OBSERVATIONS ON THE ECOLOGY AND BEHAVIOR OF GUAM PEARLFISHES (CARAPIDAE)

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ABSTRACT

From April, 1968, to January, 1969, 337 specimens of pearlfishes and 1,776 host specimens were collected on the fringing reefs of Guam. Five species of pearlfishes are represented and their distinguishing characteristics discussed. These specimens were taken from five holothurian species and from the cushion starfish, Culcita novaeguineae. The percentage of infestation of the pearlfishes varied with the host species and the reef zone occupied by the hosts.

Carapus homei tenuis larvae were collected in May, September and October. Observations on the tenuis larvae indicate that metamorphosis into the juvenile stage probably occurs within a host. The total length of the adults appears to be dependent upon the size of the host species. C. homei may have an annual reproductive cycle, with spawning in the late summer, and an increase of infestation by the tenuis and juvenile forms during the winter months.

The form called Carapus mourlani is structurally similar to C. homei but differs in its behavior (host preference), superficial melanophores, size of the pectoral fins, and possibly in the seasonality of the life form. Experimental results suggest that the anatomical differences of the two forms are not due to the effects of the hosts.

It was found that adult specimens of Encheliophis gracilis form male and female pairs, each pair infesting a single host. This paired infestation is dependent upon the gonadal development of the pearlfishes and only specimens in a spawning condition were found paired. Two egg rafts from these E. gracilis pairs were spawned in aquaria, and the embryonic and larval development of the fertile eggs was observed.

Developing vexillifer larvae reached a length of 2.5 mm.

The majority of the pearlfishes were found in the coelom or respiratory trees of their hosts. Radiographs indicate that the fishes move tail first up the respiratory trees of the holothurians upon entry, and the area of breakthrough into the coelom of the hosts may be the cloaca.

Stomach contents indicate that _Encheliophis_ _gracilis_ specimens feed exclusively on the gonads of _Holothuria_ _argus_. The other pearlfish species appear to be predators, feeding upon fishes and shrimps.

The behavior of the Guam pearlfishes was observed and filmed in the laboratory. Location of the hosts, pearlfish entry and host reaction was noted.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

OBSERVATIONS ON THE ECOLOGY AND BEHAVIOR OF GUAM PEARLFISHES (CARAPIDAE)

CHAPTER I. INTRODUCTION

The Carapidae are teleostean fishes belonging to the order Percomorphi, suborder Blennioidea. They are relatively small fishes with scaleless, elongate and tapering eel-like bodies. Carapids are well known for their symbiotic existence within holothurians, asteroids, bivalve mollusks, and tunicates. The name "pearlfish" is derived from the fact that the host for some species is the pearl oyster. Pearlfishes have been found inside oysters, trapped and entombed by a covering of mother-of-pearl (Herald, 1961). They are found throughout the tropical Pacific, Atlantic, Mediterranean, and Indian oceans. A few species occur in temperate waters.

Few of the Pacific pearlfishes have been studied in detail. Much of the available information is of a taxonomic nature with little attention given to their ecology and behavior.

The purpose of this study is to investigate species of pearlfishes on Guam, Mariana Islands, with regard to distribution, host preference, food habits, the symbiotic relationship with their echinoderm hosts and other phases of their life histories. Much of the study is concerned with the population dynamics of the pearlfish species and is based upon data collected in the field. Ethological studies were conducted in laboratory aquaria using various species of pearlfishes and their hosts. Where pertinent, ecological observations of the hosts are presented and correlated with data on pearlfishes.

This study began in April, 1968, and fieldwork was concluded in January, 1969. Due to time limitations, data were not collected through a complete annual cycle. Field research centered primarily at the Gognga Beach sector of Tumon Bay (Figs. 1A and 2A). This area was selected because of physical accessibility and the dense populations of holothurian species commonly found there. In addition, the fringing reef of Tumon Bay possesses many distinct physiographic reef zones, each providing different environmental conditions and associated holothurian species.

Review of the Literature

Taxonomic and descriptive references to the family Carapidae date back to Linnaeus (1758). Generic and specific descriptions of the Pacific carapids are found in Putnam (1874); Fowler (1900, 1927, and 1928); Barnard (1927); Petit (1934); Herre (1936); Abe (1939); Edmondson (1946); de Beaufort and Chapman (1951); Smith, J. L. B. (1955); Bonham (1960); Gosline and Brock (1960); Schultz (1960); and Smith, C. L. (1964).

Descriptions of pearlfishes occurring specifically in the Micronesian area of the Pacific are found in Abe (1939); Bonham (1960); Schultz (1960); and Smith (1964).

The larval development and life history of Carapus acus (Brunnich), a Mediterranean species, is considered in Emery (1880); Raffaele (1888); and Padoa (1947 and 1957). Strasburg (1961) discusses the larval development of Carapus homei (Richardson) from Hawaii and Smith (1964) considers the larval development of C. homei from Guam.

Observations on the behavior and ecology of pearlfishes are found in Doleschall (1858 and 1861); Anderson (1859); Emery (1880); Linton (1907); Parker (1926); Mukerji (1932); Arnold (1953, 1956 and 1957); Leiner (1960); and Klingel (1961). Smith (1964) presents notes on the ecology of the Guam pearlfishes. Hipeau-Jacquotte (1967) studied the ecology of *Carapus homei* in Madagascar.

Emery (1880), in his monograph on *Carapus acus*, a Mediterranean species, presented the first detailed anatomical description of a pearlfish.

Arnold (1956), provides a systematic revision of the family Carapidae on a world-wide basis which includes three genera, four subgenera and 23 species.

Smith (1964) recorded four species of pearlfishes from Guam including *Carapus homei* (Richardson), *C. mourlani* (Petit), *C. parvipinnis* (Kaup), and *Encheliophis gracilis* (Bleeker). In addition to these four species, Schultz (1960) reports *E. vermicularis* Müller from Guam.

Life History of the Carapidae

The eggs of pearlfishes are spawned in gelatinous masses known as egg-rafts. Each raft contains hundreds of small, ellipsoidal eggs. The raft drifts seaward and the eggs hatch into pelagic larvae known as vexillifers. This remarkable larval stage is characterized by a long, lobate, dorsal appendage called the vexillum, a modification of the first dorsal fin ray (Padoa, 1957); and a long caudal filament which extends posteriorly. The function of the vexillum is thought to be sensory, perhaps guiding the larvae to areas where acceptable hosts reside (Marshall, 1964). These vexillifer larvae live and feed in the plankton.

Vexillifers captured in plankton tows were originally and mistakenly described as adults under the name Vexillifer dephilippii Gasco (1870). The larvae grow to a size of approximately 85 mm in length before metamorphosing into the second larval stage (Padoa, 1947).

The second larval stage of pearlfishes is considered to be benthic in habitat initially and inquiline later (Arnold, 1956). As in the case of the vexillifers, these larvae were originally described as adults of a species known as Encheliophis tenuis by Putman (1874). They were later recognized as preadult stages and called tenuis larvae (Padoa, 1947). The tenuis stage is characterized by its small head, immense body length, and lack of vexillum. Tenuis larvae lack pigment and are transparent. The pectoral fins are small and rounded at this stage, and the vent is at the rear of the body cavity or abdomen. Tenuis larvae have been described for very few pearlfish species, the lengths of which are all within 150-200 mm.

Pearlfishes transform into juveniles within their hosts and there is at this time a tremendous reduction in total body length, as much as 75 percent. The vent migrates forward reaching the gular area directly under the pectoral base and the pectoral fins enlarge (with the exception of Encheliophis vermicularis which has no pectoral fins).

From the juvenile to the adult stage, there is a general lengthening of body proportions. The length of the head increases, compared to the total length of the fish. The pectoral fins continue to enlarge, and the width of the head at the pectorals increases.

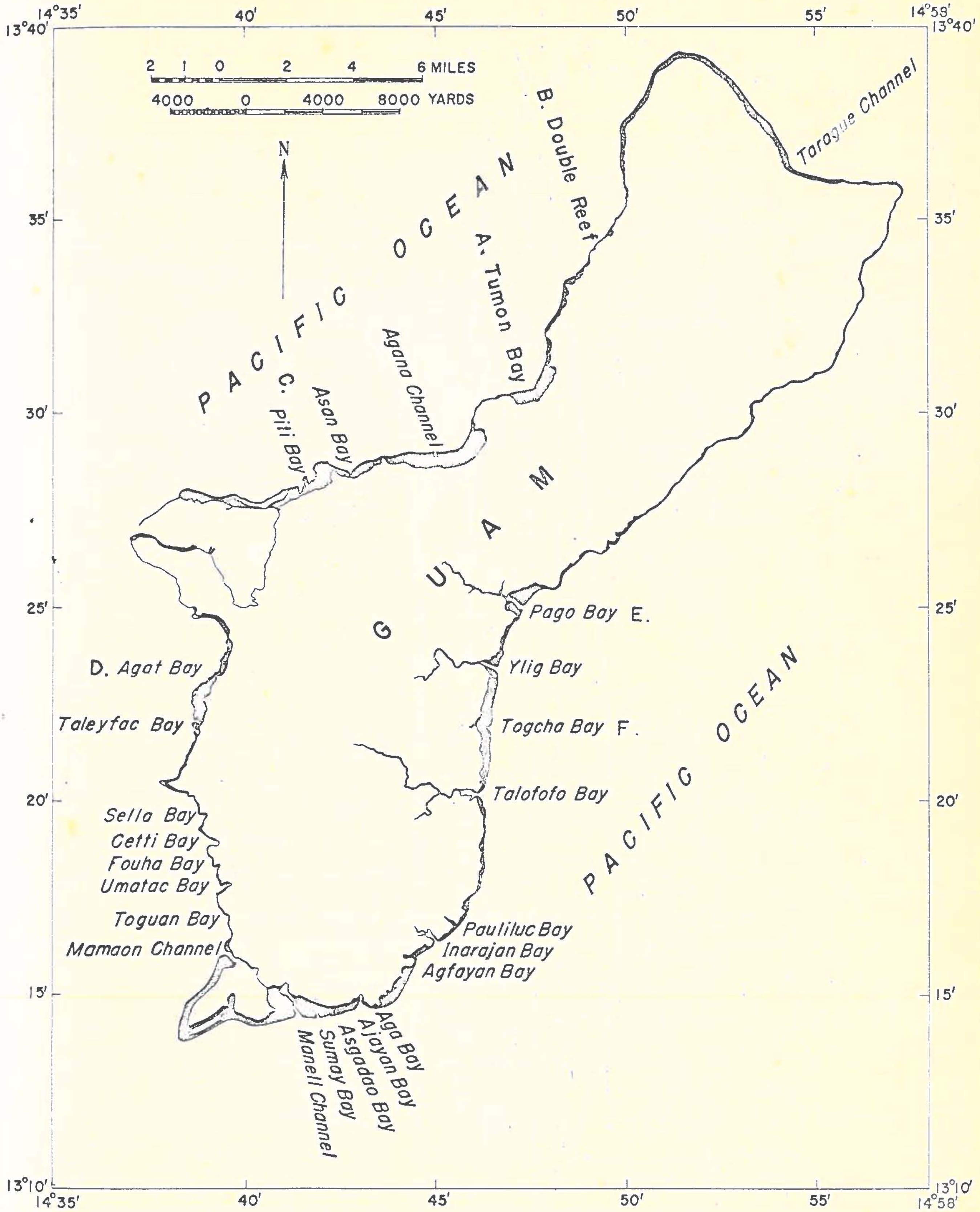
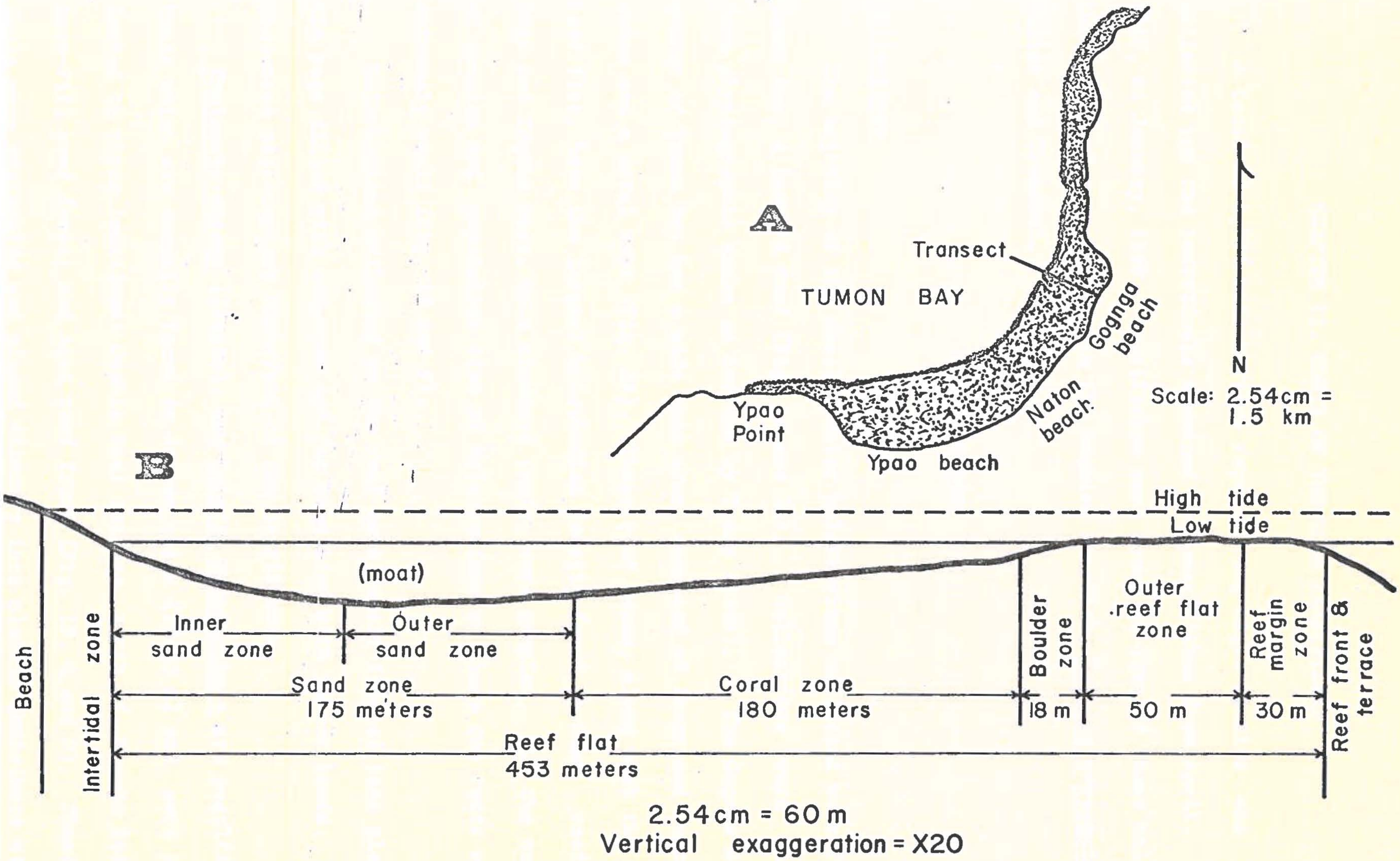


Fig. 1. Map of Guam showing collection areas (modified from Emery, 1962). A. Tumon Bay; B. Double Reef; C. Asan-Piti reef; D. Agat reef; E. Pago Bay;

Fig. 2. A, location of the reef transect and collecting area (Gognga Beach) in Tumon Bay; and B, reef zones across the transect line.


A
Transect
TUMON BAY
Gognga beach
Naton beach
Ypao Point
Ypao beach
N
Scale: 2.54cm = 1.5 km
B
High tide
Low tide
(moat)
Beach
Intertidal zone
Inner sand zone
Outer sand zone
Sand zone 175 meters
Coral zone 180 meters
Boulder zone
18 m
Outer reef flat zone
50 m
Reef margin zone
30 m
Reef front & terrace
Reef flat 453 meters
2.54cm = 60 m
Vertical exaggeration = X20

CHAPTER II. HOSTS OF THE GUAM PEARLFISHES

A total of 1,776 holothurians representing eight species was collected and the individuals examined for pearlfishes from April, 1968, to January, 1969. Pearlfishes were found in five of these holothurian species as well as in the cushion starfish, <u>Culcita</u> <u>novaeguineae</u> Müller and Troschel.

Methods

<u>Collections</u>

The majority of the shallow-water holothurians collected were from Tumon Bay, a leeward reef (Figs 1A and 2A). Collections were made from physiographic zones as defined by Tracy (1964). The Gognga Beach sector of Tumon Bay has a wide moat area composed of inner and outer sand zones and a coral zone (Fig. 2B). These reef zones, along with the outer reef flat, have the largest holothurian density found on Guam reefs. Pago and Togcha Bays, both windward reefs, were sampled, but the number of species and population size of holothurians on these two reefs were extremely small (Fig. 1E and F).

Holothurians collected on the reef were gently floated into plastic bags filled with seawater and the bags tied with rubber bands. This prevented post-capture transfers of the pearlfishes.

<u>Thelenota</u> <u>ananas</u> (Jaeger), and <u>Culcita</u> <u>novaeguineae</u> are pearlfish hosts which are normally found in deeper water (10-35 m) and were collected in three locations: Double Reef terrace, north of Haputo Point; Asan-Piti reef front; and Agat reef front (Fig. 1B, C, and D). These animals were segregated and placed directly into plastic containers with

seawater. Plastic bags were not suitable for use with these two organisms due to their large size.

Holothurians used in behavior experiments were collected in large plastic containers with seawater and transported in an inner tube fitted with a plywood bottom. These animals were taken immediately to the laboratory and placed in aquaria.

In most instances, those holothurians not collected for behavioral work were dissected within an hour of collection. In a few cases, holothurians were left in plastic bags for longer periods of time, and in one case, overnight. In these instances, the infesting pearlfishes abandoned their hosts, presumably because of lack of oxygen.

Reef transect

A transect was established across the Gognga Beach reef to quantitatively determine the zonal distribution of holothurians. This was done to delimit holothurian species within their habitats, and to determine the distribution of pearlfishes. This transect extended from the intertidal zone of the beach to the seaward edge of the reef front. The line was established by driving iron pipes into the reef flat at the intertidal zone, the reef margin zone, and at a point midway between them. A 100-pound test monofilament line was stretched between the pipes. A rope grid, nine meters square with concrete blocks attached to each corner, was laid down randomly five times in each reef zone across the transect line while moving progressively seaward. Within each grid area a complete search for all holothurians present was made. This search included overturning rocks and coral heads and fanning the sand to uncover the burrowing forms. In instances where large areas of staghorn coral

(Acropora sp.) occurred, the coral colony was broken into small pieces which could be lifted from the water and examined. The total number of holothurian species found within each grid was recorded, along with habitat data. Since boundaries of one reef zone may gradually grade into the next zone, the five random samples were taken well within each zone. Holothurians occurring in boundary areas were not counted.

Specimens of each holothurian species encountered on the transect were collected, photographed, and preserved. Efforts were made to identify those species which were infested by pearlfishes, and those which might act as pearlfish hosts. Identifications were based primarily on the microscopic dermal ossicles of each species, as described by Fisher (1907) and Panning (1944). Distinguishing characteristics of these animals are summarized in Table I.

Description of Reef Zones and Holothurians

Eighteen holothurian species were found on the transect representing the families Holothuriidae, Stichopodidae, and Synaptidae. Figure 3 is indicative of the total number of holothurians within each reef zone. A brief zonal analysis of those species which were collected follows:

Sand zone of the inner reef flat

This reef zone was 175 meters wide and can be divided into two subzones, an inner region composed primarily of sand with fleshy algae, and an outer sand zone composed of fleshy algae and a few scattered clumps of coral (Fig. 2B). At mean low tide, this zone and the coral zone form a moat approximately one meter deep behind the exposed boulder zone and foraminifera tests, and fragments of calcareous algae, coral, and mollusks. The entire zone is relatively flat. The predominant holothurian found within this zone was Holothuria atra (Jaeger), (Fig. 4). H. atra does not burrow, and was seldom found under rocks or coral heads. Often H. atra was found with a thin covering of sand and foraminifera over its dorsal surface.

Holothuria sp. 1 (Fig. 5) was commonly found in the sand zone, usually extending from the base of coral heads. This holothurian is often mistaken for H. atra, but differs in habitat, texture of body wall, Cuvierian organs, and dermal ossicles.

Specimens of Holothuria argus (Jaeger), (Fig. 6) were occasionally found in the sand zone. During the day, H. argus was normally found exposed, often with a covering of algae fragments held to its back by dorsal podia. At night, H. argus was often found partially buried in the sand, leaving its anus and last fourth of its body exposed. These holothurians were often found in pairs, and two color phases could be easily recognized.

The large, white burrowing Holothuria sp. 2 (Fig. 7) was very abundant in the sand zone and was not normally found elsewhere. This animal was found completely buried by day, but was often found exposed in the late afternoon and evening. Holothuria sp. 2 burrows in open sand, and was never found under coral or rocks.

An occasional Stichopus chloronotus Brandt (Fig. 8) was found in the sand zone, usually exposed but near clumps of coral. Specimens of S. chloronotus found in this zone were larger than those found on the outer reef flat, and a greater percentage of them were infested with the

Carapus homei than their outer reef flat counterparts.

Specimens of Stichopus variegatus Semper (Fig. 9) were also found in the sand zone, often in pairs and always on the underside of coral heads and rocks.

Coral zone

This zone was 180 meters wide and grades gradually from the outer portion of the sand zone toward the outer reef flat. The coral zone was deepest at the shoreward side (one meter) and decreased in depth seaward until, at the boulder zone, it was exposed at the lowest low tide. The coral zone consists of a sandy bottom and large colonies of corals, of which Acropora sp. was most abundant. The most common holothurian in this zone was Holothuria atra. Stichopus chloronotus and S. variegatus were more abundant here than in the sand and coral zones. A few specimens of Actinopyga mauritiana (Quoy and Gaimard), (Fig. 10) were found in the coral zone in areas where the reef pavement is exposed from the sand.

The coral zone was the main habitat of Holothuria argus. This holothurian was always found in open sand and never under rocks or coral. These animals were often found in aggregations with the individuals numbering 15 to 20 in each group.

Boulder zone

This zone was 18 meters wide on the transect and was usually exposed at low tide. The outer region of this zone contained large, scattered coral boulders resting on sand. Between these boulders there were patches of relatively barren reef pavement, covered in places by a thin mat of fleshy algae and foraminifera.

Although this was the smallest reef zone, four unidentifiable species of *Holothuria* and two of *Stichopus* were found under rocks, buried in the sand. These species were found nowhere else along the transect and none were infested with pearlfishes.

Holothuria atra was again the dominant holothurian. *Stichopus chloronotus* and *S. variegatus* continued to be more abundant as the collecting moved seaward.

Outer reef flat

This zone was 50 meters wide and like the boulder zone, was usually exposed or awash during low tides. The outer reef was a flat, barren pavement covered with a thin turf of fleshy algae overlying crustose red algae. Living foraminifera were very abundant on this algal turf. Holothurians resided under rocks and in small depressions on the reef flat. *Stichopus chloronotus* was the most abundant species found here, either exposed or under rocks. *Holothuria atra* was also abundant on the reef flat and was always found exposed. The outer reef flat was the primary habitat of *Actinopyga mauritiana*, which was normally found exposed or against the base of coral clumps. *Holothuria argus* was found in sand patches on the reef pavement. In areas of the outer reef flat where no depressions in the reef pavement exist, very few holothurians were found due to exposure at low tide and wave action at high tide.

Reef margin and terrace

The reef margin is the living and actively growing part of the fringing reef. Here the waves break against the reef flat and there is holothurians were found in this zone due to wave action, especially in the shallow area near the reef flat.

The large holothurian, Thelenota ananas (Jaeger), (Fig. 11) was occasionally found in the reef margin area, but it was normally found in sand on the reef terrace, down to a depth of at least 35 meters.

The cushion starfish, Culcita novaeguineae Müller and Troschel (Fig. 12), distinctive in possessing no identifiable arms as an adult, was found on the reef flat, but more commonly on the reef margin and the reef terrace. Culcita was normally found exposed and inactive during the day. Several color variations of Culcita were seen ranging from entirely yellow, to yellow on the aboral surface and purple on the oral surface. Adult specimens often measured 30 cm across the oral surface.

Analysis

From observations along the transect, it appears that most holothurian species inhabit specific zones on the reef, with the exception of Holothuria atra. H. atra was found abundantly in all shallow reef zones, with its greatest density in the coral zone. H. spp. 1 and 2 were concentrated in the sand zone. H. argus had its greatest density in the coral zone. Stichopus chloronotus increased in density seaward and was found in the greatest numbers on the outer reef flat. S. variegatus was concentrated in the boulder zone. Actinopyga mauritiana was found in the greatest numbers on the outer reef flat.

Generally, the density of holothurians along the transect increased from the intertidal zone to the boulder zone and outer reef flat. Holothuria argus and Stichopus variegatus were often found in pairs. Aggregations of S. chloronotus, S. variegatus, and Holothuria argus were common.

Holothuria atra, and often *Stichopus chloronotus* and *Actinopyga mauritiana* remained exposed, whereas the other holothurian species remained hidden during daylight hours.

The narrowest reef zone, the boulder zone, revealed the greatest diversity of holothurian species.

Pearlfish Infestation

The numbers of hosts examined and pearlfishes found are summarized in Table II.

Of the holothurian species found, *Holothuria argus*, *Stichopus chloronotus*, and *Thelenota ananas* each acted as hosts for two pearlfish species. *Holothuria* sp. 1, *Stichopus variegatus*, and *Culcita novaeguineae* were found to be infested by single species of pearlfishes.

Eighty-eight percent of *Thelenota ananas* collected were infested, a percentage far higher than the other hosts (Table II). Infestation of *Culcita novaeguineae* was 44 percent; *Holothuria argus*, 22 percent; and *Stichopus chloronotus*, 18 percent.

Over 150 specimens of *Holothuria atra* were collected from the various reef zones and none were found to be infested with pearlfishes. Smith (1964) collected 107 *H. atra* from Tumon Bay and he also found no pearlfishes. Infestation of *H. atra* by *Encheliophis gracilis* juveniles was observed in aquaria, but in each instance the pearlfish would leave the host within a few hours. It appears that there is some internal condition (possibly chemical) which repels pearlfishes. Strasburg (1961) [illegible] *Holothuria atra* in Hawaii and Schultz

(1960) reports Encheliophis vermicularis infesting Holothuria atra in Guam.

Forty-five specimens of Holothuria sp. 2 (a burrowing, stout bodied, thread producing form, whitish in color with two broad brownish transverse bands on the dorsal surface) were collected and none were found to be infested. Smith (1964) records Encheliophis gracilis infesting this species on Guam. A possible reason why Holothuria sp. 2 was not a host is that it buries itself completely under the sand for long periods of time. During these periods of inactivity, the oxygen need of the holothurian is apparently slight as very little water is drawn into its respiratory trees. This would tend to restrict the movement and respiration of an inquiline. H. argus is a preferred host and also buries in the sand; however, this species normally leaves its anus exposed above the sand and continues to fill and empty its respiratory trees, thus, pearlfish respiration and movement would not be restricted.

Thirty-eight specimens of Actinopyga mauritiana were collected, and none were infested. Mukerji (1932) reports Carapus homei infesting a species of Actinopyga in the Andaman Islands. The possibility exists that the entrance of Carapus homei into Actinopyga mauritiana is normally restricted due to the holothurian's enlarged anal teeth. Infestation was observed in aquaria by this investigator, but penetration appeared extremely difficult for the pearlfish to negotiate.

Culcita novaeguineae was found to be infested by Carapus mourlani. Doleschall (1861) collected Culcita specimens infested by Encheliophis gracilis. The present investigator observed E. gracilis entering Culcita in an aquarium but such occurrences were rare.

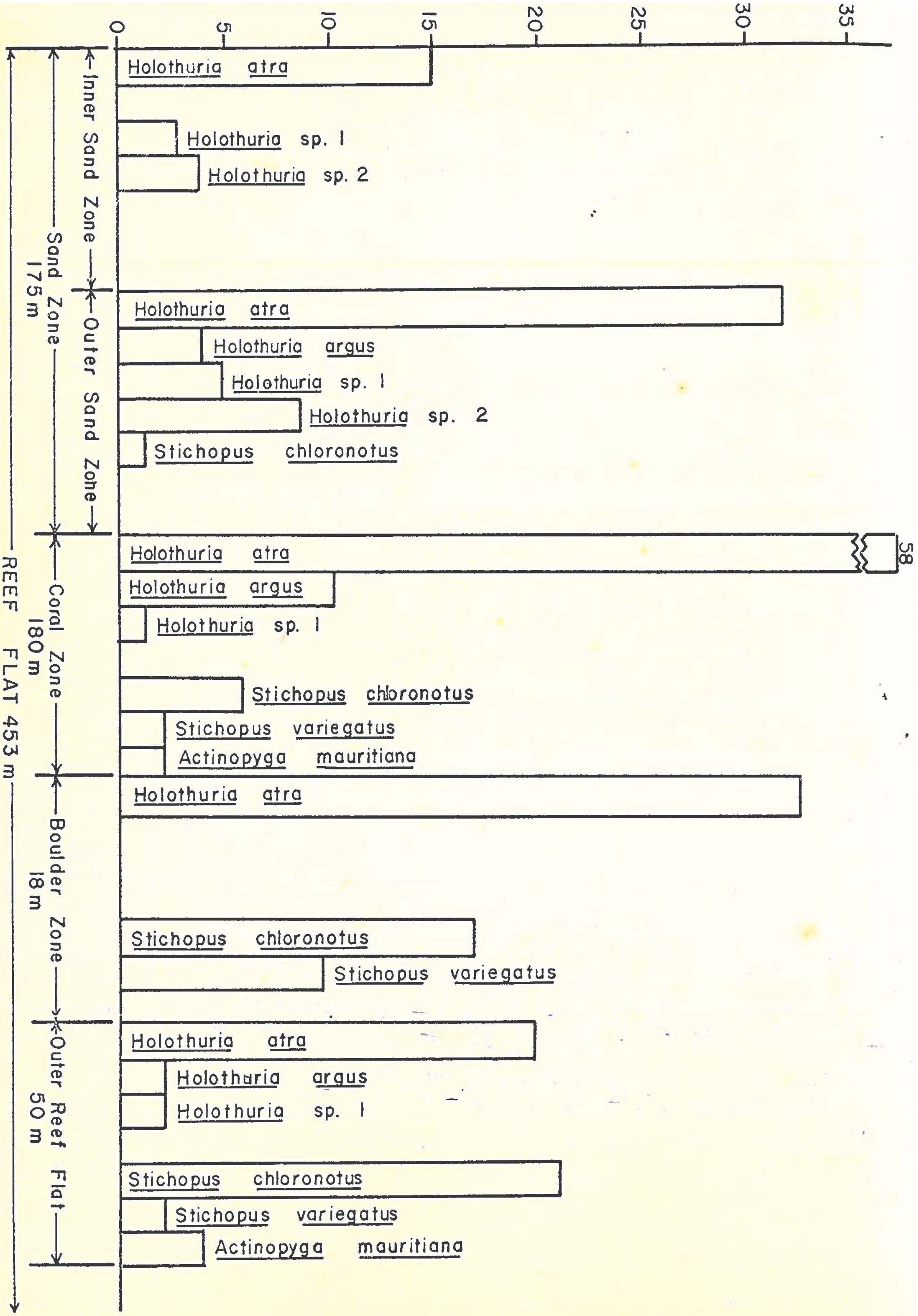
TABLE I. TAXONOMIC CHARACTERISTICS OF SOME HOLOTHURIAN HOSTS OF GUAM PEARLFISHES

	HOLOTHURIAN							
ACTERISTIC	Holothuria atra Figure 4	Holothuria sp. 1 Figure 5	Holothuria argus Figure 6	Holothuria sp. 2 Figure 7	Stichopus chloronotus Figure 8	Stichopus variegatus Figure 9	Actinopyga mauritiana Figure 10	Thelenota ananas Figure 11
r	black	black	brown, with orange spots, sole yellow	cream, with dark transverse bands	greenish-black	yellowish-grey	brown	brown, with orange sole
llae	none	dense	scattered	scattered	on warts	on warts	on warts	on warts
a (sole)	scattered	scattered	scattered	scattered	scattered	3 bands	3 bands	scattered
s	none	none	none	none	2 rows	scattered	scattered	scattered
ierian bules	none	very thin	medium thickness	very thick	none	none	none	none
e	± 180 mm	± 200 mm	± 220 mm	± 220 mm	± 180 mm	± 130 mm	± 130 mm	± 600 mm
ads	1 dense tuft	1 tuft	1 dense tuft	1 dense tuft	2, finely branching	2, finely branching	1 dense tuft	2, densely branching
cal entacles	20	20	18	18	18	18	20	18
mal ssicles	tables, rods	tables, buttons, plates	rosette rods	rosette rods	tables, chela rods	tables, rosette rods	rosette rods	tables rosette rods
itat	exposed, all reef zones	partially extended from coral	exposed, sandy areas	buried or exposed, sand zone	exposed, coral zone & reef flat	under rocks boulder & coral zone	reef pavement, reef flat	sand, reef margin & terrace

TABLE II. HOST SPECIFICITY OF GUAM PEARLFISHES

PEARLFISH	N	HOST					
		Holothuria argus	Holothuria sp. 1	Stichopus chloronotus	Stichopus variegatus	Thelenota ananas	Culcita novaeguineae
Carapus homei	185	57	---	127	1	---	---
Carapus mourlani	22	---	---	---	---	---	22
Carapus parvipinnis	46	---	---	---	---	46	---
Encheliophis gracilis	84	48	---	8	---	13	---
Encheliophis vermicularis	1	---	1	---	---	---	---
None	---	363	159	600	59	8	27
TOTAL	338	468	160	735	60	67	49
Percentage of infested hosts	---	22.4	0.6	18.4	1.7	88.1	44.9

Fig. 3. Total number of common holothurian species in each reef zone of Gognga Beach, along a transect line.


0
5
10
15
20
25
30
35
Inner Sand Zone
Outer Sand Zone
Sand Zone 175 m
Coral Zone 180 m
Boulder Zone 18 m
Outer Reef Flat 50 m
REEF FLAT 453 m
Holothuria atra
Holothuria sp. 1
Holothuria sp. 2
Holothuria atra
Holothuria argus
Holothuria sp. 1
Holothuria sp. 2
Stichopus chloronotus
Holothuria atra
58
Holothuria argus
Holothuria sp. 1
Stichopus chloronotus
Stichopus variegatus
Actinopyga mauritiana
Holothuria atra
Stichopus chloronotus
Stichopus variegatus
Holothuria atra
Holothuria argus
Holothuria sp. 1
Stichopus chloronotus
Stichopus variegatus
Actinopyga mauritiana

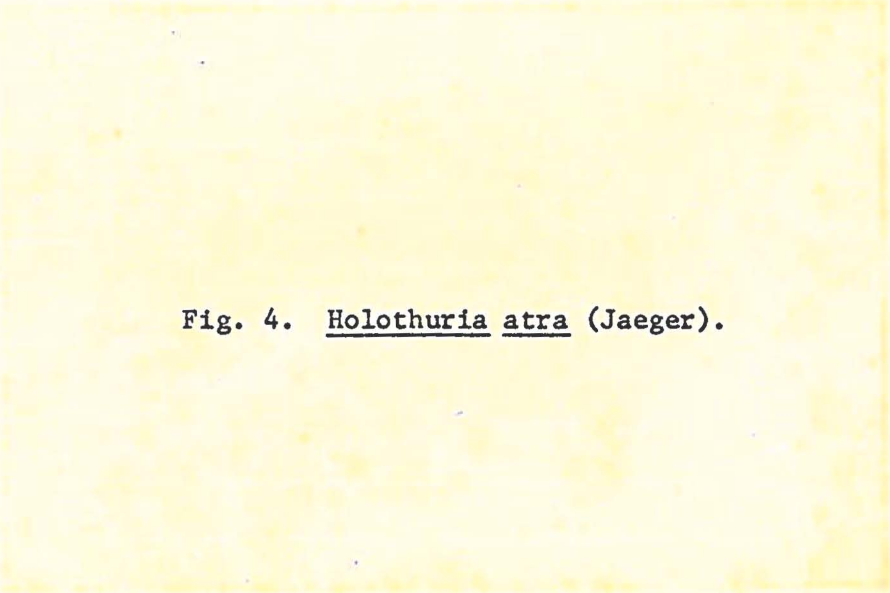


Fig. 4. *Holothuria atra* (Jaeger).

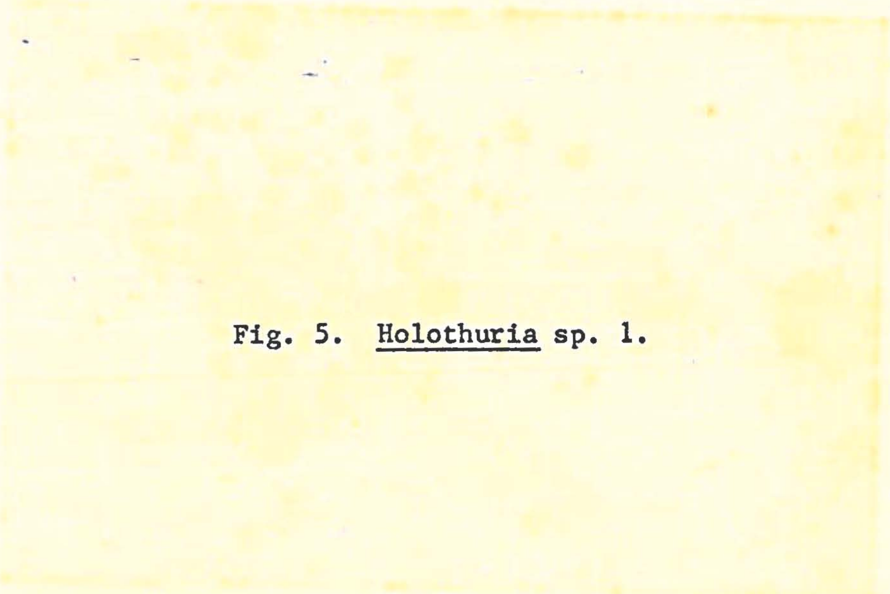


Fig. 5. *Holothuria* sp. 1.

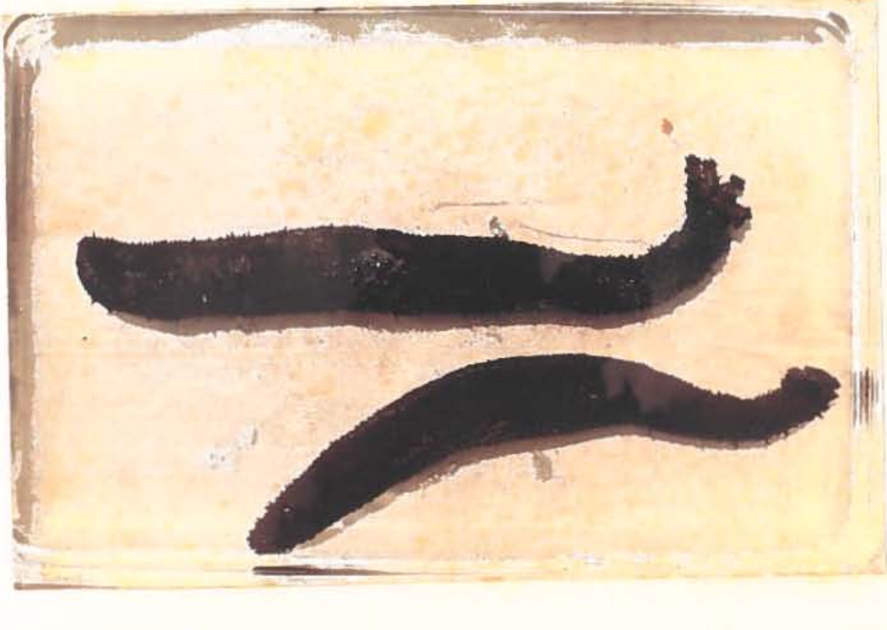


Fig. 6. Dark and light color phase of Holothuria argus (Jaege

Fig. 7. Holothuria sp. 2. This specimen is partially constricted and has liberated its Cuvierian organs.

Fig. 8. *Stichopus chloronotus* Brandt.

Fig. 9. *Stichopus variegatus* Semper.

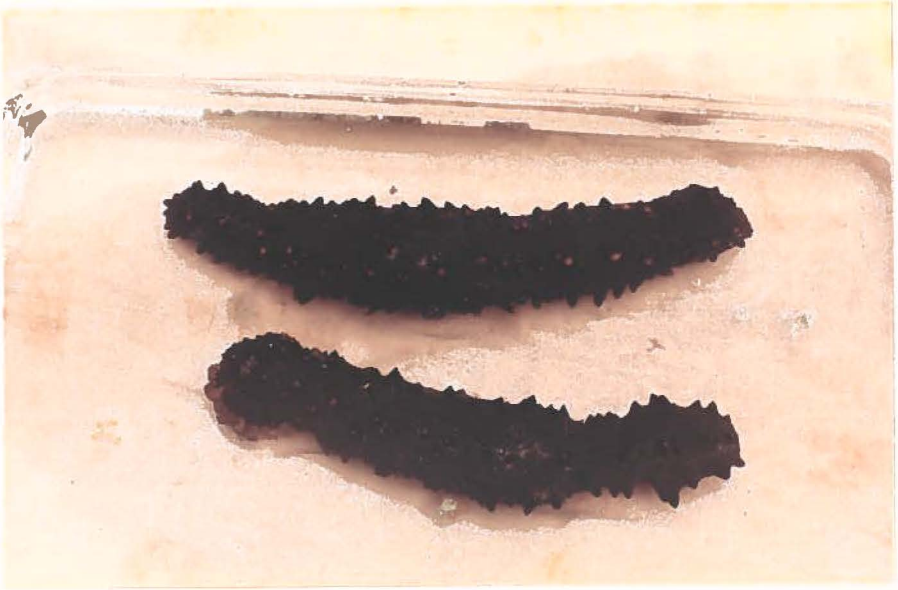
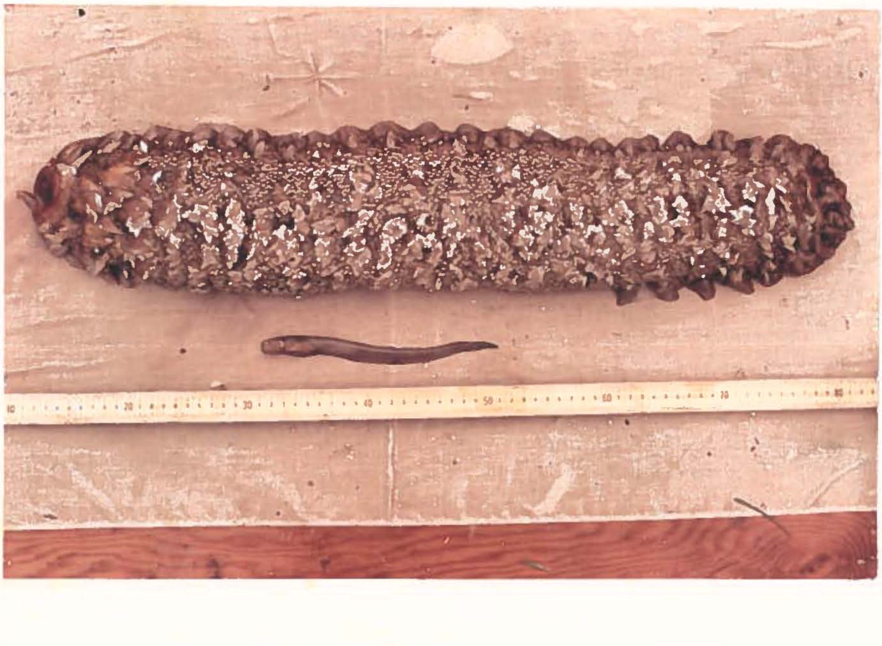


Fig. 10. *Actinopyga mauritiana* (Quoy and Gaimard).

Fig. 11. *Thelenota ananas* (Jaeger). A specimen of the pearlfish, *Carapus parvipinnis*, which infests this holothurian is included in the photograph.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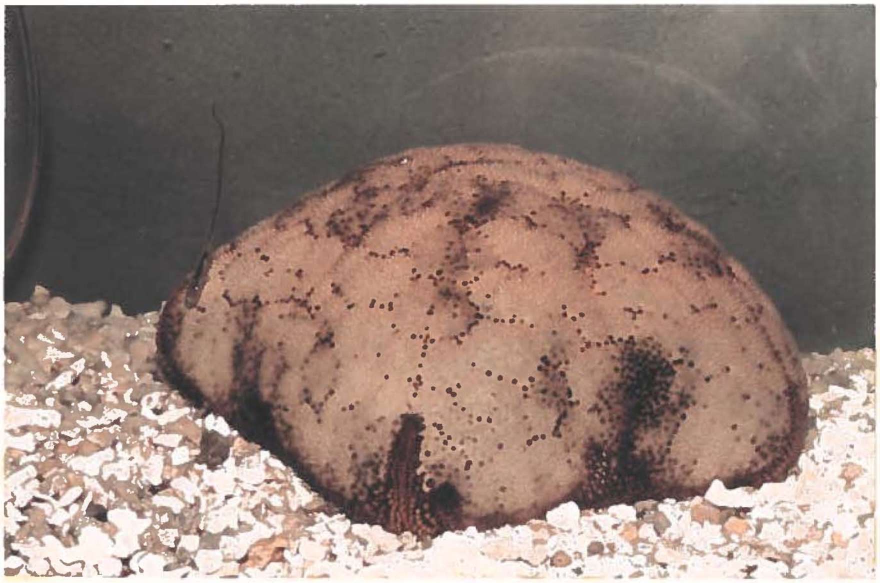
70
80

Fig. 12. *Culcita novaeguineae* Müller and Troschel. A specimen of the pearlfish, *Carapus mourlani*, which infests this asteroid is included in the photograph.



CHAPTER III. GUAM PEARLFISHES

Five species of pearlfishes, representing two genera, have been collected by this investigator. These species may be differentiated by the following key, line drawings of their heads (Fig. 13), and by other taxonomic characteristics listed in Table III.

Key to the Pearlfishes of Guam

(Modified from Smith, C. L., 1964)

1. Maxillary bound to the head and immovable; lower lip concealed under skin. Teeth on lower jaw widely separated. 30-31 trunk vertebrae....................*Encheliophis* 2

   Maxillary free from the skin of snout and not bound to the head; lower lip distinct. Teeth on lower jaw slightly separated. 17-18 trunk vertebrae................*Carapus* 3

2. Body with dark rectangular pigment spots (melanophores) arranged randomly on the head, becoming vertical bars above the abdomen, and myomer-shaped on the trunk; last eighth of the body becoming black. Pectoral fins present and well developed.......*Encheliophis* (*Jordanicus*) *gracilis*

   Body with randomly scattered melanophores becoming more numerous posteriorly; last fifth of the body becoming black. Pectoral fins absent...
   ...*Encheliophis* (*Encheliophis*) *vermicularis*

3. Body everywhere covered with fine, reddish melanophores, including skin covering the eye. Pectoral fins reduced, almost vestigial......................*Carapus parvipinnis*

   Melanophores not as above. Pectoral fins well developed..........4

4. Body without superficial melanophores other than small pigmented areas on the back of the skull, the nasal capsule, and the lower and upper jaws. Peritoneum is pigmented..............................*Carapus homei*

   Body with large round superficial melanophores in addition to those of the head and peritoneum......*Carapus mourlani*

TABLE III. TAXONOMIC CHARACTERISTICS OF FIVE SPECIES OF ADULT PEARLFISHES FROM GUAM

CHARACTERISTIC	PEARLFISH				
	Carapus homei	*Carapus mourlani*	*Carapus parvipinnis*	*Encheliophis gracilis*	*Encheliophis vermicularis*
Pectoral fins	large, 1/2 head	large, 1/2 head	small, 1/4 head	large, 1/3 head	absent
Teeth on jaws	2 series, 2 enlarged, up. jaw	2 series, 2 enlarged, up. jaw	2 series, uniform	1 row, large vomerines	1 row, uniform
Maxillary bone	free	free	free	adnate	adnate
Color	translucent	translucent	reddish brown	blue - grey	light brown
Body melanophores	absent	1 layer	several layers	1 layer	2 layers
Body shape	compressed	compressed	terete	compressed	cylindrical
Eye	large	large	small	large	medium
Vent	anterior to pectorals	anterior to pectorals	anterior to pectorals	even with pectoral base	pectorals absent vent in throat area
Gill opening above pectoral base	yes	yes	yes	no	pectorals absent
Head pigment:					
eye	no	no	yes	no	no
nasal area	yes	yes	yes	no	yes
lower jaw	yes	yes	yes	yes	no
Dorsal & anal fin darken posteriorly	no	no	yes	yes	yes
Host	holothurians	asteroid *Culcita*	holothurians	holothurians	holothurians

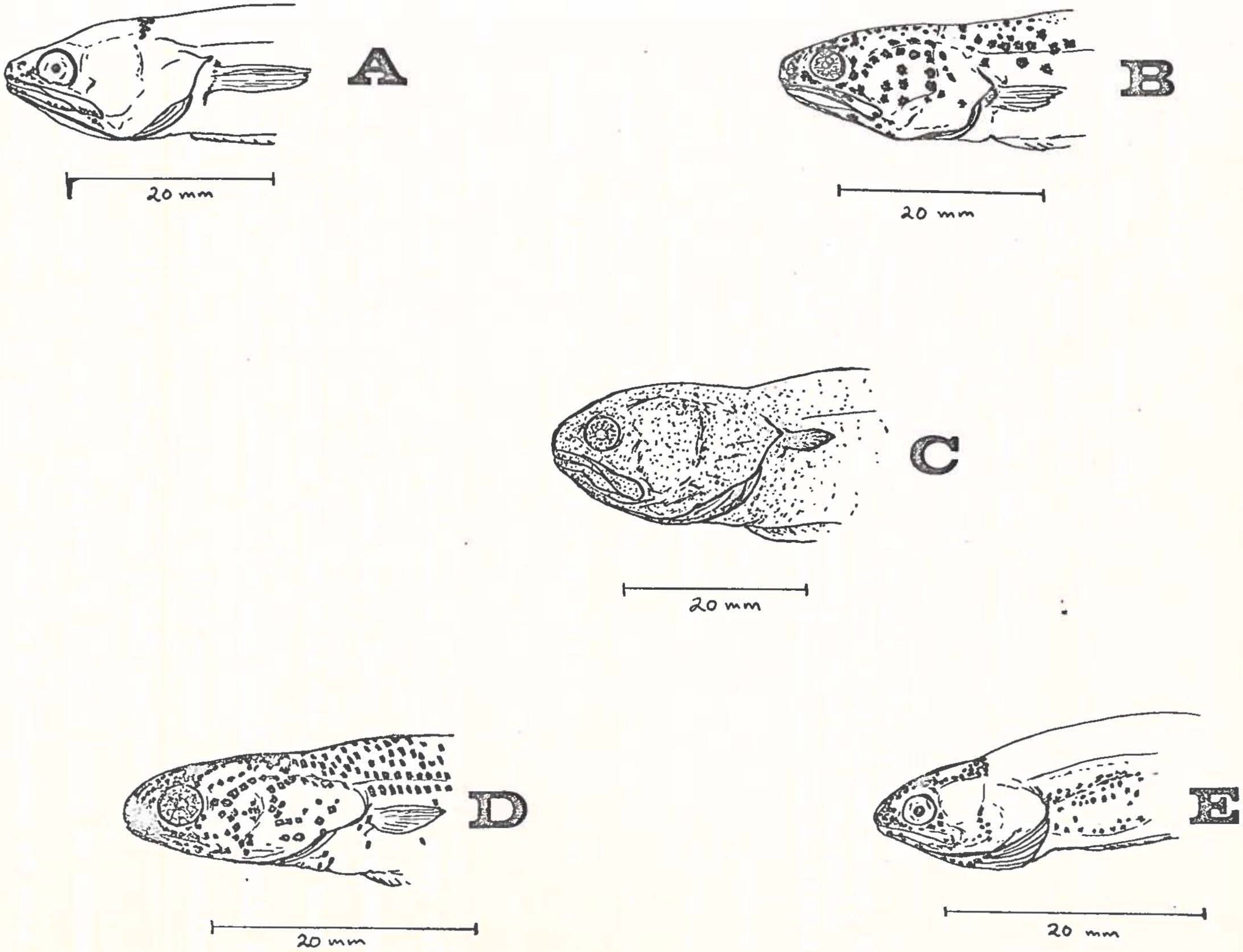


Fig. 13. Heads of five species of pearlfishes from Guam. A, *Carapus homei*; B, *Carapus mourlani*; C, *Carapus parvipinnis*; D, *Encheliophis gracilis*; and E, *Encheliophis vermicularis*.

Carapus homei (Richardson), 1844

(Figures 14A, 15, and 16)

Synonymy (abbreviated)

Oxybeles Homei Richardson, 1844:73.

Fierasfer Homei Kaup, 1856:233.

Fierasfer homei Gunther, 1862:381. Mahadevan, 1959:129.

Fierasfer microdon Gilbert, 1905:655.

Carapus homei Fowler, 1925:263 & 1959:524. Abe, 1939:574. Arnold, 1956:273. Bonham, 1960:255. Gosline and Brock, 1960: 280. Schultz, 1960:395. Strasburg, 1961:478. Marshall, 1964:397. Smith, C.L., 1964:34. Hipeau-Jacquotte, 1967:141.

Fierasfer neglectus Barnard, 1927:884.

Pirellinus lumbricoides Whitley, 1928:226.

Fierasfer Mourlani Petit, 1934:393.

Carapus neglectus Smith, J. L. B., 1955:413.

Host specificity

One hundred and eighty-five specimens of *Carapus homei* were collected from three holothurian species in Tumon Bay. These data are summarized in Table II. *Stichopus chloronotus* proved to be the preferred host with 17 percent of the specimens infested by *Carapus homei*. *Holothuria argus* also acted as a host for *Carapus homei* and 12 percent of the specimens contained this pearlfish species. Weber (1913) reports that *Stichopus variegatus* is commonly infested by *Carapus homei* in Borneo, but only one specimen of *C. homei* was found in 60 specimens of this holothurian on Guam. Hipeau-Jacquotte (1967) reports *C. homei* infesting *Thelenota ananas* in Madagascar, but none were found in 67 specimens of *T. ananas* collected locally.

Zonal variation of infestation

The largest population of Stichopus chloronotus occurred on the outer reef flat (Fig. 3). It was noted in chapter II that the population decreases in number while the individuals increase in size from the outer reef flat shoreward. Very few specimens of S. chloronotus were found in the sand zone. Those which were found here were often two to three times larger than those on the outer reef. The percent infestation of S. chloronotus by Carapus homei seemed to be directly related to the habitat of the host (Table IV). Only ten percent of the Stichopus chloronotus specimens from the outer reef flat and boulder zone were infested by Carapus homei. Infestation increased to 33 percent in the coral zone, and 44 percent in the sand zone. Variation of infestation is further demonstrated by C. L. Smith (1964) who found 63 percent infestation of Stichopus chloronotus by Carapus homei in Tumon Bay during the months of November, 1960, through June, 1961. The present investigator found only 18 percent total infestation of these two organisms during a comparable time period in Tumon Bay. The area of Tumon Bay and the reef zone where Smith made his collections is not known. Aronson and Mosher (1951) report different levels of pearlfish infestation in different parts of Bimini harbor, but they include no supporting data. Hipeau-Jacquotte (1967) reports 16 percent infestation of Stichopus chloronotus by Carapus homei on a Madagascar reef, which agrees well with the percent infestation during the present investigation.

The percent infestation of Holothuria argus by Carapus homei also varied by reef zone (Table IV). Only seven percent of the Holothuria argus specimens from the outer reef flat were infested by

Carapus homei. Infestation increased to ten percent in the boulder zone and 22 percent in the coral zone. There was, however, no difference in size or maturity of the Holothuria argus specimens encountered in the various reef zones.

Life forms

Of the 185 specimens of Carapus homei collected in Tumon Bay, the following forms were identified:

Tenuis (130-185 mm)	6 specimens
Juvenile (60-99 mm)	136 specimens
Adult (100-195 mm)	43 specimens

Identification of tenuis larvae was made on the basis of head and jaw pigmentation, and by the structure of the lower jaw (Fig. 17). In Carapus homei tenuis larvae, the lower jaw becomes wide (high) posteriorly and bears a long bony ridge laterally (Strasburg, 1961). Also, there are small pigmented areas on the head and lower jaw identical to those of the C. homei juveniles and adults.

There is a considerable reduction in length during the transformation from the tenuis to the juvenile form. The longest tenuis larva collected was 185 mm, and the smallest juvenile 60 mm, a reduction of 68 percent. Smith (1964) collected a Carapus homei tenuis larva from Tumon Bay which was 197 mm in length. Strasburg (1964) collected two C. homei tenuis larvae from Hawaii, measuring 157 mm and 200 mm.

Approximately 74 percent of the Carapus homei specimens collected were juveniles. The mean length of all specimens collected was 89 mm

(Fig. 18). Since no C. homei specimens under 100 mm in length were found to have developing gonads, the division between juvenile and adult forms was made at this length (100 mm). Hipeau-Jacquotte (1967) also considers the 100 mm length a valid division point between these two C. homei life forms.

Maturation of gonads

A total of 80 specimens of Carapus homei were dissected and examined for sexual maturity (Table V). No specimens were found with fully mature gonads. The immature females had ovaries with developing eggs but the ovaries did not appear ripe. The eggs were not separate and distinct as they were in the pearlfishes with fully mature ovaries. The immature males were identified with some uncertainty as the testes were extremely small. Smith (1964) found no mature C. homei in 185 specimens examined.

Seasonality

One Carapus homei tenuis larva was collected in May and five each in September and October. Smith (1964) collected several C. homei tenuis and post-tenuis larvae in Tumon Bay during the months of October through February.

The greatest percentage of Carapus homei infestation occurred during the months of November and December (Fig. 19). The specimens collected during these months were primarily juveniles (Fig. 20).

Carapus homei adults were collected during the months of June and July. The mean length of the specimens collected was over 100 mm during these months (Fig. 10). Smith (1964) found C. homei adults from October through July, but he did not distinguish the juvenile form. Arnold (1953) states that adults of *C. acus* are much harder to find than juveniles of this species.

It appears, then, that *Carapus homei* has an annual reproductive cycle. Spawning probably takes place during the late summer. Repeated collections in Tumon Bay indicate a decrease of infestation from December through May of *C. homei* in both *Stichopus chloronotus* and *Holothuria argus* (Fig. 19). C. L. Smith (1964) found the same decrease in his collections during the same approximate time period. He suggests that there is a high rate of infestation during the winter months as the tenuis larvae enter their hosts. Because of loss through juvenile mortality, this incidence decreases until summer, when only the mature adults remain, and spawning occurs.

Carapus homei size vs. host species

The average size of *Stichopus chloronotus* (195-239 mm) was observed to be approximately 21 percent less than the average size of *Holothuria argus* (250-300 mm). An attempt was made to determine statistically if the size of *Carapus homei* specimens was dependent upon the host species. A total of 122 specimens of *C. homei* collected from *Stichopus chloronotus* were measured, and their average length was 87 mm. Measurements were made of 56 *Carapus homei* specimens from *Holothuria argus*, and these had an average length of 96 mm (nine percent larger than the average length of *Carapus homei* specimens from *Stichopus chloronotus*). To determine if the length of *Carapus homei* is independent of host species, the Wilcoxon test for unpaired data was used (Alder and Roessler, 1968). Using a normal distribution, the value obtained was -2.23 which yields a probability of 1.29 percent. Thus, these data are significant at the five percent level and the length of *C. homei* and the type of host species are not independent. The size of the host is directly related to the size of the infesting pearlfish. Large holothurians tend to be infested by large pearlfishes. This may be the result of host size at infestation, as the pearlfish remains and matures within a single host. More likely, however, specimens of *C. homei* move from host to host in the process of feeding, and there may be active host selection, i.e., large pearlfishes select large holothurians.

TABLE IV. *CARAPUS HOMEI* INFESTATION BY REEF ZONE

HOST	Reef zone											
	Sand zone			Coral zone			Boulder zone			Outer reef flat		
	No. coll.	No. infes.	% infes.	No. coll.	No. infes.	% infes.	No. coll.	No. infes.	% infes.	No. coll.	No. infes.	% infes.
Stichopus chloronotus	9	4	44	212	71	33	163	17	10	351	35	10
Holothuria argus	--	--	--	146	32	22	88	9	10	234	16	7

TABLE V. MATURATION OF GONADS - <u>CARAPUS HOMEI</u>

MONTH	N	*Carapus homei*		
		undeveloped	immature male	immature female
April	8	8	---	---
May	3	3	---	---
June	20	18	1	1
July	13	11	1	1
August	0	---	---	---
September	2	2	---	---
October	2	1	---	1
November	12	12	---	---
December	8	6	1	1
January	12	12	---	---
TOTAL	80	73	3	4

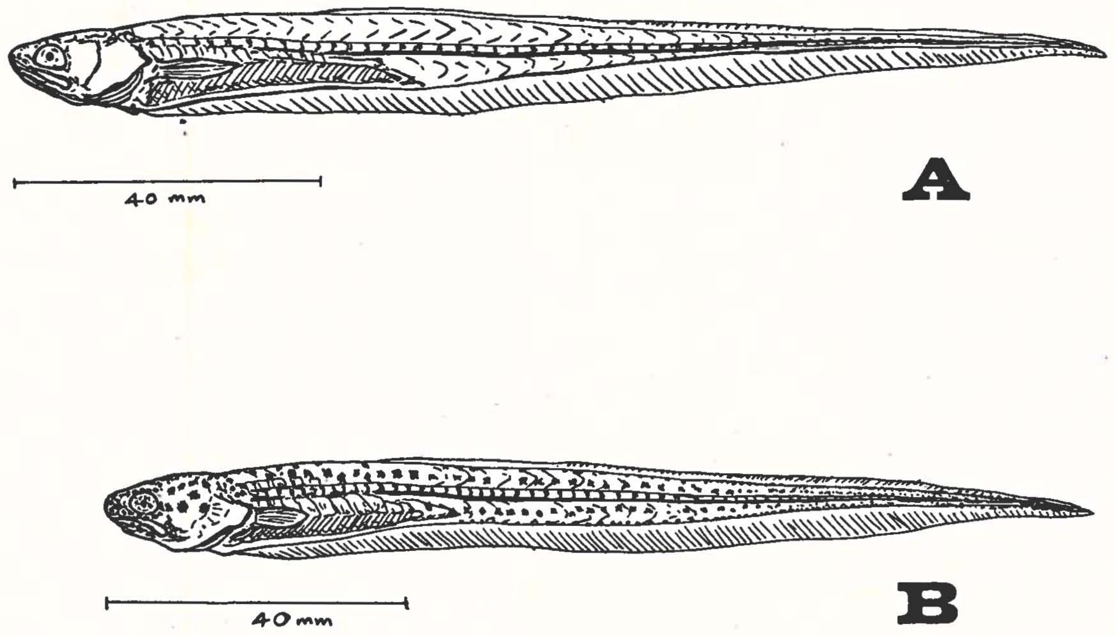
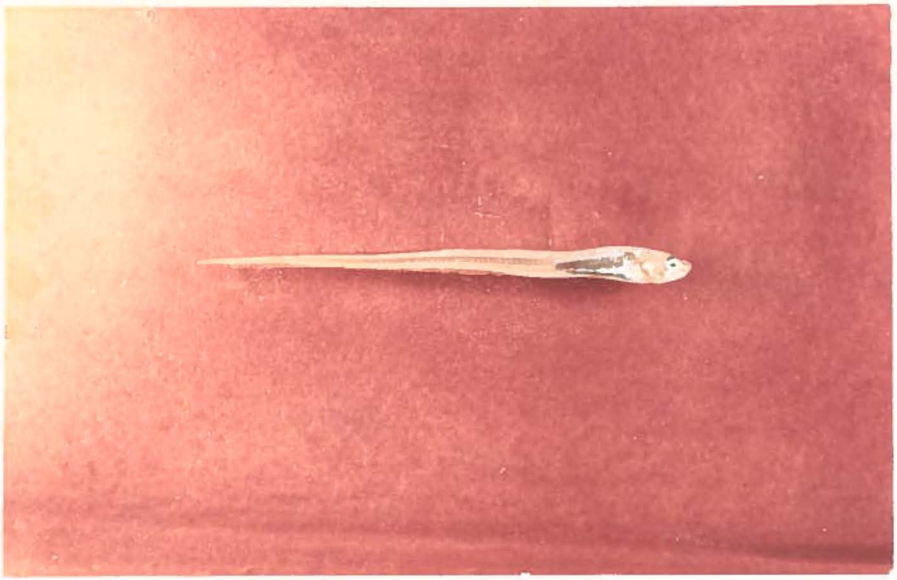


Fig. 14. A, *Carapus homei* (Richardson); and B, *Carapus mourlani* (Petit).

Fig. 15. Juvenile specimen of *Carapus homei* (Richardson)

Fig. 16. Adult specimen of *Carapus homei*, investigating *Holothuria argus*.



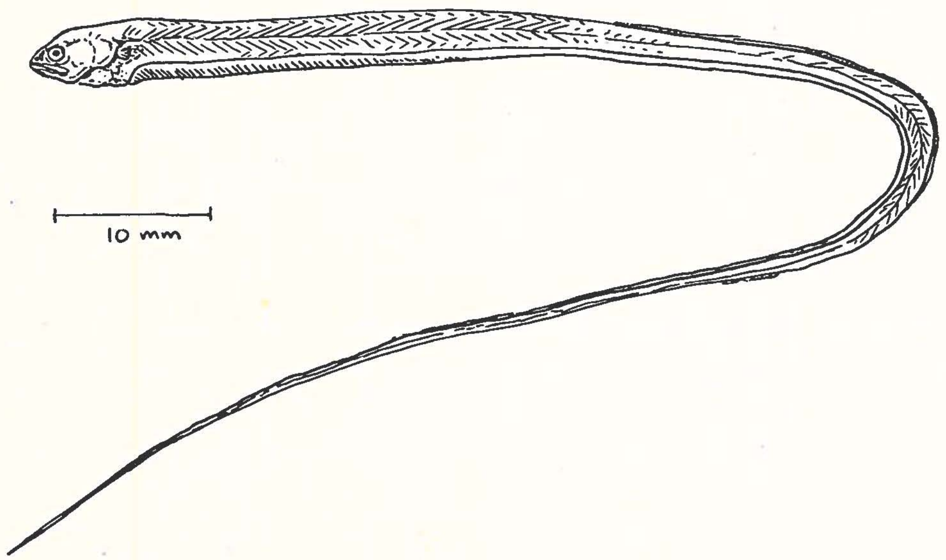


Fig. 17. Carapus homei (Richardson) tenuis larva.

Fig. 18. Length-frequency histogram of <u>Carapus</u> <u>homei</u>.

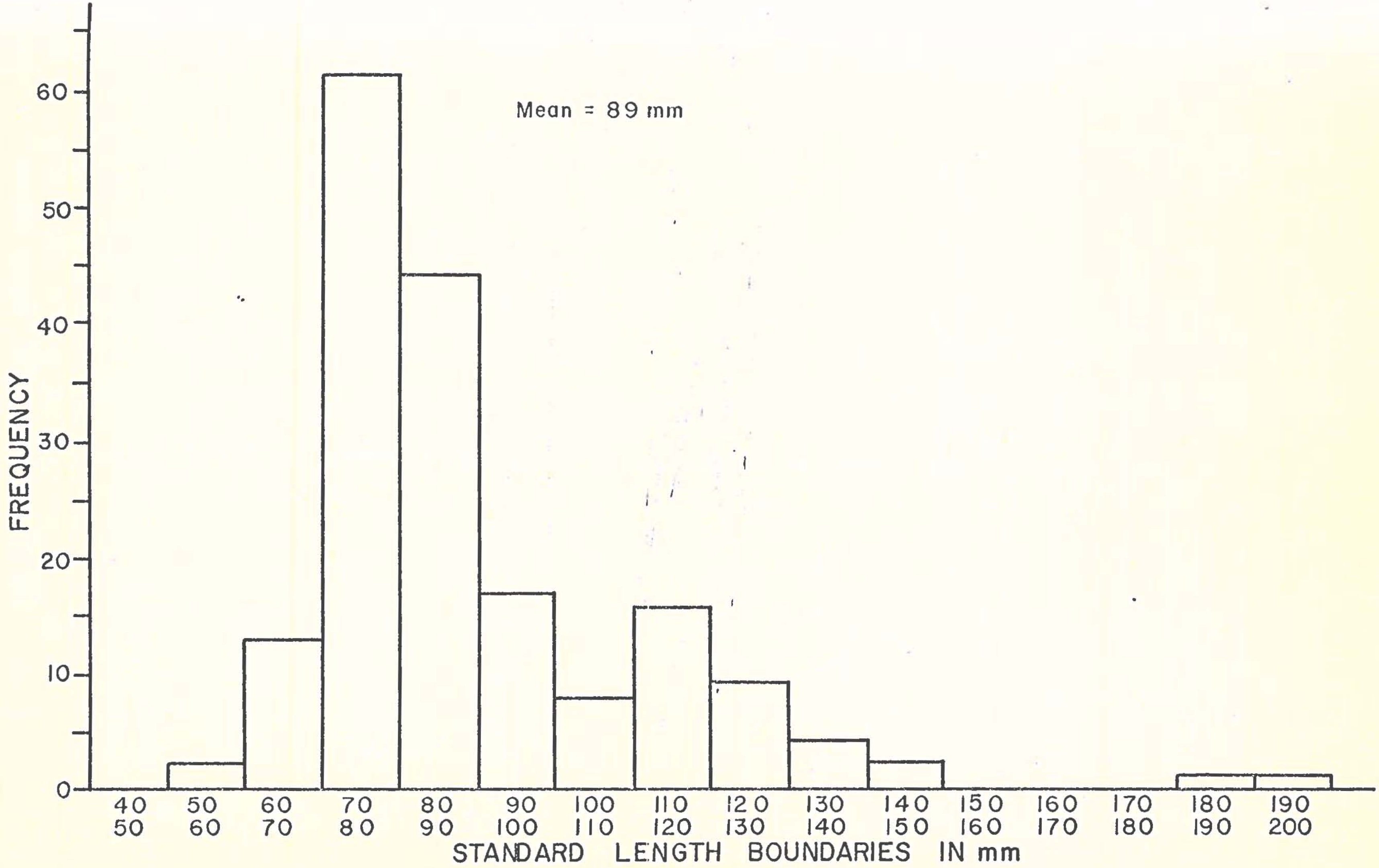

Mean = 89 mm
FREQUENCY
0
10
20
30
40
50
60
40 50
50 60
60 70
70 80
80 90
90 100
100 110
110 120
120 130
130 140
140 150
150 160
160 170
170 180
180 190
190 200
STANDARD LENGTH BOUNDARIES IN mm

Fig. 19. Seasonal variation of infestation of *Carapus homei* in Tumon Bay.

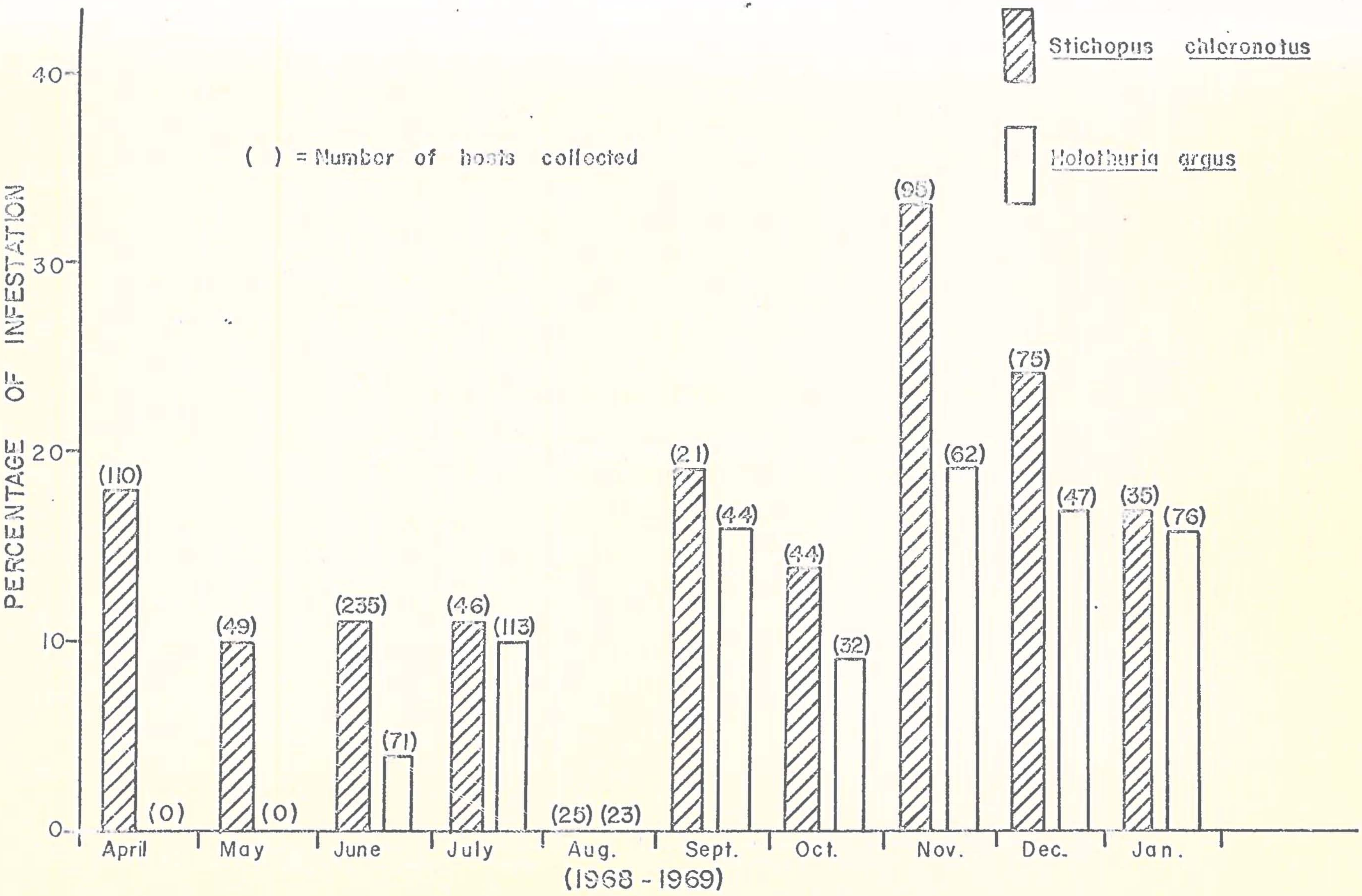
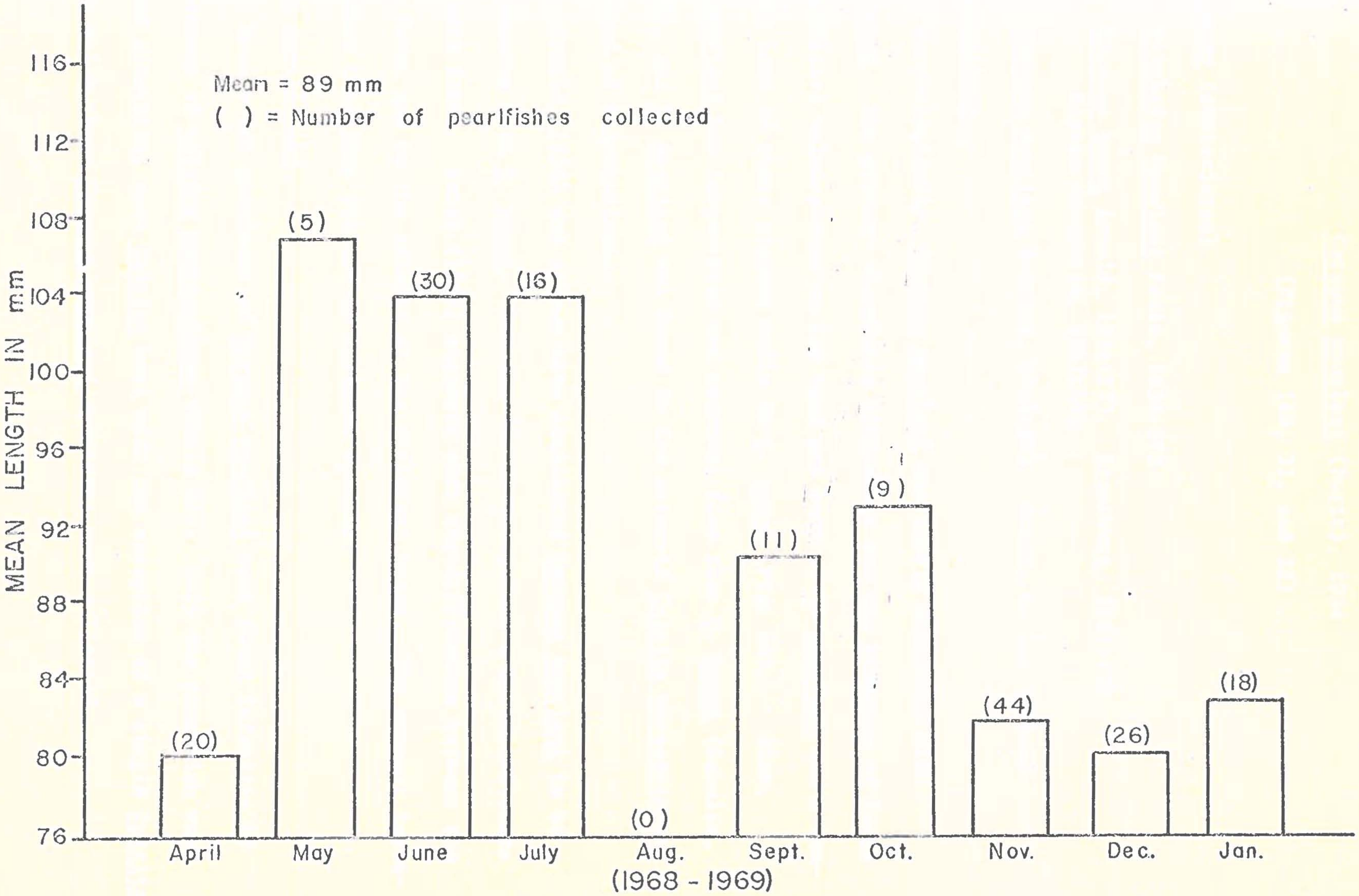

Stichopus chloronotus
Holothuria argus
( ) = Number of hosts collected
PERCENTAGE OF INFESTATION
40
30
20
10
0
(110)
(0)
(49)
(0)
(235)
(71)
(46)
(113)
(25) (23)
(21)
(44)
(44)
(32)
(95)
(62)
(75)
(47)
(35)
(76)
April
May
June
July
Aug.
Sept.
Oct.
Nov.
Dec.
Jan.
(1968 - 1969)

Fig. **20**. Mean length of *Carapus homei* by month.



Mean = 89 mm
( ) = Number of pearlfishes collected
MEAN LENGTH IN mm
116
112
108
104
100
96
92
88
84
80
76
(20)
(5)
(30)
(16)
(0)
(11)
(9)
(44)
(26)
(18)
April
May
June
July
Aug.
Sept.
Oct.
Nov.
Dec.
Jan.
(1968 - 1969)

Carapus mourlani (Petit), 1934

(Figures 14B, 21, and 22)

Synonomy (abbreviated)

Fierasfer Mourlani Petit, 1934:393.

Carapus homei Arnold, 1956:273. Strasburg, 1961:478.
Hipeau-Jacquotte, 1967:141.

Carapus mourlani Schultz, 1960:393. Smith, C. L., 1964:34.

Nomenclature

Morphologically, Carapus mourlani was nearly identical with C. homei. The primary observable difference was the presence of large superficial melanophores on the head and body of C. mourlani and a complete lack of these pigment cells in C. homei (Fig. 14). Also, C. mourlani had larger eyes and pectoral fins than C. homei (Schultz, 1960), and C. mourlani was found in the asteroid Culcita novaeguineae instead of holothurians.

Arnold (1956) and others have considered Carapus mourlani to be synonomous with C. homei. Schultz (1960) recognizes a distinctiveness of both forms. Smith (1964) suggests that the differences between the two forms may be the effects of the different hosts. The present investigator considers the two as distinct species.

Host specificity

Twenty-two specimens of Carapus mourlani were found infesting 49 specimens of Culcita novaeguineae (Table II). Only one pearlfish was found inhabiting each Culcita host with the exception of a single Culcita from which three Carapus mourlani specimens were collected.

Life forms

One tenuis larva, identified as *Carapus mourlani*, was found. This larva (140 mm long) is similar in appearance to the *C. homei* tenuis larvae collected, with the exception of slight, but clearly detectable superficial pigmentation. There is no mention of tenuis larvae being found in starfish hosts in any of the carapid literature.

Fourteen juvenile *Carapus mourlani* specimens (60-85 mm) and seven adult specimens (100-135 mm) were collected. The division point between these two life forms was considered to be 100 mm for the same reasons stated for the *C. homei* specimens. The mean length of these juvenile and adult specimens was 90 mm (Fig. 23), which closely approaches the mean length of the *C. homei* specimens (89 mm). The range of body lengths between these two species was also similar.

Maturation of gonads

a total of 17 *Carapus mourlani* specimens were dissected and the gonadal development noted with the following results:

May	1 immature female (115 mm)
July	1 immature female (120 mm)
October	2 undeveloped
November	8 undeveloped
December	5 undeveloped

The immature females had definite developing ovaries but the eggs were not yet distinct. The majority of the sexually immature *C. homei* specimens were also found during the summer months.

Seasonality

The *Carapus mourlani* tenuis larva was collected in December, as were those of *C. homei*.

The greatest percentage of infestation of Carapus mourlani occurred in July (Fig. 24), but this may be the result of small samples as only two hosts were collected during this month. Only 31 percent infestation occurred during the month of December, and the majority of the fishes were adults (Fig. 25). C. homei adults were collected during the summer months. It is possible that the seasonal life cycles of C. mourlani and C. homei are similar; however, more collections of C. mourlani are needed in order to determine any similarities of seasonality and maturation.

Carapus mourlani vs. C. homei

Smith (1964) examined the stomachs of five specimens of Carapus mourlani and found them to be empty. He also noticed that these same specimens appeared emaciated. From this, Smith suggests that Culcita may be an abnormal host which is accidentally infested by Carapus homei and in which the pearlfishes become trapped, without possibility of escape. Smith also suggests that the pigmentation of C. mourlani is part of a starvation syndrome of those homei individuals which chance to enter the wrong host. This hypothesis seems improbable as 41 percent of the Culcita specimens collected in the course of the present research were infested. Such a high percentage does not suggest chance infestation. Secondly, evidence presented later in this paper indicates that the pearlfishes are not trapped within Culcita but may leave the host under certain conditions. Thirdly, host preference experiments indicate that Carapus mourlani may actually choose Culcita and attempt entrance, when given a choice between the asteroid and a holothurian (see Chapter IV). Finally, introductions of Carapus homei tenuis larvae, juveniles, and adults into Culcita specimens, and eventual retrieval of these pearlfishes have produced no changes in pigmentation.

The following evidence, along with behavioral observations of Carapus mourlani and C. homei in Chapter IV indicates that the two forms are separate species.

A series of experiments was set up to test Smith's (1964) starvation syndrome hypothesis. Living specimens of Carapus homei were isolated in aquaria and starved to death (average time of death was three weeks). One juvenile specimen was placed in total darkness during this starvation period, and one tenuis larva was starved. A total of six C. homei specimens were isolated and observed and none showed evidence of developing melanophores at the time of death.

A second series of experiments were designed to test the hypothesis that Carapus mourlani are simply those specimens of C. homei which enter Culcita and become pigmented as a result of the influence of the host. An artifical method of introducing a living specimen of Carapus homei into Culcita was developed. A pearlfish was tagged by clipping off approximately three millimeters of its tail. Using a number four cork borer, a hole was bored through the muscular wall of a Culcita. This hole was made on the side of the asteroid, approximately 45° from vertical. Care was taken to withdraw the center portion of the bore, rather than forcing it into the asteroid's coelom. The tagged Carapus homei was introduced head first through the hole into the coelom of the Culcita. The hole was then plugged with the original boring of the muscular wall. On two occasions this plug was accidentally pushed inside the coelom during the boring. In these cases, small corks were substituted for the natural plugs.

A series of *Culcita* specimens with introduced pearlfishes were prepared in the above manner. One tenuis, four juveniles, and one adult specimen were used in the series. A *Culcita* was dissected every four days from the time of pearlfish introduction, and the longest period of infestation was 24 days. The tagged fishes were retrieved along with the original inhabitants, if any, and the specimens of *Carapus homei* were observed for pigmentation.

The tagged *Carapus homei* tenuis larva was eaten by a *C. mourlani* specimen originally infesting the asteroid (determined by stomach contents). An adult *C. homei* which was introduced ate a juvenile *C. mourlani* specimen which was present beforehand. One juvenile *C. homei* disappeared. It is presumed that it died and decomposed within the *Culcita*. In all, four *Carapus homei* and two *C. mourlani* specimens (original inhabitants) were retrieved. None of the retrieved *C. homei* had become even slightly pigmented.

In order to determine whether the pigmentation of *Carapus mourlani* is permanent, two specimens were starved to death, and a single long-term infestation (three weeks) of *C. mourlani* in *Stichopus chloronotus* produced no observable differences in pigmentation of the pearlfishes.

Based on the evidence presented in this section and a following chapter, it would seem preferable to recognize *Carapus mourlani* and *C. homei* as distinct species.

Fig. 21. Adult specimen of Carapus mourlani (Petit).

Fig. 22. Adult specimen of Carapus mourlani photographed in an aquarium. A row of imbedded vestigial scales are clearly seen above the abdomen of the pearlfish.

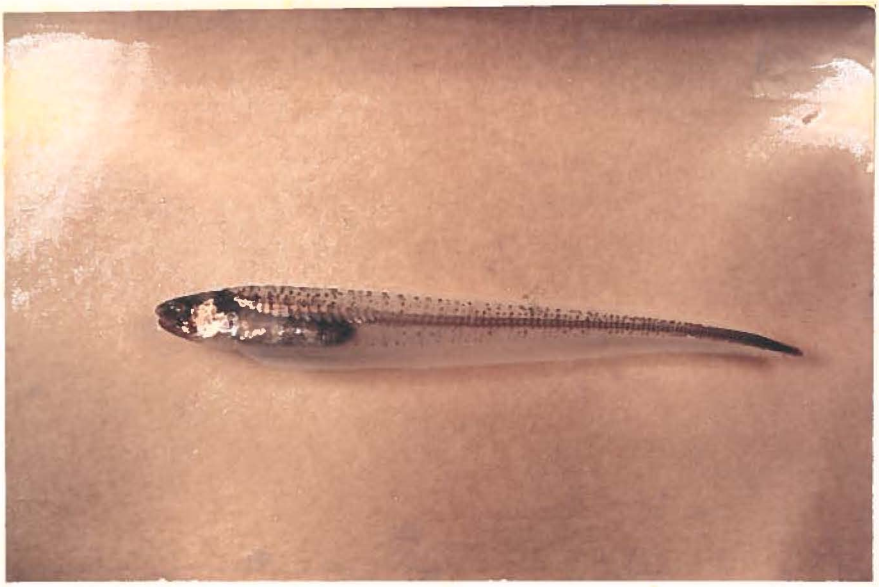


Fig. 23. Length-frequency histogram of <u>Carapus</u> <u>mourlani</u>.

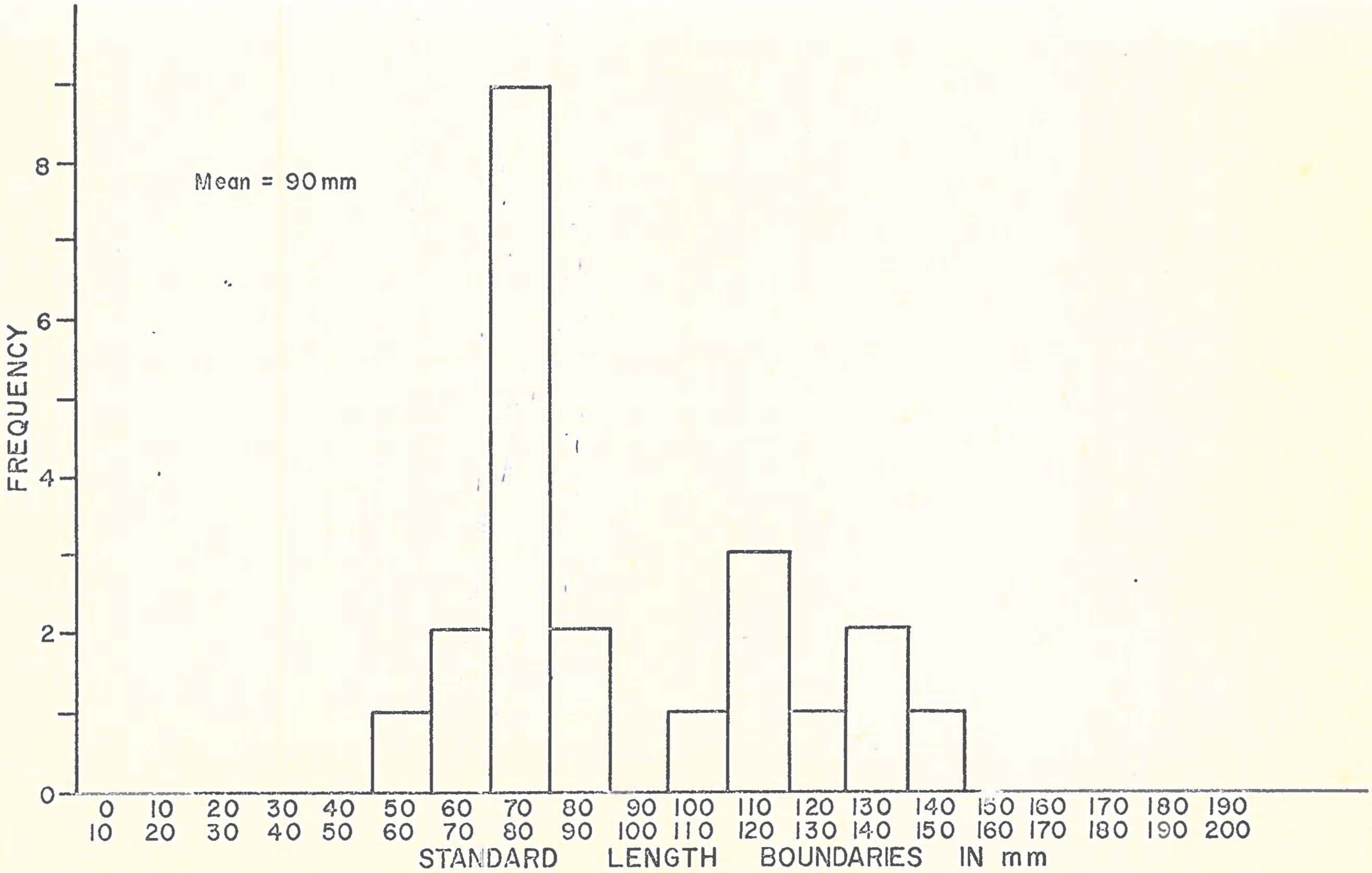
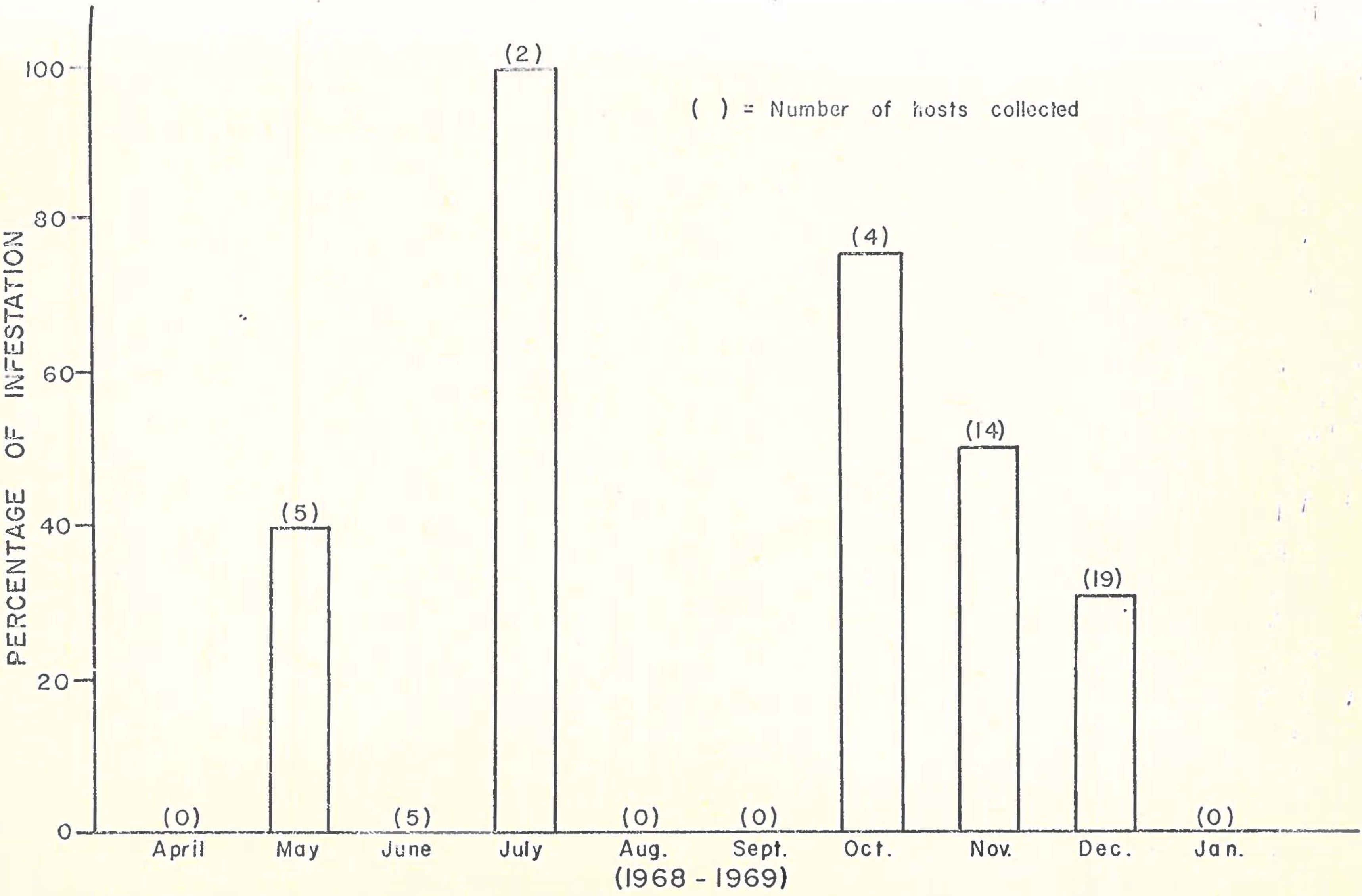
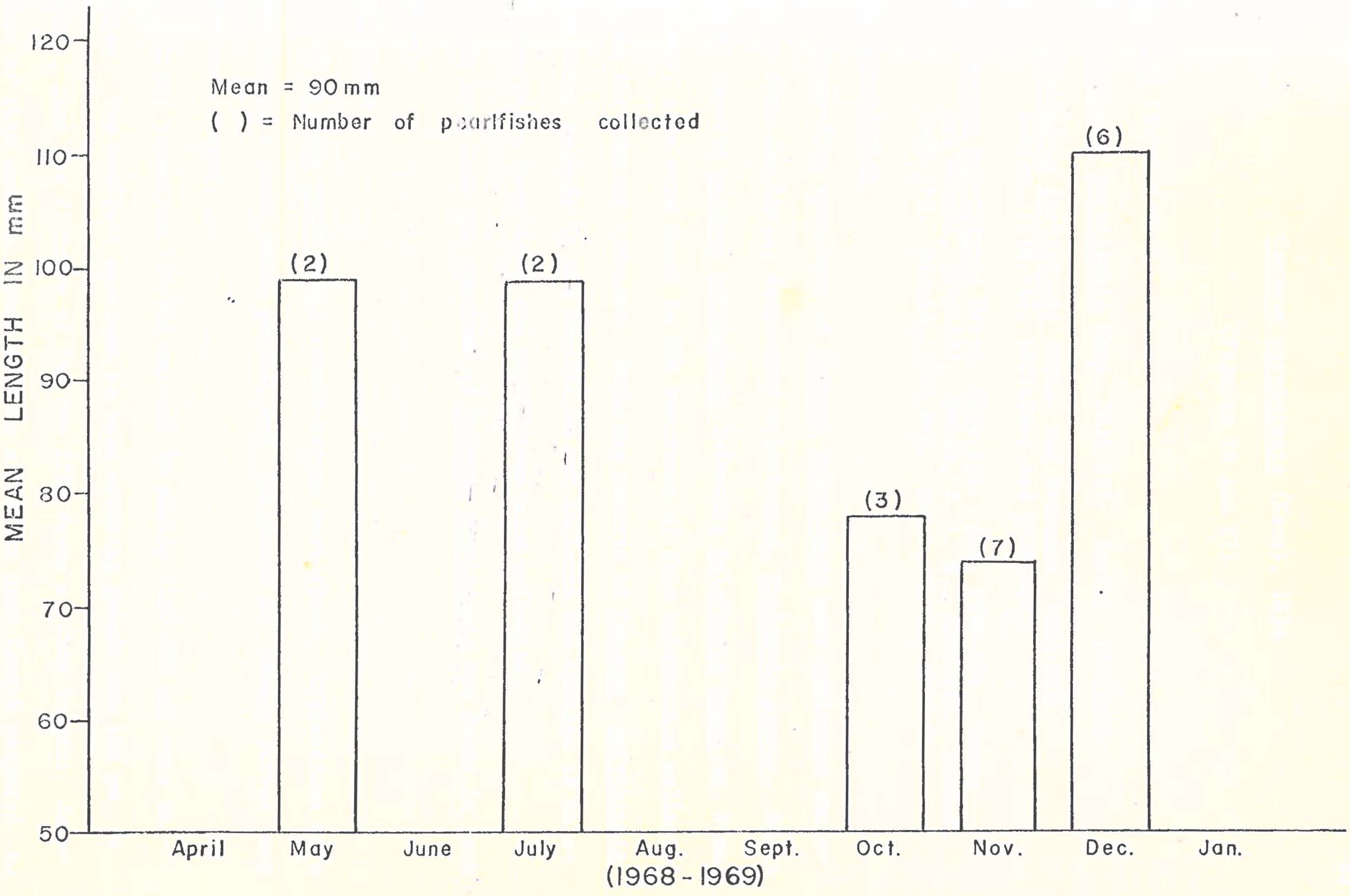

Mean = 90mm
FREQUENCY
0
2
4
6
8
0 10
10 20
20 30
30 40
40 50
50 60
60 70
70 80
80 90
90 100
100 110
110 120
120 130
130 140
140 150
150 160
160 170
170 180
180 190
190 200
STANDARD LENGTH BOUNDARIES IN mm

Fig. 24. Seasonal variation of infestation of *Carapus mourlani* in *Culcita novaeguineae*.



( ) = Number of hosts collected
PERCENTAGE OF INFESTATION
100
80
60
40
20
0
(0)
(5)
(5)
(2)
(0)
(0)
(4)
(14)
(19)
(0)
April
May
June
July
Aug.
Sept.
Oct.
Nov.
Dec.
Jan.
(1968 - 1969)

Fig. 25. Mean length of *Carapus mourlani* by month.


Mean = 90 mm
( ) = Number of pearlfishes collected
MEAN LENGTH IN mm
120
110
100
90
80
70
60
50
(2)
(2)
(3)
(7)
(6)
April
May
June
July
Aug.
Sept.
Oct.
Nov.
Dec.
Jan.
(1968 - 1969)

Carapus parvipinnis (Kaup), 1856

(Figures 26 and 27)

Synonomy (abbreviated)

Fierasfer parvipinnis Kaup, 1856:233. Fowler, 1900:523.

Jordanicus parvipinnis Jordan and Evermann, 1903:505. Fowler, 1928:445, 1931:364, and 1934:447.

Carapus parvipinnis Herre, 1936:416. Abe, 1939:574. Herre and Herald, 1950:337. Smith, J. L. B., 1955:412. Arnold, 1956:279. Herald, 1961:242. Smith, C. L., 1964:34.

Host specificity

Forty-five specimens of Carapus parvipinnis were collected exclusively from the large, deep-water holothurian, Thelenota ananas. Smith (1964) reports Carapus parvipinnis infesting Holothuria argus on Guam, however, none were found in 468 specimens of this holothurian collected during the present research. Existing reports of Carapus parvipinnis from other islands in the Pacific do not indicate the species of holothurian hosts from which the pearlfishes were collected.

No more than one specimen of Carapus parvipinnis was found in each host.

Life forms

No Carapus parvipinnis tenuis larvae were found. Collections were made during a seven month period, and instances of tenuis larvae may have been missed.

Eight juveniles (55-105 mm) and 38 adults (120-280 mm) were collected. The smallest specimen to have maturing gonads was 120 mm in length, thus the division of the two life forms at this length. Juveniles of this species appear to have a shorter head length compared to total body length than the adult form.

There is a tremendous difference in body length between the smallest juvenile and the largest adult collected. The smallest specimen of *Carapus parvipinnis* previously recorded is 63 mm in length (Smith, 1964). Maximum length for this species is reported to be 315 mm (Herre and Herald, 1950). Herald (1961) states that *C. parvipinnis* is the largest of all pearlfish species. The mean body length of the specimens collected during the present research is 177 mm (Fig. 28).

Two definite color phases of *Carapus parvipinnis* were recorded. The majority of the adult specimens were a dark reddish-brown color (Fig. 27), while a few adults were very pale, approaching white in color (Fig. 64). Herre (1936) also noticed this color variation in four specimens of *C. parvipinnis* collected from Tahiti. These variations are not associated with life forms or sexual dichromatism.

Maturation of gonads

Thirty-nine specimens of *Carapus parvipinnis* were dissected and examined for maturing gonads. The results of this examination are recorded in Table VI. The greatest percentage of mature specimens occurred in August, however, some mature specimens were found among those collected each month (with the exception of July).

The mature females had nearly ripe ovaries, distended by large numbers of eggs.

Seasonality

The greatest percent of infestation of *Carapus parvipinnis* occurred in January (Fig. 29). The mean length of these specimens found during January was 170 mm (Fig. 30), indicating that the increased infestation during this month was not caused by invading larval forms. Indeed, the majority of juveniles were found in August, the month which also contained the largest percentage of mature adults. No correlation of these data with maturation of gonads seems possible. Additional collecting through the course of a complete year must be done in order to establish recruitment and mortality rates for this species.

TABLE VI. MATURATION OF GONADS - <u>CARAPUS PARVIPINNIS</u>

MONTH	N	*Carapus parvipinnis*				
		undeveloped	immature male	immature female	mature male	mature female
April, 1968	-	-	-	-	-	-
May, 1968	-	-	-	-	-	-
June, 1968	-	-	-	-	-	-
July, 1968	3	2	1	-	-	-
August, 1968	15	5	-	3	5	2
September, 1968	-	-	-	-	-	-
October, 1968	8	3	1	3	-	1
November, 1968	3	1	-	1	1	-
December, 1968	3	1	-	1	1	-
January, 1969	7	4	1	1	-	1
TOTAL	39	16	3	9	7	4

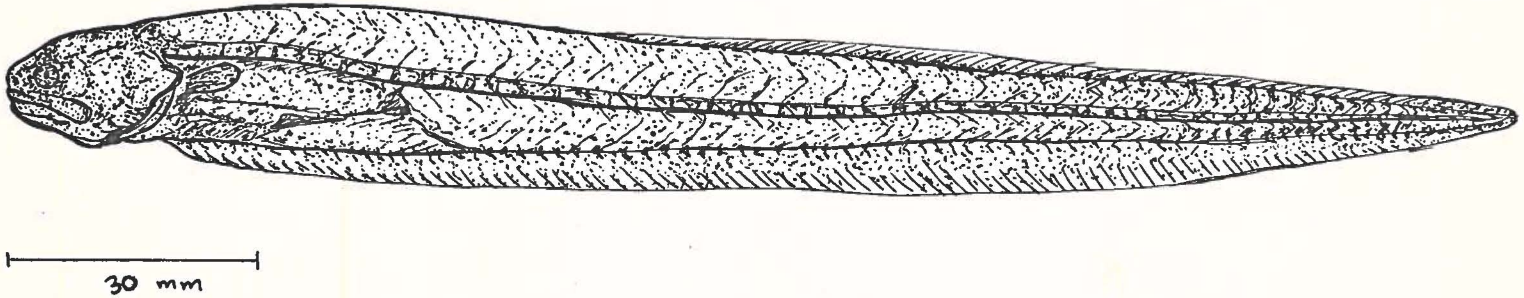


Fig. 26. Adult specimen of the pearlfish, Carapus parvipinnis (Kaup).

Fig. 27. Adult specimen of <u>Carapus parvipinnis</u> (Kaup
The specimen pictured here is of the dark color phase.


9 40 1 2 3 4 5 6 7 8 9 50 1 2 3 4 5 6 7 8 9 60 1

Fig. 28. Length-frequency histogram of *Carapus parvipinnis*.

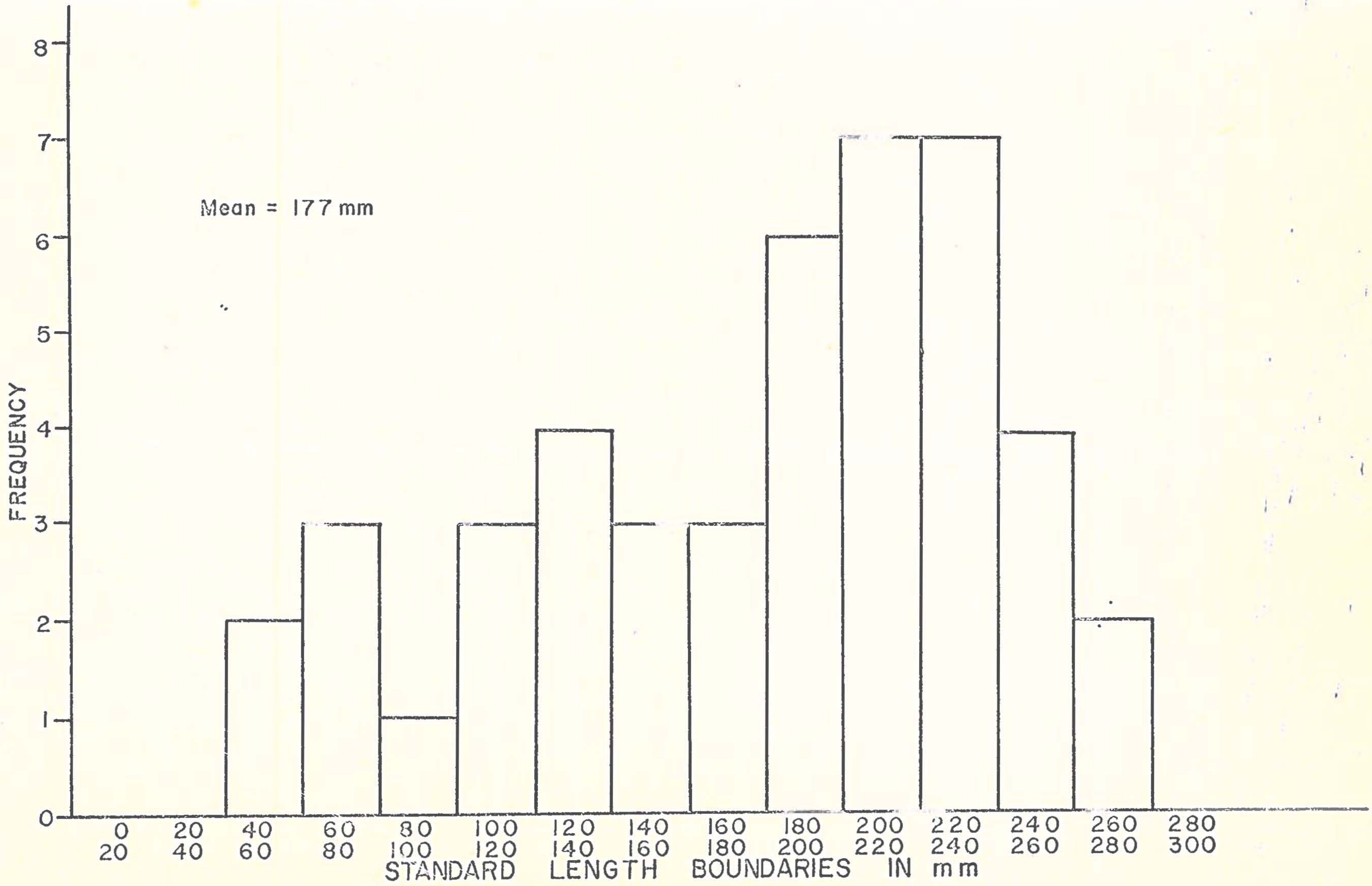

Mean = 177 mm
FREQUENCY
0
1
2
3
4
5
6
7
8
0 20
20 40
40 60
60 80
80 100
100 120
120 140
140 160
160 180
180 200
200 220
220 240
240 260
260 280
280 300
STANDARD LENGTH BOUNDARIES IN mm

Fig. 29. Seasonal variation of infestation of *Carapus parvipinnis* in *Thelenota ananas*.

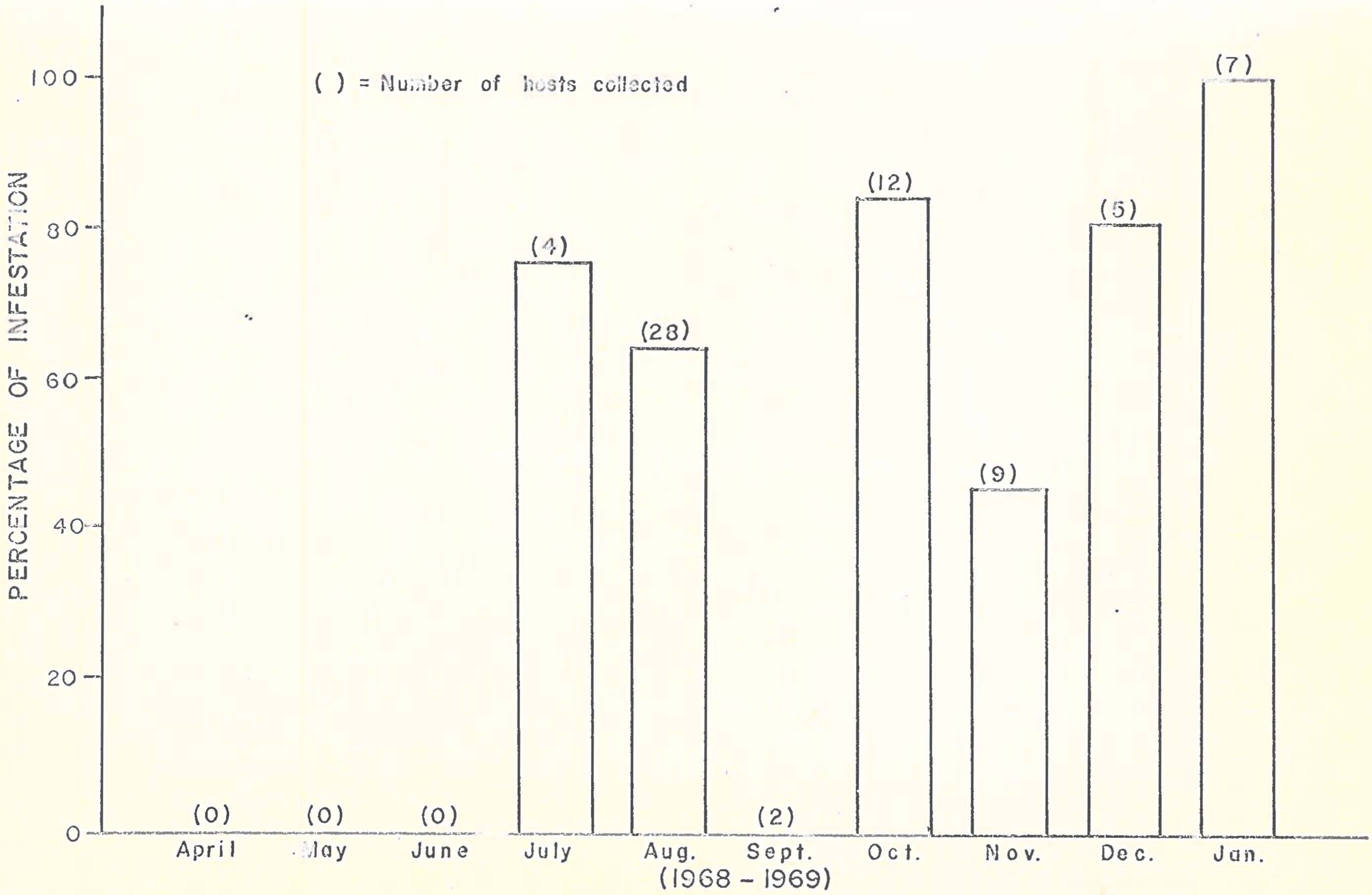
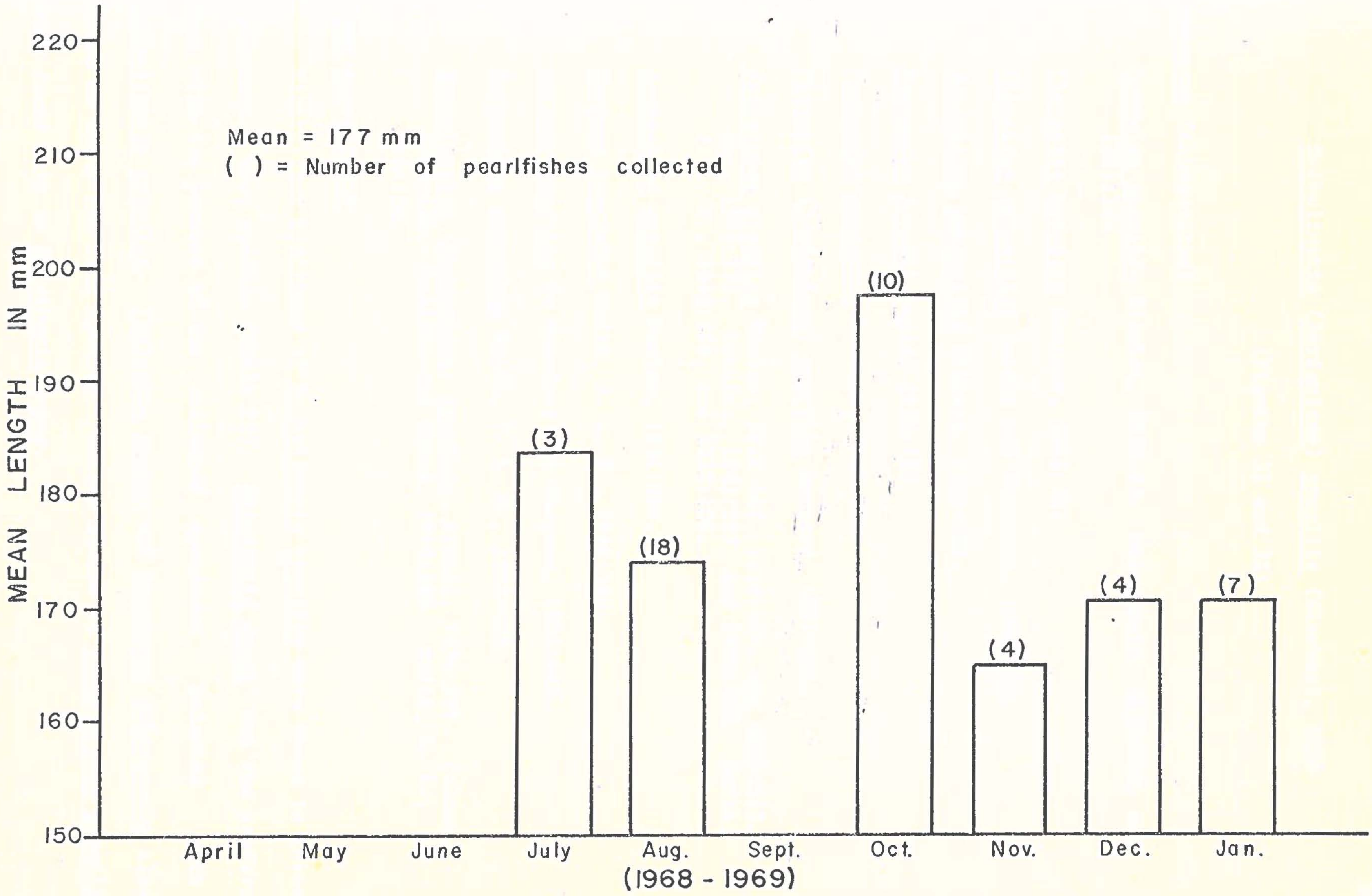

( ) = Number of hosts collected
PERCENTAGE OF INFESTATION
100
80
60
40
20
0
(0)
(0)
(0)
(4)
(28)
(2)
(12)
(9)
(5)
(7)
April
May
June
July
Aug.
Sept.
Oct.
Nov.
Dec.
Jan.
(1968 - 1969)

Fig. 30. Mean length of *Carapus parvipinnis* by month.


Mean = 177 mm
( ) = Number of pearlfishes collected
MEAN LENGTH IN mm
220
210
200
190
180
170
160
150
(3)
(18)
(10)
(4)
(4)
(7)
April
May
June
July
Aug.
Sept.
Oct.
Nov.
Dec.
Jan.
(1968 - 1969)

Encheliophis (Jordanicus) gracilis (Bleeker), 1856

(Figures 31 and 32)

Synonomy (abbreviated)

Oxybeles gracilis Bleeker, 1856:93. Doleschall, 1858:153, and 1861:340.

Fierasfer gracilis Gunther, 1862:382.

Fierasfer umbratilis Jordan and Evermann, 1902:206.

Jordanicus umbratilis Gilbert, 1905:655.

Fierasfer frantii Popta, 1912:185.

Fierasfer punctatus Barnard, 1927:884.

Fierasfer (Jordanicus) gracilis Barnard, 1927:884.

Jordanicus gracilis Fowler, 1928:445, 1934:447, and 1959:525. Abe, 1939:575. Schultz, 1943:287, and 1960:391. Smith, J. L. B., 1949:359, and 1955:404.

Jordanicus garcilis Fowler, 1931:364.

Carapus punctatus Smith, J. L. B., 1949:360.

Carapus gracilis de Beaufort and Chapman, 1951:445.

Jordanicus punctatus Smith, J. L. B., 1955:405.

Encheliophis (Jordanicus) gracilis, Arnold, 1956:299. Palmer, 1958:486. Gosline and Brock, 1960:280. Smith, C. L., 1964:34.

Host specificity

Eighty-four specimens of Encheliophis gracilis were found in three holothurian host species (Table II). Holothuria argus was the preferred host with ten percent of the collected specimens infested with Encheliophis gracilis. Thelenota ananas and Stichopus chloronotus also acted as hosts for this species. Mature adults of Encheliophis gracilis were found only in Holothuria argus.

Along with a difference in habitat, these three holothurian species differ in their ability to produce adhesive threads or Cuvierian organs (Fig. 63). *Holothuria argus* is a thread producer, whereas *Stichopus* and *Thelenota* are not. C.L. Smith (1964) suggests that the presence of these threads may attract certain species of pearlfishes. *Encheliophis gracilis* preferred a holothurian species which produced these threads. The affect of the Cuvierian organs upon pearlfishes is discussed in Chapter IV.

Smith (1964) found two specimens of *Encheliophis gracilis* infesting *Holothuria* sp. 2 (Fig. 7), but none were found by the present investigator in 45 specimens of the holothurian. *H.* sp. 2 is also a thread producer.

Doleschall (1861) and Arnold (1956) report *Encheliophis gracilis* infesting the pillow starfish, *Culcita discoidea*. None were found in 49 specimens of *Culcita novaeguineae* during the present investigation, however, entry of an *Encheliophis gracilis* specimen in *Culcita* was observed in the laboratory (see Chapter IV). Edmondson (1946) records *Encheliophis gracilis* in the asteroid *Asterope carinifera* from Hawaii, as well as in *Stichopus* sp. and *Actinopyga* sp.

Life forms

No *Encheliophis gracilis* tenuis larvae were found. Ten juveniles (76-106 mm), and 74 adults (110-264 mm) were collected. The division between the juvenile and adult form was made on the basis of sexual maturity and body shape. No sexually mature individuals were found with a body length of less than 110 mm. The juveniles have a shorter head length compared to total body length than the adults, and the body form of a juvenile is more elongate and eel-like than the adults.

Arnold (1956) lists the greatest recorded length of Encheliophis gracilis at 236 mm. Guam specimens seem substantially larger, with six collected above 236 mm. The mean length of the specimens collected during the present research was 175 mm (Fig. 33).

Seasonality

No seasonal infestation pattern of Encheliophis gracilis could be determined from the data collected over a ten month period. Infestation of Holothuria argus remained relatively stable throughout the period with the exception of August and November (Fig. 34). No E. gracilis specimens were found in 23 Holothuria argus collected during August, and the greatest percentage of infestation in this holothurian was in November. Infestation of Thelenota ananas varied considerably but this variation was probably the result of small samples. The average sample size was approximately seven holothurians.

The mean length of the collected Encheliophis gracilis specimens was over 110 mm (adult size) in all months except May and August (Fig. 35). Juveniles were found throughout the collecting period and there was no obvious increase of juvenile infestation during any single month.

Maturation of gonads

Sixty-three specimens of Encheliophis gracilis were dissected and the maturation of gonads noted (Table VII). Sexually mature specimens were collected from June through January, with the exception of August.

It was found that mature specimens of Encheliophis gracilis often form pairs, each pair infesting a single holothurian host. Fifteen E. gracilis pairs were collected, and all were found infesting

Holothuria argus (Table VIII). These pairs were found throughout the collecting period. Pairing of mature pearlfishes has not been previously recorded.

Each of the pairs comprised a mature female with a ripe ovary. The second member of each pair appeared to be male. The location of the reproductive structures within these pearlfishes is shown in Fig. 36. Gonadal material from the males of four of these pairs was histologically sectioned and identified as mature testes, with seminiferous tubules, spermatids and sperm present (Figs. 37 through 40).

The females from these pairs were normally longer than the males, (Table IX) and, due to their ripe ovaries, had enlarged abdomens (Fig. 31). Also, the female had melanophores in the abdominal region, whereas the males did not.

In order to determine if this instance of paired infestation was independent of the gonadal development of *Encheliophis gracilis*, a chi-square test (Alder and Roessler, 1968) was applied to the data in Tables VII and VIII. Since the tabular value of chi-square at the one percent level of significance equals 6.64, and the calculated value equaled 23.66, the hypothesis that pair formation was independent of gonadal development was rejected and the data indicates that paired infestation was dependent upon the gonadal development of *E. gracilis*.

The Wilcoxon test for paired cases (Alder and Roessler, 1968) was applied to the data in Table IX to determine if the females of the pairs were significantly longer than the males. The probability that pairs of *Encheliophis gracilis* with the same lengths as those listed in Table IX would be found, assuming their lengths were not significantly different, would be 0.7 percent which is significant at the one percent level. These data indicate that the female E. gracilis was significantly longer than the paired male.

In figure 41, the scatter diagram and regression lines relating to the lengths of the paired male and female Encheliophis gracilis specimens are represented. The regression lines indicate a significant degree of correlation (correlation coefficient of 0.42) and that the length of the pair members is dependent on the sex of the pearlfishes.

Pairing of male and female Encheliophis gracilis specimens is undoubtedly for reproductive purposes. It would be of great advantage for the male to be within the same host as the female at the time of spawning. Indications are that this pairing occurs only at the time of spawning since 15 unpaired mature specimens were found. No juveniles or immature specimens were found paired.

Larval development

Pearlfish spawning has never before been observed and was thought to take place at sea and not within the host (Arnold, 1956). Fertilized eggs from Carapus acus have been collected in the Mediterranean during the summer months by Emery (1880), and Padoa (1947). Emery brought the eggs into the laboratory and was able to hatch and rear the larvae for a period of one week.

On two occasions during the present research (October and December), egg rafts were spawned in aquaria by pairs of Encheliophis gracilis. On the first occasion, a single Holothuria argus was brought from Tumon Bay and placed in an aquarium by itself. It was not known at the time that the holothurian was infested by an Encheliophis gracilis pair. The holothurian remained in the aquarium overnight, and the next morning an egg raft was discovered floating at the surface of the water. The holothurian was removed and immediately dissected, and the E. gracilis pair was found infesting a single respiratory tree of the host (Fig. 42). The female of the pair had a partially spent ovary, where the ovary wall had collapsed and become wrinkled, even though there were eggs remaining inside. The male's testes were histologically sectioned and found to be producing sperm (Fig. 40).

The egg raft was oval, approximately 60 mm in length, 35 mm wide, and 35 mm deep (Fig. 43). Its color was light yellow, as a result of the yellow oil droplets within each egg. This color changed to a blackish hue as the eggs began their development. The gelatinous envelope containing the eggs was transparent, and it appeared to be divided into many sections, each radiating out from the center. The upper middle of the raft was hollow, with the eggs forming a doughnut-shaped mass within the envelope. The raft contained approximately 1,000 eggs. Each egg was ellipsoidal and approximately 0.8 mm in diameter. The oil droplet within each egg was spherical and approximately 0.22 mm in diameter.

The egg raft was left in the aquarium and for a five day period the embryonic development of the fertile eggs, and the development of the hatched prelarvae and larvae was observed. The following is an account of these observations:

0 hours + X: Appearance of the egg raft (Figs. 44 and 45). It was not possible to determine if the eggs were fertile at this time. The raft was spawned sometime during the preceding 12 hours.

24 hours + X: The egg raft was still intact and it had a blackish

appearance due to pigment cell formation in the developing embryos. The eggs on the outer surface of the raft, and those lining the central depression were fertile and developing. Eggs more deeply embedded in the envelope appeared to be infertile. Each developing embryo had a crescent-shaped strip of pigment (neural crest cells) originating near the oil droplet and extending half way around the egg (Figs. 47 and 48). At this time, approximately five percent of the eggs were developing. Small copepods were observed swimming around the egg raft. Some had entered the envelope and were moving over the eggs themselves.

<u>32 hours + X</u>: In most of the embryos, the pigment cells had differentiated into a definite head and body, and the eyes had begun development (Fig. 49). Some pigment cells were forming posteriorly near the pharyngeal area. Approximately ten percent of the eggs were now developing.

<u>40 hours + X</u>: The egg raft was not as compact as it was originally. In one area near the bottom of the raft, the envelope began to break apart and some of the eggs were liberated. The infertile eggs seemed to be disintegrating. Some eggs developed comparatively large white masses over the oil droplet (Fig. 46). All of the embryos had a definite head with developing eyes (Figs. 50 and 51). In these, myomeres were evident in the tail region. The formation of the heart may be seen, and the yolk sac has become reduced in size. In some, hatching is about to occur (Fig. 52).

<u>48 hours + X</u>: The egg raft had now completely disintegrated, however, a few clumps of the envelope containing a few eggs remained floating at the surface. The infertile eggs were lying scattered on the bottom of the aquarium. The developing embryos and larvae were suspended

in the water and moving due to the currents created by the aquarium air stone. Most of the embryos had hatched, and the developing prelarvae measured approximately 1.5 mm (Fig. 53).

56 hours + X: No trace of the egg raft remained. Approximately 200 larvae were still suspended in the water (Fig. 54). In all, the heart was pumping blood. In a few of the more mature larvae, a small dorsal bud appeared at the anterior end of the dorsal fin (Fig. 55). This bud was the beginning of the vexillum.

64 hours + X: Approximately 100 larvae remained. All had reached the stage where the dorsal bud had appeared. In a few of the larvae, the dorsal bud had elongated into a definite appendage (Fig. 56). The larvae became more streamlined as the yolk sac reduced in size. Movement of the larvae by tail flicking began.

72 hours + X: Approximately 30 larvae remained and they were transferred to a smaller aquarium in order that they might be more easily observed. In all, the vexillum had elongated and the average size of the larvae was approximately 2 mm.

80 hours + X: Seven larvae remained. They all had developed a swelling or bulb at the end of the vexillum (Fig. 57). The yolk sac had almost completely disappeared in each. The larvae were capable of moving through the water in a jerky fashion. The size of these larvae was approximately 2.5 mm.

88 hours + X: All of the larvae were dead. The most plausible cause of death was lack of food, however, other environmental conditions such as oxygen, water circulation, salinity and temperature may have been partially responsible.

Emery (1880) was able to keep his *Carapus acus* larvae alive for seven days, and they reached a size of three to four millimeters. Emery's figures of his three and four day larvae are nearly identical to the photographs of the *Encheliophis gracilis* larvae of the same age, suggesting a definite similarity of the larval development of *E. gracilis* and *Carapus acus*. Emery also includes a line drawing of the egg raft of *C. acus*, and it is similar to the *Encheliophis gracilis* egg raft. The *Carapus acus* larvae at the sixth day of development have a fully developed vexillum with a highly branched or lobate process as described by Emery. This lobate process is characteristic of all vexillifer larvae, and it is unfortunate that the *Encheliophis gracilis* larvae could not be reared to this stage.

A plankton tow was conducted shortly after the appearance of the *Encheliophis gracilis* egg raft as an effort to collect pelagic vexillifer larvae which must have been present at the time. The tow was made across Tumon Bay, above the reef terrace in approximately 30 meters of water. A surface tow and a tow at 10 meters (both at night) produced no larvae. Emery (1880) and Padoa (1947) have both collected vexillifer larvae of *Carapus acus* in plankton tows conducted at the time of the appearance of the egg rafts.

A second *Encheliophis gracilis* egg raft was acquired under slightly different conditions than the first. A fresh specimen of *Holothuria argus* was dissected and an *Encheliophis gracilis* pair was found. This pair was isolated in an aquarium for the purpose of behavior observations. The following day an egg raft, identical to the first raft, was found floating in the aquarium. It was noticed that the abdomen of the female was reduced in size compared to the day before. After a day of observation it became apparent that the raft was not fertile. An attempt to fertilize the eggs by stripping the male proved unsuccessful. At the first sign of disintegration, the egg raft was preserved in five percent formaldehyde.

Discussion

Arnold (1956) and Smith (1964) state that the life history of Encheliophis gracilis is unknown. As a result of the present research, the following has been ascertained.

Host infestation data indicates that Encheliophis gracilis is not seasonal in its breeding habits. Mature adults were found throughout the collecting period, and no substantial variation of infestation by the juveniles was observed. The percent infestation and mean length of the collected E. gracilis specimens remained fairly constant.

Upon reaching sexual maturity, male and female Encheliophis gracilis pair, and, the pair members reside together in a holothurian host (in this case, Holothuria argus). This pairing is probably temporary, lasting only to the completion of spawning. Only fully mature fishes were found paired.

Three possibilities regarding the actual spawning exist: 1) the egg raft may be spawned within the host and carried out through the holothurian's anus by the action of its respiratory currents; 2) the pearlfishes may simply extend their heads out through the holothurian's anus, keeping the major part of their bodies within the host (spawning is possible in this manner due to the location of the vent in the gular area); and 3) the pearlfishes may leave the host and spawn, either returning to the same host together or perhaps splitting up and seeking new hosts. It would seem that the second possibility is the most likely, as part of the pearlfish's relationship with a host may be for protection, and, to leave the host at a most critical time, as when spawning, would be unlikely. Similarly, the possibility of spawning inside the holothurian should not be overlooked. Data presented later in this paper show that the position of the pearlfish pairs within the holothurian was, in every case, in a single respiratory tree (Fig. 63). Observations on the egg raft indicate that the raft is able to withstand a great deal of contraction and twisting. It would be quite possible for the raft to be expelled through the anus of the holothurian as the animal respires.

The fact that the second egg raft was spawned outside of the host is not indicative of natural conditions, since the female was taken from the host at a critical time.

The possibility exists that at spawning time, each female may release more than a single egg raft. The two females from which the egg rafts originated did not have completely expended ovaries.

The egg rafts are pelagic but because of the very rapid embryonic and larval development, the rafts undoubtedly do not get far offshore. Emery (1880) noted that in the case of Carapus acus, vexillifers metamorphose into bethnic tenuis within a month of hatching. Since the embryonic and larval development of Encheliophis gracilis appears similar to Carapus acus, there may not be any significant difference in the length of life of the vexillifer larvae.

No Encheliophis gracilis tenuis larvae were found. It is possible that they infest deep-water holothurians such as Thelenota ananas. The sampling of *Thelenota* was relatively small (67 specimens), and instances of tenuis larvae may have been missed. There is little doubt that the tenuis larvae do not reach the reef flat and instead metamorphose into juveniles in deeper water. Because approximately 1,700 holothurians were sampled on the reef flat, and because of the number of mature adult *E. gracilis* specimens on the reef, instances of tenuis larvae should not have been missed.

None of the *Encheliophis gracilis* specimens infesting *Thelenota* had maturing gonads, even though a few were within the adult size range. This may indicate that *Thelenota* is a temporary host and that there is a shoreward migration of the juveniles until infestation in *Holothuria argus* on the reef flat can be accomplished. Only one juvenile *Encheliophis gracilis* was found in 468 specimens of *Holothuria argus*. The juveniles infest *Stichopus chloronotus* possibly because it is the first acceptable holothurian species encountered on the reef flat. Adults move further shoreward and infest the preferred host, *Holothuria argus*.

Other aspects of the natural history of *Encheliophis gracilis* (behavior, location in the host, and food habits) are discussed in Chapter IV.

TABLE VII. MATURATION OF GONADS - *ENCHELIOPHIS GRACILIS* IN *HOLOTHURIA ARGUS*

MONTH (1968-1969)	N	Paired mature	Unpaired mature		Unpaired immature		
			male	female	undeveloped	male	female
April	--	--	--	--	--	--	--
May	--	--	--	--	--	--	--
June	11	6	--	--	--	1	4
July	15	6	3	2	--	1	3
August	--	--	--	--	--	--	--
September	6	--	1	1	2	1	1
October	4	4	--	--	--	--	--
November	13	10	--	2	--	1	--
December	6	2	2	--	2	--	--
January	8	2	4	--	2	--	--
TOTAL	63	30	10	5	6	4	8

TABLE VIII. *ENCHELIOPHIS GRACILIS* PAIR FORMATION IN *HOLOTHURIA ARGUS*

MONTH (1968-1969)	*Holothuria argus*		*Encheliophis gracilis*	
	Collected	Infested with *E. gracilis*	Pairs	Percent of paired infestation
April	---	---	---	---
May	---	---	---	---
June	71	8	3	38
July	113	12	3	25
August	23	---	---	---
September	44	6	---	---
October	32	2	2	100
November	62	8	5	63
December	47	5	1	20
January	76	7	1	14
TOTAL	468	48	14	31

TABLE IX. STANDARD LENGTH OF *ENCHELIOPHIS GRACILIS* PAIRS

FIELD NUMBER	*Encheliophis gracilis* pairs		FIELD NUMBER	*Encheliophis gracilis* pairs	
	sex	standard length (mm)		sex	standard length (mm)
68-11	male	187	68-34	male	170
	female	208		female	233
68-12	male	175	68-35	male	185
	female	200		female	202
68-12	male	239	68-35	male	180
	female	240		female	183
68-13	male	240	68-36	male	205
	female	223		female	210
68-14	male	211	68-36	male	173
	female	233		female	225
68-15	male	200	68-39	male	190
	female	218		female	215
68-29	male	185	68-48	male	245
	female	205		female	205
68-29	male	145	AVERAGE	male	195
	female	195		female	213

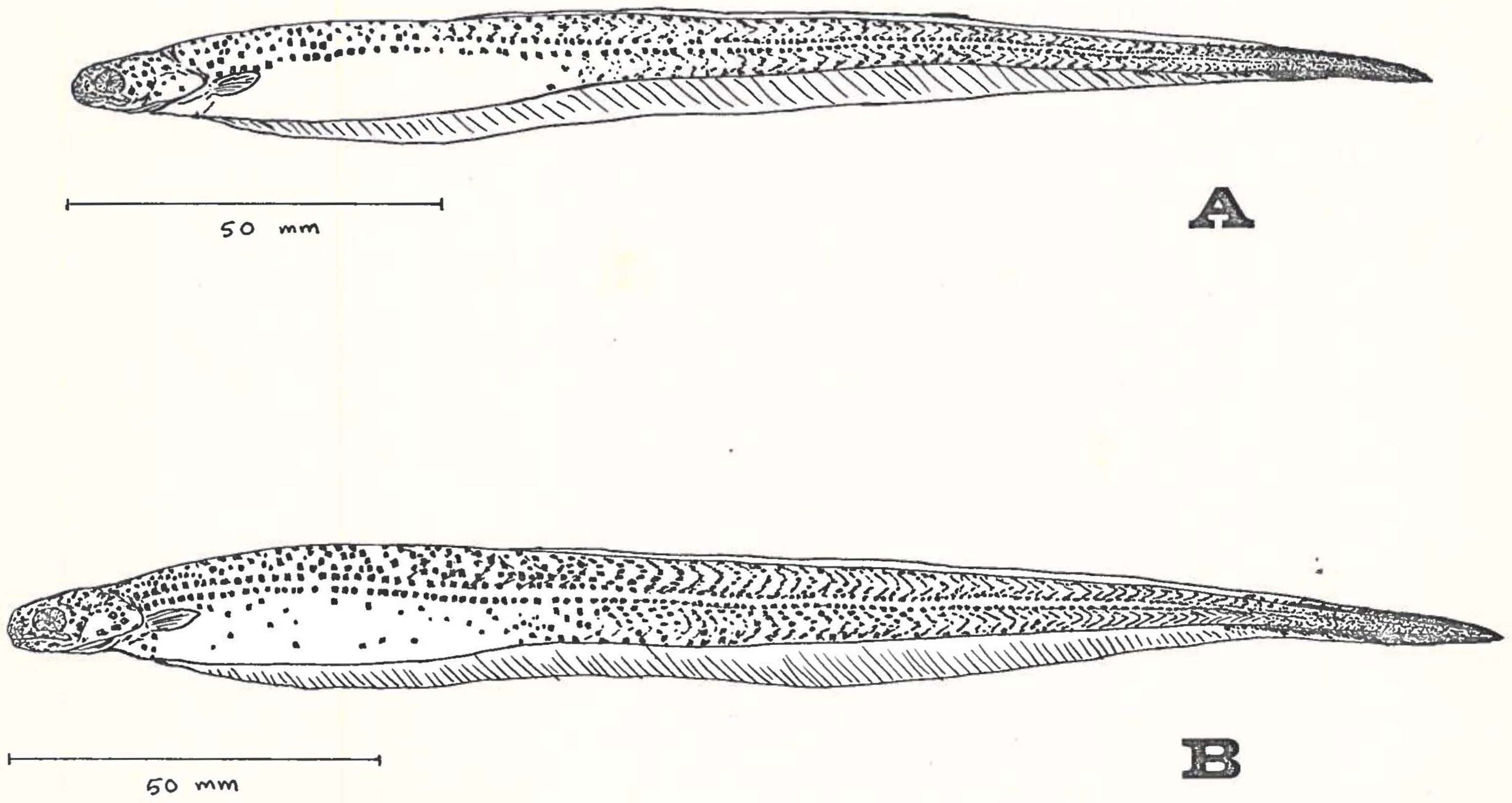


Fig. 31. *Encheliophis gracilis* (Bleeker) pair. A, male; B, female.

Fig. 32. Male (above) and female (below) specimen of *Encheliophis* (*Jordanicus*) *gracilis* (Bleeker). A parasitic fungus is attacking the anal fin of the female.

Fig. 33. Length-frequency histogram of *Encheliophis gracilis*.

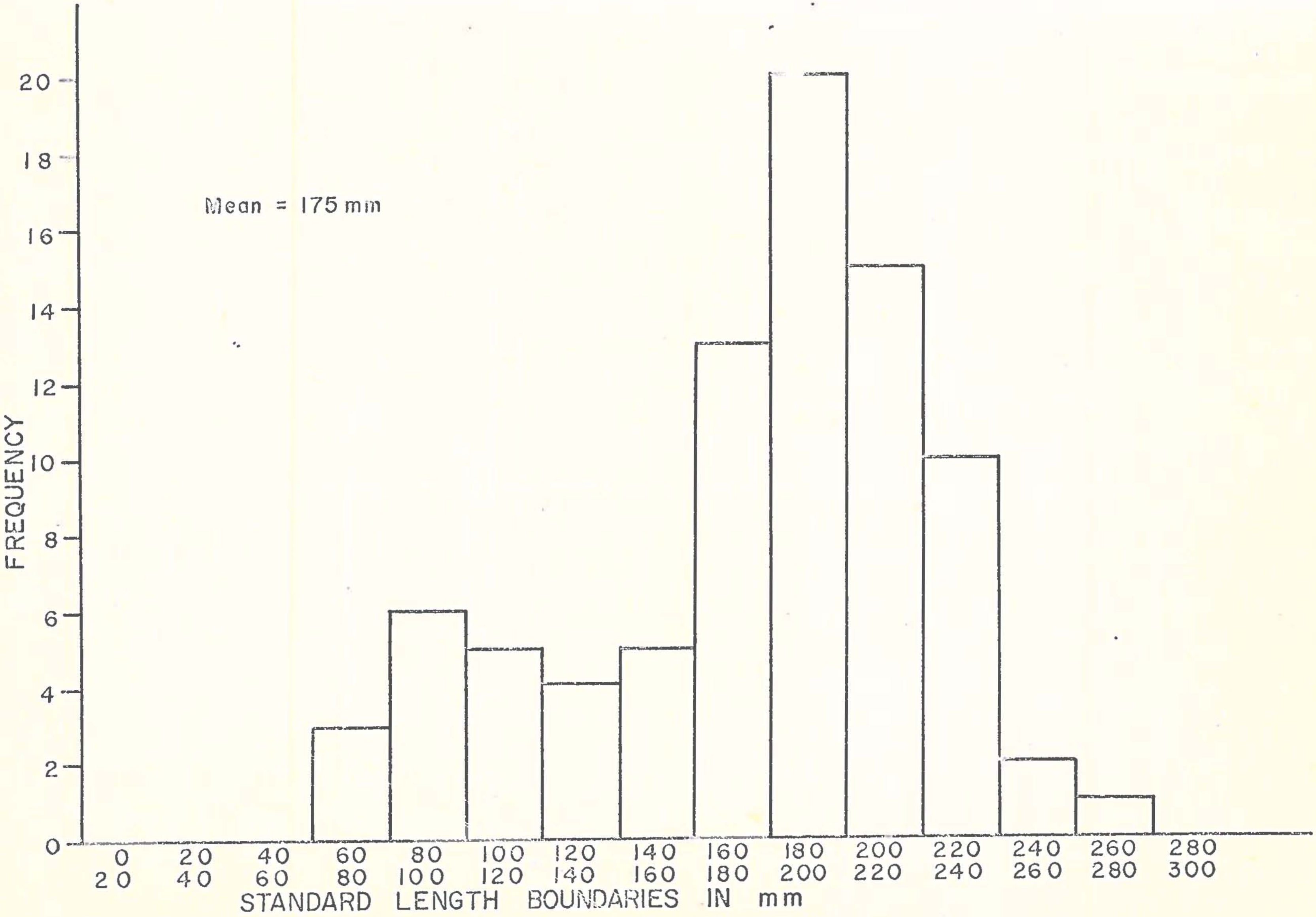

Mean = 175 mm
FREQUENCY
0
2
4
6
8
10
12
14
16
18
20
0 20
20 40
40 60
60 80
80 100
100 120
120 140
140 160
160 180
180 200
200 220
220 240
240 260
260 280
280 300
STANDARD LENGTH BOUNDARIES IN mm

Fig. 34. Seasonal variation of infestation by *Encheliophis gracilis*.

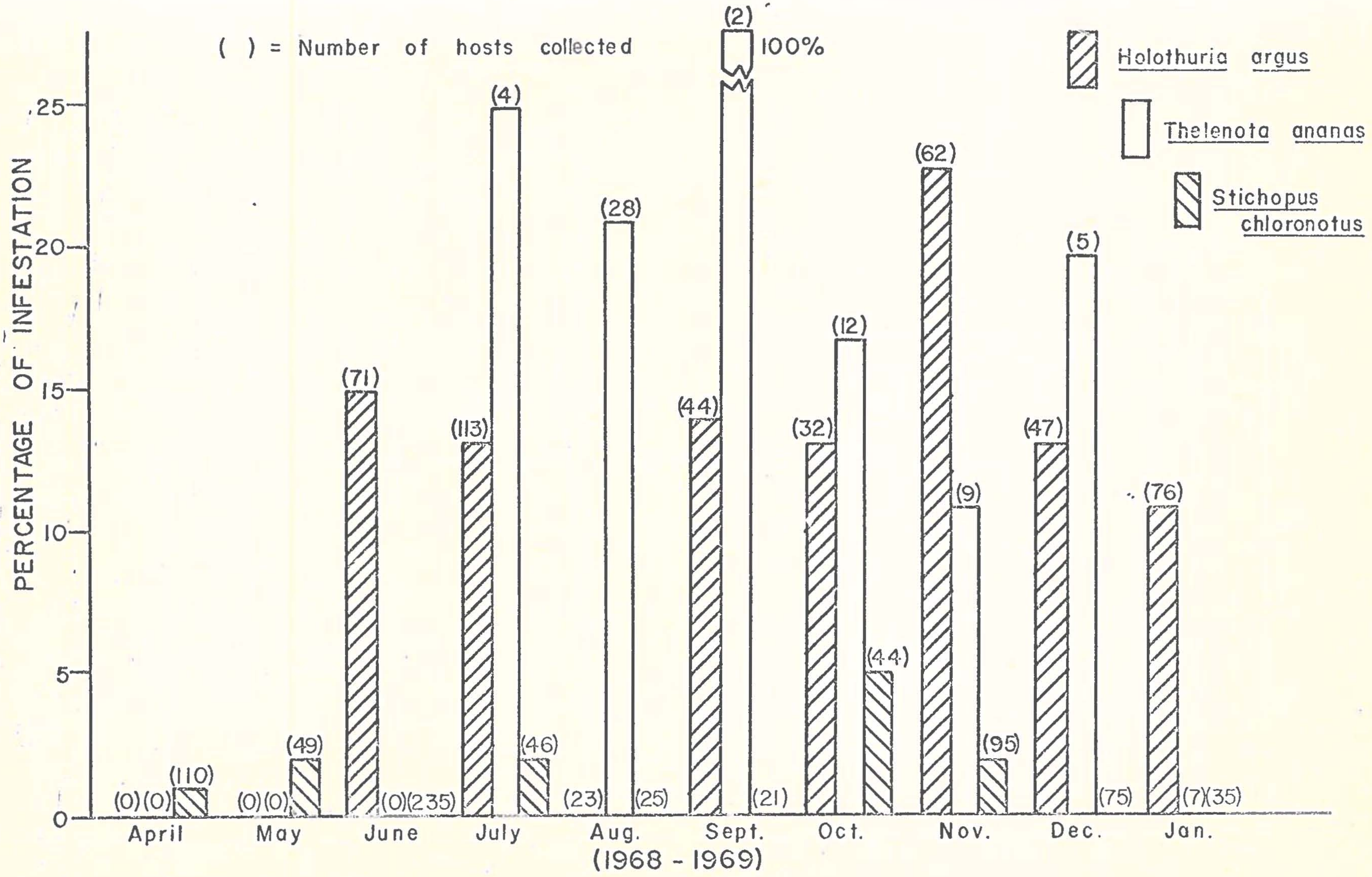
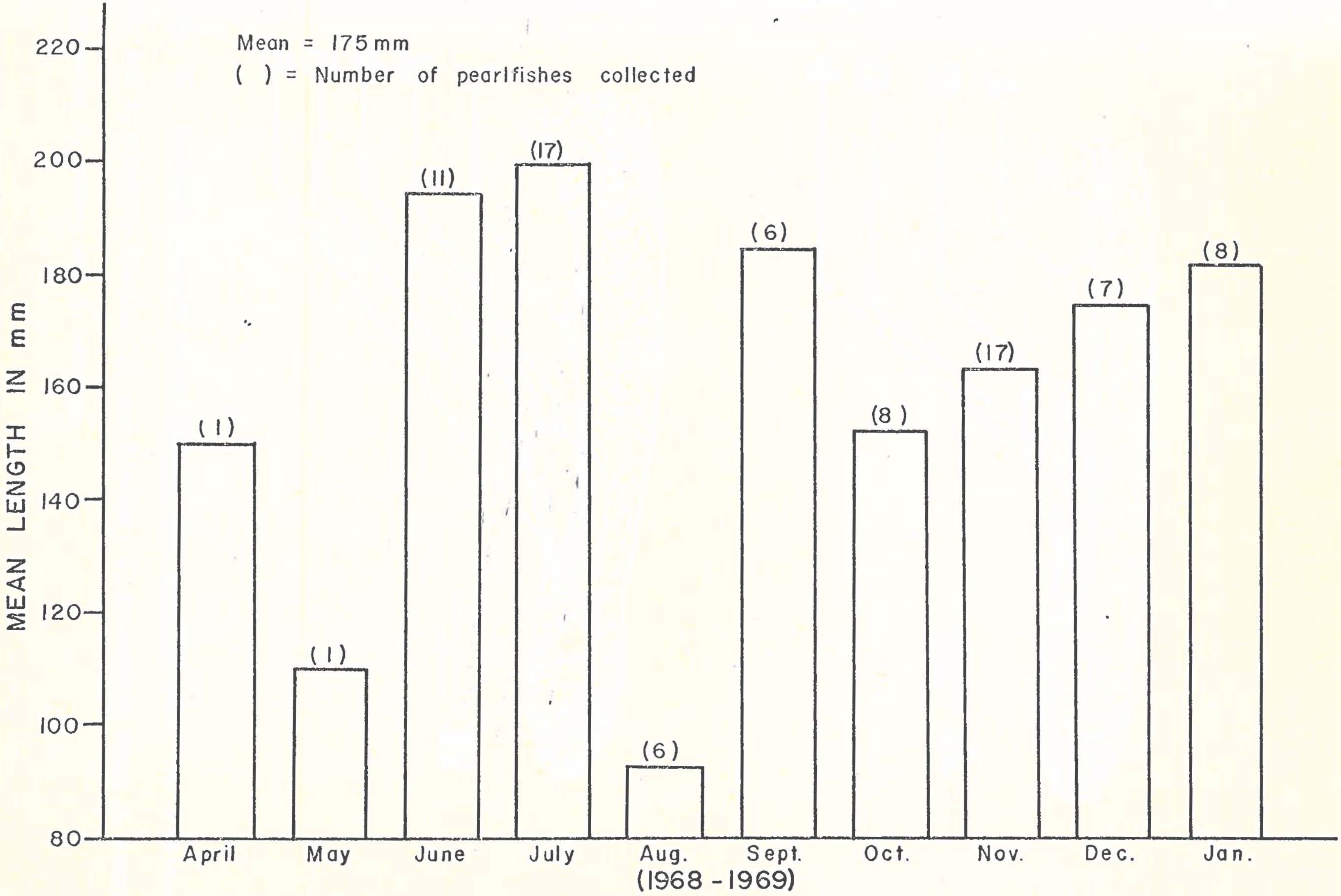

( ) = Number of hosts collected
(2)
100%
Holothuria argus
Thelenota ananas
Stichopus chloronotus
PERCENTAGE OF INFESTATION
0
5
10
15
20
25
(0)(0)
(110)
(0)(0)
(49)
(71)
(0)(235)
(113)
(4)
(46)
(23)
(28)
(25)
(44)
(21)
(32)
(12)
(44)
(62)
(9)
(95)
(47)
(5)
(75)
(76)
(7)(35)
April
May
June
July
Aug.
Sept.
Oct.
Nov.
Dec.
Jan.
(1968 - 1969)

Fig. 35. Mean length of *Encheliophis gracilis* by month.


Mean = 175 mm
( ) = Number of pearlfishes collected
MEAN LENGTH IN mm
220
200
180
160
140
120
100
80
(1)
(1)
(11)
(17)
(6)
(6)
(8)
(17)
(7)
(8)
April
May
June
July
Aug.
Sept.
Oct.
Nov.
Dec.
Jan.
(1968 - 1969)

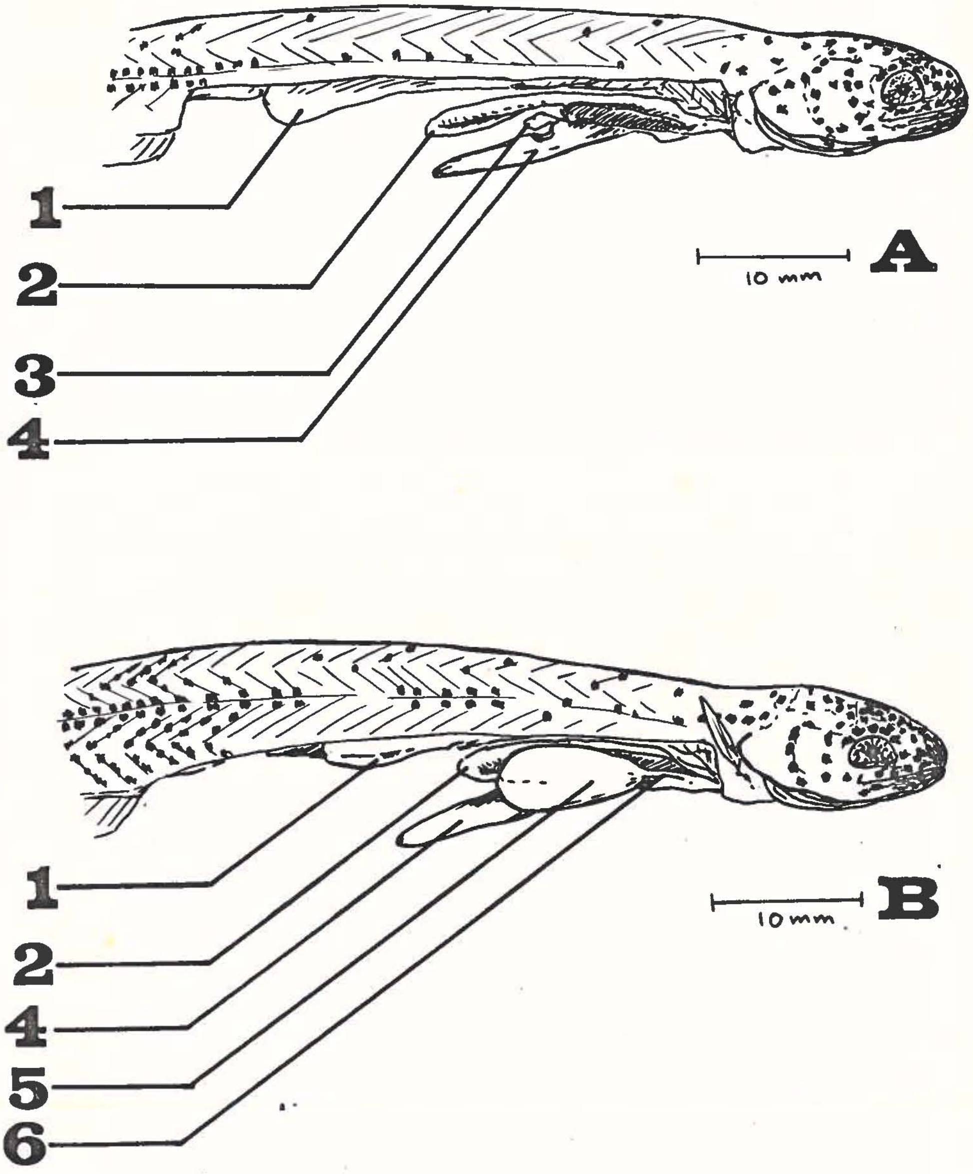
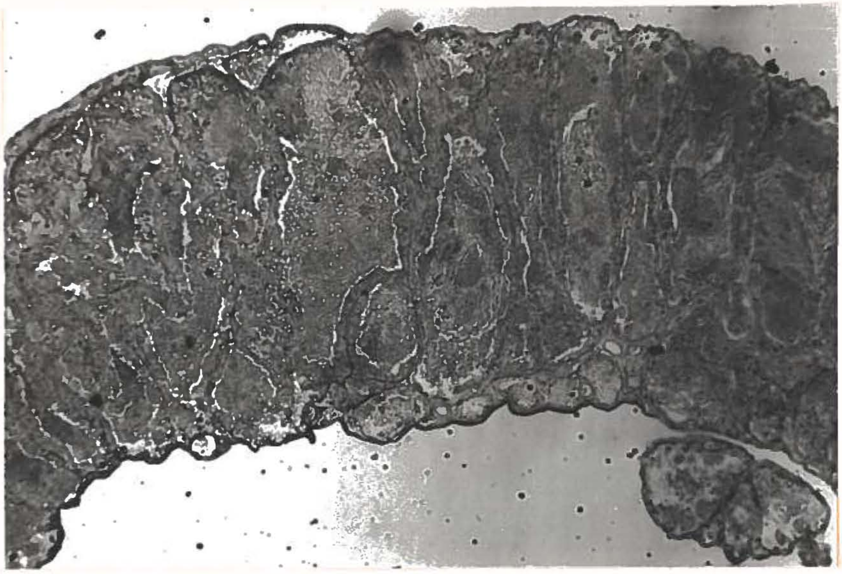


Fig. 36. Internal anatomy of an Encheliophis gracilis pair. A, a mature male; and B, a mature female. 1, air bladder; 2, intestinal loop; 3, testes; 4, stomach; 5, ovary; and 6, oviduct.

Fig. 37. Section of the male *Encheliophis gracilis* testis (40X).

Fig. 38. Enlarged view of the testis showing several seminiferous tubules (100X).



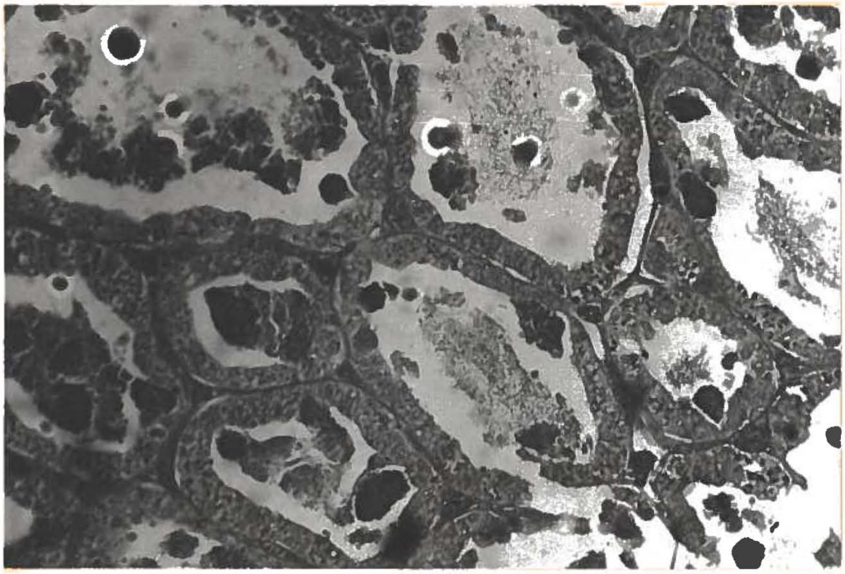
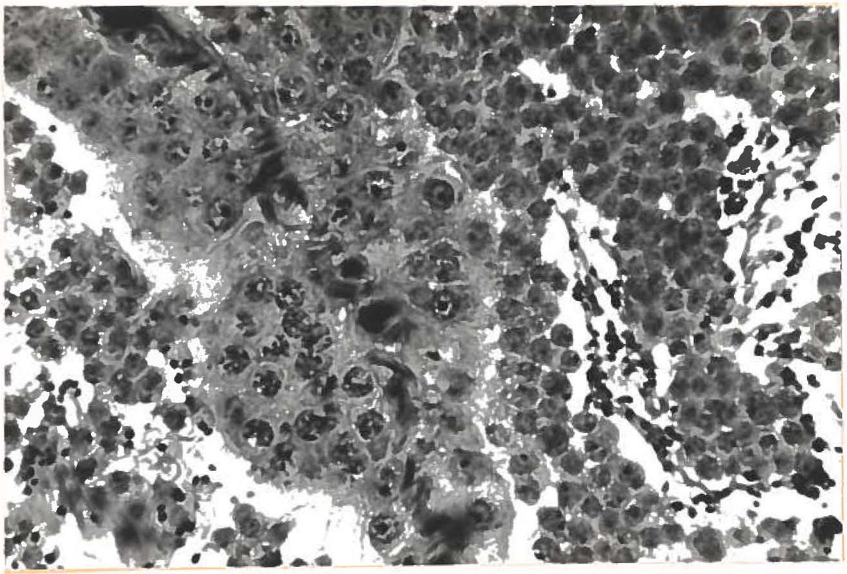


Fig. 39. Lumen of a single seminiferous tubule showing the characteristic stages of spermatogenesis (400X).

Fig. 40. Enlarged view of the lumen showing spermatids and sperm (970X).



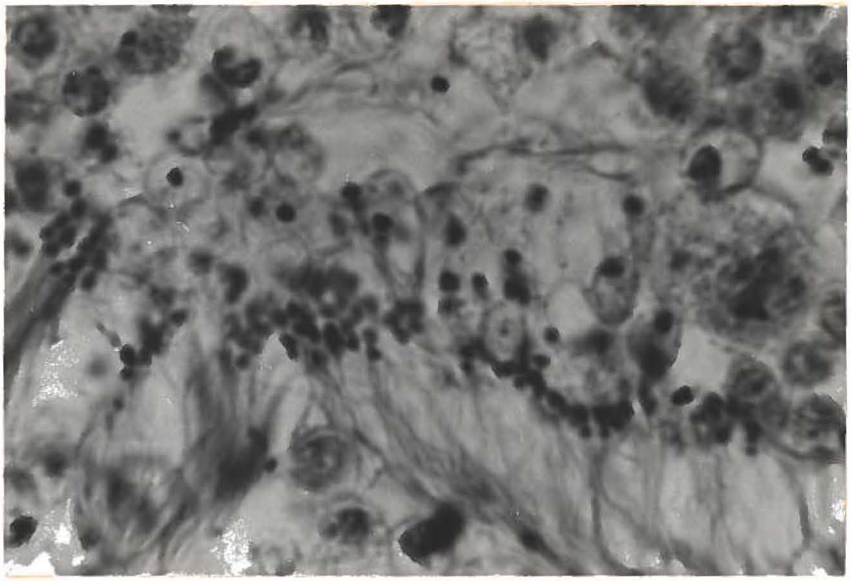


Fig. 41. Scatter diagram and regression lines relating to the lengths of the male and female *Encheliophis gracilis* pairs.

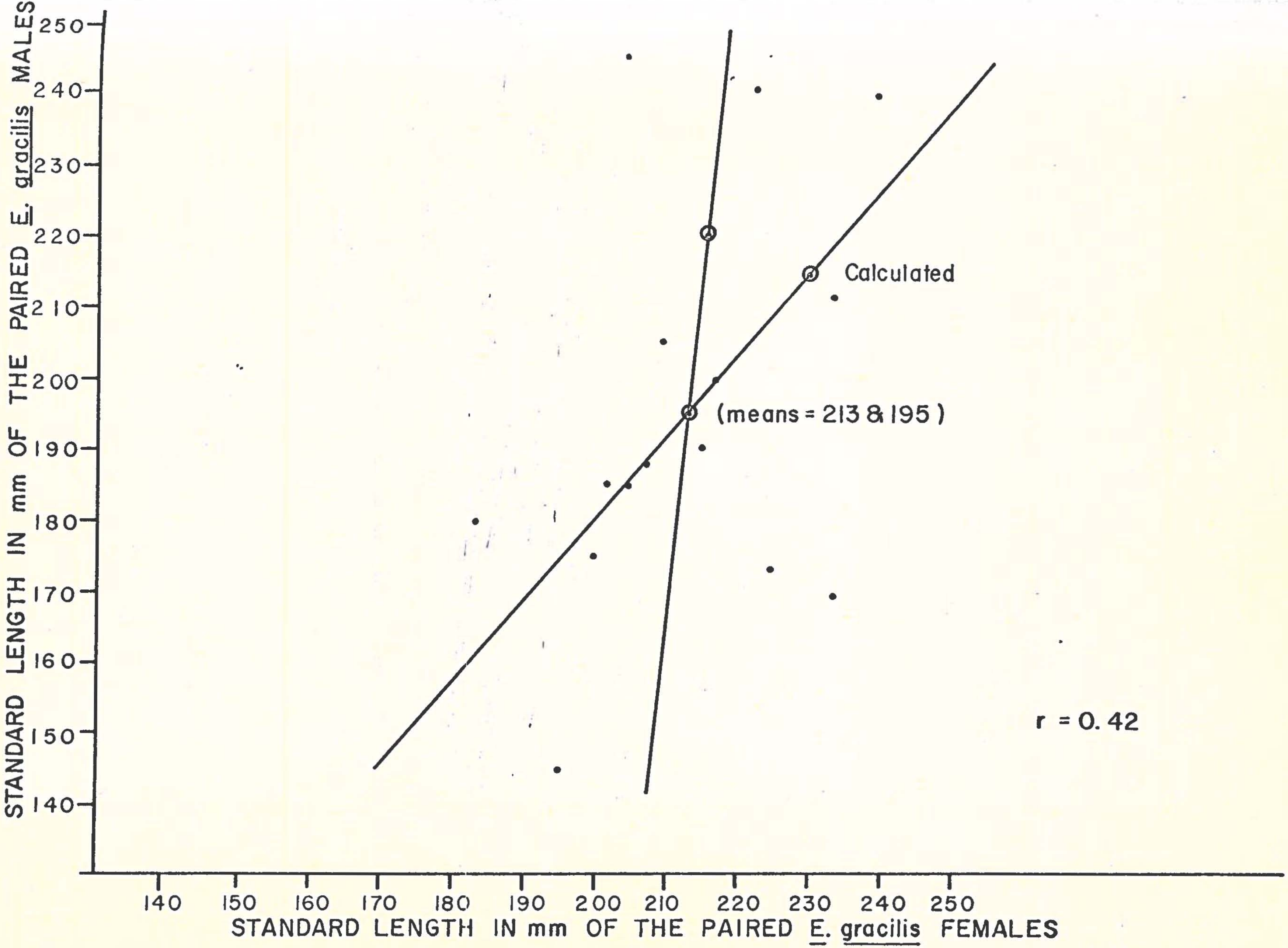

STANDARD LENGTH IN mm OF THE PAIRED E. gracilis MALES
250
240
230
220
210
200
190
180
170
160
150
140
140
150
160
170
180
190
200
210
220
230
240
250
STANDARD LENGTH IN mm OF THE PAIRED E. gracilis FEMALES
Calculated
(means = 213 & 195)
r = 0.42

Fig. 42. Female (above) and male (below) <u>Encheliophis gracilis</u> pair which produced a fertile egg raft. They are pictured with their host, <u>Holothuria argus</u>.


50
60
70

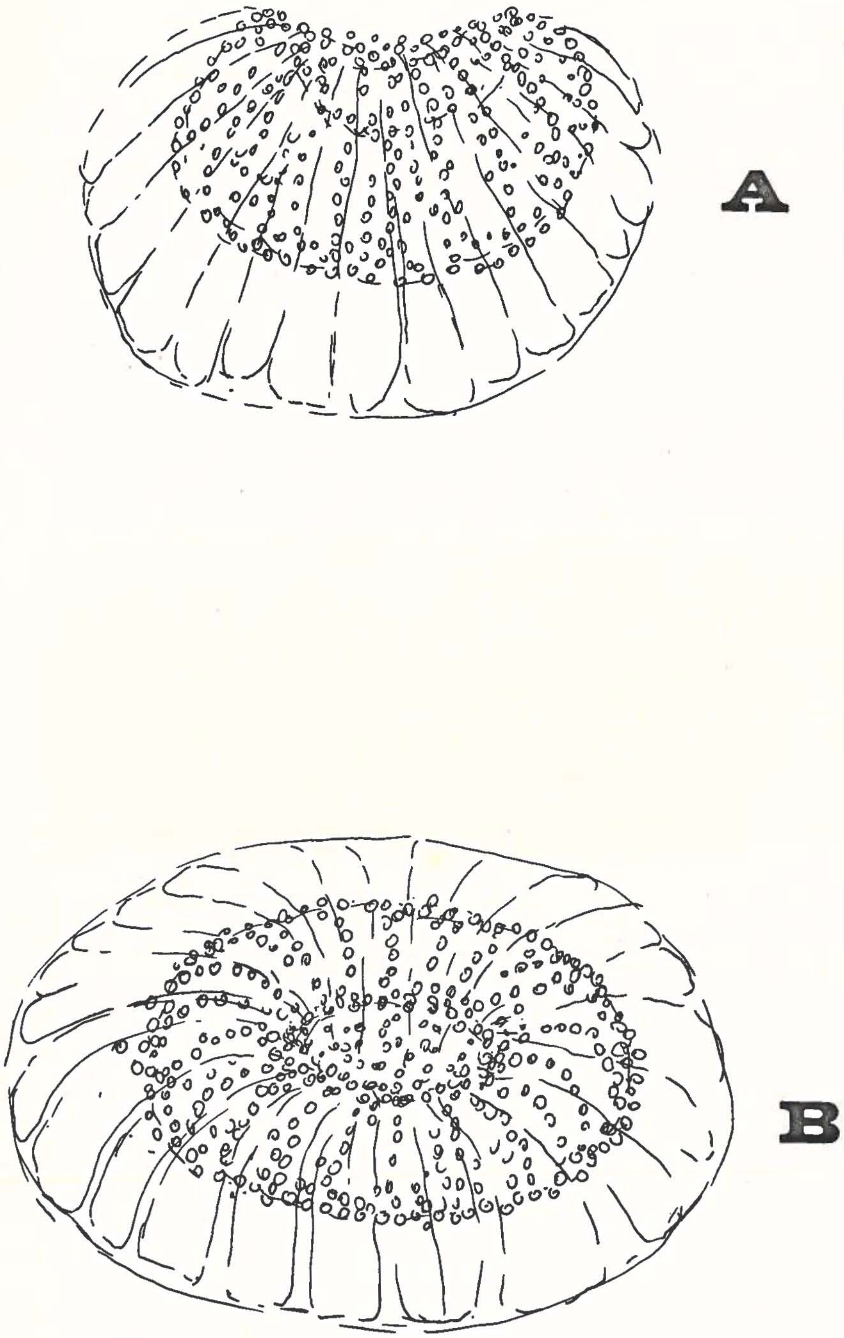
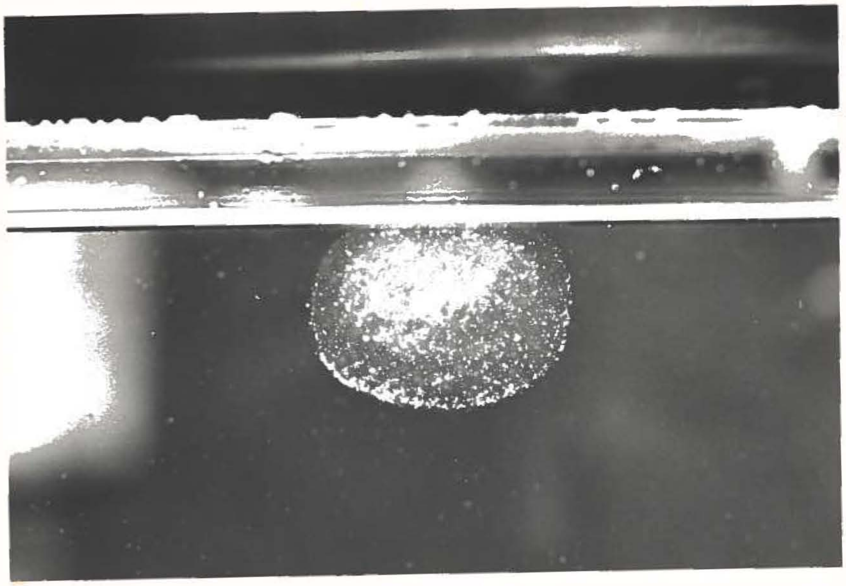


Fig. 43. Diagrammatic view of the egg raft produced by an *Encheliophis gracilis* pair. A, a side view, showing the mass of eggs and the mucus striations; and B, an oblique view showing the doughnut-shaped mass of eggs and the center depression of the egg raft. The sketch is approximately three times life size.

Fig. 44. Side view of the Encheliophis gracilis egg raft floating at the surface of the aquarium. The eggs are in a dense mass in the center of the raft, and the glistening spherical objects near the periphery of the raft are air bubbles.

Fig. 45. End view of the egg raft.



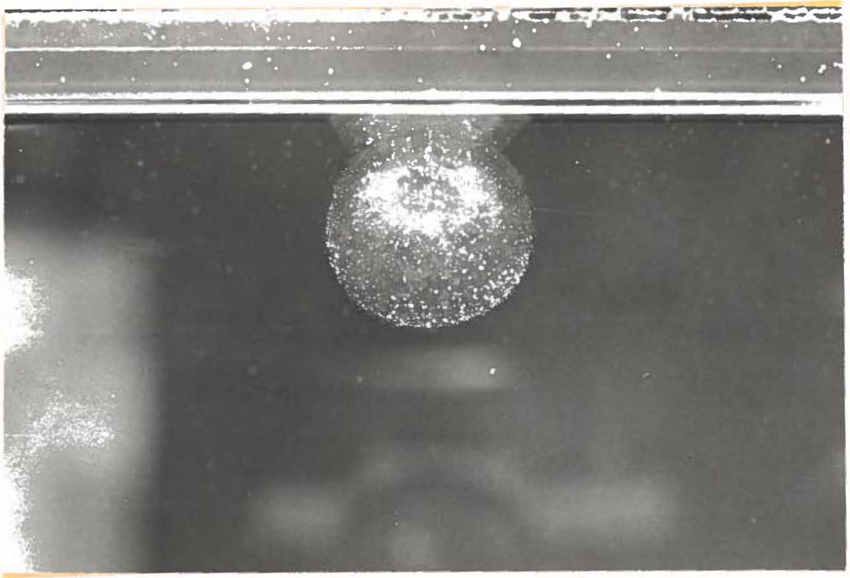


Fig. 46. Infertile egg which has begun to deteriorate (40X).

Fig. 47. A 24-hour + X embryo of <u>Encheliophis gracilis</u> (40X). The oil globule and yolk sac are distinct. Neural crest cells are present, and the eyes of the embryo are developing.

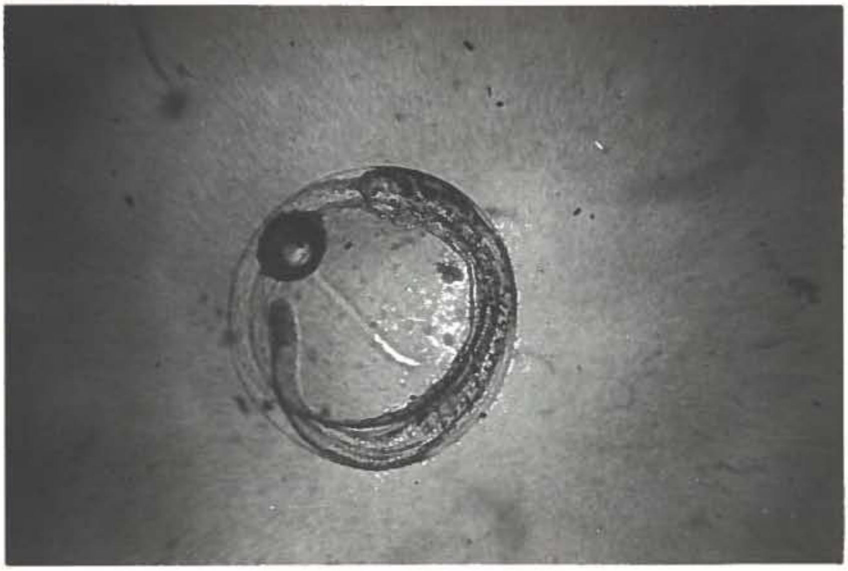
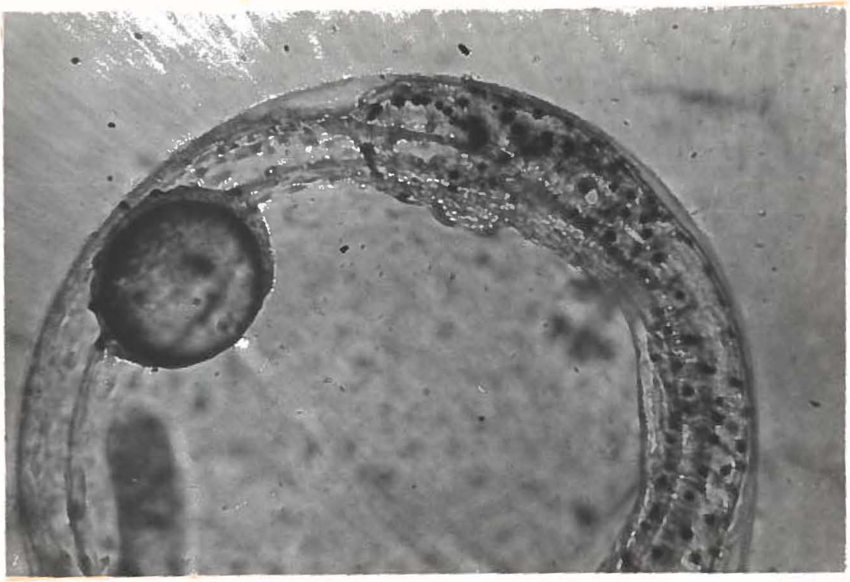


Fig. 48. Enlarged view of the anterior of the 24-hour + X embryo (100X).

Fig. 49. A 32-hour + X embryo (40X). The eyes of the embryo show further development.



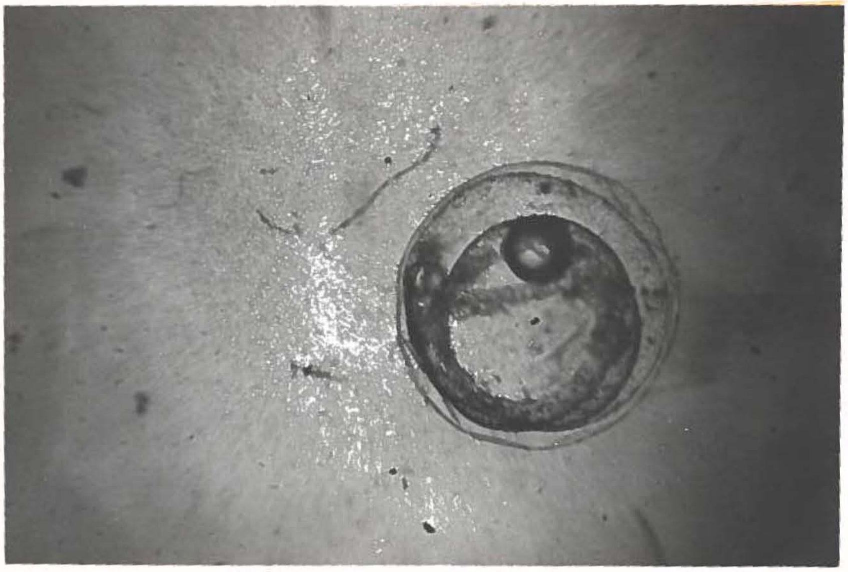


Fig. 50. A 40-hour + X embryo (40X). The formation of the heart may be seen. Myomeres are evident in the tail region. The yolk sac is becoming reduced in size.

Fig. 51. Enlarged view of the head of a 40-hour + X embryo (100X). The development of the eyes may be seen.

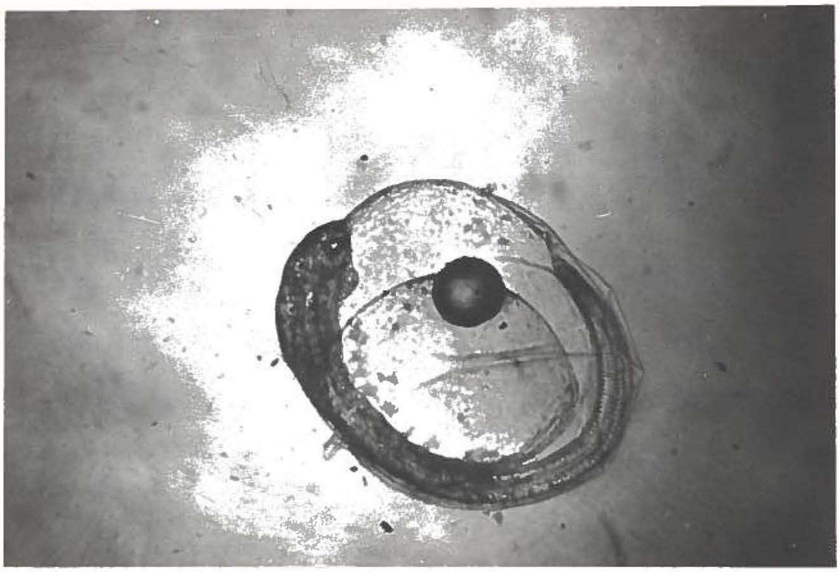
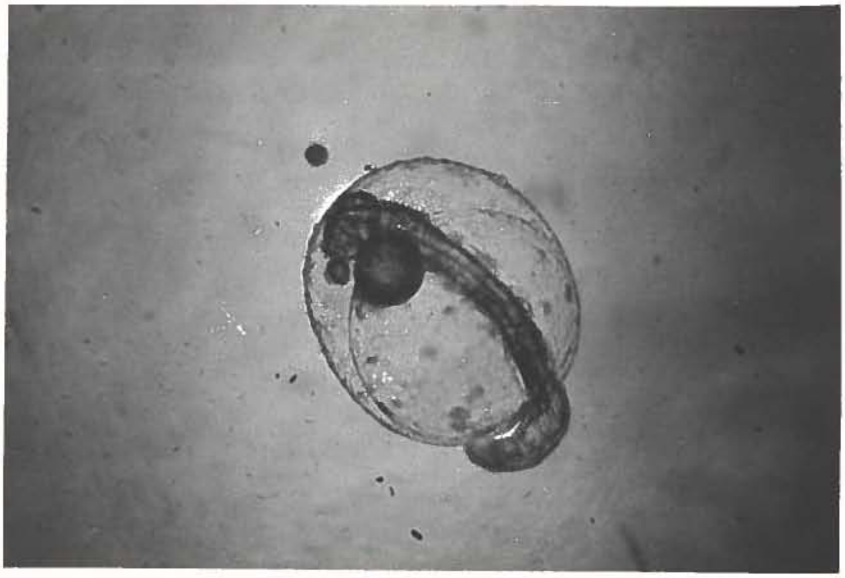


Fig. 52. Slightly dorsal view of a 40-hour + X embryo which is almost ready to hatch (40X).

Fig. 53. A 48-hour + X prelarva which has just hatched (40X).



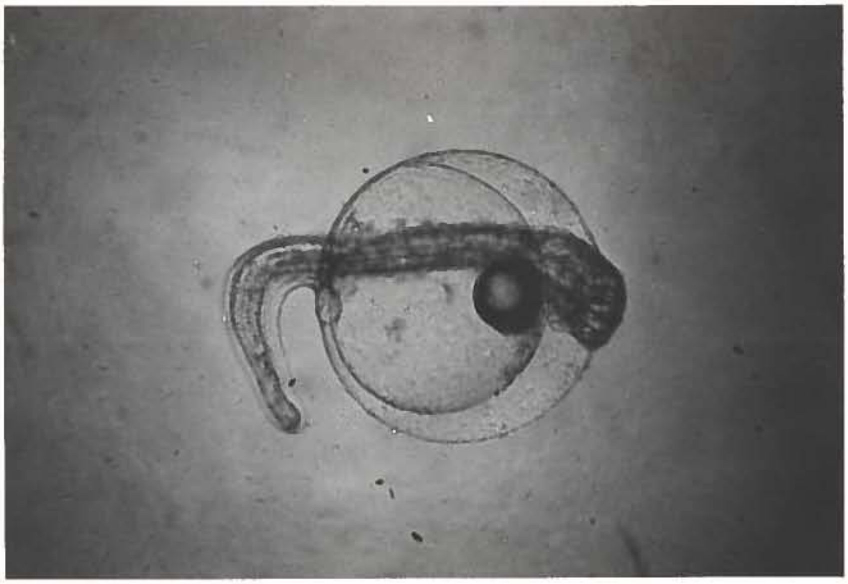


Fig. 54. A 56-hour + X larva measuring approximately 1.5 mm (40X).

Fig. 55. A 56-hour + X larva (40X). Notice the small dorsal bud which has appeared at the anterior end of the dorsal fin. The yolk sac has become considerably reduced. The dorsal and anal fins are now well developed.

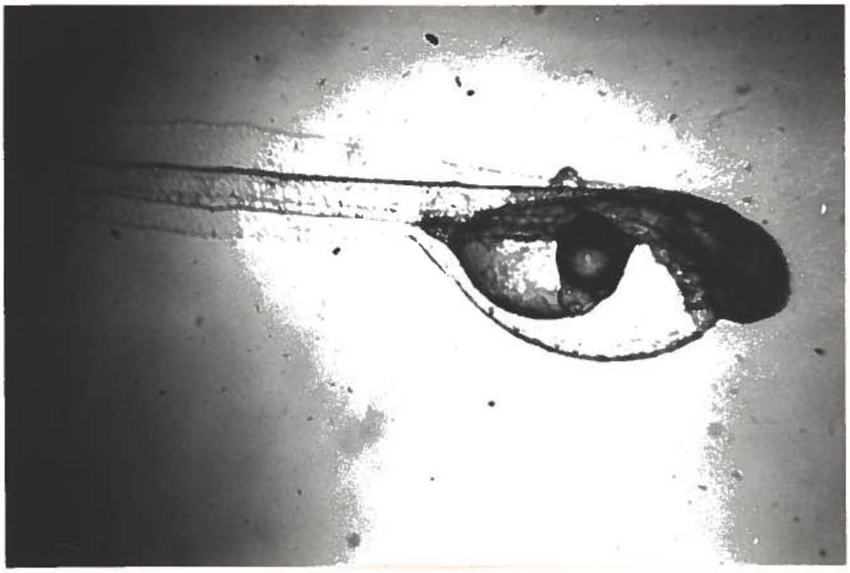
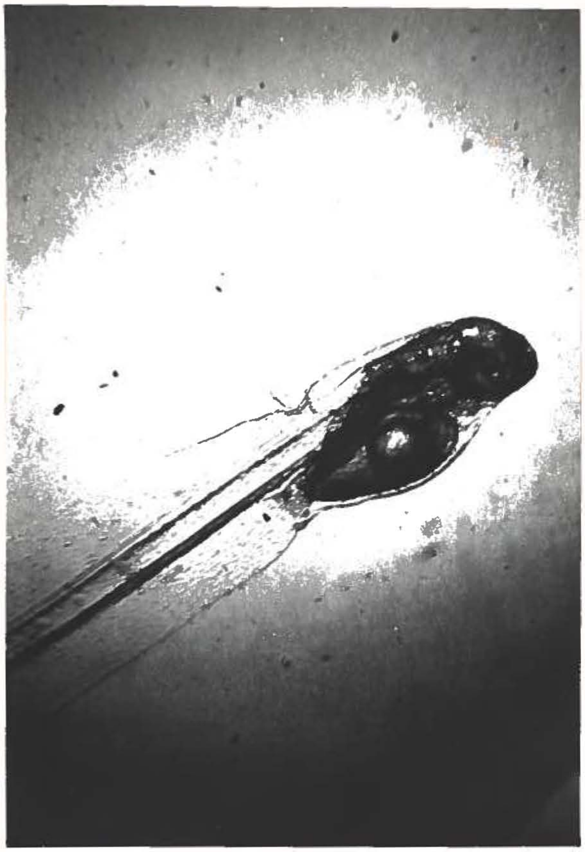


Fig. 56. A 64-hour + X larva (40X). The dorsal bud has elongated into a definite appendage. The yolk sac is nearly gone. The larva is approximately 2 mm in length.

Fig. 57. An 80-hour + X larva (40X). The dorsal appendage (now called the vexillum) has a swelling or bulb at its end. The larva is now approximately 2.5 mm in length.



Encheliophis vermicularis Müller, 1842

(Figures 58, 59, and 60)

Synonomy (abbreviated)

Encheliophis vermicularis Müller, 1842:205. Herre, 1936:416. Abe, 1939:574. Smith, J. L. B., 1955:416. Arnold, 1956: 296. Schultz, 1960:392.

Host specificity

An attempt was made to collect Encheliophis vermicularis, a species of pearlfish last collected from Guam in 1945 by Schultz (1960). Schultz identifies the host of the two specimens of E. vermicularis found in Tumon Bay, as the common Holothuria atra. Initial samplings of H. atra in Tumon Bay produced no pearlfishes. The 1945 Guam specimens (USNM 124249) were obtained from the Smithsonian Institution, Division of Fishes, to verify the identification of the pearlfishes and check the accompanying host description. The specimens were of the correct species, but a study of the enclosed data indicated that the host for this pearlfish species may not be H. atra, but another common holothurian species which resembles it (Holothuria sp. 1). This second holothurian species was collected (160 specimens) and a single specimen of Encheliophis vermicularis was found (Table II).

Two specimens of Encheliophis vermicularis were collected by the present investigator from Palau, Caroline Islands. They were found infesting an unidentified species of Holothuria. Abe (1939) reports Encheliophis vermicularis infesting holothurians from Palau.

Life form, maturation of gonads, and seasonality

The single, sexually undeveloped specimen of Encheliophis vermicularis was collected in January and measured 117 mm in length. The 1945 Guam specimens were collected in June and measured 95 and 125 mm total length. These were also sexually undeveloped. The two specimens from Palau were collected in August and measured 84 and 133 mm, the larger specimen had a nearly ripe ovary. Arnold (1956) states that the greatest recorded length for this species is 175 mm.

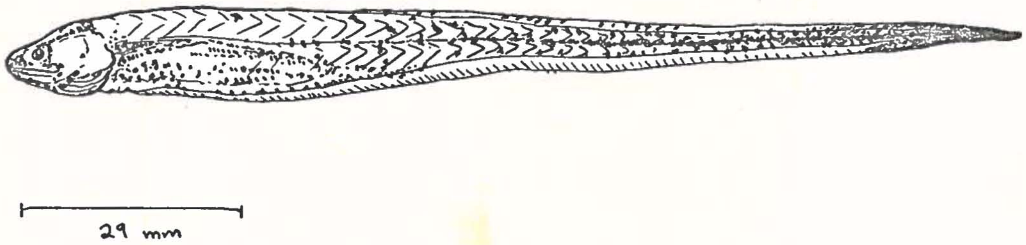
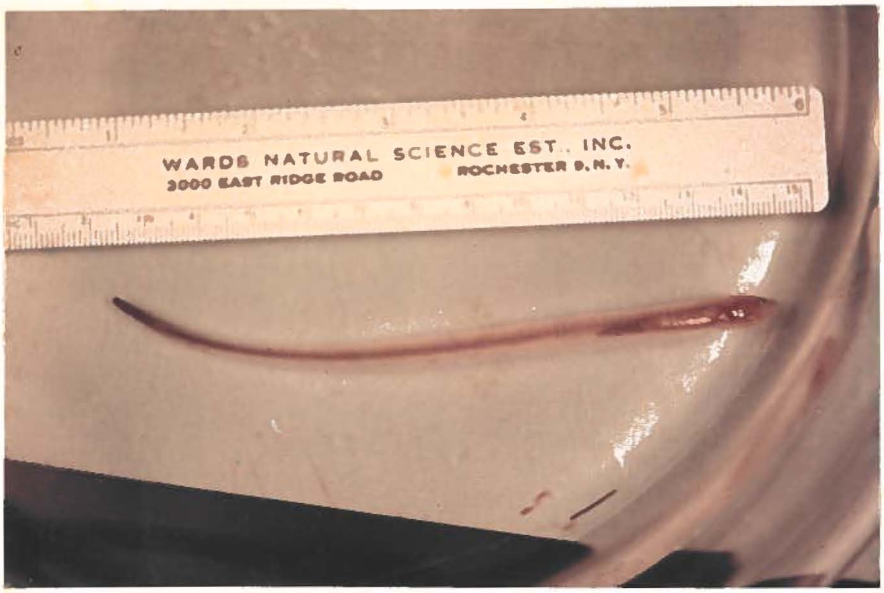


Fig. 58. *Encheliophis vermicularis* Müller.

Fig. 59. Encheliophis vermicularis Müller.

Fig. 60. Encheliophis vermicularis (same specimen as above).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3000 EAST RIDGE ROAD
ROCHESTER 9, N.Y.


PYREX

CHAPTER IV. NATURAL HISTORY OF THE GUAM PEARLFISHES

Behavior

Methods

Seven salt water aquaria ranging in size from two to 20 gallons were maintained from September 21, 1968, to January 20, 1969. Sand and coral rubble taken from the area of holothurian collections in Tumon Bay was used as bottom material for the aquaria. The aquaria were equipped with subsand filters and air stones. Seawater collected from Tumon Bay was used to fill the aquaria and to maintain water levels. Periodic checks on the salinity in each aquarium were made with a hydrometer, and each was found to be within acceptable limits (1.025-1.040 specific gravity) throughout the four month period. The water temperature in the aquaria averaged 23°C.

Two aquaria, two and 15-gallon capacities, were designated as holding tanks for the pearlfishes used in the behavior observations. The holothurian and Culcita specimens were maintained in the other tanks. Observations were made of the pearlfishes while they were segregated from their hosts, as well as when they were placed together with their hosts. Approximately 200 pearlfish entries were observed during the four month period. Upon entry, some pearlfishes were allowed to remain with the hosts for various lengths of time, these infested hosts being used in other behavior experiments. Most holothurians, however, were dissected immediately after pearlfish entry and the fishes returned to the holding tanks after their position within the host was noted.

Most fishes and hosts were rotated once a week. Those pearlfishes used the previous week were preserved and replaced by new specimens added to the holding tanks. New hosts were added each week to replace those that were dissected.

During the course of the behavior observations, an 8 mm movie was made of the entry behavior of *Carapus homei*, *C. mourlani*, *C. parvipinnis*, and *Encheliophis gracilis* with several different holothurian hosts and with *Culcita*. Nearly all aspects of pearlfish behavior can be seen in this film, and references to the film will be made in the following discussion. A copy is deposited in the Division of Biosciences and Marine Studies, University of Guam.

Pearlfish locomotion

The youngest pearlfish specimens observed were *Carapus homei* tenuis larvae. When isolated from their hosts, these larvae appeared agitated, swimming actively for long periods. During these swimming periods, their bodies were inclined upwards, and often their heads were thrust out of the water. Movement was accomplished by undulation of the entire body, including the head. Arnold (1953) observed the same type of movement with *C. acus* tenuis larvae. During periods of quiescence, the larvae stretched out on the bottom, usually with their heads in dark corners of the aquarium.

Carapus homei and *C. mourlani* juveniles and adults swam with their heads always directed downward. Body undulations were not nearly as intense in amplitude as in the tenuis specimens except at times of excitement (when frightened, or entering a host). Instead, movement primarily occurred by the action of the paired fins, which worked alternately.

Carapus parvipinnis adults moved in a manner similar to *C. homei* and *C. mourlani* adults. Their bodies were normally inclined downward at an angle greater than 45°. Forward movement was a result of slight undulations of the last quarter of the body, and of the paired fins. Lateral movement seemed to be governed by the paired fins only. C. parvipinnis specimens had shorter pectoral fins relative to body length than C. homei and C. mourlani, and more movement of the median fins appeared necessary during normal swimming than with the latter species.

Encheliophis gracilis juveniles swam head down with their entire bodies undulating, excluding their heads. E. gracilis adults often swam in a near vertical position, with undulations restricted to the last quarter of the body length during normal activity. Lateral movement appeared to be the result of the paired fins, but removal of these fins did not seem to affect the swimming ability of the adult. When frightened, E. gracilis adults assumed the tenuis type of locomotion and violent body undulations would often propel the pearlfish out of the aquarium.

The single specimen of Encheliophis vermicularis, which had no pectoral fins, was observed swimming in a shallow dish of water for a short time. Its locomotion was tenuis in nature, with undulations of the entire body excluding the head.

When not in the presence of a host, the pearlfishes seemed photonegative and remained in the dark corners of the aquaria. Carapus homei and C. mourlani specimens would lie vertically in a corner with their heads down, or flat on the bottom with their heads in a corner. Encheliophis gracilis specimens curled up in a corner and on the bottom. Carapus parvipinnis specimens would usually lie in a corner with their heads toward the surface. Two *C. parvipinnis* specimens seemed to have damaged air bladders and could lie motionless only at the surface. Occasionally they would swim to the bottom only to float back up upon relaxing.

Locating the host

The behavior involved in locating and entering a host was identical for the four pearlfish species observed; therefore, the behavior is discussed in general terms.

The sight of the pearlfishes seemed poor, possibly due to the layer of skin which covers their eyes. Klingel (1961) found that juvenile and adult *Carapus acus* specimens approached black strips of plastic on a white bottom, if the strips were large. Using different sizes of black plastic caps from specimen jars, the present investigator found that *C. homei* specimens approached the larger caps more readily than those of smaller sizes. Aronson and Mosher (1951) found that blinded pearlfishes could locate and enter their hosts, indicating that other stimuli must be involved besides sight. On four occasions during the present research, single adult specimens of *Carapus homei* and *Encheliophis gracilis* were placed in aquaria containing specimens of *Holothuria argus* in total darkness. In every case, the pearlfish located and entered the holothurian within an hour.

It was observed that if a holothurian was placed in the pearlfish holding tank, the fishes became excited and aware of its presence only after a period of time (up to one minute). On the other hand, when a pearlfish was placed in a tank which had contained a holothurian for some time, even if the holothurian was removed first, the pearlfish became excited immediately and began swimming as if in search of the host. A control, using an aquarium of fresh seawater in which no holothurians had been, proved not to excite the pearlfishes except for a brief period of swimming after introduction. Armold (1957) found that when specimens of *Carapus acus* were separated from their hosts by a glass partition, around which interchange of water could not take place, the fishes paid no attention to the holothurians. When the water was allowed to freely interchange around the partition, the fishes would become excited and attempt to reach the hosts by bumping against the glass. Thus, preliminary excitation of the pearlfishes and recognition of the hosts must depend partly upon a chemical stimulus in the water. Hipeau-Jacquotte (1967) demonstrated that this chemical stimulus, at least in the case of *C. homei* and *Stichopus chloronotus*, was the mucus secreted by the body wall of the holothurian.

During the present research, it was found that specimens of *Encheliophis gracilis* became excited in the presence of fluid extracted from the body wall, respiratory trees, Cuvierian organs, or coelom of *Holothuria argus*. These various tissues were not washed before being masticated and the pearlfishes may have been reacting to a single chemical stimulus such as the coelomic fluid.

Upon awareness of the presence of a host, the juvenile or adult pearlfish would increase its body angle to near vertical and begin swimming. Occasionally a fish would deviate from its path when near the host and swim directly to the host. More often, the pearlfish would not locate the host until, apparently, quite by chance, its swimming path caused near collision with the holothurian.

Entry into a holothurian

Once a pearlfish located a host, it began a ritual which this investigator calls "nose-bumping" (Fig. 61a). During this process the pearlfish remained nearly vertical and by using its pectoral fins and slight movements of its tail, bumped its snout repeatedly against the dorsal surface and sides of the holothurian. It appeared that this behavior may be an aid to host recognition through chemical stimulus, as the integument of the holothurians was rich in mucus. Also, nose-bumping was a means of locating depressions in the host's body, one of which was the anus. As nose-bumping occurred, the pearlfish moved slowly along the length of the holothurian, until reaching the end. During this process, when the fish was at the side of the host, depressions may be discovered under the sole of the holothurian due to the irregular substratum. Upon locating such a depression, the pearlfish would normally force its body under the holothurian, either head-first or with the characteristic tail-first stab, and it normally remained in this position until the holothurian moved. Eventually, the pearlfish reached the holothurian's anterior or posterior end where it remained for some time, nose-bumping in search of the anus. It appeared that the pearlfishes became more excited upon reaching either end of the host, and the nose-bumping became more rapid. Should the pearlfish be at the anterior end, it often attempted to enter the holothurian's mouth. This involved forcing the body under the sole of the holothurian since the mouth was located ventrally. In one instance, an adult _Encheliophis gracilis_ actually entered a _Stichopus chloronotus_ specimen through its mouth. The fish was allowed to remain in the host for several days, and upon dissection, was found in the coelom of the holothurian. Leiner (1960) states that specimens of Carapus acus were unable to enter the mouth of Stichopus regalis, however, Arnold (1953) observed mouth entry of a Carapus acus specimen in Holothuria tubulosa.

Normally, the pearlfish was unable to enter through the mouth of the holothurian, and after a short period of time (one minute or less), the fish began to move toward the posterior end, nose-bumping the dorsal surface or sides of the host. Once at the posterior end of the holothurian, nose-bumping continued as the fish searched for the anus. Occasionally, the pearlfish missed the anus and started nose-bumping back toward the anterior end of the host.

Often, the holothurian's anus was open upon the pearlfish's arrival at the posterior end, and the fish detected the presence of the anus and entered immediately. Usually, however, the anus was closed, although a slight depression remained to which the pearlfish was attracted. In this case, the pearlfish would place its snout near the closed anus, reduce its body angle to nearly horizontal, and remain in this position until the anus opened and entry could be accomplished.

The stimulus of entry was evidently not the sight of the open anus, but rather the presence of the current of water expelled through it during the process of respiration. Hipeau-Jacquotte (1967) has stimulated specimens of Carapus homei to enter a piece of glass tubing through which a current of water was passed. Arnold (1957) suggests that the current of water may contain some chemical which attracts the pearlfish. In any case, the water current did seem to provide the necessary stimulus for entry. Several times during the present research, a pearlfish would hover slightly above or below a holothurian's anus which was wide open, and yet the fish would not sense the presence of the open anus unless it happened to move into the water current. Once the water current was felt, entry behavior was immediate - there was no hesitation of the pearlfish.

Entry into a holothurian occurred head-first, or, tail-first. In making a tail-first entry, the pearlfish pushed its snout into the open anus, whipped its tail around and slid it along the body, past the head, stabbing it through the anus and into the holothurian's cloaca (Fig. 61b). The moment the tail passed the head of the pearlfish, the head was pulled from the anus, thus the position of the fish became completely reversed. At this point, the pearlfish remained still for a short period, with approximately one-fifth of its tail in the host's cloaca and its head slanting upward (Fig. 61c). During this time, it would seem that the pearlfish was searching for the opening of the respiratory trees by moving its tail along the inner wall of the cloaca. Within a few seconds to one minute, the pearlfish curved its body into a slightly spiral form, and using a clockwise, corkscrew motion, began insinuating its body into the host. This was often rapid, with smooth and continuous motion, or it occurred as a series of movements with complete entry taking as long as 15 minutes. The rapidity of tail-first entrance seemed to be determined by the size of the host, the size of the fish, and if the host was reacting to the pearlfish's presence. Entry was often as rapid as five to ten seconds.

As the pearlfish entered, it twisted its body through 360° or more. The movement of the fish into the holothurian is puzzling as there was no apparent fin motion during the process. During entry, a large pearlfish formed a loop with the part of its tail which was in the cloaca of the holothurian. This loop was pushed against the cloaca wall, and the loop traveled anteriorly, the length of the pearlfish's body, by a wave of muscular contraction. There was a sharp lateral motion of the fish's head as it entered the anus, and this motion was a result of the body loop reaching an end. The movement can be compared to that of a snake as its body loops against immovable objects and waves of muscular contraction push the animal forward. In the case of the pearlfish, these waves were reversed and the fish pushed its body backwards, into the holothurian.

After complete entry had been accomplished, the head of the pearlfish often remained visible within the cloaca of the holothurian when the anus was open. Eventually (a few minutes or a few hours) the fish completely disappeared from sight. The average entry time of the pearlfishes was approximately five minutes. It was noted that the juvenile fishes took slightly longer to locate the anus than the adults.

Head-first entry of a pearlfish into a holothurian involved swimming against the respiratory current and into the cloaca in one swift action. Quite often, pearlfishes attempting head-first entry have become momentarily trapped by the holothurian as it rapidly closed its anus. A pearlfish caught in such a manner normally whipped its tail around, stabbed it into the anus, and pulled its head out all in one swift motion. Normal tail-first entry then occurred. A second form of behavior was often expressed by the larger pearlfishes under the same circumstances. The pearlfish, with powerful lateral movements of its body, simply pushed its head through the anus. If the holothurian was small, the fish may push it across the aquarium in this process. On one occasion, a large specimen of *Encheliophis gracilis* got its head stuck in the anus of an average sized *Stichopus chloronotus* and the fish pushed the holothurian around the aquarium with violent movements. Eventually, the fish entered head-first when the holothurian was pushed into a corner.

Arnold (1956) states that *Carapus acus* tenuis larvae would not enter or respond to any holothurian. He suggests that the larvae must metamorphose into juveniles before the typical entry behavior becomes manifest. During the present research, several *C. homei* tenuis larvae were observed to respond to the presence of a holothurian in the typical manner, and in five instances, head-first entry was observed.

Leiner (1960) states that *Carapus acus* tenuis larvae will not live for more than a day outside of a host. During the present research, a single *C. homei* tenuis larva was observed in a small aquarium for a two week period before it was allowed to enter a holothurian. During these two weeks, there seemed to be no change in the body proportions of the larva. After entering a *Stichopus chloronotus* specimen, this same larva was left for 13 days before being removed from the host. At the time of removal, its body length had shortened considerably and the fish was now more juvenile in appearance. These tenuis larvae probably do not metamorphose unless they are within their hosts.

It has been recognized that juvenile specimens of *Carapus acus* normally entered holothurians head-first, whereas adults of the same species normally entered tail-first (Arnold, 1956). This was not necessarily true of the Guam pearlfishes. The majority of the entrances of *C. homei* juveniles were tail-first. Often, *C. homei* adults entered head-first, as did the adults of C. parvipinnis and Encheliophis gracilis. E. gracilis juveniles entered either way, and it seemed to depend upon the size of the fish and the relative size of the holothurian's anus. If the anal opening was large enough to permit head-first entry, and the holothurian did not close its anus during the entry process, then the majority of the pearlfishes observed would enter head-first. Should conditions not be as such, the pearlfishes would corkscrew in, using their tapered bodies as wedges to force open the anal sphincter. Holothuria sp. 2 has an unusually large anal opening and the majority of the pearlfish adults exposed to this holothurian entered head-first. It is possible that Arnold (1956) did not have a series of holothurians to work with and it would be interesting to see if a higher percentage of Carapus acus adults would enter head-first when confronted with larger hosts. During the present investigation, it was found that movement of a pearlfish up the respiratory tree of a holothurian was always tail-first, and the pearlfishes which entered the cloaca head-first must contort their bodies in order to provide for this. Much more movement of the larger fishes within the cloaca of the holothurians was observed for those entering head-first, whereas the fishes entering tail-first moved directly up the respiratory trees. It would seem that tail-first entry is a great deal more efficient than head-first entry.

On several occasions, adult specimens of Encheliophis gracilis actually entered specimens of Stichopus chloronotus which were shorter in length than the pearlfishes. Entry of this nature was normally tail-first. In one instance, the size of the pearlfish must have been too great for the holothurian and host eviseration occurred. The holothurian subsequently died, but the pearlfish remained alive within the decomposing body until it was taken from the aquarium.

Specimens of *Carapus homei* and *Encheliophis gracilis* which had the tips of their tails clipped off had a very difficult time entering a host. Repeated attempts at tail-first entry occurred, but they were usually unsuccessful; and the fishes eventually gained entrance head-first. Klingel (1961) has demonstrated the importance of the lateral line system of *Carapus acus* during tail-first entry. The nerve to the lateral line was severed, and the fish continued to swim normally. Tail-first entry was not possible as the tail could not be brought along side of the body and forced into the anus of the holothurian. The tail was always pushed past the anus and along side of the anus. The pearlfishes which had their tails clipped behaved in a similar manner.

Multiple entries of pearlfishes were observed during the behavior experiments. Up to five juvenile *Carapus homei* specimens would enter a single holothurian, without the host showing ill effects. Upon dissection, the fishes would be found in the respiratory trees and the coelom of the host. No more than two of the larger pearlfishes (*Encheliophis gracilis* and *Carapus parvipinnis*) would enter a single holothurian. Should a third large fish attempt entry, only a small part of its body could be wedged into the cloaca. In one instance, two large *Encheliophis gracilis* specimens and three juvenile *Carapus homei* specimens infested a single *Holothuria argus*. The holothurian did not respond to this multiple entry.

Holothurian reaction

Normally, when the holothurian sensed the presence of a pearlfish, it closed its anus and kept it closed for as long as possible. Sensitive anal papillae became stimulated during the pearlfish's nose-bumping and were responsible for this reaction. The holothurian was able to keep its anus closed for as long as two minutes, but ultimately it opened to expel the oxygen-deficient water from the respiratory trees.

During the pearlfish entrance, the holothurian normally constricted its anal sphincter, thus entry was made difficult. Once inside the cloaca, the pearlfish was often expelled by violent contractions of the holothurian's body. In order to prevent this expulsion, the pearlfish braced itself inside the cloaca and blocked the anus with a loop of its body. Should the fish be completely expelled, it immediately re-entered, head-first, before the holothurian closed its anus.

The holothurian, once infested, also reacted by raising its posterior end and doubling it back over the dorsal anterior region of its body, thus becoming U-shaped. Often, violent movements of the fish were detected within the holothurians' body by the rapid, pulsating movement of the host's body wall.

Holothuria argus and *H.* sp. 2 are thread producers and often extruded their Cuvierian organs in response to the entrance of a pearlfish. Usually, the entry of a small pearlfish did not provoke the release of these organs, but the entry of a large fish might. Nigrelli (1952) reports that an extraction from the Cuvierian organs of a holothurian found in the Bahama Islands was poisonous to *Carapus bermudensis* Jones which infests it. A 1:1000 suspension of this poison (called holothurin) and seawater has been demonstrated to kill an adult *C. bermudensis* in eight minutes. Death occurred by capillary constriction within the gills of the fish. Stephen's (1968) suggests that no pearlfishes are immune to this poison.

During the present research, it has been demonstrated that *Carapus homei*, *C. mourlani*, and *Encheliophis gracilis* were not harmed by the release of the Cuvierian organs and were actually attracted to the sticky threads. Should a specimen of *Holothuria argus* release its Cuvierian organs in the presence of these pearlfishes, the fishes would immediately swim to the threads and even become entangled within them in an attempt to locate and enter the holothurian's anus. Specimens of *Carapus homei* have been isolated in small bowls of seawater and the Cuvierian organs from dissected holothurians ground up and added to the water. The pearlfishes reacted by swimming to the masticated threads and nose-bumping against them. No ill-effects of the pearlfishes were observed, and after 30 minutes or longer, the fishes were returned to the holding tank to be used in future experiments. Often, pearlfishes were found within the coelom of their hosts, and it is within the coelom that these Cuvierian organs lie. Obviously, the fishes were not affected by their presence in such confined quarters. Hipeau-Jacquotte's (1967) work agrees that the Cuvierian organs attract specimens of *C. homei*, and she has noticed no ill-effects of the pearlfishes exposed to the threads.

When a holothurian becomes extremely irritated by the infestation of one or more pearlfishes, it may eviserate its intestine and other internal organs. Eviseration was only observed twice during the course of the behavior experiments and both times when large pearlfishes entered relatively small holothurians. The pearlfishes were not affected by the eviseration, and they remained within the bodies of the holothurians.

In one instance, two specimens of Encheliophis gracilis were infesting a specimen of Actinopyga mauritiana which eviserated (Fig. 62). After eviseration, the heads of the pearlfishes were observed extending from the holothurian's anus, while their tails extended from the host's mouth. The fishes remained in this position even after the eventual death of the host.

Eviseration was induced in a specimen of Holothuria argus by prodding its body with a piece of stiff plastic tubing. Three specimens of Carapus homei remained within the holothurian throughout the process and were only removed by dissection.

It is obvious, then, that the holothurian resists the entrance of a pearlfish. Barnard (1927) and others have indicated that the holothurian is a passive host, but this certainly is not true.

Entry into Culcita novaeguineae

Attempts to observe the entry of Carapus mourlani into its normal host, Culcita novaeguineae, failed. A pearlfish would nose-bump the asteroid, eventually locating the ambulacral groove, and then move up the groove head-first, toward the mouth area. This was possible only when the asteroid had its podia extended through the open ambulacra. Such behavior was observed repeatedly and in each case, the pearlfish reached the asteroid's mouth but did not enter. Upon lifting the asteroid from the bottom, the pearlfish would be found curled-up directly under the mouth. Hipeau-Jacquotte (1967) was also unable to observe the entrance of Carapus homei (the "mourlani" form) in Culcita schmeideliana (Retzuis). She suggests that a sandy bottom is needed in the aquarium so that the pearlfishes may burrow underneath the mouth of the asteroid in order to enter. During the present research, a sandy bottom was provided and the pearlfishes were not observed burrowing. It is probable that the fishes cannot enter *Culcita* unless the asteroid has its stomach everted when feeding. Perhaps in the natural environment, the presence of a pearlfish under the asteroid is a stimulus and invokes the feeding response, thus allowing the pearlfish to enter. The *Culcita* specimens kept in aquaria were never observed to evert their stomachs. They remained almost totally inactive except to extend their podia, moving only occasionally at night.

In one instance, several specimens of *Carapus mourlani* and *Encheliophis gracilis* were placed in an aquarium with a single *Culcita*. Upon lifting the asteroid 24 hours later, it was found that a young adult *Encheliophis gracilis* had partially entered, evidently tail-first, and the pearlfish's head and major part of its body were extending from the asteroid's mouth. The asteroid was turned over so that its oral surface was exposed, and the corkscrew entrance of the pearlfish was observed and recorded on movie film. This infested *Culcita* was isolated and observed for three weeks. During this time, the pearlfish left the asteroid and re-entered twice. Re-entry was not observed as it took place at night (the fish was observed swimming in the aquarium during the day). The asteroid was in an aquarium which had a hard, slate bottom with no sand. After a three week period, the asteroid was dissected and the *Encheliophis gracilis* specimen was retrieved from its coelom. Yosii (1928) reports a pearlfish from the coelom of another asteroid species, *Nardoa semiregularis*, and the figure of the fish resembles *Encheliophis gracilis*.

Doleschall (1861) reports the only other entrance of a pearlfish into a Culcita specimen ever recorded. Upon lifting a specimen of Culcita discoidea he observed an Encheliophis gracilis specimen partially extended from the asteroid's mouth. He also observed the pearlfish withdraw into the stomach of the asteroid.

Long term infestation

A few holothurians which were infested during the course of the behavior observations were segregated until their death or ultimate dissection. The longest term of infestation was eight weeks, and when the host, Stichopus chloronotus finally died, a Carapus homei specimen was retrieved from the host's respiratory tree in an apparently healthy condition.

Those pearlfishes kept from their hosts remained alive from a few days to several weeks. A Carapus parvipinnis specimen was kept alive for seven weeks outside of a host. It was found that Encheliophis gracilis specimens were highly susceptible to fungus disease when left in aquaria apart from their hosts. Many of these pearlfishes died within two days of getting the parasite on their median fins. E. gracilis specimens left within their hosts never suffered from the disease. Diseased specimens allowed to enter their hosts were found improved or completely rid of the parasite after a few days of infestation.

Pearlfishes kept apart from their hosts for long periods (a week or more) did not react as quickly in response to a host as did freshly liberated fishes. In the case of the Carapus parvipinnis specimen kept from a host for seven weeks, the fish was repeatedly given the opportunity to enter after four weeks of isolation. Even when offered its preferred host, Thelenota ananas, it would nose-bump the holothurian but did not locate the anus. Eventually, it would stop and swim back to a corner of the aquarium.

Host preference experiments

Host preference experiments were conducted using each of the Guam pearlfish species (with the exception of *Encheliophis vermicularis*) and the host species discussed and figured in Chapter II. The following is a summary of the results of these experiments.

Carapus homei

Carapus homei tenuis larvae entered both *Holothuria argus* and *Stichopus chloronotus* readily, but showed no preference between the two hosts. When given a choice between *S. chloronotus* and a holothurian other than *Holothuria argus*, the larvae chose the former in every instance.

Arnold (1957) found that *Carapus acus* juveniles were attracted more often to larger holothurians than to smaller ones. During the present research, attempts were made to determine if *C. homei* juveniles were also selective in this manner. Pairs of *Stichopus chloronotus* were used, one member of each pair being quite small, and the second member of average size. In eight trials, the fishes located and entered the larger holothurian six times.

When pairs of holothurians consisted of *Stichopus chloronotus* and *Holothuria argus* (both of which are normally infested by *Carapus homei*), both juveniles and adults showed definite preference for *Stichopus chloronotus*. When *Holothuria argus* was used in combination with any other holothurian species, *H. argus* became the preferred host.

Evidence already presented suggests that *Carapus homei* is attracted to black objects. When the holothurian pairs consisted of *Stichopus chloronotus* and *Holothuria atra*, both black and both of the same size, the pearlfishes often swam to *H. atra* first. Entry, however, was never attempted and the fishes would eventually locate *Stichopus chloronotus* and enter.

When the pairs consisted of *Holothuria atra* (black), and *H. argus* (brown), the former was always visited first, but eventually *H. argus* was entered. It appears that sight may play an important part in host location, if the holothurian is dark in color and fairly large, but the chemical stimulus from the holothurian excites the pearlfishes for entry.

Both *Carapus homei* juveniles and adults would enter *Holothuria atra* when offered no other choice. In each instance (four trials), the pearlfish would leave the host within a few hours and not re-infest the holothurian. Evidently, internal conditions of *H. atra* were not suitable for the pearlfishes. There was no physical barrier which prevented entry.

When given a choice between *Stichopus chloronotus* and *S. variegatus*, the former was always chosen by *Carapus homei* specimens. Entry into *Stichopus variegatus* was observed when no other choice was possible.

Carapus homei adults were attracted to specimens of *Actinopyga mauritiana* and entry was attempted. The pearlfishes were able to insert the tips of their tails into the holothurian's anus, but they were unable to wedge their bodies past the anal teeth of the holothurian, even with the anus partially open.

Specimens of *Thelenota ananas* were never entered by *Carapus homei*. The anus of this holothurian is surrounded by large, fleshy warts which partially cover it and C. homei specimens, although attracted to the holothurian, were never able to locate its anus.

When given a choice between Culcita and a holothurian, Carapus homei juveniles and adults would always choose the latter. When offered only Culcita, the pearlfish's behavior was the same as Carapus mourlani.

Carapus mourlani

When given a choice between Culcita and Stichopus chloronotus, Carapus mourlani investigated the asteroid first in nine out of eleven trials. After discovering a Culcita, only once did a Carapus mourlani specimen leave the asteroid and enter a holothurian. When confronted with various holothurians only, C. mourlani showed the same preferences as C. homei.

The fact that, in nine out of eleven trials, Carapus mourlani chose Culcita over Stichopus chloronotus, and Carapus homei disregarded Culcita when given a choice between it and a holothurian, suggests a basic behavioral difference between the two pearlfish species. This difference may be the result of previous infestation experience, or, it may involve specific behavioral differences between the two. Behavior is often accepted as a good taxonomic character, and perhaps these two pearlfishes may be differentiated into species on the basis of their behavior, i.e., host selection. Certainly the two would be reproductively isolated as a result of this host preference, which would make them good species regardless of their anatomical similarities.

Carapus parvipinnis

Host preference experiments between Thelenota ananas and the other holothurian species indicate that the former is preferred by

Carapus parvipinnis adults. Whenever Thelenota was present in a choice experiment, this pearlfish would swim directly to the holothurian. This attraction could be due to size alone, as Thelenota is much larger than any of the other holothurian species. When confronted with other holothurians, Carapus parvipinnis specimens entered only Holothuria argus and H. sp. 2, both of which have a large anus. Actinopyga mauritiana and Stichopus chloronotus attracted Carapus parvipinnis specimens, but entry was never attempted as the pearlfishes could not locate the anus of either. C. parvipinnis could, however, locate the partially hidden anus of Thelenota.

Culcita specimens did not appear to excite Carapus parvipinnis. Only once did a fish deviate from its swimming path in order to inspect an asteroid.

Encheliophis gracilis

Encheliophis gracilis juveniles showed a definite preference for Stichopus chloronotus, Holothuria argus, and Thelenota ananas, but no priority between the three could be determined. The juveniles were also attracted to Stichopus variegatus and Holothuria atra. Two juveniles which entered specimens of H. atra did not remain infested for more than an overnight period. Attempted entry into Actinopyga mauritiana failed repeatedly, and the pearlfishes would eventually find a depression under the sole of the holothurian, corkscrew in, and remain there.

On one occasion, a live map cowrie, Cypraea mappa Linnaeus, was placed in an aquarium with a juvenile Encheliophis gracilis. The pearlfish proceeded to enter the mantle cavity of the cowrie by corkscrewing tail-first through the shell aperture. The pearlfish's head remained barely visible within the aperture, and it could be seen only when the cowrie was clinging to the glass of the aquarium. The pearlfish left and re-entered the cowrie on several occasions thereafter.

Encheliophis gracilis adults showed a definite preference for *Holothuria argus* and *H.* sp. 2. Entry also occurred in *Stichopus chloronotus* and *Thelenota ananas*. The adults were attracted to *Actinopyga mauritiana* but entry was never observed, although a male and female *Encheliophis gracilis* pair did infest a single specimen.

As previously mentioned, *Culcita* specimens attracted *Encheliophis gracilis* adults, and one entrance was partially observed. Full-sized *E. gracilis* adults would often push a large *Culcita* around the aquarium in an apparent effort to get underneath the asteroid. The fishes were too large to wedge their bodies up through the ambulacra of the asteroid.

Discussion

The behavior of the Guam pearlfishes seems similar to that of *Carapus acus*, as described by Arnold (1953, 1956, and 1957). Arnold, however, found definite behavioral patterns in the locomotion, location of a host, and in entry with each life form of *C. acus*. These behavioral differences were not as pronounced in the life forms of the Guam pearlfishes. *C. homei* tenuis larvae did exhibit the tenuis locomotion, but the larvae were able to live outside of their hosts for a much longer period than Arnold suggested possible. Also, *C. homei* tenuis larvae repeatedly entered their hosts, whereas the *C. acus* larvae failed to recognize holothurians.

The juveniles and adults of the Guam pearlfishes swam in the typical manner as described for *Carapus acus*, but no difference between the two life forms were observed as far as host location is concerned. Arnold found that *C. acus* juveniles located hosts by sight, whereas the adults were unable to locate objects visually and found their hosts by accident. The Guam pearlfishes, upon becoming aware of the presence of a holothurian, seemed to actually locate the host by sight (if the host was large and dark-colored) regardless of the life form. Smaller and lighter colored holothurians seemed to be located by accident, again regardless of life form.

Finally, Arnold describes tail-first entry as a specific behavioral pattern of the adult *Carapus acus*, and he found that the method of entry did not bear any relation to the relative dimensions of the fishes and holothurians. The opposite was observed with the Guam pearlfishes. The method of entry seemed to depend entirely upon the relative sizes of the pearlfishes and the holothurians. Adults and juveniles were commonly observed to enter large holothurians head-first, and smaller holothurians tail-first.

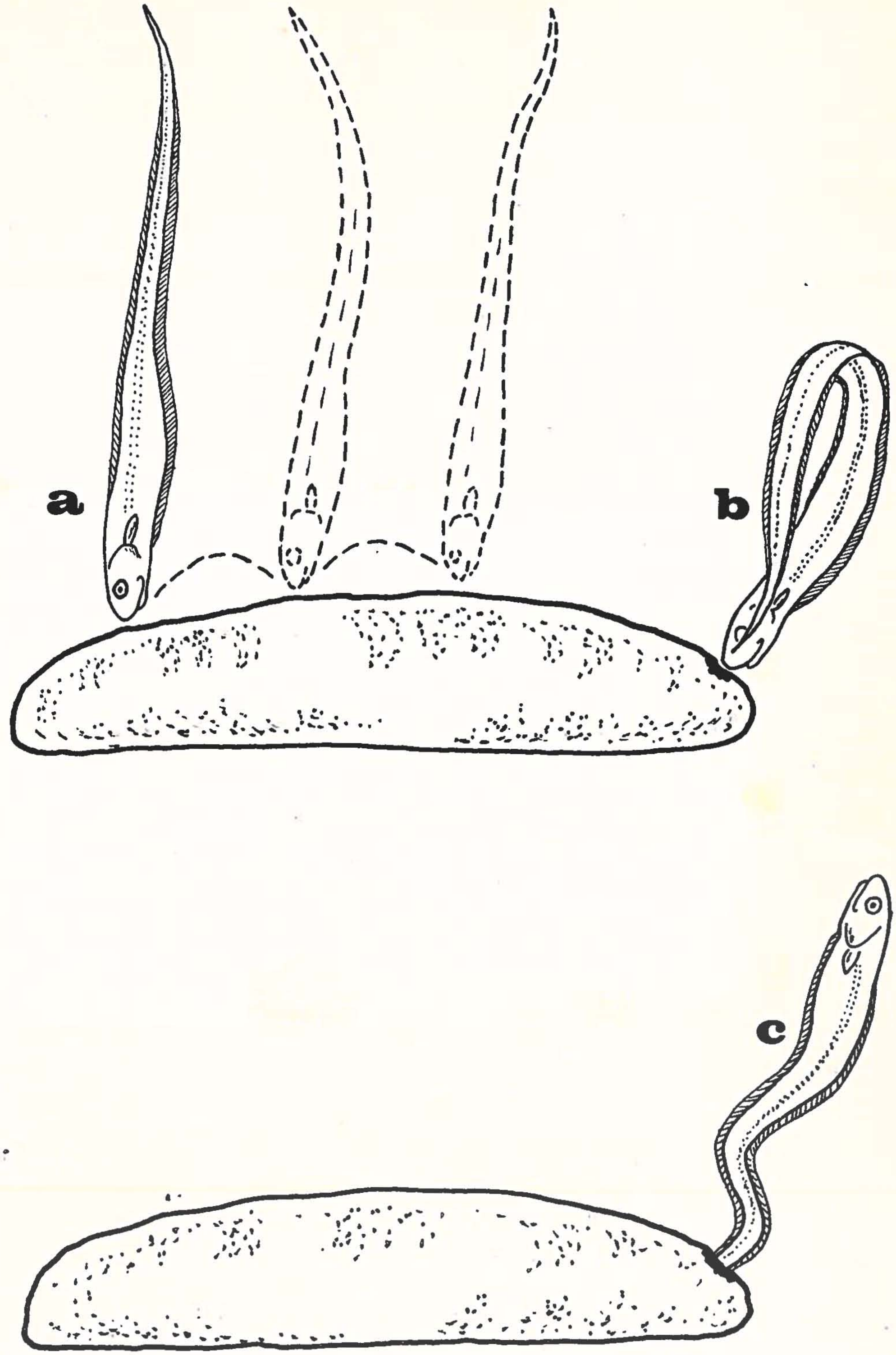
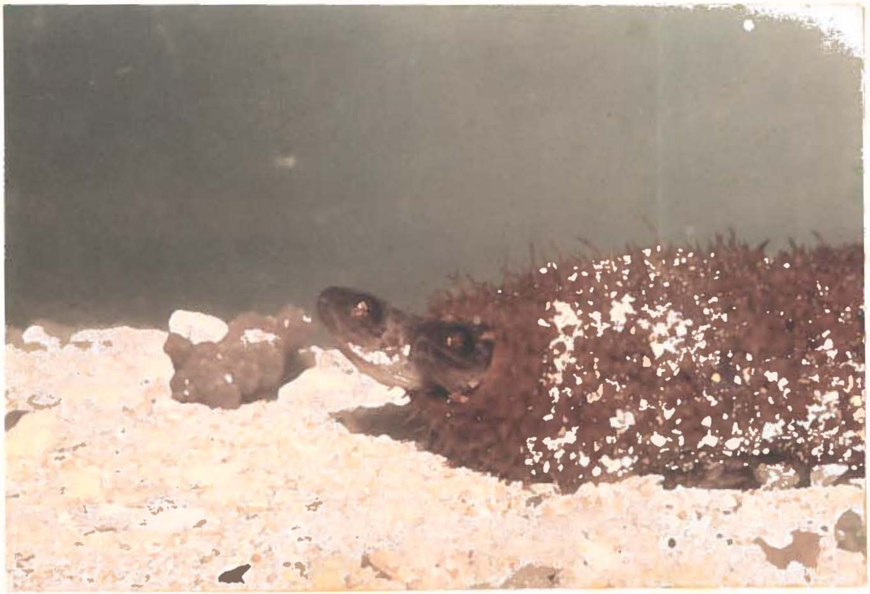


Fig. 61. Entry behavior of the Guam pearlfishes. a, nose-bumping of the pearlfish as a means of host recognition and location of the anus; b, the start of a typical pearlfish tail-first entry through the anus of a holothurian; and c, position of the pearlfish immediately after the tail is inserted through the anus.

Fig. 62. Male and female *Encheliophis gracilis* pair infesting an eviserated specimen of *Actinopyga mauritiana*. Note the anal teeth of the holothurian.



Relationship of the Pearlfish and the Host

Hypotheses

There is little agreement in the carapid literature as to the exact relationship of the pearlfish and its host. Anderson (1859), Doleschall (1861), Emery (1880), Mukerji (1932), and Hyman (1955) describe the pearlfish as a simple inquiline, and the holothurian as a passive host which provides shelter for the pearlfish, and which is not harmed, but receives nothing in return.

More recent researchers such as Leiner (1960), Strasburg (1961), Hipeau-Jacquotte (1967), and Stephens (1968) describe the pearlfish as an obligate parasite, feeding off the internal organs of its host.

Arnold (1956) believes that only the pearlfish tenuis larva is an obligate parasite, and the juvenile and adult (of Carapus acus) is a simple inquiline which may, at certain times, parasitize the host.

It appears to the present researcher that three hypotheses concerning this relationship can be postulated: that the pearlfish is 1) a simple inquiline which resides in the cloaca, intestine, or respiratory trees of its host, and which leaves its host to feed, as a predator, returning later for shelter; 2) an obligate parasite which originally breaks into the coelom of the host through the cloaca, intestine, or respiratory trees, and remains indefinitely, feeding upon the host's internal organs; and 3) an internal predator which enters a host, breaks into the coelom, feeds and shelters, and then leaves to prey upon another host.

Undoubtedly, there are differences in the mode of life of the different pearlfishes, and, possibly, each of the hypotheses may be applicable. Also, as Arnold (1956) suggests, the different life forms of a pearlfish species may differ in their dependence upon a host, and the distance to which they penetrate the host's body.

Evidence for the mode of life of the Guam pearlfish species has been gathered through a determination of the location of the pearlfishes within the holothurians by dissection and use of radiographs, and by stomach analyses of the pearlfishes. Also, a comparison of the gonadal mass of a holothurian species infested by two species of pearlfishes has provided evidence as to the relationship of these pearlfishes with their hosts.

Location of pearlfishes within the holothurians

Holothurians were dissected from mouth to anus along the ventral surface (sole). Figure 63 depicts the internal anatomy of a typical holothurian as viewed when dissected in the above manner. The intestine and respiratory trees remained intact, but the cloaca was normally cut open in the process. Upon dissection, the location of the pearlfish, if present, was recorded. Pearlfishes found lodged in a respiratory tree were often preserved *in situ*, with the membranous tree enveloping the specimen (Fig. 64). Occasionally, holothurians were frozen before dissection. This was done with the hope of determining the exact position of the pearlfish within the host. When a holothurian is cut open, the violent movements of the fish make it difficult to determine its original location (Smith, C. L., 1964).

As collections were made, the holothurians were placed in plastic bags with seawater. Often, the pearlfishes abandoned their hosts for the water within the bag. In these instances, the fishes would be listed as being found "free" from the host. In all probability, the pearlfishes found free were originally inhabiting the respiratory trees of the host, as no damaged organs in these holothurians were found, indicating that there was no breakthrough from the coelom.

It was determined by dissecting 214 infested holothurians that the pearlfishes were located in either the body cavity (coelom), or respiratory trees of the hosts (Table X). Approximately 47 percent of the pearlfishes were found free, indicating a much higher percentage of respiratory tree infestation, particularly in the case of Holothuria argus and Carapus homei.

A large percentage of the Carapus homei specimens collected from Stichopus chloronotus, including the six tenuis larvae, were found in the coelom of the hosts. The position of the fish within the coelom was normally posterior, with the head of the fish facing the cloaca of the holothurian.

Of the specimens of Carapus homei found in the respiratory trees of Stichopus chloronotus, twenty-three were juveniles and three were adults. The heads of these pearlfishes were always found facing the cloaca of the holothurians, with the tails of the fishes extending anteriorly up the respiratory trees. Mukerji (1932) states that pearlfishes reside only in the cloaca and intestine of the holothurian. He found a single Carapus homei specimen in the intestine of Actinopyga sp. Bonham (1960) found a single specimen of Carapus homei in the body cavity of Stichopus chloronotus in the Marshall Islands. Hipeau-Jacquotte (1967) found the majority of her Carapus homei specimens in the body cavity of the holothurian hosts. Smith, C. L., (1964), in his work on

Guam, also found the majority of *C. homei* spetimens in the coelom of *Stichopus chloronotus*.

Although *Encheliophis gracilis* is often reported from holothurians, the area of infestation within the host is seldom indicated (nor is the species of the holothurian). Palmer (1958) reports *E. gracilis* from the body cavity of a holothurian in the Maldive Islands.

Five of the *Encheliophis gracilis* pairs were found in the respiratory trees of *Holothuria argus*. In each case, the two members of the pair were found together in the same branch of the respiratory tree. Needless to say, the respiratory tree of this holothurian must be extremely elastic to accomodate two adult *Encheliophis gracilis* specimens. The fishes were side-by-side within the tree; however, the head of one pair member was slightly forward of the other.

It was found that under experimental conditions, *Encheliophis gracilis* specimens would occasionally enter the intestine of a holothurian, particularly if the holothurian was already infested with another pearlfish. In one instance, an *E. gracilis* adult was found to have the anterior half of its body in the host's intestine, with its head in the cloaca. The fish's tail was stuck through a break in the intestinal wall, and the posterior half of its body was found in the coelom of the host.

The majority of the *Carapus parvipinnis* specimens were found in the coelom of *Thelenota ananas*. The two specimens found in the respiratory trees were wedged in so tightly that forward movement of the fishes within the trees seemed impossible. When a holothurian was taken from water, it would close its anus and retain whatever water was present within the respiratory system. Eventually, however, the holothurian relaxed and the water was expelled. Judging from the amount of water which could be expelled, the respiratory trees of the holothurian must be tremendously expanded when full, thus allowing free movement of a pearlfish even as large as *C. parvipinnis*. When the water was expelled, the respiratory trees deflated and trapped the pearlfish in position until inflated again by the rhythmic respiration of the holothurian.

Efforts were made to determine the area of breakthrough of the pearlfish into the body cavity of the holothurian. Pearlfishes were permitted entry, and the holothurians were dissected shortly thereafter. It was found that very few of the pearlfishes entered the coelom of the hosts under laboratory conditions. Of those that did so, it was not possible to find the area of breakthrough. Leiner (1960) states that *Carapus acus* definitely pierces the respiratory tree of its host in order to enter the coelom. The present investigator has found that the respiratory trees of the Guam holothurians are finely branched structures, and no breaks were observed in them. The respiratory trees were inflated with water, but no leakage was observed. Leiner also states that *C. acus* specimens cannot break through the intestine of their hosts. As previously mentioned, *Encheliophis gracilis* is evidently capable of doing just this. During the present investigation, *E. gracilis* specimens have been repeatedly observed to chew through thick plastic bags used to collect the holothurians. The pearlfishes would leave their hosts, locate a corner of the bag, and then chew a small hole through the plastic. The fishes would then bring their tails around, inserting them through the holes, and finally wedge their bodies through in a corkscrew motion. Certainly then, *E. gracilis* specimens could break through the cloaca, intestine, or respiratory trees of a holothurian in order to enter the coelom, as all of these organs are composed of delicate tissue. This, of course, implies serious damage to these organs, particularly if the fish is entering and leaving the coelom often. The effects of this damage on the entire holothurian population may be slight, due to the large number of holothurians available for pearlfish infestation on the Guam reefs.

Location of the pearlfish within *Culcita novaeguineae*

A special method of dissection was necessary for specimens of *Culcita novaeguineae* which had extremely tough, muscular body walls. A coarse-cut pruning saw was used to halve the asteroids. The cut was made vertically, approximately eight centimeters from the center of the organism in order to leave the stomach intact. With this precaution, all of the specimens of *Carapus mourlani*, and the single specimen of *Encheliophis gracilis* were found in the coelom of the asteroids. Mukerji (1932) suggests that the pearlfishes reside in the stomach of *Culcita* specimens and that the fishes are liberated into the coelom at the time of dissection. There is no doubt that this is not the case. The pearlfishes must pierce the stomach of the asteroids in order to enter the coelom. *Culcita* specimens have no respiratory trees.

Radiographs and fluoroscopy

Through the use of X-rays, an attempt was made to determine 1) the initial area of infestation of pearlfishes in their holothurian hosts; and 2) the area and method of breakthrough of the pearlfishes into the body cavities of the hosts.

Two aquaria were set up in the X-ray department of the Guam Memorial Hospital. A rotating complement of pearlfishes and holothurians was maintained over a three week period. The aquaria were on movable carts so that they could be pushed into the X-ray rooms.

Several series of radiographs were made of holothurians infested with pearlfishes. Holothurians in which pearlfish entry had just occurred were repeatedly X-rayed over a period of five to thirty minutes. For each X-ray series, a holothurian was taken out of water and placed on a stiff plastic sheet on the X-ray table. The average time out of water for each X-ray was 20 seconds. Attempts to X-ray a holothurian in a container of water were unsuccessful. The water proved to be of the same radiographic density as the holothurian's body, thus no differentiation of the two could be achieved.

Before a pearlfish was allowed to enter a holothurian to be X-rayed, the fish was placed in a 50 percent solution of Hypaque (radiopaque sodium) and seawater. This solution did not seem to harm the fish as it diffused through the gills, entered the blood and concentrated in the tissues. The holothurian was treated in a similar manner, being allowed to respire in a Hypaque solution for up to ten minutes before the entry of the pearlfish. The Hypaque solution allowed X-ray differentiation of the various organs of the holothurian (cloaca, respiratory trees, intestine, and coelom), as well as the position of the fish within the holothurian. Several other techniques were tried with other radiopaque substances (barium, and iodine) but the use of the Hypaque proved the most successful. Barium was so dense, it masked the presence of the fish within the holothurian, and the holothurian also reacted to the barium by eviserating. Iodine proved fatal to the pearlfishes.

Medical film was used in cardboard film holders during the X-ray process. Each holder was positioned on the X-ray table under the plastic sheet upon which the holothurian rested. The settings on the X-ray machine varied with the size and density of the holothurian, and the following represent mean values:

Milliamps: 200 ma
Exposure time: 1/20th second
Kilovolt Peak: 50 KV
X-ray tube to film distance: 64 centimeters

It was hoped, through the use of the fluoroscope, to actually observe and photograph the movements of a pearlfish within a holothurian. Several attempts were made to do this, employing a variety of techniques using various radiopaque substances and X-ray values. None were successful. The holothurians were so easily penetrated by the X-rays that they were not differentiated on the bright fluoroscopic screen. With the use of barium sulfate, some internal organs of the holothurians could be seen (Figs. 65 and 66), but because of the great density of the barium, the presence of the pearlfish within the holothurian was masked, as it was in the radiographic process, and the value of the fluoroscopy was negated.

Attempts to X-ray several specimens of *Culcita*, particularly the specimen infested by the adult *Encheliophis gracilis*, were unsuccessful. It was hoped that the position of the fish in the asteroid could be determined on X-ray film. The pearlfishes, being the same radiographic density as the coelomic fluid of Culcita, were not differentiated (Fig. 67). Hypaque could not be used in this instance as the pearlfishes were already within the asteroids.

Contact prints of several radiographs were made, using standard darkroom techniques (Figs. 65 through 73).

The results of this radiographic process show that the pearlfishes generally move up the respiratory trees upon entering a holothurian. This is always a tail-first process, and the fish may move as far anteriorly as possible (Fig. 68), indicating that breakthrough into the coelom may occur at the terminal ends of these trees in some cases. Occasionally, the pearlfish will move just far enough anteriorly to permit it to keep its head in the cloaca of the holothurian (Fig. 69). This may not be the natural position of the pearlfish as it would be difficult for the sandy waste material of the holothurian to pass through the cloaca if it were. The fish may assume this position as a response to oxygen deficiency. Two or more pearlfishes may share the same respiratory tree (Fig. 69), or both of the trees may be occupied (Fig. 70).

It has been determined that the pearlfishes will move tail-first up the intestine of a holothurian (Fig. 71), but this is probably the result of a stress condition during entry, since no pearlfishes were found in the intestines of holothurians which were infested in the natural environment.

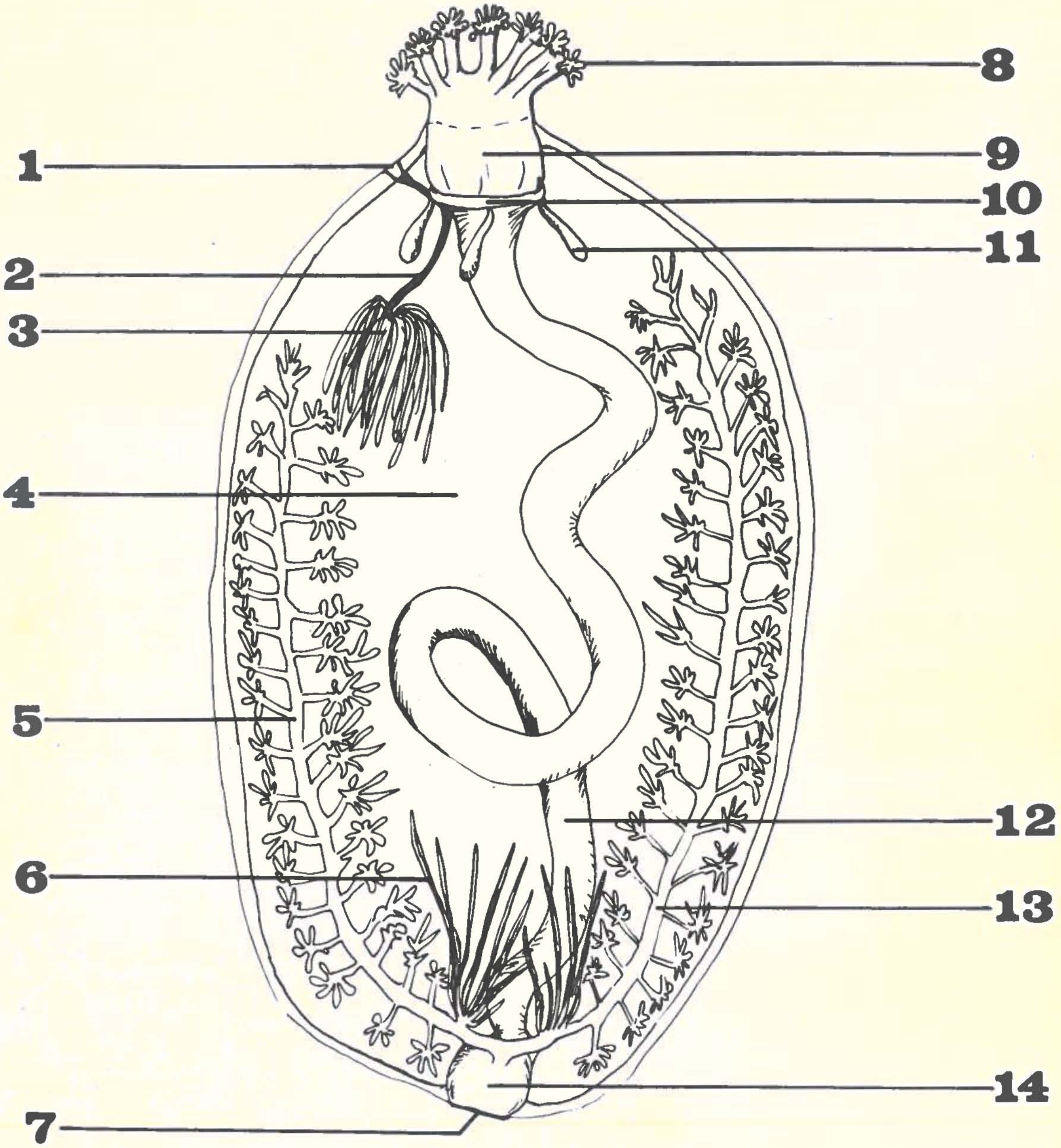
Although the radiograph is difficult to interpret, Figure 72 shows a pearlfish with the major part of its body in the coelom of the holothurian. Only the fish's head is outside of the coelom, in the cloacal area. It appears that the fish has broken through the wall of the cloaca, either in the process of entering or leaving the coelom.

TABLE X. LOCATION OF THE PEARLFISHES IN THE HOLOTHURIAN BODY

PEARLFISH	N	*Stichopus chloronotus*			*Holothuria argus*			*Thelenota ananas*		
		coelom	respir. tree	free*	coelom	respir. tree	free	coelom	respir. tree	free
Carapus homei	184	92	26	10	9	8	39	--	--	--
Carapus parvipinnis	46	--	--	--	--	--	--	29	2	15
Encheliophis gracilis	84	5	--	1	13	22	28	7	--	8
TOTAL	214	97	26	11	22	30	67	36	2	23
Percentage within each holothurian species	--	72	19	9	19	25	56	59	3	38

*Pearlfishes which abandoned their hosts for the water in the collection bags.

Fig. 63. A diagrammatic view (after Hyman, 1955) of a typical holothurian belonging to the order Aspidochirota. 1, gonopore; 2, gonoduct; 3, gonadal tuft; 4, coelom; 5, left respiratory tree; 6, Cuvierian organs; 7, anus; 8, buccal tentacles; 9, pharyngeal bulb; 10, ring canal; 11, polian vesicle; 12, intestine; 13, right respiratory tree; and 14, cloaca.


8
1
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11
2
3
4
5
12
6
13
14
7

Fig. 64. *Carapus parvipinnis* (white color phase) lodged within the membranous respiratory tree of *Thelenota ananas*. The tissue of the main branch of the respiratory tree is transparent, and forms a tight sleeve around the pearlfish.

Fig. 65. Radiograph of the respiratory organs of *Stichopus variegatus* using barium sulfate as a radiopaque substance. a, mouth; b, parasitic gastropod; c, ring canal; d, left respiratory tree; e, beginning of the right respiratory tree; f, cloaca; and g, anus.

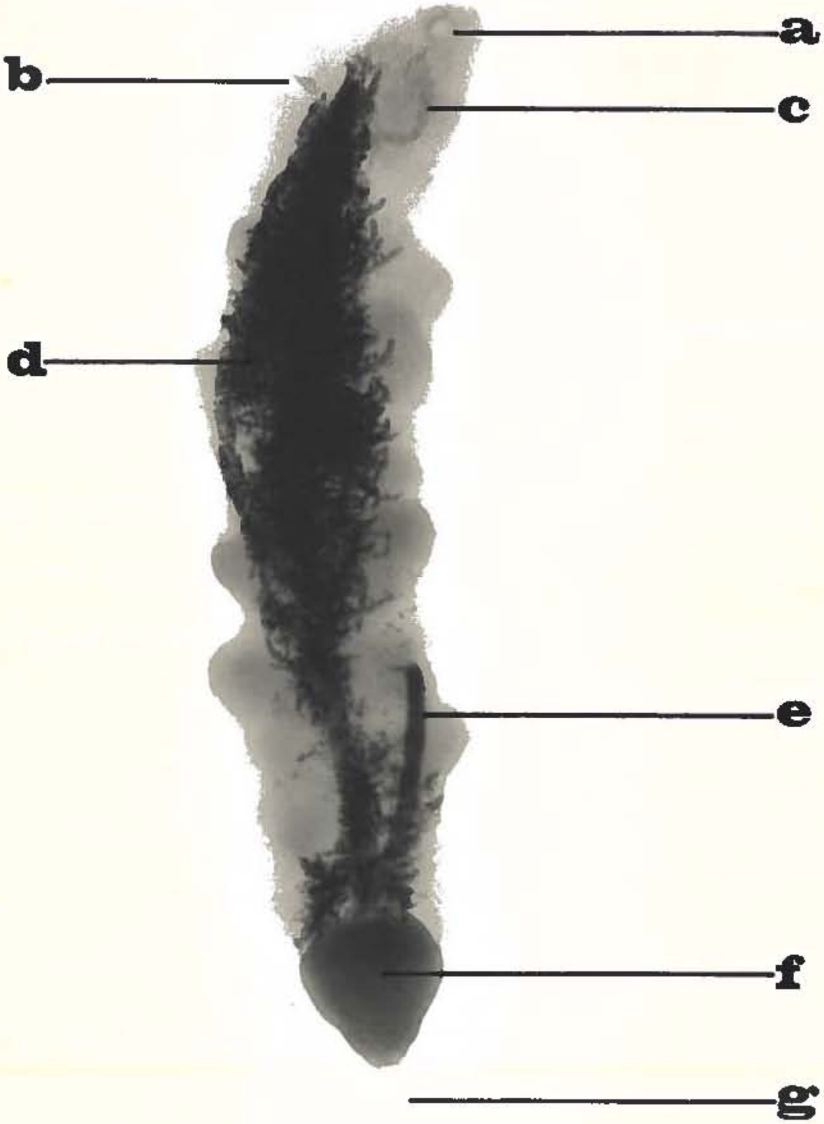
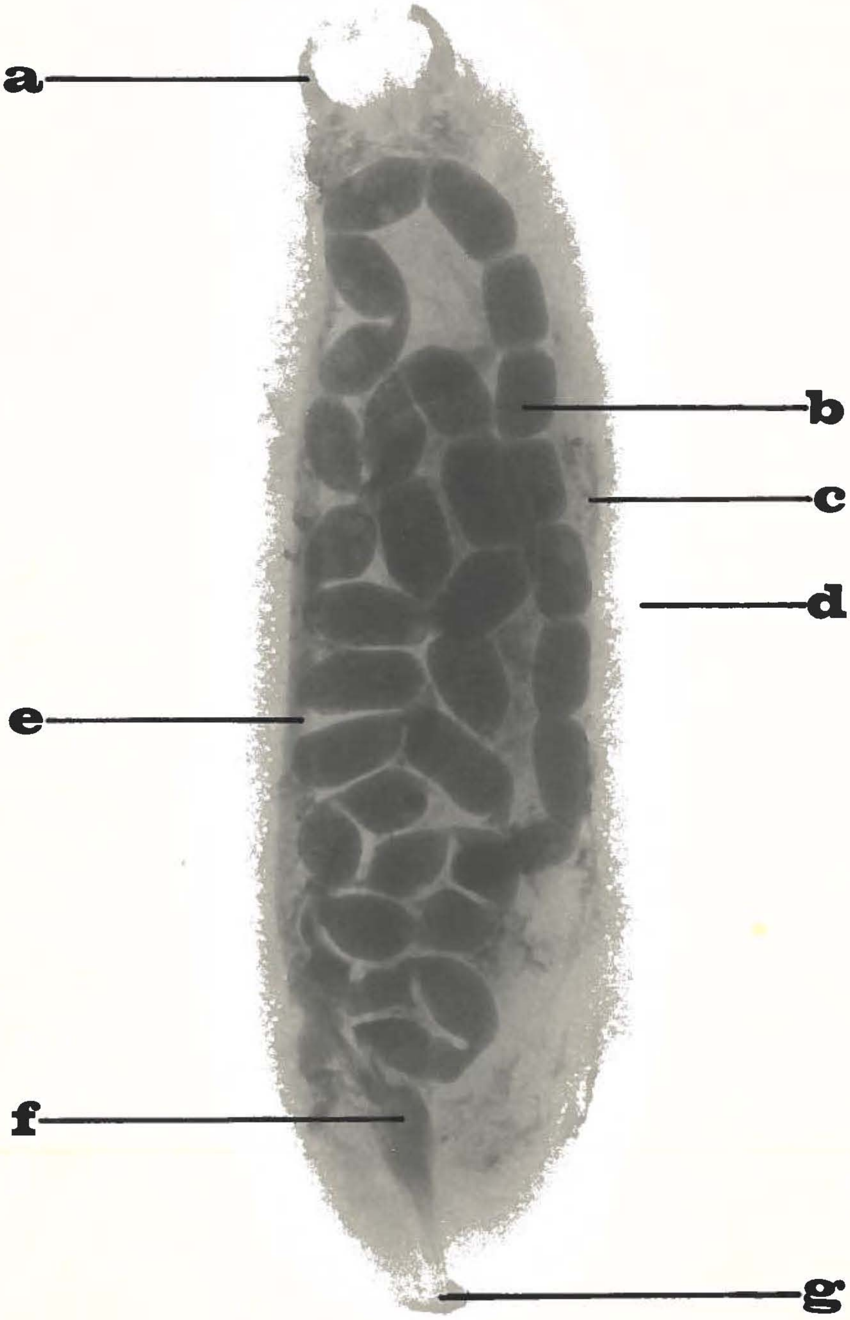

a
b
c
d
e
f
g

Fig. 66. Radiograph of the internal organs of Holothuria argus using barium sulfate as a radiopaque substance. a, ring canal; b, intestine; c, right respiratory tree; d, podia on sole; e, left respiratory tree; f, cloaca; and g, anus.


a
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e
f
g

Fig. 67. Radiograph of Culcita novaeguineae, infested by an adult specimen of Encheliophis gracilis (pearlfish not seen). a, ambulacral groove; b, muscle striations of the inner body wall; and c, mouth.

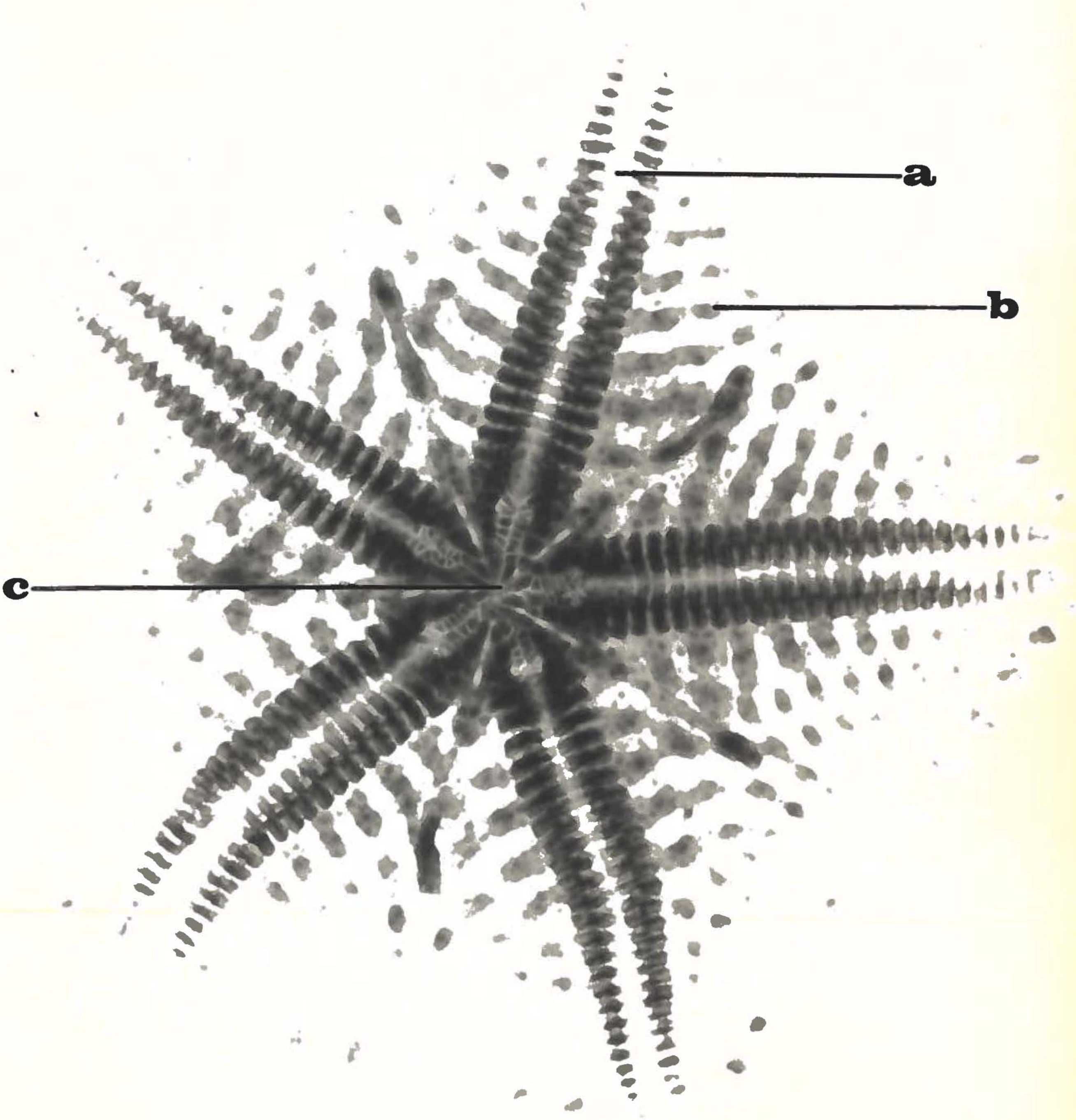

a
b
c

Fig. 68. Radiograph of an infested specimen of *Stichopus chloronotus*. a, mouth; b, parasitic gastropod; c, ring canal; d, parasitic gastropod; e, juvenile specimen of *Carapus homei* in a respiratory tree of the host; f, intestine; g, cloaca; and h, anus.

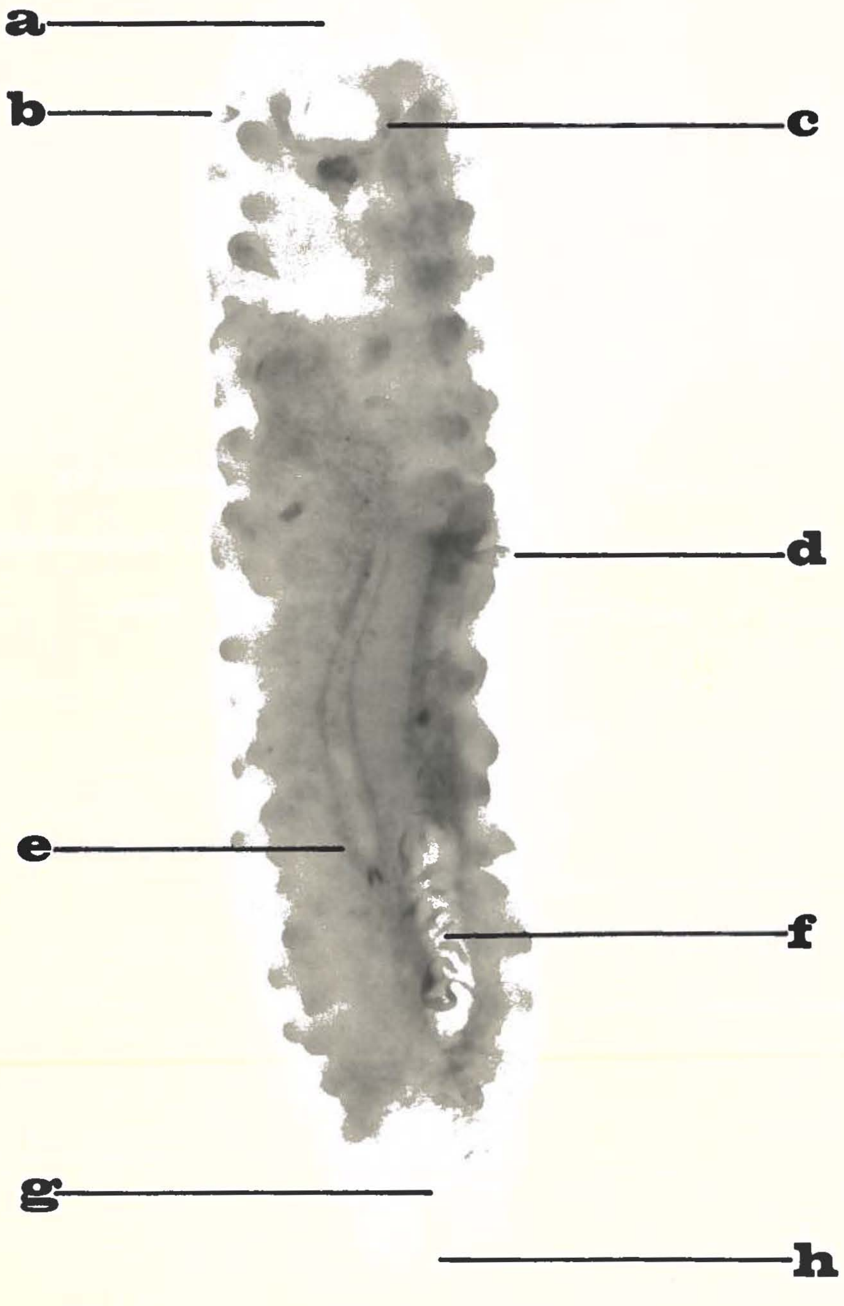

a
b
c
d
e
f
g
h

Fig. 69. Radiograph of Stichopus variegatus infested by two specimens of Carapus homei inhabiting a single respiratory tree. a, ring canal; b, pearlfish; c, pearlfish; and d, anus.

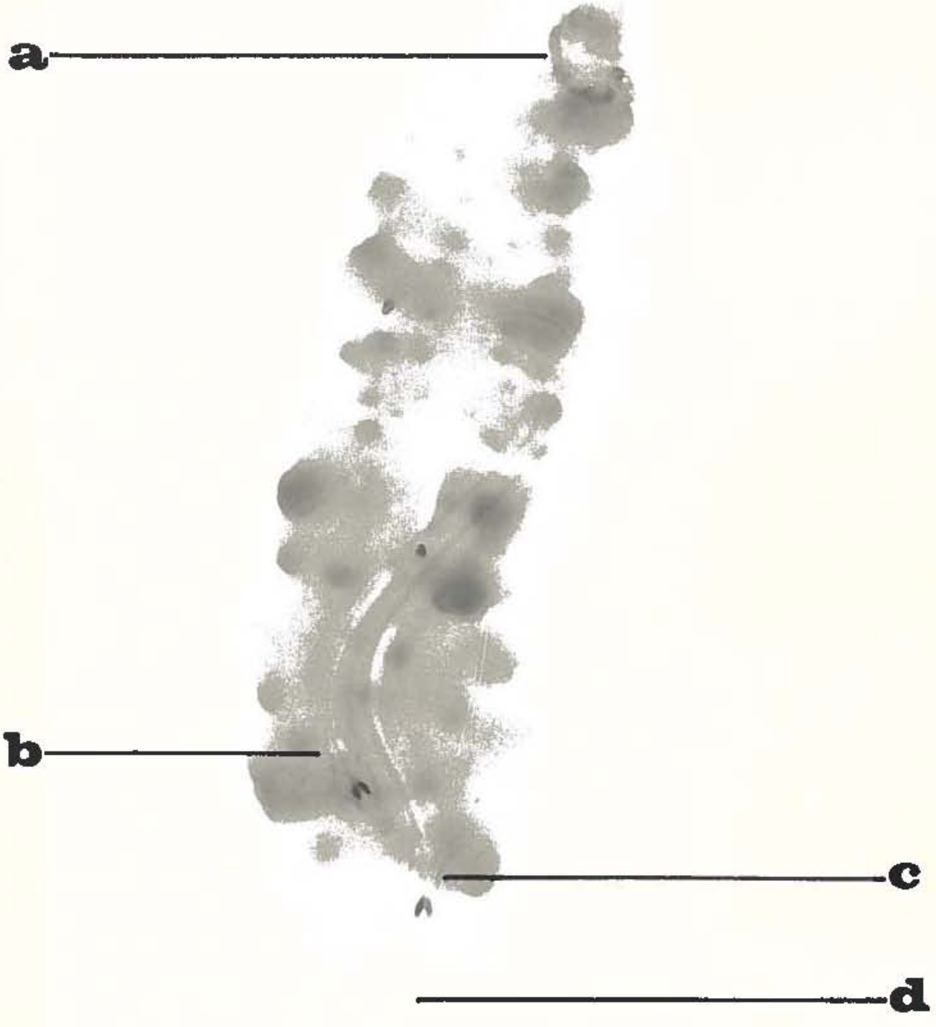
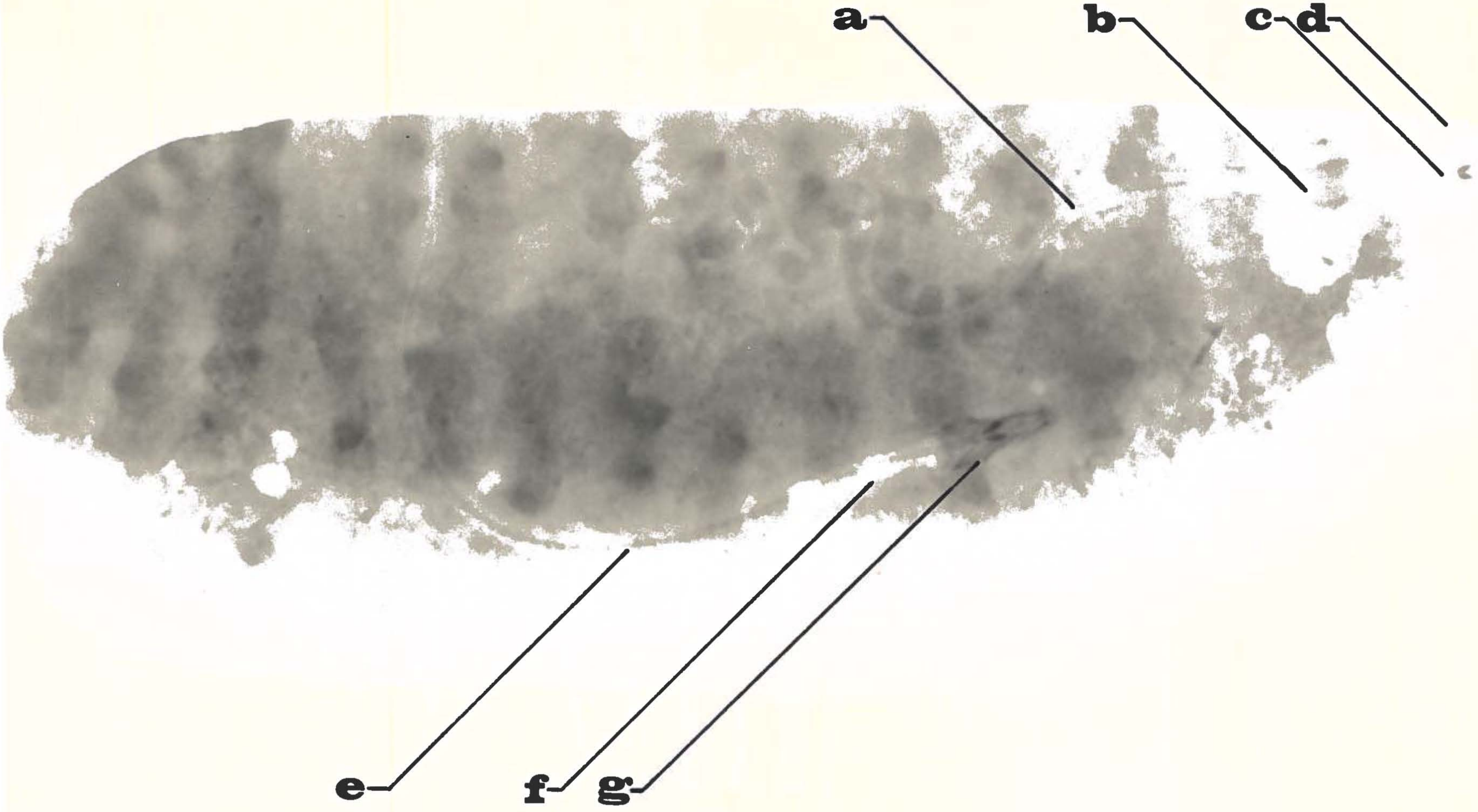

a
b
c
d

Fig. 70. Radiograph of *Thelenota ananas* (posterior only) infested by three pearlfishes. a, tail of juvenile *Encheliophis gracilis* in right respiratory tree; b, abdomen of juvenile *E. gracilis*; c, head of juvenile *E. gracilis*; d, head of a *Carapus homei* specimen sharing the right respiratory tree; e, tail of an adult *Encheliophis gracilis* specimen inhabiting the left respiratory tree; f, abdomen of adult *E. gracilis*; and g, head of adult *E. gracilis* (with gills).


a
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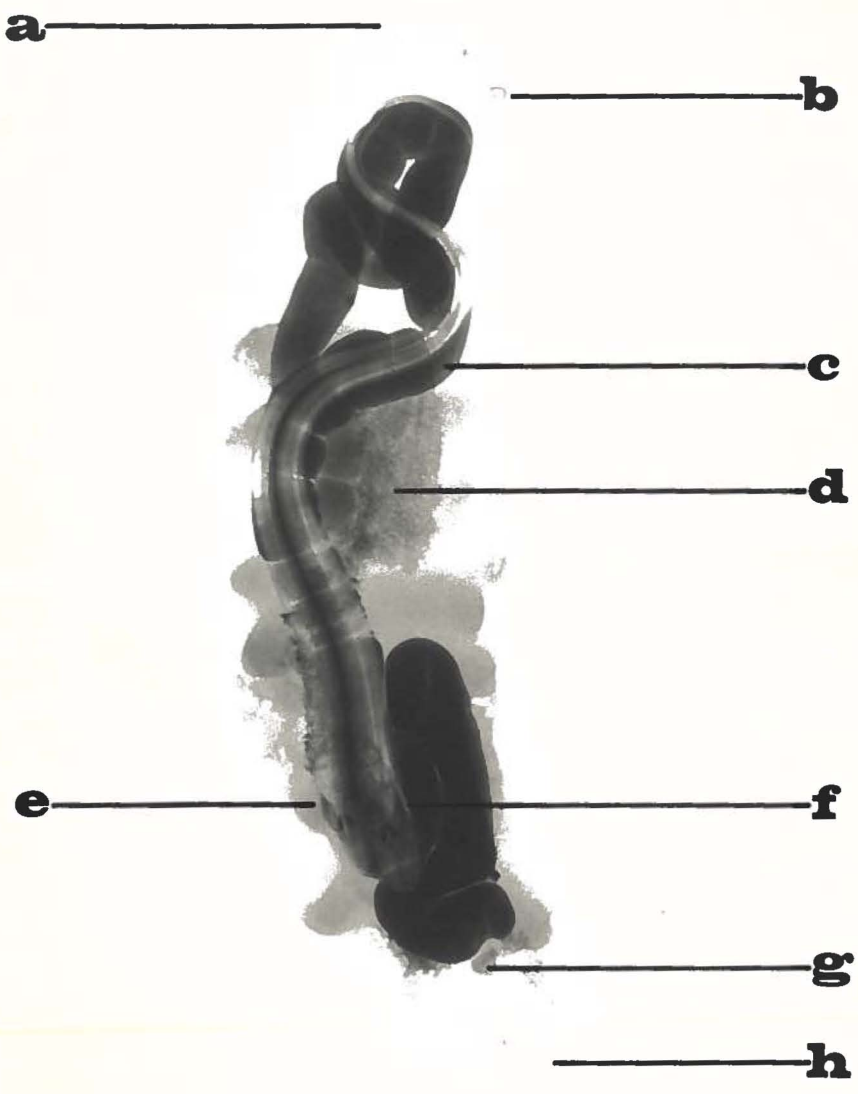
f
g

Fig. 71. Radiograph of an infested specimen of _Stichopus variegatus_. a, ring canal; b. parasitic gastropod; c, intestine; d, respiratory tree; e, adult specimen of _Encheliophis gracilis_ in the host's intestine; f, a second specimen of _E. gracilis_ sharing the host's intestine; g, cloaca; and h, anus.


a
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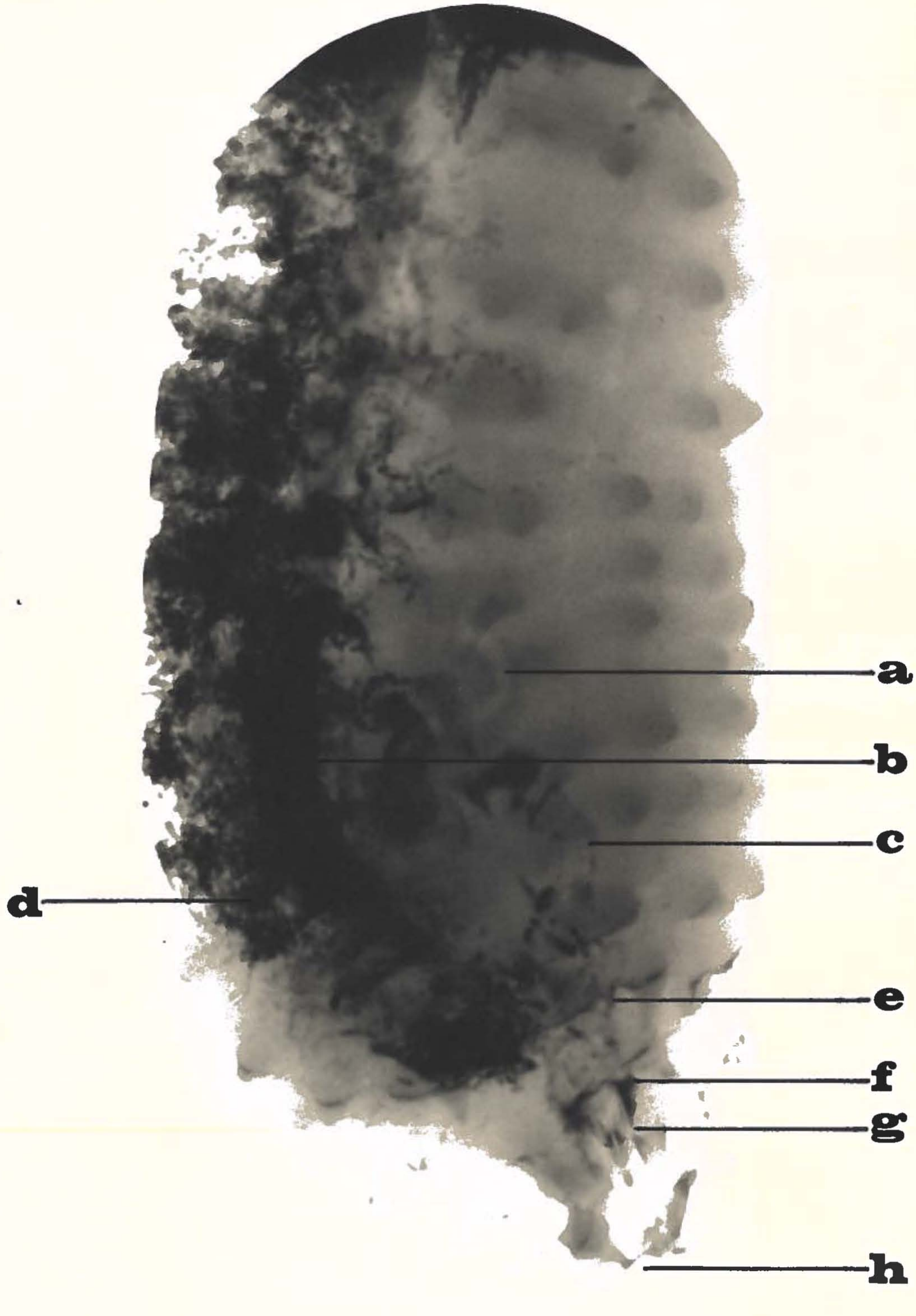
d
e
f
g
h

Fig. 72. Radiograph of Thelenota ananas (posterior only) with a Encheliophis gracilis young adult partially in the coelom of the holothurian. a, tail of pearlfish; b, intestine; c, body of pearlfish; d, left respiratory tree; e, base of right respiratory tree; f, cloaca (split open); g, head of pearlfish; and h, anus.


a
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e
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g
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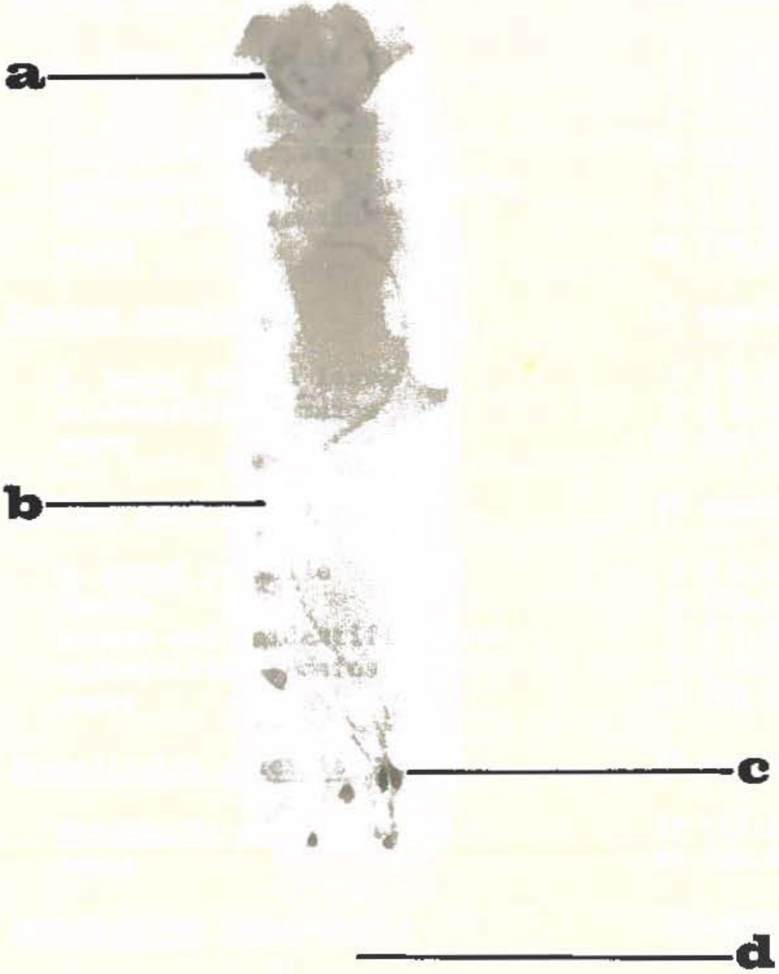
Fig. 73. Radiograph of Stichopus chloronotus infested by a single Encheliophis gracilis juvenile inhabiting a respiratory tree. a, ring canal; b, abdomen of the pearlfish; c, head of the pearlfish; and d, anus.


a
b
c
d

Food Habits of the Guam Pearlfishes

Stomach contents

The following data were obtained from the examination of 216 pearlfish stomachs:

TABLE XI. STOMACH CONTENTS OF 216 GUAM PEARLFISHES

Carapus homei	80 examined
C. homei tenuis larva	2 (2.5%)
C. homei juvenile	4 (5.0%)
C. mourlani juvenile	1 (1.3%)
unidentified fish	11 (13.7%)
unidentified fish plus shrimp	1 (1.3%)
unidentified remains	1 (1.3%)
empty	60 (75.0%)
Carapus mourlani	16 examined
C. homei tenuis larva	1 (6.3%)
unidentified remains	1 (6.3%)
empty	14 (87.4%)
Carapus parvipinnis	39 examined
C. homei juvenile	1 (2.6%)
shrimp	6 (15.3%)
shrimp and unidentified fish	1 (2.6%)
unidentified remains	3 (7.7%)
empty	28 (71.8%)
Encheliophis gracilis	78 examined
Holothuria argus gonads	29 (37.2%)
empty	49 (62.8%)
Encheliophis vermicularis	3 examined
empty	3

One immediately notices the high percentage of empty stomachs. This may be the result of biased sampling, as the majority of the collecting was done during daylight hours. However, a small sampling made at midnight (18 specimens of *Carapus homei*) revealed no increase in food found in the pearlfishes' stomachs. In 168 specimens of *C. homei* examined, C. L. Smith (1964) also found 75 percent of the stomachs empty.

Although *Carapus homei* has been reported to feed upon the gonads of its host (Hipeau-Jacquotte, 1967), neither C. L. Smith (1964) nor the present investigator found any gonadal tissue in the stomachs of 248 specimens examined. It was observed that only the very large specimens of *Stichopus chloronotus*, the preferred host of *C. homei*, had any gonads at all. The vast majority of the *Stichopus chloronotus* specimens examined (approximately 700) had no identifiable gonads. There may be a definite seasonal reproductive cycle of *S. chloronotus* which was missed during the sampling period (April through January), or, very few specimens of this holothurian actually reach maturity. In any case, the absence of holothurian gonads may have a direct influence upon the feeding habits of *Carapus homei*. Stomach contents indicated that members of this species were predators, feeding upon other fishes and possibly crustaceans.

Leiner (1960) states that *Carapus acus* is primarily a parasite, feeding upon the gonads of its holothurian host, and that occasionally, crustacean remains are found in the stomachs of this species. He suggests that the crustaceans enter the holothurian with the intake of water during respiration. This seems unlikely as the crustacean remains in the stomachs of *C. homei* and *C. parvipinnis* indicated that the shrimps were much larger than planktonic organisms, and the respiratory currents of holothurians did not seem strong enough to draw in an actively swimming shrimp larger than planktonic size. The possibility exists that the shrimps may have crawled in through the anus of the holothurians. It was found, however, by both C. L. Smith (1964) and the present investigator, that *C. homei* juveniles and adults would cannibalize individuals of their own species which entered holothurians already infested by these pearlfishes. Smith (1964) found that 11 percent of his *C. homei* specimens contained tenuis larvae in their stomachs. During the present research, *C. homei* tenuis larvae were kept isolated when it was found that juveniles and adults of the same species were preying upon them (outside the hosts).

C. L. Smith (1964) states that *Carapus homei* leaves its hosts regularly at night. He has seen several specimens swimming in open water, and a single specimen was captured in a commercial fisherman's beach seine. During the present investigation, the catch of several night fishermen at Tumon Bay was periodically examined for pearlfishes, but none were found. Aronson and Mosher (1951) made a night collection of pearlfishes in the Bahama Islands which did not reveal a lower percentage of infestation of the fishes than what was found during the daylight collections, but, they did occasionally observe pearlfishes swimming far from their hosts at night. A night sampling of *Holothuria argus* in Tumon Bay also failed to reveal a lower percentage of infestation than the normal collections.

Experiments were conducted to determine any night activity of the *Carapus homei* specimens infesting holothurians within aquaria. Several pearlfishes were tagged by clipping off approximately 3 mm of their tails. These tagged fishes were allowed to enter holothurians which were identified by size and color (*Holothuria argus* has two distinct color variations). These infested holothurians were placed in aquaria with other, possibly uninfested, potential hosts. After one or two overnight periods, the holothurians which were originally infested were dissected to determine if the tagged pearlfishes were present. In three series of experiments, it was found that the Carapus homei specimens remained with their original hosts. No night activity was observed so it is assumed that the pearlfishes did not leave their hosts at all, rather than leaving and returning to the same host. The behavior of the pearlfishes under these experimental conditions, however, may not be the same as in the natural environment.

It is difficult to understand why Carapus homei specimens break into the coelom of their hosts if they do not feed on any of the holothurian's organs. As predators, the fishes must leave their hosts occasionally, if not daily, in order to feed. It would seem more efficient for the fishes to simply reside in the respiratory trees of the holothurians if this is the case. It is also possible that the pearlfishes may feed upon the coelomocytes within the coelomic fluid of the holothurians. Such small particles of food might be digested rapidly, and may not be obvious in the stomachs of the pearlfishes.

Arnold (1953) states that Carapus acus also feeds upon the respiratory trees and polian vesicles of its host. No damage to these organs was observed in the case of C. homei and Stichopus chloronotus.

Carapus homei specimens were also found infesting Holothuria argus. H. argus differs from Stichopus chloronotus in that the average-sized specimens had well-developed gonads throughout the collecting period. In order to determine if Carapus homei specimens fed upon the gonads of this host, the following test was performed. The gonads of 136 adult *Holothuria argus* (250-300 mm) were examined and measured. In each, the gonadal tuft was removed by cutting the gonoduct (Fig. 63), and the organs measured by water displacement. A 100 ml graduated cylinder was partially filled with seawater, and the gonadal tuft was dropped into the cylinder. The displacement of the gonads was recorded in cubic centimeters. This method gave an approximate measure of the gonadal mass. The measurements are correlated with the infestation of *Carapus homei* in Table XII. When these data are plotted as a histogram (Fig. 74), it becomes obvious that there is a higher percentage of *C. homei* infestation in those holothurians with gonads intact. This indicates that there is no error in the stomach content sampling, and that specimens of *C. homei* do not feed upon the gonads of *Holothuria argus*. A comparison of this histogram with a similar one plotted for *Encheliophis gracilis* (Fig. 75) is helpful in interpreting the data. *E. gracilis* does feed upon the gonads of *Holothuria argus*.

The feeding habits of *Carapus parvipinnis* have not been reported previously. It appears that *C. parvipinnis* was similar to *C. homei* in that shrimp and fish constituted the main part of the diet of this species. Although *C. parvipinnis* specimens were often found in the coelom of *Thelenota ananas*, the gonads and other organs of the host were always intact.

C. L. Smith (1964) and Strasburg (1961) found nothing in the stomachs of nine specimens of *Carapus mourlani*. Smith observed that the specimens appeared emaciated, and as a result, suggested that *Culcita novaeguineae* is an abnormal host from which the pearlfish has no escape. As previously mentioned, Smith suggests that specimens of *Carapus mourlani* are actually starving *C. homei* specimens, and that the pigmentation of the former is a "starvation syndrome". The specimens of *C. mourlani* collected during the present research did not appear emaciated, although most had empty stomachs. The largest specimens of *C. mourlani* which were collected from *Culcita* were in excess of 200 mm in total length, longer than any *C. homei* specimen collected. Four of these large fishes were found decomposing within their hosts, after the *Culcita* specimens were placed in aquaria for two days. Evidently, the *Culcita* specimens were left too long out of water before being transferred to the aquaria. *Culcita* is quite hardy and withstood this treatment, but the pearlfishes died, presumably from lack of oxygen. These large *Carapus mourlani* specimens were not preserved due to their advanced state of decomposition when discovered, but enough of the body structure remained to estimate their lengths. A *C. mourlani* specimen reaching 200 mm in length must certainly be feeding and not starving.

Although Hipeau-Jacquotte (1967) found nothing in the stomachs of two specimens of *Carapus mourlani*, she suggests that the fishes probably eat the gonads or other organs of *Culcita*. The major organs within the coelom of this asteroid are the pyloric ceca (digestive glands) and the podia ampullae. Arnold (1953) states that *Carapus acus* eats the polian vesicles of its holothurian host, and these vesicles are similar to the large ampullae within *Culcita*. No damage to the ampullae or pyloric ceca was ever observed in the dissected specimens of *Culcita*.

Culcita novaeguineae is evidently seasonal in its reproductive habits, and only a few adult specimens (all infested) had gonads. No damage to these gonads was observed. *Carapus mourlani* may feed upon the coelomocytes of its host, as the coelomic fluid of *Culcita* is rich with these cells.

As previously discussed, a specimen of *Encheliophis gracilis* has been observed entering *Culcita*, and the same pearlfish was seen outside of the asteroid on two occasions. There is no reason to believe that specimens of *Carapus mourlani* could not leave *Culcita* (for feeding purposes) as well.

There is little doubt that *Encheliophis gracilis* fed exclusively on the gonads of *Holothuria argus*. Approximately 37 percent of the collected specimens of this pearlfish species had stomachs which were distended with thread-like material easily identified as the gonadal tissue of *H. argus*. C. L. Smith (1964) found the same material in four out of eleven specimens of *Encheliophis gracilis*. Through histological sections, Smith identified the gonadal material as holothurian testes tissue. Strasburg (1961) found specimens of *E. gracilis* from Hawaii which had fed upon the gonads of *Holothuria atra*. He states that the pearlfishes apparently feed by swallowing the free ends of the attenuate gonad, working forward to its attached base.

A few of the *Encheliophis gracilis* specimens found infesting *Stichopus chloronotus* had stomachs distended with the gonads of *Holothuria argus*. The gonads of these two holothurian species were completely different in structure and appearance, and they were easily differentiated. This would indicate that *Stichopus chloronotus* was only a temporary host and that specimens of *Encheliophis gracilis* moved from host to host to feed. When *Holothuria argus* was not available, the pearlfishes may have infested Stichopus chloronotus only for shelter.

As in the case of Carapus homei, the gonadal mass of the specimens of Holothuria argus infested by Encheliophis gracilis was measured and compared to the gonadal mass of the uninfested holothurians (Table XII). When these data are plotted as a histogram (Fig. 75), it is found that the specimens of Holothuria argus infested by these pearlfishes had much less gonadal mass than those which were uninfested. Thus, the greater the percentage of infestation by Encheliophis gracilis, the less the gonadal mass of Holothuria argus. The data indicated that Encheliophis gracilis did indeed, feed upon the gonads of its host.

Three specimens of Encheliophis vermicularis were examined (two specimens were collected in Palau), and their stomachs found empty. Arnold (1956) reports that E. vermicularis has been found in the body cavity of holothurians, feeding upon the host's viscera.

Attempts were made to feed the gonads and other organs of freshly dissected holothurians to specimens of Carapus homei, C. parvipinnis, and Encheliophis gracilis. None of the pearlfishes were observed to eat these organs, although all of the fishes tested were attracted to the tissue. Specimens of E. gracilis would curl their bodies around the organs, but they would not feed upon them. The fishes were left with the organs during three overnight periods with the hope that feeding would occur in the darkness, but the various organs remained untouched.

Discussion

Because of the facility of their entrance behavior, it would seem that the pearlfishes were capable of entering and leaving their hosts often. Pearlfishes which were kept from their hosts for long periods of time had difficulty in entering when finally provided the opportunity, whereas freshly liberated fishes could enter a host quickly. If this is true in the natural environment, pearlfishes which remained in a host indefinitely would not react so quickly when brought into the laboratory, liberated, and provided with the opportunity to re-enter a second host.

In the case of Carapus homei, the tenuis larvae may be obligate parasites, living and feeding in the coelom of their hosts. Observations of the tenuis larvae indicate that metamorphosis into the juvenile stage probably occurred within a host.

The stomach contents of Carapus homei juveniles and adults suggest that the pearlfishes fed outside of their hosts, returning to the hosts for shelter. C. L. Smith's (1964) observations on the night activity of these fishes supports this possibility. The diet of C. homei specimens may be supplemented by coelomocytes or other internal organs of the holothurian hosts. It appears that in other areas of the Pacific, C. homei may have different feeding habits than observed with the Guam specimens.

The behavior of Carapus mourlani suggests that this species is distinct from C. homei and that the infestation of Culcita is a deliberate act. The pearlfishes probably leave their hosts in order to prey upon other organisms, but supplementary feeding may occur within the coelom of Culcita. There is also the possibility that the pearlfishes break into the stomachs of the asteroids and feed upon the host's partially digested food. Pearlfish movement in and out of the Culcita specimens may be governed somewhat by the feeding activity of the host. Pearlfish entry into *Culcita* would be relatively simple if the asteroid was feeding and had its stomach everted.

It appears that *Carapus parvipinnis* was also a predator, feeding outside of its host, but a good deal more collecting must be done with this species in order to fully understand its relationship with the holothurian host.

Encheliophis gracilis was an internal predator, probably moving from host to host and feeding upon the gonads of *Holothuria argus*. The term "predator" is used here in preference to "parasite" to indicate that a single host could probably not provide enough food energy to support the life of a large pearlfish such as *Encheliophis gracilis*. Therefore, the fish must search for its prey in the conventional manner. The feeding, of course, is rather unconventional. The castration which results from this feeding process probably does not affect the holothurian, due to its remarkable powers of tissue regeneration. The holothurian population may be limited somewhat by the action of the pearlfishes, but the total ecological effect as a result of this feeding behavior is slight. The great numbers of *Holothuria argus* on the reefs of Guam, and the fairly low percentage of infestation indicate this.

TABLE XII. *HOLOTHURIA ARGUS* GONADAL MASS vs. *CARAPUS HOMEI* AND *ENCHELIOPHIS GRACILIS* INFESTATION

**Holothuria argus* (250-300 mm)		*Carapus homei*		*Encheliophis gracilis*	
Gonadal mass, cc	Number collected	Number infested	Percentage of infestation	Number infested	Percentage of infestation
0-2	42	8	19	14	31
2-4	29	8	28	5	17
4-6	26	7	27	2	8
6-8	15	3	20	2	13
8-10	10	2	20	1	10
10-12	6	2	33	0	0
12-14	4	2	50	0	0
14-16	3	0	0	0	0
16-18	1	0	0	0	0

*All holothurian specimens of adult size.

Fig. 74. *Holothuria argus* gonadal mass vs. *Carapus homei* infestation.

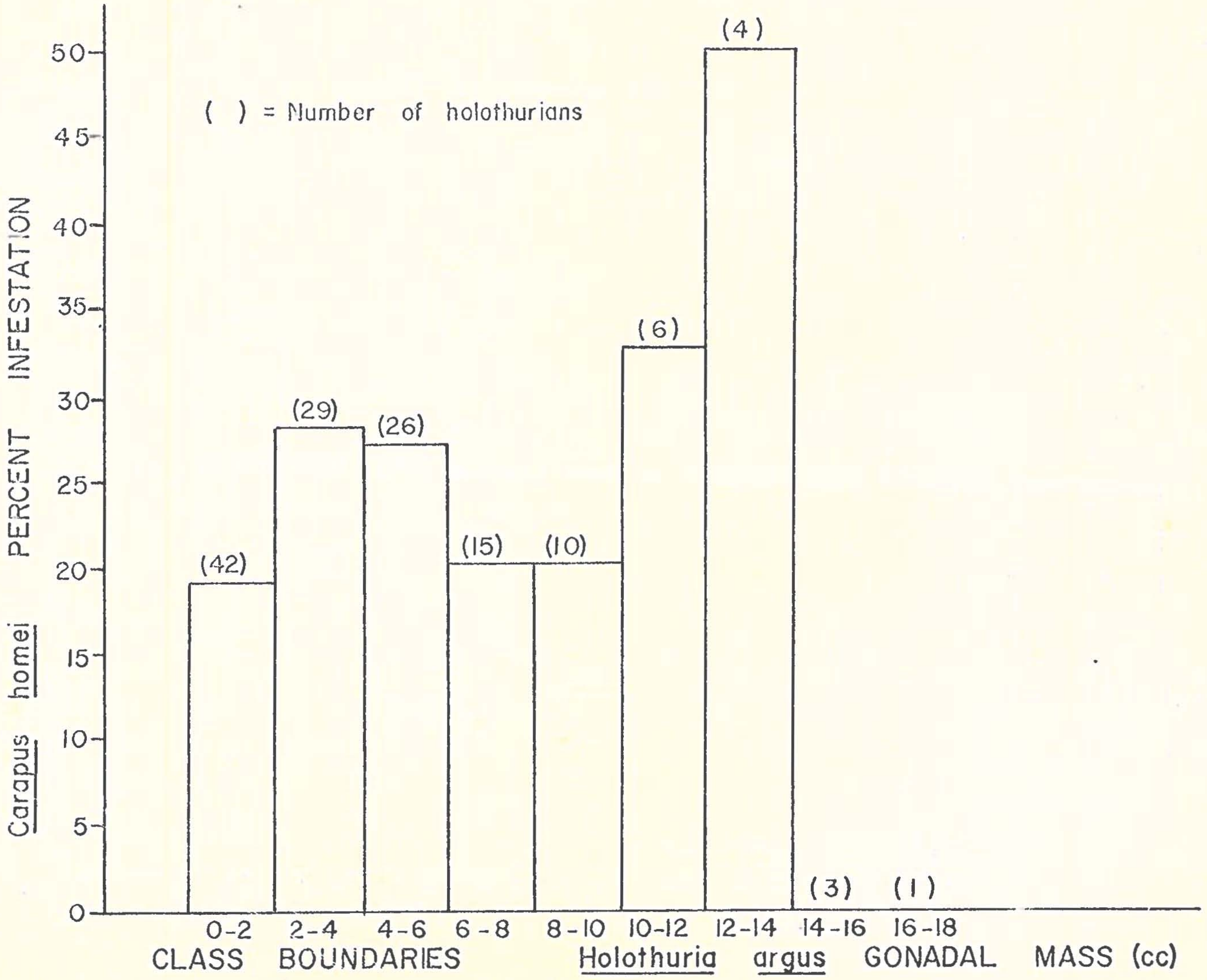
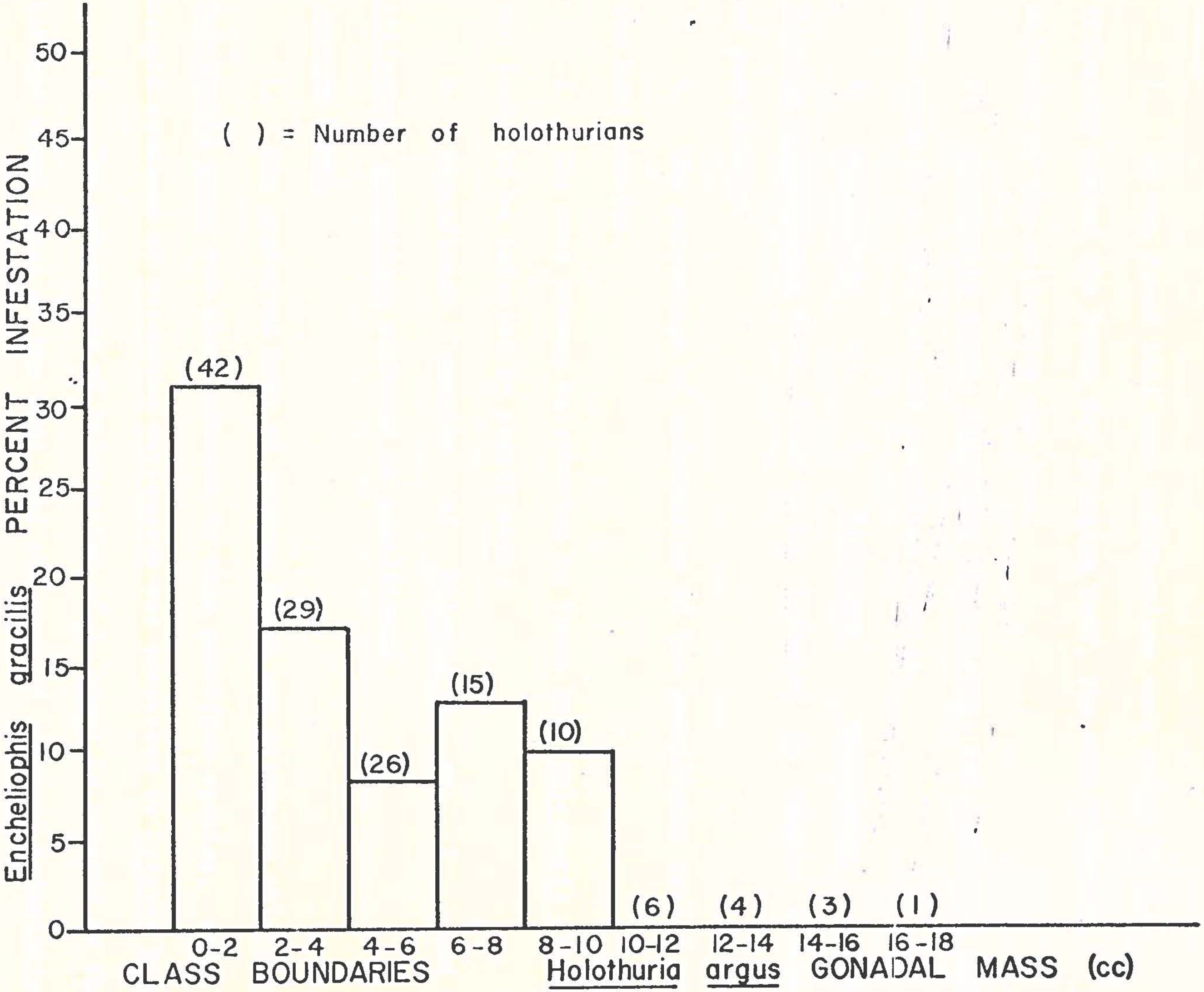

( ) = Number of holothurians
Carapus homei PERCENT INFESTATION
0
5
10
15
20
25
30
35
40
45
50
(42)
(29)
(26)
(15)
(10)
(6)
(4)
(3)
(1)
0-2
2-4
4-6
6-8
8-10
10-12
12-14
14-16
16-18
CLASS BOUNDARIES Holothuria argus GONADAL MASS (cc)

Fig. 75. Holothuria argus gonadal mass vs. Encheliophis gracilis infestation.


( ) = Number of holothurians
Encheliophis gracilis PERCENT INFESTATION
50
45
40
35
30
25
20
15
10
5
0
(42)
(29)
(26)
(15)
(10)
(6)
(4)
(3)
(1)
0-2
2-4
4-6
6-8
8-10
10-12
12-14
14-16
16-18
CLASS BOUNDARIES Holothuria argus GONADAL MASS (cc)

CHAPTER V. SUMMARY

Hosts

1. It was found that most holothurians were zonal in distribution across the Tumon Bay fringing reef, the area where the majority of the collecting was done.

2. *Holothuria argus*, *Stichopus chloronotus*, and *Thelenota ananas* each acted as a host for two pearlfish species.

3. *Holothuria* sp. 1, *Stichopus variegatus*, and *Culcita novaeguineae* were found to be infested by single species of pearlfishes.

4. The percentage of infestation of the pearlfishes varied with the host species.

5. The most common holothurian in Tumon Bay, *Holothuria atra*, was not found to be infested by pearlfishes.

6. Significant samples of *Holothuria* sp. 2, and *Actinopyga mauritiana* produced no pearlfishes.

Carapus homei

1. The preferred host of *Carapus homei* was *Stichopus chloronotus*. *Holothuria argus* was also infested by this pearlfish species, and a single specimen of *Stichopus variegatus* was inhabited by *Carapus homei*.

2. The percentage of infestation of *C. homei* is related to the habitat (reef zone) of the host, and the area of greatest infestation occurred on the inner reef flat.

3. *C. homei* tenuis larvae were collected in May, September, and October.

4. The majority of the *C. homei* specimens collected were juveniles, and the greatest percentage of infestation of this life form occurred in November and December. Observations of the tenuis larvae indicate that metamorphosis into the juvenile stage probably occurs within a host.

5. *C. homei* adults were collected in June and July.

6. No adult specimens were found with fully mature gonads. Immature males and females were collected in June and July.

7. *C. homei* may have an annual reproductive cycle, with spawning in the late summer and an increase of infestation by the tenuis and juvenile forms during the winter months.

8. The length of *C. homei* specimens appears to be dependent upon the (size of the) host species.

9. A large percentage of the collected *C. homei* specimens were found in the body cavity (coelom) of their hosts while others were found in the respiratory trees of the holothurians.

10. Radiographs of the pearlfishes and hosts indicate that the fishes moved tail-first up the respiratory trees of the holothurians upon entry. The area of breakthrough into the coelom of the holothurian was not determined.

11. *C. homei* specimens appeared to be predators, feeding upon fishes and crustaceans. These pearlfishes often preyed upon members of their own species, particularly tenuis larvae. No gonadal or other host tissue was found in the stomachs of these fishes.

12. The relationship between specimens of *C. homei* and their hosts was not clear. The pearlfishes may supplement their diet with the coelomocytes or other tissues of the hosts.

13. No nighttime activity of *C. homei* specimens outside of their hosts was observed. Night sampling did not indicate a lower percentage of infestation than during the daytime, nor more food in the stomachs of the pearlfishes.

Carapus mourlani

1. *Carapus mourlani* may be a distinct species of pearlfish, differing from *C. homei* in behavior (host preference) and in slight anatomical differences such as the presence of melanophores.

2. The anatomical differences between *C. mourlani* and *C. homei* appear not to be due to the affects of their different hosts, i.e., asteroids and holothurians.

3. The host for *C. mourlani* was *Culcita novaeguineae*. All specimens of the pearlfishes were found in the coelom of the hosts.

4. A single *C. mourlani* tenuis larva was found in the coelom of a *Culcita* specimen

5. The majority of *C. mourlani* specimens found were juveniles.

6. Immature *C. mourlani* adult females were found in May and July.

7. The majority of the *C. mourlani* specimens had empty stomachs, however, sampling may have been done at the wrong time of day. The pearlfishes may leave their hosts to feed, or, they may feed upon the internal organs or partially digested food of their hosts. No damage to the stomachs or internal organs of the asteroids was observed.

Carapus parvipinnis

1. The host of *Carapus parvipinnis* was *Thelenota ananas*.

2. No tenuis larvae of this pearlfish species were found.

3. Juveniles and adults of *C. parvipinnis* were found throughout the collecting period.

4. The greatest percentage of mature specimens occurred in August, however, some mature specimens were found throughout the collecting period.

5. The majority of the *C. parvipinnis* specimens were found in the coelom of their hosts.

6. Stomach contents indicate that *C. parvipinnis* was a predator, feeding outside of the host on shrimps and fishes.

7. The gonads and other internal organs of *Thelenota ananas* were not found to be damaged by the infesting pearlfishes.

Encheliophis gracilis

1. The preferred host of *Encheliophis gracilis* was *Holothuria argus*. *Thelenota ananas* and *Stichopus chloronotus* also acted as hosts for this pearlfish species.

2. No *E. gracilis* tenuis larvae were collected.

3. Juveniles and adults were found throughout the collecting period. No seasonal infestation pattern of *E. gracilis* could be determined.

4. Sexually mature adults of *E. gracilis* were found throughout the collecting period and only in *Holothuria argus*.

5. Adult specimens of *E. gracilis* formed pairs, each pair infesting a single host. Each of the pairs consisted of a sexually mature male with sperm producing testes and a sexually mature female with a ripe ovary.

6. Paired infestation was dependent upon the gonadal development of *E. gracilis*. Only fishes in a spawning condition were found paired.

7. The female of each pair had a significantly greater body length than the paired male, and the females had enlarged abdomens and a slightly different melanophore pattern than the males.

8. Pairing was for reproductive purposes and probably occurs close to the time of spawning.

9. Two egg rafts from two different *E. gracilis* pairs were spawned in aquaria, and the embryonic and larval development of the fertile eggs from one of the rafts was observed.

10. Developing vexillifer larvae were kept alive for a five day period, and observations were made every eight hours. The most highly developed vexillifer reached a length of 2.5 mm.

11. The embryonic and larval development of *E. gracilis* seems similar to that of *Carapus acus*.

12. The egg rafts of *E. gracilis* are pelagic, and a shoreward migration of the vexillifers, tenuis larvae, and juveniles occurs. *Thelenota ananas* seems to be a temporary host, and the migration shoreward continues until the adults can infest *Holothuria argus* on the reef flat.

13. *E. gracilis* specimens were found in the coelom and respiratory trees of their hosts. Pair members normally shared the same respiratory tree.

14. Under laboratory conditions, *E. gracilis* specimens would occasionally enter the intestine of a holothurian.

15. A radiograph shows an E. gracilis specimen broken through the cloaca of its host and partially in the coelom of the holothurian.

16. Stomach contents indicate that E. gracilis was an internal predator, feeding exclusively on the gonads of Holothuria argus.

17. A measurement of the gonadal mass of infested vs. uninfested specimens of Holothuria argus confirmed the fact that E. gracilis fed upon the gonads.

18. E. gracilis specimens must move from host to host in order to feed, as a single host could probably not provide enough food energy to support the life of this large pearlfish.

19. The total ecological effect of this castration of the Holothuria argus population was probably slight.

Encheliophis vermicularis

1. Although Holothuria atra has been reported as the host for Encheliophis vermicularis on Guam, it was found that this holothurian has probably been confused with another holothurian species. A single specimen of E. vermicularis was found infesting Holothuria sp. 1 in January.

2. A total of three specimens of E. vermicularis were examined (two from Palau), and a single mature female, 133 mm in length, was found with a ripe ovary.

3. The stomachs of the three E. vermicularis specimens were empty.

Pearlfish behavior

1. Two types of pearlfish locomotion were observed. The tenuis larvae swam with their heads pointed upward and with undulations of their entire bodies, including their heads. Juveniles and adults swam with their heads pointed downward and their body undulations were not as

intense as the tenuis larvae. Movement was primarily by the action of the paired fins.

2. The pearlfishes seemed photonegative and remained in the dark corners of the aquaria. Dark objects attracted the pearlfishes.

3. The sight of the pearlfishes seemed poor. Preliminary excitation of the pearlfishes and recognition of the hosts depended upon a chemical stimulus in the water.

4. This chemical stimulus may be centered in the mucus of the holothurian's integument; however, fluid from other organs of the holothurian also excited the pearlfishes.

5. Once a host was located, the pearlfish repeatedly bumped its snout against the dorsal surface and sides of the host's body. This behavior may be an aid to host recognition through chemical stimuli.

6. The pearlfish moved along the length of the holothurian until an end was reached and the host's anus was located.

7. Entry stimulus was the presence of the current of water expelled during the process of holothurian respiration.

8. Entry may occur head-first or tail-first, depending upon the size of the holothurian's anus and the relative size of the fish. Tail-first entry involved a corkscrew motion of the fish.

9. The behavior of the Guam pearlfishes was similar to the behavior of Carapus acus as described by Arnold (1953, 1956, and 1957), with the exception of three areas: 1) Carapus homei tenuis larvae remained alive outside of their hosts, and re-entered their hosts; 2) juveniles and adults of the Guam pearlfishes seemed to locate their hosts by sight; and 3) juveniles and adults of the Guam pearlfishes were found to enter their

hosts both head-first and tail-first with no difference in the behavior of the two life forms.

10. Holothurian reaction of the entry of a pearlfish consisted of closing the anus, contracting the body, and releasing the Cuvierian organs, if any.

11. It was found that the Guam pearlfishes were attracted to the Cuvierian organs of the holothurians and were not harmed by these organs.

12. Pearlfish entry into Culcita novaeguineae involved locating an ambulacral groove, swimming up the groove to the asteroid's mouth, and entering, probably when the asteroid was feeding and had everted its stomach.

13. Host preference experiments showed that the pearlfishes preferred the hosts normally infested and that they would infest other hosts when provided with no other choice.

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APPENDICES

Summary of field data - April, 1968 through January, 1969

APPENDIX A - HOSTS

APPENDIX B - PEARLFISHES

APPENDIX C - SEASONAL VARIATION OF PEARLFISHES

Key

Pearlfish species--

1 *Carapus homei*
2 *Carapus mourlani*
3 *Carapus parvipinnis*
4 *Encheliophis gracilis* (**E. gracilis* pair member)
5 *Encheliophis vermicularis*

Host species--

1 *Culcita novaeguineae*
2 *Holothuria argus*
3 *Stichopus chloronotus*
4 *Thelenota ananas*
5 *Stichopus variegatus*
6 *Holothuria* species 1
7 *Holothuria atra*
8 *Holothuria* species 2
9 *Actinopyga mauritiana*

Reef zone--

1 sand
2 coral
3 boulder
4 outer reef flat
5 reef margin
6 reef front
7 terrace

Life form--

1 tenuis
2 juvenile
3 adult

Reef location--

1 Tumon Bay
2 Asan-Piti
3 Agat
4 Double Reef

Location of fish in host--

1 coelom
2 respiratory tree
3 cloaca
4 intestine
5 free in collection bag

APPENDIX A - HOSTS

Field number	Host species	Reef zone	Total collected	Total infested
68-1	3	4	50	13
68-2	3	4	60	8
68-3	3	4	25	3
68-4	3	4	24	3
68-5	1	4	5	2
68-6	3	4	50	0
68-6	1	4	5	0
68-7	3	3	50	2
68-8	3	1	6	3
68-9	3	2	27	10
68-9	8	2	14	0
68-9	7	2	33	0
68-10	3	4	80	6
68-10	2	4	28	3
68-11	2	4	17	2
68-11	3	2	14	5
68-15	2	4	28	4
68-16	3	2	3	1
68-16	2	2	6	4
68-17	4	7	1	1
68-17	1	7	2	2
68-18	4	7	2	2
68-19	4	7	1	1
68-20	3	3	25	2
68-20	2	3	28	5
68-21	4	7	13	13
68-22	4	7	15	11
68-23	3	4	25	0
68-23	2	4	23	0
68-23	9	4	13	0
68-24	2	2	16	7
68-24	9	3	9	0

Field number	Host species	Reef zone	Total collected	Total infested
68-12	3	3	8	1
68-12	2	4	26	6
68-12	7	2	3	0
68-13	3	4	5	1
68-13	2	4	25	3
68-14	3	3	10	1
68-14	2	3	26	7
68-15	3	1	3	1
68-29	3	3	20	6
68-29	2	3	12	3
68-30	4	7	4	4
68-30	1	7	1	1
68-31	3	3	24	3
68-31	2	4	20	2
68-31	9	4	7	0
68-32	1	5	3	2
68-25	4	7	2	2
68-26	2	4	10	2
68-26	3	4	8	0
68-26	9	4	9	0
68-27	2	2	18	4
68-27	3	2	13	6
68-27	5	2	23	0
68-28	4	6	8	8
68-40	2	2	8	4
68-40	8	3	4	0
68-41	1	7	12	3
68-42	3	4	14	1
68-42	2	4	20	5
68-43	4	7	5	5
68-43	1	7	7	3
68-44	3	2	20	6

Field number	Host species	Reef zone	Total collected	Total infested
68-33	1	4	2	2
68-34	3	2	37	16
68-34	2	3	22	5
68-34	5	3	8	1
68-35	3	2	32	13
68-35	2	2	17	5
68-35	5	3	3	0
68-36	2	2	23	10
68-36	3	3	26	5
68-36	5	3	14	0
68-37	1	5	12	5
68-38	4	6	9	5
68-39	3	2	21	6
68-39	2	2	19	4
68-39	8	3	8	0
68-40	3	2	20	7
68-44	8	3	2	0
69-45	4	7	7	7
69-46	3	2	25	4
69-46	5	3	12	0
69-46	8	2	7	0
69-47	7	2	75	0
69-48	3	4	10	2
69-48	2	4	25	6
69-49	6	2	29	1
69-50	6	2	53	0
69-51	2	2	39	12
69-52	2	4	12	2
69-52	8	4	10	0
69-53	7	2	43	0
69-54	6	2	78	0
TOTAL			1,776	321

APPENDIX B - PEARLFISHES

Date	Field number	Specimen number	Pearlfish species	Life form	Standard length mm	Host species	Reef location	Reef zone	Location in host
4-29	68-1	1	1	2	85	3	1	4	1
4-29	68-1	2	1	2	80	3	1	4	1
4-29	68-1	3	1	2	74	3	1	4	1
4-29	68-1	4	1	2	88	3	1	4	1
4-29	68-1	5	1	2	85	3	1	4	1
4-29	68-1	6	1	3	101	3	1	4	1
4-29	68-1	7	1	2	83	3	1	4	1
4-29	68-1	8	1	2	79	3	1	4	1
4-29	68-1	9	1	2	76	3	1	4	1
4-29	68-1	10	1	2	75	3	1	4	1
4-29	68-1	11	1	2	84	3	1	4	1
4-29	68-1	12	1	2	72	3	1	4	1
4-29	68-1	13	1	2	83	3	1	4	1
4-30	68-2	14	1	2	77	3	1	4	1
4-30	68-2	15	1	2	75	3	1	4	1
4-30	68-2	16	1	2	72	3	1	4	1
4-30	68-2	17	1	2	87	3	1	4	1

Date	Field number	Specimen number	Pearlfish species	Life form	Standard length mm	Host species	Reef location	Reef zone	Location in host
4-30	68-2	18	1	2	80	3	1	4	1
4-30	68-2	19	1	2	68	3	1	4	1
4-30	68-2	20	1	2	80	3	1	4	1
4-30	68-2	21	4	3	150	3	1	4	1
5-1	68-3	22	1	2	79	3	1	4	1
5-1	68-3	23	1	2	82	3	1	4	1
5-1	68-3	24	1	1	185	3	1	4	1
5-4	68-4	25	4	2	106	3	1	4	1
5-4	68-4	26	1	2	95	3	1	4	1
5-4	68-4	27	1	2	98	3	1	4	1
5-13	68-5	28	2	3	115	1	1	4	1
5-13	68-5	29	2	2	72	1	1	4	1
6-12	68-6	---	-	-	---	-	-	-	-
6-13	68-7	30	1	2	89	3	1	3	1
6-13	68-7	31	1	3	100	3	1	3	1
6-20	68-8	32	1	3	118	3	1	1	1

Date	Field number	Specimen number	Pearlfish species	Life form	Standard length mm	Host species	Reef location	Reef zone	Location in host
6-20	68-8	33	1	3	114	3	1	1	1
6-20	68-8	34	1	3	118	3	1	1	1
6-22	68-9	35	1	3	122	3	1	2	2
6-22	68-9	36	1	3	105	3	1	2	2
6-22	68-9	37	1	2	95	3	1	2	2
6-22	68-9	38	1	3	125	3	1	2	1
6-22	68-9	39	1	3	133	3	1	2	1
6-22	68-9	40	1	3	118	3	1	2	1
6-22	68-9	41	1	3	109	3	1	2	1
6-22	68-9	42	1	3	101	3	1	2	1
6-22	68-9	43	1	2	65	3	1	2	2
6-22	68-9	44	1	2	78	3	1	2	2
6-23	68-10	45	1	3	100	3	1	4	2
6-23	68-10	46	1	2	95	3	1	4	2
6-23	68-10	47	1	2	90	3	1	4	1
6-23	68-10	48	1	3	105	3	1	4	1
6-23	68-10	49	1	3	126	3	1	4	1

Date	Field number	Specimen number	Pearlfish species	Life form	Standard length mm	Host species	Reef location	Reef zone	Location in host
6-23	68-10	50	1	3	115	3	1	4	1
6-23	68-10	51	1	3	115	3	1	4	5
6-23	68-10	52	4	3	191	2	1	4	5
6-23	68-10	53	4	3	180	2	1	4	5
6-26	68-11	54	4	3	196	2	1	4	1
6-26	68-11	55*	4	3	187	2	1	4	1
6-26	68-11	56*	4	3	208	2	1	4	5
6-26	68-11	57	1	3	117	3	1	2	1
6-26	68-11	58	1	3	121	3	1	2	1
6-26	68-11	59	1	3	103	3	1	2	1
6-26	68-11	60	1	2	83	3	1	2	1
6-26	68-11	61	1	2	85	3	1	2	1
6-30	68-12	62	1	3	100	3	1	3	5
6-30	68-12	63*	4	3	200	2	1	4	5
6-30	68-12	64*	4	3	175	2	1	4	5
6-30	68-12	65*	4	3	240	2	1	4	5

Date	Field number	Specimen number	Pearlfish species	Life form	Standard length mm	Host species	Reef location	Reef zone	Location in host
6-30	68-12	66*	4	3	239	2	1	4	5
6-30	68-12	67	4	3	201	2	1	4	5
6-30	68-12	68	4	3	175	2	1	4	5
6-30	68-12	69	1	3	130	2	1	4	5
6-30	68-12	70	1	2	70	2	1	4	5
7-1	68-13	71*	4	3	223	2	1	4	5
7-1	68-13	72*	4	3	240	2	1	4	5
7-1	68-13	73	1	3	120	3	1	4	1
7-1	68-13	74	1	3	118	2	1	4	5
7-1	68-13	75	4	3	198	2	1	4	5
7-6	68-14	76	1	2	90	3	1	3	1
7-6	68-14	77	1	3	122	2	1	3	5
7-6	68-14	78	1	3	116	2	1	3	5
7-6	68-14	79	4	3	240	2	1	3	5
7-6	68-14	80	4	3	235	2	1	3	5
7-6	68-14	81	4	3	145	2	1	3	5

Date	Field number	Specimen number	Pearlfish species	Life form	Standard length mm	Host species	Reef location	Reef zone	Location in host
7-6	68-14	82*	4	3	233	2	1	3	5
7-6	68-14	83*	4	3	211	2	1	3	5
7-6	68-14	84	4	3	220	2	1	3	5
7-14	68-15	85	1	3	120	3	1	1	1
7-14	68-15	86	4	3	165	2	1	4	5
7-14	68-15	87*	4	3	200	2	1	4	5
7-14	68-15	88*	4	3	218	2	1	4	5
7-14	68-15	89	1	3	121	2	1	4	5
7-14	68-15	90	1	3	111	2	1	4	5
7-15	68-16	91	1	2	95	3	1	2	5
7-15	68-16	92	1	3	120	2	1	2	5
7-15	68-16	93	1	3	112	2	1	2	5
7-15	68-16	94	1	2	95	2	1	2	5
7-15	68-16	95	1	3	113	2	1	2	5
7-19	68-17	96	3	3	211	4	4	7	1
7-19	68-17	97	2	2	80	1	4	7	1

Date	Field number	Specimen number	Pearlfish species	Life form	Standard length, mm	Host species	Reef location	Reef zone	Location in host
7-19	68-17	98	2	3	120	1	4	7	1
7-20	68-18	99	3	3	200	4	4	7	5
7-20	68-18	100	4	3	214	4	4	7	5
7-28	68-19	101	3	3	145	4	4	7	1
7-30	68-20	102	4	3	175	3	1	3	1
7-30	68-20	103	1	2	80	3	1	3	1
7-30	68-20	104	4	3	178	2	1	3	5
7-30	68-20	105	1	2	55	2	1	3	5
7-30	68-20	106	4	3	195	2	1	3	5
7-30	68-20	107	4	3	187	2	1	3	5
7-30	68-20	108	1	3	100	2	1	3	5
8-4	68-21	109	3	3	190	4	2	7	2
8-4	68-21	110	3	3	280	4	2	7	1
8-4	68-21	111	3	3	228	4	2	7	1
8-4	68-21	112	3	3	187	4	2	7	1
8-4	68-21	113	4	2	92	4	2	7	1
8-4	68-21	114	3	3	230	4	2	7	1

Date	Field number	Specimen number	Pearlfish species	Life form	Standard length, mm	Host species	Reef location	Reef zone	Location in host
8-4	68-21	115	3	3	250	4	2	7	1
8-4	68-21	116	3	3	160	4	2	7	1
8-4	68-21	117	3	2	95	4	2	7	1
8-4	68-21	118	3	2	105	4	2	7	1
8-4	68-21	119	4	2	76	4	2	7	1
8-4	68-21	120	4	2	80	4	2	7	1
8-4	68-21	121	3	2	55	4	2	7	1
8-24	68-22	122	3	3	232	4	2	7	1
8-24	68-22	123	3	3	257	4	2	7	5
8-24	68-22	124	3	3	231	4	2	7	5
8-24	68-22	125	3	3	137	4	2	7	5
8-24	68-22	126	3	3	122	4	2	7	5
8-24	68-22	127	3	2	76	4	2	7	5
8-24	68-22	128	4	3	139	4	2	7	5
8-24	68-22	129	4	2	93	4	2	7	5
8-24	68-22	130	3	3	215	4	2	7	5
8-24	68-22	131	3	2	101	4	2	7	5

Date	Field number	Specimen number	Pearlfish species	Life form	Standard length mm	Host species	Reef location	Reef zone	Location in host
8-24	68-22	132	4	2	78	4	2	7	5
8-31	68-23	---	-	-	---	-	-	-	-
9-14	68-24	133	4	3	200	2	1	2	5
9-14	68-24	134	4	3	224	2	1	2	5
9-14	68-24	135	4	3	185	2	1	2	5
9-14	68-24	136	4	3	210	2	1	2	1
9-14	68-24	137	1	2	70	2	1	2	5
9-14	68-24	138	1	2	71	2	1	2	5
9-14	68-24	139	1	2	72	2	1	2	5
9-15	68-25	140	3	3	245	4	2	7	5
9-15	68-25	141	3	3	141	4	2	7	5
9-21	68-26	142	1	2	95	2	1	4	1
9-21	68-26	143	4	3	162	2	1	4	1
9-29	68-27	144	1	2	86	2	1	2	1
9-29	68-27	145	1	2	95	2	1	2	1
9-29	68-27	146	1	2	70	3	1	2	1
9-29	68-27	147	1	1	142	3	1	2	1

Date	Field number	Specimen number	Pearlfish species	Life form	Standard length mm	Host species	Reef location	Reef zone	Location in host
9-29	68-27	148	1	1	---	3	1	2	1
9-29	68-27	149	1	1	---	3	1	2	1
9-29	68-27	150	1	2	85	3	1	2	1
9-29	68-27	151	1	2	80	3	1	2	1
9-29	68-27	152	4	2	98	2	1	2	1
9-29	68-27	153	1	2	89	2	1	2	1
10-5	68-28	154	3	3	---	4	2	6	5
10-5	68-28	155	3	3	207	4	2	6	5
10-5	68-28	156	3	3	192	4	2	6	5
10-5	68-28	157	3	3	247	4	2	6	5
10-5	68-28	158	3	3	226	4	2	6	5
10-5	68-28	159	3	3	170	4	2	6	5
10-5	68-28	160	4	3	110	4	2	6	5
10-5	68-28	161	4	3	115	4	2	6	5
10-6	68-29	162	1	2	82	3	1	3	2
10-6	68-29	163	1	2	72	3	1	3	2

Date	Field number	Specimen number	Pearlfish species	Life form	Standard length mm	Host species	Reef location	Reef zone	Location in host
10-6	68-29	164	1	2	73	3	1	3	1
10-6	68-29	165	1	2	82	3	1	3	2
10-6	68-29	166	1	1	---	3	1	3	1
10-6	68-29	167	1	1	130	3	1	3	1
10-6	68-29	168	1	1	65	2	1	3	2
10-6	68-29	169*	4	3	185	2	1	3	1
10-6	68-29	170*	4	3	205	2	1	3	1
10-6	68-29	171*	4	3	195	2	1	3	1
10-6	68-29	172*	4	3	145	2	1	3	1
10-12	68-30	173	3	3	223	4	3	7	1
10-12	68-30	174	3	3	250	4	3	7	5
10-12	68-30	175	3	3	120	4	3	7	1
10-12	68-30	176	3	3	135	4	3	7	2
10-12	68-30	177	2	2	75	1	3	7	1
10-13	68-31	178	4	3	170	3	1	3	1
10-13	68-31	179	4	2	90	3	1	3	1

Date	Field number	Specimen number	Pearlfish species	Life form	Standard length mm	Host species	Reef location	Reef zone	Location in host
10-13	68-31	180	1	2	73	3	1	3	1
10-13	68-31	181	1	3	145	2	1	4	2
10-13	68-31	182	1	3	104	2	1	4	1
10-13	68-32	183	2	2	80	1	2	5	1
10-13	68-32	184	2	2	85	1	2	5	1
11-5	68-33	185a	2	2	75	1	1	4	1
11-5	68-33	185b	2	2	80	1	1	4	1
11-9	68-34	186	4	2	85	3	1	2	1
11-9	68-34	187	1	2	70	3	1	2	2
11-9	68-34	188	1	2	75	3	1	2	1
11-9	68-34	189	1	2	77	3	1	2	1
11-9	68-34	190	1	2	84	3	1	2	1
11-9	68-34	191	1	2	72	3	1	2	1
11-9	68-34	192	1	2	90	3	1	2	1
11-9	68-34	193	1	2	78	3	1	2	1
11-9	68-34	194	1	2	83	3	1	2	1
11-9	68-34	195	1	2	86	3	1	2	1

Date	Field number	Specimen number	Pearlfish species	Life form	Standard length mm	Host species	Reef location	Reef zone	Location in host
11-9	68-34	196	1	2	80	3	1	2	5
11-9	68-34	197	1	2	75	3	1	2	5
11-9	68-34	198	1	2	80	3	1	2	5
11-9	68-34	199	1	2	87	3	1	2	5
11-9	68-34	200	1	2	81	3	1	2	5
11-9	68-34	201	1	2	77	3	1	2	1
11-9	68-34	202	1	2	75	5	1	3	1
11-9	68-34	203*	4	3	233	2	1	3	2
11-9	68-34	204*	4	3	170	2	1	3	2
11-9	68-34	205	1	2	94	2	1	3	2
11-9	68-34	206	1	2	80	2	1	3	5
11-9	68-34	207	1	2	80	2	1	3	5
11-9	68-34	208	1	2	84	2	1	3	5
11-10	68-35	209	4	2	91	3	1	2	5
11-10	68-35	210	4	3	130	3	1	2	1
11-10	68-35	211	1	2	78	3	1	2	1

Date	Field number	Specimen number	Pearlfish species	Life form	Standard length mm	Host species	Reef location	Reef zone	Location in host
11-10	68-35	212	1	2	80	3	1	2	1
11-10	68-35	213	1	2	70	3	1	2	5
11-10	68-35	214	1	2	75	3	1	2	1
11-10	68-35	215	1	2	95	3	1	2	5
11-10	68-35	216	1	2	88	3	1	2	1
11-10	68-35	217	1	2	81	3	1	2	1
11-10	68-35	218	1	2	81	3	1	2	1
11-10	68-35	219	1	2	80	3	1	2	1
11-10	68-35	220	1	2	82	3	1	2	5
11-10	68-35	221	1	2	70	3	1	2	2
11-10	68-35	222	1	3	140	2	1	2	5
11-10	68-35	223	1	2	79	2	1	2	5
11-10	68-35	224	1	3	135	2	1	2	5
11-10	68-35	225*	4	3	202	2	1	2	2
11-10	68-35	226*	4	3	185	2	1	2	2
11-10	68-35	227*	4	3	180	2	1	2	5
11-10	68-35	228*	4	3	183	2	1	2	2

Date	Field number	Specimen number	Pearlfish species	Life form	Standard length mm	Host species	Reef location	Reef zone	Location in host
11-17	68-36	229*	4	3	210	2	1	2	1
11-17	68-36	230*	4	3	205	2	1	2	1
11-17	68-36	231	4	3	178	2	1	2	2
11-17	68-36	232	1	3	123	2	1	2	2
11-17	68-36	233	1	2	80	2	1	2	1
11-17	68-36	234	4	3	212	2	1	2	1
11-17	68-36	235	1	2	80	2	1	2	1
11-17	68-36	236	1	2	84	2	1	2	1
11-17	68-36	237	1	2	70	2	1	2	1
11-17	68-36	238	4	3	195	2	1	2	2
11-17	68-36	239*	4	3	225	2	1	2	2
11-17	68-36	240*	4	3	173	2	1	2	2
11-17	68-36	241	1	2	72	3	1	3	2
11-17	68-36	242	1	2	80	3	1	3	1
11-17	68-36	243	1	2	74	3	1	3	1
11-17	68-36	244	1	2	84	3	1	3	1
11-17	68-36	245	1	2	69	3	1	3	1

Date	Field number	Specimen number	Pearlfish species	Life form	Standard length mm	Host species	Reef location	Reef zone	Location in host
11-23	68-37	246a	2	2	75	1	2	5	1
11-23	68-37	246b	2	2	70	1	2	5	1
11-23	68-37	247	2	2	72	1	2	5	1
11-23	68-37	248a	2	2	65	1	2	5	1
11-23	68-37	248b	2	2	83	1	2	5	1
11-28	68-38	249	3	2	70	4	2	6	1
11-28	68-38	250	3	3	205	4	2	6	1
11-28	68-38	251	3	3	205	4	2	6	1
11-28	68-38	252	3	3	180	4	2	6	1
11-28	68-38	253	4	3	110	4	2	6	1
12-7	68-39	254	1	2	80	3	1	2	2
12-7	68-39	255	1	2	80	3	1	2	2
12-7	68-39	256	1	2	80	3	1	2	2
12-7	68-39	257	1	2	---	3	1	2	2
12-7	68-39	258	1	2	---	3	1	2	1
12-7	68-39	259	1	2	73	3	1	2	1
12-7	68-39	260	1	2	85	2	1	2	5

Date	Field number	Specimen number	Pearlfish species	Life form	Standard length mm	Host species	Reef location	Reef zone	Location in host
12-7	68-39	261	1	3	135	2	1	2	5
12-7	68-39	262	1	2	78	2	1	2	5
12-7	68-39	263*	4	3	190	2	1	2	2
12-7	68-39	264*	4	3	264	2	1	2	2
12-8	68-40	265	1	2	80	3	1	2	2
12-8	68-40	266	1	2	71	3	1	2	2
12-8	68-40	267	1	2	75	3	1	2	2
12-8	68-40	268	1	2	76	3	1	2	1
12-8	68-40	269	1	2	71	3	1	2	1
12-8	68-40	270	1	2	77	3	1	2	1
12-8	68-40	271	1	2	60	3	1	2	1
12-8	68-40	272	1	2	75	2	1	2	5
12-8	68-40	273	1	3	115	2	1	2	5
12-8	68-40	274a	4	3	135	2	1	2	5
12-8	68-40	274b	4	3	130	2	1	2	2
12-22	68-41	275	2	3	125	1	2	7	1
12-22	68-41	276	2	3	135	1	2	7	1

Date	Field number	Specimen number	Pearlfish species	Life form	Standard length mm	Host species	Reef location	Reef zone	Location in host
12-22	68-41	277	2	1	140	1	2	7	1
12-26	68-42	278	1	2	75	3	1	4	2
12-26	68-42	279	1	2	76	2	1	4	2
12-26	68-42	280	1	2	72	2	1	4	2
12-26	68-42	281	1	3	195	2	1	4	2
12-26	68-42	282	4	3	200	2	1	4	2
12-26	68-42	283	4	3	203	2	1	4	2
12-28	68-43	284	3	2	80	4	2	7	1
12-28	68-43	285	3	3	190	4	2	7	1
12-28	68-43	286	3	3	200	4	2	7	1
12-28	68-43	287	3	3	240	4	2	7	1
12-28	68-43	288	4	3	110	4	2	7	1
12-18	68-43	289	2	3	110	1	2	7	1
12-28	68-43	290	2	3	145	1	2	7	1
12-28	68-43	291a	2	2	60	1	2	7	1
12-28	68-43	291b	2	2	75	1	2	7	1
12-28	68-43	291c	2	3	120	1	2	7	1

Date	Field number	Specimen number	Pearlfish species	Life form	Standard length mm	Host species	Reef location	Reef zone	Location in host
12-29	68-44	292	1	2	84	3	1	2	1
12-29	68-44	293	1	2	85	3	1	2	1
12-29	68-44	294	1	2	93	3	1	2	2
12-29	68-44	295	1	2	70	3	1	2	1
12-29	68-44	296	1	2	82	3	1	2	1
12-29	68-44	297	1	2	75	3	1	2	1
1-1	69-45	298	3	3	270	4	4	7	1
1-1	69-45	299	3	3	212	4	4	7	1
1-1	69-45	300	3	3	205	4	4	7	1
1-1	69-45	301	3	3	160	4	4	7	1
1-1	69-45	302	3	3	173	4	4	7	1
1-1	69-45	303	3	3	140	4	4	7	1
1-1	69-45	304	3	2	60	4	4	7	1
1-17	69-46	305	1	2	76	3	1	2	2
1-17	69-46	306	1	2	80	3	1	2	2
1-17	69-46	307	1	3	110	3	1	2	1
1-17	69-46	308	1	2	68	3	1	2	2

Date	Field number	Specimen number	Pearlfish species	Life form	Standard length mm	Host species	Reef location	Reef zone	Location in host
1-17	69-47	---	-	-	---	-	-	-	-
1-18	69-48	309	1	2	84	3	1	4	1
1-18	69-48	310	1	2	80	3	1	4	1
1-18	69-48	311	1	2	81	2	1	4	5
1-18	69-48	312	1	3	---	2	1	4	5
1-18	69-48	313	1	2	96	2	1	4	5
1-18	69-48	314	1	2	85	2	1	4	2
1-18	69-48	315	4	3	160	2	1	4	2
1-18	69-48	316*	4	3	245	2	1	4	2
1-18	69-48	317*	4	3	205	2	1	4	2
1-18	69-49	318	5	3	117	6	1	2	5
1-20	69-50	---	-	-	---	-	-	-	-
1-25	69-51	319	4	3	205	2	1	2	2
1-25	69-51	320	4	3	195	2	1	2	2
1-25	69-51	321	4	3	200	2	1	2	2
1-25	69-51	322	1	2	96	2	1	2	2
1-25	69-51	323	1	2	87	2	1	2	5

Date	Field number	Specimen number	Pearlfish species	Life form	Standard length mm	Host species	Reef location	Reef zone	Location in host
1-25	69-51	324	1	2	84	2	1	2	5
1-25	69-51	325	1	2	86	2	1	2	5
1-25	69-51	326	1	2	85	2	1	2	5
1-25	69-51	327	1	2	76	2	1	2	5
1-25	69-51	328	1	2	84	2	1	2	5
1-25	69-51	329	1	2	83	2	1	2	5
1-25	69-51	330	1	2	76	2	1	2	5
1-26	69-52	331	4	3	194	2	1	4	2
1-26	69-52	332	4	3	170	2	1	4	2
1-27	69-53	---	-	-	---	-	-	-	-
1-27	69-54	---	-	-	---	-	-	-	-

APPENDIX C - SEASONAL VARIATION OF PEARLFISHES

SEASONAL VARIATION OF INFESTATION BY *CARAPUS HOMEI* IN TUMON BAY

MONTH	*Stichopus chloronotus*			*Holothuria argus*		
	Collected	Infested	% infestation	Collected	Infested	% infestation
April, 1968	110	20	18	---	---	---
May, 1968	49	5	10	---	---	---
June, 1968	235	27	11	71	3	4
July, 1968	46	5	11	113	11	10
August, 1968	25	---	---	23	---	---
September, 1968	21	4	19	44	7	16
October, 1968	44	6	14	32	3	9
November, 1968	95	31	33	62	12	19
December, 1968	75	18	24	47	8	17
January, 1969	35	6	17	76	12	16
TOTAL	735	122	17	468	56	12

SEASONAL VARIATION OF INFESTATION OF *CARAPUS MOURLANI*

MONTH	*Culcita novaeguineae*		
	number of hosts	number of pearlfishes	percent infestation
April, 1968	---	---	---
May, 1968	5	2	40
June, 1968	5	---	---
July, 1968	2	2	100
August, 1968	---	---	---
September, 1968	---	---	---
October, 1968	4	3	75
November, 1968	14	7	50
December, 1968	19	6	31
January, 1969	---	---	---
TOTAL	49	20	41

SEASONAL VARIATION OF INFESTATION BY *CARAPUS PARVIPINNIS*

MONTH	*Thelenota ananas*		
	number collected	number infested	percent infestation
April, 1968	---	---	---
May, 1968	---	---	---
June, 1968	---	---	---
July, 1968	4	3	75
August, 1968	28	18	64
September, 1968	2	---	---
October, 1968	12	10	83
November, 1968	9	4	44
December, 1968	5	4	80
January, 1969	7	7	100
TOTAL	67	46	69

SEASONAL VARIATION OF INFESTATION BY *ENCHELIOPHIS GRACILIS*

MONTH (1968-1969)	*Holothuria argus*			*Thelenota ananas*			*Stichopus chloronotus*		
	number collected	number infested	percent infest.	number collected	number infested	percent infest.	number collected	number infested	percent infest.
April	---	---	---	---	---	---	110	1	1
May	---	---	---	---	---	---	49	1	2
June	71	11	15	---	---	---	235	---	---
July	113	15	13	4	1	25	46	1	2
August	23	---	---	28	6	21	25	---	---
September	44	6	14	2	2	100	21	---	---
October	32	4	13	12	2	17	44	2	5
November	62	14	23	9	1	11	95	2	2
December	47	6	13	5	1	20	75	---	---
January	76	8	11	7	---	---	35	---	---
TOTAL	468	64	13	67	13	19	735	7	1

Holothurians in James Branch, 1969. MS Thesis.

Nomen in Branch	Corrected as per Rowe 1969 and Clark and Rowe 1971
Holothuria atra (Jaeger)	*Holothuria* (*Halodeima*) *atra* Jaeger
Holothuria sp. 1	*Holothuria* (*Mertensiothuria*) *leucospilota* (Brandt)
Holothuria argus (Jaeger)	*Bohadschia argus* Jaeger
Holothuria sp. 2	*Bohadschia bivittata* Mitsukuri
Stichopus chloronotus Brandt	*Stichopus chloronotus* Brandt
Stichopus variegatus Semper	*Stichopus horrens* Selenka
Actinopyga mauritiana (Quoy & Gaimard)	see note below
Thelenota ananas (Jaeger)	*Thelenota ananas* (Jaeger)

Note: Branch does not distinguish *Actinopyga echinites* (Jaeger) which is similar to *A. mauritiana* (Quoy & Gaimard) except that it does not have white or cream markings, is more papillate, and occurs generally more shoreward. *A. mauritiana*, on the other hand is smoother, has white or cream markings (usually) and occurs on the outer reef flat and seaward. It is likely that the *A. mauritiana* reported from the "reef pavement" (table 1) and "coral zone" (Figure 3) was in fact *A. echinites*. J. Doty 7-VIII-75.

Fig. 62. shows *A. echinites* not "*A. mauritiana*."
Fig. 10. " " " "
white probably sand.