colonies which settle and develop upon suitable exposed surfaces at Millport differ in certain respects from the characters of this species as listed by Allman (1872). He gives the average height of a colony of this species as $1\frac{1}{2}$–2 in. and states that the stems bear 'more or less distinctly marked annulations at intervals'. Colonies of this species at Millport generally exceed 3 in. and may reach 4 in. in height, and annulations are very difficult to detect on the stems; they are rarely clearly visible on the stems of a living colony, but a few shallow annulations can be seen on the bare stems after the polyps have been shed and the colony has died down to the condition in which it lives through the winter. It is felt, however, that differences of this character do not warrant the formation of a separate species for this material, though it is perhaps important that their existence should be mentioned. One further difference may be added. Mr J. Corlett (personal communication) has recently found, in the course of studies on the settlement of marine organisms in the Mersey, that there *T. larynx* does not flourish unless the water temperature exceeds 60° F. As at Millport this temperature is attained, under favourable conditions, only for a few days in the year, the temperature requirements of *T. larynx* at Millport appear to differ from those elsewhere, since there is no doubt that this species is able to settle in abundance and grow rapidly when the water temperature is well below 60° F.

Elmhirst (1923) states that the breeding period of *T. larynx* at Millport extends from May to September; our observations, made in greater detail over the past 5 years, are in general agreement with this estimate. Settlement is rarely at all heavy, however, until late in July. This period of heavy settlement continues through August and September and may extend into the first weeks of October. Some settlement may occur until the end of December.

During August and September, any suitable surface soon acquires a number of young colonies which grow rapidly. All the experimental material used in the present survey was taken from surfaces immersed from rafts moored close inshore. Sometimes colonies were removed from their substratum and brought into the laboratory for experiment. At other times, when it seemed essential to disturb the material as little as possible, colonies were used which had grown from settlements on ground-glass microscope slides immersed in the sea.

The use of settlement surfaces immersed from a raft is in some ways advantageous, in others the reverse. It is possible, using this method, to provide a surface, uncolonized by other organisms, for the attachment of the settling larva. Thus it is possible to obtain large numbers of settled individuals but, because of the conditions of immersion of these surfaces hung from a raft, the environment is not wholly normal. Continuous immersion is assured, but the immersed specimens remain at a constant depth below the surface of the water and consequently may be exposed to high light intensities for longer periods than are individuals growing normally just below low-water mark on a fixed object.

Liberation of the Actinula Larva

As Lowe (1926) has shown, the developing actinula is orientated with its aboral pole pointing towards the opening of the gonophore and its oral pole, sheathed in the aboral tentacles, pointing inwards. Allman (1872, p. 407) has stated that in *T. larynx* the oral tentacles are not formed until after liberation, but it is clear from our material and from *T. larynx* from Plymouth that this statement is erroneous. The aboral pole thus emerges first as the actinula is expelled and the tips of the aboral tentacles last; as each aboral tentacle is freed, it stands out from the body of the larva, so that the newly liberated actinula has a number of aboral tentacles radiating stiffly outwards (Fig. 1A). Allman (1872) for *T. indivisa* L. and Ciamician (1879) for *T. mesembryanthemum* Allman, state that in these species the oral pole of the larva emerges first.

The factors affecting the liberation of the actinula larva are still largely unknown, but two possibilities have been investigated in the course of this work. Early laboratory experiments suggested that change in light intensity stimulated the liberation of actinulae, since the rate of liberation was increased immediately after transfer from light to darkness or from darkness to light. Later work, however, has not wholly substantiated this result, the average rate of liberation (i.e. the number of larvae liberated per hour) is greater in darkness than in light, but a change of light intensity does not invariably increase the rate of liberation of actinulae. This point requires fuller investigation under carefully controlled conditions.

The other factor investigated which affects the rate of liberation of actinulae was variation in current speed. The range of current speed that could be used was limited, and was generally low in comparison with that to which colonies are exposed during the ebb tide at Millport, but the results obtained strongly suggest that larval liberation is markedly reduced in quite moderate currents. At a water speed of 1 cm./sec. the rate of liberation was greater than that which occurred in still water, but if the speed of the current was increased to 3 cm./sec. liberation of actinulae practically stopped. These preliminary results suggest that liberation may only take place in the sea over slack-water periods. This point is of some importance in the distribution of larval settlement round a mature colony and will be discussed more fully later (p. 35).

Large numbers of actinula larvae can readily be obtained for experimental purposes by suspending mature colonies, polyps downwards, in fresh sea water. If the sea water is frequently renewed, liberation of actinulae will continue for several days. Thus, a group of colonies containing approximately 150 polyp heads liberated about 1000 larvae over a period of 6 days in the laboratory, an average of about seven larvae per polyp. This is almost certainly an underestimate, as it is reasonable to assume that some larvae had been shed before the colonies were collected and that the full development of others was

doubtless affected by the artificial conditions of the laboratory. Further, this estimate assumes that all the polyps in the colony liberated actinulae during the period of observation; as will be shown later, this is not likely to be true, so that it can be safely assumed that one polyp produces many more than seven larvae.

The Actinula Larva

Morphological changes before settlement

Immediately after liberation from the gonophore, the actinula larva of *T. larynx* consists of an ovoid body with the mouth, surrounded by four or five short oral tentacles, at one pole, and some distance below it a number of long aboral

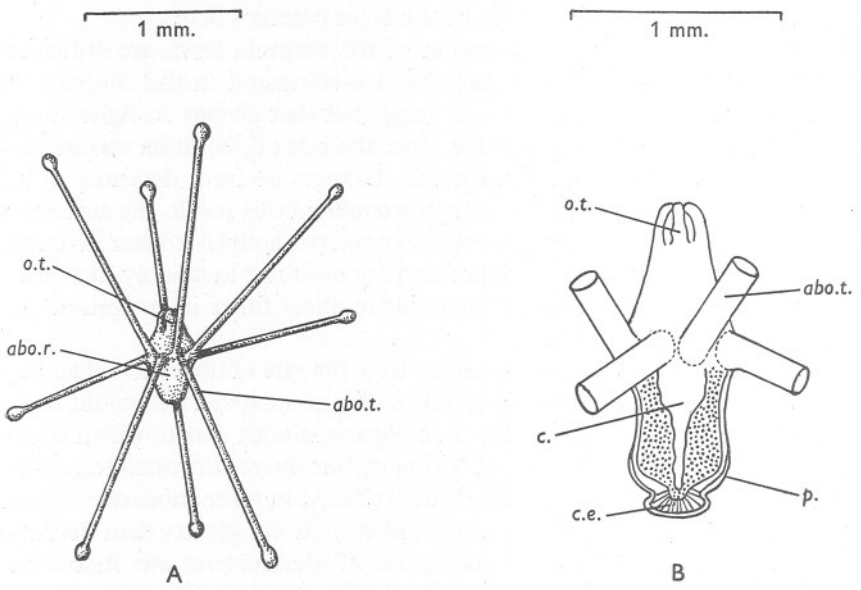

Fig. 1. A, actinula larva of *Tubularia larynx*, immediately after liberation. B, semi-diagrammatic sketch of the body of the actinula larva of *T. larynx*, shortly before settlement. The aboral region is shown in optical section. *abo.r.*, aboral region; *abo.t.*, aboral tentacle; *c.*, coelenteron; *c.e.*, columnar ectoderm; *p.*, perisarc; *o.t.*, oral tentacle.

tentacles which radiate stiffly outwards (Fig. 1A). The tips of these tentacles are swollen and within the superficial tissues of these swellings are large numbers of nematocysts. The number of these aboral tentacles varies. In a series of some 200 actinulae, the number of aboral tentacles varied from six to thirteen, most (32 %) had ten aboral tentacles and over 75 % had nine, ten or eleven aboral tentacles.

Shortly after liberation, that part of the body below the aboral tentacular ring (the aboral region) begins to elongate. Immediately after liberation this aboral

region measures 0·3 mm. in length and is 0·32 mm. in diameter at its widest part. When settlement occurs, this part of the larval body has increased in length to 0·43 mm. and its diameter has decreased slightly, now being 0·28 mm.; it is thus distinctly cylindrical in form (Fig. 1B).

The surface of the aboral pole of the actinula can be seen to be covered by a thin membrane immediately after liberation. By the time this region of the body has elongated, this membrane, which is the first appearance of the perisarc, can readily be distinguished (Fig. 1B). Some of the properties of this sheath were investigated using larvae which had settled on the substratum, since the sheath is then attached and can be manipulated more easily. If such larvae are placed in 10% NaOH, rapid and extensive maceration of the tissues takes place and the body of the larva can be drawn out of the sheath, leaving the latter as a short cylinder, closed at one end, attached to the substratum. The sheaths may then be washed several times and can be stored either in tap water or in 4% sea-water formalin.

When treated with Mallory's triple stain, the sheath stains a deep blue; it dissolves slowly in cold concentrated HCl; it is not wholly soluble in 50% NaOH at 60° C., and it gives a faint positive reaction to the chitosan test (the difficulty in obtaining a more satisfactory result to this test lies in the manipulation of the material). These properties are such as strongly to suggest that this sheath is chitinous in nature. One further point of interest emerges from these experiments; when treated with warm, concentrated alkali, the sheaths do not dissolve (though they become appreciably more delicate), but they always become detached from the substratum, suggesting that they are attached by a substance soluble in concentrated alkali. It is, therefore, possible that attachment is secured by means of an extra-chitinous cement similar to that found in arthropods. Mr B. W. Sparrow (personal communication) has demonstrated the existence of dihydroxyphenols in the attachment cement of *T. larynx*, an observation which strongly supports this possibility. There are indications (see also p. 29) that the cement used for the attachment of a number of marine animals belonging to different phyla may consist of a mucoprotein, and that this mass of cement hardens in a manner similar to that described for the arthropod cuticle by Pryor (1940). Ciamician (1879) suggested that the chitinous perisarc itself served to attach the actinula larva of *T. mesembryanthemum* to the substratum, but this seems unlikely to be true for the recently settled actinula of *T. larynx*.

General biology of the larva

After liberation, the actinula larvae sink slowly through the water. Measurements indicate that they sink at a rate of about 1 mm./sec., so that, although they may be carried in a slight current a little distance from the parent colony, it is probable that many will come into contact with a substratum not far from their point of liberation.

The aboral tentacles of the unattached actinula are held alternately pointing away from the substratum and towards it (Fig. 1A) and the larva can move slowly on the tips of the latter set of tentacles. Progress in this way is slow, but appears faster in light (of the order of 0·3 cm./hr.) than in darkness (roughly 0·1 cm./hr.). Critical experiments have not yet been made, but an examination of the results of these preliminary experiments suggests that the larvae are photokinetic rather than phototactic.

During this period of free movement over the substratum the orientation of the larva is mainly that which is eventually assumed, namely with the aboral pole towards the substratum. This point was specially examined as other accounts of the orientation of actinula larvae (e.g. Allman, 1872, for *T. indivisa*) suggest that the oral pole is directed towards the substratum and that a reversal of this orientation takes place just before settlement.

Temporary attachment to the substratum can take place by means of the tips of the aboral tentacles, but it would seem that these tentacles do not become attached immediately the larva comes into contact with a substratum. An actinula larva, placed in a dish of sea water and allowed to sink to the bottom, can at first be moved by the slightest current, but if larvae are left in contact with the substratum for a period of 2 hr., and are then subjected to a current which is very gradually increased in speed, they can withstand much faster currents. Most of the larvae became dislodged when the current speed was increased to 1–2 cm./sec., but some could withstand currents up to 8 cm./sec. before being dislodged. These results were obtained using smooth glass surfaces; under natural conditions, where the substratum may be more suitable for attachment and where the rate of increase of current speed is probably less, it may be that much stronger currents can be withstood without the larva becoming dislodged. It was frequently noted that when a larva became dislodged in a weak current it did not move far, but became attached again within a short distance (e.g. 3 cm.) of its original point of attachment. Much stronger currents were then needed finally to dislodge such larvae.

In the laboratory a considerable time interval may elapse before an appreciable proportion of actinulae become attached permanently to the substratum. Table I gives typical examples of the progress of attachment.

TABLE I. SETTLEMENT OF ACTINULAE OF *TUBULARIA LARYNX*

First-crop larvae		Second-crop larvae	
Estimated time after liberation (hr.)	Percentage attached	Estimated time after liberation (hr.)	Percentage attached
14	5	6	44
18	12	11	84
22	39	16	96
28	71	25	100
37	96		
46	100		

To obtain actinula larvae for these experiments, mature colonies of *T. larynx* were collected during the morning and inverted in fresh sea water for approximately 6 hr. The actinulae shed during this period were then collected and form the 'first-crop larvae' of Table I. The sea water bathing the colonies was then changed and the colonies were allowed to stand overnight. Actinulae were again collected the next morning and form the 'second-crop larvae'. There is clearly a very considerable difference in the rate at which these two batches of larvae become attached; this point is discussed more fully in the next section. It is evident, however, that the period for which the larvae are unattached may be greater than that recorded by Ciamician (1879) for the actinulae of *T. mesembryanthemum* (4–6 hr.). It will be noted that all the larvae eventually succeeded in becoming attached. Such results were typical of these larvae; under normal environmental conditions the proportion which settled successfully was rarely less than 90%. Other factors affecting settlement are discussed in the next section.

Settlement

It is not possible to give an account of the actual process of settlement, since this stage in the life history is apparently accomplished without the appearance of any marked structural changes or the initiation of any characteristic reactions. Sometimes the aboral tentacles lose their rigidity at about the time that settlement occurs; but this does not always seem to be so, and it is possible that this change is due to the artificiality of laboratory conditions. Larvae have frequently been watched carefully over the whole period between liberation and settlement, and it has not been possible to observe any marked or invariable change in structure or habit which would indicate that a larva was about to settle.

The effect of a number of factors on settlement has been investigated. The results obtained are briefly summarized below.

(i) *Hypotonic Sea Water.* Actinula larvae of *T. larynx* are not capable of withstanding hypotonic conditions for prolonged periods but, although degenerative changes were evident in the mixtures containing smaller proportions of sea water, there was also a tendency for settlement to be accelerated in such solutions (Table II).

Table II. Settlement in Full and Diluted Sea Water

Estimated time after liberation (hr.)	Percentage attached		
	Sea water	70% sea water	60% sea water
7	0	24	20
9	3	42	20
20	7	43	36

(ii) *Copper and Mercury.* Grave & Nicoll (1939) have recorded that copper stimulated the attachment of ascidian larvae, and Prytherch (1934) considered that increased amounts of copper present at low water caused the settlement

of the larvae of *Ostrea virginica* in Milford Harbour, though his conclusions have been severely criticized by Korringa (1940). A number of rough experiments on the effect of the addition of small amounts of copper and of mercury to sea water on the attachment of actinula larvae were carried out and the results suggest (Table III) that both of these poisons stimulate attachment to some extent.

TABLE III. EFFECT OF COPPER AND MERCURY ON SETTLEMENT

(1) Copper

Estimated time after liberation (hr.)	Percentage attached				
	Sea water	0·05 mg./l. Cu	0·075 mg./l. Cu	0·1 mg./l. Cu	0·25 mg./l. Cu
6	0	0	3	14	?81
9	3	0	6	16	80
19	7	0	39	46	100
28	24	35	50	60	100

(2) Mercury

Estimated time after liberation (hr.)	Percentage attached				
	Sea water	0·01 mg./l. Hg	0·05 mg./l. Hg	0·1 mg./l. Hg	0·25 mg./l. Hg
10	0	36	40	82	100
27	80	53	66	90	100

The range of copper and of mercury concentrations used in these experiments includes concentrations that are toxic. The median lethal concentration of copper (for an exposure period comparable with the total length of the experiment recorded in Table III) is approximately 0·09 mg.Cu/l., whereas the corresponding figure for mercury is 0·03 mg.Hg/l. Attachment is, therefore, stimulated at concentrations below the median lethal concentration for both poisons and, though the larvae in the highest concentrations were dead at the end of the experiment, attachment had taken place before death occurred.

(iii) *Benzoquinone and Ascorbic Acid.* If small amounts of these compounds are added to sea water in which actinula larvae have been placed, there is, up to concentrations which are toxic, a stimulatory effect on attachment. Table IV shows the results of one experiment using benzoquinone.

TABLE IV. EFFECT OF BENZOQUINONE ON SETTLEMENT

Estimated time after liberation (hr.)	Percentage attached				
	Sea water	0·01 mg./l. BQ	0·1 mg./l. BQ	1·0 mg./l. BQ	10·0 mg./l. BQ
15	23	36	37	0	0
27	40	74	91	0	0

At concentrations higher than 0·1 mg. benzoquinone/l. this compound killed the larvae very shortly after immersion in the solution.

Comparable results were obtained using ascorbic acid.

A brief explanation should perhaps be given of the reasons why the effect of these two rather unusual reagents was tested. Observations by one of us (K. A. P.), referred to in Harris (1946), suggest that the cement which is used to attach a number of sedentary marine animals (barnacles, mussels) to the substratum is formed from a mucoprotein basis, which is 'tanned' by the action of a quinone, in a manner similar to that described by Pryor (1940) for the cuticle of insects. It seemed possible that the cement which attaches the stolonial perisarc of *Tubularia* to the substratum was of a similar nature and the effect of these compounds was therefore investigated to test this possibility. Benzoquinone was used to provide a quinone already present in solution (in the insect cuticle the quinone is formed by oxidation from the corresponding dihydroxyphenol) and so possibly to 'tan' the mucoprotein rapidly and so prevent attachment because the mucoprotein cement had not spread sufficiently. The effect of ascorbic acid was investigated because this compound has been found (Evans & Raper, 1937) to be capable of reducing dopa quinone to dopa in the tyrosinase—tyrosine reaction (which in some respects parallels the reactions which occur as a mucoprotein becomes 'tanned'). The effect of the addition of ascorbic acid might, therefore, be to prevent 'tanning' of the mucoprotein of the cement by the prevention of oxidation of the dihydroxyphenol to the corresponding quinone. Apart from the stimulatory effects noted in Table IV, no particular effect on attachment was produced by either of these reagents.

(iv) *The presence of bacterial slime.* All the experiments on the rate of settlement of the actinula larva so far described were carried out in clean glass vessels. Such a substratum is probably highly abnormal, and an attempt was made to produce a substratum which was slightly more normal by carrying out settlement experiments in glass dishes in which sea water had been allowed to stand for a week. When this was poured away, a thin slime film, probably bacterial in origin, was present over the bottom and sides of the dish. Settlement was appreciably heavier on this surface than on a clean glass surface 24 hr. after the addition of freshly liberated actinula larvae.

The general impression gained from this series of experiments is that a variety of conditions can stimulate attachment. In fact, the reaction of an actinula larva to a disadvantageous environment seems to be that it becomes permanently attached. The reason for this may well be in the sequence of changes that takes place between liberation and settlement. The ectoderm at the tip of the aboral pole of the actinula larva is deeply columnar and its cells are packed with dark granules. The general appearance of this tissue, which is clearly discernible before the larva is liberated (Lowe, 1926), suggests that it is secretory in function. It would seem likely that this region of the ectoderm secretes the attachment cement, and it begins to do so when the aboral pole of the larval body comes into contact with the substratum. Normally this cannot happen until this region of the body has elongated, since the stiff aboral tentacles hold it away from the substratum. If, however, the aboral tentacles were to lose their rigidity for any reason, this 'fixation area' would prematurely be brought into contact with the substratum. As one of the first effects of a disadvantageous environment is to cause the aboral tentacles to lose their rigidity, it seems plausible to suggest that this is the reason why such a diverse series of conditions produce a similar effect.

The suggestion advanced in the preceding paragraph involves the assumption that the secretion of cement is possible whenever the aboral pole of the larva comes into contact with the substratum. Direct experimental proof that this assumption is correct is not yet available, but a number of observations, both under laboratory and under field conditions, suggest that this is so. Under laboratory conditions the high percentage settlement almost invariably obtained suggests that attachment is a passive process in the sense that it occurs as soon as the requisite part of the body comes into contact with a substratum, and, under field conditions, the wide range of substrata upon which settlement can occur also supports this suggestion. Settlement will not only take place readily on smooth surfaces such as glass or bakelite, and not only on rather rougher and more irregular surfaces such as ground glass or the compartments and opercula of barnacles, but also on substrata which are unstable and upon which practically no other sedentary animal seems able to settle. For example, the surface of certain types of resin-containing compositions soon becomes coated with a thick layer of jelly-like slime, which is probably bacterial in origin and which forms a most unstable substratum; actinula larvae settle in numbers on such slimes, though the young colonies are lost as soon as the slime layer peels away from the paint surface beneath. Again, anti-fouling compositions containing cuprous oxide which have a leaching rate which lies between 10 and 20 mg. copper/m.^{2}/day, acquire a felt-like algal settlement (formed, at least in part, by *Ectocarpus*) which soon completely covers the paint surface: *Tubularia larynx* is one of the few organisms which can settle and grow on this type of surface.

Algal growth of other kinds, such as *Enteromorpha* or the more normal tufted growth form of *Ectocarpus*, generally prevents settlement, but this effect is only evident if the algae exceed a certain length. In the course of exposures made to study the settlement and growth of *Tubularia larynx*, which are described more fully in the next section, it was found that settlement of the actinulae occurred readily in the presence of these algae if the latter did not exceed 2 mm. in length. This suggests that sporelings of greater length, provided their settlement was sufficiently dense, were perhaps capable of preventing contact between the actinula and the substratum. Occasionally actinula larvae succeed in settling *on* the filaments of well-grown *Ectocarpus*, and settlement will occur readily on the fronds of *Laminaria*, observations which suggest there is little inimical in an algal surface *per se*.

Post-Settlement Changes

Growth and maturity

The changes which take place in the actinula larva immediately after settlement are shown in Fig. 2. Fig. 2A shows a young form very shortly after settlement has taken place; Fig. 2B the same individual just over 7 hr. later, and Fig. 2C

its appearance after a lapse of a further 12 hr. These figures emphasize the extremely rapid elongation which occurs immediately after settlement; over a period of some 19 hr. the overall length increased from 0·98 to 1·65 mm. The cell layers of the body wall become much thinner during this process and it would, therefore, seem that this elongation is due rather to the redistribution of tissue already formed than to the production of new tissue.

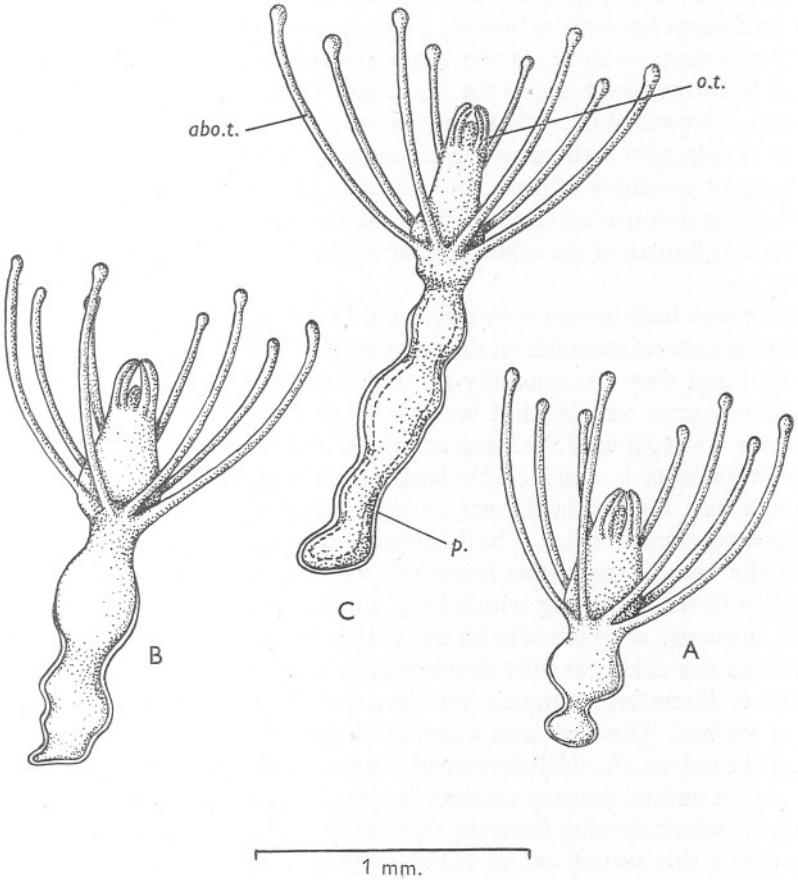

Fig. 2. Newly settled individuals of *Tubularia larynx*. A, shortly after settlement. B, approximately 7 hr. after settlement. C, approximately 19 hr. after settlement; the broken line within the aboral region indicates the inner boundary of the endoderm. *abo.t.*, aboral tentacle; *p.*, perisarc; *o.t.*, oral tentacle.

The lower part of the stem of the young individual and the short length of primary stolon are both formed from the aboral region of the actinula larva. It is difficult to decide what relation these two derivatives bear to the original point of attachment, but it seems plausible to suggest that elongation on one side of this point leads to the production of stolon, whereas elongation on the

other side leads to the production of stem. For the latter, it must be presumed that elongation is initially not equal at all points, since the stem bends sharply away from the substratum. The aboral tentacles, it will be noted, are now all directed away from the substratum, but their tips are still swollen at this stage; they lose this characteristic later.

Following this stage of elongation there is one in which growth of the stolon predominates. This part of the colony may measure 0·3 mm. in length shortly after settlement has been achieved; 2 days later its length is 0·8 mm., and after a further 2 days 1·3 mm. At this stage a second polyp often appears, usually arising from the tip of one of the branches of the stolon and growing rapidly upwards. Growth of the stolon continues to predominate for some time longer, so that 11 days after settlement a total length of stolon of 7 mm. may be present (made up of a number of branches) associated with about eight polyps. Once a branching stolonial system has been established in this way, it is probable that further growth of the colony chiefly results in the production of stems and polyps.

Gonophore buds appear very early. For example, buds were visible between the oral and aboral tentacles on the primary polyp by the time the second polyp appeared and they are generally present some 7 days after settlement. The gonophores grow rapidly and just over 3 weeks (24 days) after settlement a number are capable of shedding actinulae. This estimate of the period taken to reach maturity is considerably longer than that found by Orton (1929) at Cawsand Bay, Plymouth, though comparable with the estimate he gave in an earlier paper (Orton, 1914). The diameter of the polyps is then 2 mm. measured across the base of the aboral tentacles. Growth of the polyps continues for a further fortnight during which large numbers of actinulae are shed, after which autotomy of practically all the polyps occurs. It is of interest to note that, when the colony is fully developed, only a proportion of the polyps are capable of liberating actinulae, yet when autotomy occurs, practically all the polyps are lost. This indicates a certain degree of differentiation of function among the polyps of a well-developed colony, in that while all can presumably feed, only a limited number produce larvae. The polyps lost are later replaced by others, which develop from the tips of the stems of the established colony. Presumably this second set of polyps may themselves grow, develop gonophores, shed actinulae and finally be lost, but it has not yet been possible to carry out field observations for long enough to prove this point. Successive 'generations' of polyps certainly appear under laboratory conditions, though their gonophores may not reach full development.

Factors affecting the growth and development of the colony

Observations have been made on the general biology of colonies of *Tubularia larynx* by exposing standard microscope slides (3 × 1 in.), with one face slightly roughened (using 80-mesh carborundum powder), on painted steel plates

adapted to act as carriers for these slides. Each side of each plate can accommodate 27 slides, arranged in three rows, 9 slides to a row. These steel carriers can readily be bolted to the standard frames used for the exposure of painted steel plates; extensions to the frames allow carriers to be immersed at a number of depths. Exposures of this kind were made in 1946 and 1947, four carriers being used on each occasion; they were immersed at depths of 27, 45, 64 and 84 in. (approximately 0·7, 1·1, 1·6 and 2·1 m.) below the water surface. One slide was withdrawn from each row of each face of each carrier at approximately weekly intervals (the period between withdrawals was varied slightly according to the amount of change that was taking place at the time) and replaced by a clean slide. These slides which were withdrawn at intervals over the whole period of the experiment are termed the basic series in the description below. The slides which replaced them were themselves later withdrawn, some at approximately weekly and some at approximately 3-weekly intervals—these slides formed the weekly and the 3-weekly series mentioned later. After withdrawal, each slide was examined in detail and its various settlements and their characteristics recorded.

The slide series were immersed on 31 July both in 1946 and 1947. In 1947 immersion was continued until 10 October, but in 1946 the exposure period only lasted until 19 September.

Fig. 3 gives the general results of growth-rate measurements in 1946 and 1947. It was clearly impossible to attempt to measure growth by measurements of individual colonies, as only a few colonies could be dealt with in this way at the expense of a great amount of labour. As a rough estimate, therefore, the volume of *Tubularia* on each slide was determined. Using this method the growth on a number of slides could be rapidly estimated; the disadvantage was that reliable measurements were not possible until some time after settlement, as volumes less than 0·2 ml. could not be measured with sufficient accuracy. The results shown in Fig. 3 indicate that the initial growth rates were substantially the same in both years.

When, however, the numbers of new settlements per slide on the basic series of slides are compared for the 2 years (Fig. 4), considerable differences are evident. In 1946 the numbers of new settlements recorded showed a sharp increase between 4 and 5 weeks after the experiment began, whereas in 1947 the increase was much smaller, though it occurred at approximately the same time after immersion. Evidence will be presented later which suggests that the number of new settlements depends upon the state of maturity of the polyps in the immediate vicinity, i.e. those on the same slide or on its neighbours. The results obtained in 1946, therefore, indicate that considerable numbers of actinulae were released at almost the same time; but those obtained in 1947 may mean either that the numbers were much smaller (owing to poor development of the polyps) or simply that the actinulae were not able to settle.

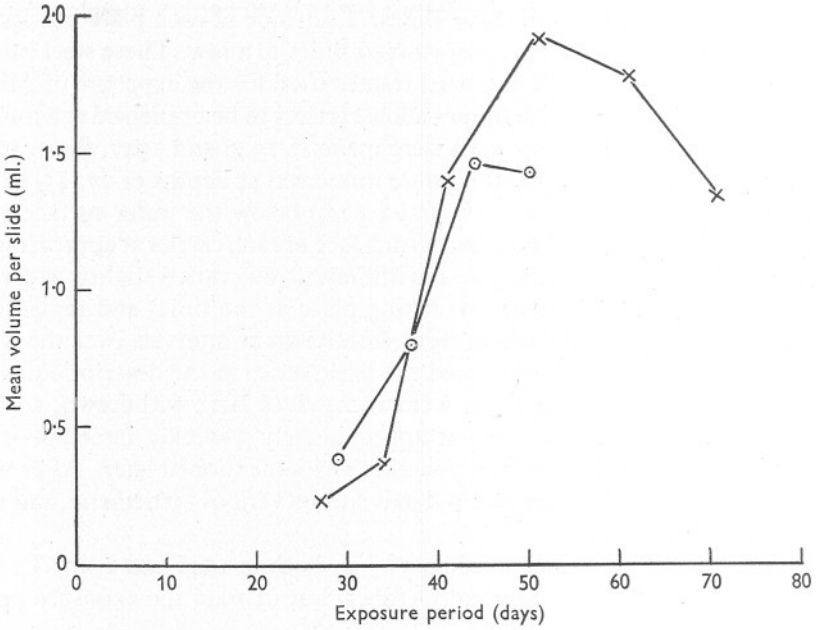

Fig. 3. Growth rates of *Tubularia larynx* in 1946 and 1947 (as indicated by increase of volume of the colonies) on the basic series of slides. —⊙—, 1946; —×—, 1947.

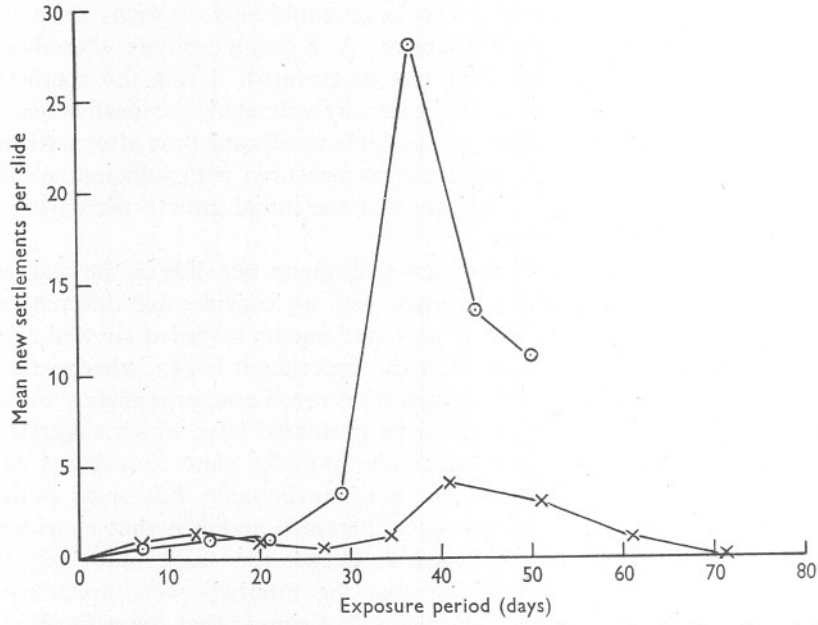

Fig. 4. New settlements of *Tubularia larynx* in 1946 and 1947, on the basic series of slides. —⊙—, 1946; —×—, 1947.

Other evidence suggests that the second explanation is probably correct. The first 5 weeks of the exposure period in 1947 were characterized by an unusually high proportion of sunny, calm days and the light intensity down to the depth covered by these slide exposures was probably greater than that for the corresponding period in 1946. This difference in environmental conditions would favour the growth of algae and, as is shown in Table V, their settlement was certainly heavier at all depths, and growth, at least of *Ectocarpus*, greater at all depths in 1947 than in 1946.

TABLE V. SETTLEMENT AND GROWTH OF ALGAE AT VARIOUS DEPTHS, AUGUST 1946 AND AUGUST 1947

Depth below sea level		*Enteromorpha* sp.		*Ectocarpus* sp.	
In.	M.	1946	1947	1946	1947
27	0·7	V. Fr. (4·8)	V. Fr. (2·5)	V. Fr. (6·6)	V. Fr. (7·6)
45	1·1	Occ. (s)	Fr. (s)	Occ. (s)	Fr. (5·6)
64	1·6	Rare (s)	Fr. (s)	Rare (s)	Fr. (3·8)
84	2·1	V. Ra. (s)	Fr. (s)	V. Ra. (s)	Rare (1·0)

V. Fr., Very Frequent; Fr., Frequent; Occ., Occasional; V. Ra., Very Rare.
The figures in parentheses are the mean lengths (in mm.) of the algal filaments. (s) indicates present only as sporelings.

These results refer to the basic series of slides; the 3-weekly and the weekly series, since their exposure periods are shorter and their chances of extensive algal colonization therefore less, should, if this suggestion is correct, indicate numbers of settlements which are more comparable for the 2 years. The results shown in Fig. 5 are in agreement with this suggestion. As might be expected, the difference between the 3-weekly series of 1946 and that of 1947 is greater than that between the weekly series; the numbers of new settlements on the latter are, within the limits of accuracy of an experiment of this sort, closely similar in 1946 and 1947.

Counts of the number of new settlements on slides exposed at various depths in 1946 showed that settlement did not take place predominantly at any of the depths investigated, but measurements of the mean volume of colony per slide at different depths suggested that the colonies attained a greater size when growing at depths greater than 4 ft. (1·2 m.) than they did when growing nearer the surface.

The presence of a mature colony contributes substantially to the amount of settlement which takes place in its immediate vicinity. When the experiments described earlier in this section were started, surfaces only a few feet away from the slide carriers bore large numbers of mature colonies of *Tubularia larynx*, yet settlement on the slides did not become heavy until some weeks later. This result might have been due to one of two causes; either the surfaces were, for some reason, not suitable for settlement immediately after immersion, or heavy settlement must be dependent upon the close proximity of mature colonies.

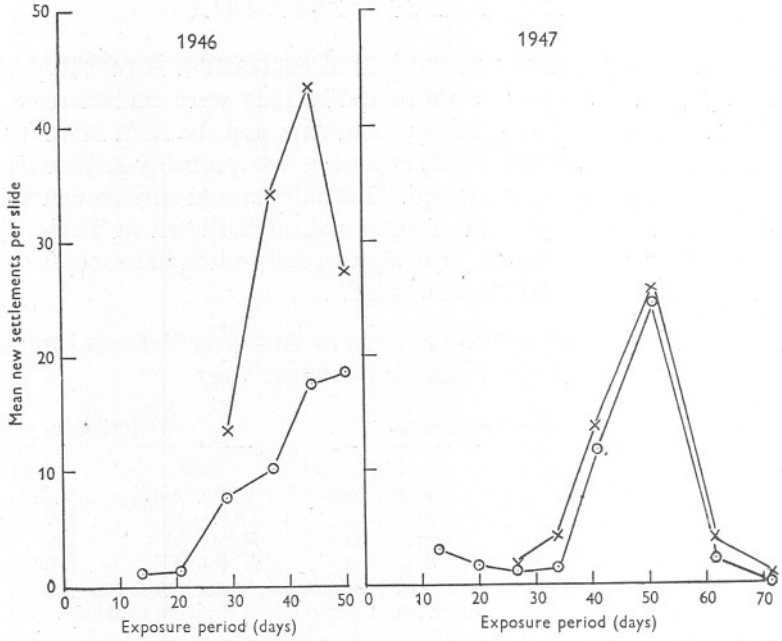

Fig. 5. New settlements of *Tubularia larynx* in 1946 and 1947, on the weekly and 3-weekly series of slides. —⊙—, weekly series; —×—, 3-weekly series.

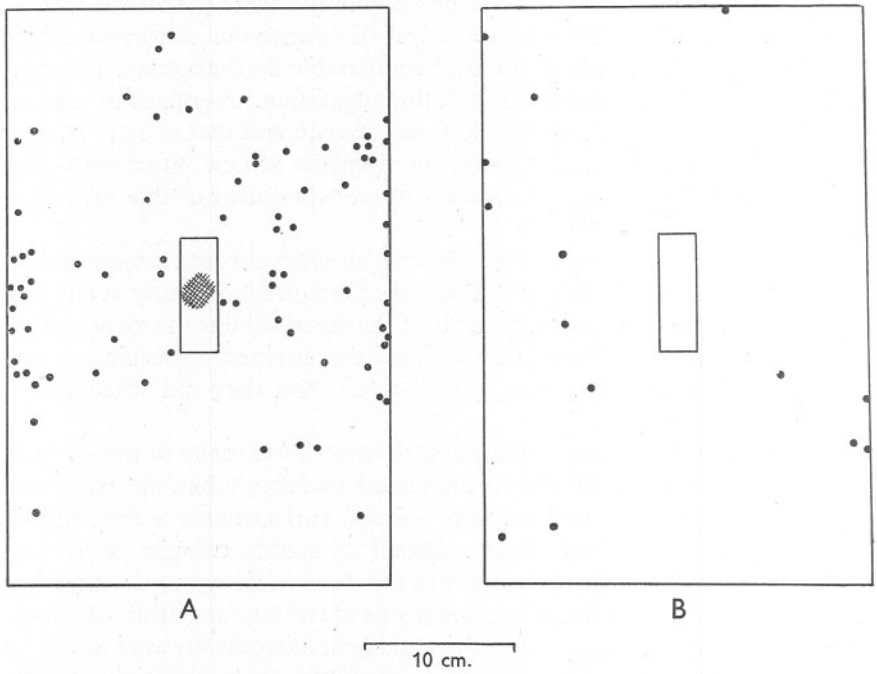

Fig. 6. New settlements of *Tubularia larynx* on (A) a wooden panel with a glass microscope slide fixed centrally bearing a colony of *T. larynx* and on (B) the same with the microscope slide clean. The position of each new settlement is indicated by a black circle.

Moore (1939) has described the lag in colonization of a newly prepared artificial surface, and it seemed conceivable that newly immersed glass slides might show a comparable lag. That this was not the factor which delayed settlement of the actinulae of *T. larynx* was clearly shown by an experiment in which freshly prepared slides were immersed beside slides which had been in the sea for some weeks, but which had their gross fouling removed. Settlement and growth on the two series was found to be closely similar.

This result suggests that the second possibility is the more likely and this is supported by the following experiment. A glass slide, bearing a mature colony of *T. larynx*, was set in the middle of a wooden panel, 10 × 15 in., and immersed from a raft which itself bore practically no *Tubularia*; a control exposure consisting of an uncolonized slide set in the middle of a similar panel was immersed close beside it at the same time. The amount of settlement on the two surfaces after a period of 11 days' immersion is shown in Fig. 6, and clearly indicates the heavier settlement which occurs in the vicinity of a mature colony.

Biotic Environment

Once settlement has been achieved and growth begun other organisms soon begin to be associated with, or to prey upon, the colony of *Tubularia larynx*. Under the conditions in which most of the colonies were studied in this investigation, where the colony of *T. larynx* was the largest colonizing form, the bases of the colonies soon began to accumulate the muddy tubes of *Jassa* sp., and also frequently bore clusters of small mussels (*Mytilus edulis* L.) which are usually settling in some abundance at the time that settlement of *Tubularia larynx* is becoming intense. The stems of the colonies quickly became colonized by Suctorians, chiefly *Ephelota* sp. and *Acineta* sp. The settlement of these Protozoa appeared to be successive in 1946, as *Ephelota* sp. predominated until early September, whereas *Acineta* sp. was more common later in the season. Diatoms, especially *Licmophora* spp. and *Striatella* sp., were also conspicuous on the stems of well-grown colonies, especially towards the end of September or early in October; the colonies of *Tubularia larynx* were then sometimes dark brown in colour due to the dense covering of these diatoms.

All these forms are merely associated with the colonies of *T. larynx*, and do not appear to have any predatory or parasitic relationships with the hydroid. Others, however, are predators or parasites.

The pycnogonid, *Phoxichilidium tubulariae* Lebour, is commonly to be found among colonies of *Tubularia larynx*, a habitat similar to that recorded by Lebour (1945) for *Phoxichilidium tubulariae* at Plymouth. The larvae of this pycnogonid occur within the gastral cavity of *Tubularia larynx* and, though a few larvae can usually be detected in this situation late in September, they seem only to be really abundant if well-developed colonies of *Tubularia* are present in some abundance at this time. Normally *T. larynx* is beginning to die down at the

end of September, but if, as in 1945, it is flourishing early in October, practically every colony has several polyps which contain the larvae of *Phoxichilidium tubulariae*. Development of the latter must be rapid, as on one occasion colonies of *Tubularia larynx*, collected from a surface that had been immersed for only 24 days, contained advanced larvae of *Phoxichilidium tubulariae*. As some part of this immersion period must have been occupied by settlement and initial growth of the *Tubularia* colony, it is reasonable to suppose that the larvae of *Phoxichilidium* must have developed to this stage in less than 20 days. Lebour (1945) states that these larvae do not appear to harm the polyp in which they occur. We have found that such a polyp is usually abnormally distended, but that it seems otherwise normal. Because colonies of *Tubularia larynx* are so resistant to copper, experiments have been carried out in which this hydroid was immersed in copper solutions of different concentrations; on some occasions colonies with polyps containing the larvae of *Phoxichilidium tubulariae* were used for these experiments, but the sensitivity of infected colonies to this poison did not differ appreciably from that of colonies in which the polyps were uninfected.

The Holotricha, *Choenia* sp. and *Loxophyllum* sp., are also commonly found on colonies of *Tubularia larynx*. *Choenia* sp. is the more abundant: it feeds on newly settled forms, and is to be found in large numbers on mature polyps shortly before these are lost and also on and in moribund stems.

Numbers of nudibranch molluscs, particularly *Cratena aurantia* (Alder & Hancock) and *Dendronotus frondosus* (Ascanius), occur about the time that settlement and growth of *Tubularia larynx* reaches its maximum. Both feed on the polyps; the numbers of *Cratena aurantia* which occur suggest that this species may play a significant part in the reduction in the numbers of polyps which takes place at the end of the season. It would seem unlikely, however, that *C. aurantia* is the primary cause of this reduction.

Discussion

General observations in the field, as well as the results of experimental work, indicate that settlement of actinula larvae is heaviest in the immediate vicinity of a mature colony, which suggests that settlement occurs shortly after liberation. Laboratory observations, on the other hand, indicate that a period of the order of 24 hr. must elapse before an appreciable proportion of the larvae become attached. This discrepancy may occur because of the unnatural conditions under which laboratory observations must be carried out, but as observations suggest that in a healthy actinula larva elongation of the aboral pole of the larva must occur before the attaching surface can come into contact with the substratum, it would be expected that an interval should elapse between liberation and attachment in the sea. In fact, it is conceivable that under field conditions the interval might be slightly longer, since the water temperature is lower in the sea than in the laboratory. It is not known how the larva

remains in contact with the substratum during this interval, but experiments carried out in the course of this investigation suggest that it is capable of remaining attached, even to a smooth, clean glass surface, in moderate water currents. In a more natural environment, attachment is likely to be more secure.

The actual means whereby this initial attachment is effected are not yet certainly known. It is possible that the tips of the aboral tentacles adhere to the substratum by means of an adhesive secretion (Orton (1929) speaks of the actinula larva of *Tubularia larynx* as 'highly adhesive'), but the experimental results do not agree well with such a hypothesis. For, if an adhesive secretion is produced, it is perhaps possible that the amount of this secretion might be increased under the stronger stimulus of faster water-flow, but it is more difficult to see why a decrease in the current, followed by a more sudden increase, should almost always cause the larvae to be dislodged. Another possibility is that attachment may be effected by means of nematocysts. The swollen tips of the aboral tentacles contain large numbers of nematocysts which, to judge by the extreme ease with which this part of these tentacles becomes attached to a needle, may be very sensitive to mechanical stimuli. Ewer (1947) has recently shown that the tentacles of the buds of *Hydra vulgaris attenuata* become attached temporarily to the substratum by means of atrichous isorhizas which lie in the nematocyst batteries in the tentacles; it would seem possible that at least some of the nematocysts in the tips of the aboral tentacles of the actinula larva have a similar function.

The results of the experiments on the effect of different current speeds on the rate of liberation of actinulae (though these experiments can only be regarded as preliminary in nature) are of interest, in that they suggest that larval liberation takes place only at periods of the tide when the larvae are least likely to be swept away in rapid tidal currents. They are in agreement with observations made on plankton hauls taken in the vicinity of mature *Tubularia* colonies. Tow-nettings have regularly been taken, during the ebb tide, from a point only a few yards from a raft bearing hundreds of mature colonies of *T. larynx*, but only individual larvae have been found when the haul was examined. Even if a plankton net is placed close against the side of a plate bearing large numbers of mature colonies, the result is much the same. This suggests that the number of larvae present in the water when the tide is running strongly is very small but, whilst this supports laboratory observations on the effect of current speed on the liberation of actinulae, these field results must be cautiously interpreted since many larvae may be caught up in the meshes of the net.

If further work substantiates the preliminary results of the effect of different current speeds on the rate of liberation of larvae, the question is then raised of the nature of the stimuli which initiate and stop liberation. Liberation of actinulae can be almost completely inhibited (in still water) by the addition

of small amounts (0·5 %) of NaCl, KCl and $CaCl_2$; the effect of potassium in this respect can partly be antagonized by the simultaneous addition of $MgCl_2$. These results, which are closely parallel to the effects of these metallic ions on the contraction of smooth muscle, suggest that the immediate cause of liberation is contraction of the muscular elements of the gonophore, but do not indicate the nature of the stimulus responsible for initiation of the contraction. Further work is needed on this point.

A number of the observations made in the course of this work suggest that the colony of *T. larynx* should be regarded as a unit to a degree greater than that which is sometimes accorded to a hydroid colony. Some degree of functional differentiation among the individual polyps (in that only a small proportion bear mature reproductive organs) and the possibility that the life period of a single polyp is shorter than that of the colony both suggest that the unit is the colony rather than the polyp, and that the activities and development of the latter depend upon the general state of the former. When the colonies die down at the end of September or at the beginning of October, it is not easy to discover any external cause of this change. No rapid changes are taking place in water temperature and, though the depredations of *Cratena aurantia* (A. & H.) and other nudibranchs cause the loss of some polyps, such effects are commonly no greater then than they have been for some time earlier. Further, the fact that, at the time when the main mass of *Tubularia* colonies which have provided the bulk of the season's growth are dying down, a small number of new settlements are growing makes it unlikely that any external factor is the cause of the general decline. Hammett (1946) concludes that endogenous factors play an important part in the general economy of *Obelia* colonies towards the end of their period of abundance, and it is difficult to resist the conclusion that such factors also play an important part in causing the degeneration of colonies of *Tubularia larynx* at the end of the summer.

Settlement and growth of *T. larynx* at Millport normally reach their maximum during August and September, but the general seasonal sequence is not always regular. In 1945 heavy settlement began early rather than late in July, but at the beginning of August all the colonies became moribund, and autotomy of the polyps occurred. Regeneration did not take place until the middle of September and then the main mass of the *Tubularia* settlement persisted until towards the end of October. There were some signs that a similar phenomenon was about to take place early in August 1947, but the process of decline was arrested at an earlier stage and the period of vigorous growth and development was not substantially interrupted. The causes of such variations in the normal seasonal sequence are unknown, since no disadvantageous environmental factor, either edaphic or biotic, could be detected over the period when the interruption occurred. The observation that vigorous growth can persist through October, if the normal seasonal sequence has been

interrupted by a period when the colonies are dormant does, however, offer some support for the suggestion that endogenous factors play a part in the autumnal decline of this hydroid.

We are indebted to the Marine Corrosion Sub-Committee of the British Iron and Steel Research Association for permission to publish this work, which was carried out in the course of an investigation of the general biology of fouling organisms. One of us (F. S. D.) was assisted by an expenses' grant from the Research Association, for which grateful acknowledgement is made. We should especially like to record our acknowledgement of the interest shown by Prof. J. E. Harris, Chairman of the Sub-Committee, and by the late Mr R. Elmhirst, Director of the Marine Station, Millport, in the progress of this work. We should also acknowledge the help of the Director of the Plymouth Marine Laboratory in providing a sample of *Tubularia larynx* for comparison with our own material.

Summary

A description is given of a number of observations and experiments on the larvae and colonies of *Tubularia*. The experimental material could best be referred to the species *T. larynx* Ellis & Solander. Its characters are not wholly consistent with the diagnosis of this species, but the differences which exist are not great enough to warrant the erection of a new species.

Liberation of the actinulae takes place more freely in darkness than in light, and there is evidence which suggests that it only occurs in still water or in weak currents.

Immediately after liberation the body of the actinula is spherical and it rests on the tips of those aboral tentacles which point away from the mouth. Growth of the aboral region takes place and permanent attachment is possible when this region is long enough to come into contact with the substratum. This elongation takes place over a period of 24–48 hr. under laboratory conditions.

Temporary attachment can take place by means of the tips of the aboral tentacles; it seems likely that this is accomplished by the eversion of nematocysts.

A thin sheath of perisarc is present over the aboral region of the actinula when it is liberated. The perisarc is chitinous in nature, and permanent attachment is secured by means of an extra-chitinous cement, probably secreted by the glandular ectoderm of the aboral pole.

Exposure of the actinula larva to hypotonic sea water, low concentrations of copper and mercury and some organic compounds stimulates permanent attachment. These abnormal environmental conditions cause a decrease in the rigidity of the aboral tentacles; this allows the aboral pole of the larva to come into contact with the substratum prematurely.

Growth is rapid once permanent attachment has taken place. An extensive stolonial system is first established, followed by the production of a number

of stems and polyps. Gonophore buds appear on the primary polyp 2 days after settlement and some polyps can liberate actinulae 24 days after settlement. Most of the actinulae produced by a colony settle, if suitable substrata are available, in its immediate vicinity.

Only some of the polyps in any one colony liberate actinulae. Liberation continues for some days and is followed by autotomy of all the polyps in the colony. These are later replaced.

The biotic environment of the grown colony is briefly described.

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