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THE BREEDING OF *CREPIDULA FORNICATA* (L.) IN THE RIVER BLACKWATER, ESSEX

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(Text-figs. 1–4)

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Introduction

The chain-forming prosobranchiate gastropod *Crepidula fornicata* is not indigenous to British waters, but has been introduced during the last 70 or 80 years, probably from North America, upon imported oysters, and, during this period, has spread to most of the oyster beds on the south-east and south coasts of this country (see Orton, 1950*b*, for a recent discussion of its present distribution). In spite of the interest shown in this species because of its detrimental effect upon oyster culture, and because of its well-known change of sex from male to female, little is known in the literature of its breeding habits here, or in North America. Conklin (1897) states that the spawning period in New England lasts from early summer to about mid-August, no larvae being found in late August. Murie (1911), in discussing the introduction of this species, elaborates upon Conklin's observations, and gives evidence that females, in samples of *Crepidula* taken from the River Colne in May and September 1898, contained spawn beneath their shells. Orton (1912*a*, p. 438) states that he is 'informed by Professor Conklin that American Crepidulae begin to spawn in May and possibly in April, whilst English Crepidulae begin

to spawn in early March'. In a footnote, he adds 'Crepidula spawned in tanks at Plymouth in early February'.

Later workers upon *Crepidula* have added little to our knowledge of the breeding period, although Coe (1936, 1938) has investigated the sex change in several species, in greater detail than Gould (1919). Orton (1909) has described the occurrence of protandric hermaphroditism in *C. fornicata* and (1912*b*) the method of feeding. Thorson (1946), whilst conducting extensive plankton investigations in Danish waters, recorded the early free-swimming stages of *C. fornicata* off north-west Jutland, in July 1940, but observed no later stages.

The author is indebted to Messrs Wombwell and French, of the Tollesbury and Mersea Native Oyster Co., for arranging the collection of regular samples of *Crepidula*. The author is also indebted to Emeritus Professor J. H. Orton, of Liverpool University, for generous advice and criticism, to his present colleague Dr D. J. Crisp for much useful advice, particularly upon statistical problems, and to the Directors of Imperial Chemical Industries Paints Division, under whose auspices the investigation was conducted.

Summary of the Life History of *Crepidula fornicata*

The anatomy and histology of the reproductive system of *C. fornicata* are well known (Orton, 1909; Gould, 1917; Coe, 1936).

Briefly, the gonad consists of an irregular lobulated organ lying along the intestine and between the lobes of the digestive gland. In the functional males it is reddish brown in colour, whilst the female gonad is bright yellow. The diverticulae of the gonad give rise to the gonoduct anteriorly. The gonoduct in the functional male consists of a very convoluted seminal vesicle, which is continued as a narrow vas deferens to open upon a ciliated groove, which runs forward, on the right side of the animal, to a large grooved muscular penis, situated behind the base of the right tentacle.

On the completion of the functional male phase, the penis degenerates, cells being sloughed off from the brown tip (Gould, 1917). As the penis becomes smaller, the width of the gonoduct increases and the inner walls become folded longitudinally. The distal part of the gonoduct develops into a prominent uterus with folded walls, into which a number of seminal receptacles open.

Whilst these changes are taking place, the penis becomes greatly reduced, and is finally represented by a minute brown scar. The gonad, which even in the male phase contains small ova, develops rapidly as oogenesis takes place, becoming a bright yellow when fully functional. Coe (1938) found that the period of sexual transformation from male to female in isolated individuals was 61 days, but was considerably longer for males forming part of a 'chain'.

The eggs are laid in a bunch of stalked balloon-shaped capsules, united basally to a common stem, fastened to the substratum and, as in *Vermetus*,

protected by the parent whilst development is in progress. The capsule has thin, cream-coloured walls, and contains *c.* 250 eggs, each 150–170 μ in diameter when initially spawned, floating in the intra-capsular fluid.

The embryonic development has been described in great detail by Conklin (1897). At the end of embryonic development, the larvae escape as advanced veligers through an apical split in the egg sac, to lead a free-swimming life until settlement occurs. The free-swimming veliger larvae possess shells 260 μ long, 190 μ broad and 170 μ high (mean of 100 measurements), with a two-third to three-quarter whorl.

In the adults, the functional male phase can be readily distinguished externally by the development of a coiled seminal vesicle, a long black penis, and a red brown gonad, whilst the functional female possesses a prominent uterus and a yellow gonad. Not infrequently a female is found with a small brown rudimentary penis. Orton (1909) gives diagrams of the functional male and female phases, with several intermediate stages.

Materials and Methods

The investigation of the breeding of *Crepidula fornicata* was commenced in May 1946, and was continued until December 1947. In 1946, samples of about 50 'chains' (i.e. *c.* 150 females) of *Crepidula* were obtained fortnightly from the oyster beds in Thirslet Creek, near Tollesbury (River Blackwater), Essex, although larger samples from Mersea Quarters (West Mersea) were examined when personal visits were made to the locality. No samples could be obtained from the River Blackwater during February and early March 1947, owing to the presence of extensive ice floes. From mid-March, however, until November, regular weekly samples of the same size (i.e. *c.* 50 chains) as employed in the previous year (1946), were obtained from Mersea Quarters (see Admiralty Charts 3740–3741). Except when personal collections were made, all samples were wrapped in damp sacking, packed in a wicker-work basket, and were dispatched by rail on the afternoon of the day of collection. They were usually received at Liverpool late the following morning, in good condition, even during the period of high temperatures during the summer of 1947. A few observations were made as opportunity arose at Burnham-on-Crouch in 1948, 1949 and 1950, but no systematic study was attempted.

The samples were examined for sex, gonad development, and presence of, and stage of development of the embryos. Shell lengths were usually recorded. Complete chains (i.e. those unbroken with the basal member present) only were employed.

The embryos were referred to seven categories, each corresponding to a readily distinguished stage in development as follows:

Stage 0. Comprising those females with well-developed ovaries, containing ripe ova, and which are ready to spawn.

Stage 1. 1- to 8-celled stage, usually an orange or deep yellow in colour (Fig. 1*a*).

Stage 2. 8-celled stage, to the end of gastrulation (Fig. 1*b*). The colour of the embryos in this stage is predominantly yellow but is sometimes cream. Owing to the amount of albuminous fluid contained within the egg cases, the embryos are normally difficult to separate for examination.

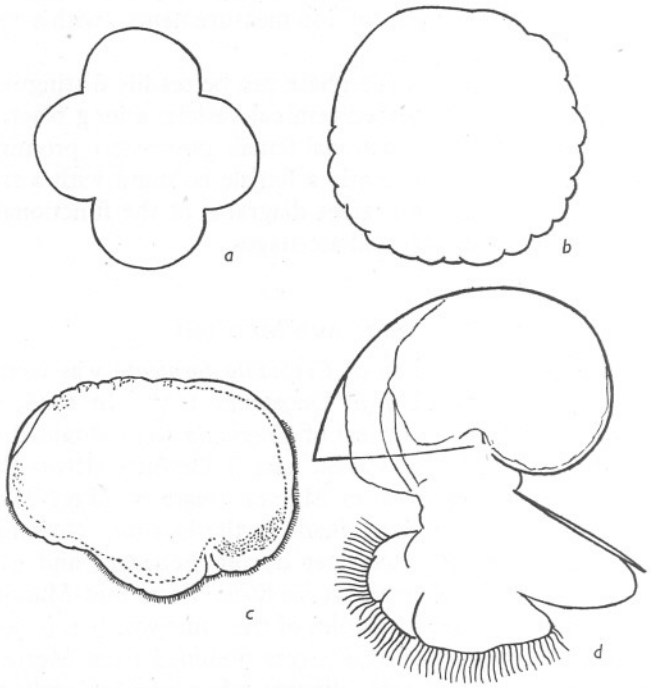

Fig. 1. *Crepidula fornicata*. Stages of embryonic development: *a*, Stage 1; *b*, Stage 2; *c*, Stage 3; *d*, Stage 5.

Stage 3. The 'pre-veliger' stage, in which gastrulation is complete, and the archenteron and stomodeum are well formed, the latter usually being prominent. The shell gland can usually be observed. The embryo is well ciliated (Fig. 1*c*). The colour is usually yellow to light yellow and the embryos are normally more readily separated than in the previous stage.

Stage 4. Early veliger stage, with well-developed velum, and shell, but with a less advanced internal organization than the next stage. The colour is usually a light yellow to yellow-brown. The embryos are readily separable in sea water.

Stage 5. Later veliger stage, ready to hatch (Fig. 1*d*). The shell is well formed, of a broad tumid appearance. The velum is slightly 4-lobed and very strongly ciliated. The margin of the velum is usually darkly pigmented. The foot is well developed and ciliated, with a dark pigment spot anteriorly. The

gut is filled with granules, and is darkly pigmented. The embryos at this stage appear brown or dark grey to the naked eye.

Stage 6. Hatched stage, represented by empty egg capsules.

The stage of development of the embryos was determined microscopically, the criterion of colour alone not being sufficiently definite to ensure accurate classification into the appropriate category.

During both seasons, the percentage of females bearing embryos in Stage 1, except at the beginning of the period of spawning in 1947, was low, and followed the general course of spawning closely. It is possible that these embryos were the result of forced spawning, extrusion of the ova occurring during the process of dredging, and hauling the trawl. As 24 hr. normally elapsed before examination could be commenced, there was ample opportunity for early segmentation to occur. It is doubtful whether such premature spawning occurred in transit (unless the material was very roughly handled), as samples collected on the same ground at West Mersea, part of which were examined immediately, and part after 24 hr. storage in air, showed no significant difference in numbers of Stage 1 embryos, although many more advanced Stage 1 embryos were present after 24 hr. than immediately after collection. Also, unsegmented ova were never observed in the material examined at Liverpool, or 24 hr. after collection at West Mersea. However, the fact that most of these Stage 1 embryos were fertilized and actively segmenting suggests that the female *Crepidula* bearing them were ripe and ready to spawn.

Sea temperatures, etc. In order to investigate a possible correlation between sea temperature and the breeding of *Crepidula* records of the bottom temperature were made twice daily (early morning and early afternoon) in Thirslet Creek, near Tollesbury, from September 1946 until December 1947. From the end of February until December 1947 sea temperatures were also taken twice daily in Thornfleet (West Mersea) at 7 a.m. and 1 p.m. The daily means of the 1947 Thornfleet bottom temperatures are shown in Fig. 4. Standard sea-water thermometers (checked against an N.P.L. standard) with small buckets around the bulbs were employed in both localities. During 1947 the daily mean bottom temperatures in the two localities differed on no occasion by more than $\pm 2{\cdot}0^{\circ}$ F.

No salinity or pH measurements were made.

Spawning in 1946 and 1947 on the Blackwater Oyster Beds

The results of the observations upon the spawning of *C. fornicata* made in 1946 and 1947 are given in Tables I and II respectively. In Table I, the numbers of females bearing Stages 2 and 3, and 4 and 5 embryos are combined, but in Table II (for 1947) each category of embryonic development is given separately. The information obtained in the two years of study is shown graphically in Figs. 2 and 3 for 1946 and 1947 respectively. With the exception of the total

TABLE I. *CREPIDULA FORNICATA*. SPAWNING AT TOLLESBURY (THIRSLET CREEK) AND WEST MERSEA (MERSEA QUARTERS) IN 1946

Date, 1946	Locality	No. of chains examined	No. of females	No. of females with: Spawn (total)	Stage 1 spawn	Stages 2 and 3 spawn	Stages 4 and 5 spawn	Cumulative percentage, i.e. percentage of females with spawn which has developed beyond: Stage 0	Stage 1	Stages 2 and 3	Mean nos. of females per chain
8. v.	Toll.	44	136	110	22	42	46	80·9	64·7	33·8	3·10
17. v.	Toll.	35	108	102	18	31	52	94·4	77·0	48·1	3·09
20. v.	W.M.	100	298	276	15	88	173	92·7	87·6	58·0	2·98
29. v.	Toll.	35	123	104	21	37	51	84·5	71·6	41·5	3·54
11. vi.	Toll.	50	184	166	26	124	16	90·2	76·1	8·7	3·68
19. vi.	W.M.	50	147	112	5	38	69	76·0	72·8	46·9	2·94
29. vi.	Toll.	50	180	138	24	73	41	76·7	57·7	22·8	3·60
10. vii.	Toll.	36	120	62	12	42	8	51·6	41·6	6·7	3·34
17. vii.	W.M.	50	151	59	18	15	26	39·0	27·2	17·2	3·02
29. vii.	Toll.	28	79	14	2	10	2	17·2	15·2	2·5	2·82
2. ix.	Toll.	51	177	24	15	6	3	13·5	5·1	1·7	3·47
19. ix.	Toll.	50	164	3	0	3	0	1·8	1·8	0	3·38
8. x.	Toll.	30	97	0	0	0	0	0	0	0	3·23
26. xi.	Toll.	25	79	0	0	0	0	0	0	0	3·16

Toll. = Tollesbury. W.M. = West Mersea.

TABLE II. *CREPIDULA FORNICATA*. SPAWNING AT WEST MERSEA (MERSEA QUARTERS) IN 1947

Date, 1947	No. of chains examined	No. of females	Nos. of females bearing spawn: Total	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Cumulative percentage, i.e. percentage of females with spawn which has developed beyond: Stage 0	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Mean nos. females per chain
19. iii.	40	105	0	0	—	—	—	—	—	0	0	—	—	—	—	2·62
17. iv.	35	101	0	0	—	—	—	—	—	0	0	—	—	—	—	2·88
23. iv.	40	92	25	25	0	—	—	—	—	27·0	0	0	—	—	—	2·30
29. iv.	45	135	89	84	5	0	—	—	—	66·0	3·7	0	—	—	—	3·00
6. v.	45	112	82	52	30	0	—	—	—	73·0	36·6	0	—	—	—	2·50
14. v.	32	75	56	1	34	21	0	0	0	74·6	73·4	28·0	0	0	0	2·34
20. v.	46	105	100	15	13	13	24	27	8	95·2	81·0	68·6	56·1	33·4	7·6	2·28
4. vi.	56	121	115	7	16	21	28	22	21	95·0	90·1	76·0	58·7	35·5	17·3	2·16
16. vi.	47	111	106	19	34	16	19	3	15	95·5	78·4	47·7	33·1	16·2	13·5	2·36
25. vi.	44	78	67	1	19	9	4	4	30	85·9	84·6	60·3	48·7	43·6	38·5	1·66
2. vii.	45	100	90	7	55	4	4	2	18	90·0	83·0	28·0	24·0	20·0	18·0	2·22
9. vii.	46	104	80	0	30	9	19	10	12	77·0	77·0	48·1	39·4	12·1	11·5	2·26
17. vii.	27	68	27	0	15	1	3	2	6	39·7	39·7	17·7	16·2	11·8	8·8	2·52
28. vii.	45	128	56	0	26	0	9	6	15	43·7	43·7	23·4	23·4	16·4	11·7	2·84
12. viii.	25	80	4	0	0	1	1	0	2	5·0	5·0	5·0	3·7	2·5	2·5	3·20
20. viii.	25	69	4	0	1	1	2	0	0	5·8	5·8	4·3	2·9	0	0	2·76
3. ix.	37	119	19	0	8	3	6	1	1	16·0	16·0	9·2	6·7	1·6	0·8	3·22
18. ix.	44	153	7	0	0	0	0	0	7	4·6	4·6	4·6	4·6	4·6	4·6	3·48
7. x.	26	86	0	0	0	0	0	0	0	0	0	0	0	0	0	3·30

percentage of females spawned in each sample, the results are given as cumulative percentages, i.e. in each sample the proportion of females carrying spawn which has developed beyond each category is plotted as a percentage of the total females present in the sample. This type of cumulative frequency distribution, because of the grouping of results, enables the temporal relationships in such biological behaviour as spawning and development to be presented more clearly. The method is similar to that employed in survival investigations in insecticide studies.

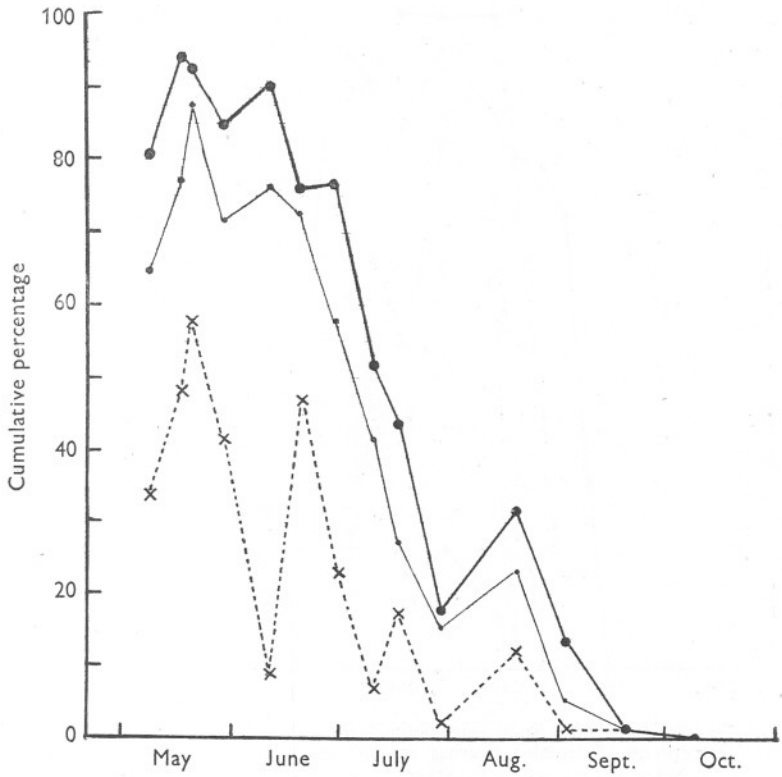

Fig. 2. *Crepidula fornicata*. Spawning behaviour at Tollesbury (Thirslet Creek) in 1946. Percentage of females bearing spawn developed beyond: ●———●, Stage 0; ·———·, Stage 1; ×– – – –×, Stage 3.

Significance of Results

The samples of *C. fornicata* employed in this investigation can be considered random, as (i) they were not chosen for any particular attribute, other than that the chains should be complete, i.e. broken chains were rejected, and (ii) each sample was made up of chains taken from several hauls upon the same bed.

In order that the significance of the differences between successive samples

might be assessed, the data obtained from the examination of the samples were treated statistically (Table III).

In column 6 of Table III the standard error of the percentage of females bearing spawn which has developed beyond Stage 2 (i.e. the sum of all females with embryos of the pre-veliger and later stages) for each sample in 1947 is given. By taking this stage of development for statistical treatment, the inclusion of possibly prematurely spawned females is avoided.

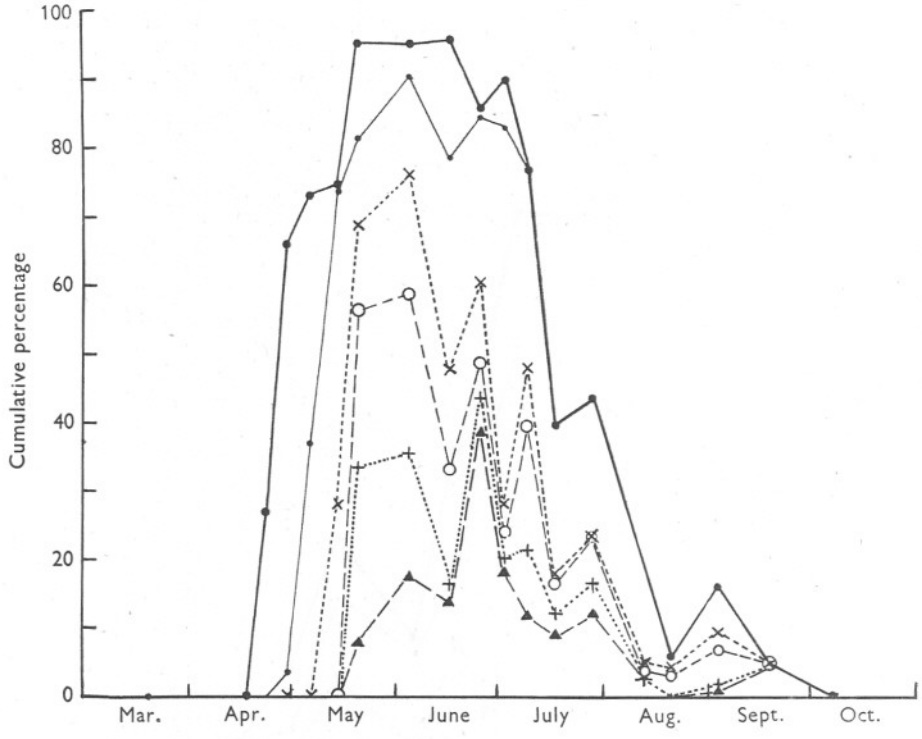

Fig. 3. *Crepidula fornicata*. Spawning behaviour at West Mersea (Mersea Quarters) in 1947. Percentage of females bearing spawn developed beyond: ●——●, Stage 0; ·——·, Stage 1; ×– – –×, Stage 2; o– – –o, Stage 3; +– – –+, Stage 4; ▲— — —▲, Stage 5.

In column 7 are given the values of χ^2 and the corresponding probability calculated from the actual numbers of females with spawn developed beyond Stage 2 in successive pairs of samples, employing the 2×2 Contingency Table method. No allowance has been made in this treatment for the association of females in chains, and the assessment of significance of the differences between pairs of samples is the most favourable obtainable, i.e. the assumption is that the females were sampled at random. As only complete chains were sampled at random, a stricter analysis, representing the most unfavourable condition,

in which the numbers of females in each sample were reduced in proportion to the number of chains in the sample, was employed, and the significance assessed by the method for small samples drawn from a Binomial Distribution. The values for χ^2 thus obtained are given in column 8 of Table III.

The differences which are significant upon a basis of $P=0{\cdot}05$ are underlined in columns 7 and 8 of Table III. Use will be made of these significant differencesi n the discussion upon possible periodicity in spawning (see p. 59).

Length of Breeding Period

The first sample of *Crepidula* examined in 1946 (8 May) was obtained after the breeding period had commenced, about two-thirds of the females bearing

TABLE III. *CREPIDULA FORNICATA*. SIGNIFICANCE OF SPAWNING RESULTS, 1947

(1) Date 1947	(2) No. of chains examined	(3) No. of females	(4) No. of females with spawn passed through Stage 2	(5) Percentage of females with spawn which has passed through Stage 2	(6) Percentage standard error of females in '5' (Binomial) + or −	(7) 2 × 2 contingency table		(8) Binomial sampling on basis of no. of chains	
						χ^2	approx. P	χ^2	approx. P
23. iv.	40	92	0	—	—	—	—	—	—
29. iv.	45	135	0	—	—	—	—	—	—
6. v.	45	112	0	0	0				
14. v.	32	75	21	28·0	7·9	**29·3**	<0·001	**9·6**	0·003
20. v.	46	105	72	68·6	6·9	**29·6**	<0·001	**13·1**	<0·001
4. vi.	56	121	92	76·0	5·7	1·42	0·14	3·05	0·69
16. vi.	47	111	53	47·7	7·3	**18·0**	<0·001	8·9	0·004
25. vi.	44	78	47	60·3	7·4	3·15	0·08	0·44	0·52
2. vii	45	100	28	28·0	6·7	**18·3**	<0·001	**6·10**	0·014
9. vii.	46	104	50	48·1	7·4	**8·30**	0·006	2·80	0·09
17. vii.	27	68	12	17·7	7·4	**16·9**	<0·001	**13·1**	<0·001
28. vii.	45	128	30	23·4	6·3	0·89	0·36	2·60	0·11
12. viii.	25	80	4	5·0	4·4	**12·1**	<0·001	**9·1**	0·003
20. viii.	25	69	4	4·3	3·9	0·04	0·84	0·00	1·00
3. ix.	37	119	11	9·2	4·7	1·34	0·25	1·04	0·29
18. ix.	44	153	7	4·6	3·3	2·16	0·15	0·21	0·66
7. x.	26	86	0	0	—	—	—	—	—

$\chi^2 g = 1 (P = 0{\cdot}05) = 3{\cdot}841$.
Values of χ^2 in heavy type indicate those significant on the $p = 0{\cdot}05$ criterion.

spawn and of which a half contained veliger larvae. Spawning had thus begun some time previously. Subsequently, egg capsules, containing embryos in various stages of development, were present in all samples until mid-September (see Fig. 1), after which date none was seen until the following season.

In 1947 the first females bearing spawn were observed on 23 April, initial spawning having thus occurred between 17 and 23 April. All the embryos were, however, in Stage 1, and may thus have been spawned prematurely. The first females with Stage 2 embryos were observed on 29 April. From this time developing embryos were present until mid-September (18th) when empty egg cases only were observed. At Burnham-on-Crouch in 1948 and 1949, female *Crepidula* with developing embryos were observed from April until September.

Thus, on the Essex oyster beds, spawning of *Crepidula* would appear to commence in early spring, and to continue until September in most years,

giving a breeding period of about 5½ months' duration. The breeding period on the Essex oyster beds is therefore rather longer than that for the same species upon the New England beds as described by Conklin (1897). Orton's (1912*a*) observation that *C. fornicata* in English waters begins to spawn in March was not borne out by the 1947 observations, but, in that year, water temperatures in the Blackwater were very low (30° F. at the beginning of the month, rising to 42° F. at the end), following the abnormal weather in February, when ice was present on many parts of the east coast. In 1948, a single sample from Tollesbury (Thirslet Creek), taken on 6 April, showed that spawning had commenced a few days previously, whilst *Crepidula* began to spawn at Burnham-on-Crouch in 1950 immediately before 30 March. It would thus seem that *Crepidula*, in a warm spring, spawns on the Blackwater oyster beds at least in late March or early April. This view is supported by Prof. J. H. Orton who, in a personal communication, states that there is little doubt that spawning commences in a normal spring in March.

Sea Temperature and the Commencement of Spawning.

In 1946, no observations of the onset of spawning in *Crepidula* in relation to sea temperature could be made. In 1947, however, the onset of spawning was carefully followed. Initial spawning (possibly premature) took place between 17 and 23 April, whilst the first Stage 2 embryos were found in the sample collected on 29 April. During this period the mean daily sea temperature remained at a little above 50° F. until about 24 April, having risen from 45° F. to this value between 12 and 17 April, and rose to 52° F. between 24 and 30 April. Thus spawning commenced at a period when the sea temperature had risen slightly above 50° F.

No systematic observations were possible in following years, but the sea temperature at Tollesbury (Thirslet Creek) rose above 50° F. at the end of March 1948, and, as has been stated previously, a sample of *Crepidula* obtained on 6 April of that year supplied data suggesting that spawning had commenced a few days previously. No observations were made in 1949, but in 1950 spawning commenced at Burnham-on-Crouch at the end of March, when the sea temperature had risen to *c.* 51° F. It is therefore possible that *Crepidula* does not spawn until the sea temperature rises to, or above, 50° F.

Temperature and Duration of Spawning Period

Although in 1947 at West Mersea initial spawning took place shortly after the sea temperature rose above 50° F., spawning ceased a considerable time before the occurrence of the autumnal homothermal epoch, the ovaries of the female *Crepidula* showing exhaustion during July. Thus, in *Crepidula* from the Blackwater beds, as in *Ostrea* (Korringa, 1947), temperature does not appear to determine the duration of the breeding period directly, and it would seem that the length of the breeding period is determined, rather, by the rate of

exhaustion of the gonads, and the rate of regeneration of ova and sperm. Spawning in *Crepidula* does not appear to be limited by the high maximum temperatures attained during the summer, but there is some evidence that the higher summer temperatures in the Blackwater in 1947 caused a more rapid spawning, and consequently an increased rate of exhaustion of the gonads, than in 1946.

Periodicity in Spawning

The results obtained in 1946, owing to rather infrequent sampling, do not provide definite evidence of periodic spawning, in the sense that a proportion of females in the population tend to release their ova in concert several times during the breeding period. Two well-defined maxima in the proportion of females bearing spawn of Stages 4 and 5 (i.e. veligers) occurred in the samples from West Mersea collected on 20 May and 19 June (See Table I and Fig. 2), whilst two smaller ones were recorded on 17 July and 19 August, although these are probably not significant statistically. On the latter date, however, a distinct

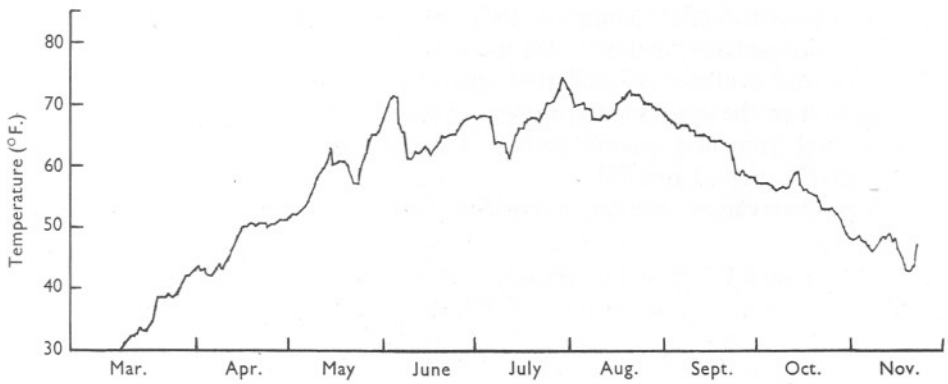

Fig. 4. Bottom temperatures at West Mersea (Thornfleet Creek) in 1947.

maximum in early stages occurred. It would thus seem that, from the beginning of May until the end of the spawning period in September, three concerted spawnings took place, one before 20 May, one immediately before 11 June (before a new moon, or during the last quarter phase) and a final one, rather distinct from the main spawning period, immediately before 19 August (at or after a new moon).

The greater frequency of sampling adopted in 1947 provides more definite evidence of concerted spawning (see Fig. 3). In this year, the first true spawning probably occurred immediately before 29 April, the first Stage 2 embryos being observed in a sample collected on this date (Table II). As the sea temperature had risen to and just above 50° some 10 days before (Fig. 4) it is unlikely that the temperature rise in itself acted as a spawning stimulus. This first spawning occurred contemporaneously with a first quarter lunar

period. Spawning continued, and on 14 May, all embryos found, except in 1·3% of the females, were in Stage 2 or Stage 3 of development, to be followed in the next sample on 20 May with a maximum of females bearing veliger larvae (Stages 4 and 5).

No evidence of a further concerted spawning was obtained until 16 June, when an increase in the proportion of females bearing Stages 1 and 2 embryos was recorded, followed in the next sample (25 June) by a significant peak in females with embryos developed beyond Stage 3, although the peak for females with embryos later than Stage 2 is not quite significant on the 5% level ($P=0{\cdot}08$) (see Table III). This spawning took place at a time when the sea temperature was *c.* 62° F., during a slight rise, after a rapid fall from 70° F. at the beginning of June (see Fig. 4), and also when the moon was waning (new moon, 18 June).

Spawning continued over the new-moon period, a maximum of Stage 2 embryos occurring in the next sample (2 July) to be followed by a significant maximum in older embryos 7 days later. This spawning, which included *c.* 7% of females with Stage 1 embryos, took place when the sea temperature was *c.* 68° F., immediately before a full moon (3 July).

No further evidence of concerted spawning was recorded until the end of August, when the sea temperature was declining below 70° F., and the moon was waxing from first quarter to full. This spawning is possibly significant, although $P=0{\cdot}15$ (Table III).

These observations are summarized in Table IV.

TABLE IV. POSSIBLE PERIODIC SPAWNING OF *CREPIDULA FORNICATA* AT WEST MERSEA, 1947

Approx. date of spawning	Moon's phase	Sea temp. at spawning maximum (° F.)	Remarks
Between 17 and 23 April	New moon, 21 April	50	Probably premature spawning
Between 23 and 29 April	New moon, 21 April	51·5	First true spawning
Immediately before 16 June	New moon, 18 June	62	Max. in Stage 2 embryos followed 25 June by significant peak in Stage 4 and older embryos
Immediately before 2 July	Full moon, 3 July	68	Max. in Stage 2 embryos followed 9 July by significant peak in Stage 3 and older embryos
Between 17 and 28 July (approx. 25th)	New moon, 18 July	67·5	Not significant
About 25 August	Full moon, 31 August	68	Final spawning

Although there is no definite indication of cyclic periodicity in spawning, the results obtained in 1947 show a marked tendency for a proportion of female *Crepidula* to spawn in concert, in a manner unassociated with temperature, or

temperature fluctuations. Spawning, however, is evidently not limited to these periods for the mimima of the incidence of females with Stage 2 (i.e. young embryos) do not touch the zero ordinate until the end of the observed breeding period.

Of the four definite spawnings, two (first and last) occurred during a first-quarter lunar period, one immediately before or at the new moon, and one just before or at the full moon. Thus, three of the four concerted spawnings can be fitted into a first-quarter lunar period, and one to a last-quarter period. Similarly, three spawnings (all except the first) occurred at times when the tidal range was increasing, whilst, although the first spawning probably took place at a period of neap tides, the intensity of spawning greatly increased as the tidal range became greater (see Fig. 3). Unfortunately, the sampling frequency was too low to provide definite evidence for or against spawning towards the end of the lunar quarter periods when the tidal range and tidal currents would increase daily. The date of commencement of spawning at Burnham-on-Crouch in 1950 (29–30 March) lends some weight to this view, as spawning commenced from 1–2 days after the occurrence of the bottom of neap tides, i.e. when the tidal range was increasing.

Lunar periodicity in spawning has been fully discussed in the past by Fox (1924), Orton (1926, 1936), Amirthalingam (1928), Battle (1930, 1932), Wheeler & Brown (1936), Korringa (1947) and others. These writers have quoted many instances of spawning of marine invertebrates at spring tides, but there are few records of spawning at neap tides or at periods when the tidal range is increasing from the minimum at neaps, although Orton (1936) states that in 1927 there was a distinct tendency for *Ostrea edulis* from the Fal and Blackwater beds to spawn upon the neap tides preceding full moon tides. Some evidence of the spawning of *Pomatoceros triqueter* during periods of neap tides at Cullercoats was obtained in 1947 (Chipperfield, 1948).

The specific inducements which cause spawning in relation to lunar phases are obscure. Briefly, the factors which may be responsible are probably either (*a*) nutritional or (*b*) concerned with intrinsic rhythms.

There has been little investigation into the possibility that cyclic changes in nutrition related to the lunar and tidal periods induce spawning, although Loosanoff & Nomezko (1946) have found that feeding of *Ostrea virginica* shows no relationship to tidal ranges or periods of light and darkness. It seems possible that hitherto unknown factors such as the 'external metabolites' of Lucas (1947) may have an important bearing on these problems. Further, in some organisms, the rhythm of spawning appears to be intrinsic, e.g. in *Convoluta* the spawning of eggs takes place during the neap tides even *in vitro*.

There is little evidence that moonlight, or its absence, or the greater illumination of the sublittoral zone at the occurrence of low water during the hours of daylight, has any effect on immersed animals as a spawning induce-

ment (Amirthalingam, 1928). Amongst the hydrographical effects of tides are the effect of alternating hydrostatic pressures, the greatest range being produced at spring tides; the variation in water temperature due to the more rapid warming and cooling of the intertidal zone at low water; and the variations in the magnitude of the tidal currents flowing at springs and neaps. Amirthalingam (1928) feels there is little evidence that hydrostatic pressure influences spawning in *Aequipecten*, whilst Orton (1936) suggests that a decrease in hydrostatic pressure with an increase in temperature may be responsible for initiating spawning in *Ostrea* on spring tides. Korringa (1947) also favours the effect of rhythmic variations in hydrostatic pressure as spawning inducements, whilst Battle (1932) suggests that the increase in pressure at spring tides causes spawning in *Yoldia sapotilla*. The presence of pressure-sensitive organs has not, as yet, been demonstrated in marine invertebrates. Variations in the velocity of tidal currents at springs and neaps may influence spawning, either directly or indirectly, e.g. via nutritional channels.

No precise spawning stimulus or series of factors contributing to the spawning stimulus in *Crepidula* can be elicited from the observations made in 1947. Spawning in *Crepidula* appears to be independent of temperature once a value of 50° F. has been attained. Tidal currents run swiftly both in the Blackwater and the Crouch, whilst the difference between the tidal range at springs and neaps is fairly large (about 5 ft. mean). Thus either of these factors may be responsible for the tendency towards concerted spawning of *Crepidula* in 1947.

Duration of Embryonic Life

Owing to the low sampling frequency adopted in 1946, and the commencement of observations when the spawning period was well advanced, no reliable estimate of the duration of embryonic life of *Crepidula* can be made from the data. The data obtained from the more frequent observations in 1947, however, can be utilized for this purpose.

If it is assumed that the first normal true spawning occurred between 23 and 29 April, the first female *Crepidula* bearing Stage 3 embryos (pre-veligers) were observed in the sample taken on 14 May (28%), and the first fully developed veligers (Stages 4 and 5), and the first empty egg capsules on 20 May. Of the Stage 3 embryos observed on 14 May, a number were very advanced, and, if these may be considered as the first spawned embryos, they may be represented in the sample of 20 May by the recently emptied egg capsules. Similarly, the bulk of the veliger spawn (Stages 4 and 5) in the sample of 20 May probably represent those embryos which were spawned between 29 April and 6 May. These relationships are clearly illustrated in Fig. 2.

Thus, at the beginning of the spawning period, when sea temperatures range between 50 and 55° F., the first pre-veligers appear about 14 days after spawning, and the first true veligers in 14–21 days, whilst hatching occurs in

21–28 days. This estimate of the period of incubation is of the same order as Orton's (1912*a*) estimate that the eggs are 'protected by the mother *Crepidula* for about a month'.

The sequence of embryonic stages is less obvious later in the season, but it is possible that the spawning which occurred on about 18 June (see Table II) is represented in the sample of 28 June by the maximum of recently emptied egg capsules, whilst the peak in early veligers in the sample taken on 9 July represents those eggs spawned immediately before 2 July (see Fig. 2). Thus, during the period of higher water temperatures, development to the early veliger stage apparently takes place in about 7 days, and the hatching stage in about 10–14 days.

Thus, at the beginning of the spawning period, embryonic development in *Crepidula* would appear to take about twice as long as in June and July. The shorter duration of the veliger stages, particularly Stage 5, as compared with the younger stages, would explain the low incidence of females bearing Stages 4 and 5 embryos in the periodic samples.

Intensity of Spawning in 1946 *and* 1947

The proportion of female *Crepidula* from the Blackwater bearing developing embryos and hatched egg cases (i.e. the total proportion spawned in each sample) in the 2 years are plotted in Figs. 1 and 2 for 1946 and 1947 respectively.

Even if allowances are made for the lower frequency of sampling in 1946, the gross spawning curves for 1946 and 1947 are very similar. In both years the maximum proportion of females bearing developing embryos or empty egg cases was *c.* 95%, or if those bearing Stage 1 (i.e. possibly premature) embryos are excluded as forced spawners, both maxima are reduced to about 90%. In both years these maxima were attained in May and were maintained during early June, the proportion of spawn-bearing females decreasing rapidly in July, the zero ordinate being approached in 1947 in mid-August. In both years a further spawning occurred at the end of the principal period, in August, and was rather earlier and almost twice as heavy in 1946 as in 1947 (*c.* 30% females with spawn in 1946, and 15% in 1947).

The females bearing embryos in Stages 4 and 5 (i.e. veligers) in one sample are unlikely to be represented by others in the next sample with embryos of the same stage of development, particularly during June and July, when embryonic development has been shown to be more rapid than at the beginning of the spawning period. Now, the aggregate percentage of females bearing Stages 4 and 5 embryos (i.e. veligers) in all samples from Tollesbury in 1946 is 179, or 230 if all samples from West Mersea are included except the one collected on 20 May (see Table I) only 3 days after a collection at Tollesbury, and in which, if spawning occurs more or less simultaneously throughout the Blackwater, veliger embryos arising from ova spawned at the same time are represented. The corresponding aggregate proportion for 1947 is 183%.

In view of the rapid development of the veliger embryos the aggregates given above would suggest the probability that the majority of the fully functional females of *Crepidula* spawn at least twice in a season. Further, the fact of periodic concerted spawning would suggest multiple spawning by some females at least, particularly as exhaustion of the ovaries was not of common occurrence until July, i.e. towards the end of the main spawning period.

Sequence of Spawning in the Chain

The spawning of female *Crepidula* in 1947, during the initial spawning period, was analysed to show the percentage without spawn with Stages 2 and 3, and Stages 4 and 5 spawn and with empty capsules in each of the first two or three positions in the chains. (Note: the first individual in the chain is taken to be the basal member which adheres to the substratum, and which is designated the 'A' position. The next individual occupies the second or 'B' position and so on.) The proportion of chains with more than three functional females (i.e. fully developed ovary, well-developed uterus and with either a very rudimentary penis or none at all) is small, and has not been considered.

Out of a total of sixty-nine chains with two females, the basal member possessed more advanced embryos in thirty-one cases, the second member bore older embryos in twenty-three cases, and in the remaining fifteen examples, both the first and the second female members of the chain possessed embryos of the same stage of development.

Only twenty-eight chains possessing three females occurred in the samples of *Crepidula* taken during the early part of the breeding season. Of these twenty-eight chains, in eleven the basal females (i.e. the female in the 'A' position) possessed more advanced embryos, in nine those in the second or 'B' position, and in three only those in the third or 'C' position. Of the remaining chains, in one the females in the 'A' and 'B' positions bore embryos in the same stage of development, and in two those in positions 'B' and 'C' did so. The number of chains with four females was too small to allow any useful analysis to be made.

The numbers involved are not very large, and the statistical error is consequently high, but in the initial spawning period, females occupying the basal or first position appear to bear somewhat more advanced embryos than those in the second position, and that these, in turn, are somewhat more advanced than in the third position. Thus, there seems to be a very slight tendency in all chains for the basal and therefore oldest female to spawn first, with the next following closely. Females in the third position appear to spawn later than those in the first two (i.e. proximal) positions, this being especially noticeable towards the end of the spawning period. It is not known whether this is due to earlier copulation, which, in any case, occurs early in the year (Orton, 1912*a*). It is possible that the proximity of one or more males may

delay the onset of maturity of the distal females, or that these spawn later, because they are younger females, maturing later normally.

The Final Spawning. In both 1946 and 1947, a small spawning occurred in August and September, as previously stated. The significance of this is rather obscure, for this spawning does not appear to be a further 'peak' of spawning in the main period, but one taking place after the termination of the latter. In view of Coe's (1938) work upon the sex change in *C. fornicata* from American beds, there is a possibility that the final spawning observed in 1946 and 1947 was due to the ripening of individuals which had changed their sex to become functional females during the summer. On the other hand, it is more likely that this final spawning took place in females which had spawned out earlier in the season, and which had, during the intervening period, re-developed ovaries sufficiently well to allow a further emission of ova to occur, whilst environmental conditions were still favourable to continued spawning.

TABLE V. *CREPIDULA FORNICATA*. POSITION IN CHAIN AND FINAL SPAWNING, 1947

Position in chain (see p. 64)	Total no. examined	Stage of embryonic development				
		Stage 2	Stage 3	Stages 4 and 5	Stage 6	Total
(*a*) 20. viii. 47:						
C	25	0	0	0	0	0
B	25	1	1	1	0	3
A	13	0	1	1	0	2
	63	1	2	2	0	5
(*b*) 3. ix. 47:						
C	37	0	1	5	0	6
B	37	4	0	0	1	5
A	28	4	2	2	0	8
	102	8	3	7	1	19

If the final burst of embryos were due to the spawning of those individuals which had changed their sex during the earlier part of the season, then the presence of embryos should tend to be confined to the distal females in the chain, i.e. to those females immediately adjacent to the males.

In Table V the distribution of females bearing embryos in two samples taken in 1947 during the final spawning period is analysed.

Thus, from the table, although it is possible that some of the distal females which had spawned were those which had changed their sex during the season, there is no evidence that the final spawning is confined to any particular position in the chain, and the figures would indicate that the final spawning is a question of adequate re-development of the ovaries whilst conditions for spawning are favourable.

Incidence of Females with a Rudimentary Penis

During both 1946 and 1947, a number of females, with a well-developed uterus, and a yellow gonad filled with abundant oocytes but with a rudimentary (degenerating) penis, often 3–4 mm. in length, were a constant feature of the samples. These were invariably[1] the most distally situated females (i.e. those nearest the males), and obviously the youngest in the chain, having been the last to change from male to female.

The incidence of these females in samples examined 1947 from West Mersea between 29 April and 28 July is analysed in Table VI.

TABLE VI. *CREPIDULA FORNICATA*, 1947. INCIDENCE OF FEMALES WITH A RUDIMENTARY PENIS

Date, 1947	Total females in sample	Females with rudimentary penis: (*a*) Total	(*b*) Without spawn	(*c*) With spawn	(*d*) Percentage of total females
29. iv.	135	9	8	1	6·7
6. v.	112	9	9	0	8·0
14. v.	75	5	5	0	6·7
20. v.	105	1	0	1	0·9
4. vi.	121	3	3	0	2·5
16. vi.	111	3	3	0	2·7
25. vi.	78	1	1	0	1·3
2. vii.	100	7	4	3	7·0
9. vii.	104	3	3	0	2·4
17. vii.	68	7	7	0	10·3
28. vii.	128	2	2	0	1·6
Total	1137	50	45 (4·0%)	5 (0·4%)	— (4·4%)

It is apparent that the mean incidence of females with a rudimentary penis is low, although it is possible that more are present at the beginning and at the end of the main spawning period than during the period of most intense spawning. The low occurrence of females with a rudimentary penis bearing spawn is very significant, and would suggest that the bulk of such females are immature, in short chains at least.

Number of Female Crepidula *in a Chain*

In Tables I and II, the mean number of females per chain in each sample is recorded in the final column.

Cursory examination of these figures suggests that, in 1946, the chains collected from Thirslet Creek (Tollesbury) contain rather more females than those collected from Mersea Quarters (West Mersea), i.e. a position nearer the open sea, whilst in 1947 rather more females occurred in those chains from Mersea Quarters collected during the latter part, and after the end, of the breeding period than during the early part.

These conclusions can be subjected to a strict test, employing the method based upon the distribution of '*t*'.

[1] I am informed by Prof. Orton that this is not always applicable to long chains of *Crepidula*.

From the 1946 observations, the mean number of females in the chains from West Mersea is 2·98 (three samples), and, in chains from Tollesbury, 3·31 (eleven samples). Comparison of these samples, on the null hypothesis that they were drawn from the same population, gives a value for 't' of 2·14. As, from the tables (Fisher & Yates, 1948, p. 32) 't' for $P=0{\cdot}05$ for 12 degrees of freedom is 2·18, it can be concluded that the probability of the Tollesbury *Crepidula* samples possessing more females per chain than the West Mersea *Crepidula* in 1946 is high, the difference between the sample means being significant on the $P=0{\cdot}06$ level. A heavier settlement of spat, owing to the more restricted size and rate of flow of the water mass, or a lower mortality in Thirslet Creek may provide possible explanations.

In 1947 figures were similarly treated, the mean of the number of females per chain from 23 April to 17 July being compared with the mean for the period 28 July to 7 October. The sample taken on 25 June was omitted, as a large proportion of the longer chains were broken. The mean number of females per chain is 2·34 (ten samples) for the earlier period, and 3·13 (six samples) for the later period. From the comparison of these means by the normal methods a value for 't' of 5·34 was obtained. From the tables the values of 't' for 14 degrees of freedom for $P=0{\cdot}05$ and $P=0{\cdot}001$ are 2·145 and 4·140 respectively. Thus, the difference between the means is highly significant, and it would seem that, in 1947, the mean number of females per chain of *Crepidula* increased at the end of the spawning period owing, possibly, to a lower mortality at the end of the season compared with the period when spawning was intensive, or to a real increase in the number of females by the change of sex from male to female. As there was little evidence in the 1947 samples of a higher mortality during the spawning period, it is probable that the sex change is responsible for the increase in the number of females per chain.

Settlement in 1946 and 1947 in the Blackwater

During the summer and early autumn of 1946, samples of culch and of adult *Crepidula* were examined carefully for the presence of *Crepidula* spat, but very few indeed were found, in spite of the high intensity of spawning, and the fact that abundant veligers were seen in plankton collected at West Mersea on 20 June.

In 1947, however, settlement was heavy. On 28 June, Mr French, who collected the samples at West Mersea, reported the presence of a few spat on the oyster culch, whilst a few minute *Crepidula* were taken from shells collected at Tollesbury on the 25th. By 2 July, spat was thickly clustered on old shells, particularly on the inner surfaces of dead *Crepidula* shells, and on the sheltered areas of oyster shells and culch. Few were found upon exposed surfaces of the shells and upon living *Crepidula* in the samples. A week later very large numbers of spat were present, and settlement continued until mid-August,

thereafter declining rapidly. A small settlement, indicated by the presence of minute spat, took place in September, at a time when spat which had settled earlier were growing rapidly. Orton (1950*a*) has recently discussed the variations in the spatfall of *Crepidula* on the Essex oyster beds in 1945–1947.

Duration of larval life in Crepidula.

Estimates of the duration of planktonic larval life based upon observations of release of larvae from, or the spawning of, the adults of sessile marine invertebrates, and of the initial settlement of spat upon appropriate substrata are notoriously inaccurate, depending, as they do, upon the assumption that the stock of larvae settling is the same as the stock which was spawned previously. Examples are well known in which, after a normal spawning, larvae developed beyond a certain stage are not seen in the plankton for a considerable period, e.g. *Verruca*. Thus, all estimates of larval life derived in this way must be interpreted with care.

Such an estimate for the duration of planktonic larval life in *Crepidula* can be obtained from the data obtained in 1947 for the Blackwater. The first hatched egg cases appeared in the sample taken on 20 May, whilst the first settled spat were observed at the end of June, thus giving a possible larval life of about 35 days.

Growth of Spat

When a length of 3–5 mm. is reached, the young *Crepidula* become highly motile and migrate from their sheltered positions under shells, etc. and settle upon existing chains, usually taking up a terminal position, or one upon the side of an adult female. A few small individuals occasionally form short chains of two or three members. It is not known whether these chains of spat are permanent or whether the basal member can migrate, carrying the remainder of the chain. Few solitary *Crepidula* over 6 mm. in length were found on culch from the Essex oyster beds in 1947.

During this period of migration, and when permanent positions are taken up on existing chains, mortality is apparently high, for the number of small individuals (*c.* 6–12 mm.) observed on chains during the autumn is only a very small fraction of the total settlement on culch, etc., in the summer.

On 29 April 1947 some fifty smallest individuals from the chains in the sample were removed and measured. The range was from 7 to 26 mm. with a mean of 18 mm., which probably represents a fair growth of the previous season's spat and is in accordance with the observations of Orton (1914). Coe (1942) states that the growth rate on New England coasts is such that, at 1 year of age, *C. fornicata* are 18–28 mm. in length. Gould (1917), on the other hand, states that *C. fornicata* grew to 10–15 mm. in 2 months at Woods Hole. Such high growth rates do not appear to be attained amongst normal populations in

the Blackwater, but no accurate information is available from measurements of individuals of unknown age in a chain owing to the impossibility of distinguishing the age groups. Investigations employing known individuals, such as Orton's (1914) constitute the only practicable method of obtaining reliable information upon growth rates, particularly of older individuals. During 1948 and 1949, a few *Crepidula* spat settled upon glass and Tufnol panels exposed at Burnham-on-Crouch in a general investigation of the season of settlement, rates of growth, etc., of sedentary marine organisms. These *Crepidula* were measured on the withdrawal of the panels. The sizes recorded lay between 10 and 22 mm.,[1] the panels having been exposed for two months (July and August) in each year. Such information must be interpreted with care, owing to the motility of the very young spat but these figures do represent the rates of growth in shell length of individuals from the time of initial settlement under favourable conditions, upon flat surfaces. As the length of a *Crepidula* shell is dependent upon the size and shape of the substratum, linear dimensions constitute poor criteria of shell growth, and an increase in length can only be considered an indication of growth.

Summary

The breeding of *Crepidula fornicata* from the Blackwater oyster beds was investigated during 1946 and 1947. A few observations were also made at Burnham-on-Crouch in 1948, 1949 and 1950. The methods of sampling, presentation of the results, and of assessing the significance of the results statistically are described.

In 1946 and 1949, developing embryos were found from April until early September, giving a breeding period of about $5\frac{1}{2}$ months' duration. The intensity of spawning was similar in both years.

In 1947, spawning commenced shortly after the sea temperature had risen to and above 50° F. Observations made in 1948 and 1950 confirmed this.

A suggestion of periodic concerted spawning of the female *Crepidula* was obtained in 1946 and 1947. The results suggest that there is an irregular periodic spawning at or immediately after neap tides.

Observations strongly suggest that most female *Crepidula* spawn at least twice in a season.

Estimates are given of the duration of embryonic and larval life. At the beginning of the breeding period, embryos develop to the pre-veliger stage in about 14 days, and to veligers in 14–21 days hatching in 21–28 days. A tentative estimate of the duration of larval life of *c.* 35 days is made. The effect of temperature on embryonic development is discussed.

The sequence of spawning in the chain is considered at the beginning and

[1] For example, on a glass panel exposed 12 July 1949 and withdrawn 9 September 1949, were two *Crepidula*, one 11 mm. and the other 22 mm. in length.

the end of the breeding period. There is some evidence that the older females spawn a little in advance of the younger ones.

The incidence of females (throughout the season) with a rudimentary penis is considered.

The mean number of females per chain at Tollesbury and West Mersea, and during and at the end of the spawning period, is discussed.

The settlement and growth of the spat of *Crepidula* are briefly described, and discussed.

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