which fatally affected some individuals, was to some extent affecting the majority, but it remained somewhat faster than in A. In each of the curves C and D the initial rate of setting is faster than the ultimate rate, which would be expected if the more vigorous larvae settled first.

TABLE II. FATE OF LARVAE IN THE EXPERIMENTS WHICH FORMED THE BASIS OF FIG. 1

	Associated larvae				Isolated larvae			
	A	B	C	D	A	B	C	D
Settled on:								
Fucus	99	91	78	52	72	70	61	35
Glass	1	9	16	13	15	16	15	18
Failed to settle	0	0	6	35	13	14	24	47
Totals	100	100	100	100	100	100	100	100

The curves relating to the isolated larvae indicate a low rate of setting during the first 3 hr., followed by a somewhat higher rate which was maintained until 7 or 8 hr. after liberation. Thereafter the rate was lower. Probably the food reserves of many larvae become low after 8 hr. of swimming, though there seems to be considerable variation in this respect. Fatal weakness appeared in a small proportion of the larvae which had been kept swimming for 6 hr., yet a few exceptionally vigorous larvae were observed to settle after having been kept swimming for several days. As exhaustion approached the larvae probably found difficulty in completing the minimum searching routine necessary to induce setting, and therefore settled at a lower rate. The movements of many larvae in batches C and D had been observed to be abnormally slow, and they often spiralled aimlessly, or became stuck to obstructions.

Under natural conditions the danger of exhaustion seems slight, for most larvae which succeed in setting probably do so within a few hours. Their negative reaction to light, appearing so soon after liberation, will generally lead them to rocky or algal surfaces before they are carried away from the neighbourhood of the main parent stocks. This and gregariousness account for the fact that *Spirorbis* populations are well concentrated. The brief periods of surface swimming, which recur during the first few hours of searching, give larvae which descend into sandy or muddy coves, or creeks, repeated chances of being carried by currents over neighbouring rocks.

SUMMARY

Laboratory experiments showed that freshly liberated *Spirorbis* larvae settle on surfaces which bear previously settled individuals, in marked preference to bare controls, but that larvae which have been kept swimming for 3 hr., or longer, exercise less choice when setting.

When larvae are freshly liberated there is a great difference between the rate of setting gregariously, and the rate of setting in isolation; but after they have been kept swimming for 3 hr. this difference becomes less, owing to the larvae setting more rapidly both gregariously and in isolation. After swimming for about 8 hr. rates of setting fall off, and many larvae fail to settle, apparently through weakness.

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