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GREGARIOUSNESS AND SOME OTHER ASPECTS OF THE SETTING BEHAVIOUR OF *SPIRORBIS*

By E. W. Knight-Jones

Marine Biological Station, Bangor

(Text-figs. 1, 2)

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Introduction

Further study of the phenomenon of gregariousness during settlement, demonstrated in the oyster *Ostrea edulis* (Cole & Knight-Jones, 1949; Knight-Jones, 1951) and the barnacle *Elminius modestus* (Knight-Jones & Stephenson, 1950), seemed to require laboratory experiments, for which a viviparous animal with a brief, lecithotrophic, pelagic stage appeared to be needed.

In the Bangor area tube-worms of the genus *Spirorbis* are amongst the most abundant and easily obtained of such animals. They have proved to be excellent material for investigations on setting behaviour. This account deals mainly with *S. borealis*, the species which settles abundantly on *Fucus*, but some field observations and a single series of experiments on *Spirorbis pagenstecheri* are included.

Previous accounts of the behaviour of *Spirorbis* larvae are very incomplete and occasionally misleading. Shively (1897) observed attachment, but gave few details, Neu (1933) counted the numbers setting on plates of various colours, and Garbarini (1936*b*) made some observations which are referred to later (p. 214). Thorson (1946) gives further references.

This work was carried out in the Department of Zoology at the University College of North Wales, where Prof. F. W. Rogers Brambell, F.R.S., has kindly given us rooms until our Station is built. I am very grateful to him and to his Staff for their hospitality and help. Dr H. A. Cole, of the Fisheries Experiment Station, Conway, encouraged me at an early stage with the information that his records of sessile organisms competing with oyster spat had given evidence of gregariousness in *Spirorbis*.

BREEDING PERIODICITY AND LIBERATION OF LARVAE

Fucus serratus covered with *Spirorbis borealis* was collected frequently between late May and early October from near Bangor Pier at about low-water mark. Breeding was in progress throughout this period. Adults on pieces of *Fucus* were put into dishes containing about 200 ml. of sea water, into which the larvae were liberated.

Breeding periodicity in *S. borealis* was first demonstrated by Garbarini (1933), who found that liberations of larvae at Concarneau and Roscoff occurred only during a few days at each quarter of the moon. Later (1936*a*) he found that growth of oocytes to ripeness lasted about 14 days, that the incubation period was of similar duration, and that breeding was synchronized so that at each quarter a batch of larvae was liberated, a batch of eggs was spawned and a batch of oocytes began to grow. Liberations of larvae at Bangor showed similar periodicity. During July and August mass liberations occurred regularly during the few days before and after the moon's quarters. Very few larvae were obtained in the intervening periods. During June (when the weather was hot) breeding was less regular and less well synchronized. Many tubes were opened at various times and practically all contained embryos, which tends to confirm that a fresh spawning follows closely upon liberation.

Synchronization of breeding appeared to be less perfect than in Garbarini's material, for larvae were rarely unobtainable, but it proved to be a waste of time to attempt experiments except during the week in which each liberation peak occurred.

No larvae appeared for the first few minutes after adults were put into a dish. It seemed that a short period was needed to recover from disturbance, even if the adults were over-ripe for liberation. In freshly collected material only occasional individuals liberated their larvae, even during peak periods, but in material which had been in the laboratory for a few hours mass liberations of larvae could be obtained by (i) exposing to light dishes which had been kept for a few hours in the dark, (ii) changing the water of dishes, whether in the light or in the dark, (iii) immersing adults which had been kept out of water for a few hours or overnight. Such changes were various in their effects. The oxygen tension and pH were usually raised, but when the change was to fresh sea water from sea water which had contained brightly illuminated *Fucus*, both were lowered. Probably over-ripe adults will react by liberation to any improvement in their surroundings. Liberation appeared to be a voluntary act on the part of the adults because it was accompanied by marked protrusion of the body and by rocking movements, and because the larvae appeared helpless when they first emerged from the parent tube. They often remained motionless for about a quarter of a minute, entangled in the parent's tentacles. Then they gradually freed themselves and swam away towards the light. Usually twenty or thirty larvae, probably the whole brood, were liberated at the same time by each adult. Occasionally only two or three larvae made their appearance in a dish, suggesting that a few may be liberated before or after the majority of the brood.

The Pelagic and Searching Phases

After liberation the larvae swam straight towards the light, each following in the path of the one in front. Swimming was relatively rapid, about 3 mm./sec. (the larvae being about 0·4 mm. long). It was largely or entirely effected by the large cilia of the prototroch. These showed metachronal waves which moved anticlockwise, seen from the apical end. The body rotated in the opposite direction.

The types of behaviour shown by larvae are summarized diagrammatically in Fig. 1. On reaching the lighted side of the dish the larvae swam backwards and forwards against the glass, usually near the surface of the water. They generally kept a straight course except when they hit an obstruction or found themselves going away from the light, when they turned and swam towards the light again, orientating directly towards it if free to do so. The length of this purely pelagic phase varied in different broods, but was usually between 15 min. and 2 hr.

After this period larvae frequently left the lighted side of the dish. Their swimming appeared rather random, with a photo-negative tendency. They generally swam straight, but if their course took them near a piece of *Fucus* they often turned and swam towards it as though able to perceive its dark surface. When kept for some hours in a clean glass vessel containing nothing suitable for attachment the majority collected near the bottom on the side remote from the light.

On making contact with a suitable surface a larva started to crawl on its ventral side at a speed of about 1 mm./sec., keeping a straight course unless it met an obstruction. On reaching the edge of a *Fucus* frond it generally crawled round on to the other side. Such a period of crawling rarely lasted more than a few minutes, and was sometimes very brief. The larva then swam off again. It often became positive to light for a further short period, before seeking another surface to crawl upon. Brief periods of swimming and crawling followed one another thus for 1 or 2 hr. During this time the larvae explored the dish thoroughly. They were often seen to encounter previously settled individuals, turn, crawl onwards, and eventually swim off again. They seemed to hurry about, with their entire output of energy given up to visiting as many different surfaces as possible.

The pelagic and searching phases were often much shorter in larvae liberated by parents which had been kept in crowded dishes or out of water for 1 or 2 days. Such larvae sometimes settled immediately they reached the glass side of the dish, if this bore a bacterial film. This behaviour appeared to be the result of long overdue liberation.

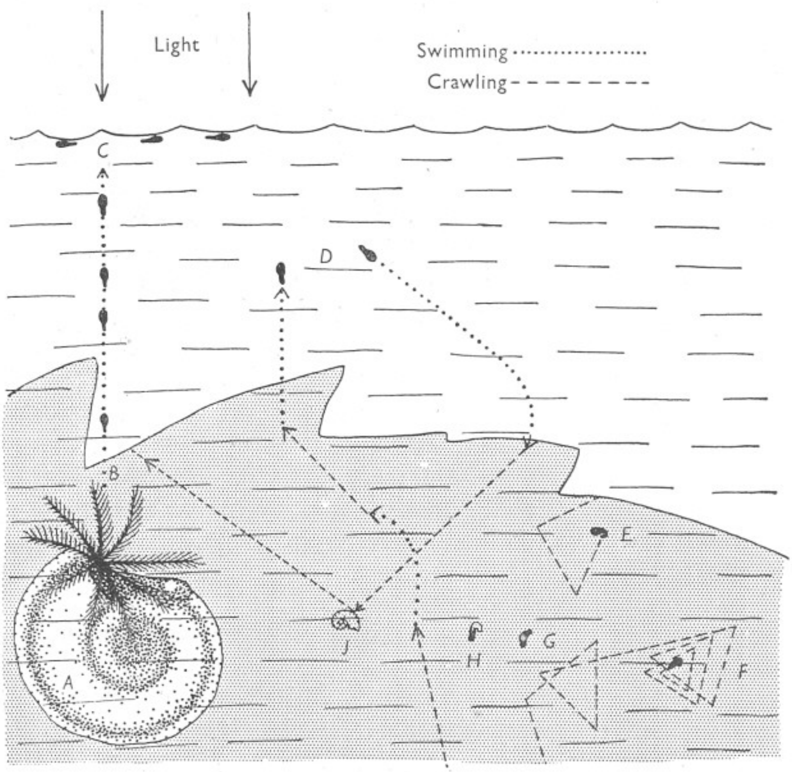

Fig. 1. Successive phases in behaviour of larvae of *Spirorbis borealis* illustrated diagrammatically. Stippled area represents frond of *Fucus serratus*. *A*, incubation within parent's tube; *B*, liberation and positive phototaxis; *C*, pelagic phase at surface; *D*, searching phase, with larvae swimming at random, or alternately negative and positive to light, interspersed with periods of crawling in straight lines; *E* and *F*, larvae about to settle and just settled, to show frequent changes of course during crawling; *G* and *H*, metamorphosis and formation of calcareous tube; *J*, individual 2 weeks after settlement.

Settlement

A larva which was about to settle was recognizable by the fact that it frequently changed direction whilst crawling. During the straight crawling of the searching phase the narrow, tail-like abdomen remained projecting longitudinally, or occasionally twitched from side to side. When about to settle the posterior end of the abdomen was frequently pressed against the substratum, to which it appeared to adhere, and the abdomen was strongly flexed so that the trunk turned sideways, usually through an angle of about 120°. The larva crawled a short distance in the new direction before again using its abdomen to steer itself through another change of course.

A larva which had begun to change direction rarely swam off again, but often continued to crawl for many minutes and sometimes covered a few centimetres in its wanderings. Eventually it began to cross backwards and forwards many times over the area within 2 or 3 mm. radius of the spot upon which it was finally to settle. Crawling became gradually slower and turning more frequent until progress ceased and attachment took place. Very similar behaviour is shown by oyster larvae which are preparing to settle (Prytherch, 1934; Cole & Knight-Jones, 1939).

In the free-swimming larva of *Spirorbis borealis* there is a superficial white spot in the dorsal mid-line at the posterior end of the thorax, sufficiently large to be seen easily with a hand-lens. Under a low-power microscope this was seen to alter in shape when the body was flexed violently, as though it was a sac full of fluid. It may conveniently be termed the attachment gland from the part it plays during setting.

Immediately before setting movement in each direction was no more than about a millimetre before a flexure of the abdomen turned the body round. As crawling ceased the abdomen continued to wave from side to side and the whole body wriggled and alternately stretched and contracted, the muscles apparently taut and very active, but the movements slight. After a few seconds a cloud of milky liquid issued from the attachment gland, apparently through a narrow pore at its posterior end. The gland rapidly emptied and the fluid spread over the surface of the trunk and abdomen, losing its opacity during dispersion, and apparently forming the initial tube. The body continued to wriggle and rock about, as though to aid the dispersion of the fluid and to ensure adhesion to the substratum. Anteriorly, the folds of the collar, which had been pressed inconspicuously against the sides of the trunk in the free-swimming larva, became erected almost perpendicularly (see figures in Fewkes, 1885) and then bent backwards again. They appeared to be beginning to enclose and mould the anterior lip of the tube which was being formed. This is probably their function, for glands secreting the calcareous tube have been demonstrated under the collar of serpulids (Swan, 1950). Posteriorly, the body became shorter and broader, so that the previously abrupt demarcation between the broad trunk and narrow abdomen disappeared. The worm, with unceasing small movements, rolled first one way and then the other, and finally completely over ventral side upwards. The large, ventro-lateral eyes, which had been next to the substratum in the crawling larva, became very conspicuous as they faced upwards. The rudiment of the operculum, which was difficult to distinguish in the larva, now appeared on the left side of the head and grew with almost visible speed, whilst the head shrank so that the eyes, formerly widely separated, approached one another closely.

Fixation, shortening of the abdomen, and rolling ventral side upwards took place within 1 or 2 min. after crawling had ceased. Very striking growth of the operculum at the expense of the larval head took a further 5 or 10 min. The change was sufficiently rapid to be termed a cataclysmic metamorphosis. The trunk and abdomen were then enclosed in an almost transparent, straight tube, which, being only about 0·25 mm. long, could not accommodate the anterior end. This initial length of tube, derived wholly or in part from the secretion of the attachment gland, remained straight and semi-transparent, but a white, opaque, calcareous extension was added to it anteriorly. This gradually elongated and immediately started to bend to the occupant's left as the inner whorl of the future tube. About 5 hr. after settlement growth at the mouth of the tube had carried its axis through 90°. The occupant then had a large operculum and rudimentary tentacles, which it could withdraw completely into the tube.

Need for Bacterial or Algal Filming of the Substratum

Surfaces immersed in sea water become covered with a film of micro-organisms after periods varying from a few hours to a few days (see ZoBell, 1946). There is evidence that larvae of a variety of sessile animals settle more readily on filmed surfaces than on clean surfaces (ZoBell & Allen, 1935; ZoBell, 1938, Scheer, 1945; Miller, Rapean & Whedon, 1948; Pyefinch & Downing, 1949; Cole & Knight-Jones, 1949).

Although previous accounts had described *S. borealis* as setting on glass, it was soon found that larvae in clean glass beakers metamorphosed only after a considerable delay. A series of experiments was carried out in which batches of larvae were divided between pairs of 50 ml. beakers. Each pair had contained sea water, with or without the addition of an algal culture (*Chlamydomonas* or *Synechococcus*) during the previous few days. One beaker of each was wiped clean with cotton-wool before use, the other was left with a visible film, which always included bacteria and was often coloured green with algal cells. The *Spirorbis* were examined after they had been in the beakers 24 hr. (Table I). In the aggregate 91 % had metamorphosed in the filmed beakers, only 15 % in the beakers which had been wiped. The results were consistent throughout with films of various origins.

A high proportion of larvae kept in clean beakers eventually became abnormal, showing some of the changes of metamorphosis, but a few retained the larval form for 2 or 3 days. The majority of these aged larvae lay motionless but some swam when disturbed. Five such larvae were placed, 48 hr. after liberation, in a flask which had contained a culture of the alga *Prasinocladus* and which bore a green film on the glass inside. After a further 24 hr. two of the five had metamorphosed and secreted tubes, showing that a greatly prolonged planktonic life may sometimes be followed by successful metamorphosis.

It was then found that the great majority of larvae would seek out and settle upon a filmed cover-slip in a clean beaker, and would not settle upon a clean cover-slip in a filmed beaker. Larvae encountering a filmed glass surface behaved differently from those encountering a clean one. On striking clean glass larvae either swam off again with no more than momentary hesitation,

TABLE I. EARLIER METAMORPHOSIS OF *SPIRORBIS BOREALIS* IN FILMED BEAKERS THAN IN CLEAN BEAKERS

(In each experiment a batch of larvae was divided between a pair of 50 ml. beakers, one with the inside surface filmed, the other wiped clean. After 24 hr. the numbers metamorphosed and unmetamorphosed were counted. In all, 91 % had metamorphosed in the filmed beakers, 15 % in the clean ones.)

Exp. no.	Origin of film	Nos. metamorphosed/unmetamorphosed in: Filmed beakers	Clean beakers
1	Beakers had contained sea water, to which a culture of *Chlamydomonas* had occasionally been added, for 14 days	14/4	0/20
2		16/2	4/15
3		16/1	0/16
4		21/5	1/21
5		11/0	3/7
6		30/0	6/24
7	A culture of *Synechococcus* for 4 days	12/1	0/17
8	Sea water in dim light for 14 days	21/0	9/10
	Totals	141/13	23/130

or swam over the surface with scarcely diminished speed. On striking a filmed surface they stuck to the film as soon as they touched it and then started crawling upon it at less than half the swimming speed. They appeared to have some difficulty in leaving a filmed surface. A larva about to swim away was often seen with its anterior end clear, its prototroch vigorously active, and its posterior end apparently attached to the surface by an invisible, gradually lengthening thread. Suddenly the larva would accelerate to normal swimming speed.

It seemed possible that clean glass might be too smooth for the larvae to crawl upon, so paired pieces of slate and granite, carefully matched, were selected as offering surfaces which were mechanically rough. One of each pair was immersed for 48 hr. in an algal culture (*Synechococcus* or *Prasinocladus*), the other was left dry. Then each pair was exposed to larvae in a 250 ml. dish. Many larvae encountered the freshly immersed stones, but none were seen to crawl upon them. Larvae that encountered the other stones, which were tinted green from the cultures in which they had been lying, immediately started to crawl. Eighty larvae settled on these stones, only two on the freshly immersed ones. (Table II).

It seems highly probable that *Spirorbis* settles on filmed surfaces rather than clean surfaces because it can crawl on them more easily, and that crawling is due to cilia sticking to bacterial films or algal surfaces. There is practically no interval between contact with a filmed surface and adhesion to it, so there is scarcely time for deliberate choice, but mechanical roughness is unlikely to be one of the factors involved.

TABLE II. LARVAE OF *SPIRORBIS BOREALIS* SOUGHT OUT FILMED SURFACES IN PREFERENCE TO FRESHLY IMMERSED ONES

(In each experiment larvae in a clean 250 ml. dish were presented with a pair of stones, one of which had been immersed in an algal culture for 48 hr., the other left dry for the same period immediately prior to the experiment.)

			Nos. settled on stones which were	
Exp. no.	Stone	Culture	Filmed	Freshly immersed
1	Granite	*Synechococcus*	36	0
2	Granite	*Prasinocladus*	18	0
3	Granite	*Prasinocladus*	4	2
4	Slate	*Synechococcus*	22	0
Totals			80	2

EFFECT OF PREVIOUSLY SETTLED *SPIRORBIS* ON CHOICE OF SUBSTRATUM

Fucus was found to be suitable for settlement whether its surface had been wiped or not. When larvae were put into thoroughly wiped 250 ml. bowls with pieces of *F. serratus*, all the larvae settled on the *Fucus*, unless the bowls were placed in the dark, when many settled on the surface film. Similar behaviour during darkness has been recorded in *Bugula* (Grave, 1930; Lynch, 1949*a*). To avoid it most experiments were carried out in the light.

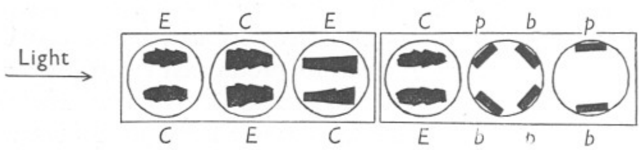

Fig. 2. In laboratory tests for gregariousness similar pieces of *Fucus*, some (*E*) bearing previously settled *Spirorbis borealis*, others (*C*) bare, were exposed side by side to larvae in 250 ml. dishes. These were contained in boxes which were white inside, both sides being equally illuminated. The diagram shows two boxes, each with three dishes. Results are given in Table III. Two dishes are shown with slate blocks, some bearing *S. borealis* (*b*), others *S. pagenstecheri* (*p*), which were used to test whether larvae could distinguish between the two species. Double pairs of blocks were used in Exps. 1–10 (see Table V), single pairs in Exps. 11–20.

As the larvae were photosensitive the dishes were placed in narrow boxes, white inside, the long axes of which were directed towards a window so that both sides appeared equally illuminated. For each experiment two similar pieces of *Fucus* were obtained from immediately above a dichotomy and placed on opposite sides of a dish, one bearing previously settled *Spirorbis*, the other a bare control. Experimental and control pieces were placed on alternate sides in adjacent dishes to guard against there being an undetected difference in

TABLE III. SETTLEMENT OF *SPIRORBIS BOREALIS* ON TWO SEPARATE SIMILAR PIECES OF *FUCUS*, ONE WITH PREVIOUSLY SETTLED *SPIRORBIS*, THE OTHER BARE

(In every experiment, except those marked with asterisks (in which the larvae showed little discrimination or appeared to have been influenced by light), at least twice as many settled amongst the previously settled *Spirorbis* as on the bare *Fucus*.)

		No. of larvae settling on	
Exp. no.	No. of previously settled *Spirorbis*	Experimental *Fucus*	Bare control
1	20	18	1
2	10	14	0
3	4	4	1
4	16	31	2
5	25	22	2
6	15	50	5
7	10	25	3
8	9	52	2
9	10	6	9*
10	10	44	0
11	8	51	1
12	12	12	16*
13	20	11	2
14	19	17	8
15	19	22	0
16	12	29	2
17	43	25	0
18	16	44	4
19	70	27	2
20	49	34	23*
21	84	7	0
22	46	15	7
23	29	4	2
24	31	13	1
25	35	43	11
26	22	24	59*
27	35	85	2
28	15	7	1
29	143	61	27
30	151	64	25
31	123	113	23
32	115	83	25
33	102	74	38*
34	111	112	49
35	46	29	5
36	56	10	21*
37	28	48	7
38	47	15	23*
	Totals	1345	409

illumination between the two sides (Table III). Freshly liberated larvae were then pipetted into the dishes in varying numbers.

During this series of experiments the following conditions were varied, but the changes did not affect the results, which are given in Table III. At first, previously settled *Spirorbis* were removed from the controls. This often involved damage to the *Fucus* so similar damage was done to the surface of the other piece of each pair, either by removing tubes or by picking pieces away with the point of a needle. A few tests with bare pieces of *Fucus* indicated that damage discouraged settlement, so some experiments (nos. 8–11) were carried out in which the experimental pieces were more damaged than the controls. Later (nos. 17–24 and 29–34) the *Fucus* used was entirely undamaged, apart from the scissor cuts by which the pieces were obtained, and *Spirorbis* was allowed to settle on the experimental pieces shortly before the experiments, whilst the controls remained in neighbouring vessels which contained no larvae. In some experiments (nos. 12–16 and 29–34) the water in the dishes was kept circulating by air bubbles, in the remainder it was left unstirred.

In thirty-one out of the thirty-eight experiments the results were consistent, with a ratio of at least 2:1 in favour of the *Fucus* which bore previously settled *Spirorbis*. It is probable that some of the remaining seven experiments involved larvae which were in poor condition, for they were from adults which had been kept for 1 or 2 days in the laboratory. In the three experiments of these (nos. 26, 36 and 38) which showed a clear majority settling on the controls, it appeared that the dishes had not been equally illuminated, and that the larvae had been collecting towards their darker sides.

Altogether 1345 larvae settled on the *Fucus* which bore previously settled *Spirorbis*, 409 on the bare control pieces. In the earlier experiments (nos. 1–24) when the numbers of larvae in each dish varied between 5 and 57, over six times as many settled on the experimental pieces as on the controls. In the later ones, in most of which between 50 and 100 larvae were used, the ratio was only about 2·5:1. This recalls results obtained with *Ostrea edulis* (Cole & Knight-Jones, 1949), which showed that gregariousness tended to be masked when settlement was heavy, probably because a few less discriminating larvae settled early upon the control surfaces, thereby encouraging further settlement upon them.

Prolongation of the Searching Phase in Isolated Individuals

The movements of larvae did not appear to be directed towards previously settled individuals, so it seemed probable that gregariousness might be accounted for by random swimming with settlement taking place more readily on *Fucus* which already bore *Spirorbis* than on bare *Fucus*. The possibility that metamorphosis might be delayed in isolated larvae was therefore investigated.

In each of three preliminary experiments 10 larvae were isolated in 50 ml. beakers, and 20 were put together into another similar beaker. To ensure, as far as possible, that the isolated and crowded larvae in each batch were comparable genetically, they were pipetted from a small liberation and put out alternately, one into isolation and two into the crowded beaker. All thirty may have come from the same parent. A piece of *Fucus* which bore five previously settled individuals was presented to the crowded larvae, pieces of bare *Fucus* to the isolated ones. The beakers were left under similar conditions of illumination and examined at intervals of about 20 min. When it was seen that

TABLE IV. *SPIRORBIS BOREALIS*. PERCENTAGES METAMORPHOSED IN EXPERIMENTAL BATCHES OF LARVAE WHICH WERE CROWDED AND/OR WITH *FUCUS* BEARING PREVIOUSLY SETTLED INDIVIDUALS AND IN CONTROL BATCHES OF LARVAE WHICH WERE ISOLATED WITH BARE *FUCUS*

(Differences of less than 20 % are marked by asterisks. The other, more considerable, differences indicate consistently earlier metamorphosis in the experimental batches.)

Exp. no.	Period (hr.)	No. of crowded larvae	Percentages metamorphosed: Experimental	Control
	Crowded with previously settled *Spirorbis*			Isolated
1	3	20	90	60
2	1	20	95	70
3	1	20	85	40
		Mean	90	57
	Crowded with initially bare *Fucus*			Isolated
1	2½	25	60	50*
2	2	20	90	50
3	2	50	100	33
4	1½	100	95	75
5	1½	100	85	80*
6	1½	45	98	90*
7	1½	50	30	30*
8	2	50	90	44
9	2	48	87	60
10	6	49	58	20
		Mean	79	53
	Single larvae with previously settled *Spirorbis*			Isolated
1	2	—	44	10
2	1	—	78	70*
3	1	—	89	20
4	1	—	90	60
5	1	—	100	70
6	1	—	50	60*
7	3	—	100	60
8	3	—	70	30
9	3	—	80	56
10	1½	—	56	50*
		Mean	76	49

most of the larvae in the crowded beaker had settled, the numbers which had metamorphosed and which were still moving were recorded, first in the crowded beaker and then in the other beakers. Because they were examined later the isolated larvae had a few minutes longer in which to metamorphose than the others. Nevertheless, it was found in each experiment that 85–95 %

of the crowded larvae had metamorphosed, but only 40–70 % of the isolated ones (Table IV).

Using similar methods, a series of ten experiments was then carried out, in which the crowded larvae, between 20 and 100 in number, were presented with bare *Fucus*. In the aggregate 79 % of the crowded larvae metamorphosed, but only 51 out of the 96 isolated ones (the 4 remaining isolated larvae could not be found, having probably adhered to the inside of the pipette). Consistently in six out of the ten experiments 20–67 % more of the crowded larvae metamorphosed than of the others. In the remaining four experiments the differences were 10 % or less. This does not prove that the presence of unmetamorphosed larvae promotes settlement. The earlier settlement in the crowded beakers may possibly have been due to a few less discriminating larvae attaching themselves and metamorphosing, thus encouraging further settlement.

In each of another series of ten experiments twenty isolated larvae were divided into two batches, one of which was presented with pieces of *Fucus* bearing from 5 to 10 previously settled *Spirorbis*, the other with similar bare pieces. A total of 73 out of 96 larvae in the former batch metamorphosed, 48 out of 99 in the latter (the remaining larvae were lost). In seven out of the ten experiments 25–70 % more larvae metamorphosed in the former than in the latter. In the remaining three experiments the differences were 10 % or less.

The length of the period of larval life appeared to be rather similar in larvae from the same parent, but it varied widely in different batches. As Table IV shows, 100 % of one batch metamorphosed within an hour, but only 60 % of another in 6 hr. The results show that the searching phase was prolonged by isolation, but do not show by how much it was prolonged. As a mere conjecture, the prolongation often appeared to be about an hour. This is probably sufficient to account for the gregariousness shown in the laboratory experiments.

Ability of Larvae to Distinguish their own Species from an Allied Species

S. pagenstecheri is abundant on stones and shells in the lower half of the tidal zone on most shores near Bangor. Its tube coils so that the mouth faces anticlockwise from an observer's viewpoint, whereas that of *S. borealis* is clockwise. Its larvae are colourless (apart from their red eyes) and very small, whereas those of *S. borealis* are much larger and reddish brown in colour.

S. borealis appeared to be rather more common on stones than *S. pagenstecheri* was on *Fucus*, so smooth slate was chosen as providing an experimental surface suitable for both species. Eight similar blocks measuring 24 × 24 × 4 mm. were prepared. These were immersed in sea water for a day, to allow a bacterial film to develop, and were then divided into two batches, with each block in a separate, freshly wiped beaker. Larvae of *Spirorbis borealis* were added to one batch, and larvae of *S. pagenstecheri* to the other. These soon settled on the blocks. The blocks were then exposed in pairs (Fig. 2), one of each pair bearing *S. borealis*, the other *S. pagenstecheri*, in 250 ml. dishes to which larvae of both species were added. After 24 hr. the numbers of both species which had settled on each block were recorded. The few *S. borealis* which had settled on the *S. pagenstecheri* blocks were then removed, whilst the *S. pagenstecheri* were removed from the other blocks. This was done with the point of

TABLE V. *SPIRORBIS* LARVAE SETTLED AMONGST THEIR OWN SPECIES IN PREFERENCE TO AN ALLIED SPECIES

(In a series of experiments larvae of *S. borealis* and *S. pagenstecheri* were put together in a 250 ml. dish containing paired blocks of smooth slate, one of each pair bearing previously settled *S. borealis*, the other *S. pagenstecheri*. Inconsistent results are marked by asterisks.)

			Nos. of larvae of			
	Nos. of previously settled		*S. borealis* setting on blocks bearing		*S. pagenstecheri* setting on blocks bearing	
Exp. no.	*S. borealis*	*S. pagenstecheri*	*S. borealis*	*S. pagenstecheri*	*S. pagenstecheri*	*S. borealis*
1	18	58	20	6	26	10
2	34	65	6	3	29	14
3	40	90	21	3	43	5
4	58	132	30	10	16	7
5	92	70	12	7	7	5*
6	4	24	38	14	24	29*
7	32	35	56	6	40	13
8	83	71	15	4	3	14*
9	93	72	18	33*	—	—
10	30	49	1	0	16	0
			Blocks changed round			
11	4	7	9	0	7	1
12	13	14	12	0	7	1
13	25	21	17	1	—	—
14	5	12	8	1	7	1
15	13	19	5	2	12	1
16	12	4	7	0	9	1
17	19	13	9	0	17	3
18	5	1	6	0	1	0
19	11	2	6	3	10	3
20	14	12	11	1	8	0
	Totals		307	94	282	108

a needle to avoid disturbing the bacterial films, which were probably similar on all the blocks since they had all been exposed under similar conditions for the same period. The experiments were then repeated.

After ten experiments the results seemed conclusive (Table V). In the aggregate, and in seven or eight out of ten separate experiments, more than twice as many larvae of each species settled on the blocks which bore their own species than on the other blocks. To guard against the possibility of there being some intrinsic difference between the blocks of each pair, the blocks were all wiped clean and changed round, so that those which had previously borne *S. borealis* bore *S. pagenstecheri* and vice versa. Another similar series of ten experiments was then carried out, using small numbers of larvae and taking care to obtain these only from freshly collected adults. The results which followed were still more conclusive. About 90 % of the larvae of each species sought out their own species in preference to the other.

It is scarcely conceivable that the larvae could have distinguished mechanically between their own species and the allied one. The tubes of the previously settled individuals grew quickly, so that the size ranges of the two species overlapped one another considerably. Those searching larvae which were seen to encounter tubes never paused to explore their shape or size, but always moved away immediately. It seems highly probable that some chemical sense played the main part in recognition of their own species.

Further Aspects of the Behaviour during Settlement

Garbarini (1936*b*) carried out experiments on larvae from *S. borealis*, which he kept in an aquarium on a clump of *Fucus*. With circulating sea water he was able to keep them thus in good condition for more than 2 months. During the initial photopositive phase larvae attached themselves to every object which they could not go round, whatever its nature. Later they appeared to be attracted or repelled by neighbouring objects. Then, if a larva passed near *Fucus*, it went straight to the surface, crawled upon it and attached itself. Few larvae settled on *Laminaria* and still fewer on *Himanthalia*, *Ascophyllum*, *Rhodymenia* and stones and other inanimate objects. None attached to ascidians. *Botryllus* seemed repellant. He visualized the contrasting behaviour towards *Fucus* and *Botryllus* as due to substances secreted into the water by these organisms.

Similarly rapid settlement, without a searching phase, was observed at Bangor in larvae liberated by adults which had been kept in the laboratory for more than 1 or 2 days (p. 204). The apparent attraction towards *Fucus* may be a response of photonegative larvae towards the dark fronds (p. 203). A few experiments tended to confirm that *Fucus* is much more favourable for settlement than *Ascophyllum*. It has already been shown that freshly immersed inanimate surfaces are very unfavourable (p. 207).

Fucus is not overwhelmingly favourable for settlement. When larvae were put into a dish, the glass of which bore a bacterial film and some previously settled *Spirorbis* on its lighted side, they all settled on the glass amongst the *Spirorbis* though there was *Fucus* in the dish. Settlement on glass cover-slips bearing a bacterial film and 10 to 50 *Spirorbis*, which had settled a short time previously, was compared with settlement on a bare piece of *Fucus* of similar size. In a single experiment in the light 25 larvae settled on the *Fucus*, 5 on the cover-slip. It seemed likely that the searching larvae found the dark surface of the *Fucus* visually during their repeated reconnaissances, so four other experiments were carried out in the dark. In these 76 larvae settled on the cover-slip, none on the *Fucus*. Light and photosynthesis are complicating factors, but in the dark gregariousness was clearly stronger than a possible predilection for *Fucus*.

Fewkes (1885) noted that *Spirorbis* larvae in aquaria sometimes settled, metamorphosed and floated on the surface film 'until the increasing specific gravity of their bodies sinks them to their future homes', but it is very unlikely that re-attachment to another surface can occur after metamorphosis. At Bangor settlement on the surface film took place particularly readily in the dark and when there was a bacterial scum there.

TABLE VI. PROVIDED THE SURFACE WAS NOT CROWDED ($<10/\text{cm.}^2$) MORE LARVAE OF *SPIRORBIS BOREALIS* SETTLED ON *FUCUS* WHICH BORE MANY *SPIRORBIS* THAN ON SIMILAR PIECES WHICH BORE FEWER

(In each experiment larvae were presented with two similar pieces of *Fucus* of surface area 4–8 cm.^{2}, one bearing many more recently settled *Spirorbis* than the other.)

	Fucus with many *Spirorbis*		*Fucus* with few *Spirorbis*	
Exp. no.	(Nos. previously settled)	Larvae setting	(Nos. previously settled)	Larvae setting
1	(80)	49	(20)	29
2	(83)	11	(18)	2
3	(54)	15	(21)	1
4	(120)	16	(18)	0
5	(32)	13	(7)	3
6	(26)	14	(5)	12
7	(120)	8	(10)	0
8	(38)	14	(5)	1
Totals		140		48

Settlement took place very frequently in concavities, such as the slight grooves alongside the midrib of *Fucus* or the angle at the periphery of a beaker's base. This was probably a response to contact stimuli. It could not be sheltering from currents or light when it occurred in still water and with a transparent substratum.

In eight experiments (Table VI) larvae attached in far greater numbers to *Fucus* which bore many recently settled *Spirorbis* (5–10/cm.^{2}) than to pieces which bore fewer (1–2/cm.^{2}), but the crawling larvae left areas which were crowded with 10–20 recently settled *Spirorbis* per cm.^{2} and spaced themselves out very evenly on adjoining less crowded areas. In a further five experiments (Table VII), pieces bearing 1–4/cm.^{2} proved to be more favourable for settlement than similar pieces crowded with 20–50/cm.^{2}. Apparently the gregarious tendency operates until it has led to a crowding of about 10/cm.^{2}, but larvae arriving thereafter try to find less crowded areas nearby.

This spacing out tendency is not due to physical lack of space because larvae proved to be capable of settling at over 40/cm.^{2} when they were given no other choice. It must involve discrimination, which is probably exercised during crawling. The behaviour immediately prior to settlement is well suited to ensuring, as far as possible, that there is a clear area of 1 or 2 mm. radius round the place of attachment (p. 205, and Fig. 1).

A spacing-out tendency on very crowded surfaces has also been recorded in the barnacle *Elminius modestus* (Knight-Jones & Stephenson, 1950).

TABLE VII. MORE LARVAE SETTLED ON *FUCUS* WHICH BORE FEW *SPIRORBIS* THAN ON SIMILAR SMALL PIECES WHICH WERE VERY CROWDED (>20/CM.^{2})

(In each experiment larvae were presented with two similar pieces of *Fucus* of surface area 1–2 cm.^{2}, one with few or no recently settled *Spirorbis*, the other crowded to an artificially high degree (the previously settling larvae having been given no other surface for attachment).)

	Fucus with crowded *Spirorbis*		*Fucus* with few *Spirorbis*	
Exp. no.	(Nos. previously settled)	Larvae setting	(Nos. previously settled)	Larvae setting
1	(53)	10	(4)	37
2	(62)	9	(2)	16
3	(59)	11	(3)	11
4	(50)	14	(0)	20
5	(63)	11	(5)	30
	Totals	55		114

SOME FIELD OBSERVATIONS

Sample patches of *Fucus serratus* at different levels on the shore were examined during one of the periods in which *Spirorbis borealis* was liberating larvae. Recently settled individuals were numerous about low-water mark, particularly on the new growth bordering on old stems which were thickly covered with old *Spirorbis*. Very few were found on the abundant *Fucus* at about half-tide, which bore no older stages of *Spirorbis*, though a few laboratory experiments had indicated that *Fucus* collected from above half-tide was as favourable for settlement as some from low-water mark. One may speculate that liberations occurred during the ebb tide or at low water, so that larvae, settling within a few hours, got little opportunity of reaching the higher levels of the shore, but so far there is no evidence for such behaviour. It is probable that the heavy settlement at low-water mark, where adults were numerous, was largely due to the gregarious tendency, and that this usually prevents large numbers of larvae from settling at an unfavourably high level on the beach.

Very few *Spirorbis* were found on *Ascophyllum*, even in areas where this was intermingled with *Fucus* bearing abundant *Spirorbis* (see p. 214).

S. pagenstecheri was comparatively rare on *Fucus*, but when one individual was noticed on a frond a search generally revealed several others nearby. It was common on stones and shells in damp places in the lower half of the tidal zone. It is probably short-lived, for in many places the stones bore only tubes which were empty or occupied by *Polydora*. When the collection of adults for laboratory experiments was begun, much time was wasted in the difficult task of examining the tubes to see whether or not they contained live *Spirorbis*. Then it was noticed that amongst live tubes there were usually many very small but conspicuously white tubes of recently settled individuals, whilst no such concentrations of young individuals were to be found round dead tubes. This might suggest that the larvae had settled immediately after liberation, but laboratory observations showed that larvae from freshly collected adults were positively phototactic for at least a quarter of an hour after liberation, and usually for longer than this. Probably they are so in the field, which would lead to their being widely dispersed.

Shells of *Nucella lapillus* were often covered with *Spirorbis*, either with the dextral tubes of *S. pagenstecheri* or the sinistral tubes of *S. granulatus* or *S. borealis*. Whilst some *Nucella* bore tubes which were all dextral, others from the same area bore only sinistral tubes. Mixtures of two species on such a small area as a *Nucella* shell appeared to be comparatively uncommon. *Spirorbis* on rocks and stones occurred in clumps, often of a single species. Isolated individuals were comparatively rare.

These observations left a strong impression that the specifically gregarious tendency demonstrated in the laboratory was of great importance in the field.

Gregariousness as Probably a General Feature of the Settlement Behaviour of Marine Larvae

Thorson (1950) found that about 70–80 % of bottom-living invertebrates have planktonic larvae, and reviewed the evidence that many of these larvae are able to postpone metamorphosis for days or even weeks, and to search actively for a suitable substratum. 'In a water area with a bottom current of only half a knot the larvae forced towards the bottom by their photonegativity and testing the substratum at intervals may be carried over a distance of 24 km. in 24 hr., i.e. 170 km. per week, and their chance of finding a suitable substratum for settling and metamorphosis seems to be great.' It is usually possible to relate the patchiness of the benthos to differences in the substratum, but sometimes it is difficult. *Pecten maximus*, for example, lives on a sandy bottom which may extend for vast areas, yet Priol (1930) described this species as essentially gregarious. During dredging near Bangor, in the wide sandy area of Red Wharf Bay, it was found commonly in some places but not at all in hauls from neighbouring, apparently similar, ground.

The distribution of marine animals is probably greatly influenced by the settlement behaviour of their planktonic larvae. In general, little is known about this behaviour. No evidence for gregariousness during settlement had been revealed when Allee (1931), having described crowded populations of certain littoral species, which appeared to be far below the level of organization at which a 'social appetite' was likely to occur, concluded that aggregations of primitive animals form frequently as the result of forced movements. This must be true. Berrill (1950) may well have been justified in describing the aggregations, which are usually typical of the ascidian *Styelopsis grossularia*, as due to the viviparous development and insensitivity of the tadpoles to light, factors tending to 'keep the children at home', though he gave the free-swimming period as of many hours.

In laboratory experiments on ascidian larvae (Grave, 1935, 1941, 1944; Grave & Nicoll, 1940) metamorphosis was hastened by crowding or by aqueous extracts of the adults or larvae.[1] This is suggestive. The distribution of ascidians often suggests specific gregariousness. In 1948 *Ascidiella aspersa* was abundant and *Ascidia conchilega* was comparatively sparse on certain oyster grounds worked from the Fisheries Laboratory at Burnham-on-Crouch, Essex. *Ascidia conchilega* occurred in clumps, with few isolated individuals, as though the larvae were gregarious and could distinguish their own species from the other.

So far tests for gregariousness have been carried out only on *Ostrea edulis*, *Elminius modestus* and the two species of *Spirorbis* dealt with here. These animals were not chosen for research because they were obviously more gregarious than other marine invertebrates, but because of their economic importance or convenience. The fact that the tests gave positive results in each species, representing three main phyla, suggests that gregariousness will prove to be a rather general feature of settlement behaviour. Though it has been demonstrated only in sessile species, in which it is likely to be particularly important, it will probably occur in others also, since most marine invertebrates have limited powers of locomotion as adults.

Andrews (1949), having observed that Folliculinidae constantly occurred in clumps, suggested that the behaviour could hardly serve for protection or feeding, but that breeding might be aided by clumping, if a process of conjugation occurred in the imperfectly known life cycle of the group. Breeding is likely to be the main advantage to be derived from gregariousness, but it is not the only one. Another is that larvae will probably find, in places where the species is already established, suitable conditions for their own survival.

[1] Glaser & Anslow (1949) have suggested that this may have been due to copper, which they found to be present in ascidians and which has been shown to accelerate their metamorphosis. The grounds for this idea are weakened by the facts that (i) Grave (1935) found that tissue extracts which rapidly and consistently induced metamorphosis in larvae of the same species had at most only a slight effect on another species, and (ii) a variety of unfavourable conditions and dilute solutions of other poisons besides copper have been shown to accelerate metamorphosis, not only in ascidians (Zinkin, 1938), but in *Bugula* (Lynch, 1949*b*) and *Tubularia* (Pyefinch & Downing, 1949). The same criticism may now be made of Prytherch's conclusion that copper is necessary for metamorphosis in larvae of the American oyster. Prytherch (1934) put forward, in support of this, a controversial idea regarding the function of the pigment spots in the larval mantle (Cole, 1938) and an interpretation of some results, showing peak settlement at low water, which has also been questioned (Korringa, 1940). It should not yet be accepted that copper has a peculiar or necessary effect on metamorphosis in either oysters or ascidians.

This will be particularly important in species with closely restricted habitats. Often the factors which make places unsuitable for a species are complex or do not operate all the time. The presence of adults is a simple test of suitability. Without gregariousness many larvae of *S. borealis* would probably settle, during high water, on the *Fucus* in the upper half of the tidal zone, where they would be doomed to desiccation during subsequent ebbs (see p. 216).

Gregariousness in oysters proved to be most marked when settlement was light and to be masked when settlement was very heavy, probably because a few less discriminating larvae soon settled on bare surfaces, stimulating others to follow (Cole & Knight-Jones, 1949; see also p. 210). This phenomenon seems bound to accompany gregariousness in general and to have the following results, beneficial to the species. In poor breeding years settlement of larvae will be concentrated round the parent stocks, replenishing these as far as possible. This happened during the poor oyster spatfall of 1947 in the River Crouch, Essex (Knight-Jones, 1951). In normal breeding years old stocks will be built up and the range of the species may be extended to new areas, where pioneer larvae will be followed by others. In exceptionally good breeding years larvae will settle abundantly in every area to which they drift in sufficient numbers. Great increases in the range of the species will occur and many larvae will settle in unusual and often unsuitable places. Oyster larvae are said to have settled abundantly (and most unusually) on reeds in flooded ditches during the exceptionally good spatfall of 1935 in the River Crouch, Essex. Settlement in such places is mass suicide, but population pressure on the old beds is thereby reduced. This pressure may become too great in exceptionally good breeding years. At Conway, in 1940, practically all *Mytilus edulis* of marketable size were smothered and killed by a continuous covering of young mussels (Ministry of Agriculture and Fisheries, 1946, p. 57). In *Elminius* and *Spirorbis* the spacing-out tendency on crowded surfaces (p. 216) and the normally short span of life guards against such a disaster.

SUMMARY

Spirorbis borealis and *S. pagenstecheri* were chosen for laboratory experiments on settlement behaviour because they are viviparous, with larvae which soon settle.

S. borealis liberated larvae mainly at about the moon's quarters.

After liberation larvae were positively phototactic for a period (15 min. to 2 hr.), then swam more at random. For a further period of 1 or 2 hr. they visited a large number of different surfaces, crawling upon them and then swimming off again.

When about to settle the crawling larvae changed direction with gradually increasing frequency by flexing the abdomen. Eventually they attached themselves, with wriggling movements, by the milky secretion of a gland on the

dorsal surface, which formed the initial, semi-transparent tube. Within 1 or 2 min. they rolled over on to the dorsal side and embarked upon a cataclysmic metamorphosis.

Larvae settled on surfaces of glass or stone only if the surfaces bore bacterial or algal films. In clean vessels larvae did not metamorphose, except abnormally or after a considerable delay, unless a filmed surface or a piece of *Fucus* was provided for attachment. Searching larvae did not stick to wiped or freshly immersed surfaces and appeared to be unable to crawl upon them, but immediately they touched filmed surfaces they stuck to them and started to crawl.

Many more larvae settled on pieces of *Fucus* which bore previously settled *Spirorbis* than on bare controls.

Settlement occurred earlier in larvae which were crowded, or accompanied by previously settled individuals, than in isolated larvae.

Paired slate blocks, one bearing previously settled *S. borealis*, the other *S. pagenstecheri*, were exposed to larvae of these two species. The great majority of the larvae settled on the blocks which bore their own species rather than on those which bore the other.

Fucus was very favourable for settlement of *S. borealis*, but in the dark bare *Fucus* proved less favourable than filmed glass surfaces which bore previously settled *Spirorbis*. Larvae often settled in concavities, probably in response to contact stimuli. Gregariousness soon led to a crowding of from about 10 to 20 recently settled individuals per cm.^{2}, but larvae arriving thereafter sought less crowded areas nearby, on which they spaced themselves out.

In the field settlement of *S. borealis* occurred mainly about low-water mark, on *Fucus* which already bore *Spirorbis*. Stones which bore live adult *S. pagenstecheri* could easily be distinguished from others which bore dead tubes, because many small, white tubes of recently settled individuals occurred amongst the adults.

Gregariousness during settlement has been demonstrated in each of the only four species so far investigated (*Ostrea edulis*, *Elminius modestus* and two species of *Spirorbis*), representing three main phyla. It is probably a general feature of the settlement behaviour of planktonic larvae, helping them to find suitable habitats, to maintain old breeding stocks and to form new ones.

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