SYMBIOSIS BETWEEN *ECHINOECUS PENTAGONUS* (CRUSTACEA, BRACHYURA) AND ITS HOST IN HAWAII, *ECHINOTHRIX CALAMARIS* (ECHINOIDEA)

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ABSTRACT

The symbiosis between the parthenopid crab, Echinoecus pentagonus, and its host in Hawaii, the diadematid sea urchin, Echinothrix calamaris, was studied to show how physiological, behavioral, and morphological adaptations are involved in the establishment of a dynamic equilibrium between the partners.

Males and juvenile females live on the host's peristome. Adult females are confined to the rectum, where calcification of the periproct produced a gall-like structure. The carapace and rostrum of adult females become curved and rounded, apparently as a result of living in the rectum. It appears that the rectum of two other diadematids is not sufficiently large to accomodate the adult females.

Feeding habits and nutrition of the symbiont were investigated by analyzing feeding behavior, stomach contents, and the ingestion and assimilation of host material by use of tracer techniques. The crab can be considered as a parasite since it is metabolically dependent on the host. The males and juvenile females meet their energy requirements by ingesting epithelial tissue and tube feet from the peristomial region. The resulting damage, however, is rapidly repaired as the result of the remarkable regenerating capacities of the host. Adult females ingest coelomocytes and material from the fecal pellets of the host.

Host coelomocytes, mostly pigmented eleocytes, migrate across the inner epithelium of the rectum and accumulate in the lumen. They appear to be involved in the deposition of pigments in the normally unexposed tissue. The fecal pellets are composed primarily

of sediment, but there is a relatively large amount of partially digested algae and encrusting organisms, as well as ciliates, nematodes, and bacteria. Typical samples of dry feces contain 0.51% lipid, 0.20% alcohol- and TCA-soluble carbohydrates, and 0.10% protein. Organic material from the feces is selectively removed by the modified maxillipeds of the crab. The introduction of large females outside the rectum results in severe damage and eventually causes the death of the host.

Estimates of the energy budget of sea urchins experimentally fed with algae indicate that 39.5% (431.8 calories/day) of the energy input is retained. Minimal energy requirements of males are supplied by a relatively small amount of epithelial tissue (3.95 mg/day); whereas, almost three times this amount (10.29 mg/day) is required for adult females placed outside the rectum. Caloric values for the coelomocyte aggregations are almost twice as high as those for epithelial tissue.

Larvae of the crab were reared in the laboratory. Development was completed after three zoeal and one megalopal stages. Positive orientation to gravity and a negative response to light coupled with a chemical attraction to host material provide the behavioral mechanism which enables the megalopa to find a host and thereby establish the association.

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SECTION I

INTRODUCTION

Symbioses may be defined as heterospecific associations during which a number of adaptations enable the partners to live in close contact or to interact in ways other than predation. A symbiotic association involves the establishment of a dynamic equilibrium as a result of adaptive interaction between the members. As a consequence, relative benefit or harm (both anthropomorphic concepts that cannot always be defined) result. The aim of this study is to reveal how physiological, behavioral, and morphological adaptations are involved in the establishment of the dynamic equilibrium during the association of the brachyuran crab, Echinoecus pentagonus, and its host in Hawaii, the echinoid, Echinothrix calamaris.

Symbiotic forms are common among the decapod crustaceans (Balss, 1956; Patton, 1967a). Work has been carried out on the life history and habits of some of the pinnotherid crabs (see review by Cheng, 1967) and in the gall crab, Hapalocarcinus (Potts, 1915; MacNamee, 1961). Some behavioral aspects of the symbioses between hermit crabs and sea anemones have been investigated (see review by Ross, 1967). Surprisingly little is known on the remaining forms, generally referred as "commensals."

SECTION II

THE SYMBIONT

INTRODUCTION

Echinoecus pentagonus (A. Milne Edwards) is a member of the subfamily Eumedoninae of the family Parthenopidae (Brachyura, Oxyrhyncha). Nine genera, with a total of 25 species, are placed in this subfamily (Serène, Duc and Luom, 1958; Serène and Romimohtarto, 1963). All the known species assigned to the Eumedoninae appear to be associated with crinoids and echinoids except one species, Rhabdonotus pictus A. Milne Edwards, which has been reported from crinoids as well as from the pennatulacean Virgularia (Serène and Romimohtarto, 1963). The subfamily is known only from the Indo-West Pacific region. All other parthenopids are free-living.

In Hawaii the adult females of the symbiont are found living in the rectum of Echinothrix calamaris (Pallas) a diadematid sea urchin. Males and small females live on the host's peristome but move freely on the surface of the test. Males may also be found in the rectum.

TAXONOMY

The systematic position and nomenclature of E. pentagonus was in a state of confusion until the revision by Serène et al. (1958). Four generic designations and a total of 13 combinations have been used to designate it. The complete list of synonyms and references is given below.

Eumedon pentagonus A. Milne Edwards, 1879. Bull. Soc. Philomath. Paris, 3:104.

Echinoecus pentagonus M. Rathbun, 1894. Proc. U.S. Nat. Mus., 17:66.

Eumedon convictor Bouvier and Seurat, 1905. C. R. Hebd. Séanc. Acad. Sci., Paris, 140:629.

Echinoecus pentagonus Rathbun, 1906. Bull. U.S. Fish Comm., 23:880, text fig. 37.

Liomedon pentagonus Klunzinger, 1906. Krabben Roten Meeres, 57, pl. 2, fig. 11.

Eumedon convictor Nobili, 1907. Mem. Accad. Sci. Torino, 57:382.

Eumedonus convictor Laurie, 1915. J. Linn. Soc. London, Zool., 31:411.

Eumedonus petiti Gravier, 1922. Bull. Mus. Hist. Nat. Paris, 28:484.

Eumedonus pentagonus Balss, 1922. Arch. Naturgesch., 88A:137.

Eumedonus pentagonus Balss, 1924. Arch. Naturgesch., 90A:70.

Eumedonus pentagonus Flipse, 1930. Siboga Exped., 39c(2): 80, 90.

Eumedonus pentagonus Sakai, 1936. Crabs of Japan, 113, pl. 30, fig. 2.

Eumedonus pentagonus Miyake, 1937. Biogeographica, 2:29, fig. 3.

Eumedonus pentagonus Monod, 1938. Mem. Inst. Egypte, 37:112.

Eumedonus pentagonus Sakai, 1938. Studies Crabs Jap., 3:349, 360, pl. 33, fig. 3.

Echinoecus pentagonus Miyake, 1939. Annot. Zool. Jap., 18:84.

Echinoecus rathbunae Miyake, 1939. Annot. Zool. Jap., 18:84, 88, figs. 1A, 2A, 3A.

Echinoecus rathbunae convictor Miyake, 1939. Annot. Zool. Jap., 18:84.

Echinoecus petiti Miyake, 1939. Annot. Zool. Jap., 18:85.

Echinoecus petiti nipponicus Miyake, 1939. Annot. Zool. Jap., 18:86, 90, figs. 1B, 2B, 3B.

Echinoecus klunzingeri Miyake, 1939. Annot. Zool. Jap., 18:85.

Eumedon convictor Mortensen, 1940. Monogr. Echinoidea 3(1):171,294.

Echinoecus petiti japonicus Mortensen, 1943. Monogr. Echinoidea, 3(3):334.

nec Eumedonus pentagonus Buitendijk, 1950. Bull. Raffles Mus., 21:71.

Eumedon convictor Caullery, 1952. Parasitism and Symbiosis, 5.

Eumedonus convictor Holthuis, 1953. Atoll Res. Bull., 24:6.

Eumedonus convicter (sic) Morrison, 1954. Atoll Res. Bull., 34:6.

Eumedon convictor Hyman, 1955. The Invertebrates IV, 588.

Echinoecus rathbunae Balss, 1956. Bronn's Kl. Ordn. Tierreichs, 7:1415.

Echinoecus rathbunae convictor Balss, 1956. Bronn's Kl. Ordn. Tierreichs, 7:1415.

Eumedon convictor Dales, 1957. Mem. Geol. Soc. Amer., 67(1):407.

Echinoecus pentagonus Serène et al., 1958. Treubia, 24:152, figs. II, 2, 3; plate IV, fig. A.

Eumedon convictor Green, 1961. Biology of Crustacea, 102.

Eumedon convictor Cheng, 1964. Biology of Animal Parasites, 4.

Echinoecus pentagonus Sakai, 1965. Crabs Sagami Bay, 102, plate 43, fig. 3.

A. Milne Edwards (1879) described the species in question as Eumedon convictor, the name "Eumedon" probably being the French translation of Eumedonus. This genus was established by H. Milne Edwards (1834) for a species (E. niger) that has not been found since then. Miers (1879) used this name for his definition of the subfamily. After four additional species had been described, Gravier (1922) proposed a modification to the diagnosis of the genus, giving it a broader definition. It was also recognized that the species of Eumedonus could be separated into two different groups under this definition: (1)

a group with a bifurcated rostrum and a spine between the antero-lateral and posterolateral borders, and (2) a group with an entire or slightly emarginated rostrum and rounded anterolateral borders. Serène et al. (1958) restricted the name Eumedonus to the first group and proposed the use of Echinoecus Rathbun for the second. This last arrangement was based on the premise that "dans l' impossibilité d' établir une diagnose théorique exacte et valabe pour tous les spécimens de l'espèce, celle de Rathbun (1894) parait la plus valable." It is noted that this step had been suggested earlier by Barnard (1954). Ward (1934) indirectly supported this separation by reestablishing the genus Echinoecus. This interpretation was later recognized by Miyake (1939) and Barnard (1954). The genus Echinoecus was established by Rathbun (1894) with Echinoecus pentagonus as the genotype.

Nobili (1907) was the first to suggest the identical nature of Eumedon convictor Bouvier and Seurat, Echinoecus pentagonus Rathbun, and Liomedon pentagonus Klunzinger. This was later supported by other workers with the exception of Miyake (1939) who reviewed the genus Echinoecus and divided it into four species (including a new name for Liomedon pentagonus Klunzinger and Echinoecus pentagonus Rathbun) and two subspecies. Serène et al. (1958) concluded that this was an artificial separation, being based on individual variations and juvenile characters. That this is the case is readily appreciated by examining Miyake's illustrations. Serène's modification has been accepted by Miyake (personal communication) and is employed in the most recent taxonomic treatment of the species (Sakai, 1965).

EXTERNAL MORPHOLOGY

Echinoecus pentagonus is characterized by a smooth subpentagonal carapace and by the absence of spines on the anterolateral angles of the carapace (Figure 1). The carapace is convex in the anteroposterior direction. The rostrum is triangular and flattened and its tip is slightly indented. The lower surface of the carapace and the external surface of the third maxillipeds are strongly punctate. The eyes are small. The circular orbits are bordered below by the small and narrow basal antennal segment. The antennae are very small, being covered by the rostrum. The chelipeds are short and punctate. The ischium has a short tooth on the inner margin while the merus is armed with two strong teeth on opposite sides of the distal end. The carpus bears two strong teeth on the inner margin and the propodus has a conspicuous tooth at the upper margin and a finger armed with small, blunt teeth along the prehensile edge. The dactylus is sharp and provided with an ondulating prehensile edge. The ambulatory legs are slender, punctated and are all approximately of the same size. They are not armed with spines but the outer distal end of the merus ends in a sharp angle that resembles a tooth. The dactylus of each leg has numerous short hairs along the inner edge. All the legs end in a very sharp dactylus. The fifth pair is inserted dorsally in relation to the fourth pair. The abdomen, composed of seven segments, is much expanded in the females. The morphology of the mouthparts will be discussed in Section V.

Adult females are much larger than the males. Carapace width (measured across the anterior angles) of 86 females varies from 5.7

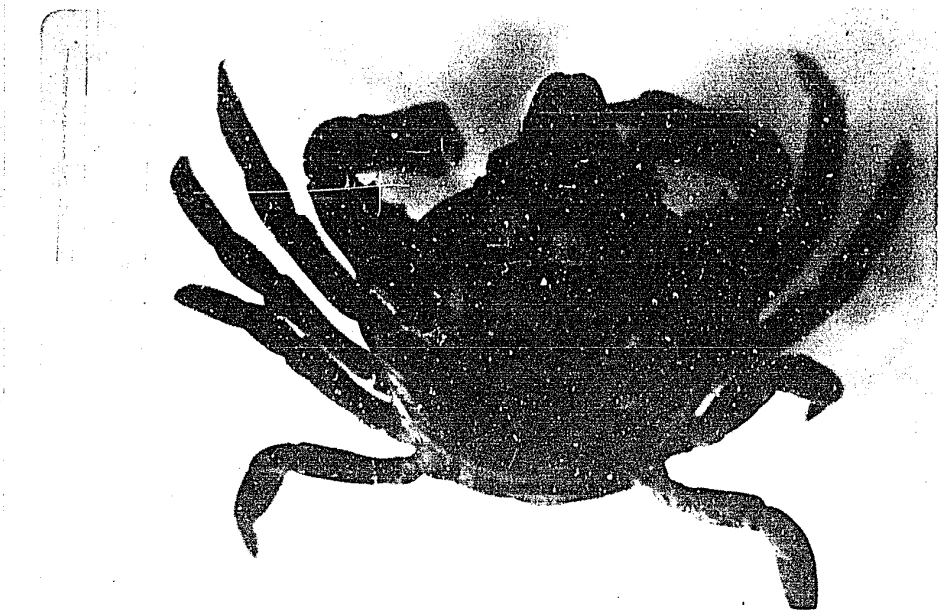


FIGURE 1. ADULT FEMALE OF ECHINOECUS PENTAGONUS. (X3.5)

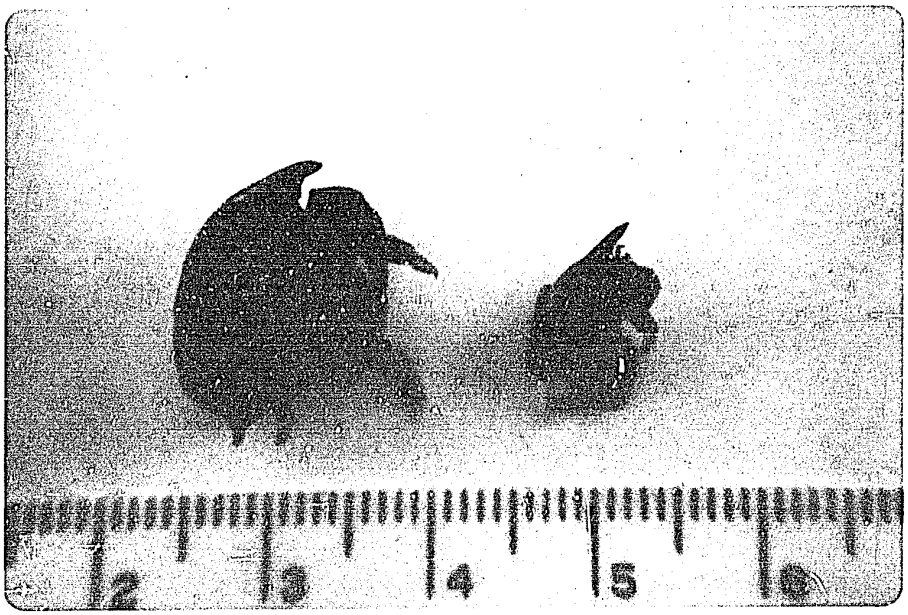


FIGURE 2. LATERAL VIEW OF A FEMALE AND A MALE OF ECHINOECUS PENTAGONUS. (SCALE IN CENTIMETERS)

to 17.0 mm; that of 35 males from 2.8 to 9.9 mm. The carapace is conspicuously inflated and the rostrum is less pronounced and a little more deflexed in the large females than in the males and small females (Figure 2). The anterolateral angles of the carapace are pointed and the eyes are visible from above the carapace is small individuals whereas in the large females the angles are rounded and the eyes are usually not visible from above. No differences were found in the serrations of the chelipeds and in the width of the posterior end of the carapace as reported by Serène et al. (1958).

It seems safe to attribute morphological differences to size rather than sex. However, when the largest males were compared with females of the same size, it was apparent that in females the carapace was broader anteriorly, the rostrum was less pronounced and more curved, and the eyes were barely visible from above the carapace. Thus, it appears that these morphological changes occur at a faster rate in females.

Specimens collected in the Hawaiian Islands were always of a dark reddish-brown color. Some males and a small female (the only crab found living on Echinothrix diadema) had a broad, white line along the anterior margin of the carapace and rostrum. A somewhat lighter border was also observed in some males and small females. Small patches of calcareous material and even small growths of the green alga Ulva were commonly observed on the dorsal surface of the carapace of females living in the rectum.

Specimens have been described in the literature as dark violet (Bouvier and Seurat, 1905), dark violet with light spots across the

carapace (Klunzinger, 1906), violet with colorless bands on each side of the posterior half of the carapace and rostrum (Gravier, 1922), and purplish with a pair of elongated white flecks on the carapace (Sakai, 1938). Dark violet crabs, some ornamented with "whitish peculiar patterns" and with a stripe on the anterior border and two longitudinal bands on the posterior half of the carapace, and a female with a pale brown ventral surface with a brown stripe running transversely on each segment were described by Miyake (1939). The color plates presented by Sakai (1936, 1938, 1965) portray a female with a purple body and a somewhat lighter stripe along the anterior border of the carapace as well as an elongated spot on each side of the carapace as described above. A similar pattern is shown in an illustration given by Miyake (1937). Serène et al. (1958) described the crabs as reddish brown-violet in color ("rouge brun violet") with pinkish-white bands along the anterior border and posterior portion of the carapace. Similar bands were observed on the chelipeds and walking legs.

It is apparent that some of the morphological characters of the species are related to its symbiotic existence. Serène et al. (1958) and Serène (1961) have pointed out that several morphological features that seem to be in common to all members of the subfamily Eumedoninae (insertion of the fifth pair of legs dorsal to that of the fourth, hook-like arrangement of the dactylopropodal articulation of the walking legs, and strong bristles along the inner edge of the dactylus of the walking legs) are adaptations that permit strong anchorage of the crabs to their hosts. The presence of a very sharp dactylus in each of the walking legs of Echinoecus pentagonus is another adaptation of this nature.

The rounded outlines and the smooth, convex surfaces of the carapace and rostrum of large females (which live in the rectum) contrasts sharply with the less rounded and convex character of the same parts in the males and small females. A condition similar to that of the females is found in the crabs of the family Pinnotheridae where the round to oval carapace is smooth and broad dorsally and its outlines are conspicuously rounded. All pinnotherids are symbiotic. One particular species, Pinnaxodes chilensis (A. Milne Edwards), lives in association with sea urchins, with females and males found free on the test (see Section IV). This situation is identical to the habit of Echinoecus pentagonus. The softening of the surface and outlines of the carapace in both groups can be suggested as having some adaptive significance by decreasing mechanical irritation to the host in those cases where the symbiont is in direct contact with the host's soft tissues.

The adaptive value of homochromism in a symbiont is obvious. The primary spines of adult Echinothrix calamaris are almost black but the secondary spines, test epithelium, and podia are of a dark reddish-brown color that matches closely that of the crabs. In many cases the primary spines bear dark brown horizontal bands. The exposed inner epithelium of the rectum is bright reddish-brown. Juvenile sea urchins have a greenish test and their spines are white with green horizontal bands.

Purple crabs have been reported in the literature from dark purple Anthocidaris crassispina (A. Agassiz) and Phyllacanthus dubius Brandt, irridescent blue Echinothrix diadema Linn., and E. calamaris. Only the crabs collected from E. calamaris and Diadema sp.

in Viet Nam (Serène et al., 1958) were of a brownish color similar to that of the Hawaiian specimens.

The color of Echinothrix calamaris appears to vary with its geographical distribution. All the individuals that I collected or observed in the Eniwetok Atoll, Marshall Islands, were more reddish in color than those from Hawaii. Mortensen (1940) commented on the "extraordinary variation in the colour of the spines" of the species ("from almost pure white to almost wholly black, but generally distinctly banded, with greenish, brownish, purplish, or even reddish bands").

The strikingly small size of the eyes, antennules, and antennae of the symbiont can be suggested as a product of a decrease in the adaptive value of sensory structures in forms which spend their entire postlarval life literally anchored to a single environment. In males and small females the eyes are relatively bigger and not hidden below the anterior border of the carapace. Males can be found in the rectum but usually move freely on the test, being even able to move between sea urchins through the spines.

A review of the adaptations in symbiotic crustaceans is given by Rioja (1950) and Patton (1967a). Pearce (1962) has discussed the adaptations in pinnotherids.

DISTRIBUTION

Accurate information on the habitat and general biology of E. pentagonus is generally lacking. Emphasis has been placed on its systematic position. Much subjectivity is found in the few efforts made to understand the nature of its association with sea urchins.

In the description of the species, A. Milne Edwards (1879) did not give any information other than the external morphology and geographical location of the crab. Rathbun (1894) described an adult female from the "anal end of the intestinal canal" of E. calamaris collected from the Bonin Islands. She observed that the crab "with its smooth broad carapace and short legs is adapted for commensalism, and resembles superficially certain of the Pinnotheridae of similar habits." Bouvier and Seurat (1905) reported this crab from a sea urchin "provisionally" identified from a spine as Echinothrix turcarum (= E. diadema). Females were found living in large "anal pockets" produced by "an invagination of tegumentary lining." Males were never collected but it was concluded that they were free living or at least never living with the females. In the atoll of the Tuamotu Archipelago where they were collected, only four sea urchins from the lagoon were found to harbor the symbiont, whereas those from the "exterior reef" never were associated with crabs. The association was described as "close commensalism" since the absence of a connection with the "cavity of the sea urchin" did not fit their definition of parasitism. The morphological adaptations of the crab were presented as evidence for this "close association."

In the first and only published record from the Hawaiian Islands Rathbun (1906) reported a male collected from a depth of 48 meters in the vicinity of Moku Manu, a small island near Kaneohe Bay, Oahu. No other information was given. It seems safe to assume that the specimen, obviously collected from dredged material, became separated from a sea urchin in the process.

Klunzinger (1906) gave no information on the habitat of a single female he collected in the Red Sea. Gravier (1922) reported on the collection from another worker with a male and a female crab living "around the mouth, over the peristomial membrane" of a diadematid sea urchin in Madagascar. According to Gravier, the crabs "circulated and concealed themselves between the spines that bordered the membrane." Furthermore, they "did not appear to leave this region, and ... their color is absolutely the same as that of the spines of the urchin." These two crabs (described as Eumedonus petiti) were seen as an intermediate state between Eumedonus pentagonus A. Milne Edwards (suggested as a "loose commensal") and Eumedonus convictor Bouvier and Seurat (also defined as a "close commensal"). All of these forms were later shown to represent the same species (Serène et al., 1958). Balss (1922) collected the crab living "commensally" with an unidentified species of Echinothrix in the Bonin Islands. Nobili (1907), Flipse (1930) and Monod (1938) reviewed some of the literature and only dealt with taxonomy. Laurie (1915) and Balss (1924) only included the species in faunal check lists.

The symbiont was first reported from Japan in association with the echinometrid sea urchin, Anthocidaris crassispina (Sakai, 1936). Miyake (1937) reported it from the Danjo Islands, southwest of Kyushu, Japan. No information on its habitat was given but in a later paper (Miyake, 1939) the same author referred to the crabs collected at this location as "found free-swimming at rocky shores." Sakai (1938) listed the species as a "commensal" of A. crassispina. Miyake (1939) recorded the finding of a female in the "anal region" of the cidaroid

Phyllacanthus dubius taken from a depth of about 100 fathoms in the Bonin Islands. A "bag" was formed where the crab was living (Miyake, personal communication). Another female was collected from the "anal tube" of E. calamaris at the same location. These two females, designated as Echinoecus rathbunae, were considered by him as "commensals," being "well supplied with food through the activities of their host." Males were reported to "swim freely." Four small individuals, three females and one male, were reported to be found "free-swimming at rocky shores" at Okino, an island between Korea and the western tip of Honshu Island, Japan. These were identified (under the name Echinoecus petiti nipponicus) as being identical to those collected by Sakai (1936, 1938) on A. crassispina. They were referred as "commensals" but "in such an extent that the crabs only obtain the benefit of shelter." Sakai (1965) reported males and females found "commensally living among the spines close by the mouth region" of A. crassispina in shallow water at Sagami Bay, eastern Honshu, Japan. Miyake (personal communication) recently observed a sea urchin (identified as "probably E. calamaris") from the southern part of Kyushu, Japan, which had a "bag in its intestinal tube" probably produced by the symbiont, although he did not observe the symbiont, in association with E. diadema, a form which "can easily be found" in Kyushu.

A single crab was recorded by Mortensen (1940) from the rectum of a specimen of E. diadema collected in Mauritius. It produced "some deformation to the apical system" of the host. Holthuis (1953) listed the crab as a "commensal on anal plate region" of E. diadema from the Tuamotu Archipelago. The sea urchins, described as banded and of "good

size" were probably E. calamaris. This record is apparently the one given from the same location by Morrison (1954).

Serène et al. (1958) found the symbiont living on E. calamaris and Diadema sp. From about 70 sea urchins of both species, a total of five males and one young female were collected from separate individuals. Two males were obtained from the "anal region" of Diadema and a male and the single female from the same region of E. calamaris. A small male was collected from the peristome of an unidentified sea urchin. No information was given on the habitat of the remaining male. Some specimens of Diadema were reported with "pulled-out spines" but no crabs were found on them. Commenting on the variety of habitats reported in the literature, they concluded that "no matter the type of habitat on the host, free or invaginated in a pocket (natural or artificial) commensalism seems to us the normal way of life of the species and the genus ... but it is not absolute and it can be temporarily interrupted." However, they favored Bouvier and Seurat's "close commensalism" as shown by the morphological adaptations of the crab rather than the "cross" between a free living existence and "commensalism" proposed by Gravier.

Johnson (1962) included the species in a list of the "commensal and semi-parasitic" decapod crustaceans of Singapore. It was reported as being a "not uncommon commensal at Singapore" (Johnson, personal communication). R. U. Gooding (personal communication) has found the crab on E. calamaris, occurring "up to 25% in some places" from the islands off the coast of Malaya, but the same sea urchin, although found in Singapore waters, according to Gooding, does not seem to be infected. He has found both sexes in the "anal cone ... always only

one to each urchin." L. Eldredge (personal communication) has observed the symbiont living in the rectum of Diadema sp. in Guam.

Caullery (1952), Hyman (1955) and Balss (1956) briefly mentioned the association. Dales (1957) referred to the "gall-forming" ability of the crab. Green (1961) mentioned that the crab "causes the host's shell to fold back to form a sort of gall." Cheng (1964) explained that "resting in this nest, the commensal does not have to exert any energy in continuously swimming around the host, yet it is on hand to share the food of the sea urchin and enjoy its protection." Hinegardner (1961) stated that a "balloon or bulb-like structure surrounding the anus of E. calamaris [is] not present ... when the anus contains a parasitic crab that is present in rare cases." His specimens were collected from shallow water in Kaneohe Bay, Oahu, Hawaii.

It seems safe to conclude from this information that E. pentagonus is an obligate symbiont of sea urchins. Only Miyake (1937, 1939) has reported free-living crabs. He could have very easily been dealing with individuals dislodged from sea urchins during their collection. The omission of the habitat in some of the records does not imply that the crabs were found in a free living condition. Unfortunately, this is the interpretation that has been given by some authors.

Five species of sea urchins have been recorded as hosts: the diadematids Echinothrix calamaris, E. diadema, and Diadema sp.; the echinometrid Anthocidaris crassispina, and the cidaroid Phyllacanthus dubius. In Hawaii I have consistently found the symbiont in association with E. calamaris. Only a small female (carapace breadth of 6.0 mm) was found living on the test of E. diadema (see Section IV).

Crabs have been reported from the rectum or "anal region" of E. calamaris, E. diadema, Diadema sp., and P. dubius, as well as from the periproct or oral spines of A. crassispina and an unidentified diadematid. Known host records and location of the symbiont in the host are summarized in Table I.

E. pentagonus, like all members of the subfamily Eumedoninae, is restricted to the Indo-West Pacific faunal region. It has been reported from the Red Sea (Klunzinger, 1906; Laurie, 1915), Madagascar (Gravier, 1922), Mauritius (Milne Edwards, 1879; Mortensen, 1940), Singapore (Johnson, 1962), Malay Peninsula (R. U. Gooding, personal communication), Viet Nam (Serène et al., 1958), New Guinea (Bouvier and Seurat, 1905), Bonin Islands (Rathbun, 1894; Balss, 1922, 1924; Miyake, 1939), Japan (Sakai, 1936, 1938, 1965; Miyake, 1937, 1939), Guam (Eldredge, personal communication), Tuamotu Archipelago (Bouvier and Seurat, 1905; Nobili, 1907; Holthuis, 1953; Morrison, 1954) and Hawaii (Rathbun, 1906).

TABLE I. RECORDED HOSTS AND MICROHABITATS OF *ECHINOECUS PENTAGONUS*

HOST	LOCATION	REFERENCE
Echinothrix calamaris	female in "anal end of intestinal canal"	Rathbun (1897)
	female in "anal tube"	Miyake (1939)
	male and female in "anal region"	Serène et al. (1958)
	males and females in rectum	Gooding (personal communication)
Echinothrix diadema	female in rectum (host "provisionally identified")	Bouvier and Seurat (1905)
	rectum	Mortensen (1940)
	"anal plate region" (host probably *E. calamaris*)	Holthuis (1953)
Echinothrix sp.	not given	Balss (1922
Diadema sp.	males in "anal region"	Serène et al. (1958)
	rectum	Eldredge (personal communication)
unidentified diadematid	male and female on periproct	Gravier (1922)
Anthocidaris crassispina	not given	Sakai (1936, 1938)
	males and females on oral spines	Sakai (1939)
Phyllacanthus dubius	"anal region"	Miyake (1939)

SECTION III

THE HOST

INTRODUCTION

Echinothrix calamaris is one of the two species representing the Indo-Pacific genus *Echinothrix* Peters of the family Diadematidae (Echinoidea, Diadematoida). A discussion of the general morphology and taxonomy of the species, including a list of synonyms and references, has been given by Mortensen (1940).

Several morphological characters distinguish it from *E. diadema* which is a close form often found living in the same habitat. In *E. calamaris* the primary spines have elongated, blade-like scales which have their free ends pointing toward the tip of the spine (see figure in Alender, 1966). This makes them hard to rub from tip to base. The lumen of these spines is larger in diameter than the thickness of their walls. In *E. diadema* the primary spines are armed with small, rounded scales arranged in longitudinal rows usually visible with the naked eye. The lumen of the spines is smaller than the thickness of their walls. The ambulacra of *E. calamaris* are raised aborally, leaving a naked and sunken interambulacral region. This condition does not occur in *E. diadema*. The periproct is large and conspicuous in *E. calamaris*. It is expanded to reveal numerous white plates (Figure 12). The periproct is small in *E. diadema*. *E. calamaris* can also be identified by its larger size.

A great deal of confusion has been created by the use of colors in distinguishing between the two species. The primary spines are banded (with alternating bands of dark and light shades of green) in

the juveniles of both species. This character, however, is more conspicuous and persists much longer in E. calamaris. A brilliant green line running along the aboral interambulacra is characteristic of juvenile E. calamaris. Mortensen (1940) has indicated that color varies geographically in both species. In Hawaii, the test and podia of E. calamaris are dark reddish-brown and the spines dark brown to black. White spines have been observed in a few adult individuals. The spines of adult E. diadema are dark irridescent blue.

A similar species, Diadema paucispinum A. Agassiz, can be distinguished from Echinothrix by the presence of black primary spines which are longer than the height of the test. The spines are reddish and unbanded in the juveniles. The species is the only representative in Hawaii of the circumtropical genus Diadema Gray (= Centrechinus Jackson).

DISTRIBUTION

E. calamaris has been recorded in the literature from littoral waters to depths of 90 meters (Mortensen, 1940). In Hawaii, I have collected it from depths of 1.5 to 30 meters.

The species was first reported from Hawaii by Agassiz (1872). It was later recorded by Agassiz and Clark (1907, 1908) from Puako Bay, Hawaii, and Penguin Bank, west of Molokai, from depths of 28 to 29 fathoms (around 51.5 meters). Only juvenile specimens were collected. Edmondson (1946) considered the species as "not widely distributed about the islands," giving only two records which appear to be those of Agassiz and Clark. Hinegardner (1961) found the species to be abundant in depths of approximately two to five meters (about

six ... to fifteen feet") in Kaneohe Bay, Oahu, Hawaii.

Most of the specimens studied during this investigation were collected from Kaneohe Bay (Figure 3). Other collections were made from additional locations in the islands of Oahu, Maui, Hawaii (Figure 4), and in Eniwetok in the Marshall Islands.

The species is found living in two general types of substrates. It typically is found in relatively large numbers among coral (mostly Pocillopora meandrina Verrill) and in ledges at depths starting at approximately 4 meters (Figure 5). Some individuals can be found in shallower water in some locations. Sea urchins have been collected or observed living under these conditions in all locations except in the shallow water reefs of Kaneohe Bay. Individuals are found here in depths of 2 to 3 meters along the upper edge of the dense colonies of the coral Porites compressa Dana growing along the seaward side of some reefs (locations designated as 1 and 2 in Figure 3). The populations are small in size. Sea urchins occupy the relatively few bare spaces that are available between the coral colonies and the massive growths of the green alga Dictyosphaeria cavernosa (Figure 6). Individuals can also be found in isolated patches of Porites compressa, P. lobata Dana, and Pocillopora meandrina (locations 4) and in some bare sections of reefs where algae are abundant (locations 3). Several juveniles have been dredged from the sandy bottom of the bay. Patches of algae and coral rubble are characteristic of the area. Large populations are found in deeper water at the entrance of the bay (locations 5 and 6).

In Eniwetok, sea urchins have been found living in a shallow

FIGURE 3. MAP OF THE EASTERN PORTION OF KANEOHE BAY, OAHU, HAWAII, SHOWING LOCATIONS WHERE DIADEMATID SEA URCHINS WERE COLLECTED OR OBSERVED.

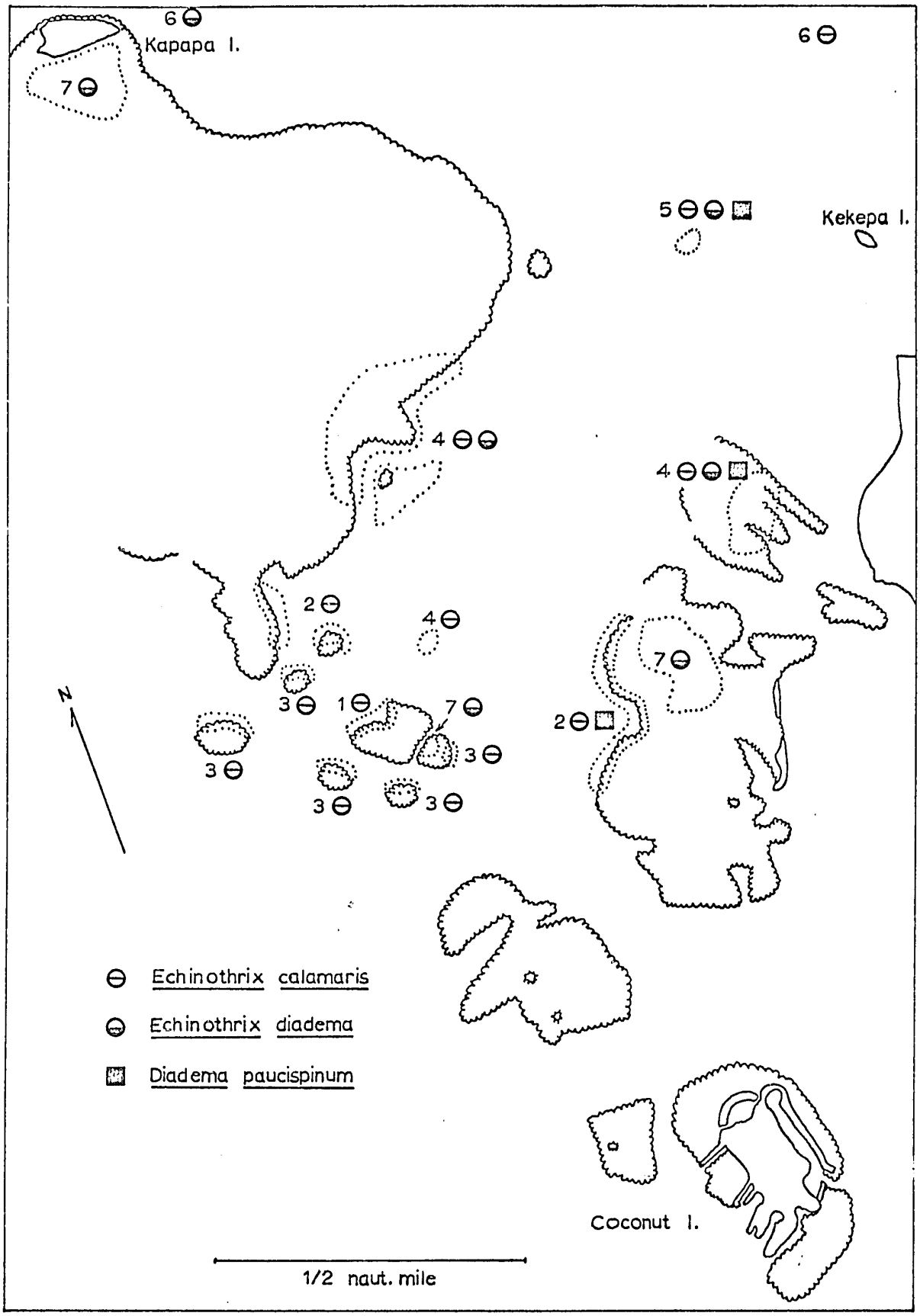
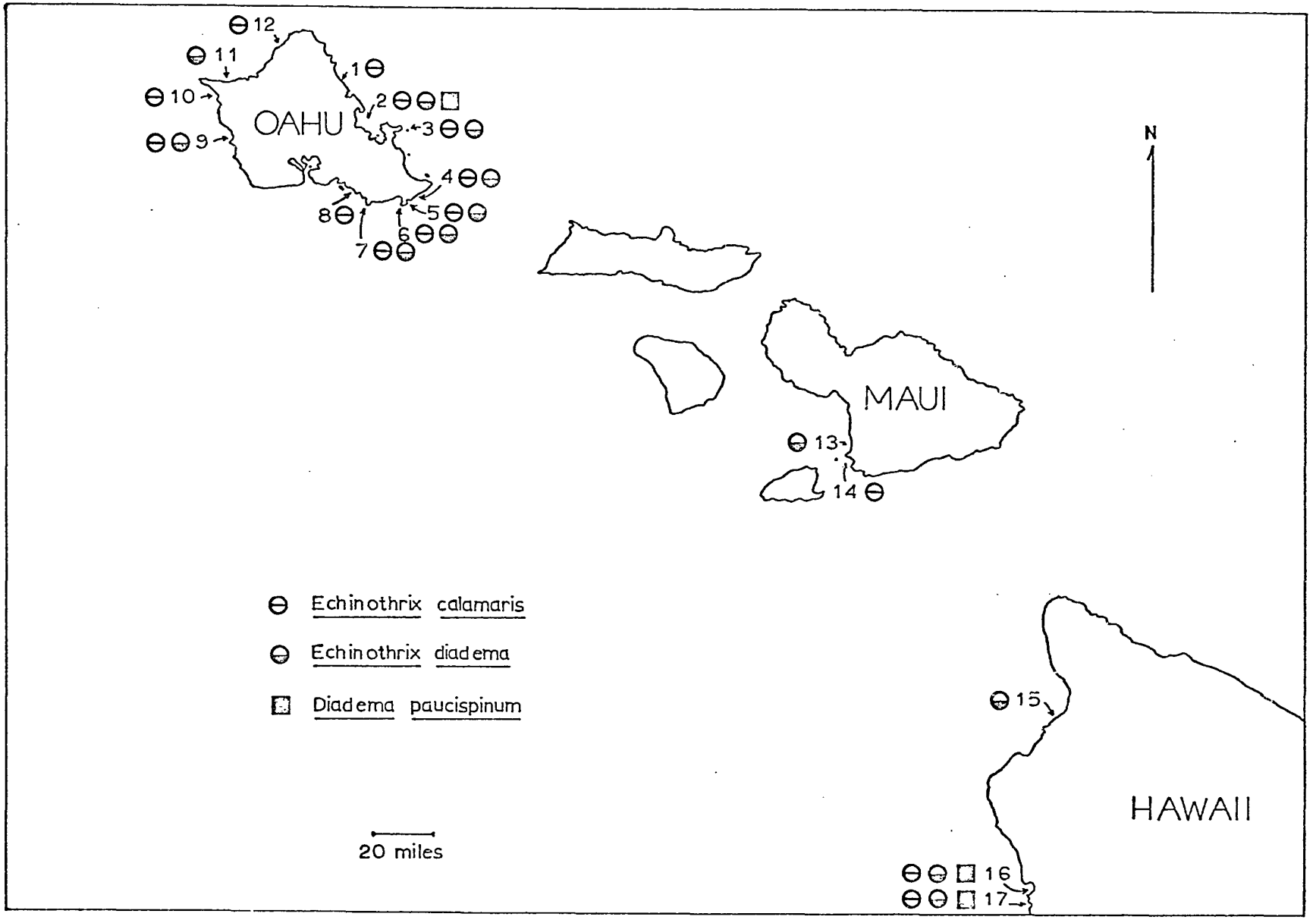

6
Kapapa I.
7
6
5
Kekepa I.
4
4
2
4
7
3
1
7
3
2
3
3
3
N
Echinothrix calamaris
Echinothrix diadema
Diadema paucispinum
Coconut I.
1/2 naut. mile

FIGURE 4. MAP OF THE HAWAIIAN ISLANDS SHOWING LOCATIONS WHERE DIADEMATID SEA URCHINS WERE COLLECTED OR OBSERVED.

1. Hauula
2. Kaneohe Bay
3. Moku Manu Island
4. Halona Blowhole and Koko Crater area
5. Hanauma Bay
6. Kawaihoa Point
7. Diamond Head Beach
8. Kewalo Basin
9. Pokai Bay
10. Makua
11. Mokuleia
12. Pupukea
13. Puu Okai Beach
14. Paako
15. Puako
16. Kealakekua Bay
17. Honaunau Bay


OAHU
MAUI
HAWAII
N
Echinothrix calamaris
Echinothrix diadema
Diadema paucispinum
20 miles
1
2
3
4
5
6
7
8
9
10
11
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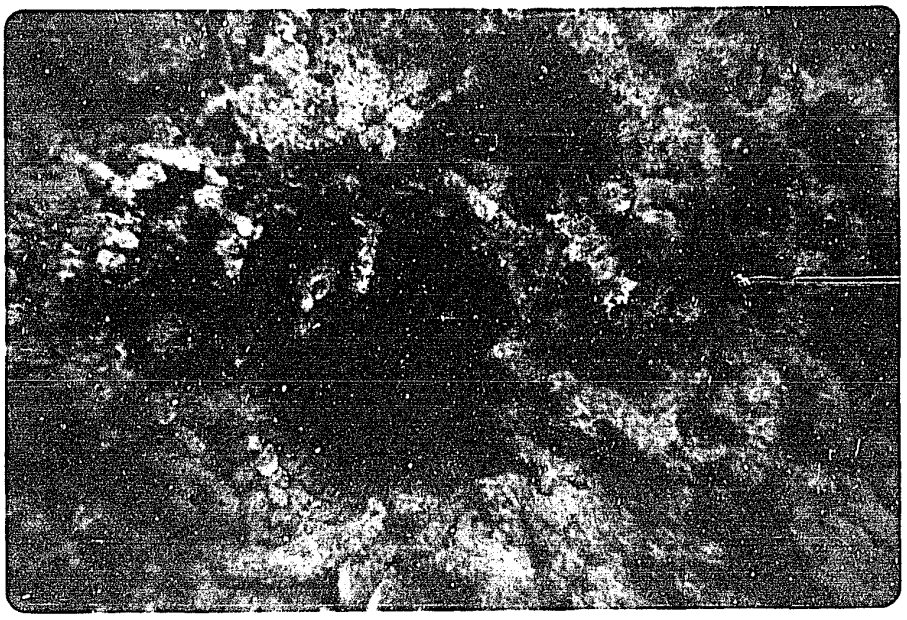


FIGURE 5. TYPICAL DEEP WATER HABITAT OF ECHINOTHRIX CALAMARIS.

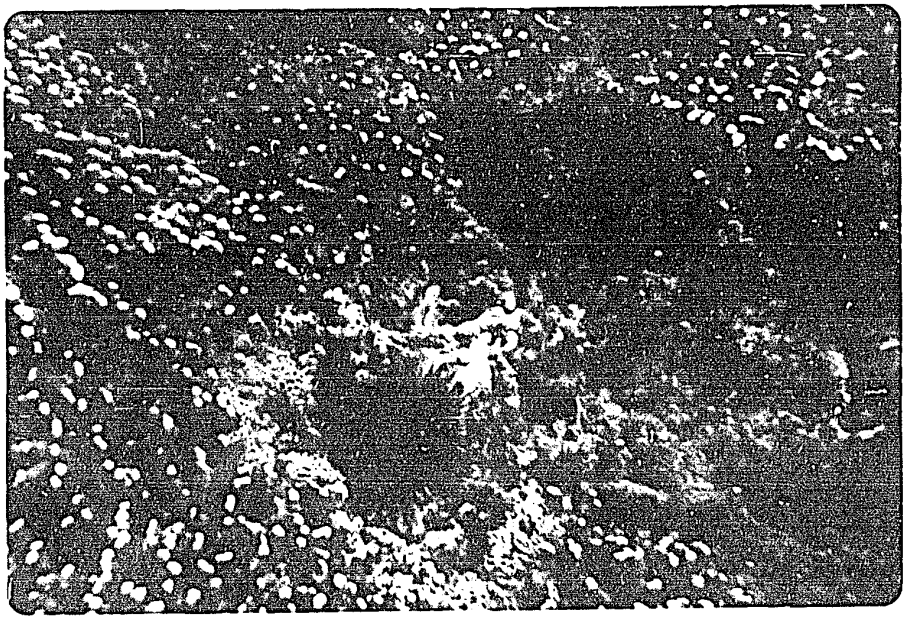


FIGURE 6. SHALLOW WATER HABITAT OF ECHINOTHRIX CALAMARIS IN KANEOHE BAY.

water quarry at a depth of approximately 2 meters. E. diadema, Diadema setosum (Leske), and D. savignyi Michelin were also present.

Specimens from the shallow water populations in Kaneohe Bay and Eniwetok show some differences when compared to those from the typical offshore situation. Sea urchins found in shallow water are conspicuously larger (Figure 7). A more striking difference is the almost complete absence of Echinoecus pentagonus in the shallow water individuals. The symbiont or the calcified periproct resulting from its presence was observed in almost all of the sea urchins collected from water deeper than 5 meters. Only three shallow water sea urchins (all collected from location 1) showed evidence of the presence of the symbiont: a small female in the rectum of one and the calcified periprocts (but no crabs) of the remaining two. No crabs or calcified periprocts were found in any of the sea urchins examined at Eniwetok. General morphological characters appear to be identical in the two size variants. Shallow water sea urchins are rapidly occupied by crabs under laboratory conditions.

This apparent variation in size was analyzed by taking measurements of specimens collected from representative localities. Shallow water sea urchins were obtained from a depth of three meters in location 1. Those from deeper water were taken from depths of 5 to 7 meters in location 5. The following measurements were taken after most of the spines were removed: test diameter (across ambitus from the madreporite interambulacrum to the opposite ambulacrum), test height (through the madreporite interambulacrum) peristome diameter (across an ambulacrum to the opposite interambulacrum), and periproct diameter (across from

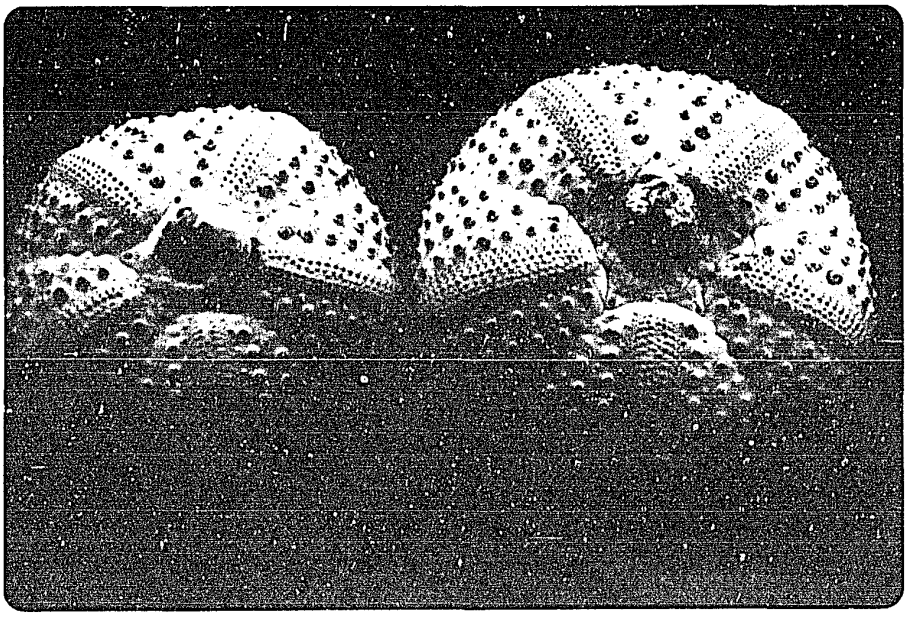


FIGURE 7. CLEANED TESTS OF ECHINOTHRIX CALAMARIS SHOWING VARIATION IN SIZE. SPECIMEN ON THE LEFT IS FROM A TYPICAL DEEP WATER LOCATION, SPECIMEN ON THE RIGHT FROM A SHALLOW WATER REEF IN KANEOHE BAY. BOTH SHOW CALCIFIED PERIPROCTS. (SCALE IN CENTIMETERS)

the madreporite to the opposite side). All measurements were made with a Vernier caliper.

General color pattern (an approximate indication of age), condition of the periproct, and measurements are given in Tables II (shallow water sea urchins) and III (deep water sea urchins). The regression of test height and peristome diameter on test diameter in both populations is depicted in Figure 8. The deformation of the periproct resulting from the presence of the crab in almost all of the deep water sea urchins did not permit the use of periproct diameters in comparing the two populations.

The regression of the available test measurements suggests that the shape of the test is similar in both populations and that the only obvious significant difference is in its size, those of shallow water individuals being larger. Juvenile characters observed in medium-sized, shallow water individuals (banded spines, green line along the aboral interambulacra) were not found in deep water forms of the same size range. The smallest individuals had identical coloration in both populations. Of the 25 shallow water sea urchins that were measured, calcified periprocts, including one with a crab living in it, were found in three individuals. On the other hand, only three of the 33 sea urchins collected at the entrance of the bay showed normal periprocts. No crabs were found in their tests or peristomes when the measurements were taken.

Test measurements of sea urchins obtained from additional collections showed a similar condition. Test diameter of 20 sea urchins collected from shallow water in Kaneohe Bay (location 1) ranged from 60.0 to 111.4 mm ($\bar{x}$ = 96.2 ± 12.2 mm). Seven sea urchins, also col-

TABLE II. TEST MEASUREMENTS (IN MM), COLORATION OF THE SPINES, AND CONDITION OF THE PERIPROCT IN SPECIMENS OF ECHINOTHRIX CALAMARIS COLLECTED FROM AN INNER, SHALLOW WATER REEF IN KANEOHE BAY, OAHU, HAWAII

SPECIMEN NUMBER	SPINE COLOR	PERIPROCT CONDITION	TEST DIAMETER	HEIGHT	PERISTOME DIAMETER	PERIPROCT DIAMETER
1	banded	normal	61.9	24.4	24.1	10.6
2	banded	normal	78.6	32.7	28.9	11.8
3	banded	normal	81.6	34.7	34.7	14.1
4	dark	normal	84.0	32.6	32.5	13.2
5	banded	normal	84.6	31.9	31.7	11.2
6	banded	normal	87.8	35.3	34.5	14.4
7	banded	normal	90.0	35.0	35.6	11.9
8	dark	normal	96.3	43.7	39.0	17.5
9	dark banded	normal	98.0	44.0	39.7	16.0
10	dark banded	calcified (with crab)	98.4	44.5	39.3	18.8
11	dark banded	normal	98.7	42.7	37.6	18.3
12	dark	normal	98.7	43.2	41.9	19.1
13	dark	calcified	99.2	47.8	42.0	15.5
14	dark	calcified	99.6	47.0	39.9	20.1
15	dark	normal	102.1	47.5	39.7	18.5
16	dark	normal	104.0	46.3	40.8	21.6
17	dark	normal	104.0	49.3	42.7	19.0
18	dark	normal	104.9	51.7	40.5	17.8
19	dark	normal	105.5	46.5	43.5	16.4
20	dark	normal	107.2	49.5	41.2	19.0
21	dark	normal	108.7	48.1	43.6	19.0
22	dark	normal	111.0	47.8	40.6	20.7
23	dark	normal	111.9	50.5	44.5	19.9
24	dark	normal	116.3	54.3	44.3	20.4
25	dark	normal	121.7	50.1	42.1	25.9
Mean ($\bar{x}$)			98.2	43.2	38.6	17.2
Standard Deviation (S.D.)			13.1	7.6	5.1	3.7

TABLE III. TEST MEASUREMENTS (IN MM), COLORATION OF THE SPINES, AND CONDITION OF THE PERIPROCT IN SPECIMENS OF *ECHINOTHRIX CALAMARIS* COLLECTED FROM A DEEP WATER REEF AT THE ENTRANCE OF KANEOHE BAY, OAHU, HAWAII

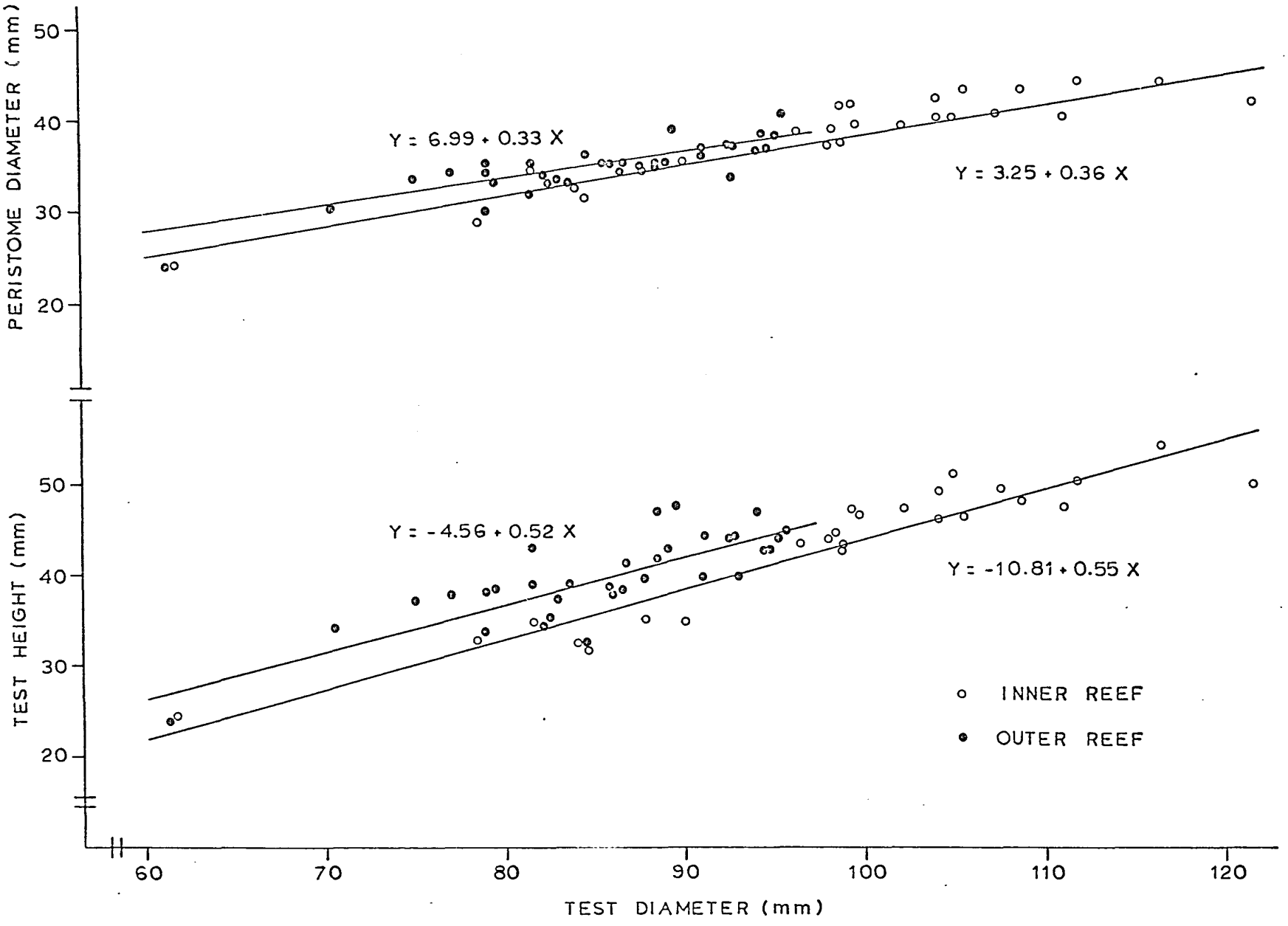
SPECIMEN NUMBER	SPINE COLOR	PERIPROCT CONDITION	TEST DIAMETER	HEIGHT	PERISTOME DIAMETER	PERIPROCT DIAMETER
1	banded	calcified	61.2	24.0	24.0	10.9
2	dark	calcified	70.5	34.2	30.5	12.2
3	dark banded	calcified	75.0	37.8	33.5	17.0
4	dark	normal	77.0	37.9	34.3	10.7
5	dark	calcified	79.0	33.9	35.5	14.5
6	dark	calcified	79.0	38.2	34.4	15.6
7	dark	calcified	79.4	38.4	33.2	17.8
8	dark	normal	81.6	39.1	32.0	11.8
9	dark	calcified	81.6	42.9	35.1	24.6
10	dark	calcified	82.1	34.3	34.1	15.0
11	dark	calcified	82.4	35.6	33.5	11.7
12	dark	calcified	83.0	37.6	33.9	17.5
13	dark	normal	83.7	39.0	33.5	15.6
14	dark	calcified	84.6	32.8	36.5	16.9
15	dark	calcified	85.9	38.8	35.6	16.2
16	dark	calcified	86.0	38.0	35.6	20.3
17	dark	calcified	86.6	38.7	34.6	17.8
18	dark	calcified	86.8	41.5	35.7	21.5
19	dark	calcified	87.8	39.8	35.1	18.8
20	dark	calcified	88.4	42.1	35.6	22.3
21	dark	calcified	88.4	47.2	35.4	22.7
22	dark	calcified	89.0	42.9	35.5	12.2

(continued)

TABLE III. (Continued) TEST MEASUREMENTS (IN MM), COLORATION OF THE SPINES, AND CONDITION OF THE PERIPROCT IN SPECIMENS OF ECHINOTHRIX CALAMARIS COLLECTED FROM A DEEP WATER REEF AT THE ENTRANCE OF KANEOHE BAY, OAHU, HAWAII

SPECIMEN NUMBER	SPINE COLOR	PERIPROCT CONDITION	TEST DIAMETER	HEIGHT	PERISTOME DIAMETER	PERIPROCT DIAMETER
23	dark	calcified	39.4	47.9	39.5	16.0
24	dark	calcified	91.0	40.0	36.2	15.7
25	dark	calcified	91.0	44.6	36.9	14.8
26	dark	calcified	92.5	44.2	37.6	21.8
27	dark	calcified	92.6	44.3	34.0	22.1
28	dark	calcified	92.9	40.0	37.6	29.0
29	dark	calcified	94.0	47.3	36.8	22.0
30	dark	calcified	94.3	43.0	38.7	21.0
31	dark	calcified	94.8	43.0	37.0	18.0
32	dark	calcified	95.1	44.7	38.3	25.0
33	dark	calcified	95.6	45.0	41.0	20.7
Mean ($\bar{x}$)			85.5	39.9	35.2	17.9
Standard deviation (S.D.)			7.7	4.9	2.9	4.5

FIGURE 8. VARIATION IN TEST MEASUREMENTS FOR INDIVIDUALS OF ECHINOTHRIX CALAMARIS COLLECTED FROM AN INNER, SHALLOW WATER REEF AND AN OUTER, DEEPER WATER REEF IN KANEOHE BAY, OAHU, HAWAII.


PERISTOME DIAMETER (mm)
50
40
30
20
Y = 6.99 + 0.33 X
Y = 3.25 + 0.36 X
TEST HEIGHT (mm)
50
40
30
20
Y = -4.56 + 0.52 X
Y = -10.81 + 0.55 X
INNER REEF
OUTER REEF
60
70
80
90
100
110
120
TEST DIAMETER (mm)

lected from shallow water (location 4), had test diameters that ranged from 90.0 to 110.0 mm ($\bar{x}$ = 95.6 $\pm$ 6.7 mm). No crabs or calcified periprocts were observed. Pooled test diameters of 50 deep water sea urchins (location 5) ranged from 79.5 to 97.4 mm ($\bar{x}$ = 87.5 $\pm$ 5.1 mm). Four showed normal periprocts. Collections were also made from two locations at depths of 30 meters (locations 6). A total of 26 individuals had test diameters that ranged from 54.2 to 98.9 mm ($\bar{x}$ = 82.7 $\pm$ 11.9 mm). Thirteen of these had a mean height of 36.6 $\pm$ 8.3 mm and a mean peristome diameter of 31.1 $\pm$ 5.3 mm. All sea urchins, including juveniles, were occupied by crabs.

There are no available data on the test measurements of sea urchins collected from locations other than Kaneohe Bay. However, the obviously larger size shown by the shallow water individuals of Kaneohe Bay has not been observed in sea urchins from any other locations in the Hawaiian Islands, even when found living in relatively shallow water, as in the case of Pokai Bay, Oahu and Kealakekua Bay, Hawaii.

Overall size of individuals collected at Eniwetok was similar to those from the shallow water reefs of Kaneohe Bay. Test diameters given by Mortensen (1940) for 15 specimens from unknown locations ranged from 5 to 130 mm.

It may be suggested that variations in size are the results of differences in growth rates. This is based purely on speculation since there is no available data on growth rates <u>per se</u>. Such phenomenon has been previously described in several other species of sea urchins. The subject has been reviewed by Swann (1966), with more recent examples given by Fuji (1967), McPherson (1968a), and Ebert

(1969). In the case of E. calamaris, this variation could be due to one or more possibilities: the presence of Echinoecus pentagonus, environmental differences, or genetic factors.

The presence or absence of the symbiotic crab does not seem to have any influence in the size of sea urchins. The only shallow water individual that was found with a crab and the other two that apparently harbored the symbiont at one time were not smaller than the other members of the same population. The dimensions of their tests were actually greater than the average (specimen Nos. 10, 13, and 14 of Table II). The opposite situation (deep water sea urchins with normal periprocts) was observed in seven out of the 83 individuals from location 5 that were measured (specimen Nos. 4, 8, and 13 of Table III and an additional four with test diameters of 79.6, 86.8, 87.1, and 95.0 mm). Of these, the last three were larger than the mean value for the 83 individuals (83.7 mm) but still smaller than the average shallow water individual. Nevertheless, it is still possible that small crabs were living on their tests. The available data indicate two points: (1) sea urchins belonging to heavily infected populations do not show any abnormal increase in size when crabs are not found living on them and (2) sea urchins of populations that normally do not harbor the symbiont do not show a smaller size when crabs (or calcified periprocts) are present. Larger samples are necessary to test for statistical significance.

A possible higher growth rate in the shallow water populations can be correlated with a higher availability of food in this habitat. E. calamaris feeds mostly on algae, but considerable amounts of encrusting organisms, sand, and detritus are also ingested (see Section V).

Diet can be determined by examining fecal pellets. The green alga, Dictyosphaeria cavernosa, that grows in large aggregations along the reefs of Kaneohe Bay is the most important food source for the sea urchins living in this substrate. Sargassum is common in the other shallow water areas of Kaneohe Bay. Large algae are usually absent in the offshore substrate. Fecal pellets from these sea urchins are mostly composed of sand, calcareous fragments, and filamentous algae.

There is no direct correlation between size variations and depth. Even when differences in depth have been used to distinguish between the typical offshore areas and the Kaneohe Bay reefs, sea urchins from the former can be found in depths equal to those of Kaneohe Bay. Parameters such as temperature, predation, and wave action do not seem to differ to the extent of being directly involved. However, differences of the substrate in Kaneohe Bay can be traced to such factors as depth, wave action, circulation, etc.

The possibility of variations in size or growth rates being determined or influenced by genetic differences could only be answered by carrying out rearing experiments.

OTHER SYMBIONTS

In addition to E. pentagonus, other organisms have been found living in close association with E. calamaris. Sea urchins, like most echinoderms, can be considered as "advantageous" habitats for symbionts (Davenport, 1966). Their large surface areas are covered, at least in part, by a ciliated epithelium, offering a clean, well aerated environment. The spines, sometimes toxic, give protection against predators. Echinothrix appears to have few predators.

Several fish, a spiny lobster, and a large gastropod are known to prey on Diadema antillarum Philippi in the West Indies (Randall et al., 1964). Hiatt and Strasburg (1960) list some wrasses and trigger fish as feeding on sea urchins in the Marshall Islands.

Two organisms have been found living on the spines of E. calamaris. Large numbers of the platyctenean ctenophore, Coeloplana willeyi Abbott, are commonly found on the primary and secondary spines of both species of Echinothrix. This, and at least two related species have been recorded from other sea urchins (Abbott, 1902; Hyman, 1955). C. willeyi was first recorded from Hawaii by Matthews and Townsley (1964). The animal extends its flattened, reddish-brown body along the sea urchin's spines. A retractile tentacle, expanding as long as 12 cm, is located at each end.

Stegopontonia commensalis Nobili, a pontoniid shrimp (family Palaemonidae, subfamily Pontoniinae), is occasionally found clinging to the spines of Echinothrix and Diadema. The shrimps are relatively large, 15 to 35 mm in total length. A conspicuous white line runs along each side of the dark purple body. It can also be identified by its broad and dorsoventrally flattened rostrum. These shrimps are usually found in pairs. The species has been previously recorded in association with Indo-Pacific diadematids (Nobili, 1907; Mortensen, 1940) but its presence in Hawaii is not recorded in the literature.

Another pontoniid shrimp, Tuleariocaris holthuisi Hipeau-Jacquotte, has been found living on the spines of E. diadema in Kaneohe Bay (Castro, 1966). This shrimp, also found in pairs, is black but its color changes to red at night. Its laterally compressed rostrum is armed

with six to eight dorsal teeth. Bruce (1967) has reported it living on the diadematid Astropyga radiata (Leske) in Hawaii. It may also be associated with E. calamaris.

A large, reddish-brown turbellarian was once observed crawling on the test of a specimen of E. calamaris. It is not known if this form is normally associated with the sea urchin. Several turbellarians have been recorded as internal or external symbionts of sea urchins (Hyman, 1955).

Parasitic gastropods of the families Stiliferidae and Eulimidae have been reported from Echinothrix and Diadema (Mortensen, 1940; Edmondson, 1946; Tinker, 1958). Three individuals, tentatively identified as Stilifer mittrei Petit, were found attached to the test of a specimen of E. diadema. Their proboscis has perforated the test of the sea urchin and reached the perivisceral cavity just above a gonad. None was found on E. calamaris.

Endosymbiotic ciliates and bacteria are found in large numbers in the gut of the sea urchin. They are especially common in fecal pellets (see Section V).

SECTION IV

THE ASSOCIATION: ECOLOGICAL ASPECTS

INTRODUCTION

This section deals with what can be termed the ecological aspects of the association, i.e. the distribution of the symbiont in the host and its microhabitat. The question of why the symbiont is associated with a particular species of sea urchin has been partially answered by analyzing its micro-habitat. The question was posed as to what "advantages" are offered by the host species in comparison with other similar species. The host's morphological responses to the presence of the symbiont have also been investigated. Emphasis has been placed on the adaptive significance of these responses rather than on the degree of pathology involved.

MATERIALS AND METHODS

Sea urchins were collected from various locations in Oahu, Maui, and Hawaii (Figures 3 and 4). SCUBA equipment was generally used during collecting. Sea urchins and crabs stayed alive for considerable time only when kept in large tanks provided with running sea water. The sea urchins were rapidly killed by stagnant conditions or by changes in temperature brought about by direct sunlight. Most of this investigation was carried out at the Hawaii Institute of Marine Biology on Coconut Island, Kaneohe Bay, Oahu. Collecting areas near the island permitted transfer of sea urchins precluding any adverse conditions. Mortality was especially high among deep water individuals as they had to be crowded together in collecting bags during transport. The crabs were less delicate but died if left in contact with dead sea

urchins. Collecting conditions did not permit keeping the sea urchins separate from each other. Sea urchins kept in the laboratory were fed with algae, mostly Laurencia sp.

For the general histology of the periproct, rectum, and peristome, tissues were fixed in 70% ethyl alcohol or 10% neutral formalin. Decalcification of periproct and peristome tissues was accomplished by immersion in Decal (Decal Chemical Corp., Pomona, New York) for 10 to 12 hours, or in a 10% aqueous solution of disodium ethylenediaminotetraacetic acid (EDTA) adjusted to pH 7.0 with sodium hydroxide, for at least 24 hours. The tissues were embedded in paraffin and sectioned at 5 to 10 microns. Frozen sections of some decalcified tissues were made on a cryostat (International Equipment Co. model CTD). Serial sections were stained with Harris' hematoxylin counter-stained with eosin, Mallory's triple connective tissue stain, or Milligan's trichrome stain, all as given by Humason (1967). The stained sections were mounted in Permount.

Histochemical determinations of polysaccharides and related compounds was carried out by using the periodic acid-Schiff stain (PAS) reaction (Humason, 1967). Glycogen was removed by treating the sections with 0.1% diastase solution maintained at 37°C for one hour prior to exposure to the periodic acid solution of the PAS staining procedure (Thompson, 1966). The presence of acid mucopolysaccharides was investigated by staining EDTA-decalcified sections with pH 3 alcian blue-yellow (Parker and Diboll, 1966). Frozen sections were stained with Sudan black B for the determination of lipids (Humason, 1967). Glycerol jelly was used as the mounting medium.

In order to get some insight into the question of host-specificity,

the orientation and general behavior of crabs were analyzed in individuals placed in contact with abnormal hosts. Crabs were removed from their host and placed in 40 gallon tanks with individual specimens of Echinothrix diadema and Diadema paucispinum.

RESULTS

Distribution of the Symbiont in the Host.

The rare occurrence of crabs in the shallow water populations of E. calamaris in Kaneohe Bay has already been indicated in Section III. Of a total of 65 shallow water individuals examined, one had a small female living in its rectum and two revealed calcified periprocts characteristic of sea urchins which harbor crabs. This represents an occupancy rate of 4.6%, a figure that does not include a considerable number of individuals that were not recorded but that showed normal periprocts.

It is difficult to estimate what percentage of deep water sea urchins had crabs living on them. Calcified periprocts were found in almost all of the individuals collected or in those examined in their natural environment; they, however, were not always occupied by crabs. Empty calcified periprocts were apparently produced by males who had moved out or by females that had died. The periproct condition of a total of 226 deep water individuals was recorded. Of these, 211 (93.3%) showed the calcified condition. Most of the individuals with normal periprocts were juveniles, but juveniles with calcified periprocts were also encountered. Incidence of calcified periprocts was observed to be higher in the deepest locations. Most of the fully grown individuals with normal periprocts were collected from a

depth of four to six meters at the entrance of Kaneohe Bay (location 5, Figure 3).

It was also difficult to determine the number of crabs living in the rectum and free on the peristome and test of each sea urchin. Males and small females tended to move from their original locations and even to other sea urchins when these came in contact during collection. Females or males living in the rectum usually moved out if the host was injured. Small crabs were commonly overlooked and some even escaped detection by moving into their hosts' intestine. Collecting conditions did not permit keeping individual sea urchins isolated from each other. Only the total number of crabs obtained from each collection was recorded. Data from nine locations on Oahu (except inside Kaneohe Bay) and Maui are summarized in Table IV. These figures do not show a significant difference in the occurrence of the symbiont in the different locations. However, differences in the size of the samples and in the time and effort spent in examining each sea urchin do not justify making any conclusions.

Microhabitat.

Large, adult females with a carapace width of at least larger than 7 mm are restricted to the rectum of the host (Figure 9). They only move out when the host is badly injured or dead. The pocket-like rectum is formed by an enlargement of the distal end of the digestive tube (Figure 13). It is surrounded by the periproct. The presence of crabs in the rectum induces heavy calcification of the periproct, leaving a relatively wide opening through which the rostrum and part of the carapace of the crab can usually be seen (Figure 10). The

TABLE IV. FREQUENCY OF OCCURRENCE OF *ECHINOECUS PENTAGONUS* IN DIFFERENT POPULATIONS OF *ECHINOTHRIX CALAMARIS*

LOCATION	DEPTH	SEA URCHINS WITH CALCIFIED PERIPROCTS	SEA URCHINS WITH NORMAL PERIPROCTS	FEMALE CRABS	MALE CRABS	CRABS PER TOTAL NUMBER OF SEA URCHINS
Koko Crater Area, Oahu	7-8 m	7	2	7	1	0.9
Halona Blowhole, Oahu	6-8 m	12	3	11	7	1.2
Portlock, Oahu	6-8 m	19	3	14	6	0.9
Pokai Bay, Oahu	5 m	3	0	2	2	1.3
Kewalo Basin, Oahu	5 m	3	0	2	0	0.7
Pupukea, Oahu	6 m	1	0	1	0	1.0
Ahihi Bay, Maui	5 m	1	0	1	0	1.0
Kaneohe Bay, Oahu (location 5, Figure 3)	5-7 m	38	3	24 (3)*	6	0.8
Off Kaneohe Bay, Oahu (location 6, Figure 3)	30 m	13	0	5 (1)*	6	0.9
TOTAL		97	11	67 (4)*	28	x = 0.9

* Small females.

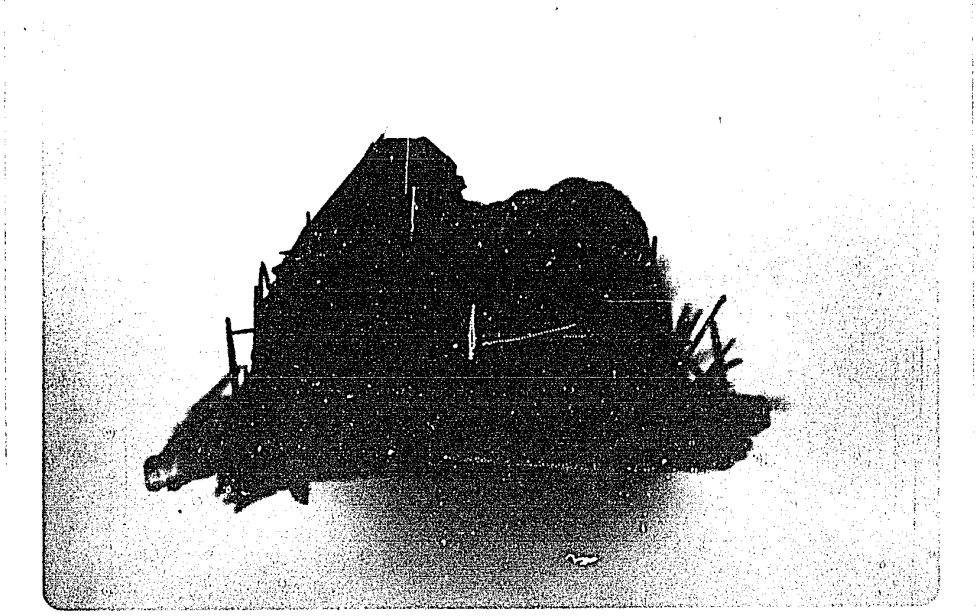


FIGURE 9. CALCIFIED PERIPROCT OF ECHINOTHRIX CALAMARIS CUT OPEN IN ONE SIDE TO SHOW A FEMALE ECHINOECUS PENTAGONUS IN ITS NORMAL POSITION. (X2.3)

FIGURE 10. ABORAL VIEW OF A CALCIFIED PERIPROCT OF ECHINOTHRIX CALAMARIS SHOWING A FEMALE ECHINOECUS PENTAGONUS INSIDE. (X2.3)

rectum is normally occupied by one female but males are sometimes found in it, alone or accompanying a female. Two females have been occasionally observed in rectums enclosed by a calcified periproct provided with two openings. Mobility of the crabs in the rectum is rather limited, especially in the case of larger females. The legs, with their sharp dactyli, are used in attachment.

Most males, as well as the postlarval and juvenile stages of both sexes, live along the outer borders of the peristome. They firmly attach themselves to the soft tissues of the peristome, their anterior ends oriented toward the mouth of the host (Figure 11). Males actively move on the test and even move across the spines to other sea urchins.

The periprocts of unoccupied sea urchins are rapidly destroyed by crabs in the process of getting established in the rectum. The degree of visible damage to the sea urchins varies, depending on the size of the periproct as well as on that of the crabs involved. In one case, the plates of the apical system were left exposed. Normal epithelial tissue was found to completely cover the whole area after ten days. Whole sections of damaged tissue are sometimes rejected. Healthy, pigmented tissue starts appearing almost immediately.

Small females and males living in the rectum have been observed to move inside the intestine when efforts are made to capture them. It is not known if this occurs under normal conditions. Large females are not small enough to be able to enter into the intestine.

Morphology, Histology, and Histochemistry of the Periproct and Rectum.

The periproct is a membrane that surrounds the rectum and contains the anal opening. It is located at the aboral pole in all regular

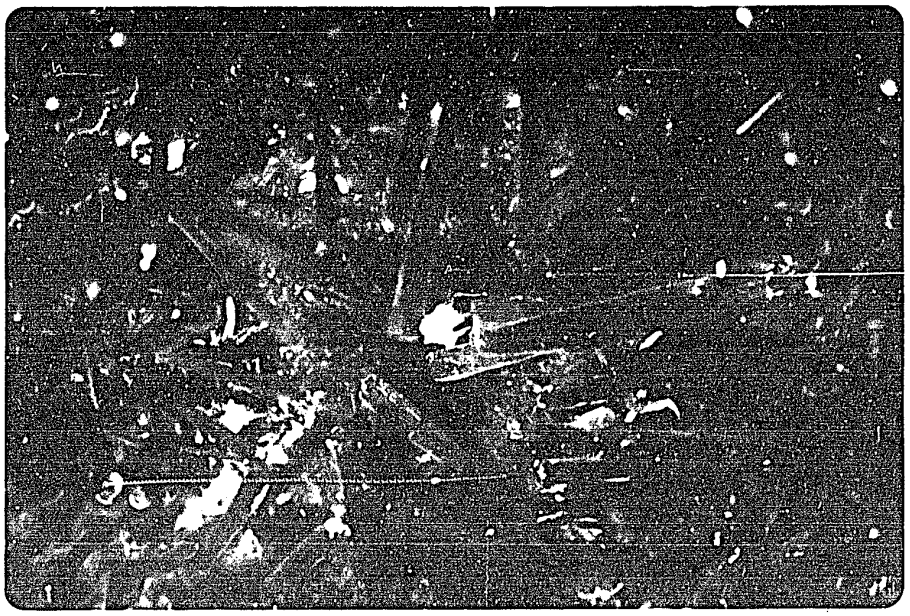


FIGURE 11. PERISTOME OF ECHINOTHRIX CALAMARIS SHOWING TWO MALE ECHINOECUS PENTAGONUS. (X1.8)

sea urchins. The periproct of E. calamaris is characterized by its large size. Its surface is studded with a large number of small, irregularly arranged endoskeletal plates (Figure 12). The anus is always located in the center of the body disc. It is capable of expanding when the fecal pellets are expelled. In juvenile individuals the periproct is light green with brown spots radiating from the dark brown anus. In older individuals the periproct is brown and not as conspicuous, being contracted part of the time.

The apical system is composed of five large genital plates with one occupied by the madreporite, and five terminal ocular plates (Figure 19). A pore in each of the terminal plates indicates the location of the terminal podium. A gonopore is located in each of the genital plates. All terminal plates are insert in relation to the periproct. A varying number of small and irregular plates is located along the inner margin of the periproct adjacent to the apical system. The number and size of these plates increase as the membranous part of the periproct decreases in size with age. The plates of the apical system, as well as those around the base of the periproct, are provided with short, secondary spines and tridentate pedicellariae. In those large individuals where the periproctal membrane is reduced, the spines of the periproctal plates cover the periproct when it is contracted.

The rectum is the expanded terminal part of the intestine. In E. calamaris it is expanded into a large, pocket-like cavity (Figure 13). It is completely encircled by the periproctal sinus which is a coelomic cavity separated from the perivisceral cavity by a thin, perforated

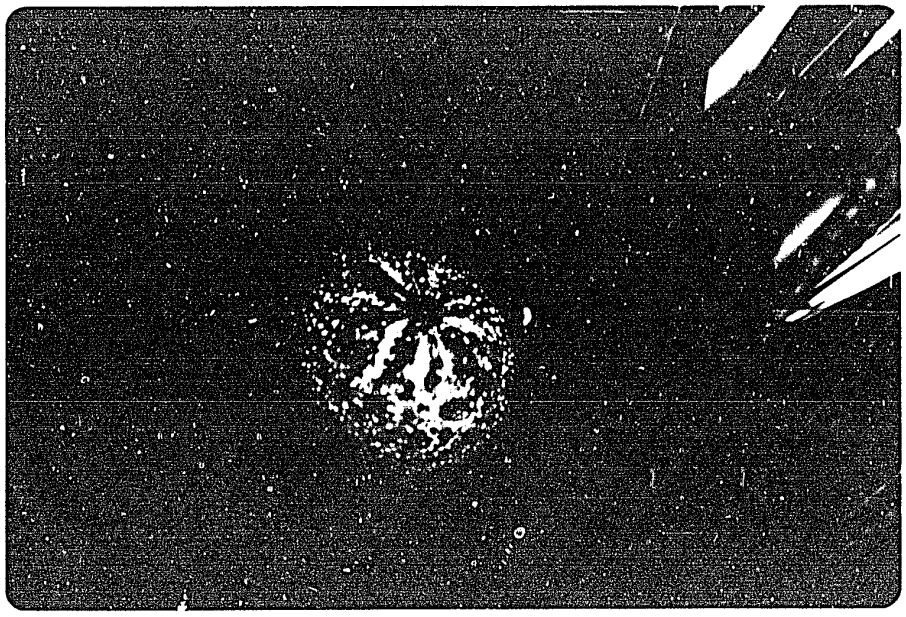


FIGURE 12. NORMAL PERIPROCT OF A JUVENILE *ECHINOTHRIX CALAMARIS*. (X1.8)

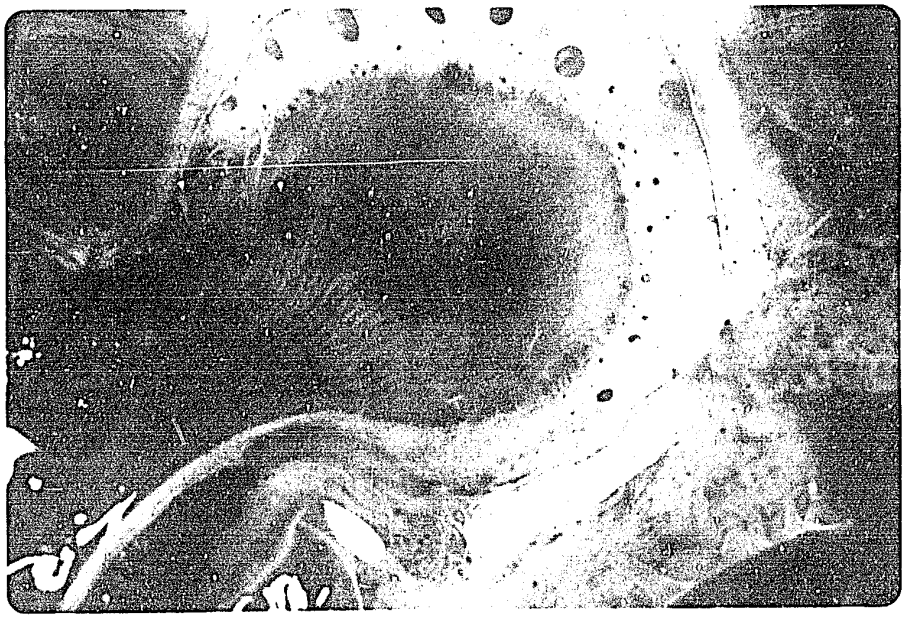


FIGURE 13. LOWER VIEW OF THE RECTUM OF *ECHINOTHRIX CALAMARIS* SHOWING THE PERFORATED PERIPROCTAL MESENTERY AND THE LOWER SURFACE OF THE PLATES OF THE APICAL SYSTEM.(X3.5)

membrane, the periproctal mesentery. This mesentery is attached to the lower surface of the rectum. Thin mesenteries connect the periproct to the outer wall of the rectum across the periproctal sinus. No evidence was found for the presence of a perianal sinus, a coelomic cavity around the anus (Hyman, 1955). The periproctal mesentery is surrounded by the ring-shaped aboral or genital coelomic sinus between the gonoducts and just below the genital and terminal plates. The long intestine curves twice around the inner side of the test before it enters into the rectum at the interambulacrum between rays III and IV (D and E). The periproct is covered by a single-layered external epithelium composed of columnar cells (Figure 14). These cells contain numerous pigment granules. No evidence of ciliation has been observed. A dermis of connective tissue is found below the outer epidermis. Its thickness varies from 80 to 270 microns. Round to oval endoskeletal elements are arranged along its outer margin. The dermis is composed of connective tissue fibers. These are more compact and regularly arranged in layers, sometimes in a wave-like pattern, around the endoskeletal granules and along the inner and outer margins. Small coelomocytes, probably lymphocytes, are commonly found among the connective fibers. Aggregations of large coelomocytes, identified as eleocytes, are present in some regions. Eleocytes, the so-called "morula cells" of some authors, are oval to spherical in shape. Their cytoplasm is filled with vacuoles. The diameter of each cell varies from 8 to 9 microns. No spicules have been observed between the loose fibers in undecalcified sections. It seems that this spongy arrangement facilitates the

FIGURE 14. CROSS SECTION OF A NORMAL PERIPROCT (ABOVE) AND RECTUM (BELOW) OF ECHINOTHRIX CALAMARIS. THE SPACE LEFT BY A SKELETAL PLATE CAN BE SEEN BELOW THE EXTERNAL EPITHELIUM (RIGHT). THE PERIPROCTAL SINUS AND A MESENTERY CAN BE SEEN IN THE CENTER. (MILLIGAN'S TRICHROME; X60)

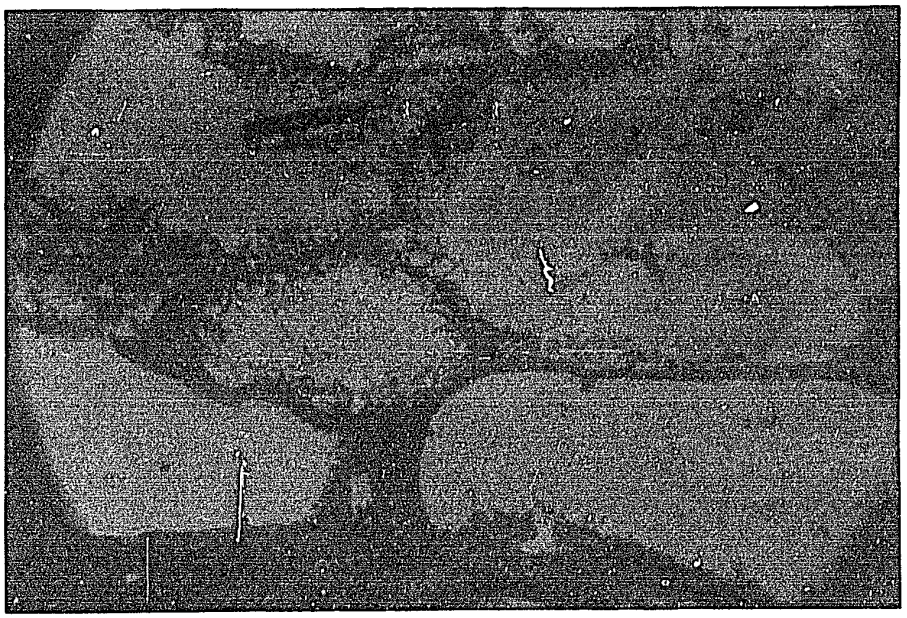


FIGURE 15. CROSS SECTION OF A CALCIFIED PERIPROCT (ABOVE) AND RECTUM (BELOW) OF ECHINOTHRIX CALAMARIS. THE PERIPROCTAL SINUS AND A MESENTERY CAN BE SEEN IN THE CENTER. (MILLIGAN'S TRICHROME; X60)

contraction and expansion of the periproct. Treatment of periproct tissue with 2% sodium hypochlorite has revealed that the round endoskeletal granules are composed of a fine meshwork of ring-shaped spicules fused to each other in a tridimensional pattern. The connective tissue layer is bordered along its inner surface by two thin muscle layers; specifically, a layer of longitudinal muscles followed by one of circular muscles, and by the coelomic epithelium or peritoneum that lines the periproctal sinus. The coelomic epithelium is composed of short cuboidal cells with large nuclei. The thin mesenteries that connect the periproct with the rectum are composed of connective tissue lined with coelomic epithelium. Their connective bands are continuous to those of the dermis of the periproct and the connective tissue layer of the rectum. Muscle layers seem to be absent.

The general histology of the rectum and the other regions of the digestive tube of sea urchins has been described by Hamann (1887), Stott (1955), Fuji (1961), and Lewis (1964). In E. calamaris, the lumen of the rectum is lined with inner epithelium composed of tall columnar cells (Figure 16). Their nuclei are arranged in a central position. This layer varies from 24 to 40 microns in thickness. In addition to the folding observed when the periproct is contracted, a secondary folding is observed along part of the surface of the inner epithelium. There is no histological or histochemical evidence for the presence of gland cells in the rectum. The inner epithelium is bounded along its inner edge by a thin basement membrane. Beneath this membrane is a layer of loose connective tissue. This layer is

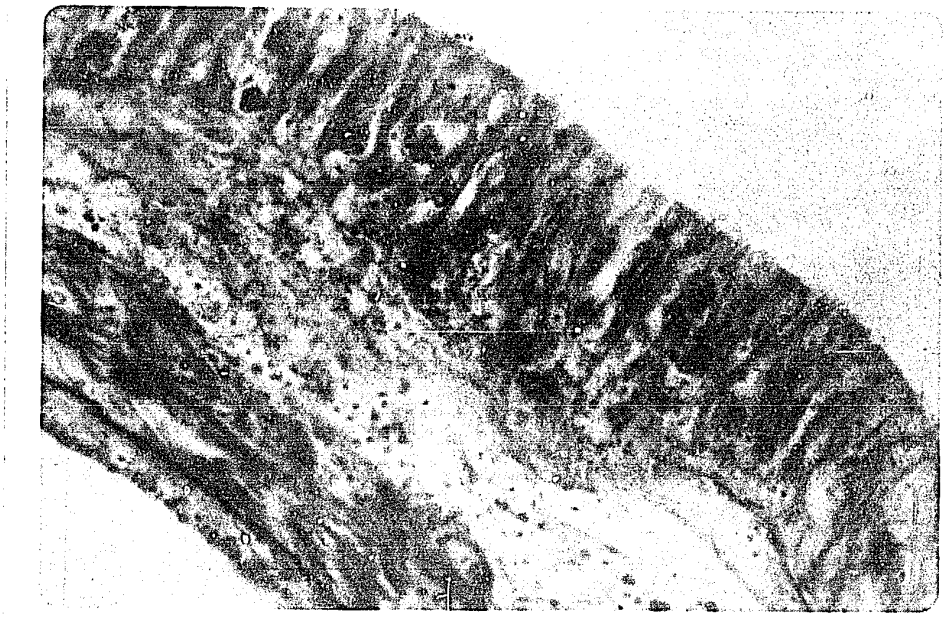


FIGURE 16. CROSS SECTION OF THE MEDIAL PORTION OF THE RECTUM OF AN INDIVIDUAL OF ECHINOTHRIX CALAMARIS PREVIOUSLY OCCUPIED BY A FEMALE ECHINOECUS PENTAGONUS. ELEOCYTES CAN BE SEEN MIGRATING ACROSS THE INNER EPITHELIUM. (PAS REACTION; X400)

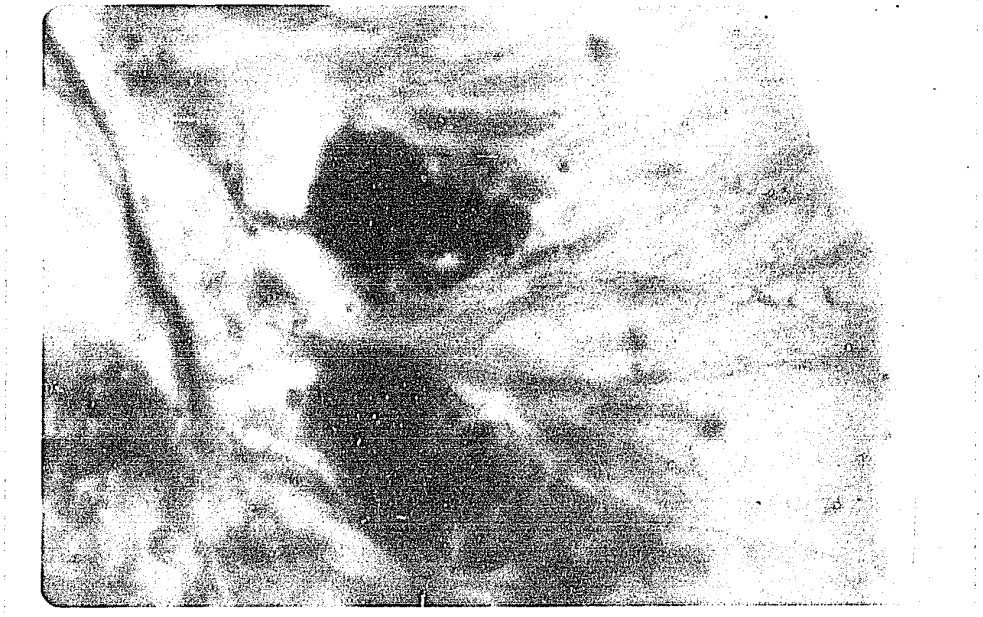


FIGURE 17. CROSS SECTION OF THE INNER EPITHELIUM OF THE MEDIAL PORTION OF THE RECTUM OF AN INDIVIDUAL OF ECHINOTHRIX CALAMARIS PREVIOUSLY OCCUPIED BY A FEMALE ECHINOECUS PENTAGONUS. TWO ELEOCYTES CAN BE SEEN MOVING BETWEEN EPITHELIAL CELLS AND ONE JUST BELOW THE BASEMENT MEMBRANE. (PAS REACTION; X1,600)

thinner than that present in the periproct. The fibers are loosely arranged and there are no dense concentrations along the margins. Coelomocytes, as well as concentrations of pigment granules, are present between the fibers. The inner border of the rectum consists of a thin layer of both circular and longitudinal muscles followed by the coelomic epithelium that lines the periproctal sinus and the perivisceral cavity.

The periproctal mesentery is composed of the same coelomic epithelium, muscle, and connective layers which are found bordering the coelomic cavities.

The anal opening is no different from the rest of the periproct. The periproctal sinus is contiguous with the anus, showing no evidence of a separate perianal sinus. The anus is bordered by a thick layer of the same external epithelium that lines the periproct. This cellular layer gradually fades as the inner epithelium of the rectum appears below the opening. There appears to be no specialized muscle layers.

Glycogen, i.e. PAS-positive, diastase-negative material, has been found to be present in the eleocytes and in intracellular granules in the inner epithelium of the rectum. Boolootian and Lasker (1964) have reported the histochemical determination of glycogen in eleocytes of Strongylocentrotus purpuratus (Stimpson). Fuji (1961) has reported no glycogen in the rectum of S. intermedius (A. Agassiz) but has demonstrated its presence in other parts of the gut. Presence of glycogen in granules of gut cells in S. purpuratus and and Dendraster, however, has been reported by Giese (1966).

Lipids, i.e. Sudan black B positive material, have been found accumulated in the inner epithelium of the rectum. Storage of neutral lipids in the gut has been shown to occur in S. purpuratus (Lawrence et al., 1966), Dendraster (Giese, 1966), and in three tropical Atlantic sea urchins (Lawrence, 1967). Fuji (1961) found lipids to be present in the intestine of S. intermedius but not in the rectum.

No acid or sulfated mucopolysaccharides occur in the rectum. The absence of mucopolysaccharides or of gland cells in the rectum of other sea urchins has been reported by Stott (1955), Fuji (1961), and Holland and Nimitz (1964).

Presence of the symbiont in the rectum induces heavy calcification of the periproct. A round to oval, cone-shaped structure replaces the soft periproct (Figures 7 and 19). It is composed of pentagonal and hexagonal plates contiguous with those of the apical system. The size of the plates gradually decreases from the proximal end. Secondary spines and tridentate pedicellariae are usually present. The total diameter of the calcified periproct in relation to the size of test appears to vary. This is probably due to differences in the relative age of the cone. Large cones with two openings have been found on several occasions. The anus is replaced by an irregularly shaped opening which varies from 6 to 12 mm in diameter.

A few changes in the morphology of the apical system are observed in sea urchins with large or oval-shaped calcified periprocts. In some cases one or two of the terminal plates are reduced or absent, with the cone reaching the edge of the ambulacra (Figure 19). The raising of part of the apical system, sometimes including the

madreporite, and surrounding interambulacral plates has been observed in a few individuals. Abnormalities in the genital plates have never been observed.

At least five sea urchins presented the peculiarity of having a regenerated periproctal membrane inside a calcified cone without a crab. The periproct in these individuals was always thin, not expanded, and dark brown. A large anal opening was observed in the center of each. The small skeletal plates of normal periprocts were usually observed. The complete calcified periproct of one of these individuals had been destroyed by a large female crab. A regenerated periproct was observed in an individual that was released without crabs in the shallow water lagoon of Coconut Island. The date of its release was not recorded but at least four months had elapsed before its recapture.

The thick skeletal plates of calcified periprocts occupy most of the connective tissue layer. Decalcified sections have revealed thin connective fibers around the large empty spaces previously occupied by the plates (Figure 15). These plates are composed of the same meshwork of ring-shaped spicules which make up the round plates of the normal periproct. They are continuous to the test skeleton. A reduction of the periproctal sinus is also evident but the thin mesenteries which connect the inner wall of the periproct with the rectum are still present. The secondary folding of the inner epithelium of the rectum is reduced or absent. No apparent change in the size or morphology of these cells is observed. There is no histological evidence of any mechanical damage to the inner epithelium.

One significant difference between abnormal and normal rectums is the conspicuous abundance of eleocytes in the inner epithelium of the former. Coelomocytes have been observed in the connective tissue layer and between the cells of the inner epithelium, apparently pushing their way to the lumen of the rectum (Figures 16 and 17). Small coelomocytes, the so-called lymphocytes (Endean, 1966) are also abundant. Smears of the reddish, mucous-like material that typically lines the lumen of occupied rectums was found to be composed almost completely of eleocytes (Figure 18). Some of the cells appear to have been disintegrating. Examination of this material under a phase microscope has revealed that pigmented as well as non-pigmented eleocytes (the latter usually referred in the literature as "colorless morula cells") were present. Non-pigmented epithelial tissue was also obtained in the smears. The lumen of normal rectums do not include any pigmented material. However, some eleocytes have been found in smears. Removal of the periproct from sea urchins not previously occupied by the symbiont appears to induce the appearance of pigmented material.

Morphology and Histology of the Peristome.

The peristome is a soft but strong fleshy membrane that extends from the edge of the oral border of the test to the mouth (Figure 11). The mouth, surrounded by a fleshy lip, is located in its center. The five teeth of the Aristotle's lantern can be seen protruding from the mouth. Five pairs of club-shaped buccal podia are located below the lip. These modified podia are believed to have a chemoreceptive function (Hyman, 1955). A large number of small oral plates are found

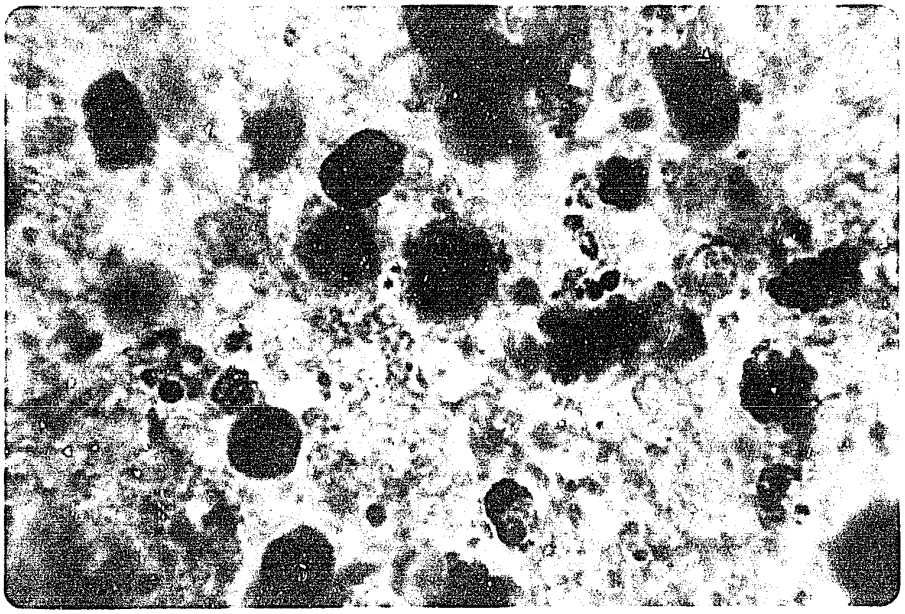


FIGURE 18. SMEAR OF THE COELOMOCYTE AGGREGATIONS IN THE RECTUM OF AN INDIVIDUAL OF ECHINOTHRIX CALAMARIS PREVIOUSLY OCCUPIED BY A FEMALE ECHINOECUS PENTAGONUS. THE COELOMOCYTES ARE PIGMENTED ELEOCYTES. (X600)

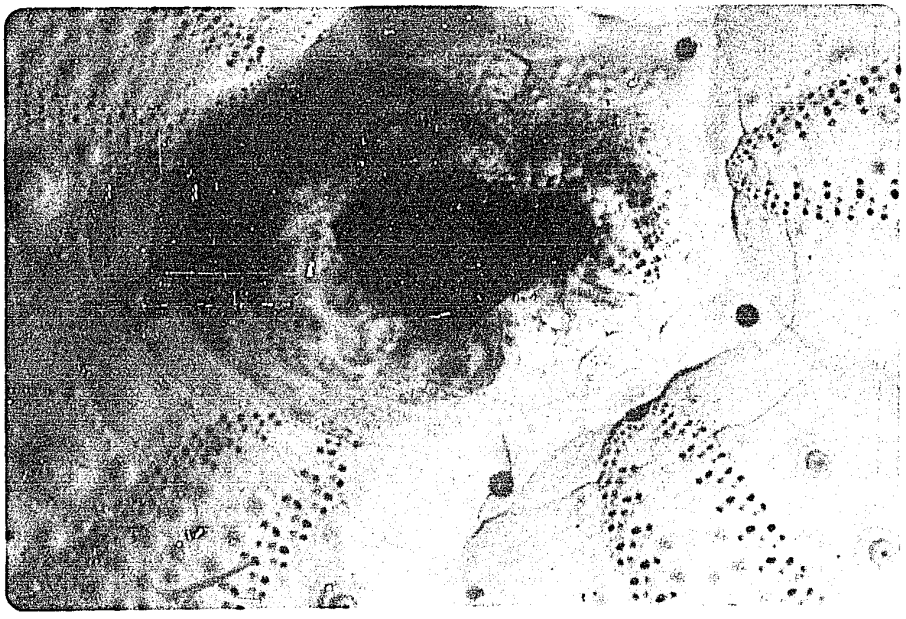


FIGURE 19. ABORAL VIEW OF THE CALCIFIED PERIPROCT AND APICAL SYSTEM OF THE CLEANED TEST OF AN INDIVIDUAL OF ECHINOTHRIX CALAMARIS PREVIOUSLY OCCUPIED BY A FEMALE ECHINOECUS PENTAGONUS. (X2.3)

embedded in the peristome and buccal lip. Plates along the ambulacral axis are much bigger than the interambulacral ones. The ambulacral plates immediately below the buccal podia bear short secondary spines. Others can bear pedicellariae and short spines. Five pairs of aborescent gills are found on the edge of the peristome, one at each of the junctions between the ambulacral and interambulacral regions.

The peristome, like the rest of the body, is covered by a heavily pigmented epithelium. The skeletal plates are embedded in the connective tissue layer. This is followed by the coelomic epithelium that lines the perivisceral cavity.

Host-Specificity

When placed in a tank with E. diadema, male and female crabs rapidly moved to the sea urchins. Crabs, especially the large females, produced visible damage to the sea urchins. The soft tissue at the base of the spines and test epithelium were torn off and ingested (Figure 20). Spines and podia were removed, leaving large exposed regions in the test. No crabs ever moved inside the rectum. The periproct was eaten or torn off in several occasions but the rectum was never occupied, even when small crabs were placed directly on the periproct. Damage to sea urchins was always observed after an overnight contact with crabs. Death usually followed, depending on the size of the sea urchins and the number of crabs present. On one occasion, about ten small sea urchins were left in a tank where two large females and several males and small females had been living in isolation for periods of six to eight days. After four days most of the sea urchins had missing spines and torn-off tissue. All sea

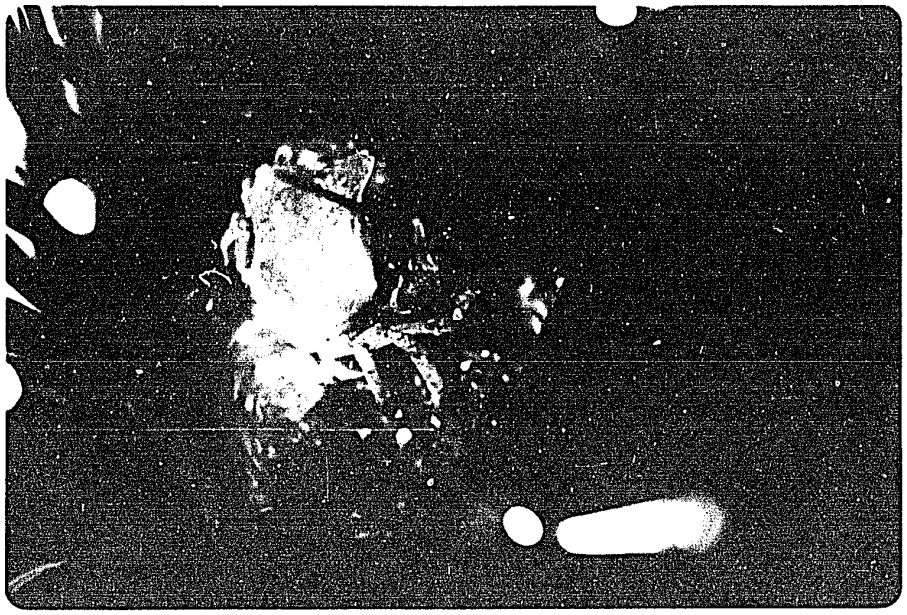


FIGURE 20. ADULT FEMALE OF ECHINOECUS PENTAGONUS LEFT OVERNIGHT ON THE TEST OF ECHINOTHRIX DIADEMA. (X1.8)

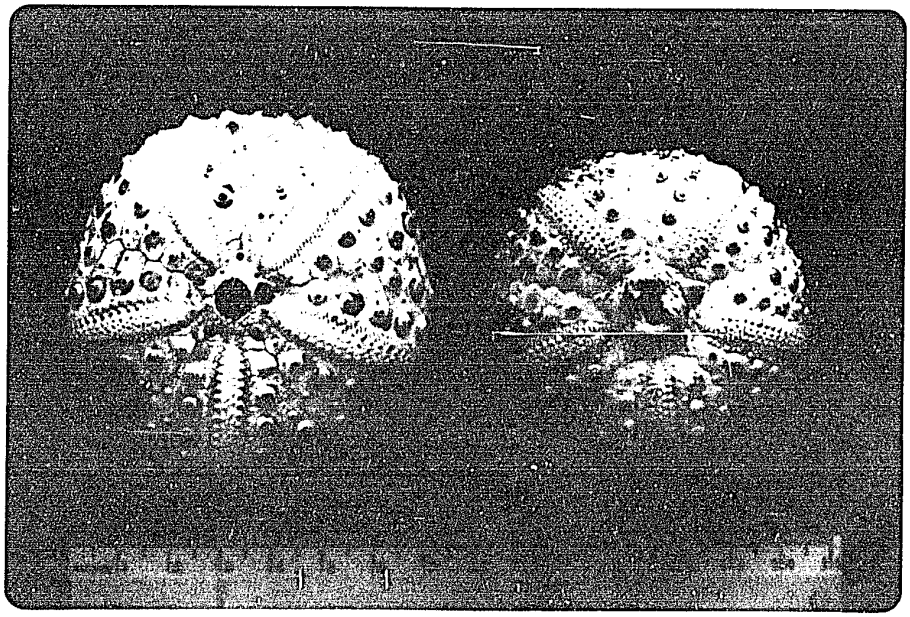


FIGURE 21. CLEAN TESTS OF ECHINOTHRIX DIADEMA (LEFT) AND OF A JUVENILE ECHINOTHRIX CALAMARIS. (SCALE IN CENTIMETERS)

urchins were dead after two days. Crabs established on E. diadema moved to any specimens of E. calamaris that were added to the tanks.

A large number of spines were missing from the only individual of E. diadema that served as host to the symbiont in nature. Its test epithelium had been torn off. The sea urchin was recorded as "dying."

Trials involving two individuals of Diadema paucispinum and several crabs suggest an apparent incompatibility between the sea urchin and the crab. The latter appears to show no active attraction to the sea urchin. Sea urchins reacted violently when crabs were placed in contact with them. The rapid movement of the long primary spines prevented the crabs from moving into the test. Crabs always moved away from the sea urchins, even when left for as long as three days. These crabs rapidly moved to either E. calamaris or E. diadema, if present. The only exception was a small male which was placed directly on the test of one sea urchin. After four days it had removed six or seven primary spines, an undetermined number of secondary spines and podia, and a large amount of epithelial tissue from an area in the ambitus of the test. The crab was removed after four days. Complete healing of the test epithelium was observed after ten days.

Epithelial tissue and tube feet of both E. diadema and D. paucispinum were eaten by isolated crabs, including postlarval stages reared in the laboratory.

Isolated crabs moved away when placed on the toxopneustid sea urchin Tripneustes gratilla (Linn.), a large brownish form with short spines.

DISCUSSION

The distribution of the symbiont in its host follows a pattern observed in other symbiotic crustaceans, i.e. adult females are restricted to gall-like structures and males moving freely on the host. This arrangement has an obvious adaptive value to both the symbiont and its host. Pathological calcification produced by the host as a defensive mechanism is at the same time exploited by the symbiont, which utilizes it as a protective enclosure. Giard (1911) gave the name "thalacies" to galls and similar tissue reactions produced as a response to the presence of symbionts.

The calcification of the periproct is the most obvious response of E. calamaris to the presence of crabs in its rectum. This response can be considered as an example of pathological calcification, a reaction of tissue to physical, chemical or living injury (Einstein, et al., 1960). Pathological calcification is seen as a defense mechanism at the tissue level. Physical contact with the crabs appears to be the main effector of calcification. A reduced, but otherwise normal, periproctal membrane is regenerated when crabs are removed, even when the calcified structure remains. In addition to physical irritation (ingestion of tissue, mechanical contact, respiratory currents), chemical factors can be involved in the inducation of calcification. The process appears to be a continuation of the calcification observed at the base of normal periprocts in large sea urchins. The calcification process in echinoderms has been reviewed by Raup (1966).

Calcification in the form of gall-like structures is a common

phenomenon among echinoderms serving as hosts to a variety of symbionts. At least four species of copepods are known to induce the production of galls in sea urchins. Pionodesmotes phormosomae Bonnier forms spherical galls in the test of the echinothurid sea urchin Hygrosoma petersii (A. Agassiz) (Bonnier, 1898), Calvocheres globosus (Hansen) produces galls in the primary oral spines of the echinothurids Calverisoma gracile (A. Agassiz) (Hansen, 1902), and Sperosoma quincunciale de Meijere (Agassiz and Clark, 1909), and Calvocheres oblongus (Stephensen) in the primary oral spines of Hygrosoma petersii (Stephensen, 1935). Galls in the test of the fossil sea urchin Collyrites dorsalis have been attributed to a copepod, Castexia douvillei (Mercier, 1937). Other copepods form similar galls in ophiuroids (Caullery, 1952; Hyman, 1955). Several symbiotic gastropods induce gall production in numerous asteroids and at least in one echinoid (Caullery, 1952). Myxostomid annelids produce calcified structures in crinoids (Clark, 1921).

Gall-producing habits are also observed among brachyuran crabs of the family Hapalocarcinidae that are symbionts of madreporarian corals (Potts, 1915). Females are imprisoned in galls produced by the coral while the males are free-living on the host.

The symbiotic association between the pinnotherid crab Pinnaxodes chilensis (A. Milne Edwards) and sea urchins presents a number of similarities to that of Echinoecus pentagonus. It is found in association with the echinometrid Coenocentrotus gibbosus (Valenciennes) and the echinid Loxechinus albus (Molina), both littoral forms found along the western coast of South America. This

crab has been recorded from Ecuador to southern Chile (Garth, 1957). Several female crabs (collected from L. albus) and two sea urchins were sent to me through the courtesy of Mr. Marco A. Retamal, Universidad de Concepción, Chile. Females live in the rectum, which expands into a thin, sac-like structure that extends almost to the oral surface of the host. The rectum is rich in a thick, pigmented material (dark brown in the alcohol-preserved specimens) very similar to the eleocyte aggregations of Echinothrix. Only a few algal filaments could be identified under a microscope. It seems that this material is composed of feces as well as cells or mucous from the host. The exoskeleton of the females is soft. They closely resemble Echinoecus in the general morphology of their carapace and chelipeds. The abdomen is much expanded. Carapace width of eight females varied from 16.5 to 20.4 mm. According to Verrill (1867), who has given one of the few accounts on the biology of this symbiont, males live among the spines of the host. Photographs presented by Rathbun (1918) show that the males have a less rounded carapace. She has indicated that their carapace is hard. The crab appears to be very common in both species of sea urchins, even when several authors have considered it to be more common in C. gibbosus. Distortion of the shell seems to be restricted to C. gibbosus, a smaller form. Verrill has reported that the test is "usually swollen on the sides over the cyst, and the anal area is depressed and distorted." A reduction of the size of the genital and terminal plates is seen in the drawings given by Jackson (1912). No pathological calcification is apparent. An irregular and excentric opening is left in the periproct through which

the legs of the crab can be seen. The gonopores seem to retain their normal size. There are no apparent changes in the morphology of the apical system of L. albus. The periproct is very small and the presence of females in the rectum is not obvious from the outside. Test dimensions for both species have been given by Mortensen (1943). In ten specimens of L. albus, the test diameter varied from 13 to 98 mm and the diameter of the apical system from 4 to 17 mm. Seven specimens of C. gibbosus had test diameters varying from 20 to 51 mm and apical systems from 5.5 to 11 mm.

The rectum and the surrounding aboral surface of echinoderms can be suggested as an advantageous microhabitat for symbionts. The feces of the host, usually rich in organic matter, possibly serve as a food source. Additional protection from predators is provided for those forms living inside the rectum or intestine.

The pontoniid shrimp, Tuleariocaris holthuisi Hipeau-Jacquotte, is found living on the aboral spines of two sea urchins in Madagascar. It has been suggested by Hipeau-Jacquotte (1965) that this shrimp ingests feces. The portunid crab, Lissocarcinus orbicularis Dana, is occasionally found in the anus of holothuroids (Needham, 1966). All seven species of the pinnotherid genus Dissodactylus live in association with the oral and anal region of several species of the sand dollars Mellita and Encope in tropical North American waters (Rathbun, 1918; Rioja, 1944). Hyman (1955) has suggested that the crabs feed on the feces of the host. Other examples among crustaceans are given by Hyman (1955).

E. pentagonus is the only eumedonid crab that is known to invade

the rectum of its host. Yet, many of the 25 species that are included in the subfamily are very rare, being known only from a few specimens obtained from dredged material with no information on their specific habitats.

Proechinoecus sculptus Ward was described from specimens collected from the oral surface of the intertidal sea urchin (Colobocentrotus atratus (Linn.) (Ward, 1934). Zebrida adamsi White has been reported from three sea urchins: the temnopleurid Salmacis bicolor rarispina L. Agassiz, Toxopneustes piloleus (Lamarck) (Serène et al., 1958), and Anthocidaris crassispina (Sakai, 1938). Mortensen (1904) has reported it as "making a path" on the test and eating tube feet, pedicellariae, and spines in the first two hosts. Another species is known from one specimen. Two species of Eumedonus have been reported from sea urchins: E. zebra Alcock from Goniocidaris biserialis (Doderlein) (Sakai, 1965) and E. granulosus MacGilchrist (as E. zebra) from an unidentified sea urchin (Lenz, 1905). Two other species are known only from a few specimens with depth as the only information given. Five out of seven species of Harrovia and four of the six species of Ceratocarcinus are known to be associated with crinoids. One particular species, H. truncata Rathbun, is known from three specimens dredged from deep water in Hawaii (Rathbun, 1906; Edmondson, 1951). Rhabdonotus pictus A. Milne Edwards has been recorded from crinoids and from the pennatulacean Virgularia (Serène and Romimohtarto, 1963). The remaining genera, Gonatonotus (with two species) and Dentoxanthus (with one species) are also known from a few dredged specimens.

The presence of E. pentagonus in the rectum raises the question of how much injury it causes to its host. Males and small females,

which ingest epithelial tissue and tube feet, appear to produce not much visible damage to the peristome and test. Furthermore, damaged tissue is rapidly regenerated. In one case, the pigmented epithelium regenerated three days after being completely removed with a spatula, leaving the white skeletal plates exposed. Heavy calcification of the periproct is the most obvious effect. However, partial calcification and reduction in the size of the periproct is observed in large, unoccupied sea urchins. No specific function can be attributed to the periproct other than to protect the rectum. Large and conspicuous periprocts are characteristic of some diadematids, especially in the juveniles. A respiratory function can be suggested but it is doubtful due to the thickness of the external epithelium. It is noted that the role of the rectum as a nutrient storage organ has been suggested by Lawrence et al. (1966).

No morphological changes appear to take place in the rectum. Only an increase in the number of eleocytes in the inner epithelium and lumen has been observed. Numerous functions have been attributed to the considerable variety of coelomocytes that are ubiquitously found in the coelomic fluid and tissues of echinoderms (Boolootian and Giese, 1958; Endean, 1966). Eleocytes have been suggested as having a role in the translocation of nutrients (Boolootian and Lasker, 1964) and even in epidermal digestion and absorption (Pecquignat, 1966). Echinochrome, a red naphthoquinone pigment characteristic of sea urchins, has been found present in eleocytes (MacMunn, 1885). The possibility of its connection with the transport and storage of oxygen has been refuted (Farmanfarmaian, 1966). Another orange-red pigment

has been found in the eleocytes of Diadema antillarum (Millot, 1957). The pigment, also found in colorless coelomocytes, changed to the red form and even to black and brown melanin-like pigments when oxidized on exposure to air. In addition, an orange-red naphthoquinone has been found in the coelomocyte aggregations of E. calamaris (see Section V). The increase in the number of eleocytes in rectums occupied by the symbiont can be associated with a deposition of pigment due to the direct exposure of the tissue to light. Nevertheless, some eleocytes have also been observed in the lumen of normal rectums. An excretory function can also be suggested. Van der Heyde (1923) has expressed the opinion that the intestine of echinoderms, especially "the terminal parts", function as an "organ of excretion." On the other hand, Lewis (1967) has found that the excretion of ammonia nitrogen in the rectum of D. antillarum averaged lower than any of the five regions of the digestive tract studied by him. It is significantly higher in the hind gut. A combination of the last two possibilities has been proposed by Fox (1953) by suggesting that the naphthoquinone pigments act as excretory products.

Ingestion of external epithelium and podia by the males and small females is offset by the remarkable regenerating abilities of the sea urchin. These small individuals are found in relatively small numbers (see Table IV) and their mobility does not restrict them to a single sea urchin. The large females live in the rectum, where abundant, mucous-like eleocyte aggregations and fecal pellets are utilized as a food source. There is no evidence for the ingestion of inner epithelium. Perforation of the thin rectum results in the

loss of coelomic fluid. Serious damage is produced only when large females are placed outside the rectum (in individuals with the rectum occupied by a female) or in an abnormal host, where the rectum is not invaded. Test epithelium, podia, and the tissue at the base of the spines are ingested (Figure 20).

It appears appropriate to conclude on the basis of morphological changes that the normal functions of the periproct and rectum appear not to be visibly impaired by the presence of the symbiont. Analysis of the available data on size differences has shown no correlation between this variation and the presence of crabs. There is also no evidence for any morphological or physiological damage to the reproductive system.

The host's mechanisms of defense against the symbiont must also be taken into consideration. Defense mechanisms may be classified as behavioral (those involving the whole individual or specialized external organs), tissue, or cellular. The activity of pedicellariae is probably the only effective behavioral defense mechanism, being involved in capturing any small swimming organisms that come in contact with the sea urchin. Their implication in the prevention of the settling of the symbiont's larvae will be discussed in Section VI. Calcification of the periproct is considered here as a defense mechanism at a tissue level. Cellular mechanisms, phagocytosis by specialized coelomocytes in the case of echinoderms (Endean, 1966; Hilgard et al., 1967; Johnson, 1969), are obviously not directly involved in any significant reactions against the symbiont under consideration. No amoebocytes were observed in the coelomocyte aggregations of the rectum. Crabs also appear not to be affected by the possible presence of enzymes

in the rectum or fecal pellets, or by toxins in the tissue that is ingested. Stages living free on the test are not big enough to be mechanically injured by the secondary spines which are known to contain a pressor substance, probably noradrenaline, associated with a sac-like structure at their tips (Alender, 1966).

Adult crabs are attracted to its host when removed from it. Since they are restricted to the sea urchin, it is in the settling larval stage (or in an early postlarval stage) that behavioral patterns that are involved in their attraction to its normal host or to the specific environment where the host is found, can be considered to have any adaptive significance. Only the possible attack on the sea urchin by predators could cause the symbiont to move out of its host. In such situations it can be assumed that the symbiont is also eaten. The response to "attractants" or "host factors" has been investigated in some symbionts (see Section VI).

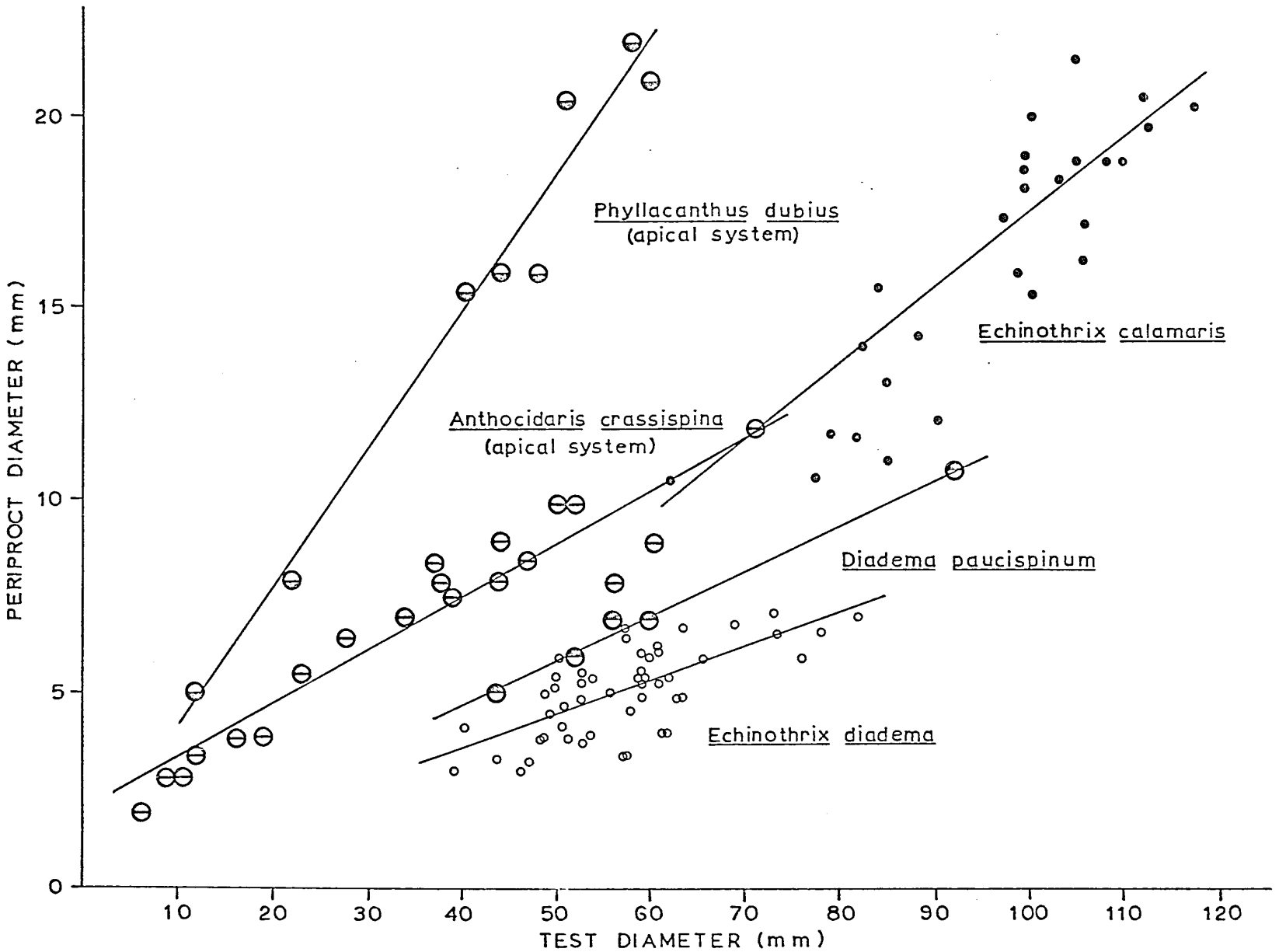
An apparent incompatibility between the symbiont and E. diadema and D. paucispinum is suggested by their behavior under laboratory conditions. Isolated crabs appear to be attracted to E. diadema. Nevertheless, heavy damage follows as a consequence of their presence in both species of sea urchins. The dynamic equilibrium that exists between the symbiont and its normal host in Hawaii is apparently never established in the two other diadematids. The presence in nature of a crab living on E. diadema is probably accidental. Even when the crab was a small female, damage to the sea urchin was considerable.

Several possible answers can be suggested to the question of why the symbiont is not normally found in E. diadema or D. paucispinum.

One obvious answer is the fact that the periproctal area in these two species is not large enough to permit its occupation by the symbiont. This has also been suggested by Miyake (personal communication). Large females thus remain on the test, ingesting more tissue than the host can regenerate. A comparison between the regression of periproct diameter on test diameter in the three species is given in Figure 22. A large periproct and a larger increase in periproct diameter with size is shown by E. calamaris. The relatively larger size of its periproct in relation to that of E. diadema is shown in Figure 21.

Crabs have been reported three times from the rectum or "anal region" of E. diadema (provisionally identified by Bouvier and Seurat, 1905; Mortensen, 1940; Holthuis, 1953), and from the "anal region" of unidentified species of Diadema (Serène et al., 1958; Eldredge, personal communication) (see Table I). Bouvier and Seurat recorded one female in a calcified periproct, Mortensen observed "calcification to the apical system," and Serène et al. recorded two small males. Some sea urchins have been described as having lost spines. The crabs recorded by Holthuis were obtained from the "anal plate region." Their host appears to be E. calamaris judging from the description. No information was given on the size of the hosts in any of these records. The possibility exists that E. diadema attains a larger size in other areas, a case similar to that of E. calamaris in Kaneohe Bay. Test diameter values given by Mortensen (1940) for non-Hawaiian specimens of E. diadema show that the largest individuals are larger than those collected in Hawaii. Values of Mortensen's

FIGURE 22. RELATIONSHIP BETWEEN PERIPROCT OR APICAL SYSTEM DIAMETER AND TEST DIAMETER IN JUVENILE AND ADULT SPECIMENS OF *ECHINOTHRIX CALAMARIS*, *E. DIADEMA*, *DIADEMA PAUCISPINUM*, *PHYLLACANTHUS DUBIUS*, AND *ANTHOCIDARIS CRASSISPINA*.
Data for *P. dubius* and *A. crassispina* taken from Mortensen (1928, 1943).


PERIPROCT DIAMETER (mm)
TEST DIAMETER (mm)
0
5
10
15
20
10
20
30
40
50
60
70
80
90
100
110
120
Phyllacanthus dubius
(apical system)
Anthocidaris crassispina
(apical system)
Echinothrix calamaris
Diadema paucispinum
Echinothrix diadema

15 specimens vary from 6.5 to 94.0 mm ($\bar{x}$ = 47.1 ± 27.1 mm), whereas those of 50 individuals collected from various locations in Kaneohe Bay vary from 39.4 to 81.7 mm ($\bar{x}$ = 57.1 ± 8.9 mm). The smaller mean diameter observed in Mortensen's specimens is due to a greater spread (standard deviation more than three times as large as that of the Hawaii specimens). However, values of the periproct diameter in Mortensen's largest specimens (9.0 to 13.5 mm) correspond to relatively small, juvenile Echinothrix calamaris (test diameter of approximately 50 to 60 mm), forms whose rectums are not normally occupied by crabs.

The symbiont is also found in two other sea urchins: Phyllacanthus dubius, a West Pacific species in which the crab has been reported as occupying the rectum, and Anthocidaris crassispina, another West Pacific form in which the oral region is occupied instead. The regression of apical system diameter on test diameter in these two species is compared to the three Hawaii diadematids (Figure 22). Only the diameter of the apical system (periproct plus apical plates) was available for comparison, with the data taken from Mortensen (1928, 1943). Judging from Mortensen's illustrations, Figures 23 and 24, the periproct proper is approximately half of the apical system diameter in both species. Even if the values are not corrected by a factor of two, P. dubius shows a situation similar to that of E. calamaris, and A. crassispina to that of the other two diadematids.

P. dubius (family Cidaridae) is a littoral form found only in the Bonin Islands, southeast of Japan. The periproct is listed as "small" by Mortensen (1928). The apical system is about 40% of the test diameter. Miyake (personal communication) reports that the symbiont

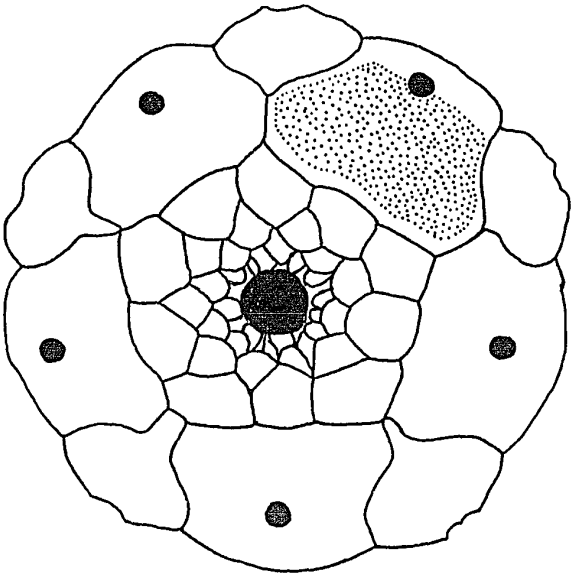


FIGURE 23. PERIPROCT (CENTER) AND THE PLATES OF THE APICAL SYSTEM IN PHYLLACANTHUS DUBIUS.
(From Mortensen, 1928; X4.5)

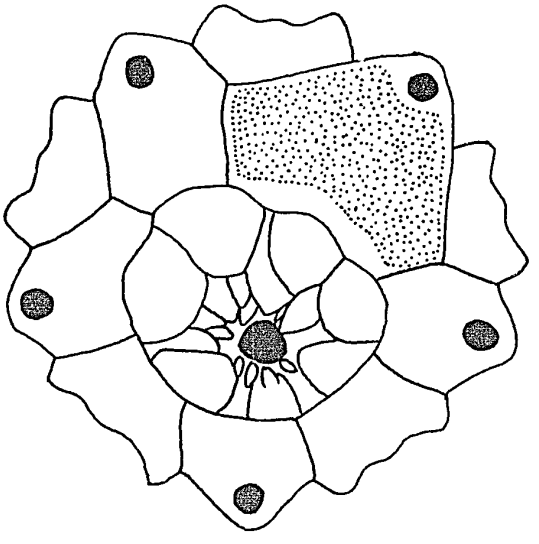


FIGURE 24. PERIPROCT (CENTER) AND THE PLATES OF THE APICAL SYSTEM IN ANTHOCIDARIS CRASSISPINA.
(From Mortensen, 1943; X6)

makes "a bag ... similar to the Hawaii's case." The symbiont is also associated with E. calamaris in the Bonin Islands (Rathbun, 1897; Miyake, 1939).

Anthocidaris (= Heliocidaris) crassispina (family Echinometridae) is also a littoral form. It has been recorded from southern Japan to Hong Kong. The apical system is approximately 20% of the test diameter. The peristome is described as "rather naked," with small plates and no spines (Mortensen, 1943). Its test is relatively small. Twenty individuals examined by Mortensen from various localities had test diameters that ranged from 6.0 to 71.0 mm ($\bar{x}$ = 34.7 ± 18.8 mm). It appears from the records in the literature (Sakai, 1936; 1938; 1965) that in Japan the symbiont is normally associated with the oral region of Anthocidaris. This would imply that no serious damage is produced to the sea urchin. The presence of smaller crabs in Japanese waters would explain such a situation. Only Sakai (1938) gives the measurements of a crab collected from the sea urchin, an individual of undetermined sex with a carapace width of 6.5 mm. The female in his illustration has a carapace width of approximately 7 mm. The "free swimming" individuals recorded by Miyake (1939) included three females with carapace widths of 5.4, 7.0, and 7.3 mm, respectively, and a male with a carapace width of 6.7 mm. The females are smaller than those collected in Hawaii ($\bar{x}$ = 12.0 ± 2.2 mm; N = 86) or in other areas of the Pacific. This, of course, is only a suggestion based on extremely limited data. Anthocidaris is also a host for the eumedonid Zebrida adamsi (Sakai, 1965).

Differences observed in the habitat of the three species of

Echinothrix and Diadema in Hawaii are not significant enough to have any effects on the distribution of the symbiont. E. diadema typically is found in crevices and similar protected areas in shallow waters ranging from 1 to 3 meters, but large populations can be found in depths of 6 to 8 meters, a habitat typically occupied by E. calamaris (see Figures 3 and 4). D. paucispinum has always been collected in areas where E. calamaris is common. Mortensen (1940) has recorded the species as "littoral down to 40 meters." All three species have been found living sympatrically in Kaneohe and Kealakekua Bays.

Nutritional requirements appear to be provided by all three diadematids. Postlarval as well as the adult stages were fed on tissue from all the sea urchins. Some postlarval crabs lived on E. diadema for some time (see Section VI).

SECTION V

THE ASSOCIATION: NUTRITIONAL ASPECTS

INTRODUCTION

Physiological, behavioral, and morphological adaptations in numerous symbiotic organisms enable them to utilize host material in order to meet at least part of their nutritional requirements. Metabolic dependency, being in the form of nutrients, enzymes, or development-inducing factors (Cheng, 1967), can be considered as a normal outcome of close symbioses. Development of metabolic dependency by one or both partners implies that critical adjustments have taken place in order that the utilization of metabolites by one side be in a dynamic equilibrium with its production and utilization by the other. In the case of the association of Echinoecus pentagonus and its host, the question of how such an equilibrium is in effect has been partly answered by estimating the energy relationships between both species.

The nutritional relationships in symbioses have been studied mostly in parasitic helminths (see review by Read, 1968) and in endozoic algae and related symbioses (see review by Smith et al., 1969). Most of the work has been restricted to the determination of nutritional requirements or the utilization of certain metabolites. The subject of energetic relationships in symbioses remains practically untouched.

MATERIALS AND METHODS

Ingestion and Assimilation of Host Tissues.

The stomach contents of numerous crabs were analyzed in order to determine their diet. Almost all individuals were removed from sea urchins which had been collected from their normal habitats a few hours before. Stomach contents of crabs isolated from their host were also analyzed. The weight and feeding habits of isolated crabs were recorded in a number of marked individuals placed in a 40 gallon tank provided with sand, algae, dead coral, and detritus.

Qualitative determination of the ingestion of host tissue by the symbiont was carried out by injecting uniformly labelled D-glucose-^{14}C (Volk Radiochemical Co., Burbank, California; specific activity of 210 mc/mM) into the perivisceral coelom of sea urchins and determining the amount of labelled material found present in the tissues of crabs found living in the rectum, peristome, and test. Six sea urchins, each with a female or male in the rectum and another crab on the peristome or test, were injected through the peristomial membrane with 100 µc (0.1 ml, equivalent to 84.6 µg of D-glucose) of the glucose solution using a disposable 1.0 ml syringe. The small amount of ethyl alcohol present in the solution as a preservative (20% per volume) was found to produce no visible effects on sea urchins after a similar non-labelled solution was injected into one individual. The injected animals were kept in a 115 gallon Fiberglass tank provided with running sea water. Two additional sea urchins, each with two crabs, were kept in the same tank as controls. All sea urchins had been starved for two to three days and few or no fecal pellets were being voided. One sea urchin was removed 3, 6, 12, 18, 24, and 48 hours after the injection

of the glucose. A control sea urchin was removed 24 hours and the second 48 hours after being in contact with labelled individuals. Crabs werethoroughly washed with sea water and frozen. After weighing (frozen dry weight), each crab was sonicated (using a Biosonik oscillator) in 10% methanolic trichloroacetic acid (TCA) over ice. The samples were subsequently placed in a water bath-shaker at 48°C for two hours, centrifuged, and the supernatant adjusted to 4.0 ml with anhydrous methyl alcohol before counting. To the TCA-insoluble material 4 ml of 2N methanolic potassium hydroxide were added, stirred, placed in a water bath-shaker at 48°C for 18 hours, centrifuged, and the supernatant similarly adjusted to 4.0 ml for counting. The insoluble material that remained was separated by centrifugation into skeletal fragments and a white precipitate that appeared to be insoluble protein. Both residues were washed with absolute methyl alcohol and dissolved in 4.0 ml concentrated perchloric acid in an ice bath in order to slow down the reaction. All samples were decolorized overnight with 5 to 10 drops of 30% hydrogen peroxide under direct light. Samples were counted in a Beckman LS-100 liquid scintillator at appropriate preset errors and counting times. Beckman's "Fluoralloy" (a mixture of naphthalene, butyl PBD, "Cab-O-Sil," and PBBO) dissolved in distilled dioxane was used as the scintillant mixture. Background corrections were made with reagent blanks. Quenching corrections were made from a quench ratio curve prepared for most of the digestions used throughout the experiments: TCA, potassium hydroxide, and formic acid. Unlabelled tissue was digested and decolorized as in the previous experiments. A ^{14}C-labelled bicarbonate solution was used as the external standard.

The rate of translocation and deposition of labelled material in the sea urchin was studied by injecting 100 μc of the same labelled glucose into each of two individuals, one with a previously occupied rectum and the other with the periproct removed. Both had been starved for approximately one week. At appropriate intervals, samples were taken from the coelomocyte aggregations along the walls of the rectum, from the external epithelium of the peristome, and from the coelomic fluid. The tissue samples were collected with small capillary tubes and transferred into individual test tubes containing 0.5 ml absolute methyl alcohol. The alcohol was evaporated in a water bath at 40° - 45°C and the tissue dissolved at the same temperature in 1.0 ml 1N aqueous sodium hydroxide. Half of the solution (0.5 ml) was counted as in the previous experiment and the remaining volume was used for the determination of total protein nitrogen using the method of Lowry et al. (1951). A standard protein curve was prepared by using frozen dry bovine serum albumen dissolved in 1N sodium hydroxide. The coelomic fluid was sampled by removing 0.5 ml of fluid through the peristomial membrane using 1.0 ml disposable syringes. An equal volume of 10% aqueous disodium ethylenediaminetetraacetic acid (EDTA) at pH 8.0 was used as the anticoagulant. To separate the coelomocytes from the fluid, the samples were immediately centrifuged at 6,000 rpm in a Servall refrigerated centrifuge for 30 to 45 minutes before being frozen for further analysis. The coelomocytes were transferred with Pasteur pipettes, washed with 10% EDTA, and idssolved in 1.0 ml of concentrated formic acid. The coelomocyte fraction and 0.1 ml of the cell-free fluid were counted as the rest of the samples. Similar coelomic fluid samples were taken from a third labelled sea urchin.

Samples from the rectum, gonads, esophagus, intestine, and axial complex of a sea urchin injected 48 hours before were digested in concentrated formic acid, decolorized with 30% hydrogen peroxide, and counted. A 1.0 ml cell-free sample of the coelomic fluid was treated and counted as in the previous experiment. Analysis of the labelled material in the rectum of another sea urchin injected 48 hours before was also carried out. The tissue was first homogenized in cold 10% aqueous TCA and centrifuged. The glycogen in the supernatant was precipitated with 95% ethyl alcohol (Clark, 1964), isolated, dissolved in concentrated formic acid, and counted. The TCA-alcohol fraction was decolorized and counted. The insoluble fraction was extracted with 1.0 ml 1N aqueous sodium hydroxide and the remaining material digested in concentrated formic acid. Both samples were decolorized and counted. Coelomocyte aggregations from the rectum of an additional sea urchin which had been injected 48 hours before were extracted in cold 10% aqueous TCA. The supernatant was decolorized and one portion counted. Two volumes of 95% ethyl alcohol were added to the remaining supernatant for the precipitation of glycogen. The TCA-alcohol supernatant was counted after centrifugation. The insoluble material was digested in concentrated formic acid, decolorized, and counted. Corrections for quenching were not made for sea urchin samples.

Autoradiographs were made of sections from the rectum of a sea urchin which had been injected 24 hours before with labelled glucose. Frozen sections 5-10 μ thick, of the tissue were made on a cryostat. These were mounted on slides which had been previously cleaned and immersed in a 0.5% gelatin-.05% chromium and potassium sulfate subbing

solution (Gude, 1968). The mounted sections were stained with Harris hematoxylin and eosin or a 0.25% solution of fast green (Humason, 1967), covered with a thin layer of celloidin and subbing solution, and coated with Kodak Nuclear track liquid emulsion (type NTP). The preparations were exposed for 62 days at 4°C, developed, and mounted in Permount.

The distribution of labelled material in crabs was analyzed in another set of experiments. In the first trial, four crabs, three females and one male, were kept for 48 hours on a sea urchin injected with labelled glucose as before. The stomach, hepatopancreas-gonads, gills, and the remaining tissues (including the skeletal parts) of each crab were individually digested in 1.0 ml concentrated formic acid, decolorized, and counted. The experiment was repeated with five crabs, three females and one male, kept in contact for 24 hours with a sea urchin injected with labelled glucose. The maxillipeds, maxillae, mandibles, and the tip of the chelipeds were removed from two of the females. Blood was removed from each crab (utilizing a calibrated capillary tube) prior to the removal of the main organ systems. Feces were also obtained from one individual. All samples were digested and counted as before.

The possible accumulation in the symbiont of the naphthoquinone pigments characteristic of sea urchins was also investigated. Pigments were extracted from the peristome epithelium, coelomocyte aggregations, tube feet, and spines of sea urchins, as well as from whole crabs with their digestive tracts removed. Absolute methyl alcohol was used in the extractions of the pigments. Acetic acid (5% of total volume) was

added to the alcohol as a stabilizing agent (Spruit, 1949). Spectra were determined in 1.0 cm cells on a Beckman DB-G spectophotometer.

The excretion of dissolved metabolites through the rectum was investigated by measuring the release of labelled materials from a sea urchin injected with labelled glucose. An individual with its rectum previously occupied by a crab was injected through the peristome with 100 μc of uniformly labelled glucose-^{14}C. It was kept in a 40 gallon tank provided with running sea water. A 1.0 ml sample of water was taken from inside the calcified periproct and from the surface of the test at a point in the ambitus. A third sample was taken from the surrounding water at the center of the tank. Samples were taken at various intervals during a period of 48 hours. Levels of radioactivity were measured by liquid scintillation as before.

Fecal Pellets.

The fecal pellets of the host were chemically analyzed in order to estimate their nutritive value. Samples were taken from individuals collected from different locations and from those with pellets of different appearance. Feces were obtained directly from the rectum or after being voided. All samples were kept frozen before analysis.

Alcohol-soluble carbohydrates were determined by extracting the fecal samples three times with hot 80% ethyl alcohol. After the alcohol was evaporated under reduced pressure, the residue was dissolved in distilled water and the sugars determined spectrophotometrically by the phenol-sulfuric acid method of Dubois et al. (1956). TCA-soluble carbohydrates in the alcohol-washed feces were extracted by slowly heating the samples with 5% aqueous TCA, centrifuging, and

measuring the sugars by the phenol-sulfuric acid method as before. A Beckman Spectronic 20 spectophotometer was used in both analyses. Determinations were carried out in triplicates and a correction was made for the presence of pigments. D-glucose standards were used.

Total protein was measured by extracting the alochol- and TCA-washed samples with hot 1.0N sodium hydroxide, centrifuging, and analyzing triplicates of the supernatant for proteins by employing the Folin-phenol method of Lowry et al. (1951). The reagents were prepared using the modifications given by Price (1965). Standards were prepared by precipitating a standard solution of crystalline bovine serum (Armour Pharmaceutical Company) with 5% TCA followed by an extraction with hot 1.0N sodium hydroxide. A Beckman Spectronic 20 spectrophotometer was used in the determinations.

Total lipid content of a different set of samples was determined by the method of Mukerjee (1956) as outlined by Strickland and Parsons (1968). Their procedure was modified by the use of a small water condenser attached to the test tubes during saponification. A Beckman DB-G spectrophotometer with 1.0 cm cells was used in the determinations.

The quantitative determination of organic carbon was carried out using the acid dichromate digestion method of Johnson (1949) as adapted to spectrophotometry by Strickland and Parsons (1968). The sodium sulfate washing suggested by these authors, however, was omitted. Absorbance was determined in 1.0 cm cells on a Beckman DB-G spectrophotometer.

For the histochemical analysis of the fecal pellets envelope (peritrophic membrane), whole, undecalcified pellets were fixed in

70% ethyl alcohol, embedded in paraffin, and sectioned. Sections were stained using the periodic acid-Schiff (PAS) stain reaction for polysaccharides, followed by pH 2.6 alcian blue counterstained with metanil yellow, and Lillie's (1928) toluidine blue O technique as given by Humason (1967). In order to maintain the metachromatic properties of toluidine blue, the stained sections were not dehydrated and glycerin was used as the mounting medium. The last two reactions are specific for acid mucopolysaccharides.

The presence of enzymes in the symbiont capable of breaking-down algal polysaccharides was investigated by incubating sugar-free algal homogenate with stomach and hepatopancreas extracts. Fresh Ulva fasciata, a shallow water green alga, was homogenized and repeatedly washed in hot 80% ethyl alcohol and cold distilled water. The washed tissues were dried in vacuum and 20.0 mg samples were incubated in 0.1N sodium phosphate buffer (pH 6.8) with filtered extracts of stomach and hepatopancreas of individual crabs. The crab tissues were previously washed in filtered sea water, homogenized in cold buffer, and centrifuged. The pH of the digestive organs of several crabs varied from 6.8 to 7.0. The crab's tissue-algae preparations, as well as digestive system and algae controls, were kept in a water bath at 30°C. One drop of toluene was added to each test tube as bacteriostatic agent. The rate of release of reducing sugars in all of the preparations was measured spectrophotometrically as assayed by the 3,5-dinitrosalicylate reduction (Clark, 1964). Absorbance was determined in a Beckman DB-G spectrophotometer.

Energetic Relationships.

The energy budget of the sea urchin was estimated by measuring food intake, production of fecal pellets, oxygen consumption, and excretion of dissolved organic matter in a number of individuals. Five medium-size sea urchins, including two with rectums previously occupied by crabs, were first kept for three weeks with the red alga Laurencia sp. as the only food source. After one day of starvation, all sea urchins were placed in individual 10 gallon tanks provided with air stones. The amount of Laurencia ingested during the period of 24 hours was determined by weighing (paper towel-dry weight) the alga present in each tank at the beginning and end of the interval of time. The fecal pellets voided during the same 24 hour period were siphoned-out with a large volumetric pipette and immediately frozen. Daily ingestion of food was recorded for each of the five individuals during a period of five days. Similar paper towel-dry samples of the alga were dried in an oven, weigh, and a correction factor determined in order to express the amounts of algae ingested in terms of dried weights. Fecal pellets were dried in an oven and their dry weight recorded. Even when the animals were starved for one day before quantifying the food intake, feces from the food ingested prior to the experiment were still being voided by at least two individuals. As a consequence, no records were taken of feces voided during a period of time equal to that during which feces were still produced after no more food was provided at the end of the five day period. Caloric values of the alga and feces were determined by using a Phillipson oxygen microbomb calorimeter (Phillipson, 1964) connected to a Beckman

potentiometric recorder. Benzoic acid was used in the calibration of the instrument.

Oxygen consumption in each of the five sea urchins was determined by using an eight liter, wood and Plexiglass respirometer provided with a stirrer and a lower compartment through which tap water flowed to maintain constant temperature. The total volume of the respirometer and the connecting tubes was approximately 8,478 ml. Absolute volumes, i.e. total volume corrected for the volume of each sea urchin, varied from 8,196 to 8,364 ml. The temperature of the ambient water varied between 26° and 27°C. The oxygen content of the water in the respirometer was measured at the beginning of the experiment (after a 20 to 30 minute period of acclimation in which running sea water was allowed to flow through) and one hour after the chamber was sealed. Oxygen concentrations were determined using the Winkler technique (Strickland and Parsons, 1968). A control determination was carried out with an empty respirometer and all values corrected for it.

Excretion in the form of dissolved organic carbon was determined in two of the sea urchins. Each individual was placed in a 3,000 ml beaker with 2,000 ml of Millipore-filtered sea water and each provided with an air stone. Water samples from these and a similar control container were taken and analyzed for dissolved organic carbon according to the method of Menzel and Vaccaro (1964) as modified by Strickland and Parsons (1968). The water used in each of the determinations was filtered by using a syringe with a Millipore filter attachment. All samples were analyzed in triplicates. The infrared absorption gas analyzer (Beckman model IR-215) was connected to a Beckman potentiometric recorder and an integrator. All five individuals had been

feeding on Laurencia prior to the measurements of oxygen consumption and release of dissolved organic carbon.

Oxygen consumption in the symbiont was measured in a respirometer built from a 500 ml glass jar and a rubber stopper provided with two sealable openings for the flowing of water. Absolute volume of the jar and connecting tubes varied from 402 to 403 ml. The respirometer was placed in a large pan with running tap water. The ambient temperature varied from 26° to 27°C. The Winkler technique was used to measure the oxygen content of the water before and one hour after the sealing of the jar. Each determination was preceded by a 20 to 30 minute period of acclimination. A control run was carried out as in the sea urchins.

Caloric values were obtained for a sample of coelomocyte aggregations taken from the rectum of three sea urchins and a similar sample of peristome epithelium from four sea urchins. A Phillipson oxygen microbomb calorimeter was used in the determinations.

RESULTS

Stomach Contents of the Symbiont.

The stomach contents of male and female crabs removed from the hosts' rectum consisted mostly or entirely of host tissues. Partly digested material takes the form of a reddish-brown, amorphous mass, but spherical cells were observed occasionally (Figure 25 and 26). These cells appear to be eleocytes, found in large numbers in the coelomocyte aggregations that line the rectum. Algae were also present in roughly 75% of the individuals that were examined. Most of the alga was in the form of empty and intact filaments, but relatively

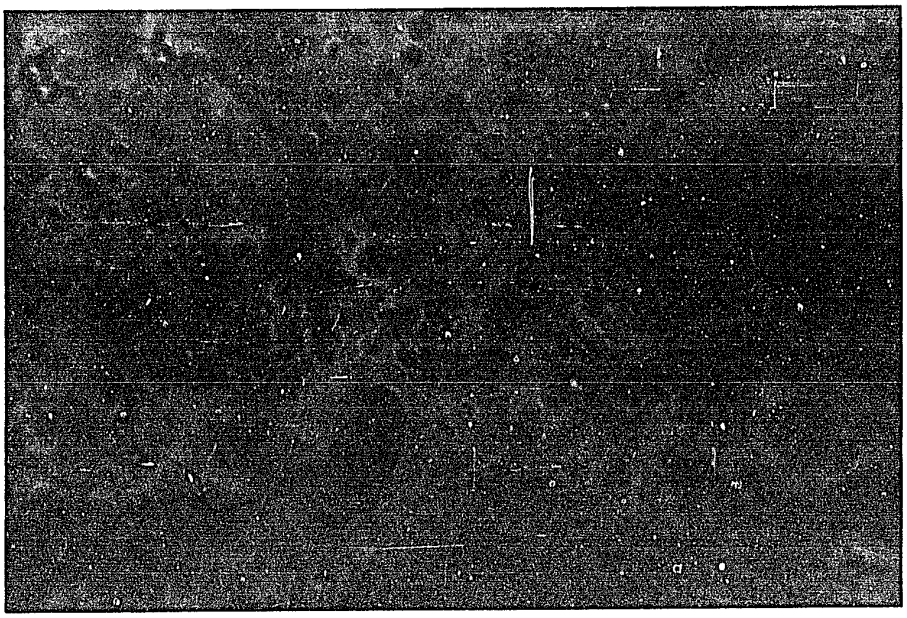


FIGURE 25. PORTION OF THE STOMACH CONTENTS OF A FEMALE ECHINOECUS PENTAGONUS FOUND LIVING IN THE RECTUM OF ECHINOTHRIX CALAMARIS. THE OVAL-SHAPED CELL APPEARS TO BE A PIGMENTED ELEOCYTE FROM THE HOST. (X600)

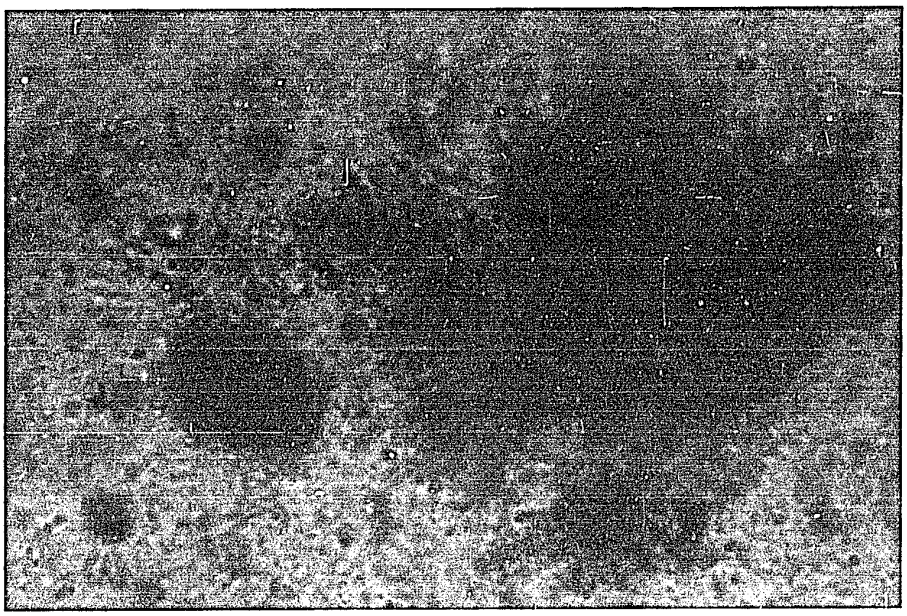


FIGURE 26. STOMACH CONTENTS OF A MALE ECHINOECUS PENTAGONUS FOUND LIVING ON THE PERISTOME OF ECHINOTHRIX CALAMARIS. THE MATERIAL IS COMPOSED OF PIGMENTED HOST TISSUES. (X60)

large fragments were sometimes observed (Figure 27). Sand grains, sponge spicules, diatom frustulae, and bristle-like skeletal fragments were also frequently found. Small oil droplets were observed in at least two individuals. A ciliate was once observed moving between the stomach contents of one female. The occurrence of bacteria was not investigated. The regular oscillation of small particles in the stomach fluids was probably due to Brownian movement. Results confirm early observations of large females ingesting coelomocyte aggregations as well as material from the host's feces (see analysis of fecal pellets).

Contents of the stomach of juvenile female and male crabs taken from the peristome and test consists of partly digested host tissues (Figure 26). Nevertheless, algal filaments and sponge spicules similar to those present in crabs living in the rectum were found in three males. These crabs could have previously been present in the rectum or ingested material from fecal pellets discharged from it.

The stomachs of crabs found on dead sea urchins were empty. A similar situation was generally observed in crabs isolated from sea urchins. A male which had been isolated for 15 days in a tank containing sand and algae had some unrecognizable detritus and algal filaments in the stomach. A female isolated for an undetermined period under similar conditions had some sponge spicules, a bristle-like fragment, and a ciliate similar to the one previously recorded. Otherwise, the stomachs of isolated crabs were empty.

A reduction in the size of the hepatopancreas was observed in isolated crabs. After dying, some even resembled exuviae. Differences in weight of 14 individuals that were isolated at the same time

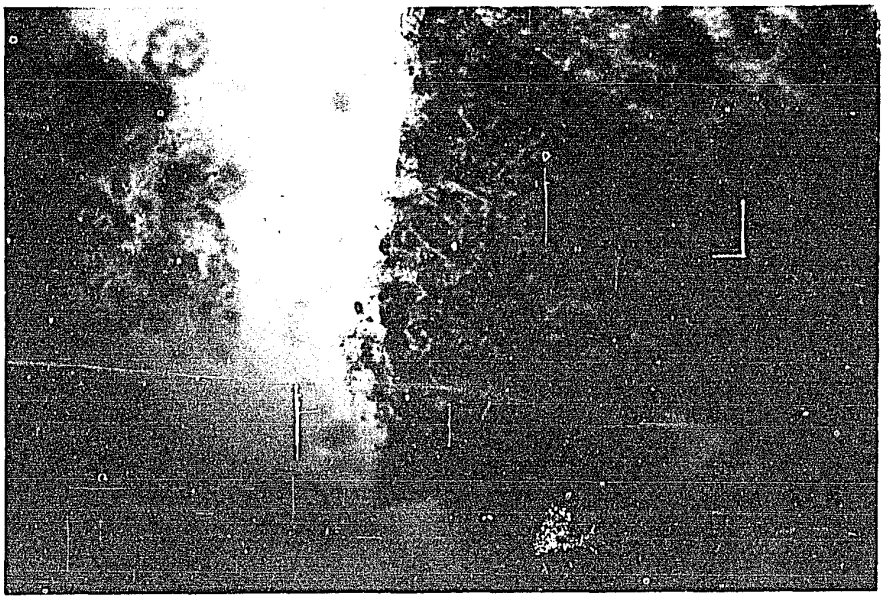


FIGURE 27. STOMACH CONTENTS OF A FEMALE ECHINOECUS PENTAGONUS FOUND LIVING IN THE RECTUM OF ECHINOTHRIX CALAMARIS. THE MATERIAL IS COMPOSED OF PIGMENTED HOST TISSUES AND AN ALGAL FRAGMENT OBTAINED FROM HOST'S FECAL PELLETS. (X40)

is given in Table V. The final weight is that taken at least three days before death. A significant increase in weight was observed in only one individual. The hepatopancreas is considered as the primary storage organ in crustaceans (Vonk, 1960).

Female crabs removed from the rectum remained motionless for long periods of time. Even the antennae and mouthparts remained tightly folded to the carapace. Yet, a strong thigmotactic orientation was shown. Shelter below hard surfaces was generally sought. Males and small females were much more active when removed from their normal habitat. Strong thigmotactic orientation was also shown. All crabs appeared to be more active at night. Cannibalism was never observed, even when dead crabs were present.

Feeding Behavior of the Symbiont.

Normal feeding behavior of large female crabs was difficult to observe due to their position inside the rectum and their relative inactivity when removed from the host. "Scooping" in the form of semicircular movements of the chelipeds are involved in gathering the coelomocyte aggregations that accumulate along the walls of the rectum. The chelipeds are slowly moved to the mouth area, where the material is removed by the maxillipeds. The endopodite of the third maxillipeds can be observed to vibrate repeatedly with the predominantly vertical movement of the maxillipeds. The remaining two pairs of maxillipeds are involved in the cleaning of the relatively long bristles of the palps. Cleaning of the ambulatory appendages also takes place.

Tissue is similarly ingested by crabs living outside of the rectum. Epithelial tissue and tube feet are scooped-out or torn-off

TABLE V. WEIGHT IN GRAMS OF INDIVIDUALS OF *ECHINOECUS PENTAGONUS* ISOLATED FROM *ECHINOTHRIX CALAMARIS*

CRAB NUMBER	SEX	TIME ISOLATED (DAYS)	INITIAL WEIGHT	FINAL WEIGHT	WEIGHT LOST (OR GAINED)	% WEIGHT LOST (OR GAINED)
1	female	14	1.223	1.224	(0.001)	(0.08)
2	male	15	0.317	0.311	0.006	1.89
3	female	15	0.689	0.636	0.053	7.69
4	female	15	0.780	0.750	0.030	3.85
5	female	21	0.850	0.816	0.034	4.00
6	female	21	0.956	0.959	(0.003)	(0.31)
7	female	26	1.005	0.928	0.077	7.66
8	female	31	0.633	0.664	(0.031)	(4.89)
9	female	31	0.971	0.902	0.069	7.11
10	female	32	0.924	0.888	0.036	3.90
11	female	33	0.604	0.558	0.046	7.62
12	female	35	0.602	0.594	0.008	1.33
13	female	35	0.948	0.904	0.044	4.64
14	female	36	0.824	0.783	0.041	4.98

by the chelipeds. Small crabs have been observed to remove secondary spines, tube feet, and probably pedicellariae from relatively large areas along the edge of their hosts' peristomes.

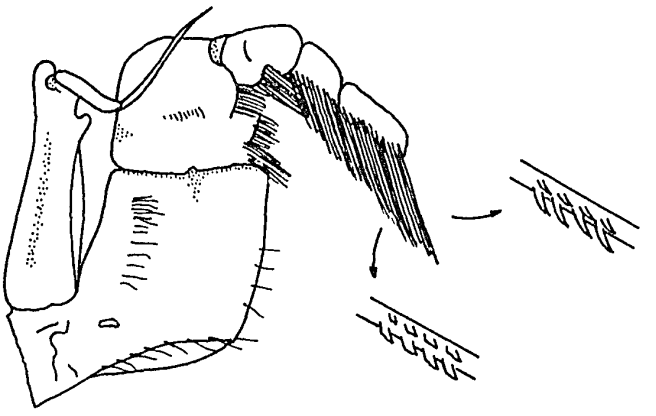
In crabs living in the rectum, the fecal pellets are handled by the chelipeds before they are moved to the mouth region. The third maxillipeds break down the pellets. The bristles of the palps are involved in sorting the particles, some of which can be seen as they are expelled from the region of the mouth. The resulting sediment often accumulates in the rectum.

A female was once observed to chase a large living amphipod that was placed on the aboral surface of the host. The chelipeds were rapidly raised, but the amphipod was not captured. Dead amphipods and fragments of algae placed in the rectum were captured with the chelipeds and eaten.

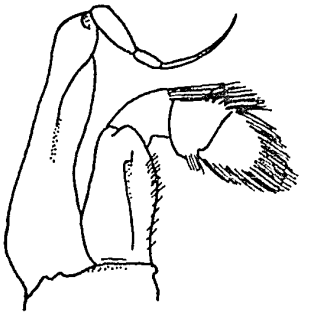
Buccal Appendages of the Symbiont.

The buccal appendages are similar in both sexes. The third maxillipeds are characterized by a well developed palp, the three distal articles of the endopodite, which bear relatively long, hair-like setae (Figure 28). Two rows of short, tooth-like bristles are found along the inner side of each setae. The size and shape of the bristles vary according to their relative position, being blunt in the middle setae and shorter at the end of each seta. Shorter setae are found along the outer border of the merus and ischium of the endopodite. The last three segments of the endopodite in the second maxillipeds form a concave, spoon-like structure. A large number of strong setae is found oriented toward the inner surface in a brush-like arrangement.

FIGURE 28. INNER SURFACE OF A THIRD MAXILLIPED (A) AND A SECOND MAXILLIPED (B) OF ECHINOECUS PENTAGONUS.



A



1 mm

B

Only minute bristles are observed in some of the more proximal setae. The first maxillipeds have a paddle-like endopod provided with a few short setae. The basis and coxa are provided with long, simple setae.

The outer border of the first and second maxillae, the basis and coxa of the exopod, are provided with short setae. The mandibles are strongly calcified. The mandibular palps have short setae.

Third maxillipeds with similarly specialized dactyli are found in Pinnaxodes chilensis and other pinnotherids (Wass, 1968), and in Trapezia, Tetralia, and Domecia, xanthid crabs associated with madreporarian corals (Knudsen, 1967; Patton, 1967b). A similar arrangement has also been observed in the parthenopids Lambrus (Platylambrus) stellata (Rathbun) and Harrovia elegans De Man. The first species, like most members of the subfamily Parthenopinae, lives on sandy to muddy bottoms. Harrovia is a eumedonid associated with crinoids.

Digestive System of the Symbiont.

The mouth leads to the stomach through a short esophagus. The stomach is divided into a typical cardiac (anterior) and a pyloric (posterior) chamber. The zygocardiac ossicles of the gastric mill are composed of three conspicuously blunt cusps and seven to eight slender ridges. A blunt tooth is located at the posterior end of the urocardiac ossicle. Rows of setae are found along the ventral and lateral walls of the stomach, as well as through the length of the pyloric valve. The stomach does not show any specialized morphological features such as those present in Domecia (Patton, 1967b) and Hapalocarcinus (Potts, 1915).

The hepatopancreas extend along both sides and below the stomach and continue posteriorly along the intestine. They open into the pyloric chamber of the stomach. No caeca were observed in the intestine.

Ingestion and Assimilation of Host Tissues.

Relatively high amounts of ^{14}C-labelled material were found present in crabs which had been in contact with sea urchins injected with labelled D-glucose (Table VI). The location indicated for each crab is that at the end of the time of contact. All females were fully grown, thus their presence outside of the rectum represents an abnormal situation. At least two males moved from their original hosts and it is possible that some moved in and out of rectums. Significant concentrations of labelled material started appearing at least six hours after a sea urchin was injected. Labelling took place faster and in larger amounts in crabs found in the rectum. The specific activity of the alcoholic potassium hydroxide fraction (composed mostly of proteins and probably glycogen) was found to be higher (except in one case) than the alcoholic TCA fraction (smaller molecules such as simple sugars and amino acids and probably lipids). It is possible that some of the label, most probably in insoluble proteins, was lost due to the strong reaction of the tissues with concentrated perchloric acid.

Labelled material was rapidly removed from the coelomic fluid in the injected sea urchins (Figure 29). It is assumed that the amount of label that was removed in the 0.5 ml samples was negligible. Labelled material started appearing in the coelomocyte aggregations

TABLE VI. ^{14}C-LABELLED MATERIAL (IN CPM/MG OF TISSUE) FROM INDIVIDUALS OF *ECHINOECUS PENTAGONUS* LIVING IN CONTACT WITH *ECHINOTHRIX CALAMARIS* (ONE SEA URCHIN FOR EACH OF THE TIME INTERVALS) INJECTED WITH 100 µc OF D-GLUCOSE-UL-^{14}C

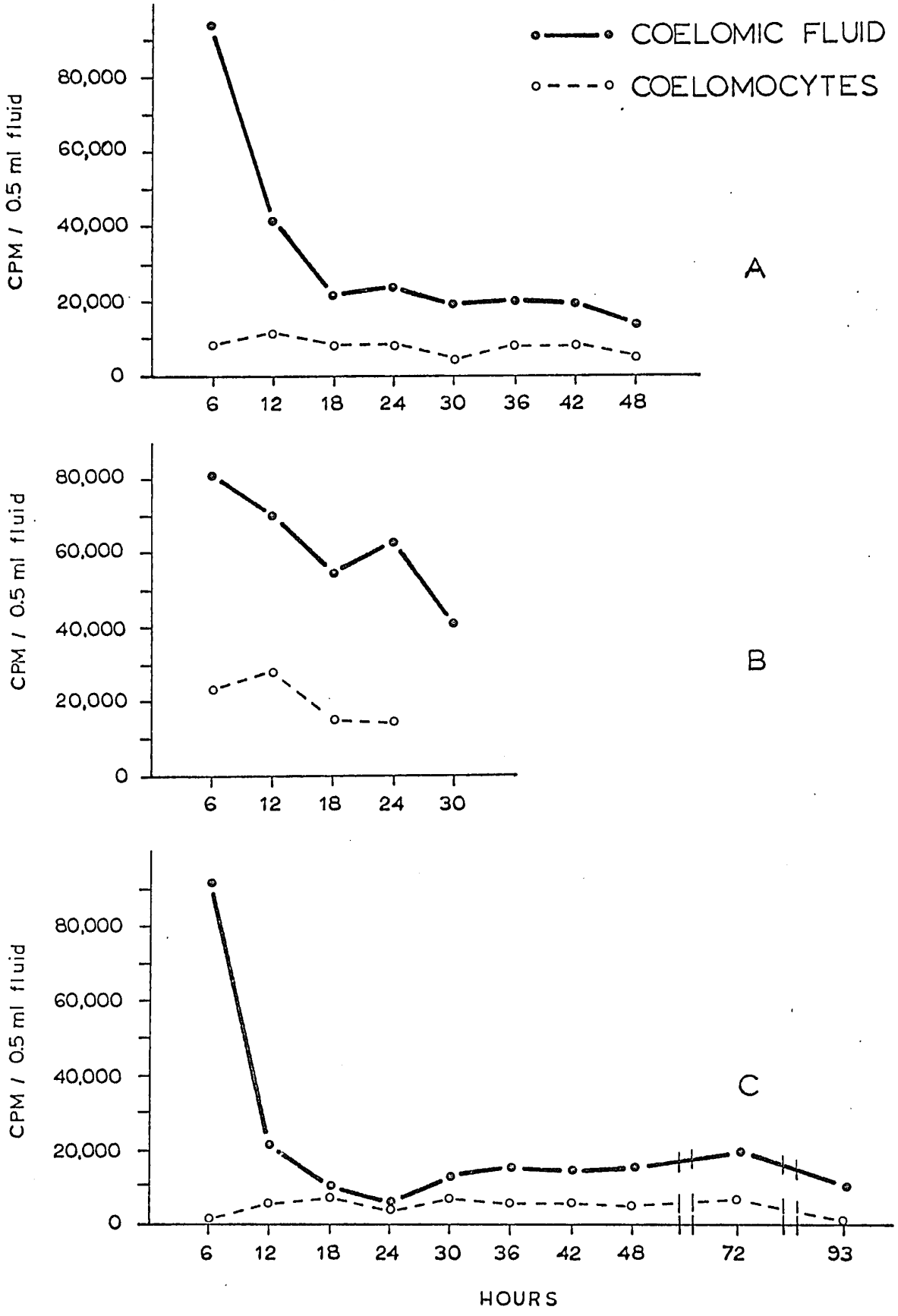
HOURS IN CONTACT WITH SEA URCHIN	SEX	LOCATION IN SEA URCHIN	ALCOHOLIC TCA FRACTION[1]	ALCOHOLIC KOH FRACTION[2]	$HClO_4$ FRACTION[3]	TOTAL SPECIFIC ACTIVITY
3	female	test	0	56.8	0.1	56.9
3	male	test	0	0	0.3	0.3
6	female	rectum	31.2	1,061.4	1.0	1,093.6
6	female	test	16.1	181.8	0.2	198.1
12	male	rectum	201.3	692.5	1.7	895.5
12	male	peristome	2.4	0	0.2	2.6
18	female	rectum	714.7	1,248.7	2.0	1,965.4
18	male	peristome	64.1	381.1	0.4	445.6
24	male	rectum	118.4	1,433.4	2.1	1,553.9
24	male	peristome	407.0	1,030.8	1.4	1,439.2
48	male	rectum	13.4	171.4	0.8	185.6
CONTROLS						
24	female	rectum	0	0	-	0
24	male	peristome	0	0	-	0
48	female	rectum	2.4	0	-	2.4
48	female	test	2.6	0	-	2.6

1 Low molecular weight molecules and lipids.

2 Proteins and glycogen.

3 Skeleton and insoluble protein.

FIGURE 29. CHANGE OF ^{14}C-LABELLED MATERIAL AS A FUNCTION OF TIME IN THE COELOMIC FLUID AND COELOMOCYTES OF INDIVIDUALS OF ECHINOTHRIX CALAMARIS INJECTED WITH 100 µc OF D-GLUCOSE-UL-^{14}C.


COELOMIC FLUID
COELOMOCYTES
CPM / 0.5 ml fluid
80,000
60,000
40,000
20,000
0
6
12
18
24
30
36
42
48
A
CPM / 0.5 ml fluid
80,000
60,000
40,000
20,000
0
6
12
18
24
30
B
CPM / 0.5 ml fluid
80,000
60,000
40,000
20,000
0
6
12
18
24
30
36
42
48
72
93
HOURS
C

of the rectum and in the epithelium of the peristome at least one hour after the labelled glucose was injected (Figure 30). Larger amounts were normally present in the rectum. This could explain why more labelled material was found in crabs living in the rectum. A comparison between the deposition of labelled material in a normal rectum and in one previously occupied by the symbiont was not possible. Abundant coelomocyte aggregations developed in the sea urchin from which the periproct had been removed, a situation never observed in rectums covered with a periproct (see Section IV). The deposition of material appears to have been greater in the sea urchin with a rectum originally occupied by a crab (individual B) but the difference is probably due to the fact that the sea urchin was smaller and the relative amount of labelled glucose in its perivisceral coelom was obviously larger. Non-quantified samples of the coelomocyte aggregations, peristome epithelium, and oral tube feet of another sea urchin (individual A of Figure 29) showed labelling of all three types of tissue six hours after the injection of labelled glucose.

The distribution of radiocarbon in different organs and in the coelomic fluid of a sea urchin injected 48 hours before with labelled glucose is given in Table VII. The relative levels of labelled glycogen and other major biochemical constituents in the rectum (without including the calcified periproct) and in the coelomocyte aggregations of similarly injected individuals are given in Tables VIII and IX, respectively. As expected, radiocarbon was translocated through the various organ systems. The axial complex shows a significantly higher specific activity. The axial gland itself has

FIGURE 30. CHANGE OF ^{14}C-LABELLED MATERIAL AS A FUNCTION OF TIME IN THE COELOMOCYTE AGGREGATIONS OF THE RECTUM AND PERISTOME EPITHELIUM OF INDIVIDUALS OF *ECHINOTHRIX CALAMARIS* INJECTED WITH 100 µc OF D-GLUCOSE-UL-^{14}C.

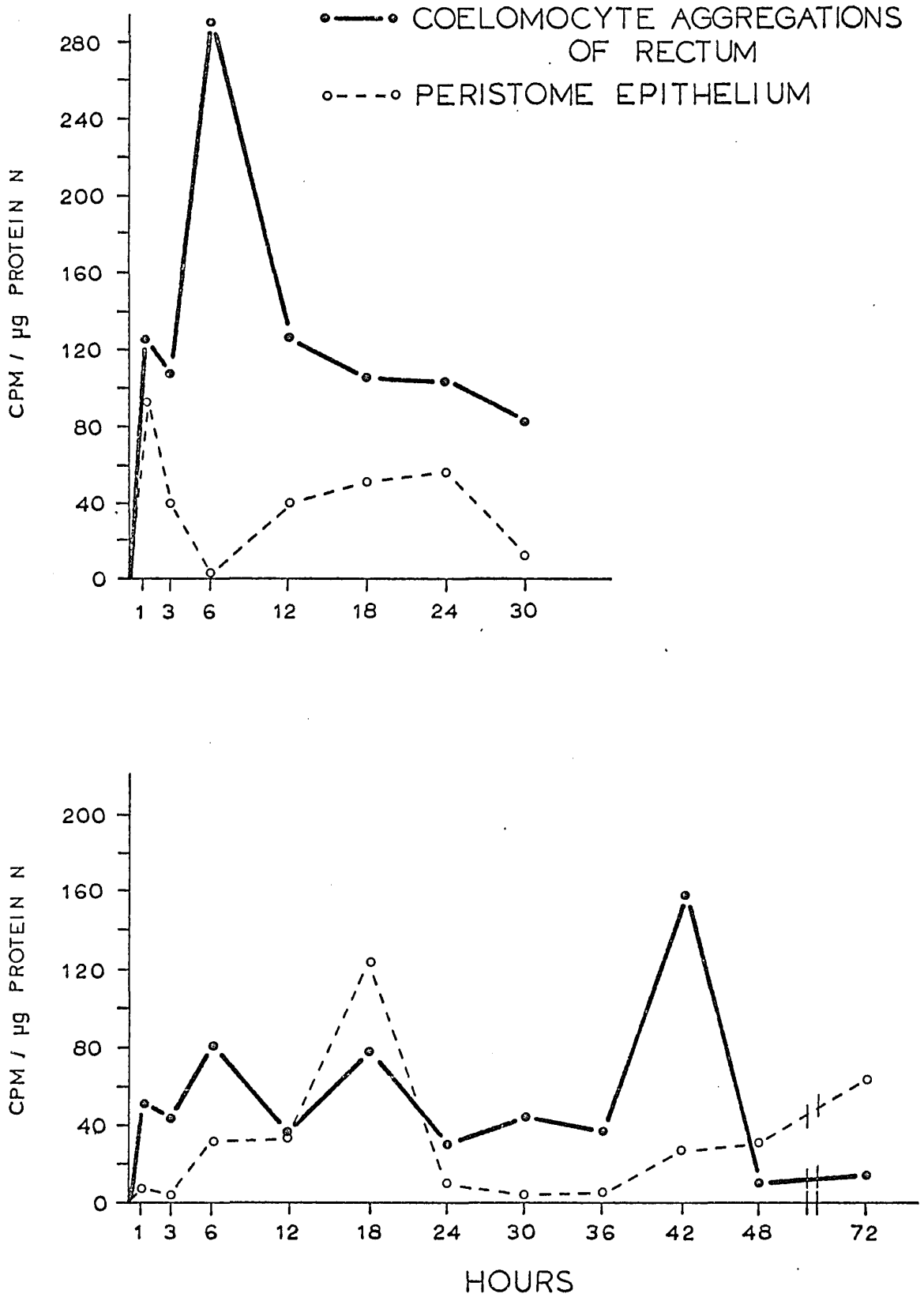

COELOMOCYTE AGGREGATIONS OF RECTUM
PERISTOME EPITHELIUM
CPM / µg PROTEIN N
280
240
200
160
120
80
40
0
1 3 6 12 18 24 30
CPM / µg PROTEIN N
200
160
120
80
40
0
1 3 6 12 18 24 30 36 42 48 72
HOURS

TABLE VII. ^{14}C-LABELLED MATERIAL IN VARIOUS ORGANS OF AN INDIVIDUAL OF *ECHINOTHRIX CALAMARIS* INJECTED WITH 100 µc OF D-GLUCOSE-UL-^{14}C 48 HOURS BEFORE SAMPLING

SAMPLE	CPM/MG	
Coelomic fluid (cell-free)	71,985.7	(CPM/0.5ml)
Esophagus	979.2	
Intestine	464.7	
Rectum	698.9	
Gonad	181.3	
Axial complex	9,611.3	

TABLE VIII. COMPOSITION OF THE ^{14}C-LABELLED MATERIAL IN THE RECTUM OF AN INDIVIDUAL OF *ECHINOTHRIX CALAMARIS* INJECTED WITH 100 µc OF D-GLUCOSE-UL-^{14}C 48 HOURS BEFORE SAMPLING

FRACTION	CPM/MG	% TOTAL ACTIVITY
Glycogen	59.5	11.3
TCA-ethyl alcohol	223.8	42.5
NaOH	151.0	28.7
Residue	92.2	17.5
TOTAL	526.6	-

TABLE IX. COMPOSITION OF THE ^{14}C-LABELLED MATERIAL IN THE COELOMOCYTE AGGREGATIONS IN THE RECTUM OF AN INDIVIDUAL OF *ECHINOTHRIX CALAMARIS* INJECTED WITH 100 μc OF D-GLUCOSE-UL-^{14}C 48 HOURS BEFORE SAMPLING

FRACTION	CPM/MG	% TOTAL ACTIVITY
TCA (aqueous)	1,622.8	-
TCA-ethyl alcohol	651.0	12.2
Glycogen (TCA fraction - TCA-alcohol fraction)	971.8	18.3
Residue	3,692.1	69.5
TOTAL	5,314.9	-

been described in echinoids as being full of coelomocytes (Hyman, 1955). The labelled carbon of the glucose was incorporated into other metabolites, at least in the case of the rectum and in the coelomocyte aggregations.

Autoradiographs of cross sections of the rectum in a sea urchin which had been injected with labelled glucose 24 hours before showed that most of the labelled material was concentrated along the inner epithelium (Figure 31). The radioactivity did not appear to be restricted to coelomocytes. Labelled glucose (as well as other sugars, amino acids, and some lipids) are assumed to have been washed out of the tissues during the process of decalcification and staining. The label was thus incorporated into large molecules not soluble in EDTA or in any of the solvents used during staining.

The amounts of labelled material in the coelomocytes and in the cell-free coelomic fluid suggest that the coelomocytes are not solely responsible for the removal of the label. The different tissues and organs surrounding the coelomic cavities appear to remove labelled material directly from the fluid. The importance of coelomocytes and the hemal system in the translocation of nutrients in sea urchins has been stressed by Boolootian and Lasker (1964). Similar work in S. purpuratus (Farmanfarmaian and Phillips, 1962) and in a starfish, Pisaster ochraceus Brandt (Ferguson, 1964a; 1964b) have shown that the coelomic fluid serves as the most important vehicle for nutrient transport. The subject is discussed by Anderson (1966).

The total amount of D-glucose injected into each of the sea urchins (84.6 µg) is small when the total volume of the coelomic cavities is considered. The concentrations of reducing sugars or glucose in the

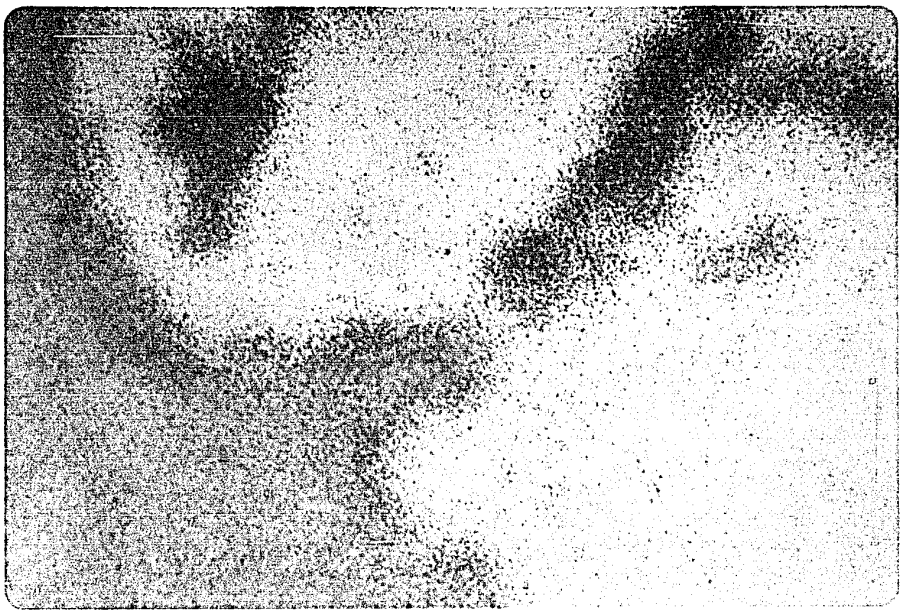


FIGURE 31. AUTORADIOGRAPH OF A CROSS SECTION OF THE RECTUM OF AN INDIVIDUAL OF ECHINOTHRIX CALAMARIS INJECTED 24 HOURS BEFORE WITH 100 μc OF D-GLUCOSE-UL-^{14}C. (EXPOSED FOR 62 DAYS AND STAINED WITH FAST GREEN; X400)

coelomic fluid of sea urchins increase to relatively high concentrations after feeding (Lasker and Giese, 1954; Boolootian and Lasker, 1964; Lawrence et al., 1966). Anderson (1966) has explained that "there is evidently no mechanism other than what might be considered supply and demand operating to regulate concentrations of reducing sugar in the body fluid of sea urchins." The labelled material found present in the coelomic fluid was not analyzed but it is assumed that it was not exclusively in the form of D-glucose. Fluctuations observed in the deposition of labelled material in the periproct and rectum (Figure 30), and even in the release of dissolved material from the rectum and test (Figure 32) are similar to those reported by Ferguson (1964a, 1964b) in Pisaster. His evidence indicates that reabsorption and release of stored material is not a uniformly regulated process.

The distribution of ^{14}C labelled material in crabs is summarized in Tables X and XI. The presence of this material outside of the stomach is an indication that host tissue is assimilated. Some of the ingested material was voided in the feces. A total of 43.6 cpm were detected in some unweighed feces from one of the crabs (female No. 1 of Table XI). Most of the label was found in the tissues remaining after the major organ systems were removed. Nevertheless, the gills showed the highest specific activity in most individuals. This can be explained, at least in part, by their excretory function (Parry, 1960). Two crabs with all of their mouthparts and the tips of their chelipeds removed showed some activity (Table XI). Even when specific activities were much smaller, the distribution of the label was similar to that

TABLE X. DISTRIBUTION OF ^{14}C-LABELLED MATERIAL IN FOUR INDIVIDUALS OF *ECHINOECUS PENTAGONUS* IN CONTACT FOR 48 HOURS WITH AN INDIVIDUAL OF *ECHINOTHRIX CALAMARIS* INJECTED WITH 100 µc OF D-GLUCOSE-UL-^{14}C

CRAB NO. AND LOCATION IN HOST	CPM PER MG TISSUE WEIGHT	CPM PER MG TOTAL WEIGHT	% TOTAL ACTIVITY
1. *female* (outside rectum)			
stomach	2.0	< 0.1	11.7
hepatopancreas-gonads	0.6	< 0.1	12.8
gills	4.3	< 0.1	16.0
remaining tissues	0.2	0.2	59.5
whole animal	-	0.3	-
2. *female* (outside rectum)			
stomach	6.3	0.1	8.9
hepatopancreas-gonads	2.2	0.1	5.7
gills	18.7	0.3	21.3
remaining tissues	0.8	0.8	64.1
whole animal	-	1.2	-
3. *female* (test)			
stomach	16.5	0.3	24.0
hepatopancreas-gonads	1.7	0.1	6.0
gills	39.7	0.3	28.4
remaining tissues	0.5	0.5	41.6
whole animal	-	1.1	-
4. *male* (peristome)			
stomach	69.9	0.9	10.1
hepatopancreas-gonads	62.5	1.5	18.1
remaining tissues	6.4	6.1	71.8
whole animal	-	8.5	-

TABLE XI. DISTRIBUTION OF ^{14}C-LABELLED MATERIAL IN FIVE INDIVIDUALS OF *ECHINOECUS PENTAGONUS* (TWO WITHOUT MOUTHPARTS) IN CONTACT FOR 24 HOURS WITH AN INDIVIDUAL OF *ECHINOTHRIX CALAMARIS* INJECTED WITH 100 µc OF D-GLUCOSE-UL-^{14}C

CRAB NO. AND LOCATION IN HOST	CPM PER MG TISSUE WEIGHT	TOTAL WEIGHT	% TOTAL ACTIVITY
1. *female* (rectum)			
blood	-	0.2	1.9
stomach	10.6	0.2	1.9
hepatopancreas-gonads	7.6	0.6	8.0
gills	73.4	1.0	12.3
remaining tissues	6.9	7.0	75.9
whole animal	-	8.0	-
2. *female* (test) NO MOUTHPARTS			
blood	-	< 0.1	1.3
stomach	0.3	< 0.1	0.9
hepatopancreas-gonads	0.7	< 0.1	9.0
gills	4.9	0.1	16.3
remaining tissues	0.3	0.3	72.5
whole animal	-	0.4	-
3. *female* (test) NO MOUTHPARTS			
blood	-	< 0.1	0.1
stomach	0.3	< 0.1	0.6
hepatopancreas-gonads	0.4	< 0.1	3.5
gills	2.8	< 0.1	4.2
remaining tissues	0.6	0.6	91.6
whole animal	-	0.7	-

(Continued)

TABLE XI. (Continued) DISTRIBUTION OF ^{14}C-LABELLED MATERIAL IN FIVE INDIVIDUALS OF *ECHINOECUS PENTAGONUS* (TWO WITHOUT MOUTHPARTS) IN CONTACT FOR 24 HOURS WITH AN INDIVIDUAL OF *ECHINOTHRIX CALAMARIS* INJECTED WITH 100 µc OF D-GLUCOSE-UL-^{14}C

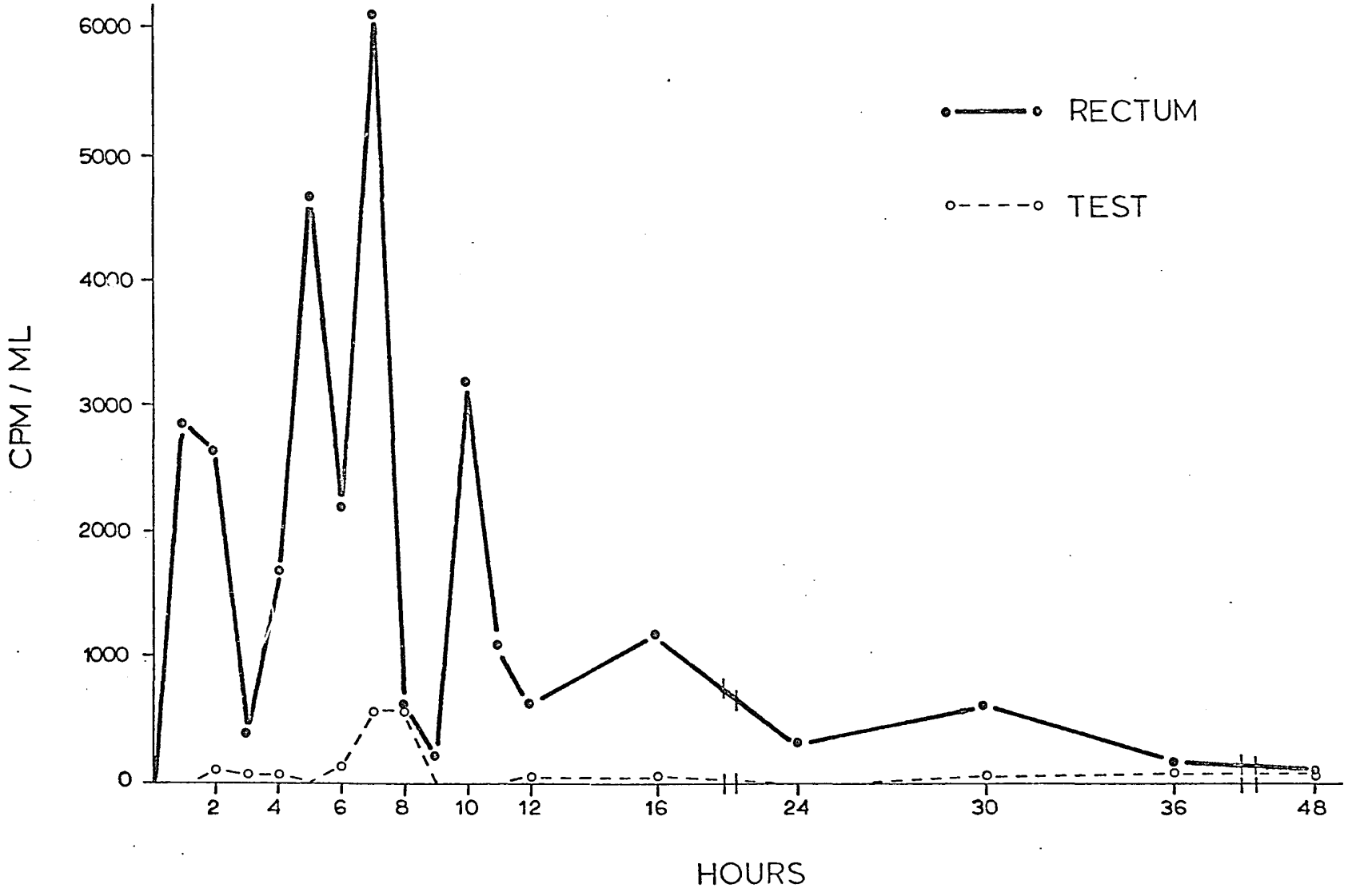
CRAB NO. AND LOCATION IN HOST	CPM PER MG TISSUE WEIGHT	CPM PER MG TOTAL WEIGHT	% TOTAL ACTIVITY
4. male (peristome)			
blood	-	0.2	0.8
stomach	130.9	2.1	7.1
hepatopancreas-gonads	114.0	5.7	19.0
gills	97.5	0.5	1.6
remaining tissues	23.2	21.5	71.5
whole animal	-	30.1	-
5. male (peristome)			
blood	-	< 0.1	0.1
stomach	5.6	0.1	1.2
hepatopancreas-gonads	6.6	0.3	2.3
gills	17.1	0.1	0.7
remaining tissues	12.4	12.4	95.8
whole animal	-	12.0	-

observed in normal crabs. It is not known if these two crabs were actually able to ingest host tissue. A small amount of labelled material was found in the stomach of both animals, but in percentages lower than in the other crabs. It was shown in an earlier trial experiment that crabs were able to ingest host tissue when only their maxillipeds had been removed since pigmented feces were observed days later. Three similarly treated crabs showed specific activities of 2.1, 0.5, and 0.6 cpm/mg (in contrast to 9.5 in a crab with all mouthparts present) when in contact with a sea urchin injected 48 hours before.

Dissolved labelled material was found to be significantly higher in water samples taken from the rectum than that in similar samples taken from the surface of the test. Values shown in Figure 32 are corrected for the specific activity of similar samples taken from the water in the tank where the sea urchin was kept, values which varied from 17.00 cpm/ml to 253.00 cpm/ml.

The alcohol-soluble pigments of the peristome epithelium and the coelomocyte aggregations of the sea urchin show the typical absorption spectrum for naphthoquinones (Millot, 1957). Absorption peaks were at 254-262, 338-344, and 480-490 mu for the coelomocyte aggregations and 240-260, 324-330, and 490-510 mu for the peristome epithelium. Pigments from both types of tissue were bright orange-red. Pigments extracted from the crab and from the spines and tube feet of the sea urchin were dark reddish-brown. They showed maximum absorption at the ultraviolet portion of the spectrum with no distinctive peak. The nature of the brown pigment in both species is unknown. No

FIGURE 32. ^{14}C-LABELLED MATERIAL IN WATER SAMPLES TAKEN FROM THE RECTUM AND TEST OF AN INDIVIDUAL OF ECHINOTHRIX CALAMARIS INJECTED WITH 100 µc OF D-GLUCOSE-UL-^{14}C.


6000
5000
4000
3000
2000
1000
0
CPM / ML
2
4
6
8
10
12
16
24
30
36
48
HOURS
RECTUM
TEST

naphthoquinone pigments were found in the tissues of crabs.

Composition and Nutritive Value of the Fecal Pellets of the Host.

The shape and general composition of the fecal pellets of Echinothrix calamaris varies considerably. They are typically spherical, measuring 3 to 5 mm in diameter, and sand-like in color. These pellets are mostly composed of sediment, sand, and small fragments of coral and calcareous algae (mostly Porolithon). Filamentous algae are found in considerable amounts. The algae were not identified, but green and red filaments are the most common. Empty filaments are also present. Large brown fragments, possibly Sargassum, are sometimes present, giving the pellets a dark brown coloration. Ciliates also commonly occur. Bright orange and light brown forms have been identified. Ciliates, mostly holotrichs, are common inhabitants of the gut of sea urchins (Hyman, 1955). Nematodes and the shells of foraminiferans have also been observed. Most of the fecal pellets of the shallow water populations of Kaneohe Bay are elongate, larger (5 to 10 mm in length), greenish-brown in color, and covered with a much thicker peritrophic membrane. They are primarily composed of large fragments of algae, most probably Dictyosphaeria cavernosa (see Section III). Sand, coral fragments, and filamentous algae are also common. Small organisms were never observed. Spherical, sand-colored pellets are also voided by these individuals. Small, bright red pellets were observed on two occasions. They were found to be composed of orange-red sponge tissue, monaxon spicules, numerous bright orange ciliates, and a small amount of sediment.

Although bacteria were never observed, their presence in pellets

has been demonstrated by Dr. Louis H. Di Salvo (personal communication). Bacteria from pellets composed primarily of sand rapidly digested agar plates containing tissue of Porolithon, a red calcareous alga. Different views have been given on the role of bacteria and other microorganisms in the process of digestion in sea urchins (Lasker and Giese, 1954; Eppley and Lasker, 1959; Farmanfarmaian and Phillips, 1962; Anderson, 1966).

Carbohydrate, protein, lipid, and organic carbon contents of fecal pellet samples are given in Tables XII and XIII. Most of the organic carbon was evidently in the form of the structural polysaccharides of algae, forms which, being insoluble in alcohol or TCA, were not measured. Monosaccharides and their derivates, and probably some di- and trisaccharides, were sampled in the alcohol soluble fraction. The longer chains, i.e. fragments of the higher polysaccharides, starches, glycogen, etc., were sampled in the TCA fraction. The polysaccharides of marine algae have been reviewed by Mori (1953) and Black (1954). The higher polysaccharides of algae (alginic acid, laminarin, agar, fucoidin, etc.) are typically polymers of hexoses or their uronic acids. Preliminary chromatographic characterization of the alcohol-soluble fraction of some fecal samples showed that glucose, and at least two other sugars, probably monosaccharides, were present. Even when the samples were partly desalted by ion exchange chromatography, remaining pigments and salts interfered with the separation of sugars.

Lipids appear to be the second most important constituents, an average of 1.4% of the organic carbon. The total organic carbon was

TABLE XII. CARBOHYDRATE AND PROTEIN CONTENTS OF FECAL PELLET SAMPLES OF *ECHINOTHRIX CALAMARIS* (SAMPLES NOS. 1 TO 5 WERE COMPOSED PRIMARILY OF SAND AND SEDIMENT, 6 OF SPONGE TISSUE)

	1	2	3	4	5	6	MEAN VALUES
Alcohol-soluble carbohydrate							
µg/mg feces	0.22	0.37	0.38	0.51	0.60	0.16	0.37
% dry weight	0.02	0.04	0.04	0.05	0.06	0.02	0.04
TCA-soluble carbohydrate							
µg/mg feces	0.69	0.72	1.25	5.40	0.48	1.21	1.63
% dry weight	0.07	0.07	0.13	0.54	0.05	0.12	0.16
Protein nitrogen							
µg/mg feces	0.63	0.78	1.07	1.78	0.51	1.29	1.01
% dry weight	0.06	0.08	0.11	0.18	0.05	0.13	0.10

TABLE XIII. ORGANIC CARBON AND LIPID CONTENTS OF FECAL PELLET SAMPLES OF *ECHINOTHRIX CALAMARIS* (SAMPLES NOS. 7 TO 9 WERE COMPOSED PRIMARILY OF ALGAE, 10 TO 12 OF SAND AND SEDIMENT)

	7	8	9	10	11	12	MEAN VALUES
Organic carbon							
µg/mg feces	650.18	494.00	-	236.05	285.03	133.59	359.77
% dry weight	65.01	49.40	-	23.60	28.50	13.35	35.97
Total lipid							
µg/mg feces	3.76	3.45	12.73	4.32	2.63	3.98	5.14
% dry weight	0.38	0.34	1.27	0.43	0.26	0.40	0.51
% organic carbon	0.58	0.69	-	1.82	0.91	3.00	1.40

not measured in the samples where carbohydrates and proteins were measured, but from their concentrations it appears that they account for less than 1.0% of the organic carbon. Carbohydrate and protein contents were relatively constant, but considerable variation was observed in the lipid and organic carbon. This larger variation was probably due in part to sampling errors, since smaller amounts of feces had to be used in the determination of lipids and organic carbon.

The peritrophic membrane of the fecal pellets gave a positive reaction with PAS, pH 2.6 alcian blue, and toluidine blue O, indicating the presence of acid mucopolysaccharides. Toluidine blue gives a positive reaction with chondroitin sulfate A, hyaluronic acid, and heparin (Brimacombe and Webber, 1964). Fecal pellets of Diadema antillarum have been described by Lewis (1964) as covered with a thin "mucous secretion secreted by the oesophagus."

There is no evidence for the occurrence of enzymes in the symbiont capable of degrading the alcohol- and water insoluble carbohydrates of the green alga Ulva fasciata. Small amounts of reducing sugars (0.01 to 0.02 mg/ml, corrected for alga and crab tissue controls) were detected in four of the seven assays of the alga incubated with hepatopancreas extract. Nevertheless, there was no gradual increase in the concentration of reducing sugars that would indicate any digestion of the algal tissue.

Energetic Relationships.

Data on the ingestion of food and production of feces in the five sea urchins utilized in the energy budget experiments are summarized in Table XIV.

Time required to digest all of the food provided during a period of

TABLE XIV. DRY WEIGHT IN GRAMS AND CALORIC VALUE OF FOOD INTAKE AND PRODUCTION OF FECES IN FIVE INDIVIDUALS OF *ECHINOTHRIX CALAMARIS*

Sea urchin number	FOOD		FECES		"ASSIMILATION EFFICIENCY"
	Total amount ingested in five days	Average amount ingested per day	Total amount voided	Average amount voided per day (in a five day basis)	
1	3.33	0.67	0.62	0.12	81.30
2	5.84	1.17	1.34	0.27	77.00
3	2.23	0.45	0.33	0.07	85.20
4	3.48	0.70	0.38	0.08	89.00
5	5.65	1.13	0.77	0.15	86.30
Mean values	4.11	0.82	0.69	0.14	83.76

Mean amount of food ingested per day 0.82 g

Energy equivalent (1.0 g *Laurencia* = 1,332.96 cal) 1,093.03 cal

Mean number of calories ingested per gram dry weight per day (mean dry weight of the five individuals = 45.07 g) 24.25 cal

Mean amount of feces voided per day 0.14 g

Energy equivalent (1.0 g dry feces = 993.63 cal) 139.11 cal

Mean number of calories per gram dry weight per day (Mean dry weight of the five individuals = 45.07 g) 3.09 cal

five days varied from seven to ten days. All feces were voided within a period of four days in one individual, whereas small amounts were voided during longer periods in others. The average amount of feces voided was calculated on a five day basis. Lewis (1964) has reported that in Diadema antillarum it took 8 to 12 hours for the food to pass through the gut when continuously provided. It is thus possible that in Echinothrix food was retained in the gut longer than normal after no food was provided at the end of the five day feeding period.

The "assimilation efficiency" (AE) calculated as:

$$AE = \frac{\text{weight of alga ingested - weight of feces}}{\text{weight of alga ingested}} \times 100$$

is given only for the purpose of comparison with previous work in other species of sea urchins. The presence in the fecal pellets of previously assimilated material (the peritrophic membrane, mucous, etc.) and the possibility of release of unassimilated food in the form of dissolved material makes this an unreliable measurement (Johannes and Satomi, 1967). Values given for Strongylocentrotus purpuratus vary from 43 to 93% (Lasker and Boolootian, 1960; Boolootian and Lasker, 1964) and from 32.4 to 83.4% in S. intermedius (Fuji, 1962; 1967). Efficiencies were found to vary with the type of alga provided as food source, the size of the individuals, and even the time of the year.

Table XV summarizes the results on oxygen consumption and release of dissolved organic carbon by the sea urchins. Relatively high oxygen consumption values for some individuals were probably a result of unusual activity inside the respirometer. Oxygen consumption values for other sea urchins have been given by Farmanfarmaian

TABLE XV. LEVELS AND CALORIC VALUES OF OXYGEN CONSUMPTION AND RELEASE OF DISSOLVED ORGANIC CARBON IN FIVE INDIVIDUALS OF ECHINOTHRIX CALAMARIS

Sea urchin number	OXYGEN CONSUMPTION				RELEASE OF ORGANIC CARBON		
	Dry weight (grams)	ml O_2/hour	ml O_2/day	ml O_2/gm/day	mg C/hour	mg C/day	mg C/g/day
1	52.26	6.809	163.416	3.127	-	-	-
2	47.80	6.157	147.768	3.091	-	-	-
3	51.35	4.208	100.992	1.967	-	-	-
4	34.15	1.391	33.384	0.978	1.20	28.80	0.84
5	39.80	4.093	98.232	2.468	4.86	116.64	2.93
Mean values	45.07	4.532	108.758	2.326	3.03	72.72	1.88

Energy equivalent of 1.0 ml of oxygen at a respiratory quotient of 0.80 — 4.801 cal

Energy equivalent of mean oxygen consumption — 522.16 cal/day

(= 11.58 cal/g/day)

(1966), Nicol (1967), McPherson (1968b) and Lewis (1968a, 1968b). Caloric values for the mean volume of oxygen consumed assume a non-protein respiratory quotient (RQ) value of 0.80, the halfway point between the extreme carbohydrate and fat oxidations (West et al., 1966). RQ values for sea urchins have been determined for Paracentrotus lividus (Buddenbrock, 1937) and for different tissues of Allocentrotus fragilis (Giese, 1961). Values for P. lividus vary from 0.81 to 0.93 and those for A. fragilis from 0.57 (gut) to 0.92 (testis).

It would have been difficult to determine the amount of energy lost in the form of metabolites being released into solution, a figure generally ignored by ecologists. A detailed qualitative and quantitative analysis of the excretory products was necessary. There is a considerable difference between the two values obtained for the release of dissolved organic carbon in an effort to estimate excretory output in the sea urchin. The high value obtained in one of the individuals is probably a result of contamination.

These measurements account only for an estimated 40 to 70% of the total excretory products. Excretion of ammonia by P. lividus has been measured as varying between 26.2 and 30.0% of the total non-protein nitrogen (Delaunay, 1931). Excretory ammonia in D. antillarum ranged between 61 to 64% (Lewis, 1967). Large amounts of amino acids (25 to 29%) were measured by both investigators. Purines, urea, and uric acids were recorded only by Delaunay. Most of the dissolved organic carbon excreted by Echinothrix can be assumed to be in the form of amino acids. For the average 72.72 mg of organic carbon

excreted per day, 227.32 cal/day (5.87 cal/gm/day) would be released if it is assumed that only glycine is excreted (heat of combustion of glycine given as 234.5 kilogram calories per gram molecular weight by Kharasch, 1929). The presence of other amino acids would increase this estimate; purines, urea, and uric acid, on the other hand, would lower it.

Oxygen consumption measurements in the symbiont are summarized in Table XVI. As in the host, the caloric equivalents for oxygen consumption assume a respiratory quotient (RQ) value of 0.80. RQ values for brachyurans listed by Wolvekamp and Waterman (1960) ranged from 0.44 to 1.34. Data on oxygen consumption for other brachyurans have been given by Wolvekamp and Waterman (1960), Prosser and Brown (1961), and Nicol (1967).

DISCUSSION

The utilization of host material as a food source by the symbiont is directly related to the microhabitat of the crabs. The ingestion of coelomocytes and fecal material by adult females, as well as of peristomial epithelium and tube feet by males and juvenile females, is in an apparent balance with the processes involved in the production of these materials by the host.

The presence of abundant aggregations of coelomocytes in the lumen of rectums occupied by crabs has already been indicated in Section IV. The accumulation of pigmented eleocytes along the rectum, which is normally covered by the periproct, can be explained as a response to its exposure to light. This is observed when the periproct is removed. An orange-red naphthoquinone pigment similar to that of D. antillarum (Millot, 1957) was found present in the coelomocyte aggregations.

TABLE XVI. VOLUME AND ENERGY EQUIVALENTS OF OXYGEN CONSUMPTION IN MALE AND FEMALE INDIVIDUALS OF ECHINOECUS PENTAGONUS

	Crab number	Dry weight (grams)	ml O_2/hour	ml O_2/day	energy equivalent* (calories/day)	ml O_2/g/day
Females from rectum						
	1	1.210	0.173	4.152	19.93	3.431
	2	0.632	0.137	3.288	15.79	5.203
Mean values		0.921	0.155	3.720	17.86	4.317
Males from periproct						
	1	0.433	0.078	1.872	8.99	4.323
	2	0.407	0.067	1.608	7.72	3.951
	3	0.249	0.034	0.816	3.92	3.277
Mean values		0.363	0.060	1.432	6.88	3.850

* Energy equivalent of 1.0 ml of oxygen at a respiratory quotient of 0.80 = 4.801 calories

Fox (1953) has also suggested that naphthoquinone pigments may function as excretory products. Radiocarbon rapidly accumulates in the coelomocyte aggregations in amounts larger than in the peristome. Release by the rectum of considerable amount of labelled material also takes place. It is not known if a similar release of material occurs in normal rectums, where only a small number of eleocytes has been found to be present. It is also possible that mechanical irritation by crabs, or by the capillary tubes utilized to remove coelomocytes, might induce an increase in the number of coelomocytes. Nevertheless, eleocytes have not been recorded as being involved in phagocytosis or in any similar defensive mechanism.

There appears to be no records of the presence of a high concentration of coelomocytes in the rectums of other sea urchins. Abundant mucous-like, pigmented material was found in the rectum of Loxechinus albus, which is occupied by the females of the pinnotherid Pinnaxodes chilensis (see Section IV). Coelomocytes are ingested by the rhabdocoel turbellarian Syndesmis franciscana, a symbiont of the gut and coelom of the sea urchin Lytechinus variegatus Clark (Jennings and Mettrick, 1968).

The sea urchin's naphthoquinone pigments are not retained in the skeleton or tissues of the symbiont. Deposition of naphthoquinones occurs in the bones of the Pacific sea otter, a form which feeds on sea urchins (Mertens, 1935). Melanins, pigments generally insoluble in alcohol, are found in crustaceans (Goodwin, 1960) and sea urchins (Vevers, 1966; Fox and Hopkins, 1966). Carotenoids, found in most crustaceans, are restricted to the internal organs in sea urchins. The presence of pigments in the feces of the symbiont indicates that

at least some of the pigments from the host are not assimilated.

Some radiocarbon was found present in two crabs which had their mouthparts removed prior to contact with a sea urchin injected with labelled glucose. This finding and the high specific activity of the gills in these and other crabs can suggest the possibility of an absorption of dissolved labelled material released by the host. The removal of labelled organic compounds from the surrounding water has been reported in numerous forms (Stephens, 1967; 1968). The gills are seen as the most probable, if not the only, place through which absorption can take place in the otherwise impermeable arthropods. Removal of labelled compounds from solution by arthropods has been reported only in an euphausid (McWhinnie and Johanneck, 1966) and in a freshwater amphipod (Zubchenko, 1966). Such removal is not a proof of a net uptake (Johannes et al., 1969; Johannes and Webb, in press). These workers found that a marine turbellarian showing an apparent uptake of dissolved ^{14}C-amino acids was actually carrying out a net release of free amino acids to the medium.

Host feces provide an additional food source to crabs living in the rectum. Considerable amounts of nutrients remain in the partly digested algae and encrusting animals which constitute the diet of Echinothrix. Organisms associated with the pellets (bacteria, ciliates, and nematodes) are probably ingested. The peritrophic membrane and any mucous present are other possible food sources. Plant and animal proteins, lipids, simple sugars, and glycogen (including glycogen-like polysaccharides of blue-green algae) are assumed to be digested by enzymes normally found present in decapods (see review by Mansour-Bek,

1954). There is no evidence for the presence of enzymes capable of digesting the sugar- and water-insoluble carbohydrates of Ulva.

The organic constituents of the fecal pellets of some marine invertebrates have been analyzed by several workers (see Table XVII). Rough estimates of inorganic material in the gut contents of Diadema antillarum are also given by Lewis (1964). Studies by Johannes and Satomi (1966), Frankenberg et al. (1967), and Frankenberg and Smith (1967) suggest that fecal pellets may have an important role as a nutrient source.

The energy budget of the host has been estimated from measurements taken from five individuals which had been acclimated to somewhat atypical conditions. The shallow water alga Laurencia is probably never ingested in nature and some elements of their normal diet were possibly absent. A starvation period after quantifying food intake probably affected the assimilation of the food still present in the gut. Even when food was provided later, respiration measurements were probably affected by the starvation period. Oxygen consumption values for some individuals were much higher than in the others. Oxygen consumption in other sea urchins has been reported as lower than normal during periods of starvation (Farmanfarmaian, 1966; McPherson, 1968b) and higher after feeding following starvation (Farmanfarmaian and Phillips, 1962). Other possible sources of nutrition that were not quantified were the utilization of previously stored material and the assimilation of dissolved organic matter taken from solution. In any case, similar individuals with crabs living on them were kept under the same conditions for over six months.

TABLE XVII. ORGANIC CONSTITUENTS (AVERAGE % DRY WEIGHTS) OF FRESH FECAL PELLETS OF MARINE INVERTEBRATES

SPECIES	ORGANIC CARBON	NITROGEN	CARBOHYDRATE	PROTEIN	LIPID	REFERENCE
Littorina planaxis (Gastropoda)	2.0	-	-	-	-	North, 1954
Cirriformia tentaculata (Polychaeta)	2.1	-	-	-	-	George, 1964
Hydrobia ulvae (Gastropoda)	10.75	0.02	-	-	-	Newell, 1965
Macoma baltica (Pelecypoda)	8	0.03	-	-	-	Newell, 1965
Palaemonetes pugio (Decapoda)	20	4.48	13	28[a]	2.5	Johannes and Satomi, 1966
Crassostrea virginica (Pelecypoda)	4.6-6.1[b]	-	-	-	-	Haven and Morales - Alamo, 1966
Balanus eburneus (Cirripedia)	5.5-6.8[b]	-	-	-	-	Haven and Morales - Alamo, 1966
Mya arenaria (Pelecypoda)	5.3	-	-	-	-	Haven and Morales - Alamo, 1966
Modiolus demissus (Pelecypoda)	4.4-5.6[b]	-	-	-	-	Haven and Morales - Alamo, 1966
Molgula manhattensis (Urochordata)	5.4	-	-	-	-	Haven and Morales - Alamo, 1966
Callinasa major (Decapoda)	2.9	0.28	-	-	-	Frankenberg et al., 1967
Echinothrix calamaris (Echinoidea)	35.97	-	0.20[c]	0.10	0.51	This work

[a] N x 6.25

[b] Seasonal variation

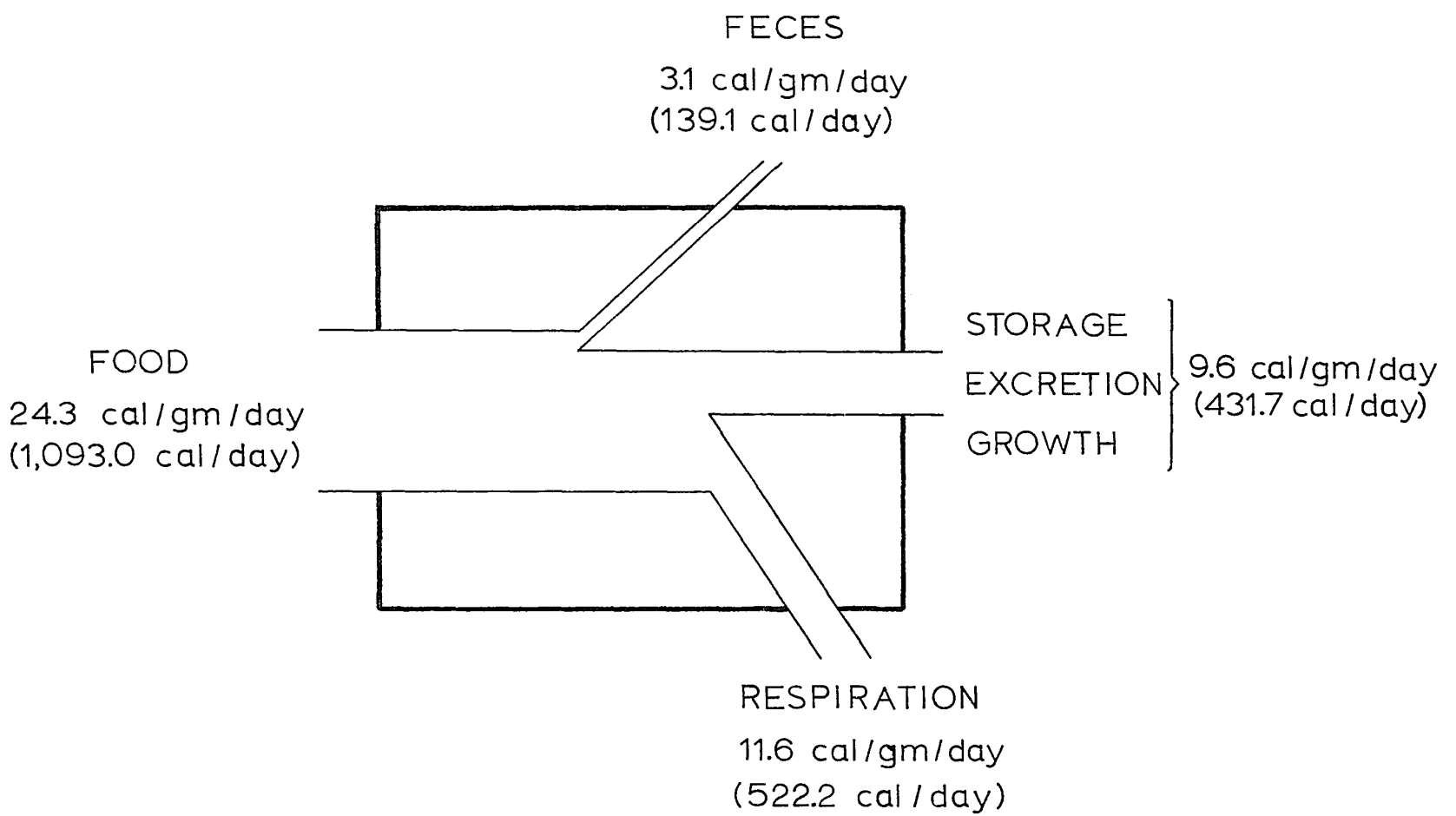
[c] Alcohol and TCA soluble

A model of energy flow in Echinothrix calamaris is given in Figure 33. An average of 431.76 cal/day (equivalent to 9.58 cal/gm/day and 39.5% of the average energy input) is left available for the storage of food energy and for the growth and repair of tissues. This figure includes the energy lost in the form of excretory products. The amount of energy utilized in growth and storage obviously depends on the age, sex, and gonadal stage of each individual. Figures given by Fuji (1967) for Strongylocentrotus intermedius show that the amount of energy required for the gorwth of gonadal tissue varies relatively little in one, two, and four year old individuals (6.8 to 7.8% of the total energy intake), whereas the energy required for growth changes from 12.0% in the one year old individuals to 1.7% in the four year old ones. Storage of food energy in Echinothrix and other sea urchins appears to be considerable. Individuals can be starved for relatively long periods of time.

It is not possible to calculate how much energy from the host is theoretically available to the symbiont. For the five individuals in question, this figure would be a total of 431.76 cal/day (9.58 cal/gm/day) minus the average amount of energy lost as excretory products, the minimal amount required for the growth and repair of tissues not available to the symbiont, and for the storage of food energy in these tissues.

Minimum daily energy requirements for three males living outside the rectum (as measured by the energy equivalent of their oxygen consumption) averaged 6.88 cal/day (Table XVII). Since peristomial

FIGURE 33. A SCHEMATIC REPRESENTATION OF THE ENERGY BUDGET OF INDIVIDUALS OF ECHINOTHRIX CALAMARIS KEPT UNDER LABORATORY CONDITIONS.


FECES
3.1 cal/gm/day
(139.1 cal/day)
FOOD
24.3 cal/gm/day
(1,093.0 cal/day)
STORAGE
EXCRETION
GROWTH
9.6 cal/gm/day
(431.7 cal/day)
RESPIRATION
11.6 cal/gm/day
(522.2 cal/day)

epithelium from four sea urchins had a caloric value of 1.74 cal/mg dry weight, a total of 3.95 mg of tissue would be required to be ingested per day in order to meet these energy requirements. Minimum energy requirements for two adult females living in the rectum averaged 17.86 cal/day. The average caloric value of the coelomocyte aggregations from three sea urchins was 3.57 cal/mg dry weight. A total of 5.00 mg of this material would be required to meet their minimum daily energy requirements.

It was not possible to quantify the ingestion of food in crabs living in their normal microhabitat. The estimated amount of epithelium that would be theoretically ingested by male crabs living in the peristome agrees with the degree of damage normally observed in sea urchins occupied by small crabs. On the other hand, a total of 10.26 mg of peristomial tissue (almost three times as much as in the males) would be required to be ingested in order to meet the minimum energy requirements of females. This agrees with the heavy damage that is observed when large females are kept outside the rectum (Figure 20). Coelomocytes and fecal pellets are constantly being emptied into the rectum. The burden of adult females on the economy of the host is thus minimized by the utilization of these materials as energy sources. The ingestion of fecal material can even be placed on a non-consumer trophic level.

SECTION VI

LARVAL DEVELOPMENT AND HABITAT SELECTION

INTRODUCTION

Searching behavior by the free-living larvae for a specific host is a most critical stage in symbioses. Behavioral adaptations enable these stages to find hosts by means other than chance, as is true of the larvae of most sessile forms (Reese, 1964; Thorson, 1964). Releasing stimuli from the host and the physical environment elicit responses enabling the larvae to differentiate their specific host.

Most of the investigations on host-finding behavior have been carried out on the larvae of trematodes (see reviews by Cheng, 1967; 1968). Work on other symbionts has been relatively scant (see reviews by Davenport, 1955; 1966a). Emphasis has been placed on the demonstration of an attraction of adult stages to "host factors," as in the case of some pinnotherid crabs (Johnson, 1952; Davenport et al., 1960; Sastry and Menzel, 1962). This behavior, however, may have little adaptive significance in forms which spend their entire adult life on their hosts.

In the present study, habitat selection by the settling larval stage of Echinoecus pentagonus has been analyzed by considering its normal host, Echinothrix calamaris, as well as two similar forms, E. diadema and Diadema paucispinum, as possible alternative hosts.

MATERIALS AND METHODS

The larvae were reared following modifications of the methods given by Costlow and Bookhout (1960) and Reese and Kinzie (1968).

A small ovigerous female was removed from her host and isolated in an eight gallon aquarium with air supplied through an air stone. The host was collected from a depth of 30 meters along a shelf forming the seaward edge of Kaneohe Bay. The eggs showed an advanced stage of development. Two nemertean worms (probably Carcinonemertes mitsukurii Takakura) were found living in the abdomen of the female. Nemerteans of the genus Carcinonemertes are known to feed on eggs of brachyuran crabs (Humes, 1942). Several hundred larvae hatched on the thirteenth day of isolation.

Covered plastic boxes, each provided with 18 compartments, each measuring 4.4 X 5.2 X 4.0 cm, were used in the rearing of the larvae. The boxes were immersed overnight in 5% hydrochloric acid followed by thorough washing with distilled water and air-dried under an ultraviolet light. Approximately 30 to 40 ml of sea water was added to each compartment. Water was first prefiltered through an Aqua-pure cartridge filter (Cuno Engineering Co., Meriden, Connecticut). The freshly filtered water was treated with penicillin (50,000 units of Potassium Penicillin G per liter; Eli Lilly & Co., Indianapolis, Indiana) and passed through filter paper. One to four larvae were placed in each of the compartments. The number was gradually reduced in the last stages to one per compartment. The boxes were kept covered in an Eberbach variable speed shaker. Temperatures of the water ranged between 27° and 29°C. The larvae were fed on freshly hatched Artemia nauplii. A generous volume of larvae which had been previously washed with penicillin-treated sea water and filtered through filter paper, was

added to each compartment every day. Some larvae were initially fed on cultures of the diatom Phaeodactylum tricornutum. Larvae were transferred every day by use of Pasteur pipettes into clean boxes containing freshly treated sea water. The original compartments were examined for the presence of molted exoskeletons. The frequency and date of molts were recorded for each of the larvae.

General orientation of megalopae to light was studied by placing individual larvae in square, two liter containers which were darkened in all but one side. Light was provided by a microscope lamp. Responses to gravity and light in a water column was studied by using a 73 cm high chromatography column. Trials were carried out in a dark room with a microscope lamp as a light source.

The relative strength of phototactic responses was correlated with age and a possible attraction to host material. Larvae were individually placed in a one liter beaker containing host material (spines, tube feet, and fecal pellets) on the bottom. Measurements were taken of the time required for each larva to move in the direction of the light of a microscope lamp (directed from above to the surface of the water) after the lamp had been turned off in a dark room for at least one minute and the larva had settled to the bottom. The experiment was carried out with megalopae of different age and with those which emerged from zoea at a later date ("late megalopae").

Orientation of megalopae to its normal host's tissues and those of E. diadema was analyzed by taking time-lapse movies of larvae under different sets of conditions: (1) freshly removed E. calamaris spines versus similar, thoroughly washed spines; (2) E. calamaris

spines versus E. diadema spines, both freshly removed; (3) freshly removed and washed E. diadema spines; (4) freshly removed E. calamaris tube feet versus black, chemically inert carbon chips; (5) E. calamaris tube feet versus E. diadema tube feet; (6) E. diadema tube feet versus black carbon chips; (7) water from an eight gallon aquarium which had contained an individual of E. calamaris for the preceding 24 hours versus water from a control aquarium. Two larvae of the same age were used in the trials, one for each of the two contrasting subjects. Larvae were placed individually in the compartments of a plastic box similar to those used in rearing. Contrasting primary spines, two per compartment, were approximately of the same length, width, and color. Three tube feet or carbon chips were used. The latter were larger in overall size since the tube feet contracted to about one third of their original size.

A photographic apparatus consisted of a Bolex H16M camera connected to a Sage series 500 time-lapse apparatus and set at three frames per minute. Actual measurement showed this to be 1 frame every 26 seconds. Kodak 16 mm Tri-X reversal film was used. Light was provided by two 58 cm fluorescent lamps placed 28 cm above the plastic box. Recordings started immediately after the larvae were placed in the compartments and lasted from 12 to 14.7 hours.

The films were analyzed by recording with a stopwatch the total time of contact of the larvae with the objects. The paths of individual larvae were traced directly from the screen. The movie of larvae placed in sea urchin and control seawater was analyzed by

determining the average speed of five pairs of larvae. The movie projector was stopped every second (equivalent to 7.91 minutes of the actual time) and the position of each larva recorded in sheets of paper taped to the screen. The distance between the points was measured with a ruler. The average speed for the actual distance covered during a period of 237.46 minutes was calculated assuming a straight path between the points.

The possibility of host tissue influencing metamorphosis in megalopae was investigated by adding freshly removed and dead spines, tube feet, and tissue from the test, rectum, or peristome to larvae in separate compartments. Host material was changed daily. Artemia nauplii were not added to some of the compartments.

Orientation of larvae to live sea urchins was studied by placing one sea urchin in an eight gallon aquarium provided with sand, coral fragments, and molluscan shells. General behavior of the larvae was recorded before and after placing a sea urchin in the aquarium.

Forty megalopae, 15 to 21 days old, were added to a 140 gallon tank containing three specimens of E. calamaris in each, including one with a female crab in its rectum, three of E. diadema, and three of D. paucispinum. Sand, shells, coral fragments, and algae (mostly Laurencia sp.) were also present. All sea urchins were removed daily and individually examined for the presence of megalopae or postlarval stages. If dead sea urchins were encountered, they were replaced with living ones.

Postlarval crabs were kept in plastic boxes and fed on tube feet. All crabs were eventually placed on specimens of E. calamaris

and E. diadema. Sea urchins, including one large specimen of D. paucispinum, were kept in the 140 gallon tank previously used. Movements of the crabs on the sea urchins were recorded for the following four months.

RESULTS

Larval Development.

Larval development under laboratory conditions was completed after three zoeal stages and one megalopa.

The first zoeal stage lasted from four to eight days. Molting was more common during the fifth and sixth day after hatching. Mortality during the early stages was relatively low, averaging four to five per day, out of 175-200 larvae. It was noticeably higher, however, during the third and fourth days after hatching due to the clogging of branchiae in some of the larvae which had been feeding on diatoms. Molting appears to have been retarded in other diatom-fed larvae. Mortality was also somewhat higher in the boxes where more than one larva were placed in each of the compartments. The duration of the second zoeal stage was two to five days, that of the third stage was two to eight days.

The total length (including the spines of the telson) of alcohol preserved zoeae is 1.6 mm and carapace length is 0.7 mm. There is a conspicuous, blunt-tipped dorsal spine and a small rostral spine. Reddish-brown chromatophores were observed on the carapace of live larvae. The second and third zoeal stages are similar in general morphology, with the exception of a large reddish spot on the dorsal spine of the latter.

Zoeae showed a strong attraction to light. They were generally very active. Strong movements of the abdomen were involved in locomotion.

A total of 111 megalopae was obtained from 10 to 19 days after hatching. Most appeared during the fourteenth day but a second peak was observed during the seventeenth day. Perhaps the later peak was composed of those showing a retarded development due to inadequate food.

Megalopae have a carapace roughly rectangular in shape, measuring 1.2 mm long by 0.9 mm wide in specimens preserved in glycerin-alcohol. The anterior border of the carapace is conspicuously broadened. The rostrum is small. A small spine is located on each of the anterolateral borders of the carapace. The absence of these spines in the adult is an important diagnostic character for the species. The abdomen is 0.9 mm long. The chelipeds are very well developed, closely resembling those of adults. The antennules and antennae are also well developed. Live larvae were light reddish-brown during daytime and almost transparent at night.

Habitat Selection.

From many hours of observation during rearing and the experimental phase of the study, the following observations can be made with respect to qualitative differences in the behavior of megalopae.

Megalopae were generally less active than the zoeae. They spent a good part of their time resting or crawling on the bottom of their compartments. Resting, and sometimes feeding, was usually in an

upside-down position. Movements were usually along the edges of the compartments. Fast, circular movements were common.

Megalopae showed a positive response to light, although not as strongly as in the zoeae. Strength of the response was gradually lost with age. They began orienting toward the light source rather than moving in its direction. Definite positive responses could no longer be observed in larvae at least six days old. Responses to light in the late megalopae was highly variable.

A positive response to gravity was always observed. The megalopae slowly sank or swam to the bottom when placed in a water column in large containers. Young megalopae placed in a water column moved away from the bottom only when a light beam was directed above. The same larvae did not always follow the light beam when the water column was placed in a horizontal position.

The presence of host material (spines, tube feet, and fecal pellets) did not seem to have any immediate effects in reversing the normal positive response to light of one to five day old megalopae. These almost immediately left the bottom of the container when a light was directed to the surface. Late megalopae and those older than seven days generally stayed in contact with host material for periods ranging between 1.0 to 5.5 minutes.

Table XVIII summarizes results obtained from the analysis of the time-lapse movies. There is a significant difference ($P = 0.014$) between the time that the megalopae spent in contact with freshly removed spines of E. calamaris and the time spent on similar but washed spines. Significance was tested according to the Sum Rank Test

TABLE XVIII. ACTUAL TIME IN MINUTES SPENT BY MEGALOPAE IN CONTACT WITH VARIOUS SEA URCHIN AND BLANK SUBJECTS. VALUES WERE OBTAINED FROM THE ANALYSIS OF 16 MM TIME-LAPSE MOVIES.

AGE IN DAYS OF THE LARVA IN EACH SITUATION	SITUATION AND LENGTH OF TIME SPENT IN CONTACT	
	E. calamaris living spines	*E. calamaris* washed spines
12	722.4 (100%)	65.7 (9.1%)
13	722.4 (100%)	0
14	722.4 (100%)	295.5 (40.9%)
15	309.3 (42.8%)	4.2 (0.6%)
	E. calamaris living spines	*E. diadema* living spines
13	0	135.6 (15.3%)
14	884.4 (100%)	884.4 (100%)
15	819.8 (92.7%)	833.6 (94.3%)
16	364.4 (41.2%)	884.4 (100%)
	E. diadema living spines	*E. diadema* washed spines
15	863.2 (100%)	618.6 (71.7%)
16	863.2 (100%)	326.2 (37.8%)
17*	647.2 (75.0%)	675.8 (78.3%)
18*	521.1 (60.4%)	2.1 (0.2%)

* Larva previously used in *E. calamaris* versus *E. diadema* tube feet trials.

(Continued)

TABLE XVIII. (Continued) ACTUAL TIME IN MINUTES SPENT BY MEGALOPAE IN CONTACT WITH VARIOUS SEA URCHIN AND BLANK SUBJECTS. VALUES WERE OBTAINED FROM THE ANALYSIS OF 16 MM TIME-LAPSE MOVIES.

AGE IN DAYS OF THE LARVA IN EACH SITUATION	SITUATION AND LENGTH OF TIME SPENT IN CONTACT	
	E. calamaris tube feet	carbon chips
14	621.7 (82.7%)	15.9 (21.%)
15	588.9 (78.3%)	223.5 (29.7%)
	E. calamaris tube feet	*E. diadema* tube feet
15	767.9 (86.8%)	9.5 (0.7%)
16	262.7 (29.7%)	0
	E. diadema tube feet	carbon chips
17	0	6.4 (0.7%)
18	843.1 (97.9%)	101.7 (11.7%)

(Dixon and Massey, 1957), a non-parametric method. The megalopae normally moved along the edges of the compartments. Contacts with living spines resulted in the larvae remaining attached to them (Figure 34). They remained stationary, moved along or across the spine, or even pushed it back and forth. Contacts with washed spines were sometimes frequent but of short duration.

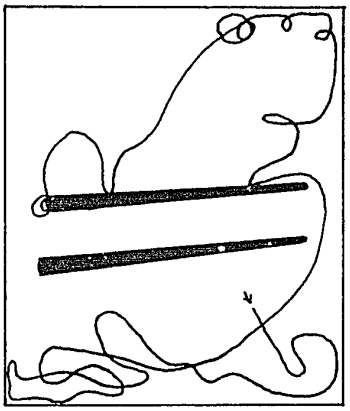
There is obviously no significant difference between the time spent in contact with living spines of E. calamaris and E. diadema. All larvae remained on both types of spines after only one contact. Differences in the orientation to living and washed spines of E. diadema are not as distinctive as in E. calamaris. The larvae made several contacts (three to at least seven) with the washed spines but ended up staying attached to them. Difference in the amount of time in contact can be considered as being not significant ($P = 0.100$).

Trials with tube feet were too few to be of any significance. Megalopae always remained on tube feet of E. calamaris after one contact, a situation observed only once in tube feet of E. diadema.

The possible effects of age and previous contacts with sea urchin material cannot be analyzed with the present data.

There is no significant difference between the rate of movement of megalopae in sea urchin and control sea water (Table XIX). It was assumed that they followed a straight course between the recorded points. This, of course, does not represent the actual situation, but it is a satisfactory way to quantitize an aspect of the behavior of the larvae. The films did not show any indications of differences in speed (orthokineses) or in the frequency of turning (klinokineses) between the megalopae under different conditions, situations which

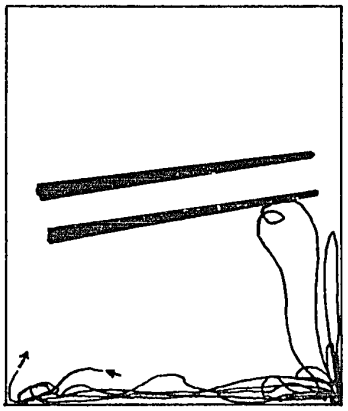
FIGURE 34. EXAMPLES OF THE MOVEMENT OF MEGALOPAE IN COMPARTMENTS CONTAINING LIVING OR WASHED PRIMARY SPINES OF ECHINOTHRIX CALAMARIS. ACTUAL DIMENSIONS ARE SHOWN. TOTAL TIME OF THE RECORDING WAS 12 HOURS AND 2 MINUTES.



living spines

15 day old larva

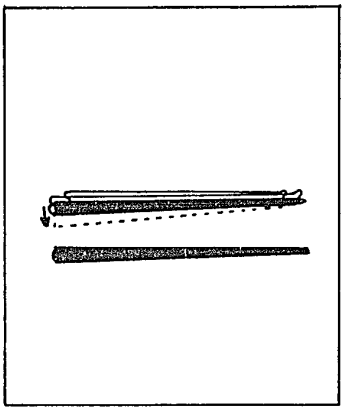
309.3 min (42.8%)
in contact



washed spines

15 day old larva

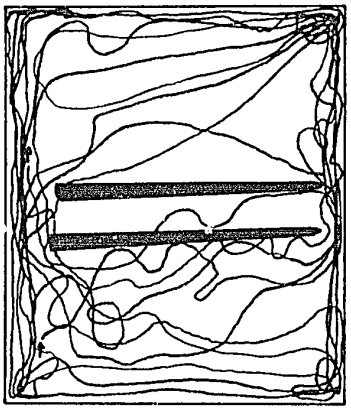
4.2 min (0.6%)
in contact



living spines

12 day old larva

722.4 min (100%)
in contact



washed spines

12 day old larva

65.7 min (9.1%)
in contact

TABLE XIX. RATE OF MOVEMENT OF MEGALOPAE PLACED IN SEA WATER FROM AN AQUARIUM CONTAINING AN INDIVIDUAL OF ECHINOTHRIX CALAMARIS AND IN CONTROL SEA WATER. DATA WERE OBTAINED FROM A SEGMENT OF A 16 MM TIME-LAPSE MOVIE EQUIVALENT TO AN ACTUAL PERIOD OF 237.46 MINUTES.

AGE IN DAYS OF THE LARVA IN EACH SITUATION	E. CALAMARIS WATER		CONTROL WATER	
	Actual distance (mm)	Speed (mm/min)	Actual distance (mm)	Speed (mm/min)
9	577.4	2.43	696.2	2.93
9	400.2	1.68	223.8	0.94
11	684.3	2.88	578.1	2.43
13	425.1	1.79	517.9	2.18
15	312.8	1.31	299.4	1.26

would suggest the triggering of appetitive searching behavior.

Reactions of megalopae to live specimens of E. calamaris were tested on several occasions. A three day old larva swam directly to a sea urchin when placed on the surface of the water approximately 20 cm from the subject. Four and a half hours later, it was observed attached to a primary spine with its anterior end oriented toward the distal end of the spine. Three hours later, the larva was found captured by a tridentate pedicellaria near the ambitus. It was still moving its legs but part of the carapace was crushed and the right cheliped was missing.

A six day old megalopa was added later to the same aquarium. This time a light was placed directly above the aquarium. The larva first oriented toward one of the sides of the aquarium, then swam back to the center, turned around in a small circle, and swam directly to the sea urchin. It was subsequently seen crawling on the test but it could not be found the next day.

Two 11 day old megalopae were placed directly above another sea urchin. One was observed attached to a fleshy base of a primary spine. The sea urchin died three days later. One larva was found dead on it but the second survived and subsequently metamorphosed after three days in contact with tube feet (larva No. 5 of Table XX).

Fourteen day old megalopae did not show any apparent changes in their general behavior when a sea urchin was placed in their vicinity. They settled to the bottom and were active, lifting sand grains with their chelipeds and moving around coral fragments and shells.

Only six of the 40 megalopae that were placed in the large

140 gallon tank were found in contact with sea urchins. The first larva was found near the periproct of a dead E. calamaris 48 hours after they were added. A dead larva was found two days later on a dead E. calamaris and another on a dead D. paucispinum. The other three larvae were observed on a moribund E. calamaris six days after the larvae were added to the tank. One larva was dead but the other two were found crawling on the test. The live larvae were placed back in the tank. No additional megalopae or postlarval stages were found in any of the sea urchins that were present in the tank. Some megalopae were still seen moving on the sand as long as four days after their transfer.

Of the remaining megalopae that were kept in the plastic boxes, only eight metamorphosed into juvenile crabs (Table XX). One of these (No. 6) was part of the late batch of megalopae. Three had no recorded contact with sea urchin material. It is possible that they were involved in the trials recorded in the time-lapse movies. On the other hand, some of the megalopae that were kept in contact with spines or tube feet died without undergoing metamorphosis. Others, with or without previous contact with sea urchin material, were kept on a diet of Artemia nauplii for as long as 37 days.

Most of the megalopae which did not metamorphose after 20 or more days underwent peculiar morphological and behavioral changes. A thin white line developed along the anterior border of the carapace. White spots also appeared on the chelipeds, a characteristic of postlarval crabs. These megalopae did not swim and remained attached to tube feet, spines or any objects placed in their compartments. They readily ate E. calamaris and

TABLE XX. DEVELOPMENT OF MEGALOPAE AND POSTLARVAL CRABS OF *ECHINOECUS PENTAGONUS*

LARVA NO.	MEGALOPA	CRAB I	CONTACT WITH HOST MATERIAL	FURTHER DEVELOPMENT
1	April 9	April 20	none	Crab II (April 27), dead (May 13)
2	April 6	April 22	rectum (two previous days)	Crab II (April 24), crab III (April 28), crab IV (May 7), crab V (May 21), crab VI (June 2) crab VII (June 25); placed on *E. diadema* (July 5) but moved to *E. calamaris* (late July)
3	April 5	April 25	tube feet (5 prev. days)	Crab II (May 3), crab III (May 15); dead (May 15)
4	April 7	April 25	primary spines (15 prev. days)	Crab II (April 27), crab III (May 4), crab IV (May 15); dead (May 15)
5	April 8	April 25	sea urchin (3 days), tube feet (3 prev. days)	Crab II (April 27), crab III (May 3), crab IV (May 15); dead (May 15)
6	April 12	April 27	none	Crab II (May 6), crab III (May 16), crab IV (June 1), crab V (June 27); placed on *E. diadema* (July 5) but moved to *E. calamaris* (late July)
7	April 7	April 28	tube feet (18 prev. days)	Crab II (May 6) } both placed on *E. calamaris* (May 21), one entered into rectum (late July)
8	April 8	May 5	none	Crab II (May 17) }

E. diadema tube feet and spines. Some megalopae were also observed to pick and reject material from fecal pellets. Cannibalism took place when larvae were placed together.

Postlarval crabs were characterized by the presence of a conspicuous white to cream band along the anterior third of the carapace (Figure 35). With the exception of white bands and spots on the chelipeds, the body of live crabs was light to dark reddish-brown. The width of the anterior part of the carapace in live crabs was approximately 1.0 mm. The carapace was straight. The eyes, antennae, and antennules were proportionally larger than in the adults. Sex could not be determined during the first instars.

Like adult crabs, postlarvae showed strong attraction to host material. They were fed mostly on tube feet, consuming up to four per day. Tube feet, as well as epithelial tissue from spines and test of *E. calamaris*, *E. diadema*, and *D. paucispinum*, were immediately ingested. Fecal pellets were never placed in the compartments. Food was handled as by the adults (see Section V).

Postlarval crabs were kept in plastic boxes for as long as 71 days (see Table XX). As many as six molts were recorded in one crab. Nevertheless, growth was not as rapid as in those kept on sea urchins (Nos. 7 and 8 of Table XX). The largest carapace width attained by any of the isolated crabs was 3.0 mm (No. 2 of Table XX), half the size of those living on *E. calamaris* for approximately the same length of time (see below).

Two crabs (Nos. 7 and 8 of Table XX) were placed after their first molt (crab II stage) on separate specimens of *E. calamaris*.

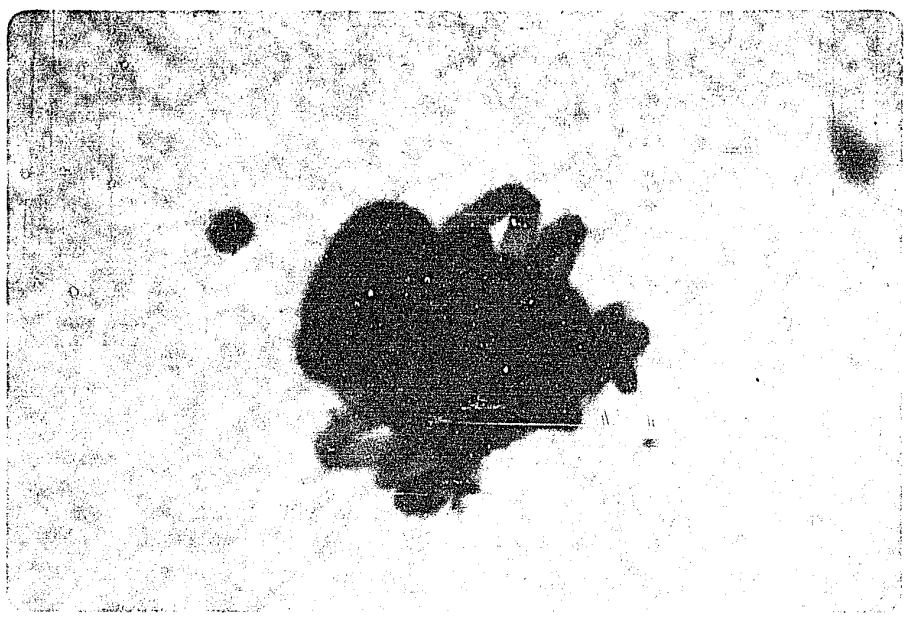


FIGURE 35. CRAB I POSTLARVAL STAGE OF *ECHINOECUS PENTAGONUS* ATTACHED TO A TUBE FOOT OF *ECHINOTHRIX CALAMARIS*. (X30)

Forty days after, both crabs were observed in their original hosts. Both were females, with carapace widths of 6.0 and 6.5 mm, respectively. They showed the typical adult coloration with no white bands or spots. Both were found living on the peristome of their respective hosts where tube feet and epithelial tissue had been removed. One of the crabs was found 25 days later inside the rectum of a large E. calamaris. Its carapace had been recorded as measuring 8.0 mm in width six days before. The sea urchin had shown a normal periproct prior to the invasion.

A female (No. 2 of Table XX) with a carapace width of 3.0 mm, and a male (No. 6 of Table XX) with a carapace width of 2.4 mm were placed on separate specimens of E. diadema. They spent most of the time on the peristome. Epithelial tissue and tube feet were ingested. The female was once observed on the aboral side close to the periproct where two primary spines had been removed. The crabs showed a considerable increase in size after 11 days. Specifically, the carapace of the female measured 3.8 mm wide and that of the male measured 3.1 mm wide. Both crabs were observed two weeks later near the periproct of one specimen of E. calamaris. One was seen shortly afterwards inside the rectum. The crab apparently moved out from the rectum. Four sea urchins, two E. calamaris and two E. diadema, died one month later and no crabs were found on the remaining diadematids.

DISCUSSION

Contrary to observations made on other symbiotic brachyurans, ovigerous females of E. pentagonus are very rare. Of approximately

150-200 females that were examined during a period of four years, only six were found with eggs. Their distribution in time suggests the absence of a defined breeding season. An abbreviated larval development, a characteristic of other Oxyrhyncha (Gurney, 1942), appears to be correlated with a prolonged embryonic stage. Among the Parthenopidae, larval stages have been described for only a few members of the free-living Parthnopinae (Bourdillon-Casanova, 1960; Heegaard, 1963; earlier literature reviewed by Gurney, 1939).

The type and number of experiments that were carried out on the behavior of megalopae were limited by the relatively small number of larvae that were available at one time, the effect of unfavorable conditions in the development of some, and aging. Nevertheless, the information obtained helps to answer the questions of how the symbiont finds its host.

It appears that the free-swimming megalopae, rather than the postlarvae, are involved in the active search for a suitable habitat. Contact of megalopae with host material appears to trigger their metamorphosis into the juvenile stages, which show the same behavioral, physiological, and morphological adaptations that enable the species to live in symbiosis with sea urchins.

A positive orientation to gravity, acting in conjunction with a loss of an initial positive reaction to light, can be involved in bringing the megalopae to the neighborhood of the host.

The capture of one megalopa by a pedicellaria of E. calamaris is an indication of their role as a defensive mechanism. The possible effect of pedicellariae on the settling of larvae has been discussed by Mendes (1965).

Attraction to living material from E. calamaris but not to similar, tissue-free surfaces suggests the presence of chemical rather than visual orientation to the host. Results from trials involving spines of E. diadema may also suggest that the megalopae are attracted to them. Adult crabs also appear to be attracted to the sea urchin (see Section IV). The symbiont has been reported as living in association with this species in other regions, and one small female was once found living on it in Hawaii (see Section II). The incompatibility, however, between the symbiont and E. diadema (as well as with D. paucispinum) has already been demonstrated (see Section IV). Under laboratory conditions, crabs were able to live on this species but eventually moved to the normal host. Similar movements are possible in nature, but it would not explain the absence of the symbiont in most of the populations of E. diadema where no E. calamaris are present. Only juvenile crabs would then be found on these sea urchins, a situation observed only once. Distribution of the symbiont in Hawaii is difficult to explain without the presence of a behavioral mechanism that would enable the settling megalopae to specifically locate their normal host.

A negative orientation to light and a positive orientation to gravity may explain the almost complete absence of the symbiont in E. calamaris from the shallow water populations of Kaneohe Bay and its abundance in the deeper water populations of the same species (see Section III). Crabs have been found in sea urchins from similar shallow depths but in areas that are immediately adjacent to deep water. This would not explain the absence of the symbiont in E. diadema, a form which is also found in relatively deep water.

SECTION VII

CONCLUSIONS

Echinoecus pentagonus is only associated with Echinothrix calamaris in Hawaii. There is an apparent incompatibility between the symbiont and two similar diadematid sea urchins, E. diadema and Diadema paucispinum. The rectum in these two species is not sufficiently large to accomodate the adult female crabs.

It is evident that the symbiont is adapted for its symbiotic existence with sea urchins. The coloration of the adults and most of the juveniles is almost identical to that of the adult E. calamaris. The dactyli of the legs are modified for attachment. The smooth surface, softened outlines, and convex shape of the carapace in the adult females can be interpreted as adaptations that reduce mechanical irritation to the exposed tissues of the host's rectum. The well developed palp of the third maxillipeds is involved in the sorting of the host's fecal pellets.

Host material is the only demonstrable source of nutrition to the symbiont. Males and juvenile females ingest primarily host peristomial tissue. A change in diet correlated with an increase in the energy requirements of the adult females is perhaps the best example of the development of adaptations in the establishment of a dynamic equilibrium between the host and symbiont. The large females ingest coelomocytes and material from the fecal pellets instead of epithelial tissue and tube feet, a situation that normally produces heavy damage and death to the host.

A positive orientation to gravity and a negative orientation to

light coupled with a chemical preference may operate in conjunction to provide the specificity necessary in bringing the free-swimming megalopae into the neighborhood of the host. The postlarval, juvenile, and adult stages show active attraction to the host.

E. calamaris, as the host, can be considered as the "passive" member of the association. Nevertheless, adaptive responses are observed in individuals occupied by the symbiont. The unique pathological calcification of the periproct is interpreted to be a defensive mechanism elicited by the presence of crabs in the host's rectum. The migration of a large number of eleocytes into the lumen of the rectum is suggested as a response to the exposure of tissues to light. Pedicellariae, actively involved in the capture and removal of small bodies that come in contact with the sea urchin, act as a non-specific defense mechanism and may have an effect in the settling of the megalopae.

Certain features of E. calamaris are directly utilized to the "benefit" of the symbiont: namely, the large rectum and peristome, the gall-like calcification of the periproct, the rapid regeneration of epithelial tissue, and the migration and accumulation of coelomocytes in the rectum.

Numerous schemes have been suggested in trying to classify symbioses (see review by Cheng, 1967). This has resulted in a large number of meaningless categories and definitions. The use of the presence or absence of metabolic dependency in categorizing symbioses appears to be the most sensible. It is rather unfair, however, when used in dealing with cases where behavioral adaptations are pre-

dominantly involved, such as in cleaning symbioses and in the association between alpheid shrimps and burrowing gobiids. The metabolic dependency of *E. pentagonus* on its host places the association under the category of parasitism.

LITERATURE CITED

Abott, J.F. 1902. Preliminary notes on Coeloplana. Annot. Zool. Jap., 4:103-108.

Agassiz, A. 1872. Revision of the Echini. Mem. Mus. Comp. Zool. Harvard, 3:1-378.

Agassiz, A. and H.L. Clark. 1907. Preliminary report on the Echini collected, in 1902, among the Hawaiian Islands, by the U.S. fish Commission steamer "Albatross," in charge of Commander Chauncey Thomas, U.S.N., commanding. Bull. Mus. Comp. Zool. Harvard, 50:229-259.

Agassiz, A. and H.L. Clark. 1908. Hawaiian and other Pacific Echini. The Salenidae, Arbacidae, Aspidodiadematidae and Diadematidae. Mem. Mus. Comp. Zool. Harvard, 34:43-132.

Agassiz, A. and H.L. Clark. 1909. Hawaiian and other Pacific Echini. The Echinothuridae. Mem. Mus. Comp. Zool. Harvard, 34:135-203.

Alender, C.A. 1966. A biologically active substance from the spines of two diadematid sea urchins. In Animal Toxins (F.E. Russell and P.R. Saunders, eds.). Pergamon Press, Oxford, 428 pp.

Anderson, J.M. 1966. Aspects of nutritional physiology. In Physiology of Echinodermata (R.A. Boolootian, ed.). Interscience, New York. 822 pp.

Balss, H. 1922. Ostasiatische Decapoden. III. Die Dromiaceen, Oxystomen und Parthenopiden. Arch. Naturgesch., 88A:104-140.

Balss, H. 1924. Ostasiatische Decapoden. V. Die Oxyrhynchen und Schlussteil. Arch. Naturgesch., 90A:20-84.

Balss, H. 1956. Decapoda. VI. Okologie (Fortsetzung). In Dr. H.G. Bronns Klassen und Ordnungen des Tierreichs, Vol. 5, Part 1, Buch 7. Akadem. Verlag., Leipzig.

Black, W.A.P. 1954. Constituents of the marine algae. Chem. Soc. London, Rep. Progr. Chem., 50:322-335.

Bonnier, J. 1898. Note sur le Pionodesmotes phormosomae, copépode parasite du Phormosoma uranus. Résult. Camp. Scient. Prince Albert I Monaco, 12:61-66.

Boolootian, R.A., and A.C. Giese. 1958. Coelomic corpuscles of echinoderms. Biol. Bull., 115:53-63.

Boolootian, R.A., and R. Lasker. 1964. Digestion of brown algae and the distribution of nutrients in the purple sea urchin Strongylocentrotus purpuratus. Comp. Biochem. Physiol., 11:273-289.

Bourdillon-Casanova, L. 1960. Le meroplancton du Golfe de Marseille. Les larves de crustaces decapodes. Rec. Trav. Sta. Mar. Endoume, 30(18):1-286.

Bouvier, E.L., and G. Seurat. 1905. Eumedon convictor, crabe commensal d'un oursin. C.R. Hebd. Séanc. Acad. Sci., Paris, 140:629-631.

Brimacombe, J.S., and J.M. Webber. 1964. Mucopolysaccharides: chemical structure, distribution and isolation. Elsevier, Amsterdam, N.Y. 181 pp.

Bruce, A.J. 1967. Descriptions of some new genera and species from the Western Indian Ocean and the South China Sea. Notes on some Indo-Pacific Pontoniinae. III-IX. Zool. Verhand., 87:1-73.

Buddenbrock, W. von. 1937. Grundriss der vergleichenden Physiologie. Gebrüder Borntraeger, Berlin. 567 pp.

Buitendijk, A.M. 1950. On a small collection of Decapoda Brachyura chiefly Dromiidae and Oxyrhyncha, from the neighborhood of Singapore. Bull. Raffles Mus., 21:59-82.

Casselman, W.G.B. 1959. Histochemical Technique. Methuen, London. 205 pp.

Castro, P. 1966. Check-list of the symbiotic decapod crustaceans of the Hawaiian Islands. M.S. Thesis (Plan B), University of Hawaii.

Caullery, M. 1952. Parasitism and Symbiosis. Sidgwick and Jackson, London. 340 pp.

Cheng, T.C. 1964. The Biology of Animal Parsites. W.B. Saunders, Philadelphia. 727 pp.

Cheng, T.C. 1967. Marine molluscs as hosts for symbioses, with a review of known parasites of commercially important species. In Advances in Marine Biology, Vol. 5 (F.S. Russell, ed.). Academic Press, London. 424 pp.

Cheng, T.C. 1968. The compatibility and incompatibility concept as related to trematodes and molluscs. Pac. Sci., 22:141-160.

Clark, A.H. 1921. A monograph of existing crinoids. Volume 1. The comatulids. Part 2. Bull. U.S. Nat. Mus. 82:1-795.

Clark, J.M. 1964. Experimental Biochemistry. Freeman, San Francisco. 228 pp.

Costlow, J.D. and C.G. Bookhout. 1960. A method for developing brachyuran eggs in vitro. Limnol. Oceanog., 5:212-215.

Dales, R.P. 1957. Commensalism. In Treatise in Marine Ecology and Paleoecology, Vol. 1 (J.W. Hedgpeth, ed.). Geol. Soc. Am., Mem. 67:1-1296.

Davenport, D. 1955. Specificity and behavior in symbioses. Quart. Rev. Biol., 30:29-46.

Davenport, D. 1966a The experimental analysis of behavior in symbioses. In Symbiosis, Vol. 1 (S.M. Henry, ed.). Academic Press, New York. 478 pp.

Davenport, D. 1966b. Echinoderms and the control of behavior in associations. In Physiology of Echinodermata (R.A. Boolootian, ed.). Interscience, New York. 822 pp.

Davenport, D., G. Camougis, and J.F. Hickok. 1960. Analyses of the behaviour of commensals in host-factor. I. A hesionid polychaete and a pinnotherid crab. Anim. Behav., 8:209-218.

Delaunay, H. 1931. L'excrétion azotée des invertébrés. Biol. Rev. 6:265-301.

Dixon, W.J. and F.J. Massey. 1957. Introduction to statistical analysis. McGraw-Hill, New York. 488 pp.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem., 28:350-356.

Ebert, T.A. 1969. Growth rates of the sea urchin Strongylocentrotus purpuratus related to food availability and spine abrasion. Ecology, 49:1075-1091.

Edmondson, C.H. 1946. Reef and shore fauna of Hawaii. Spec. Publ. Bernice P. Bishop Mus., 22:1-381.

Edmondson, C.H. 1951. Some Central Pacific crustaceans. Occ. Pap. Bernice P. Bishop Mus., 20:183-243.

Einstein, R., R.E. Trueheart, and G.M. Hass. 1960. Pathogenesis of abnormal tissue calcifications. In Calcification in Biological Systems (R.F. Sognnaes, ed.). A.A.A.S., Washington. 511 pp.

Endean, R. 1966. The coelomocytes and coelomic fluid. In Physiology of Echinodermata (R.A. Boolootian, ed.). Interscience, New York. 822 pp.

Eppley, R.W. and R. Lasker. 1959. Alginase in the sea urchin, Strongylocentrotus purpuratus. Science, 129:214-215.

Farmanfarmaian, A. 1966. The respiratory physiology of echinoderms. In Physiology of Echinodermata (R.A. Boolootian, ed.). Interscience, New York. 822 pp.

Farmanfarmaian, A., and J.W. Phillips. 1962. Digestion, storage, and translocation of nutrients in the purple sea urchin (Strongylocentrotus purpuratus). Biol. Bull., 123:105-120.

Ferguson, J.C. 1964a. Nutrient transport in starfish. I. Properties of the coelomic fluid. Biol. Bull., 126:33-53.

Ferguson, J.C. 1964b. Nutrient transport in starfish. II. Uptake of nutrients by isolated organs. Biol. Bull., 126:391-406.

Flipse, H.J. 1930. Die Decapoda Brachyura der Siboga-Expedition. VI. Oxyrrhyncha: Parthenopidae. Siboga Exped., 39c(2):1-94.

Fox, D.L. 1953. Animal Biochromes and Structural Colours. Cambridge Univ. Press. 378 pp.

Fox, D.L., and T.S. Hopkins. 1966. The comparative biochemistry of pigments. In Physiology of Echinodermata (R.A. Boolootian, ed.). Interscience, New York. 822 pp.

Frankenberg, D., and K.L. Smith. 1967. Caprophagy in marine animals. Limnol. Oceanog., 12:443-450.

Frankenberg, D., S.L. Coles, and R.E. Johannes. 1967. The potential trophic significance of Callinassa major fecal pellets. Limnol. Oceanog., 12:113-120.

Fuji, A. 1961. Studies on the biology of the sea urchin. IV. Histological observation of the food canal of Strongylocentrotus intermedius. Bull. Fac. Fish. Hokkaido Univ., 11:195-202.

Fuji, A. 1962. Studies on the biology of the sea urchin. V. Food consumption of Strongylocentrotus intermedius. Jap. J. Ecol., 12:181-186.

Fuji, A. 1967. Ecological studies on the growth and food consumption of Japanese common littoral sea urchin, Strongylocentrotus intermedius (A. Agassiz). Mem. Fac. Fish. Hokkaido Univ., 15:83-160.

Garth, J.S. 1957. Reports on the Lund University Chile Expedition 1948-49. 29. The Crustacea Decapoda Brachyura of Chile. Acta Univ. Lund, n.s., 53(7):1-130.

George, J.D. 1964. Organic matter available to the polychaete Cirriformia tentaculata (Montagu) living in an intertidal mudflat. Limnol. Oceanog., 9:453-455.

Giard, A. 1911. Oeuvres Diverses, Réunies et Rééditées par les Soins d'un Groupe d'Elèves et d'Amis. Vol. 1. Laborat. Evolut. Etres Organis., Paris. 590 pp.

Giese, A.C. 1961. Further studies on Allocentrotus fragilis, a deep-sea echinoid. Biol. Bull., 121:141-150.

Giese, A.C. 1966. On the biochemical constitution of some echinoderms. In Physiology of Echinodermata (R.A. Boolootian, ed.). Interscience, New York. 822 pp.

Goodwin, T.W. 1960. Biochemistry of pigments. In The Physiology of Crustacea, Vol. 1 (T.H. Waterman, ed.). Academic Press, New York. 670 pp.

Gravier, C. 1922. Sur un nouveau crabe (Eumedomus petiti nov. sp.) commensal d'un oursin de Tuléar (Madagascar). Bull. Mus. Hist. Nat. Paris, 28:484-486.

Green, J. 1961. A Biology of Crustacea. Quadrangle Books, Chicago. 180 pp.

Gude, W.D. 1968. Autoradiographic Techniques. Prentice-Hall, Englewood Cliffs, N.J. 113 pp.

Gurney, R. 1939. Bibliography of the Larvae of Decapod Crustacea. Ray Society, London. 123 pp.

Gurney, R. 1942. Larvae of Decapod Crustacea. Ray Society, London. 306 pp.

Hamann, O. 1887. Beiträge zur Histologie der Echinodermen. Heft 3. Anatomie und Histologie der Echiniden und Spatangiden. G. Fischer, Jena. 176 pp.

Hansen, H.J. 1902. Echinocheres globosus n. gen., n. sp., a copepod parasitic in spines of an echinothurid. Vidensk. Meddr. Dansk Naturh. Foren., 1902:437-449.

Haven, D.E., and R. Morales-Alamo. 1966. Aspects of biodeposition by oysters and other invertebrate filter feeders. Limnol. Oceanog., 11:487-498.

Heegaard, P. 1963. Decapod larvae from the Gulf of Napoli hatched in captivity. Vidensk. Meddr. Dansk Naturh. Foren., 125:449-493.

Heyde, H.C. van der. 1923. Petites contributions à la physiologie comparée. IV. Sur l'excretion chez les echinodermes. Arch. Neerl. Physiol., 8:151-159.

Hiatt, R.W. and D.W. Strasburg. 1960. Ecological relationships of the fish fauna on coral reefs of the Marshall Islands. Ecol. Monogr., 30:65-127.

Hilgard, H.R., W.E. Hinds, and J.H. Phillips. 1967. The specificity of uptake of foreign proteins by coelomocytes of the purple sea urchin. Comp. Biochem. Physiol., 23:815-824.

Hinegardner, R.T. 1961. Studies on the sea urchin egg nucleus: its isolation, structure, physical properties and DNA content. Ph.D. Thesis. University of Hawaii.

Hipeau-Jacquotte, R. 1965. Notes de faunistique et de biologie marines de Madagascar. III. Un nouveau décapode nageur (Pontoniinae) associé aux oursins dans la région de Tuléar: Tulеariocaris holthuisi nov. gen. et nov. sp. Rec. Trav. Sta. Mar. Endoume, 37:247-259.

Holland, N.D. and Sr. A. Nimitz. 1964. An autoradiographic investigation of the gut mucopolysaccharides of the purple sea urchin (Strongylocentrotus purpuratus). Biol. Bull., 127:280-293.

Holthuis, L.B. 1953. Enumeration of the decapod and stomatopod Crustacea from Pacific Coral Islands. Atoll Res. Bull., 24:1-62.

Humason, G.L. 1967. Animal Tissue Techniques. Freeman, San Francisco. 569 pp.

Humes, A.G. 1942. The morphology, taxonomy, and bionomics of the nemertean genus Carcinonemertes. Illinois Biol. Mongr., 18(4): 1-105.

Hyman, L.H. 1955. The Invertebrates. Vol. 4. Echinodermata. The Coelomate Bilateria. McGraw-Hill, New York. 763 pp.

Jackson, R.T. 1912. Phylogeny of the echini, with a revision of paleozoic species. Mem. Boston. Soc. Nat. Hist., 7:1-491.

Jennings, J.B., and D.F. Mettrick. 1968. Observations on the ecology, morphology and nutrition of the rhabdocoel turbellarian Syndesmis franciscana (Lohman, 1946) in Jamaica. Carib. J. Sci., 8:57-69.

Johannes, R.E., and M. Satomi. 1966. Composition and nutritive value of fecal pellets of a marine crustacean. Limnol. Oceanog., 11:191-197.

Johannes, R.E., and M. Satomi. 1967. Measuring organic matter retained by aquatic invertebrates. J. Fish. Res. Bd. Canada, 24:2467-2471.

Johannes, R.E., and K.L. Webb. In press. Release of dissolved organic compounds by marine and freshwater invertebrates. Proc. Conf. Organic Matter Nat. Waters.

Johannes, R.E., S.J. Coward, and K.L. Webb. 1969. Are dissolved amino acids an energy source for marine invertebrates? Comp. Biochem. Physiol., 29:283-288.

Johnson, D.S. 1962. Commensalism and semi-parasitism amongst decapod Crustacea in Singapore waters. Proc. First Regional Symposium on Scientific Knowledge of Tropical Parasites, 282-288.

Johnson, I.S. 1952. The demonstration of a "host-factor" in commensal crabs. Trans. Kansas Acad. Sci., 55:458-464.

Johnson, M.J. 1949. A rapid micromethod for estimation of non-volatile organic matter. J. Biol. Chem., 181:707-711.

Johnson, P.T. 1969. The coelomic elements of sea urchins (Strongylocentrotus). III. In vitro reaction to bacteria. J. Invert. Pathol., 13:42-62.

Kharasch, M.S. 1929. Heats of combustion of organic compounds. Bur. Standards J. Res., 2:359-430.

Klunzinger, C.B. 1906. Die Spitz- und Spitzmund Krabben (Oxyrhyncha und Oxystomata) des Roten Meeres. F. Enke, Stuttgart. 91 pp.

Knudsen, J.W. 1967. Trapezia and Tetralia (Decapoda, Brachyura, Xanthidae) as obligate ectoparasites of pocilloporid and acroporid corals. Pac. Sci., 21:51-57.

Lasker, R., and R.A. Boolootian. 1960. Digestion of the alga, Macrocystis pyrifera, by the sea urchin, Strongylocentrotus purpuratus. Nature, 188:1130.

Lasker, R., and A.C. Giese. 1954. Nutrition of the sea urchin Strongylocentrotus purpuratus. Biol. Bull., 106:328-340.

Laurie, R.D. 1915. Reports on the marine biology of the Sudanese Red Sea. XXI. On the Brachyura. J. Linn. Soc. London, Zool., 31: 407-475.

Lawrence, J.M. 1967. Lipid reserves in the gut of three species of tropical sea urchins. Carib. J. Sci., 7:65-68.

Lawrence, J.M., A.L. Lawrence, and A.C. Giese. 1966. Role of the gut as a nutrient-storage organ in the purple sea urchin (Strongylocentrotus purpuratus). Physiol. Zool., 39:281-290.

Lenz, H. 1905. Ostafrikanische Dekapoden und Stomatopoden. Gesammelt von Herrn Prof. Dr. A. Voeltzkow. Abh. Senckenb. Naturf. Ges., 27:341-392.

Lewis, J.B. 1964. Feeding and digestion in the tropical sea urchin Diadema antillarum Philippi. Can. J. Zool., 42:549-557.

Lewis, J.B. 1967. Nitrogenous excretion in the tropical sea urchin Diadema antillarum Philippi. Biol. Bull., 132:34-37.

Lewis, J.B. 1968a. Comparative respiration of tropical echinoids. Comp. Biochem. Physiol., 24:649-652.

Lewis, J.B. 1968b. Respiration in the tropical sea urchin Diadema antillarum Philippi. Physiol. Zool., 41:476-480.

Lillie, R.D. 1928. Eine Schnellmethode zur Toluidinblau-Schleimfärbung. Z. Wiss. Mikrosk., 45:381.

Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193:265-275.

MacMunn, C.A. 1885. On the chromatology of the blood of some invertebrates. Quart. J. Micro. Soc., 25:469-490.

MacNamee, C.G. 1961. Life history, morphology, habits and taxonomy of Hapalocarcinus marsupialis (Arthropoda, Crustacea, Decapoda). M.S. Thesis. University of Hawaii.

McPherson, B.F. 1968a. Contributions to the biology of the sea urchin Eucidaris tribuloides (Lamarck). Bull. Mar. Sci., 18:400-443.

McPherson, B.F. 1968b. Feeding and oxygen uptake of the tropical sea urchin Eucidaris tribuloides (Lamarck). Biol. Bull., 135:308-321.

McWhinnie, M.A., and R. Johanneck. 1966. Utilization of inorganic and organic carbon compounds by Antarctic zooplankton. Antarctic J. U.S., 1:210.

Mansour-Bek, J.J. 1954. The digestive enzymes in Invertebrata and Protochordata. In Tabulae Biologicae, Vol. 21 (H.J. Vonk et al., eds.). Junk, The Hague.

Matthews, D.C., and S.J. Townsley. 1964. Additional records of Hawaiian Platyctenea (Ctenophora). Pac. Sci., 18:349-351.

Mendes, E.G. 1965. The pedicellariae and the settling of pelagic larvae of sessile animals on sea urchins. An. Acad. Bras. Cienc., 37(Suppl.):215-219.

Menzel, D.W., and R.F. Vaccaro. 1964. The measurement of dissolved organic and particulate carbon in seawater. Limnol. Oceanog., 9:138-142.

Mercier, J. 1937. Zoothylacies d'échinide fossile provoquées par un crustacé Castexia douvillei nov. gen., nov. sp. Bull. Soc. Géol. France, ser. 5, 6:149-154.

Mertens, R. 1935. Aus dem Leben des Seeotters. Natur Volk, 65:401-407.

Miers, E.J. 1879. On the classification of the Maioid Crustacea of Oxyrhyncha with a synopsis of the families, sub-families and genera. J. Linn. Soc. London, Zool., 14:634-673.

Millot, N. 1957. Naphthoquinone pigment in the tropical sea urchin Diadema antillarum Philippi. Proc. Zool. Soc. London, 129:263-272.

Milne Edwards, A. 1879. Description de quelques crustaces nouveaux. Bull. Soc. Philomath. Paris, ser. 7, 3:103-110.

Milne Edwards, H. 1834. Histoire Naturelle des Crustacés, Comprenant l'Anatomie, la Physiologie et la Classification de ces Animaux. Vol. 1. Roret, Paris. 468 pp.

Miyake, S. 1937. Brachyura of the Danjo Islands. (In Japanese). Biogeographica, 2:27-30.

Miyake, S. 1939. Note on crabs of the genus Echinoecus Rathbun living commensally with echinoids (Parthenopidae, Eumedoninae). Annot. Zool. Jap., 18:83-94.

Monod, T. 1938. Decapoda Brachyura. Mission Robert Ph. Dollfus en Egypte. Mem. Inst. Egypte, 37:91-162.

Mori, T. 1953. Seaweek polysaccharides. In Advances in Carbohydrate Chemistry, Vol. 8 (C.S. Hudson and M.L. Wolfrom, eds.). Academic Press, New York. 408 pp.

Morrison, J.P.E. 1954. Ecological notes on the mollusks and other animals of Raroia. Atoll Res. Bull., 34:1-18.

Mortensen, T. 1928. A Monograph of the Echinoidea. I. Cidaroidea. C.A. Reitzel, Copenhagen. 551 pp. Atlast with 88 plates.

Mortensen, T. 1940. A Monograph of the Echinoidea. III (1). Aulodonta. C.A. Reitzel, Copenhagen. 370 pp. Atlas with 77 plates.

Mortensen, T. 1943. A Monograph of the Echinoidea. III (3). Camarodonta II. C.A. Reitzel, Copenhagen. 446 pp. Atlas with 66 plates.

Mukerjee, P. 1956. Use of ionic dyes in the analysis of ionic surfactants and other ionic organic compounds. Anal. Chem., 28:870-873.

Needham, R.L. 1966. Some aspects of the ecology, ethology and larval development of the sea-cucumber crab Lissocarcinus orbicularis Dana. M.S. Thesis (Plan B), University of Hawaii.

Newell, R. 1965. The role of detritus in the nutrition of two marine deposit feeders, the prosobranch Hydrobia ulvae and the bivalve Macoma balthica. Proc. Zool. Soc. London, 144:25-45.

Nicol, J.A.C. 1967. The Biology of Marine Animals. Interscience, New York. 699 pp.

Nobili, G. 1907. Ricerche sui crostacei della Polinesia. Decapodi, stomatopodi, anisopodi e isopodi. Mem. Accad. Sci. Torino, ser. 2, 57:351-430.

North, W.J. 1954. Size distribution, erosive activities, and gross metabolic efficiencies of marine intertidal snails, Littorina planaxis and L. scutulata. Biol. Bull., 106:185-197.

Parker, B., and A. Diboll. 1966. Alcian stains for histochemical localizations of acid and sulfated polysaccharides in algae. Phycologia, 6:37-46.

Parry, G. 1960. Excretion. In The Physiology of Crustacea, Vol. 1 (T.H. Waterman, ed.). Academic Press, New York. 670 pp.

Patton, W.K. 1967a. Commensal Crustacea. Proc. Symp. Crustacea, 3:1228-1244.

Patton, W.K. 1967b. Studies on Domecia acanthophora, a commensal crab from Puerto Rico, with particular reference to modifications of the coral host and feeding habits. Biol. Bull., 132:56-67.

Pearce, J.B. 1962. Adaptation in symbiotic crabs of the family Pinnotheridae. Biologist, 45:11-15.

Pequignat, E. 1966. Skin digestion and epidermal absorption in irregular and regular urchins and their probable relation to the outflow of spherule-coelomocytes. Nature, 210:397-399.

Phillipson, J. 1964. A miniature bomb calorimeter for small biological samples. Oikos, 15:130-139.

Potts, F.A. 1915. Hapalocarcinus, the gall-forming crab, with some notes on the related genus Cryptochirus. Pap. Dept. Mar. Biol. Carnegie Instn. Wash., 8:33-69.

Price, C.A. 1965. A membrane method for determination of total protein in dilute algal suspensions. Anal. Biochem., 12:213-218.

Prosser, C.L., and F.A. Brown. 1961. Comparative Animal Physiology. W.B. Saunders, Philadelphia. 688 pp.

Randall, J.E., R.E. Schroeder, and W.A. Stark. 1964. Notes on the biology of the echinoid Diadema antillarum. Carib. J. Sci., 4:421-433.

Rathbun, M.J. 1894. Notes on the crabs of the family Inachidae in the United States National Museum. Proc. U.S. Nat. Mus., 17: 43-75.

Rathbun, M.J. 1906. The Brachyura and Macrura of the Hawaiian Islands. Bull. U.S. Fish Comm., 23:827-930.

Rathbun, M.J. 1918. The grapsoid crabs of America. Bull. U.S. Nat. Mus., 97:1-461.

Raup, D.M. 1966. The endoskeleton. In Physiology of Echinodermata (R.A. Boolootian, ed.). Interscience, New York. 822 pp.

Read, C.P. 1968. Some aspects of nutrition in parasites. Am. Zool., 8:139-149.

Reese, E.S. 1964. Ethology and marine zoology. In Oceanography and Marine Biology, Annual Review, Vol. 2 (H. Barnes, ed.). George Allen and Unwin, London. 548 pp.

Reese, E.S. and R.A. Kinzie. 1968. The larval development of the coconut or robber crab Birgus latro (L.) in the laboratory (Anomura, Paguridea). Crustaceana, Suppl. 2:117-144.

Rioja, E. 1944. Observaciones sobre algunas especies de cangrejos del género Dissodactylus Smith (Braquiuros Pinnotéridos) de las costas mexicanas del Pacífico. Estudios carcinológicos. XVI. An. Inst. Biol. Méx., 15:147-160.

Rioja, E. 1950. Observaciones ecológicas acerca del inquilinismo de algunos crustáceos. Rev. Soc. Mex. Hist. Nat., 11:141-147.

Ross, D.M. 1967. Behavioral and ecological relationships between sea anemones and other invertebrates. In Oceanography and Marine Biology, Annual Review, Vol. 5 (H. Barnes, ed.) George Allen and Unwin, London. 653 pp.

Sakai, T. 1936. Crabs of Japan. (In Japanese). Tokyo. (Not seen).

Sakai, T. 1938. Studies on the Crabs of Japan. III. Brachygnatha, Oxyrhyncha. Yokendo, Tokyo. 364 pp.

Sakai, T. 1965. The Crabs of Sagami Bay Collected by His Majesty the Emperor of Japan. East-West Center Press, Honolulu. 206 pp. (English text).

Sastry, A.N., and R.W. Menzel. 1962. Influence of hosts on the behavior of the commensal crab Pinnotheres maculatus Say. Biol. Bull., 123:388-395.

Serène, R. 1961. A note on the systematics of the Brachyura and the morphology of commensal species. Proc. Ninth Pac. Sci. Congr., 10:32-34.

Serène, R. and K. Romimohtarto. 1963. On some species of Eumedoninae from Indo-Malayan region. Mar. Res. Indonesia, 6:1-17.

Serène, R., T.V. Duc, and N.V. Luom. 1958. Eumedoninae du Viet-Nam (Crustacea) (avec un bibliographie de la sous-famille). Treubia, 24:135-242.

Smith, D., L. Muscatine, and D. Lewis. 1969. Carbohydrate movement from autotrophs to heterotrophs in parasitic and mutualistic symbiosis. Biol. Rev., 44:17-90.

Spruit, C.J.P. 1949. Absorption spectra of quinones. I. Naphthoquinones and naphthohydroquinones. Rec. Trav. Chim. Pays Bas, 68:309-324.

Stephens, G.C. 1967. Dissolved organic material as a nutritional source for marine and estuarine invertebrates. In Estuaries (G.H. Lauff, ed). A.A.A.S., Washington. 757 pp.

Stephens, G.C. 1968. Dissolved organic matter as a potential source of nutrition for marine organisms. Am. Zool., 8:95-106.

Sephensen, K. 1935. Some endoparasitic copepods found in echinoids. Vidensk. Meddr. Dansk Naturh. Foren., 98:223-234.

Stott, F.C. 1955. The food canal of the sea urchin Echinus esculentus L. and its functions. Proc. Zool. Soc. London, 125:63-86.

Strickland, J.D.H., and T.R. Parsons. 1968. A practical handbook of seawater analysis. Bull. Fish. Res. Bd. Canada, 167:1-311.

Swann, E.F. 1966. Growth, autotomy, and regeneration. In Physiology of Echinodermata (R.A. Boolootian, ed.). Interscience, New York. 822 pp.

Thompson, S.W. 1966. Selected Histochemical and Histopathological Methods. Charles C. Thomas, Springfield, Ill. 1639 pp.

Thorson, G. 1964. Light as an ecological factor in the dispersal and settlement of larvae of marine bottom invertebrates. Ophelia, 1:167-208.

Tinker, S.W. 1958. Pacific Sea Shells. Charles E. Tuttle Co., Rutland, Vt. 240 pp.

Verrill, A.E. 1867. Remarkable instances of crustacean parasitism. Am. J. Sci., ser. 2, 44:126.

Vevers, H.G. 1966. Pigmentation. In Physiology of Echinodermata (R.A. Boolootian, ed.). Interscience, New York. 822 pp.

Vonk, H.J. 1960. Digestion and metabolism. In The Physiology of Crustacea, Vol. 1 (T.H. Waterman, ed.). Academic Press, New York. 670 pp.

Ward, M. 1934. Notes on a collection of crabs from Christmas Island, Indian Ocean. Bull. Raffles Mus., 9:5-28.

Wass, M.L. 1968. A new pinnixid commensal with a holothurian (Crustacea:Decapoda). Tulane Stud. Zool., 14:137-139.

West, E.S., W.R. Todd, H.S. Mason, and J.T. van Bruggen. 1966. Textbook of Biochemistry. Macmillan, New York. 1595 pp.

Wolvekamp, A.P., and T.H. Waterman. 1960. Respiration. In The Physiology of Crustacea, Vol. 1 (T.H. Waterman, ed.). Academic Press, New York. 670 pp.

Zubchenko, I.A. 1966. Utilization of D-glucose-1-C^{14} dissolved in water by fresh-water shrimp Gammarus (R.) balcanicus. (In Russian). Nauch. Dokl. Vyssh. Shk., Biol. Nauk., 9(4):117-119. (Abstract) Biol. Abstr., 48 (103991).