THE ANNUAL GROWTH AND REPRODUCTIVE CYCLE IN FOUR ASCIDIANS

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(Text-figs. 1–11)

This study was undertaken to increase our very incomplete knowledge of the annual cycle in ascidians.

Berrill (1950) states that 'the age of ascidians, with one exception, is practically impossible to estimate, unless a certain inhabited area is followed closely through seasons and years'. In the present work the method has been to follow certain aspects of the ascidian population of a chosen area for a period of nearly two years. The area was the Old Dock in Ardrossan Harbour, Ayrshire, and the ascidian population contained four species: *Diplosoma listerianum* (Milne Edwards), *Ciona intestinalis* (Linnaeus), *Ascidiella aspersa* (Müller) and *Botryllus schlosseri* (Pallas). *Diplosoma* belongs to the order Enterogona, suborder Aplousobranchiata; *Ciona* and *Ascidiella* to the order Enterogona, suborder Phlebobranchiata; and *Botryllus* to the order Pleurogona, suborder Stolidobranchiata. Thus each of the three main suborders is represented, and the four ascidians studied are amongst the commonest British species.

There are three main aspects of the annual cycle: (i) growth, (ii) number and life-span of generations in the year, (iii) sexual reproduction.

Growth. In the simple ascidians *Ciona* and *Ascidiella* increase in length of the body suffices as a measure of growth. In the compound forms *Diplosoma* and *Botryllus*, however, the case is complicated by the existence of budding. This may involve rapid and almost continual production of new individuals which replace, or live along with, old ones in the colony. It is therefore difficult to find a significant measure of growth in these compound forms. The most convenient measurement, however, is area, since during growth the colonies of both *Diplosoma* and *Botryllus* increase in surface area but scarcely at all in thickness.

Number and life-span of generations in the year. The appearance of a new generation and the disappearance of an old one can be detected by inspection of the samples. It is difficult to see the small newly settled larvae, however, and the first evidence of a new generation is usually the presence of small individuals or colonies which have been established for a short but unknown time.

Sexual reproduction. Faunistic lists and papers sometimes give notes on the breeding season of various species, but these are usually generalized

conclusions based on observations made over a number of years. Also they fail to define the term 'breeding season', which might include a variety of conditions and times from the onset of gonadial development to the settlement of larvae. The period when an ascidian carries eggs and sperm is often considerably longer than the period of their liberation and development in nature. This latter period depends on internal and external limitations on spawning and successful development.

In the present study the progress of breeding was followed by noting the periods when animals carried eggs and sperm, developing eggs, or larvae, and by finding the period during which larvae had settled.

The quantity of eggs and sperm in the ducts can be readily seen in *Ciona* and *Ascidiella*. *Diplosoma* and *Botryllus*, however, do not have long genital ducts in which eggs and sperm are stored, and in these animals the reproductive condition was assessed by the state of development of gonad, and by the presence or absence of developing eggs and larvae in the zooids or the colony.

The Area Studied

The Old Dock, Ardrossan, is a semi-tidal sea-water dock, the water-level in which is controlled by sea gates. The amount of interchange of water between the dock and the sea is uncertain and variable, and depends partly on the traffic of ships through the gates. The predicted depth at H.W.O.S.T. is 19 ft. and the area of the dock is 4·23 acres. The walls, which are of stone, are vertical and form the substratum to which *Ciona* and *Ascidiella* are attached. *Diplosoma* and *Botryllus* are generally fixed to the test of the simple ascidians.

Methods

Samples were collected by scraping the walls of the dock at intervals throughout 1950 and 1951.

The size of samples varied with the availability of material, which depended partly on the time of year and partly on the water-level in the dock. The number of specimens measured (N) is shown on histograms of size distribution where percentages are used. The animals were narcotized with menthol, fixed in the expanded state in strong formalin and stored in weak formalin. Measurements of length in *Ciona* and *Ascidiella* were made to the nearest 1 mm. The colonies of *Diplosoma* and *Botryllus* were carefully removed from the substratum (generally the test of *Ciona* or *Ascidiella*), placed flat on the glass negative-carrier of a photographic enlarger, covered with a glass slide, and inserted in the enlarger. The outline of the projected image was traced on squared paper and the area of the colony calculated from the outline tracing.

Specimens of *Ciona* and *Ascidiella* were dissected and the genital ducts examined for eggs and sperm. A number of zooids were removed from each

colony of *Botryllus* and examined for the presence of gonad, developing eggs and larvae. In the colonies of *Diplosoma* zooids were examined for eggs, and the common test for developing eggs and larvae. In each sample of *Botryllus* and *Diplosoma* about twenty colonies were thus examined. Pieces of several colonies of the compound forms, in each sample taken during 1950, were sectioned to confirm the results of dissection.

Ascidiella aspersa (Müller)

Growth, number of generations in the year, and life-span.

The length of the specimens was taken as a measure of their size. In Fig. 1 a series of histograms shows the distribution by size of *A. aspersa* in the samples taken in 1950 (left) and 1951 (right).

The sample of 24 January 1950 showed two populations, one whose body length had the mode about 25 mm., and the other with the mode about 50 mm. The population of small animals represented rather more than 80% of the sample and consisted of animals that settled as larvae in the previous summer (1949). The remaining population, of large specimens, was rather less than 20% of the sample and represented animals that settled as larvae in the summer of 1948.

Between January and April there appears to have been some growth of the smaller animals of the 1949 group, but less amongst the larger ones. During this period the percentage of the total sample represented by the 1948 group gradually decreased until in April it accounted for only about 2% of the total sample. Thus, although a large part of the 1948 group had perhaps died before the first sample was taken in 1950, it was in the months from January to April that this group virtually disappeared.

Growth for the 1950 season became important between 21 April and 24 May and continued at a high rate until about the middle of June. From then until the middle of July growth was considerably slower, at least amongst the larger individuals.

The sample of 14 July gave the first evidence of the new generation, and from this time until about the end of September growth of animals of this 1950 group continued. During the same period there was no appreciable growth in specimens of the 1949 group, and after the end of September there appeared to be no further growth in either the 1949 or the 1950 group.

On 27 November about 20% of the sample belonged to the 1950 group and about 80% to the 1949 group.

It is evident that in 1950 only one new generation of *A. aspersa* was produced, and that this was established mainly in July but possibly also somewhat earlier and later.

The samples of 11 January and 13 April 1951 are too small to be very useful but tend to confirm the results of 1950, that at this early period of the year
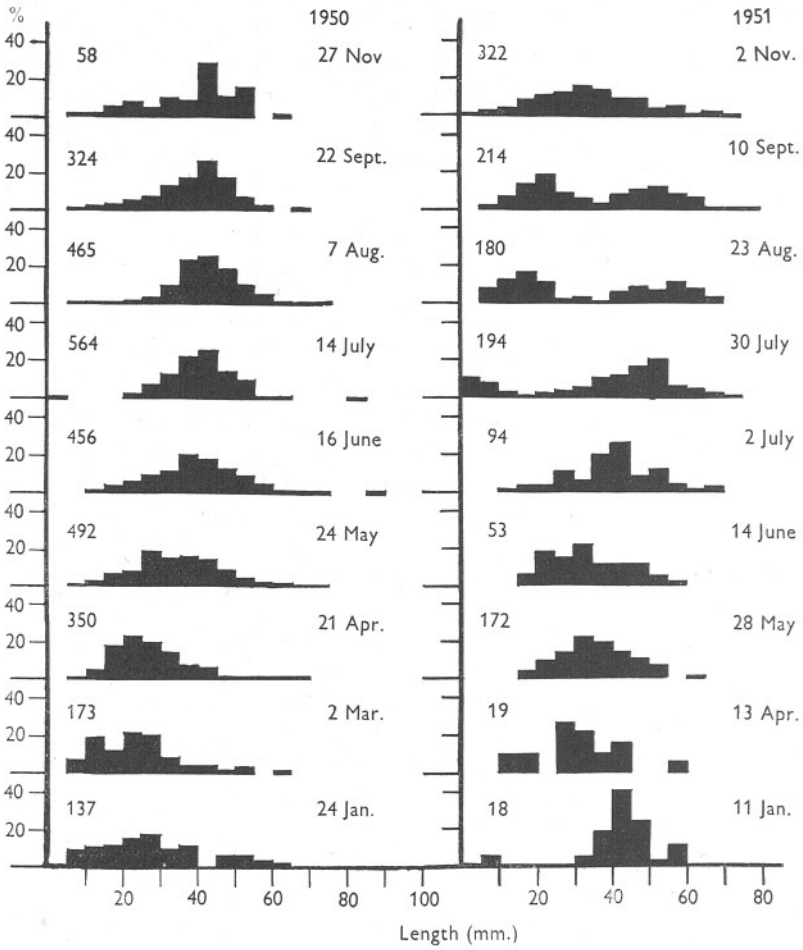
Fig. 1. Size-frequency histograms (in percentages) of *Ascidiella aspersa* in 1950 and 1951. The figures on the left of each histogram denote the number of specimens in the sample.
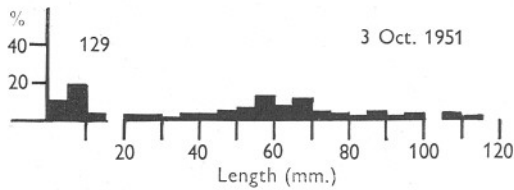

Fig. 2. Size-frequency histogram of *A. aspersa* on 3 October 1951, from Loch Sween, Argyll; as in Fig. 1.

the population represents two year-groups. The animals of these two groups had settled as larvae in the summers of 1949 and 1950 respectively. The samples also suggest that many of the larger individuals (i.e. of the 1949 group) died between 11 January and 13 April.

The figures for the rest of 1951 follow the same general pattern of growth and settlement that was found in 1950. In 1951, however, no growth was apparent until after 14 June, whereas in 1950 the animals had grown considerably before 24 May. On 2 July no newly settled individuals were found. They were present on 30 July, however, but in the sample of 23 August and in all later samples none was found. The period of larval settlement in 1951 therefore started between 2 July and 30 July and finished between 30 July and 23 August.

Animals of the new, 1951, group continued to grow from the end of July until some time between 10 September and 2 November, but during this period the animals of the old, 1950, group made no measurable growth. Only one new generation was established in 1951.

A single sample of *A. aspersa* was taken from Loch Sween, Argyll, on 3 October 1951 to provide a comparison with the autumn samples from Ardrossan. Fig. 2 shows the size distribution of the specimens. It appears that here also the population represents two clearly separate age-groups, the smaller animals, up to about 15 mm. long, belonging to the 1951 settlement and the larger ones belonging to the 1950 settlement. The size distribution of this sample suggests that the general pattern of growth and reproduction may be similar over much of the west coast of Scotland. The main differences in the sample from Loch Sween, as compared with autumn samples from Ardrossan, are: (i) the smaller size of the individuals of the 1951 group, and (ii) the larger size of those of the 1950 group.

These features may result from a later breeding season and more favourable conditions for growth in Loch Sween.

It may be deduced, from the samples of these two years, that the life-span is of the order of 18 months, extending approximately from the middle of one summer until the winter of the following year. *A. aspersa* in this area is essentially an annual, a conclusion which Hüus (1937) also reached for this species on the Norwegian coast.

Sexual maturity

Fig. 3 shows, for each of the size-groups, the percentage of sexually mature animals in the samples of 1950. The upper half (*S.*) refers to sperm, and the lower half (*E.*) to eggs, in the genital ducts. Sexual maturity is here taken to mean the presence of sperm or eggs in the genital ducts, irrespective of the quantity. The figures show two features:

(1) *A. aspersa* is hermaphrodite, but is slightly protandrous, as the sperm duct starts to fill before the oviduct. The 20–25 mm. group shows this

protandrous tendency most clearly. This finding contradicts Herdman's (1899) general statement that *Ascidia* is protogynous.
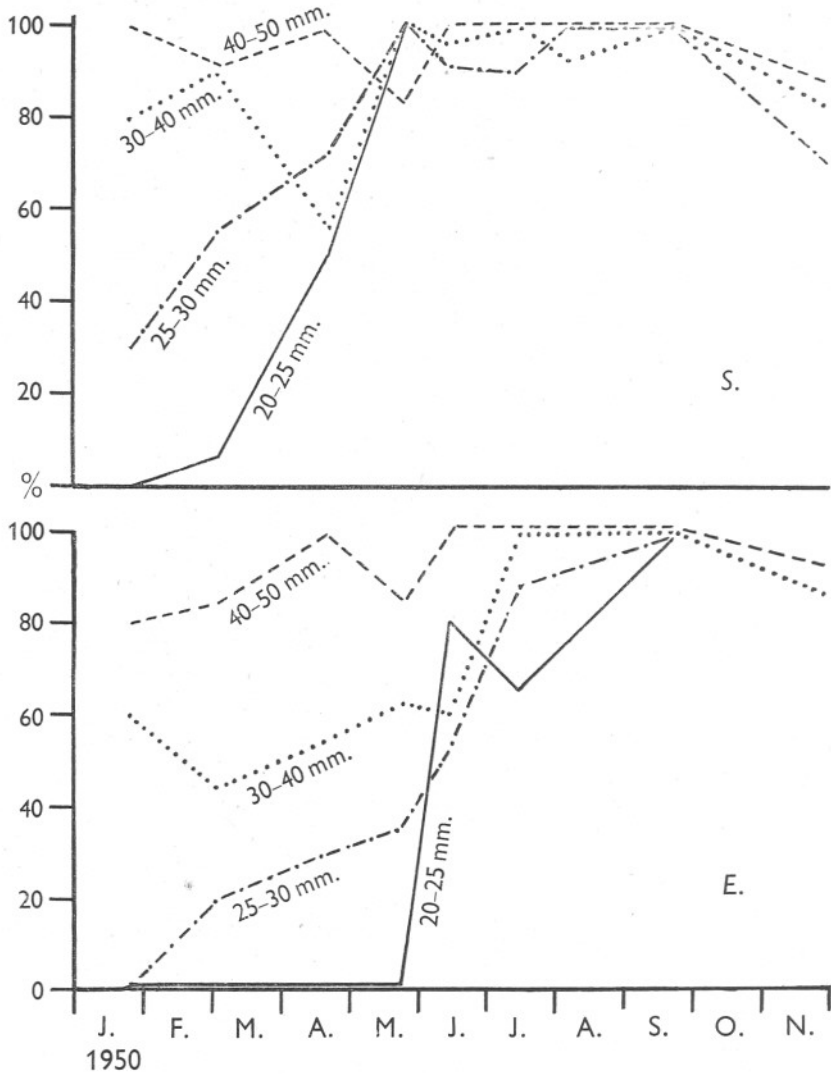

Fig. 3. Relations between the time of year and the percentage of specimens of *A. aspera* with sperm (*S.*) and eggs (*E.*) in the ducts, for each of the size groups 20–25, 25–30, 30–40 and 40–50 mm., during 1950.

(2) Sexual maturity depends primarily on the size of the animal. When an individual reaches a certain critical size sperm begins to appear in the sperm duct and at a slightly larger size eggs pass into the oviduct. The critical size, however, changes during the year. Thus practically all individuals over

40 mm. in length are mature as male and female throughout the year. In the 30–40 mm. group over 80% are mature as males during the whole year, but from January until June only about 40–60% of this group are mature as females. In the 25–30 mm. group there is a steady rise in the proportion with sperm, from January to May, when all have become mature; there is a similar but more gradual rise in the proportion with eggs. The 20–25 mm. group shows the sharpest rise in the percentage mature, both as male and as female, between January and June. In June sperm generally starts to fill the sperm duct when the animal is slightly under 25 mm. long, and eggs pass into the oviduct when the animal is rather less than 30 mm. long. This difference of about 5 mm. in the critical body length for male and female maturity is supported by observations made on a sample of *A. aspersa* taken on 20 August 1948 from a tufnol plate suspended in the waters of Loch Sween, Argyll. Fig. 4 shows, for this sample, the relation between the body length and the presence or absence of sperm and eggs in the ducts. Incidentally, the animals in Loch Sween became mature at a smaller body size than did those in any of the samples from Ardrossan. This may have resulted from environmental differences like those which appear to have influenced the Ardrossan population during the course of the season.

The time of settlement, growth rate, critical body length and life-span were such that a sample taken at Ardrossan at any time of year contained a large proportion of mature individuals. In the sample of 27 November 1950, for example, most of the 1949 group carried eggs and sperm, and the larger members of the 1950 group also did so. Of this population, however, the 1949 group died before spawning again, in 1951. Its place was taken by the 1950 group, which spawned in the summer of 1951. In the area studied, therefore, it appears that a given year group of *A. aspersa* has only one spawning season, and that is in the year after it settled as larvae.

Ciona intestinalis (Linnaeus)

Growth, number of generations in the year, and life-span

Ciona is more difficult to measure than *A. aspersa* because it is more contractile and because its body form varies more according to its contact with the substratum. It was therefore essential to narcotize the animals thoroughly before fixing and measuring them. Another difficulty was the limited supply of animals necessitating small samples.

Fig. 5 is a series of histograms showing the distribution by size of *Ciona* taken at intervals throughout 1950 and part of 1951. The samples of January, March and April 1950 showed two populations, but these were not clearly distinguishable. The numerically larger population had a small body length, and most specimens were between 10 and 30 mm. long. These animals had settled as larvae in 1949. The other population had a body length ranging
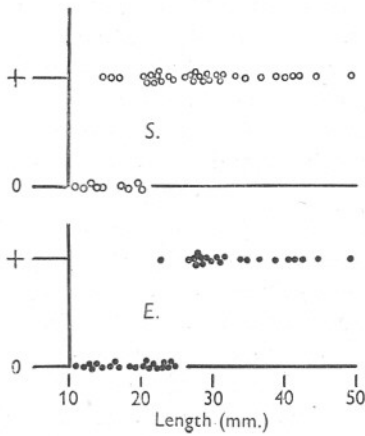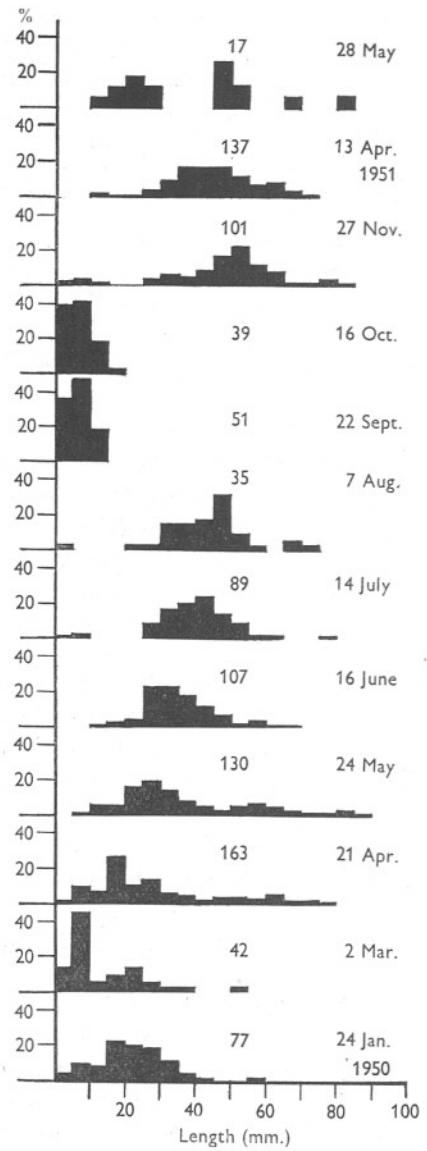
Fig. 4.

Fig. 5.

Fig. 4. Relation between the body length and the presence or absence of sperm and eggs in the ducts of *A. aspersa*, in a sample from Loch Sween, Argyll, taken on 20 August 1948. *S.* sperm in duct; *E.* eggs in duct.

Fig. 5. Size-frequency histograms (in percentages) of *Ciona intestinalis* in 1950 and early 1951. The figures denote number of specimens in the sample.

approximately from 50 to 70 mm. These animals had settled as larvae in 1948. The 1949 group tended to be bimodal, suggesting that during the previous year settlement may have been more intense at two periods separated by an interval when fewer larvae settled. Little or no growth was evident between January and 21 April, but during May, June and July the animals of the 1949 group grew considerably. By the end of May the bimodal tendency could no longer be detected in the 1949 group, the smaller individuals having grown faster than the larger ones. The animals of the 1948 group made little, if any, growth between May and July, as most of them had already reached their maximum size. There are, unfortunately, gaps in the records of the 1949 group during September and October, as continued sampling had reduced the stock. It was not until November that numbers of this population were found in a different part of the dock. These gaps in the records, however, are not serious, since it is obvious from the histograms for August and November that we are dealing with the same year-group. Between August and November there appears to have been little growth within the 1949 group.

It is more difficult to assess the proportion of the 1948 and 1949 groups in each sample of *Ciona* than in the samples of *Ascidiella*, and to say when the 1948 group died out. Most of the 1948 group had apparently disappeared before the first sample was taken in 1950, but a few were certainly still present in the sample of 24 May.

The first evidence of the 1950 settlement was in the sample of 14 July, in which a few young specimens were found. As one of these was already 7 mm. in length, settlement of larvae must have started a few weeks before this sample was taken. It was difficult to find numbers of the 1950 group sufficient to give a clear idea of their growth rate. This may have been due partly to depleted breeding stock and partly to an unsuccessful breeding season. The similarity between the histograms for 22 September and 16 October suggests that growth of the members of the 1950 group did not continue after the end of September.

Only one generation of *Ciona* was produced in 1950 in the area studied. Settlement of larvae appears to have started in July and continued through August and probably into September, as at the end of this month specimens of only 2 mm. length were still being found. The presence of individuals in the 0–5 mm. group during October and November does not necessarily indicate further settlement. As growth had already stopped for the year individuals which settled in September could still be in the 0–5 mm. group throughout the winter. This suggestion is supported by the presence of specimens only 3 and 4 mm. long in the samples of January and March 1950.

In 1951 samples were obtained up to 28 May after which the population was apparently so depleted that only occasional specimens were taken. These

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few samples, however, tend to confirm two features of the 1950 sampling: (i) growth of the animals of the recent year-group (in this case the 1950 group) started before the end of May; and (ii) most of the remaining animals of the previous year-group (in this case the 1949 group) died before the end of May.

The general pattern of growth, reproduction and replacement of generations in *Ciona* was very like that found in *Ascidiella*. Individuals settled in the summer, grew until autumn, resumed growth in the following spring, spawned in the summer of that year, and died in the following winter. They are therefore essentially annuals, although they may be 18 months old or somewhat more when they die.
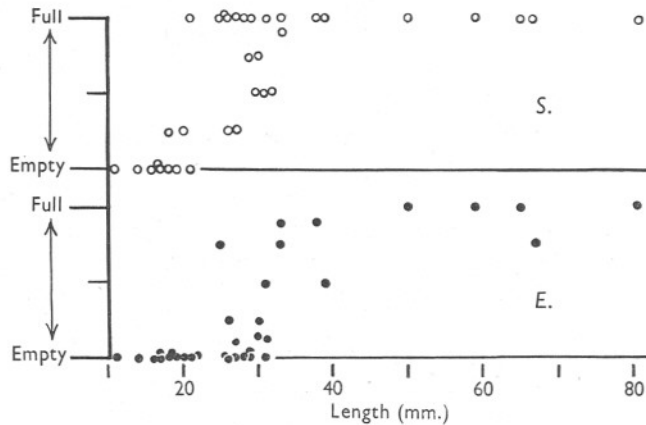

Fig. 6. Relation between the body length and the quantity of sperm (*S.*) and eggs (*E.*) in the ducts of *C. intestinalis* in the sample taken on 24 May 1950.

Orton (1914, 1920) found that in the Plymouth area *Ciona* breeds from April to November, producing two or three generations in the year. Hǔus (1937) states that on the Norwegian coast *Ciona* is sexually mature within its first year of life and dies off after breeding, having a life of about one year. In Naples, Lo Bianco (1909) records *Ciona* as breeding throughout the year. Rünnstrom (1936) found that *Ciona* has three physiological races which breed within different temperature ranges. On the west coast of Norway he found *Ciona* to spawn from May to August, after which the old generation dies off. It is replaced by the new one which becomes sexually mature by the end of August but does not, apparently, produce a further generation: eggs and sperm may be shed from October to December but fail to develop.

The population of *Ciona* studied in Ardrossan Old Dock resembles the animals of the Norwegian west coast in breeding behaviour and the annual cycle of generations, although differing somewhat in the timing of events and speed of processes.

Sexual maturity

Fig. 6 shows the relation between the length of the body and the quantity of eggs and sperm in the genital ducts of a number of specimens of *Ciona* from the sample of 24 May 1950. The remaining samples were not examined in detail, but the same general relations were found.

Ciona, like *Ascidiella*, is a slightly protandrous hermaphrodite. Sperm starts to enter the sperm duct at a critical body length and eggs appear in the oviduct later, when the body length is somewhat greater. Fig. 6 suggests that the critical body length for the appearance of sperm in the duct is about 25 mm. and of eggs in the oviduct about 30 mm., in the sample examined.

Diplosoma listerianum (Milne Edwards)

Growth

The area of the colonies was measured as described on p. 42. In Fig. 7 a series of histograms shows the distribution by area of the colonies taken at intervals throughout 1950 and 1951.

All colonies of the sample of January 1950 originated from larvae that settled in 1949. Most of the colonies of this sample were less than 50 mm.^{2} in area and the largest was 170 mm.^{2}. Growth in 1950 started between 2 March and 21 April and continued until about the end of May. Maximum size appears to have been reached by 16 June, when a number of colonies were between 1000 and 1500 mm.^{2} in area.

In the sample of 14 July a few colonies showed degeneration of the zooids, but it was in the sample of 7 August that this process first became widespread. Food was not seen in the gut of zooids in August, and this is taken as another sign of lowered vitality. By 22 September 1950 most of the 1949 generation of colonies had died and many of those remaining were degenerate.

New colonies of the 1950 generation were first observed in the sample of 14 July but were few. The new colonies were more common on 7 August. On 22 September most of the sample, and on 27 November all of it, consisted of the new, 1950, generation.

Appreciable growth of the newly established colonies took place before 22 September.

The pattern of growth and replacement of generations in 1951 was in general similar to that observed in 1950. In 1951, however, spring growth up to 28 May was much less than spring growth during a corresponding period of 1950 (up to 24 May). A similar feature was observed in the growth of *Botryllus* (see p. 58). In the autumn of 1951 colonies of the new generation continued to grow after 10 September, as shown in Fig. 7, bottom right.

Sexual reproduction

Asexual reproduction by budding leads to an increase in the size of the colony and not to the founding of new colonies, which is the function of
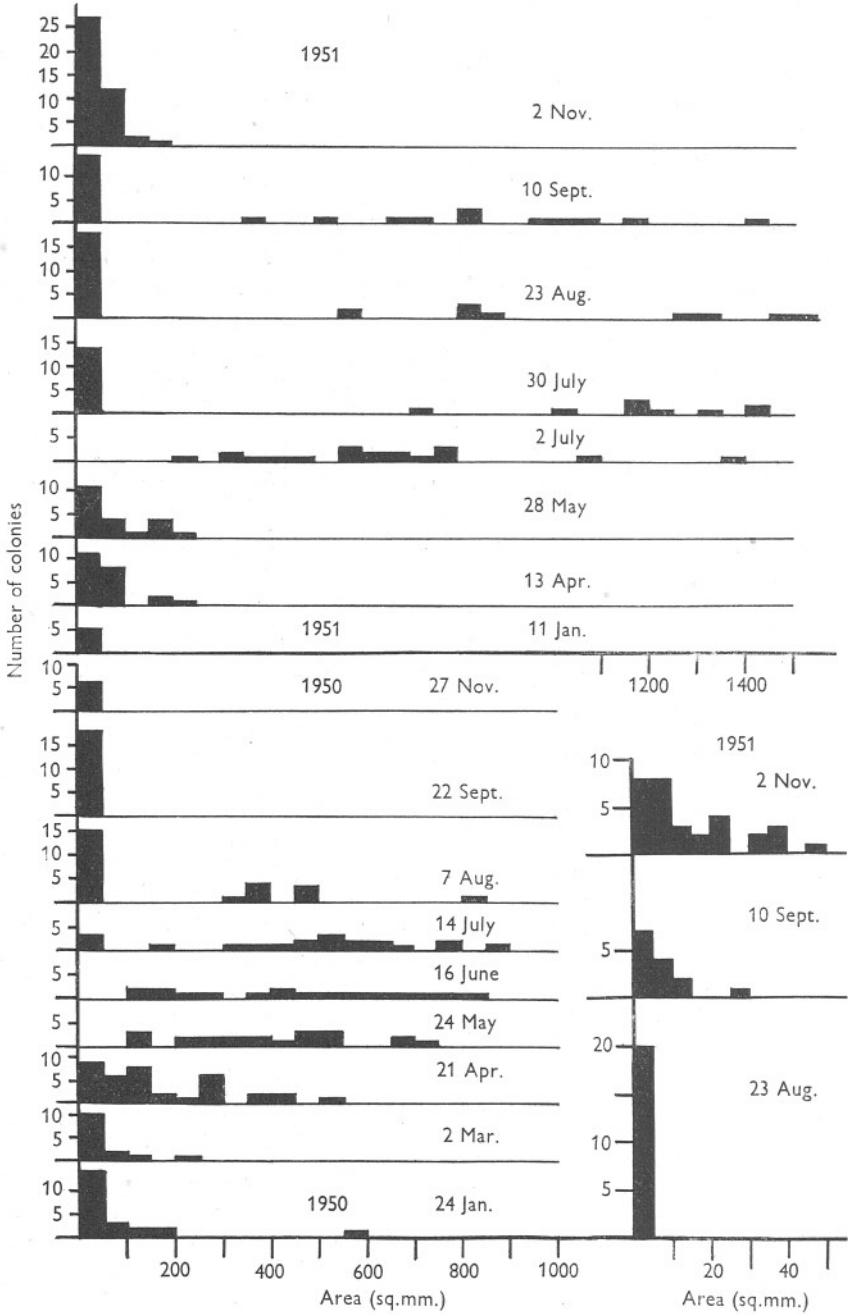
Fig. 7. Size-frequency histograms (by area) of *Diplosoma listerianum* in 1950 and 1951. The smallest specimens for three successive dates are further analysed in the inset at right bottom.

sexual reproduction. During sexual reproduction budding was reduced but it continued long after the onset of gonadial development and egg production. It only ceased, presumably, when there was serious competition for raw materials between the sexual and the asexual processes. Huxley (1921) and Berrill (1935) have drawn attention to this competition in colonial ascidians.

The ovary (Fig. 8, *Ov.*) of *Diplosoma* lies in the lower part of the abdomen, and shows only a few large eggs at any time. As the season advances the eggs enlarge and pass singly from the abdomen down into the basal layer of test. Lahille (1890) states that the egg is fertilized only after breaking through the body wall and coming to lie in the common test, but Berrill (1950) maintains that it is fertilized before leaving the abdomen. Development to the larval stage certainly takes place within the common test.

Fig. 9 summarizes the conditions found in the samples of *Diplosoma* throughout 1950, and records the percentage of colonies with: (A) neither eggs nor larvae in zooids or test; (B) eggs in zooids but not in test; (C) eggs in zooids and eggs or larvae in test; (D) eggs or larvae in test but no eggs in zooids; (E) larvae.

The graphs indicate the progress of breeding activity.

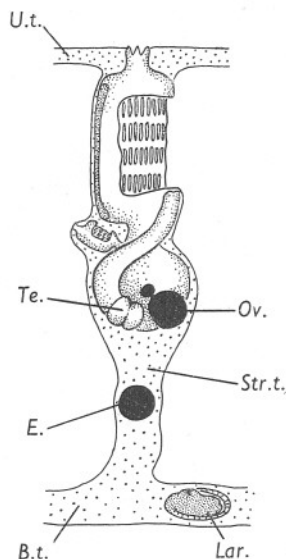January to March was a period of sexual inactivity, during which colonies

Fig. 8. Zooid of *D. listerianum* within the common test of the colony. *B.t.* basal layer of common test; *E.* egg; *Lar.* larva; *Ov.* ovary; *Str. t.* strand of common test securing zooid to basal layer; *Te.* testis; *U.t.* upper layer of common test.

of the 1949 generation had eggs neither in zooids nor in test. Some colonies, however, showed eggs or larvae, or the degenerate remains of these, in zooids or test. These eggs and larvae appear to be residual products which failed to develop completely and escape during the previous year. They may be regarded as abnormal, as they were probably held within the incompletely disintegrated basal layer of colonies of the 1948 generation. This condition was more common in 1951 (see p. 55).

Between the March and April samples there had been an increase in the proportion of colonies with eggs in the ovaries, and this period marks the onset of sexual activity for the year. During May there was a sharp rise in the percentage of colonies with eggs in the common test as well as in the zooids, indicating a large-scale passage of eggs from the zooids down into the common test. It was on 24 May that the first developing embryos were seen. All

colonies examined in the middle of June had eggs in the zooids and also eggs
or larvae in the test. Egg production started to diminish in July, as indicated
by a slight drop in the percentage of colonies with eggs in zooids as well as
test. Between mid-July and early August the ovary became inactive and the
production of eggs ceased. In the July and August samples all colonies
examined had larvae, and some had developing eggs, in the test.

The first signs of exhaustion resulting from breeding activity were seen in
July, when a few colonies were found with degenerating zooids; in August
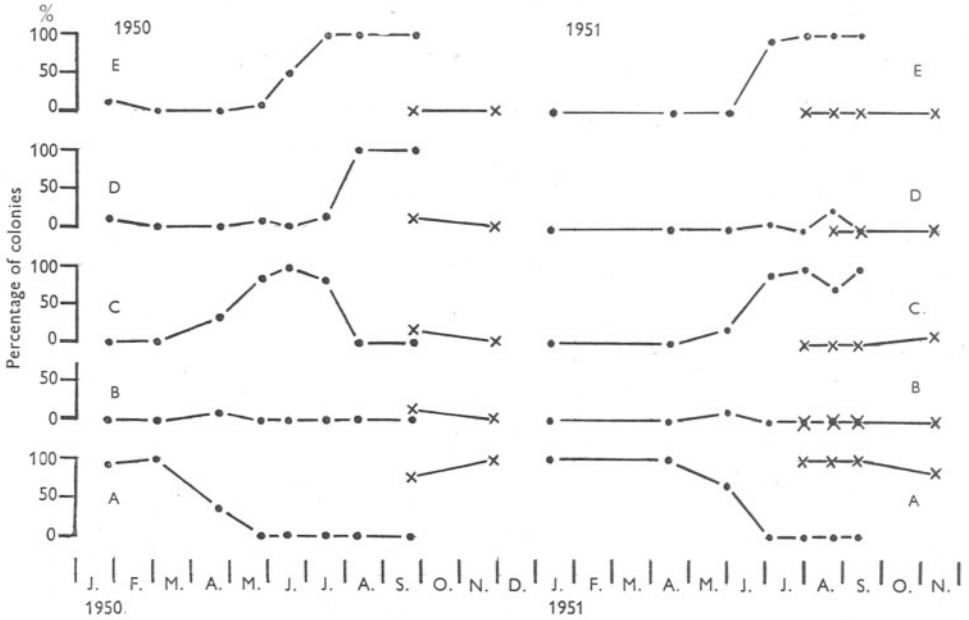this condition was common.

Fig. 9. The breeding activity of *D. listerianum* in 1950 (left) and 1951 (right). A, colonies with
no eggs and no larvae in zooids or test; B, colonies with eggs in zooids but not in test;
C, colonies with eggs in zooids and eggs or larvae in test; D, colonies with eggs or larvae
in test but no eggs in zooids; E, colonies with larvae.

Larval settlement was indicated by the presence of young colonies in the
samples of 14 July, 7 August and 22 September. The settling period was not
determined more precisely and may not have extended into the second half
of September. It is perhaps significant that the settling period coincided with
the period during which the old colonies disappeared, and it may be that the
larvae are liberated only with the disintegration of the breeding colonies.
Berrill (1950), on the other hand, states that the active tadpoles escape from
the test matrix to the common cloacal cavities of the colony and thence to
the exterior. Many of the Ardrossan colonies examined, however, showed
areas of degenerating zooids below which larvae were still retained in the

common test. The existence of many composite colonies (see below) in November 1951 also indicates prolonged retention of larvae in the test, even after the disappearance of the parent zooids.

The young colonies grew and, as the September sample showed, some of the zooids produced eggs. Developing larvae were also found in a few young colonies in September. That some of these young colonies may have given rise to a second generation is suggested by the presence of a few two-zooid colonies in late November. It is possible, however, that these small colonies in November came from late larvae of the old colonies.

A study of the breeding activities of *Diplosoma* during 1951 confirmed the general picture gained in 1950, as seen from the right-hand part of Fig. 9. It appears that the onset of breeding was later in 1951 than in 1950. This difference is shown in the later disappearance of the non-breeding condition (A), the later appearance of eggs in the zooids (B), the later rise in the percentage of colonies with eggs in both zooids and test (C), and the later transition of colonies to the final breeding stage (with eggs or larvae in the test only, D). By comparing the dates in 1950 and 1951 on which the same percentage of the samples was in a particular reproductive state, we can estimate the lateness of the 1951 season. The lateness was of the order of 6 weeks.

One other feature of the 1951 graphs deserves comment: this is the much smaller percentage of colonies during August with eggs or larvae in the test only, compared with the 1950 figures. The lower percentage in 1951 was due to the later degeneration of zooids, because as long as the zooids remain healthy some at least continue to produce eggs.

The delayed decay of the old colonies in the autumn of 1951 gave rise to another interesting condition: this was the formation of composite colonies. A composite colony consists of the basal layer of test of an old colony with its larvae still enclosed, and zooids of the new generation derived from these larvae which have metamorphosed *in situ*. No zooids of the old generation remain.

Later breeding in 1951 may have resulted from later growth, which was observed to occur, but more probably some environmental factor, such as lower water temperature, retarded both activities.

Number of generations

In the area studied *Diplosoma* produced, both in 1950 and 1951, one principal generation and possibly a minor one in 1950 late in the season. Orton (1914) states that, in the Plymouth area, the species passes through 'at least two crops in a year' and three or more in favourable seasons. Once he got larvae from a colony aged not more than 3 weeks and 5 days.

The rate of development of the colonies and the time at which larvae are liberated are the factors determining the number of generations per year.

These factors are greatly influenced by local and seasonal conditions. Berrill's (1950) statement that 'breeding occurs throughout the year' is too general, although no doubt true in specially favourable conditions.

Botryllus schlosseri (Pallas)

Growth

The colonies of *Botryllus* were removed from the substratum and their area measured by the method described on p. 42. Fig. 10 gives a series of histograms showing the distribution by area of the colonies taken at intervals throughout 1950 and 1951.

In January 1950 the sample of *Botryllus* represented a single year-group, consisting of colonies that had been established in 1949. Many colonies were under 25 mm.^{2} in area and had only one system of zooids. The few large colonies in this sample were between 200 and 300 mm.^{2} in area and these had ten to twelve systems of zooids. The onset of growth for the 1950 season occurred between 2 March and 21 April. In the April sample most specimens lay within the 25–30 mm.^{2} group and these colonies had two or three systems of zooids. Large colonies between 500 and 750 mm.^{2} had fifteen to twenty systems. Growth continued during May and by 16 June maximum size had, in general, been attained. Only two colonies in the June sample had as few as six systems of zooids and the majority had fifteen to twenty or more systems. One or two of the large colonies showed some degeneration in June, the first to be observed in 1950.

The sample of 14 July showed two important features: (1) the first new colonies resulting from larval settlement, and (2) considerable degeneration in the large colonies.

The new generation was represented by two small colonies, each of a few zooids arranged in one system.

By 7 August the change of generations was almost complete, only one colony of forty-three examined belonging certainly to the 1949 generation. The remainder of this sample consisted of small colonies, mostly of one system, but a few of two, three, or four systems. Two single zooids were found, recently metamorphosed from settled larvae. On 22 September no colonies of the 1949 generation were found.

A more detailed comparison of the samples of 7 August, 22 September, and 27 November 1950 shows that the young colonies grew during late summer and autumn (Fig. 10, inset). Table 1 suggests that this growth took place without an increase in the number of systems per colony.

It can be seen by inspection of the histograms for 1951 that they agree fairly well with those for 1950. In the sample of January 1951 all colonies belonged to the 1950 generation. Growth had begun by 13 April and continued until June. Early in July degeneration of colonies had started but was most noticeable at the end of that month. The first new colonies, of the 1951
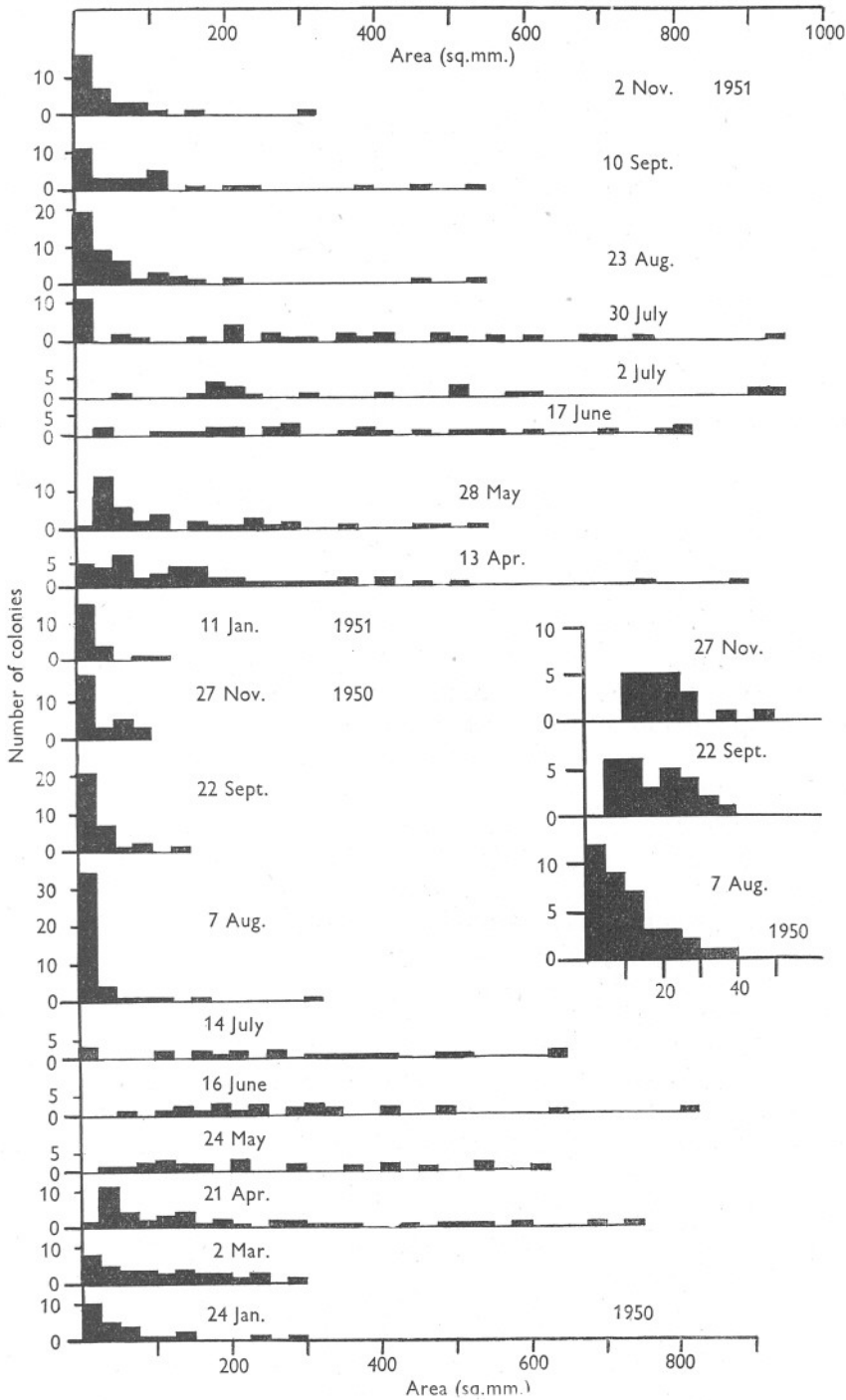
Fig. 10. Size-frequency histograms (by area) of *Botryllus schlosseri* in 1950 and 1951. The smallest specimens for three successive dates in 1950 are further analysed in the inset on the right.

generation, were seen at the end of July, and by 23 August most of the sample consisted of this new generation.

Although the general pattern of growth was similar in the two years, the onset of spring growth was later in 1951 than in 1950. This can be seen by comparing the histograms for approximately corresponding dates in the two years, viz. 21 April 1950 and 13 April 1951; 24 May 1950 and 28 May 1951. A similar retardation of growth was noticed in *Diplosoma*.

TABLE I

No. of systems per colony	No. of colonies			
	7. viii. 50	22. ix. 50	27. xi. 50	11. i. 51
A few zooids	2	0	0	0
1	24	20	15	16
2	9	5	3	1
3	4	2	4	2
4	1	2	5	0

Number of generations

The population of *Botryllus* passed through one generation in each of the years studied, and it seems unlikely that even the earliest new colonies were able to produce many larvae that settled to form a second new generation. Orton's (1914) findings that *Botryllus*, like *Diplosoma*, has at least two crops in the year applies to the Plymouth area.

Sexual reproduction

Berrill (1941, 1947, 1950) has described the process of bud formation and gonad development in *Botryllus* and *Botrylloides*. He has shown that the sex of the gonad and the state of development that it attains depend on the size of the bud bearing the gonad. Berrill (1935) also found that sexual and asexual reproduction take place simultaneously, but that budding may be relatively subdued during sexual reproduction.

The samples of *Botryllus* were examined, and Fig. 11 records the percentage of colonies in each sample during 1950 and 1951 with: (A) neither eggs nor larvae, (B) gonads, (C) large or developing eggs, (D) larvae.

Gonads were visible in a low percentage of colonies in January and March, and sections showed small developing ovaries and testes in some zooids and buds. No larvae were present in these months and only a few large eggs were found in one colony in March. Gonadial development was rapid during March and early April, but still there were only a few large eggs, and no larvae. The percentage of colonies with visible gonads was highest in May and June, dropped sharply to the level of July, and by late September had returned to the winter level. The percentage of colonies whose zooids had large or developing eggs followed a rather similar curve, but reached a high value for a shorter period.

Larvae were first found in the sample of 24 May, when some colonies had fully developed larvae and others had tailed but still incomplete larvae. The percentage of colonies bearing larvae continued to rise in June and reached a maximum in July, after which there was a rapid decrease. A few larvae were still found until the end of November. The first new colonies of the year appeared in the sample of 14 July and their number increased in August, but there was no evidence of very recent settlement in the sample of 22 September.
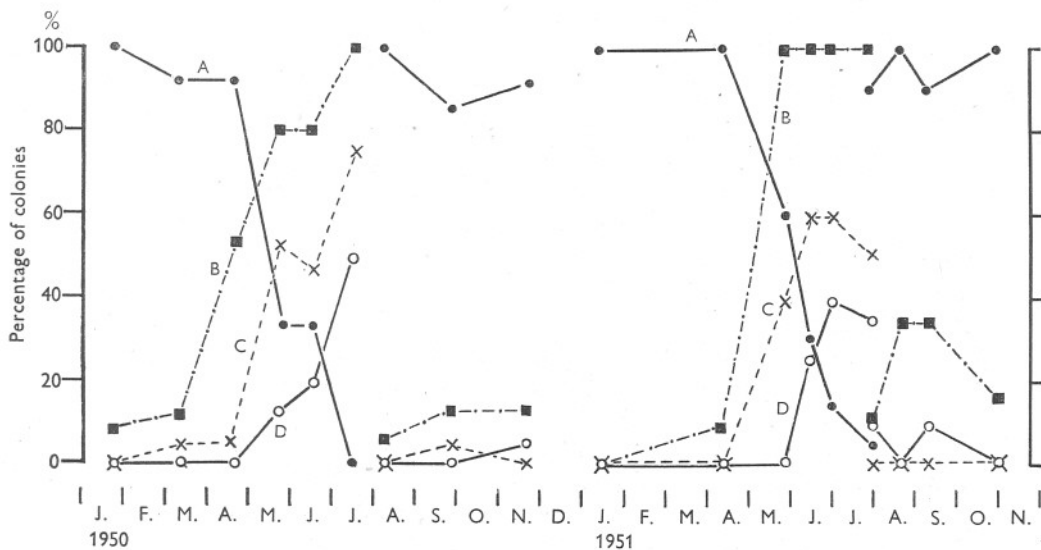

Fig. 11. The breeding activity of *B. schlosseri* in 1950 (left) and 1951 (right). A, colonies with no developing eggs and no larvae; B, colonies with visible gonad; C, colonies with developing eggs; D, colonies with larvae.

In 1951 the cycle of breeding in *Botryllus* was similar to that in 1950, but was later in most of its stages. This appears from a comparison of the graphs, for the two years, of the percentage of colonies with (1) gonad, (2) developing eggs, and (3) larvae. The period of larval settlement, however, was rather similar, starting some time between 2 July and 30 July, and extending certainly until the end of August and perhaps into September.

In both years the proportion of *Botryllus* colonies with larvae reached, at its maximum, only about 30–40% of the total examined. This is in marked contrast to the condition in *Diplosoma*, where 100% of the colonies examined in July and August had larvae. The probable explanation of the difference lies in the fact that the larvae of *Botryllus* develop in the atrial cavity of the zooid, from which they escape to the exterior when fully developed. The larvae of *Diplosoma*, on the other hand, completing their development embedded in the basal test of the colony, certainly escape less easily, and perhaps only with the dissolution of the parent colony.

Budding

Asexual reproduction by budding may, in general, occur at any season and a high proportion of all colonies examined had buds. The process appeared to be less active, however, in August and September, both in the old generation and in the newly established colonies. In *Botryllus*, as in *Diplosoma*, exhaustion follows the intense sexual activity of the summer, and reduced asexual reproduction is here the prelude to the death of the old colony. It is surprising that budding activity was not intense in the young colonies of August and September.

SUMMARY

The populations of *Ciona intestinalis*, *Ascidiella aspersa*, *Diplosoma listerianum* and *Botryllus schlosseri* in the Old Dock of Ardrossan Harbour, Ayrshire, were studied in 1950 and 1951. Samples taken at intervals of about 4–7 weeks were used to follow growth, the progress of breeding, and the number and duration of generations.

In each species fastest growth was in May, June and early July. There was no growth in winter.

In all the species studied, except *Ciona intestinalis* for which sufficient data were not obtained, growth was several weeks later in starting in 1951 as compared with 1950.

Each species behaved as an annual, which settled as a larva, grew to sexual maturity, bred, and died, within a period of 12–18 months.

Breeding in each species was confined to a few months in the summer, and took place in the year following that in which the animals settled as larvae. *Diplosoma* may possibly also have bred at the end of its first summer.

In *Diplosoma* and *Botryllus* similar stages in the breeding cycle were several weeks later in 1951 than in 1950.

In *Ascidiella* and *Ciona* critical body sizes were found at which eggs and sperm began to fill the genital ducts. These species are protandrous; after the sperm starts to fill the sperm duct the body grows about 5 mm. before eggs pass into the oviduct.

REFERENCES

BERRILL, N. J., 1935. Studies in tunicate development. IV. Asexual reproduction. *Phil. Trans. Roy. Soc. London*, B, Vol. 225, pp. 327–79.
—— 1941. Size and morphogenesis in the bud of *Botryllus*. *Biol. Bull. Woods Hole*, Vol. 80, pp. 185–93.
—— 1947. The developmental cycle of *Botrylloides*. *Quart. Journ. Micr. Sci.*, Vol. 88, pp. 393–407.
—— 1950. *The Tunicata*. Ray Society, London.
HERDMAN, W. A., 1899. *Ascidia*. L.M.B.C. Memoirs, Liverpool. 52 pp.
HÜUS, J., 1937. Tunicata: Ascidiaceae. *Handb. Zool. Kükenthal und Krumbach*. 5, 2nd half, pp. 545–672.

HUXLEY, J. S., 1921. Studies in dedifferentiation. II. Dedifferentiation and resorption in *Perophora*. *Quart. Journ. Micr. Sci.*, Vol. 65, pp. 643–97.

LAHILLE, F., 1890. *Recherches sur les Tuhiciers des côtes de France*. Toulouse. 330 pp.

LO BIANCO, S., 1909. Notizie biologiche riguardanti specialmente il periodo di maturita sessuale degli animali del golfo di Napoli. *Mitt. Zool. Stat. Neapel*, Vol. 19, pp. 513–761.

ORTON, J. H., 1914. Preliminary account of a contribution to an evaluation of the sea. *Journ. Mar. Biol. Assoc.*, Vol. 10, pp. 312–26.

—— 1920. Sea-temperature, breeding and distribution in marine animals. *Journ. Mar. Biol. Assoc.*, Vol. 12, pp. 339–66.

RÜNNSTROM, S., 1936. Die Anpassung der Fortpflanzung und Entwicklung mariner Tiere an die Temperaturverhältnisse verschiedener Verbreitungsgebiete. *Bergens. Mus. Årbok*, 1936, Nr. 3, 36 pp.