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ON THE HABITS AND ECOLOGY OF THE TUBE-BUILDING AMPHIPOD *AMPHITHOË RUBRICATA* MONTAGU.

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Introduction

During the summers of 1923 and 1924, while studying the ecology of the littoral vegetation at Otter Cliffs, on the rocky southern shore of Mt. Desert Island, Maine, the writer became interested in the tubes found penetrating the tufts of the green alga *Spongomorpha hystrix*. These tubes proved to be the nests of the amphipod *Amphithoë rubricata;* and some preliminary observations on the nesting habits of this species were made in 1924. During the following summer, he determined to investigate more thoroughly the mode of origin of these nests, and the use made of them by their builders. The nests in the *Spongomorpha* had such a definite structure that they suggested a symbiosis between the animal and plant with possible reciprocal modifications, and these researches were undertaken in anticipation of discovering such relations. It soon became evident that the problem was almost entirely of a zoological nature, but nevertheless it was deemed advisable to continue the study already begun. During the course of the investigation it became necessary to devote considerable attention to the animal's methods of food taking, and data relating to its ecology were incidentally accumulated. All of the experimental work described in this paper was done at the Mt. Desert Island Biological Laboratory at Salisbury Cove, Maine.

Distribution and Habitat

Amphithoë rubricata is a rather large amphipod, attaining 2.3 cm. in length, belonging to the tube-building family Podoceridae (Fig. 1). It is ruddy brown to olive green in color and the carapace and appendages are impressed with numerous, small, punctate dots. Each abdominal segment bears dorsally a white spot, situated posteriorally, which tends to disappear in preserved specimens. In some individuals this spot is lengthened into a white streak along the posterior margin of the somite.

The species is very widely distributed. It is common along the North American coast of the Atlantic Ocean from the Bay of Fundy to Long Island Sound. On the European side it occurs along the entire coast of Norway, and the coasts of England, France and the Azores (Holmes, '04). On Mt. Desert Island it was found in more or less abundance at every point along the exposed southern shore at which a search was made for it, from

Otter Cliffs on the east to Lopaus Point on the west; and specimens were also collected from the shore of the tidal basin near West Sullivan on the mainland (Hancock County). On the north shore of Mt. Desert Island, washed by the more quiet waters of Frenchman's Bay, it was not found. At the Mt. Desert stations it was collected only from tide pools. These ranged throughout the inter-tidal zone, but the animal is more readily found in the lower half of this zone. Along the shore of the tidal basin above Sullivan's Falls, specimens were found with homes in the *Chondrus* growing on the rocks several inches above the level of the water, when the tide was low. This would indicate that the animal does not require constant submergence, so long as the alga in which it is living remains moist. The low-water level of the basin is roughly three feet above the mean-low-water for the exposed coast. No dredgings were undertaken to search for the *Amphithoë* below the levels of the lowest tides, but Sumner, Osburn and Cole ('11) state that in the Woods Hole survey it was dredged in Buzzards Bay and Vineyard Sound "chiefly at depths of less than 6 fathoms (1 to 13) on bottoms of sand, gravel, and stones; almost exclusively confined to adlittoral stations," and according to Sars ('90–'95) it is "found in comparatively shallow water, among algae." It is therefore a species restricted to the upper edge of the littoral region, and adapted to existence in the zone of greatest wave action.

In the tide pools, the algae which serve as shelter vary according to the locality. At Otter Cliffs, during the earlier part of the summer, *Spongomorpha hystrix* was almost exclusively employed as host. Nowhere along the entire coast of Mt. Desert was our amphipod found so abundantly as in a large rock-pool, six feet above mean-low-water, the bottom of which was densely covered with this alga. It was from this pool that most of the animals used in the experiments were collected. At Seawall and along the western shore of Bass Harbor, where *Chondrus crispus* is the dominant alga in most of the tide pools, this plant was the favored habitat. In some of the lower pools on the wide, gently inclined foreshore at Seawall large numbers were found in *Corallina officinalis.* Nests were found in many other algae, *e.g.*, in *Ceramium rubrum* and *Ulva clathrata* at Otter Cliffs; in *Desmarestia viridis* at Seal and Bass Harbors; *Chordaria flagelliformis* at Seawall; *Polysiphonia urceolata* at Bass Harbor; while it is found in *Spongomorpha spinescens* at Otter Cliffs and Hunter's Head; but, with the exception of *Ulva,* it occurred only in small numbers in each of these algae. With two exceptions, all the specimens found were taken from among living, attached algae. These two, the nests of which could not be discovered, were collected from the mud around the rhizomes of the eel grass (*Zostera marina*) at Bass Harbor. Verrill and Smith ('72) also mention eel grass as a habitat for this species, and state that it may be found in tubes of gravel or seaweed attached to the under side of stones at low water. The species has also been taken from the surface of old piles. In practically all of these habitats, *Gammarus locusta* is associated with it.

The above mentioned pool at Otter Cliffs lies within the area of the survey conducted by Dr. Duncan S. Johnson and the writer, and on their charts is designated as "Pool N," which designation we shall, for brevity's sake, retain in the present article. The records of the temperature of this pool will serve to indicate the great daily fluctuations of this factor to which organisms inhabiting the tide pools are exposed. Pool N is among the largest tide pools found, it is 64 feet at greatest length, and 36 feet at greatest breadth. The elevation is 6.3 feet. The maximum depth is 3 feet, but large portions are less than a foot. It is exposed to the sun on the east and partly on the south. During July, 1923, the extreme range of temperature recorded was from 9° C., at 6:30 A.M. on July 27, to 19° C. at 1 P.M. on the 21st, a variation of 10° C. This is in marked contrast to the constancy of the ocean itself, for which the minimum temperature recorded was 9.5° C. and the maximum 12° C., or a fluctuation of only 2.5° C. The pool may cool slightly below the temperature of the ocean when exposed at low water on a cold night, and it rises considerably above the latter on every bright, summer day during which it is exposed. It is not only the great range of temperature, but also the extreme rapidity of change which distinguishes these rock pools from the ocean. As soon as the waves begin to wash into a heated pool, which was previously exposed above their reach, the water in the pool is very rapidly cooled, especially if the surf is at all violent, as it usually is along the southern coast of Mt. Desert. Thus Pool N, the temperature of which was 19° C. at 1 P.M. on July 21, began to receive the ocean wash a few minutes later. In less than half an hour the temperature fell to 13.5° C., and must soon have reached 9.5° C., the temperature of the ocean at this time. Such sudden changes cannot be without importance to the organisms subjected to them.

The mechanical action of the waves, the constant pounding of the surf into the tide pools, dominates the form of every plant and the habits of every animal. Only algae which are provided with an efficient means of attachment can maintain themselves in the majority of the tide pools, and the animals which do not directly attach themselves firmly to the rocks, as do the sea-anemone and the periwinkle, are dependent upon the plants for shelter. *Amphithoë rubricata* falls within the latter class, and its habits have been moulded by its complete dependence upon its host plant. The Otter Cliffs station, where it was most abundant, is exposed to the full force of the waves of the open Atlantic. The beach at Seawall, the next most favorable station for observing it, is somewhat protected by Great Cranberry Isle, while the enclosed waters of Bass Harbor are comparatively calm.

The breeding season of *Amphithoë* began, in the summer of 1925, early in July. On July 11 females bearing eggs were collected. A larger collection made on July 14 contained 52 females, of which all but three bore either eggs or young in the brood-sac, and over half were with young. On July 18, young, which had been released from the mother, were discovered for the first time in Pool N. Of 15 females collected on August 10 from Pool N, 6 bore eggs and 8 bore young in the brood-sac, leaving only a single individual with neither. The last collections were made on August 27, when females bearing eggs and young were present. As is the rule with amphipods, the eggs are laid in the brood-sac of the female, where they develop and the young are harbored during the earlier stages of growth. The brood-sac is made up of eight flat, elliptical, skin-like appendages, the oostegites, which are attached four on a side, at the insertions of the second pair of gnathopods and the first three pairs of periopods. Each oostegite has around the margin long hairs, by the entanglement of which with the marginal hairs of the neighboring appendages the eight segments are bound together, over the ventral surface of the thorax, into a single pouch. In the brood-sac of one large female 51 young amphipods were counted, while in a second 73 were present.

The average size of the males is slightly larger than that of the females. The average length, measured from the eye to the termination of the last uropod, of 15 males collected from Pool N was 18.1 mm.; that of 41 females was 16.2 mm., or 1.9 mm. less. The average of 10 males collected from among the *Corallina* at Seawall was 13.2 mm.; that of 18 females was 12.6 mm. or 0.6 mm. less. The largest male from Pool N measured 23 mm.; the largest female 20 mm. However, in the less numerous Seawall collection, the largest female, 16 mm., exceeded the largest male, 15 mm. The animals collected from among the *Corallina* seemed mature, and two of the females were with eggs in the brood-sac, although these were among the smallest found, and only 10 and 11 mm. long respectively. Their small size seems to be the result of the unfavorable habitat furnished by the *Corallina,* which will be discussed in a subsequent paragraph.

The females were much more numerous than the males. All collections were made without regard to sex, and the numbers determined later in the laboratory. The relative abundance of the sexes in these collections ought, therefore, to be representative of the proportions in which they occur in the general population. Out of a total of 139 animals, 100 were female and only 39 male. The females outnumber the males by almost three to one. The origin or significance of this numerical preponderance of the females cannot even be surmised, but may yield interesting results to some future investigator.

Nesting Habits

The earliest mention of the tubes inhabited by *Amphithoë rubricata* which the writer has been able to discover is that of Johnston in 1828. Here the animal is designated as *Gammarus punctatus,* but the observation of Johnston is included by Bate and Westwood under the heading of *Amphithoë littorina,* which is considered by Sars ('90–'95) as a synonym for *A. rubricata.*

The fact that *Amphithoë* inhabits nests, which it constructs by binding together sundry foreign material with threads secreted from glands situated in the periopods, is often mentioned in systematic works including this genus (Sumner, Osburn and Cole, '11, Holmes '04, etc.), and is briefly referred to in popular accounts of the crustacea (Calman, '11). The same is true of many other of the tube-building Podoceridae. Holmes ('01*b*) made a careful study of the habits of *A. longimana*, and his paper includes a more detailed description of the tube and the manner of its construction. He was interested in the animal more from the viewpoint of animal behavior than of ecology, and his observations were almost entirely confined to animals kept in the laboratory, and therefore necessarily under artificial conditions. There is much in common between the habits of *A. longimana* and *A. rubricata*, and in Holmes' article one might often be read for the other without doing violence to the truth.

We are fortunate in possessing a more complete account of the tubes built by *Cerapus tubularis*, Say. Say ('17) was the first to describe these structures, but denied that they could have been fabricated by the amphipod, because "there is no organ belonging to the Cerapus which could be adapted to a function so remarkable." He expressed the opinion that the tubes might have been constructed by an annelid, such as *Tubularia*. The amphipod, enveloped in the tube, ran rapidly about the branches of *Fucus* and *Sertularia*, using its four antennae as feet. Smith ('74) discovered the thread-secreting glands in several genera of tubicolous amphipods, and recognized ('82) the true nature of the tubes of *Cerapus*. These tubes are covered on the outside with minute, elongated particles, apparently the excrement of the animal; within they are lined with a smooth layer of cement which binds together the particles forming the exterior of the sheath. The tubes seem never to be attached, but the animal swims about within them, propelling itself and its home by the use of the antennae. It is very difficult to force the living animal from the tube, and Smith believed that it never leaves it voluntarily.

Stimpson ('53) has described the tubes of *Cerapus rubricornis*, Stimpson (= *Ericthonius rubricornis*, Holmes), which differ from those of *C. tubularis* in being closed at one end and attached for about half their length to submarine objects. The tubes occur in close colonies and are often adherent to each other.

Smith ('82) also wrote in some detail of the method of construction of the tubes of *Microdeutopus minax*, Smith. Having loosely bound together several filaments of an alga, the animal used this as a framework to support a transparent tube of its silk-like secretion, which it spun around itself. Then the pellets of excrement, as they accumulated, were broken into smaller fragments by the mouth parts and worked into the wall of the tube. Smith carefully described the method of spinning, and mentions that a species of *Amphithoë*, which he also observed, worked in a closely similar manner.

Amphipods of other families, although they do not construct their own homes, have the habit of securing for themselves various tube-like shelters. The female of *Phronima sedentaria* establishes herself in the transparent tube of a Siphonophore, having first eaten away its rightful occupant. The male, however, lives in deep water and is free swimming, without external protection. During the breeding season he mates with the female in the tube. The young, after leaving the brood-sac of the mother, remain a short while within the tube, but afterwards sink to the bottom, and do not occupy their own tubes until maturity (Brehm, '18).

Various Types of Nests

In their natural environment the most conspicuous, and structurally the most permanent of all the various types of *Amphithoë* nests found by the writer are those in *Spongomorpha hystrix*. Since the form and habit of this plant are so important in determining the character of the tube built in it, and so markedly affect the life of the amphipod inhabiting it, a brief description of its structure and mode of growth will be given. Taxonomically, *Spongomorpha* is closely related to the better known *Cladophora*, and has a similar branching habit. Each plant forms an erect cluster of coarse, stiff, dark green filaments, each 200–300 micra in diameter and bearing numerous upright, compound branches The cluster expands from base to apex and has roughly the shape of an inverted cone. In addition to these stiff, erect filaments, there are also found, especially near the base of the cluster, a second type, the comparatively slender, flexuous, rhizoidal filaments which bend downward and interweave as they descend among the coarse branches, finally reaching the substratum, to which they are anchored by their tips. The presence of these rhizoidal branches forms the essential difference between *Spongomorpha* and *Cladophora*. The rhizoids serve the double function of binding together the individual filaments into a more resistant whole, and of providing the attachment of the plant to the rocks. The plants often grow so close together that they are held erect by mutual pressure. The turf they form reaches 8 cm. in thickness. So effectively does this algal mat protect the surface of the rock on which it grows, in patches often covering many square feet, that a layer of fine sediment collects beneath it, notwithstanding the constant washing of a surf which is seldom mild. The plant reaches its best development in shallow, well-insolated tide pools in the upper littoral zone, where it is associated with *Ulva* and *Enteromorpha*. It thrives well in water so shallow that at low tide it is hardly submerged.

The nests of the amphipod are in the form of tubular canals, penetrating the cluster of filaments, usually in a longitudinal direction. The best developed nests are situated well in towards the center of the cluster. A single large cluster may contain as many as eight tubes. The walls of the tube are formed by the algal filaments, bound together and overlaid by the thread secreted by the amphipod. A cross section of the tube of a mature individual would show: (1) a circular opening 5 to 8 mm. in diameter; around this (2) a lining composed of hyaline, cobwebby strands about 1.5 micra in diameter, forming a false membrane and binding together (3) the filamentous branches of the alga, among which interweave the rhizoidal filaments. The tube is usually of approximately uniform diameter, is open at both ends, and may reach 5 cm. or more in length. Figure 2 shows the interior of a tube, though in a different alga. Occasionally a tube is found built in the form of a U, with both openings at the top of the cluster, and the bend several centimeters below this. The openings of the tube are often constricted, but are so conspicuous that they are readily recognizable by an observer standing beside the pool. Bound into the wall of the tube are often found bits of foreign algae, of species common in the vicinity of the nest. The nests present in such coarsely branching algae as *Desmarestia viridis* and *Chordaria flagelliformis* resemble in a general way those built in *Spongomorpha*, but differ in several important points. They are usually situated more or less basally, where the branching is more dense, and the upper aperture is well below the apices of the branches, whereas in *Spongomorpha* it is on a level with them. This is a result of the looser branching habit of these brown algae, which nearer the apex would not offer such effective support for the wall of the tube (see Fig. 2). The basal position of the tubes makes it more difficult for the amphipods to secure other algae as food, and accordingly these plants are not the most desirable habitats. The nests built in *Spongomorpha spinescens*, which, because of its hooked, spine-like branches in addition to the rhizoids, is an even more compact structure than *S. hystrix*, and those found in *Polysiphonia urceolata*, at Bass Harbor, very closely resemble the tubes in *S. hystrix*.

Quite different are the nests constructed among the much-divided lobes of the expanded, cartilaginous thallus of *Chondrus crispus*. These reached their best development in the tide pools at Seawall, but were also found on the more protected shore of Bass Harbor. Most of these tubes are built with one side against the flat surface of the *Chondrus*, the other made up of a heterogeneous collection of bits of various algae and fragments of the shells of molluscs and barnacles, bound together with a thick web of "amphipod silk." The use made of the *Chondrus* thallus itself is variable. At times a lobe is rolled inward, or advantage taken of its natural curvature, so that it forms one longitudinal half of the tube; again, it seems to be used merely as a support against which a tube, built of algal and shell fragments, is anchored. Often one finds a nest which is constructed between parallel surfaces of two lobes of the same thallus, bound together by transverse walls composed of silk and particles of shell. But regardless of the manner in which the *Chondrus* is employed, that portion of the plant immediately bounding the cavity of the tube is always overlaid with threads of silk applied so thickly as to form a glutinous membrane. This part of the wall is usually free of the foreign matter which enters so largely into the composition of the unsupported portion; it serves to bind the entire tube to the alga. These tubes are never so long as those built in *Spongomorpha*; they are usually between 1.5 and 2 cm. and occasionally reach 3 cm. in length.

The nests in *Chondrus*, in addition to being smaller than those in *Spongo-*

morpha, are neither so neat nor so substantial as the latter. They are more likely to collapse when the occupant is removed, and are not so regularly tubular as the *Spongomorpha* nests. On the other hand, they impress one as the work of a much more skillful artisan, more ingenious and more flexible in his methods. The amphipod in the *Spongomorpha* has only to burrow in among the filaments and to spin its thread around itself. The builder in the *Chondrus* must confront the problem of the anchorage of its tube, of arranging the pieces of foreign material it employs, and of holding them in place until they are firmly attached by the threads. At times, one finds the sinus between two lobes of a thallus neatly bridged up by a wall of silk and animal or plant fragments. The foreign algae and bits of shell are used so freely that a *Chondrus* plant with several nests bristles with such debris. Some of the pieces used are very large, for example, a branch of *Ptilota* 5 cm. long was attached to the wall of a tube of half the length. The smaller inclusions in the tube are held firmly in a matrix of silk, which is employed very liberally.

The bottoms of several of the shallow rock pools near the low water mark at Seawall bear a close dense growth of stunted coralline (*Corallina officinalis*). The stand is so compact that it forms a trap for particles of sand and fragments of shell, and the plants finally become buried almost to the top with the fine debris which has accumulated among them. In July, 1925, individuals of *Amphithoë rubricata* were numerous in this formation. Their tubes were ill-defined structures which collapsed totally when removed from the water, and most of them could be recognized only in virtue of the adhesion of the particles of debris held by the threads of silk. They impressed one as having been formed by the animal merely burrowing among the loose detritus, and spinning a web about itself, which bound together the surrounding fragments. No especial use seemed to be made of the *Corallina*, which merely served as the accumulator of the debris surrounding the canal. Bits of algae, *Delesseria* and *Ptilota*, were occasionally found within a tube. The aeration within these nests must have been very poor, and the difficulty of finding fresh, non-calcareous algae for food was great. The unfavorable environment was reflected in the small size of the amphipods, the lengths of which are given above.

Each tube is inhabited usually by a single amphipod, and never by more than two. When two animals are present in a single nest they are almost always male and female. Johnston ('28) mentions the fact that a male and female of his *Gammarus punctatus* (*A. rubricata*) dwell in each burrow. During the course of the summer, 16 pairs, each inhabiting the same nest, were collected; each pair consisted of a male and female, with the single exception of a tube inhabited by two females. The pairs were taken back to the laboratory, each in a separate vial, and the sexes determined by microscopical examination. That one of each sex dwells in the same tube was occasionally observed in the laboratory cultures. In every case examined the male was larger than his mate. Usually the female, at this season, bore eggs or young. Thus, of the eight pairs collected on July 19, seven of the females carried young in the brood-sac, while the pouch of the remaining one was empty. It is possible that the sexes were together for the purpose of fertilizing a second brood, but they were not clasping, and this point was not determined. Considering the predominance of the females, it would be interesting to know whether a male visits the nests of several females in succession. However, both males and females form tubes, and by no means every male is found within the tube of a female, and naturally most of the females are alone.

Method of Constructing the Tube

It was found possible to maintain the amphipods in the laboratory, in shallow vessels of fair capacity, for several weeks. The top or bottom of a large moist-chamber formed an aquarium very favorable for the observation of the animals' behavior—given a suitable alga, and the sea water renewed two or three times a week. The vessels were placed on a shelf out of direct sunlight. Under these conditions the animals built their nests, went through their moults, and raised their young.

When amphipods collected from the tide pools are placed in a vessel con-

Fig. 1. *Amphitoë rubricata*, showing posture when at rest within the tube (magnified 7 times).

taining no solid matter, they cling tightly together in a knot and settle down on the bottom. Each seems endeavoring to cover itself with the others, and it is difficult to free an individual from the group. If there are solid particles present, fragments of alga, or the like, the animals closely attach themselves to them. These actions are all expressions of the pronounced thigmotaxis

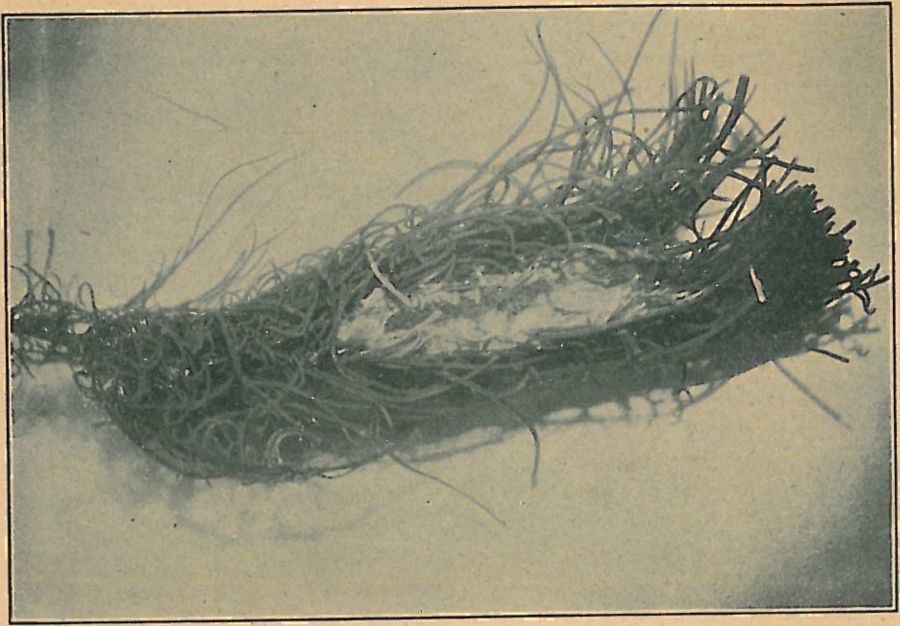

FIG. 2. A tube of *Amphitoë* built in *Chordaria flagelliformis*. The tube has been cut open, and the interior rubbed with chalk (magnified twice).

of the species. When, however, a tuft of *Spongomorpha* has been placed in the bowl, the amphipods soon take refuge among its branches, and almost immediately set to work to construct a tube. The silk glands are situated in the first two pairs of periopods. A duct leads from a sac-like dilation in the terminal segment to a pore at the very tip of the leg (Fig. 1), through which the silk-like secretion is discharged. The third to fifth pairs of periopods are not provided with glands, but terminate in sharp, outwardly directed hooks, which the animal employs to hold itself among the filaments or in the completed tube. These legs are bent backward and upward, so that they extend beyond the dorsal surface of the abdomen. The abdomen itself is bent forward beneath the body, and held in this position while the animal is spinning or resting in the tube. The animal usually spins with its body parallel to the filaments of the alga. The four gland-tipped legs move to and fro among the filaments, spinning the thread and attaching it to the alga at various points. They work with a steady, sustained motion which Smith has compared with the movement of the hands in playing a piano. Occasionally the gnathopods are brought into action, apparently to help arrange the filaments. Before long it is apparent that the branches of the *Spongomorpha* are bound together, and a web of hyaline fibers encircles the spinner. After working in one position for some time, the animal turns a somersault through 180°, and continues spinning at the other end of the incipient tube. This shift in position is accomplished rapidly and neatly by pulling the thorax backward over the incurved abdomen. At intervals it pivots sideways. This procedure has been followed for an hour at a time; after twelve hours the nest, although still rudimentary, is an easily recognizable structure. After the building of the tube has progressed to a certain stage, it becomes difficult to observe the actions of the animal within it, but a well developed tube represents certainly many days of spinning (see Fig. 2).

According to Smith's observation on *Microdeutopus minax,* and Holmes' on *Amphithoë longimana,* the nest may be completed in half an hour. It is true that after working for this period *A. rubricata* has a structure which offers some protection, but the layer of thread lining the tube by no means reaches its full thickness, nor the tube its full length. The writer has observed amphipods still spinning in tubes that were already several days old.

As an example of the results obtained in laboratory cultures, the following experiment (I) may be cited. On July 14, 33 amphipods (18 ♂, 15 ♀), collected from Pool N, were distributed among 6 moist chamber halves and given tufts of *Spongomorpha* collected from around the edges of the pool, where no tubes were present. By July 16, 30 of these had constructed nests, while the remaining three had died. On July 22, when the last census was made, 26 of the animals were still living. Meanwhile, some of the females had released their young, which had constructed tubes of their own. Some of the mature animals, instead of entering the *Spongomorpha* cluster, located their tubes between the alga and the bottom of the vessel, a variation in the usual manner of construction.

Specificity in Tube Building

The various types of nests differ so greatly from each other that it was not at all evident *a priori* whether an animal taken from one type could construct a tube for itself when supplied with the materials appropriate for the manufacture of a second type. Could an amphipod which was accustomed to working with a filamentous alga like *Spongomorpha,* and using no foreign material in the construction of its home, also employ an expanded thallus and loose particles of seaweed, and build a tube of the type found among the *Chondrus* at Seawall? Were there various hereditary strains within the species, each specialized to building in a kind of alga present in its particular habitat, but unable to make use of a strange form? The following experiments were undertaken with this question in mind.

Experiment II

On July 25, amphipods already used in experiment I were placed in moist chamber halves containing *Polysiphonia fastigiata*. The branches of this red alga are much stiffer, shorter and coarser than the filaments of *Spongomorpha*. By July 27 all of the animals had formed nests in the *Polysiphonia*. On July 31, eighteen of the animals continued to occupy these nests, the nineteenth having died. The experiment was terminated and the tubes examined; in all essentials they resembled those built by the same individuals in *Spongomorpha hystrix*.

Experiment III

On August 1, amphipods collected from tubes in *Chondrus* at Bass Harbor were placed in a vessel containing tufts of *Spongomorpha*. On the following day, animals collected from similar nests at Seawall were added, bringing the total number up to eight. The nests made by these animals were examined on August 5; they were well developed and were indistinguishable from the tubes made in the same seaweed by amphipods collected from among it. All of the eight had made nests.

Experiment IV

On August 10, 20 amphipods collected from the *Spongomorpha* in Pool N were placed in three moist chamber halves, in each of which were several large, clean plants of *Chondrus crispus*. Sea-lettuce was cut up into small pieces, and mixed among the *Chondrus*. At intervals the water was stirred around, so as to bring the sea-lettuce within reach of the amphipods working among the *Chondrus*. On the following day, 6 of the animals were dead. By August 13, 4 more had died, 9 were found within tubes built in the *Chondrus*, and 1 was unaccounted for. After this preliminary examination, various shells (clam, mussel, barnacle, etc.), which had been previously broken up into small fragments in a mortar, were sifted over the *Chondrus* plants containing nests, and the process repeated two days later. On August 16 the final examination of the nests was made. These structures, 10 in number, were as varied in detail as those found at Seawall, but resembled the latter in all essentials of structure. The walls were in part made up of the *Chondrus* thallus, and in part of *Ulva* and shell particles joined together by amphipod silk. Wherever the *Chondrus* formed part of the wall of the cavity, it was lined with a layer of silk, and free of foreign particles, as in the Seawall nests. As many as 16 shreds of *Ulva* were employed in the construction of a single tube, in addition to large shell fragments. The workmanship in these tubes was just as adept as that in the Seawall nests.

The writer also had the opportunity to observe an experiment of the same character carried out by nature on a much larger scale. Towards the end of August, *Spongomorpha hystrix* began to disappear from all of the tide pools along the base of Otter Cliffs. The loss of these plants left bare large spaces in the bottoms of the pools, overlaid by the sediment which had collected beneath them. The basal holdfasts remained attached to the rocks, a method of perannating common to many algae. A microscopical examination of the filaments of the remaining plants, and of the persistent bases, showed many of the cells shrunken and dead. These plants could in many places be floated free of their attachments merely by touching them with the finger, and at the next high water would be washed out of the tide pools. The cause of the dying off of the *Spongomorpha* has not been determined, but is possibly the expression of an autonomic periodicity of the plant; or the very warm days of the latter part of August may have been responsible. It may be mentioned that *S. spinescens*, which grows on exposed rocks in preference to the tide pools, was dying in a similar manner.

By August 25 the disappearance of the *Spongomorpha* from the western half of Pool N, where the amphipods had been most abundant, was almost complete. A search was made to determine the fate of these amphipods. Many of them, especially the younger ones, which could better find room there, had taken refuge in the persistent bases of the *Spongomorpha*, which they had honeycombed with their tubes. Large numbers of the remaining adults, however, had taken up their abode in *Ulva*, and a few built tubes in the *Ceramium rubrum* growing among the vanished *Spongomorpha*. The nest in the *Ulva* was formed in a fold of the flat thallus, and bound on the interior with silk. The remarkable point is that previous to the death of the *Spongomorpha* not a single amphipod was found in Pool N in any plant except this, although *Ulva* had been present just as abundantly throughout the summer. Tubes might have been located in other algae, but at most their number was so small that they eluded search. This indicates a marked degree of selection on the part of the animal. It cannot be stated whether many amphipods perished in the disaster, but undobtedly the versatility in nest building which they display served them in good stead in this instance.

The experiments undertaken on this point furnish good evidence of the ability of the amphipods to fabricate nests out of materials with which they have had no previous experience. What elements are involved in this capacity to work successfully with unfamiliar materials, the writer does not pretend to know, although the correct interpretation of the results might be of value from the viewpoint of animal behavior. It is possible that the form of the nest is the purely mechanical outcome of the reaction between the animal's endeavor to surround itself with a tube and the physical properties of the building material at hand to it. Another alternative is that it learns by a process of trial and error the proper way in which to work; but if this is true, it learns very rapidly, because the first nests completed in *Chondrus* by the animals taken from *Spongomorpha* apparently reached the highest degree of perfection of this difficult type. Only a detailed analysis of the behavior of the amphipods throughout the above experiments would be likely to throw light on the question of how much intelligence the animals employ in their tube-building.

Building of Tubes by Young Amphipods

The young amphipods, at the time they emerge from the brood-sac, are about 3 mm. long, still transparent and unpigmented, and already with the same conformation as the adult, since these animals go through no elaborate series of larval transformations. The first of the new brood were found leading an independent existence in the tide pools on July 18. As to the impulses associated with the departure of the young from the maternal protection there is little evidence. The writer on two occasions witnessed the forcible expulsion of young amphipods from the nest of a female. Whether this method of freeing themselves of their offspring is general to the females, he cannot state, never having been so fortunate as to witness it in the field. It is possible that the young crawled out of the brood-sac in response to some spontaneous impulse, and the female " showed them the door " only after she felt them wandering about in the nest. The notes made at the time may be of interest:

" On July 18, while examining the experimental material in the laboratory, I noticed one female come to the mouth of her nest and seem to struggle very violently, kicking about her antennae and gnathopods actively. Upon closer examination, it appeared that her young were attached to her appendages, and that her blows were directed towards them. She seemed to be trying to expel them from the nest, she shoving violently, they holding on tenaciously. She used her gnathopods merely to push, and I do not believe that she employed her chelae. The antennae were bent forward and also used in pushing. The young seemed to resist the expulsion to the best of their ability. Finally she succeeded in shoving a bunch of them over the rim, bent forward her long antennae and gave them an additional push with it, and then retreated within. The young crawled around in the branches at the mouth of the nest.

" On July 19 I saw the same performance repeated by another amphipod. She pushed about five young beyond the confines of her nest, and then retreated to the other end. A few minutes later, she inadvisedly stuck her head out of the door by which she had dismissed her offspring, and immediately they attached themselves to her in a lively fashion. She retreated with them and after a short interval reappeared at the mouth and went through the process of expulsion once more."

Three days from the time they leave the brood-sac, to set an upper limit for the time required, the young amphipods are in tubes of their own construction. They probably begin the structure almost immediately upon starting off on an independent existence, but it requires this interval before the nests can be recognized with certainty. The number of days here given is based upon evidence derived from cultures begun on August 10, and the algae used make it certain that the only young amphipods present were those placed in the aquaria. On August 13, the tubes of the young amphipods, which had left their parents in the interval, were distinctly recognized. Evidence derived from an entirely different culture (July 14–18) would set the upper limit at four days. Holmes mentions that the young of *Amphithoë longimana* begin to construct nests immediately upon leaving the brood-sac of the mother.

The tubes of the immature amphipods resemble those of the adults in everything but size. The smallest found in *Spongomorpha* were 3–4 mm. long and about 1 mm. in diameter. Some of them were clustered right on the lip of a large tube, like little fumaroles about the crater of a large volcano; others were removed from the immediate vicinity of a large tube. In the former case, the young animals probably settled down to build their own homes on the spot where their mother parted with them. Other nests found in the laboratory material were constructed of small pieces of *Ulva* rolled together or bound against the bottom of the vessel, and very small animals sometimes formed a tube by binding the excreta of larger animals against the glass.

Considering how loath to leave their nests the amphipods are, it became of interest to know whether the young animal, as it grew, enlarged its tube to keep pace with itself, or whether it periodically deserted its old nest to build larger quarters elsewhere, as a hermit crab seeks a new shell. Smith was faced by the same problem in the case of his *Cerapus*. He noticed that one end of the cylindrical tube was somewhat larger than the other, and suggested that the amphipod widened the structure at need by removing the pellets of dung from one end and building them into a larger circumference at the other; but on this point he gives no evidence. We have noticed in the case of *Phronima* that during the growing period the young animal is without external protection. Theoretically there is no reason to suppose that the young *Amphithoë* could not readily push apart the walls of its tube, the fibers readily yielding to the pressure exerted by the growing body, but experimental evidence seemed desirable on this point.

The ideal method of attacking this problem would be to observe marked amphipods in marked tubes in their natural habitat, but this presented so many difficulties that it was not attempted. The alternative method was to isolate young amphipods in the laboratory, and measure their tubes at the beginning and end of a sufficient interval of time. This was the plan adopted. In a preliminary experiment, four out of six amphipods remained in the same nest for eighteen days. During this period two of the animals moulted twice, the others once each. Encouraged by these results, a more elaborate experiment was undertaken. A number of young amphipods and their tubes were collected from Pool N. Each animal was brought back to the laboratory in a separate vial, in which was also placed the tube. The amphipod was given a large vessel containing a clean tuft of *Spongomorpha*. The tuft was chosen so that it would be small enough to examine thoroughly for any tubes it might contain, and large enough to supply the animal with food for the duration of the experiment, as well as give ample room for the nests. The water was changed frequently, and observations made several times a week. At the end of the experiment, the new nests were measured and these dimensions compared with those of the original tubes. A simple and accurate method of measuring the diameter of the tubes was devised. The ends of the tubes were trimmed off evenly with scissors, and a slender triangle cut from stiff paper inserted in one end as far as it would go under very gentle pressure, or until the sides of the tube were flattened out against it. The apex of the triangle was snipped off at the point where it entered the tube, then pulled out and the line of separation measured with a millimeter scale. The dimension multiplied by $(2 \div \pi)$ gives the diameter of the tube. Both ends of each tube were measured in this manner.

On August 14, nine young amphipods were isolated in the laboratory. Five of these deserted their old nests for new ones during the course of the observations. Desertions most often occurred at the time of changing the water. On August 30, the cultures were terminated, and the diameters of the four original tubes which were still occupied were determined. Amphipod A, which was taken from a tube of 2.5 mm. in diameter, now occupied one of 3.0 mm. diameter; B had enlarged its tube from 2.7 to 3.3 mm., C from 1.5 to 2.4 mm., D from 1.7 to 2.5 mm. During this period A, B, and C moulted once each, D three times. Assuming the new tube when first built in the laboratory to be in each case of substantially the same diameter as that from which the animal was just removed in the field, the average growth of the four tubes in 16 days was 0.7 mm. It is to be regretted that the experiment could not have given longer duration, but the *Spongomorpha* had already begun to die before the cultures were terminated.

Return to the Nest

In the tide pools, an *Amphithoë* is rarely seen outside of its tube, and in such cases it is probable that it was forcibly evicted. In the laboratory, they are more often seen uncovered, but usually these animals are moribund and will be found dead later; active animals are even here rarely found outside of their nests, except when they have been disturbed. Johnston ('28) observed that if he expelled a pair of his *Gammarus punctatus* (= *Amphithoë rubricata*) from their burrow, they repeatedly returned to it. The writer tried to repeat this observation, but without success. Violent means were necessary to eject an amphipod from its tube, but once detached from the entrance, it swam away in the tide pool and settled down in another plant, making no effort to return. When an animal is released in the pool, it usually swims more or less directly to the bottom, probably an expression of the negative phototaxis discovered in this species by Holmes ('01*a*). In the mass cultures in the laboratory an animal which has left its nest is as likely to return to another deserted tube as to its own, and as often settles down to build a new one. In the isolation cultures, the return to the nest was frequently observed, but here the possibility of finding shelter elsewhere was limited by the small amount of seaweed present. The animal designated as A left and returned to its tube at least three times during the course of the experiment. The desertions were made while the water was being changed. After the experiment was terminated, the tube, cut out from the *Spongomorpha* cluster, trimmed at the ends, and flattened on a triangle of stiff paper which penetrated it, was thrown back into the vessel with the amphipod. The animal almost immediately swam up to the tube and inserted itself beside the paper, where it remained.

Stimpson, in his description of the habits of *Cerapus rubricornis*, mentions " their awkward celerity in regaining their respective tubes after having left on short excursions." Holmes found that in *Amphithoë grandimana*, when the amphipods left their nests to wander about in the dish in which he kept them, they did not necessarily return to their own tubes, but to the first deserted one which they happened to encounter. Verrill and Smith ('72) state that *Amphithoë rubricata* may often be found free among seaweed or under stones, having deserted the tube. If this be true, the behavior of the animals they observed differs essentially from that of the strain present in the tide pools of Mt. Desert Island.

Symbionts

The living amphipod often bears numerous colonies of *Vorticella* sp. attached to various parts of the exoskeleton. Even young animals, just out of the brood-sac of the mother, are hosts to these protozoa. No other macroscopic animals were found in tubes still inhabited by *Amphithoë*, which is intolerant of any intruder in its nest. Deserted tubes are often the haunt of *Gammarus locusta*, or the annelids *Homothoë imbricata* and *Lepidonotus squamatus*, and small mussels are not infrequently found attached within them.

Food

Under favorable conditions, the food of *Amphithoë* consists entirely of vegetable matter (algae). The stomachs of 22 amphipods, collected from Pool N, were dissected out and examined, and contained only algae, *Spongomorpha, Ulva, Enteromorpha, Polysiphonia*, diatoms, etc. Under other circumstances, the animal may become in part carnivorous. Of 8 specimens collected among the *Corallina* at Seawall, 3 had eaten animal food. In the stomachs of 2 were found fragments of the exoskeletons of small crustacea, one probably an amphipod, and in the third was a ball of sponge spicules. Of the two specimens collected from the mud around the eel grass rhizomes, one had eaten a young amphipod, and the other decaying vegetable matter. The feeding habits of this species are very similar to those of other amphipods, such as the freshwater *Gammarus pulex*, which eats leaves and other vegetable matter when this is available, but in the absence of vegetable food turns carnivorous. Holmes found that *Amphithoë longimana* readily ate flesh when this was offered to it, and did not hesitate to devour another of the same species.

The stomachs of the amphipods usually contain more or less of the silk with which they line their tubes, entangled among the triturated ingesta. Seventeen out of the 22 animals taken from *Spongomorpha* had eaten more or less of this alga, and one taken from *Desmarestia* had only *Desmarestia* in its alimentary canal. It has been previously mentioned that bits of foreign algae are bound into the walls of the tube, and the same species are often found in the stomach of the amphipod inhabiting the tube. The amphipods eat material present inside of the nest or within reach of it, often the nest itself, and incidentally incorporate some of the thread with their food. When isolated with *Spongomorpha* as the sole source of diet, the animals can live certainly a month and perhaps indefinitely upon this food. If an amphipod is isolated with a nest in a small tuft of *Spongomorpha,* it will eventually consume every particle surrounding its tube, covering the latter with excrement instead.

Since *Amphithoë* is hardly ever seen swimming about in the tide pools, and probably never voluntarily leaves its nest, it seemed likely that it depends entirely upon fragments of algae which the waves waft within reach of the tube to furnish variety in its diet, and lacking this, feeds upon the plant in which the tube is situated. As a check to this conclusion, some observations were undertaken in the laboratory. On July 18 there were present in the cultures 18 amphipods with well developed nests in *Polysiphonia fastigiata.* These had been given no other food. Pieces of *Ulva* of various sizes were placed in each of the three vessels in which the nests were located. The *Polysiphonia* tufts were moved to one side of the dish, and the sea lettuce placed on the other, about 3 inches from the mouths of the tubes. Care was taken not to stir around the water. Two days later bits of *Ulva* were offered to the five amphipods in one of the vessels, and all grasped it. The thirteen other animals were killed in formalin, and their nests examined for the possible presence of pieces of *Ulva.* The color contrast between the green *Ulva* and the red *Polysiphonia* would have made the former easy to recognize, but none was found in any of the thirteen tubes. The alimentary canals were next examined. Nine contained *Polysiphonia* and four were empty, but none contained a trace of *Ulva.* Here was an acceptable article of diet, for which the amphipods were apparently eager, within a short distance of the tubes, but there is no evidence that a single one swam the three inches which separated them from it.

A comparison between the contents of the alimentary canal and the foreign algae present in the nest indicates that under natural conditions the animals do not feed outside of the nest. If an alga is present in the stomach or intestine, fragments of the same species can usually be found bound into the wall of the tube, although the converse is not necessarily true. Even the occasional absence of such an alga from the tube would be no evidence that the animal had foraged beyond the nest, since the animal may have consumed all of a fragment which had been wafted to it, without binding any of it to the wall of the tube; or it might have eaten the last morsel of a bit previously there. If no foreign alga is present within the tube, the amphipod feeds entirely upon the *Spongomorpha* in which its tube is built. Table I gives a detailed analysis of the stomach contents and tube inclusions of 11 amphipods collected from Pool N. Four of these had eaten algae other than *Spongomorpha,* and in three cases the same alga was present in the tube. In the fourth case (No. 2) the amount of the foreign alga in the alimentary canal was very small. Conversely, four of the nests contained no foreign inclusions, and the amphipods inhabiting them had eaten only *Spongomorpha.*

TABLE I. *Amphipods and tubes collected from Pool N, July 22, 1925. Algae found in both the tube and alimentary canal are printed in italics*

No.	Contents of tube	Contents of Alimentary Canal
1	Empty	Spongomorpha, threads
2	*Porphyra*	Spongomorpha, *Porphyra* (very little), threads
3	*Ceramium,* Porphyra, Polysiphonia, *Fucus*	*Fucus, Ceramium*
4	Ulva, Polysiphonia	Spongomorpha
5	Enteromorpha, Unidentified brown alga	Spongomorpha, Polysiphonia (very little), threads
6	Empty	Spongomorpha
7	Empty	Spongomorpha
8	Polysiphonia, Ulva	Spongomorpha
9	Alaria, Ulva	Spongomorpha, threads
10	*Chordaria*	Spongomorpha, *Chordaria*
11	Empty	Spongomorpha

The amphipods secure their food, other than that already in the tube, by a characteristic grasping reaction. Holmes has described a similar reaction in *Amphithoë longimana.* In the laboratory, the animals often rest in their tubes with the head at one of the openings, and the antennae protruding beyond. If a bit of *Ulva,* held in a forceps, is brought up close to the antennae, the amphipod, if it is not too well fed, will grasp it with its gnathopods, and draw it back into the tube. If the morsel is not released from the forceps, the animal will occasionally hold on so tightly that the whole alga in which the tube is built may be pulled about by it on the bottom of the aquarium. By snatching away the morsel as the amphipod grasps for it, the animal may occasionally be caused to lunge out of the tube, but never so far that the hinder periopods do not retain a firm hold at the rim of the aperture, and it cannot thus be induced to leave the nest entirely. Not only will the amphipods grasp any alga offered to them, even *Spongomorpha,* which they already have in abundance, but they have also been observed to take repeatedly bits of lens paper and small fragments of wood. The former was sometimes eaten, but the latter always rejected from the tube, either immediately, or after a short interval in which the animal tried to eat it. The reaction seems to be stereotyped, the animals grasp first and examine their catch later; they sometimes grasp at the empty forceps. If the alga taken in is not all eaten at once, the remainder is bound into the wall of the tube, where it may serve as food later. Both young and mature animals exhibit this reaction.

In the tide pools, when the tide is low and the water in the pool quiet, the amphipods usually rest far down in the tube, which is often several times their length. A shred of *Ulva* held in the forceps at the mouth of the tube elicits no response from the amphipod, which seems oblivious of it. Upon gently swaying the tuft of *Spongomorpha,* however, or agitating the water about it, the occupant, in about half the cases tried, moved upward to the aperture and waved about its antennae in the water. If the Ulva were now offered, it was usually grasped by the gnathopods and drawn back into the tube. The reaction to such a gentle swaying is quite different from the animal's behavior when the tube is violently shaken, or pinched on the outside. In the latter case, it stubbornly adheres in its nest, and it is very difficult to cause it to budge. It is not always possible by swaying to cause the amphipod to move up to the aperture, or once there to take the proffered morsel, and the reaction is probably determined by the condition of hunger.

It was not found possible to observe the behavior of the amphipods when the surf was breaking into the tide pools, but it seems probable that the action of the waves in swaying the *Spongomorpha* back and forth has the same effect as a gentle rocking by human agency. The animal might then wait at the mouth of the tube until a fragment of seaweed, and these are common in the pools, is wafted within its reach, and then draw it back into the tube. The amphipods could not be caused by mechanical swaying to remain at the aperture more than two minutes, but the accompanying conditions are so different from those of wave action that they may well have distinguished between the two.

Conclusion

The habits of *Amphithoë rubricata* have been so thoroughly modified by its sedentary mode of existence that the individuals spend their entire life in practically complete dependence upon the algae which serve them as hosts. After leaving the brood-sac of the mother, the young amphipod constructs a tube of its own, which it may enlarge to keep pace with its own growth. When the reproductive period is at hand, the mating of the sexes occurs within the tube. For food it is dependent upon the host alga itself, or what foreign algae it can secure without leaving its tube. Compared with the associated *Gammarus locusta*, the *Amphithoë* is a weak swimmer, and comparatively helpless in the tide pool. When placed on the surface of the water, even a mature animal often experiences some difficulty in breaking away from the surface film. The ability to leap after the fashion of a sand flea is completely lacking to *Amphithoë*.

Other amphipods have met the problem of maintaining themselves within the surf zone by following other lines of development. Without the protection afforded by tubes, *Gammarus*, with more slender body and greater agility, is equally successful with the comparatively bulky, slow-moving, but protected *Amphithoë*.

In conclusion the writer wishes to express his thanks to Dr. Roy W. Miner and to the United States National Museum for the determination of specimens; to Prof. Ethan Allen Andrews for reading and criticizing the text; to Prof. Duncan S. Johnson and Prof. Ulric Dahlgren for much help given throughout the course of this study.

Summary

1. The habitat of *Amphithoë rubricata* is described.

2. The breeding season begins early in July and continues through August.

3. The females are more numerous than the males, in approximately the ratio 3 to 1.

4. Several types of tubes built by this species are described.

5. The method of constructing the tube is described.

6. An individual taken from one type of host plant has been found able to construct a tube in an entirely different type of plant. There is no specificity in this respect.

7. The young animals, after leaving the brood-sac, begin almost immediately to construct their own nests.

8. The nest may be enlarged as the animal grows.

9. *Amphithoë* lives almost entirely upon algae, but is carnivorous on occasions.

10. The animal has never been seen to leave its nest for food.

11. The method of taking food is described.

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