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THE REPRODUCTION OF *HALICLONA LOOSANOFFI* AND ITS APPARENT RELATIONSHIP TO WATER TEMPERATURE [1]

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There have been few detailed studies on the reproductive periods of sponges and very few on the reproduction of particular species within different parts of their geographical ranges (Fell, 1974a and 1976). Usually studies of sponge reproduction have involved either examination of tissue samples taken at regular intervals throughout the year or observation of larval settlement on experimental surfaces. Rarely has an attempt been made to compare the results from these two methods of study (Bergquist and Sinclair, 1973).

Water temperature has been frequently assumed to be a major environmental factor regulating the reproduction of sponges. Although such regulation would not be unexpected, it has been documented in few cases (Storr, 1964; Wells, Wells and Gray, 1964; Simpson, 1968). Consequently more extensive information concerning the relationship between water temperature and reproductive activity would be of interest.

The present study defines the reproductive period of *Haliclona loosanoffi* in the Mystic Estuary, Connecticut using three different approaches: 1) examination of tissue samples from adult specimens, 2) observation of larval release from freshly collected specimens in the laboratory, and 3) study of larval settlement on mollusc shells suspended in the estuary. These studies confirm preliminary results (Fell, 1974b) which indicated that the reproductive period of *Haliclona loosanoffi* is much earlier at Mystic than at Milford, Connecticut (Hartman, 1958) and occurs at about the same time as in more southern regions (Wells *et al*, 1964; Marsh, 1970). Because of this unexpected situation, it seemed of interest to determine whether the reproductive period could be related to water temperature. The evidence is consistent with the hypothesis that water temperature is a major factor regulating reproduction in this species.

Since previous studies on the reproduction of *Haliclona loosanoffi* were almost exclusively concerned with larval settlement (Hartman, 1958; Wells *et al*, 1964), little was known about the type of sexuality, gametogenesis and embryonic development. Information on these subjects was obtained in this study by the examination of tissue samples. In addition, some new observations concerning the selection of settling surfaces by the larvae of this species were provided by the larval settlement studies.

Materials and Methods

Specimens of *Haliclona loosanoffi* were collected from the lower Mystic Estuary, Connecticut at regular intervals during five years (1969–1971, 1973, and 1974).

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In many cases the specimens were fixed immediately in Bouin's solution in sea water, but in some cases they were studied in the laboratory before they were fixed (see below). The fixed specimens were cut into pieces and examined under a dissecting microscope for the presence of large oocytes and embryos. Small samples of the specimens were then embedded in paraffin and sectioned serially at 10 μ. The mounted, deparaffinized sections were stained with hematoxylin and eosin. Usually a minimum of 5 spaced serial sections were scanned at a magnification of 1000 $\times$ to determine the presence or absence of small oocytes or spermatic cysts, and additional sections were examined at lower magnifications. In all more than 150 specimens were studied histologically.

During one summer (1974) specimens of *Haliclona loosanoffi,* collected at weekly intervals, were brought back to the laboratory and placed in finger bowls containing sea water for 6 to 12 hrs to see if they would release larvae. From 2 to 14 (usually 5 or more) specimens were examined on each date, and the sea water in which they were placed was filtered, freshly collected sea water from the Mystic Estuary. Only intact specimens, which were resting on sandy bottom, were used. On one occasion when large numbers of larvae were released by the sponges, observations on larval behavior and settlement were made in the laboratory. The sponges were carefully removed from the bowls, and the larvae were kept in the original sea water into which they were released.

In 1971 larval settlement was studied by suspending mollusc shells in the Mystic Estuary at regular intervals. Freshly shucked shells of the clam, *Mercenaria mercenaria,* were thoroughly cleaned in fresh water and completely dried. A small hole was drilled near the center of each shell, and the shells were strung in groups of 5 on nylon cord with the concave (inner) surface down and tygon tubing spacers (1.5 inches long) between successive shells. From 1 May to 16 August ten shells were placed in the estuary below the level of tidal exposure every three weeks. Each time a new set of shells was suspended in the estuary, the preceding set was removed. The shells were examined for the presence of sponges under a dissecting microscope as soon as they were brought back to the laboratory, and the identification of each small sponge was checked by preparing a wet mount of it and examining the preparation at a magnification of 430 $\times$. Skeletal characteristics were considered diagnostic.

The size index of many specimens was determined by multiplying the length $\times$ width $\times$ height. When specimens were of irregular shape, they were cut into a number of more or less regularly shaped pieces and the size indices of the individual pieces were summed.

Results

General description of reproduction

Haliclona loosanoffi apparently is gonochoric, there being a separation of the sexes. While this is true, what appear to be abortive small oocytes are found in many male specimens during the latter part of the reproductive period. The sex ratio is approximately 1 : 1. Of 71 specimens examined during the period when all specimens were reproductive (last week of May through the first week of July), 34 were males and 37 were females.

Gametogenesis and embryonic development in *Haliclona loosanoffi* are virtually identical to these processes in *Haliclona ecbasis* which has been studied in detail (Fell, 1969, 1970). The sperm develop within spermatic cysts which are scattered throughout the endosome. Each cyst is made up of one or more clusters of spermatogenic cells surrounded by a layer of pinacocytes. All of the cells of a cluster are at the same stage of development, but different clusters within the same cyst may be at different stages. The sperm have a spherical head approximately 1 μ in diameter and a tail about 10 μ in length. All of the sperm in a cluster are oriented in the same direction. Frequently all stages of spermatogenesis occur simultaneously in the same specimen.

The oocytes are individually distributed throughout the endosome. At an early stage of growth an oocyte becomes surrounded by nurse cells which it progressively engulfs. The nucleus of the engulfed nurse cells disappears but the cytoplasm appears to remain unchanged. Consequently the larger oocytes and early embryos are filled with a large number of nurse cells, and little oocyte (or blastomere) cytoplasm can be discerned. After the engulfment of nurse cells is complete, the oocyte becomes enclosed within a thin follicular envelope consisting of flattened pinacocytes.

Distinct blastomeres usually are not detectable during the initial cleavage stages, presumably because of the close packing of the cells. The early embryos are therefore difficult to distinguish from the larger oocytes at low magnification. As cleavage proceeds the engulfed nurse cells are gradually fragmented and incorporated into the cytoplasm of the blastomeres. When this process is complete, the embryo develops into a solid, flagellated larva (parenchymula). Spicules appear at the periphery of the embryo during late cleavage, but as the larva develops they become situated internally near the posterior pole. Mature larvae leave the parent sponge by way of the excurrent water system.

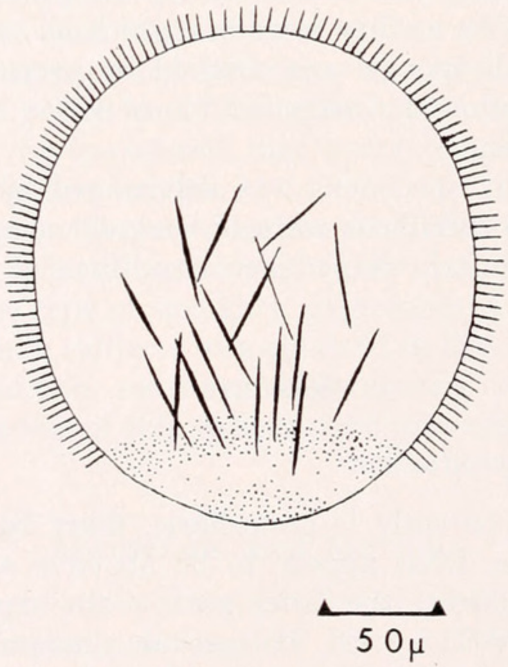

FIGURE 1. Free-swimming parenchymula larva of *Haliclona loosanoffi*.

It is not uncommon for all stages of oogenesis and embryonic development to be present in a single specimen at the same time.

The free-swimming larva of *Haliclona loosanoffi* measures approximately 188 × 172 μ. Most of the larva is light beige and a golden-brown ring encircles the posterior pole. In many cases an area of brown pigment occurs in the center of the ring. This may be sufficiently large in some larvae that the posterior pole is almost completely pigmented. Short flagella cover the entire surface of the larva except for the posterior pole. The larval spicules (oxeas) are about 50–60 μ in length (Fig. 1).

Reproductive period

The earliest that active specimens of *Haliclona loosanoffi* have been found in the Mystic Estuary is late May or early June. Already at this time all of the specimens contain large numbers of gametes and/or embryos. This situation persists through the third week of June, and then the percentage of specimens with large numbers of reproductive elements steadily declines reaching a low level by the end of July (Fig. 2A). As would be expected, female specimens contain primarily small oocytes at the beginning of the reproductive period. Then the small oocytes become less numerous and the number of embryos steadily increases. Finally, during the period when mature larvae are released (see below), the embryos progressively decrease in abundance (Table I). Growing oocytes and mature larvae never constitute a large proportion of the reproductive elements.

During the first part of the reproductive period male specimens possess a large number of large spermatic cysts. Some of the larger cysts measure about 100 × 50 μ. Then both the number and size of the cysts decline. Beginning about the fourth week of June, the larger cysts are only about half the size of those found earlier in the season. These smaller cysts generally are composed of a single cluster of spermatogenic cells. By the second week of June, some of the cysts containing mature sperm appear largely empty, suggesting that most of the sperm have been released.

Although actual reproduction (as judged by the presence of embryos and larvae in the specimens) ends by late July or early August, some specimens with spermatic cysts or small oocytes may be found until at least the end of September.

During the early part of the reproductive season there is a considerable variation in the size of the specimens of *Haliclona loosanoffi,* depending in part upon the number of gemmules which contributed to their development. Twenty four specimens collected in late May and June had size indices (length × width × height) ranging from 0.024 to 16.7 cm^3 (mean *ca.* 2.0 cm^3). The density of reproductive elements was high in all of these sponges, indicating that within broad limits size is not an important factor affecting reproductive activity.

Flagellated parenchymula larvae are found in histological sections of specimens collected from the second week of June through the first week in August (Fig. 2C). The release of larvae by freshly collected specimens in the laboratory (Fig. 2B) and larval settlement on experimental surfaces (Fig. 2A) both indicate that larvae are released throughout this period. The greatest larval settlement seems to occur in mid-July.

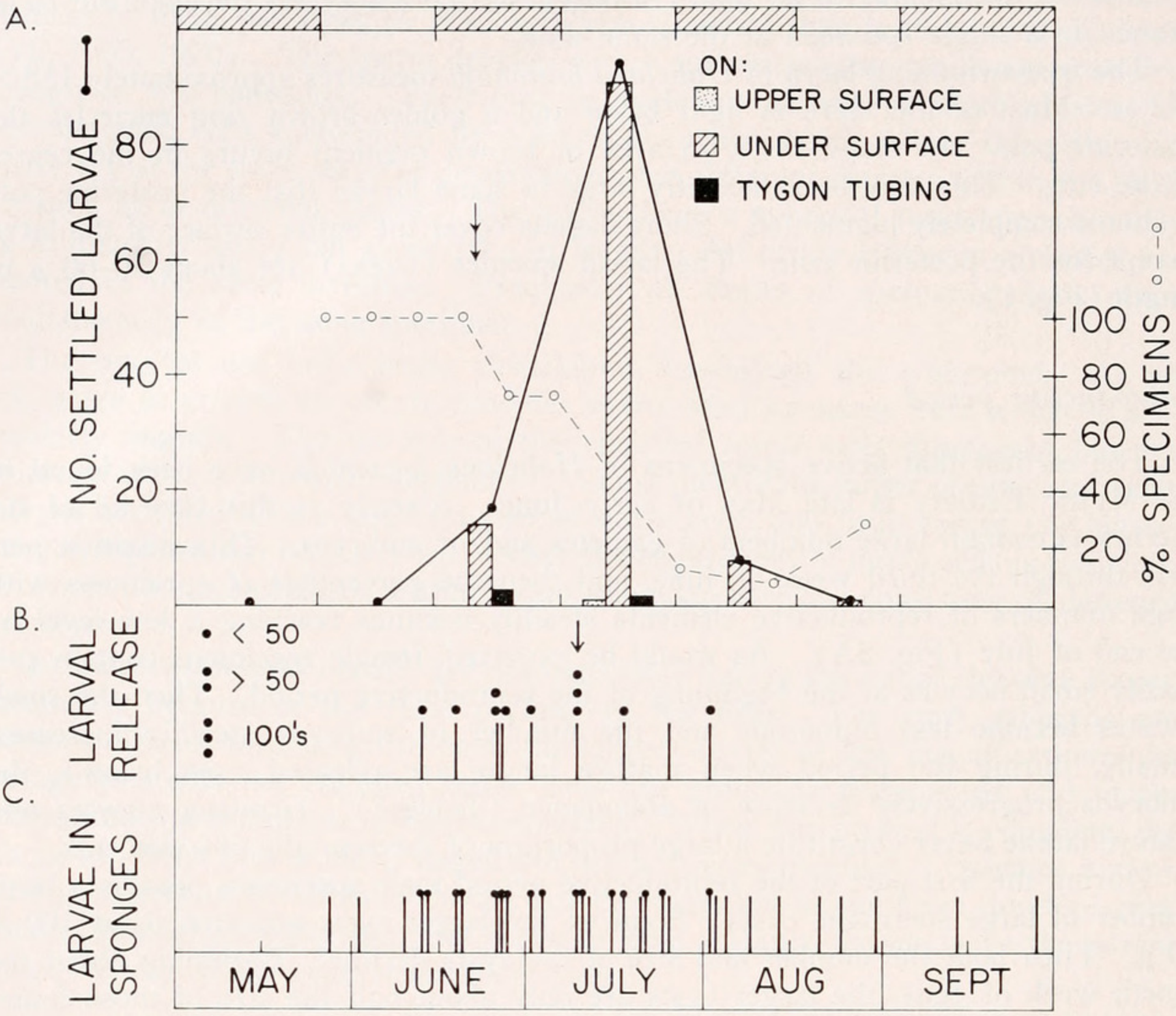

FIGURE 2. Reproduction of *Haliclona loosanoffi* in the Mystic Estuary, Connecticut. *A.* Reproductive activity and larval settlement: Right—percentages of specimens (both male and female) with large numbers of reproductive elements calculated on a weekly basis (11 to 16 specimens were examined for each point, excepting the first and last for which 5 and 7 specimens were examined, respectively). Left—the number of larvae settled on 10 *Mercenaria* shells per 3-week interval. The shells were strung with the concave surface down and tygon tubing spacers between them. The pattern of settlement on available surfaces is indicated. The double arrow marks the earliest date that large numbers of small postlarvae were observed on eel grass in the same area. *B.* The release of larvae by freshly collected specimens of *Haliclona loosanoffi* in the laboratory. Vertical lines show when observations were made, and dots indicate the greatest number of larvae released per specimen. The arrow marks the only date that a larva of this sponge was found in plankton samples. *C.* The occurrence of flagellated larvae in specimens of *Haliclona loosanoffi* collected at different times during the late spring and summer. Vertical lines represent collections of specimens made over a 5-year period; when dotted, flagellated larvae were found.

Eel grass (*Zostera marina*) in the Mystic Estuary provides a very large surface area for larval settlement, and the greatest number of larvae apparently settle on this substrate. Heavy settlement of *Haliclona* larvae on the eel grass was first observed near the end of June. The postlarvae grow rapidly and by late summer may achieve a size index of about 15 to 45 cm³.

TABLE I

Numbers of reproductive elements per cm² histological section (10 μ thick) and the relative abundance of different kinds of reproductive elements in female specimens of Haliclona loosanoffi *during different periods within the reproductive season. Means (in italics) and ranges (in parentheses) are given. (Large oocytes are defined as those containing engulfed nurse cells; embryos are defined as cleavage stages and developing larvae.)*

Period		Number of specimens examined	Number of reproductive elements/cm²	Percent total number of reproductive elements			
Month	Week			Small oocytes	Large oocytes	Embryos	Flagellated larvae
May	4	3	*432* (265–540)	*97* (94–100)	*2* (0–3)	*1* (0–3)	*0*
June	1	5	*570* (392–758)	*74* (41–100)	*3* (0–5)	*23* (0–54)	*0*
June	2	5	*572* (327–1064)	*48* (20–83)	*3* (1–7)	*48* (10–75)	*1* (0–3)
June	3	8	*388* (175–771)	*40* (20–79)	*2* (0–3)	*57* (21–75)	*1* (0–4)
June	4	7	*272* (82–934)	*44* (9–98)	*1* (0–3)	*52* (2–85)	*2* (0–4)
July	1	6	*141* (59–302)	*62* (29–90)	*1* (0–2)	*35* (10–65)	*2* (0–5)
July	4	8	*59* (4–327)	*90* (20–100)	*0.1* (0–1)	*9.8* (0–78)	*0.1* (0–1)

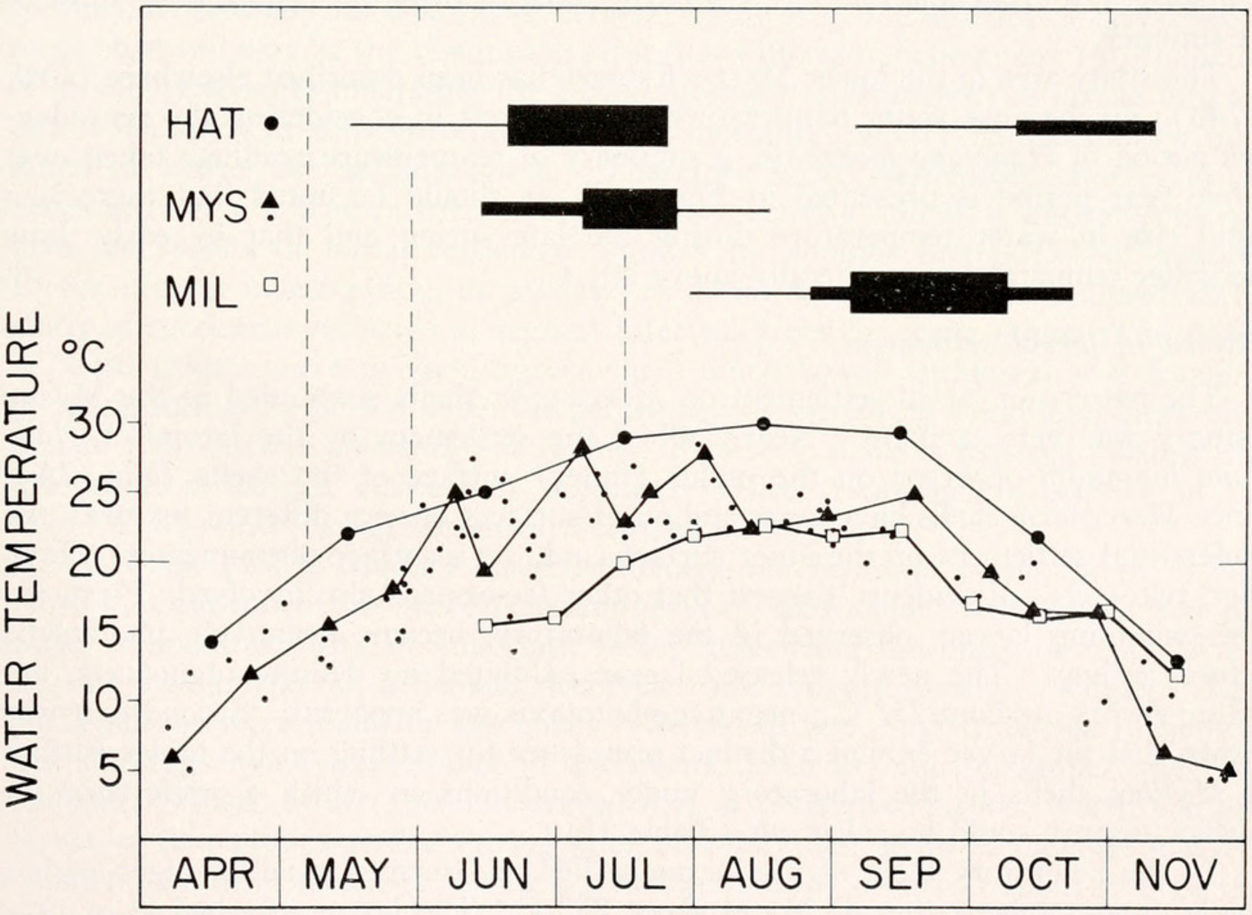

FIGURE 3. Water temperature and periods of larval settlement of *Haliclona loosanoffi* at Hatteras Harbor, North Carolina, the Mystic Estuary, Connecticut and Milford Harbor, Connecticut. The large symbols connected by lines show the surface temperatures during the years that larval settlement was studied; the dots indicate the water temperatures in the Mystic Estuary during other years. The relative numbers of larvae settling on experimental surfaces during each interval of observation are indicated by the thickness of the bars. Information from Hatteras Harbor is taken from Wells, *et al* (1964); and that from Milford Harbor is taken from Hartman (1958). Larval settlement at Milford during 1947 is shown. Vertical broken lines show the approximate time that water temperature reaches 20°C at each location.

Table II

Settlement of the larvae of Haliclona loosanoffi *on the shells of* Mytilus edulis *in the laboratory with natural photoperiod. (The development of settled larvae on the outer shell surface appeared to be somewhat superior to that of larvae on the inner shell surface. Larvae also settled in large numbers on the sides of the glass containers.)*

Shell surface	Number of settled larvae		
	When facing down	When facing up	Ratio
Outer surface	249	32	7.8
Inner surface	175	38	4.6
Both surfaces	424	70	6.0

Although free-swimming larvae of *Haliclona loosanoffi* apparently are present in the Mystic Estuary for about one and a half months, such a larva was found in plankton tows on only one occasion (in early July). On the other hand, the larvae of a species of *Halichondria* were regularly taken in plankton tows during most of the summer.

The study area in the lower Mystic Estuary has been described elsewhere (Fell, 1974b); but because water temperature is of interest in considering the reproductive period of *Haliclona loosanoffi,* a summary of temperature readings taken over a five year period is presented in Figure 3. It should be noted that there is a rapid rise in water temperature during the late spring and that by early June the water temperature is generally above 20° C.

Pattern of larval settlement

The pattern of larval settlement on *Mercenaria* shells suspended in the Mystic Estuary was very striking. Nearly all of the settlement by the larvae of *Haliclona loosanoffi* occurred on the under (inner) surface of the shells (Fig. 2A). Since *Mercenaria* shells have inner and outer surfaces of very different textures, the preferential settlement on the inner surface could be a surface phenomenon. However, two pieces of evidence suggest that other factors are also involved. First, the free-swimming larvae, observed in the laboratory, became negatively phototactic before settling. The newly released larvae exhibited no definite phototaxis, but within 24 hrs at about 25° C a negative phototaxis was apparent. Secondly, it was shown that the larvae exhibit a distinct preference for settling on the under surface of *Mytilus* shells in the laboratory under conditions in which a preference for surface texture could be ruled out (Table II).

In the laboratory most of the larvae settled and formed small sponges with a single oscular tube within 48 hrs at about 25° C. The larvae attached at or near the anterior pole with the brown pigment ring pointing out. Under conditions in which there were large numbers of larvae settling on a limited surface area, many examples of postlarval fusion (usually in twos and threes) were observed.

Discussion

Gametogenesis and embryonic development in *Haliclona loosanoffi* are essentially identical to these processes in *Haliclona ecbasis* and *Haliclona permollis* (Fell, 1969 and 1974a). Hartman (1958) described the eggs of *Haliclona loosanoffi* as ranging in size from 25 × 25 μ to 43 × 35 μ and the embryos as ranging in size from 150 × 130 μ to 215 × 185 μ. Evidently what he designated as eggs were small oocytes which had not yet begun the process of nurse cell engulfment and no distinction was made between the larger oocytes and the early-stage embryos. Growing oocytes are never very abundant and they may have been missed by him. However, the larger oocytes and early embryos contain large numbers of engulfed nurse cells and appear very similar at low magnification. This similarity is enhanced by the fact that distinct blastomeres usually can not be detected during the early cleavage stages.

In *Haliclona loosanoffi* the sexes are separate, a condition found in many other sponges (Fell, 1974a; Sarà, 1974); and the sex ratio is roughly 1 : 1. However, abortive small oocytes are seen in many male specimens. A similar situation has been described for *Hymeniacidon sanguinea* by Sarà (1961); but in the population of this species, males were much more abundant than females.

Previous studies on the reproductive period of *Haliclona loosanoffi* and a number of studies on the reproduction of other sponges have been largely or completely confined to an analysis of larval settlement. However, the amount of larval settlement observed may be the result of at least four different phenomena: 1) production of larvae by sponges inhabiting the region of study, 2) transport of larvae into and out of the study area by currents, 3) larval mortality, and 4) competition by other kinds of larvae for settling space. Therefore larval settlement curves do not necessarily give a true picture of reproductive activity and it is desirable to confirm the results of larval settlement studies by another method(s) of analysis. In the present investigation, an analysis of larval settlement was combined with a study of specimens collected at regular intervals from the same region. The results of these studies were in good agreement. Since larval settlement was found to increase during the period when the percentage of specimens containing large numbers of reproductive elements was falling (Fig. 2A), there is reason to believe that the larval settlement curve may accurately reflect reproductive activity in this case. The greatest larval settlement occurred in July.

Hartman (1958) studied larval settlement of *Haliclona loosanoffi* in Milford Harbor, Connecticut for three consecutive years and came to the conclusion that water temperature was an important factor influencing the onset of reproduction. At this location larval settlement occurred from August through October. Eggs and embryos were found in specimens collected in late August and early September and eggs alone were found in specimens taken in late September and early October. Wells, *et al* (1964) observed that at Hatteras Harbor, North Carolina larvae of this species settled during June and July and again in October and November. At both localities the first larval settlement occurred about two to four weeks after the water temperature rose above 20° C.

Although the reproductive period of *Haliclona loosanoffi* at Mystic, Connecticut may slightly overlap the reproductive period at Milford, it corresponds more closely to the major reproductive period at Hatteras Harbor (Fig. 3). The water temperature in the Mystic Estuary and Hatteras Harbor are very similar during the late spring, and this fact may account to a large extent for the similarities in the reproductive periods of *Haliclona loosanoffi* at these locations. The shallowness of the Mystic Estuary is probably the leading factor responsible for the early rise in water temperature compared to that in Milford Harbor (Pearcy, 1962). Since Mystic is only about 70 miles east of Milford on Long Island Sound, it seems highly probable that the differences in the reproductive period at these places is due to the observed differences in water temperature.

In the Mystic Estuary specimens of *Haliclona loosanoffi* with oocytes and embryos may be found in late May or early June, about two weeks earlier than those containing flagellated larvae. In addition, experiments, involving the placement of refrigerator-stored gemmules of *Haliclona loosanoffi* back into the Mystic Estuary, have shown that within about three weeks after the development of small sponges (at *ca.* 25° C) some of them may contain flagellated larvae (Fell, unpublished observations). These facts suggest that reproduction is probably initiated sometime between two and four weeks before the appearance of the first larvae and support the suggestion of Hartman (1958) that 20° C is close to the critical temperature at which the reproductive process begins (Fig. 3).

The factors responsible for the termination of reproductive activity in populations of *Haliclona loosanoffi* are probably several and depend upon local conditions. At Hatteras Harbor the first period of reproduction ends in July when the water temperature approaches 30° C (Wells, *et al,* 1964), and at Milford reproductive activity ceases in the fall when the water temperature falls below about 16° C (Hartman, 1958). In both cases the sponges are dying. The second reproductive period at Hatteras Harbor ends when the water temperature declines to below 16° C, but at this location many of the specimens of *Haliclona loosanoffi* survive the winter (Wells *et al,* 1964). In the Mystic Estuary actual reproduction ceases in late July or early August when the water temperature is about 22° C. At this time the sponges are actively engaged in gemmule production which normally continues for two more months (Fell, 1974b and 1976). There may be an antagonism between sexual reproduction and gemmule formation, perhaps in the form of competition for nutritive reserves.

A relationship between water temperature and the timing of sexual reproduction has also been demonstrated in *Hippiospongia lachne* (Storr, 1964). Reproduction of this species was studied at Cedar Keys, Florida, the Bahamas and British Honduras; and it was found that the peak of reproductive activity was reached earlier in the year in progressively more southern regions. Reproduction did not occur when the water temperature was below 23° C, and reproductive activity declined when it rose above 29° C.

It was shown here that the larvae of *Haliclona loosanoffi* tend to settle on under surfaces. This is consistent with the observed distribution of adults in the field. Hartman (1958) found that this sponge frequently encrusts the under surface of intertidal rocks, and Wells, *et al* (1964) observed that it typically occurs in obscure places where it is protected from the sun. *Haliclona loosanoffi* is sometimes found in large numbers encrusting the shells of mussels (*Mytilus edulis*) attached to the bottom of floating docks. Although the larvae become negatively phototactic before they settle, other aspects of their behavior may also be important to achieving the observed pattern of settlement. For example, in the strings of mollusc shells used in this study as spat collectors, the upper surfaces of most of the shells were shaded by the shells higher on the strings. The larvae may become negatively geotactic upon swimming into a shaded region and therefore settle almost exclusively on the under surfaces (see Crisp, 1974). There are also other possible explanations, and more extensive studies on the behavior of the larvae of *Haliclona loosanoffi* under a variety of conditions are needed.

In places where eel grass is present, *Haliclona loosanoffi* may be among those forms which settle on it in large numbers (also see Marsh, 1970). Even though the larvae may settle primarily on what are the under surfaces of the blades at the time of settlement, this orientation is obviously transient; and the postlarvae are found on upper, lower and vertical surfaces.

Appreciation is expressed to Sibyl Hausman and Jeanie Kitchen for expert assistance with the histological work and to Dr. Frances Roach and Ruth Fell for invaluable help with the field studies.

Summary

1. A general description of the reproductive elements of *Haliclona loosanoffi* is given. They were found to be very similar to the corresponding elements of other North American haliclonids.

2. *Haliclona loosanoffi* apparently is gonochoric, and the sex ratio is about 1 : 1. Abortive small oocytes are found in many male specimens during the latter part of the reproductive period.

3. The reproductive period of *Haliclona loosanoffi* in the Mystic Estuary, Connecticut is from late May or early June to late July or early August; but specimens with only small oocytes or spermatic cysts may be found until late September.

4. The reproductive period at Mystic, Connecticut is about two months earlier than at Milford, Connecticut and occurs at about the same time of year as the major reproductive period at Hatteras Harbor, North Carolina. Water temperature appears to be a major factor determining the reproductive period.

5. Very small specimens of *Haliclona loosanoffi*, as well as large ones, have high densities of reproductive elements during the early part of the reproductive period. Therefore it appears that size is not a major factor affecting reproductive activity in this species.

6. The larvae of *Haliclona loosanoffi* become negatively phototactic before they settle and exhibit a definite perference for settling on the under surface of submerged substrates. This pattern of settlement conforms to the distribution of adult specimens.

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