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A COMPARATIVE MORPHOLOGICAL AND ECOLOGICAL
STUDY OF TWO SPECIES OF THE SEA URCHIN GENUS
ECHINOMETRA IN HAWAII.

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A COMPARATIVE MORPHOLOGICAL AND ECOLOGICAL STUDY OF TWO
SPECIES OF THE SEA URCHIN GENUS ECHINOMETRA IN HAWAII

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ABSTRACT

Two species or varieties of the sea urchin Echinometra occur in Hawaii. The relationship between the two may be that of polymorphs, polyphenotypes, or separate species. One form (E. oblonga) has black spines, usually has stout, blunt spines, rarely has spindle-shaped spicules in the gonad, has a higher average number of pore-pairs per arc, and has a smaller mean and maximum test length. The other form (E. mathaei) has non-black (usually pink and/or green) spines, rarely has heavy spines, almost always has spindle-shaped gonad spicules, has a lower average number of pore-pairs per arc, and has a larger mean and maximum test length.

The abundance of the black form is greatest in areas of surf action, where it numerically dominates the pink/green form. The pink/green form predominates in areas of less turbulence in tide-pools, on shallow reef flats, and in deeper water.

No significant differences were detected in tolerance to insolation, desiccation and heating, extreme salinity changes, or spine strength per unit cross-sectional area. However, the larger mean diameter of the black spines does make the breaking resistance of the average black spine greater than that of the average pink/green spine.

Polyphenotypic variation under the different ecological conditions was investigated with respect to spine color, spine shape, and gonad spicules and judged not responsible for the observed morphological differences between the two forms.

Monthly samples collected for 2 years indicate that the spawning

period of both forms occurs in the spring (March-June) with a second, minor spawning in December. Mature gametes were found in a few individuals at all times of the year. The December spawnings were perhaps induced by extremely heavy rainfalls. Initiation of the spring spawning season appeared to be correlated with increasing temperature and internal nutrient supply.

Homogametic fertilizations (sperm and eggs from the same color form) were over 94% successful. Heterogametic fertilizations (sperm and eggs from different color forms) were about 10% successful. Sperm from an unusual urchin with intermediate morphological characteristics produced 64% fertilizations with one form and 100% fertilizations with the other. Larvae from all crosses survived normally for 6 days, after which they died from starvation. No attempt was made to rear larvae to metamorphosis.

Examination of the morphological variation in Echinometra in the rest of the Pacific Ocean shows less distinction between the varieties, with more overlap in morphological features. Stout, blunt spines and an absence of spindle-shaped gonad spicules are correlated with each other, but spine color differences (black versus non-black) are not consistent with this separation. Monomorphic populations of Echinometra occur in the Gulf of Suez, Western Australia, Japan, and the eastern Pacific.

The definite habitat specialization, extensive non-ecophenotypic morphological distinctness, and the gamete incompatibility strongly indicate that the two forms in Hawaii are separate species. The

varieties of Echinometra found in the South Pacific are evidently polymorphs of a single species. The distinctness of the two Hawaiian species is probably indicative of character displacement.

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INTRODUCTION

Characteristically, populations of animals and plants exhibit variability in color and morphology, behavior, tolerance to environmental factors, growth rate, etc. Knowledge of the fundamental sources of this variability is of great importance to the classification and comprehension of ecological interrelationships of these organisms. Two similar forms may be distinct species, polymorphs of the same species or polyphenotypes of the same species. In this paper these terms are defined as follows. Species are groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups (Mayr, 1940). Polymorphism (genetic) is the occurrence together in the same habitat of two or more discontinuous forms or phases of a species in such proportions that the rarest of them cannot be maintained merely by recurrent mutation (Ford, 1940). Polyphenism is the occurrence of several phenotypes in a population, the differences between which are not the result of genetic differences (Mayr, 1963).

One provocative example in which the extent of the gene pool is not clear has been provided by the sea urchin genus Echinometra in Hawaii. The genus is represented by two forms which have been regarded as separate species by some authors (e.g. Edmondson, 1946) and polymorphs by others (e.g. Mortensen, 1943). Both are found in the intertidal and subtidal zones. They are easily distinguished by the color of the spines: one form is black (Fig. 1) and the other is pink, green, or some combination of these two colors, but never black (Fig. 2). In other parts of their range, however,

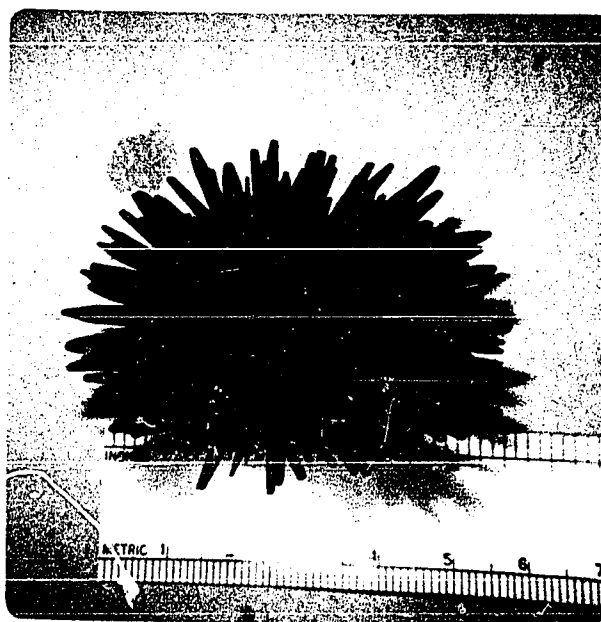


Figure 1. The black form of Echinometra in Hawaii.

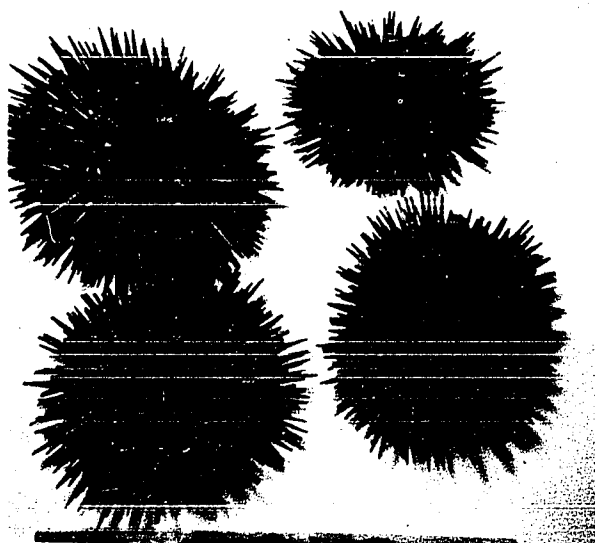


Figure 2. The pink/green form of Echinometra in Hawaii.

intergrades have been reported (Mortensen, 1943).

All of the genera of the family Echinometridae except Echinometra are confined to the Indo-Pacific, and the greatest number of genera and species are found in the Indonesian area (Mortensen, 1943). It is customary to say that most marine animals in the Indo-Pacific have had their center of evolution in the Indonesian area and have since radiated into the more remote parts of the Indian and Pacific Oceans (Ekman, 1953). However, Ladd (1960) has suggested instead that the Pacific Island basin from Hawaii to New Guinea was formerly more densely dotted with islands, and that a single well-developed fauna existed throughout this paleo-archipelago. The present rich fauna of the Indonesian area and the impoverished fauna of the Pacific Islands has resulted from extinctions in the subsiding, ever-changing environment of the Pacific Islands. Thus, the locality of origin of the family Echinometridae and genus Echinometra is open to question.

The distribution of the living members of the genus Echinometra is tropicopolitan (Fig. 3). Five species were recognized by Mortensen (1943), and the characters by which he identifies them are presented in Table 1. The species distributions as shown in Figure 3 are those principally established by Mortensen (1943), but modified by the findings of Kier (1966), Clark (1948), and F. Julian Fell (personal communication). Kier extended the range of E. viridis to Dominica, Clark extended the range of E. mathaei to the Revillagigedo and Galapagos Islands, and Fell limited the range of E. insularis to Easter Island. The limits of distribution of Echinometra in the East China Sea and in the Gulf of Mexico are estimated.

The distribution of Echinometra fossils is shown in Table 2.

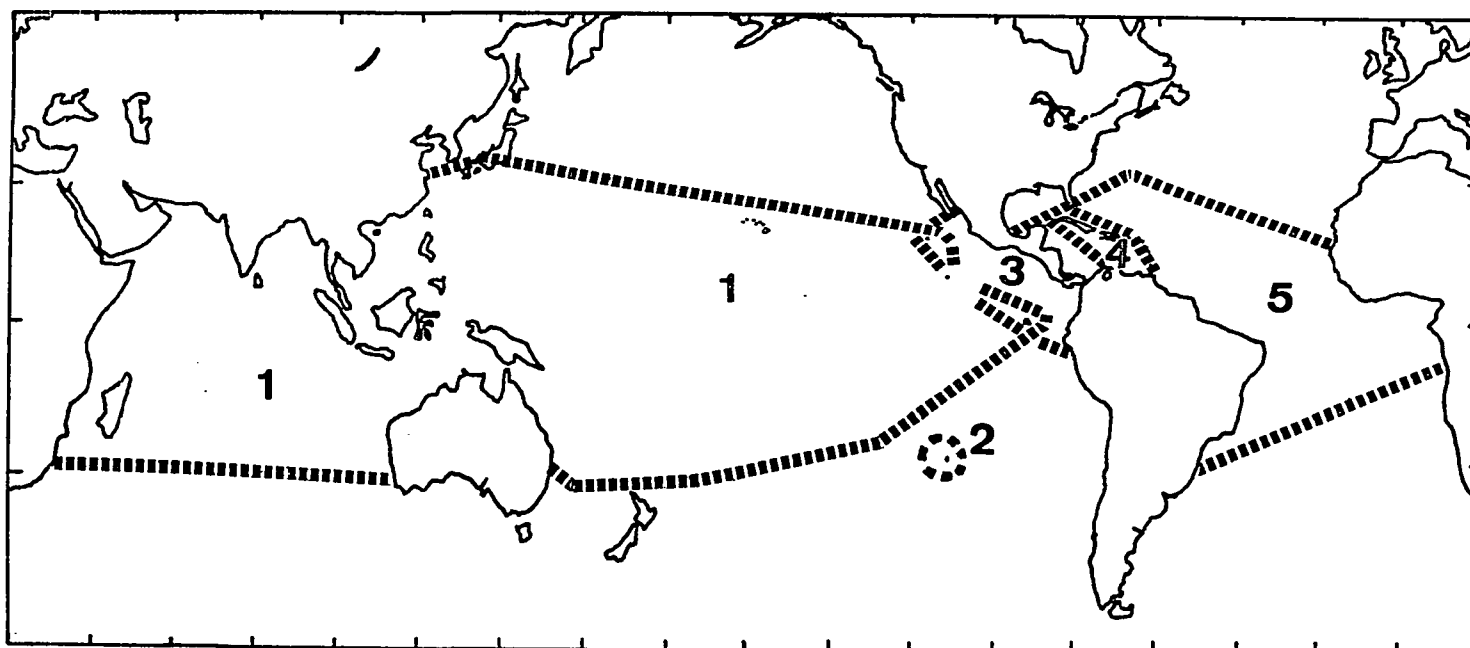


Figure 3. The distribution of the species of Echinometra recognized by Mortensen (1943). (Based on Mortensen, but modified by the findings of Kier (1966), Clark (1948), and F. Julian Fell (personal communication).)
 1. E. mathaei 2. E. insularis 3. E. vanbrunti 4. E. viridis
 5. E. lucunter.

Table 1

The Species of Echinometra Recognized by Mortensen (1943)
and Their Identifying Characters

Species	Location	Pore- pairs per arc	Spine color	Other characters
<u>E. mathaei</u>	Indo-Pacific	4-5	white to black & all intermediates, often with white tips	
<u>E. insularis</u>	Easter Island	5-6	deep purplish or reddish	apical plates with several tubercles
<u>E. viridis</u>	Caribbean Sea	5-6	brownish, green distally, usually purple-tipped	apical plates almost naked
<u>E. lucunter</u>	Atlantic	6-8	white to black & all intermediates	high auri- cular tag
<u>E. vanbrunti</u>	East Pacific	6-8	uniform dark purple or black	low auri- cular tag

Table 2

Fossil Species of Echinometra, as
Listed in Mortensen (1943)

Species	Location	Geologic epoch or period	Pore-pairs per arc
<u>E. thomsoni</u>	India	Eocene	7
<u>E. miocenica</u>	France	mid-Miocene	4
<u>E. prisca</u>	St. Barthelemy, Anguilla, & Cuba	Tertiary	3 sometimes 4
<u>E. sp.</u>	Java, Indonesia	Miocene	4
<u>E. sp.</u>	Ceram, Indonesia	Pliocene	4
<u>E. vanbrunti</u>	California	Pliocene	6-8
<u>E. mathaei</u>	Gulf of Suez	Pleistocene	4-5
<u>E. mathaei</u>	Red Sea	?	4-5
<u>E. mathaei</u>	Farasan Islands, Red Sea	?	4-5

A smaller number of pore-pairs (3 or 4) in an arc is considered the primitive condition (Mortensen, 1943). The occurrence of the most primitive form in both the Indo-Pacific and the Atlantic implies that the present species probably have arisen from a single progenitor. On the other hand, the oldest fossil yet found, E. thomsoni, has seven pore-pairs per arc and was found in India.

The Echinometra that are found in the Indo-Pacific have frequently been separated into a number of different species or even genera (Döderlein, 1906). The two most persistent separations have been into the species E. mathaei (Blainville, 1825) and E. oblonga (Blainville, 1825). A brief review of the major taxonomic studies of these two forms follows. A complete historical review can be found in Mortensen (1943).

Echinometra mathaei was described by Blainville (1825) on the basis of a single rather small urchin from Mauritius. He described it as white, with dull green spines, four pore-pairs per arc and eight interambulacral tubercle rows. Echinometra oblonga was described in the same paper on the basis of two rather small individuals the locality for which was unknown. They were described as "blanche, peut-etre violacee" (white or perhaps violet), with five pore-pairs per arc and six interambulacral tubercle rows. The white color undoubtedly referred to the denuded test in both species.

The next author to describe the species was Michelin (1845). It is not certain whether he saw the original types or not. He recognized two varieties of Blainville's E. mathaei (incorrectly

called E. lucunter by him for unclear reasons), violacea and albida, but gave no distinguishing characters. He separated E. oblonga (incorrectly called E. mathaei by him, again for unclear reasons) from the above forms on the basis of its blue-green (or sea-green), short, strong spines, more numerous large tubercles, and more ovoid form.

Agassiz (1872-1874) examined a number of specimens of both species including the original type specimens. He described E. mathaei (called E. lucunter by him, following the nomenclatural mistake of Michelin: the mistake persisted intermittently until corrected by Mortensen in 1903) as having the spines tipped with a different color than the color of the spine base, the milled ring generally brilliant white, the madreporite only slightly larger than the other genital plates, and the actinal (gill) cuts well-marked. E. oblonga was distinguished by short, thick spines which were frequently swollen in the center, uniform spine color, madreporite much larger than the other genital plates; the actinal cuts barely marked.

Döderlein (1906) agreed with Agassiz that the stout spines and the uniform spine color were characteristic of E. oblonga, but also reported that this species possessed tri-radiate spicules in the pedicels, whereas E. mathaei had none. He considered this difference so striking that he erected a new genus, Mortensenia, for the old E. oblonga.

Clark (1912), whose material came mostly from the Hawaiian Islands and the Tuamotu Islands, characterized E. oblonga as usually

black, though rarely dull purplish or even dull red and with short, stout and usually blunt spines. He described E. mathaei as very rarely black and with thin, usually pointed, spines.

Edmondson (1946), who worked exclusively with Hawaiian individuals, identified E. oblonga as dark purple or black and with typically shorter, thicker, and blunter spines than E. mathaei. Individuals of the latter species could be green, gray, or reddish brown, but not black.

Finally, Mortensen (1943) considered the two forms as varieties of the same species, although he gave them trinomial names, as if they were subspecies. Although he included no separatory key, he mentioned that E. mathaei oblonga is often black, with stout spines, tri-radiate spicules in the pedicels, and the aboral spines much longer than the others. Mortensen (loc. cit.) also suggests that the specific epithet E. oblonga should not be applied to the black variety of the Indo-Pacific Echinometra because Blainville said in his original description of E. oblonga that its color was "blanche, peut-etre violacee." However, it seems obvious to me that this description applied only to the denuded test, and the use of Blainville's name for the black form is deemed appropriate.

Mortensen also recognized Michelin's (1845) variety violacea and characterized it as having strongly developed tridentate pedicellariae and a highly characteristic purplish color. He reported that E. violacea is apparently restricted to Mauritius, Madagascar, and the Natal Coast of Africa.

It is apparent from these investigations that there is a lack

of agreement on the identity of E. oblonga and E. mathaei as species, and confusion with respect to the proper distinguishing characters. Only those character differences listed by Mortensen seem to be at least partially valid; however, as mentioned, he regarded the two forms as varieties. Nevertheless, in Hawaii the two forms can be separated readily on the basis of spine color alone. Only two specimens that were intermediate in color have been found, and these were also intermediate in other characters. It seems that either the two forms are more distinct in Hawaii or their distinctness in other areas is less obvious and has been further obscured by lumping of geographic varieties. At any rate, it is unclear whether the two color forms (both in Hawaii and in the rest of the Indo-Pacific) are members of the same gene pool or not. In presenting the results of the present study, the two forms will be referred to as black and pink/green.

There are four features of the populations of Echinometra that can be investigated and used to assist in making a decision as to the relationship of the gene pools of the two forms. The degree of morphological variation may indicate their degree of reproductive separation. Variation can be expected in both polymorphic forms and in separate species, but a high degree of variation indicates separate species. As Mayr (1969) has pointed out, "There are severe limits to the amount of genetic variation that can be accommodated in a single gene pool." The degree of habitat separation can also indicate the degree of reproductive separation, in the same fashion as the degree of morphological variation. A lack of coincidence of

reproductive periods is definite evidence of reproductive isolation, although the converse is not necessarily true. Finally, and most certainly, incompatibility of gametes indicates reproductive isolation.

COLLECTION SITES

Echinometra can be found on most hard substrates exposed to breaking waves in the intertidal and subtidal areas around the Hawaiian Islands. They also occur in tidepools that are not too isolated from the ocean. The apparent habitat requirements are for a solid, silt-free substrate in shallow water with fairly good circulation. Usually they inhabit elongate holes or channels in the rock, as is shown in Figure 4. I have observed populations on the island of Oahu at Laie Point, Kapapa Island and Checker Reef in Kaneohe Bay, Makapuu Point, Blowhole Coast, Hanauma Bay, Portlock, Black Point, Waikiki, Maili Point, Kaneilio Point, Makua, and Pupukea (Figure 5). I have been told there are also large populations at Mokapu Peninsula and Ewa Beach. On the island of Hawaii I have observed populations of Echinometra at Koaie, Kealakekua Bay, and Honaunau Bay (Figure 6). There are only two locations where I would expect the urchins to occur, but where I could find none, at Kahe Point, Oahu and outside Palemano Point (Kealakekua Bay), Hawaii.

At locations where populations have been seen, the substrate may be basalt, tuff, eolianite, fossil coral reefs, living coral (Pocillopora meandrina Verrill, Porites compressa Vaughn), dead coral, calcareous rubble, and igneous rubble. Generally the individuals are found in shallow cavities or crevices, or occasionally under large rocks. It is almost always necessary to break the rock to extract the urchins. In calcareous material, especially, the cavities they inhabit are so rounded and

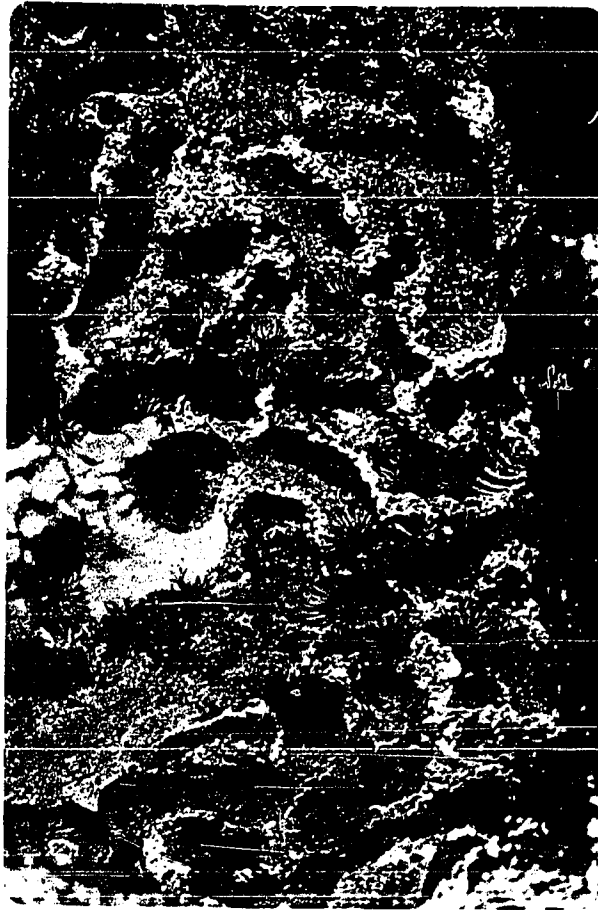


Figure 4. Echinometra in holes on Blowhole Coast, Oahu.

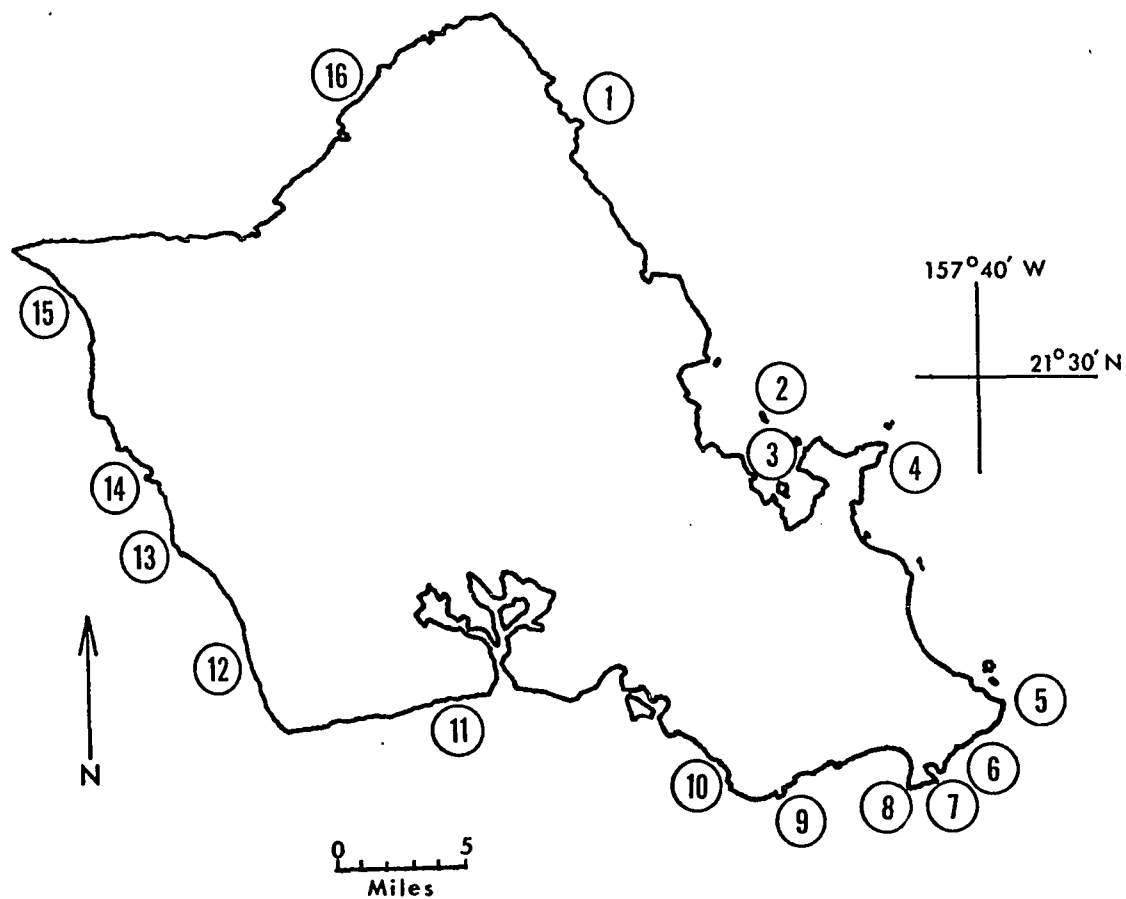


Figure 5. Locations on the Island of Oahu Mentioned in the Text.

- | | |
|---------------------|--------------------|
| 1. Laie Point | 9. Black Point |
| 2. Kapapa Island | 10. Waikiki |
| 3. Kaneohe Bay | 11. Ewa Beach |
| 4. Mokapu Peninsula | 12. Kahe Point |
| 5. Makapuu Point | 13. Maili Point |
| 6. Blowhole Coast | 14. Kaneilio Point |
| 7. Hanauma Bay | 15. Makua |
| 8. Portlock | 16. Pupukea |

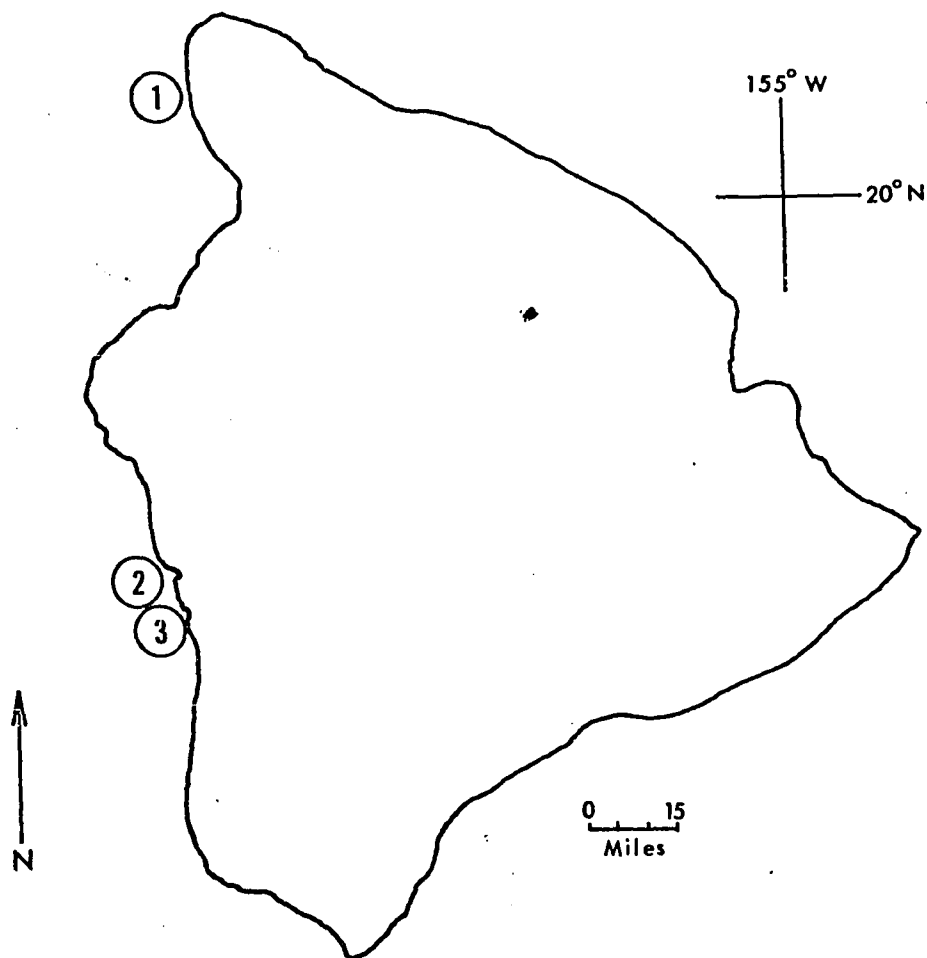


Figure 6. Locations on the Island of Hawaii Mentioned in the Text.

1. Koaie (Mahukona)
2. Kealahou Bay
3. Honaunau Bay

smooth and consistently of average-urchin-size that it seems impossible that the urchins are not responsible for deepening shallow natural depressions, or at least trimming the sides of natural holes. It is also obvious that a hole is not formed by a single urchin, but could only be the result of activity of generations of urchins. Unfortunately, I know of no experimental work to substantiate the numerous references in the literature to rock-boring by Echinometra.

Quantitative studies of the habitat of the two species were made on transects at Kealakekua Bay, Honaunau Bay, Koaie, Kaneilio Point, Pupukea, Maili Point, and Kapapa Island. The transects at Kealakekua Bay, Honaunau Bay, and Koaie were all on coralline algae (Porolithon sp.)- encrusted basalt of prehistoric lava flows. The surf at these leeward locations is generally a non-breaking swell; however, winter Kona storms in particular can form breaking waves there. Kaneilio Point, at Waianae on the lee coast of Oahu, is subject to the same surf conditions. The substrate is composed of the consolidated calcareous marine sediments of an emerged reef. Pupukea, on the north shore of Oahu, is of emerged reef material too. During the summer it frequently is washed by only a gentle swell, but during the winter it experiences regular heavy surf. Maili Point, on the lee coast of Oahu also, consists of a calcareous reef veneer resting on a shallow lava base. The substrate inside the surf zone is calcareous material; beyond this the substrate is predominantly lava. A constant low surf breaks on the offshore reef edge. The

Kapapa area will be described fully later. (The geologic compositions of the Oahu sites were taken from Stearns (1939). Additional notes on Hawaiian waves can be found in Moberly and Chamberlain (1968).)

The reproductive cycles of the two species were studied at Kapapa Island and Black Point. These two areas were selected because of the large populations of both species found there, and the relative ease with which they could be collected. Sampling was made at approximate 30-day intervals, beginning in January 1967, and ending in January 1969. Twenty animals including both color forms were collected at each of the two localities. The mean monthly collecting date at Black Point was the 23rd, while that at Kapapa was the 29th. Most of the urchins were average to large size (30-60mm test length), but a few smaller individuals (14-29mm test length) were included.

The Kapapa site is located on the southeast (lee) side of Kapapa Island, which stands on the seaward side of a large shallow flat in Kaneohe Bay. The tradewind-driven surf strikes heavily on the outside of the island, but only secondary waves of a meter or less in height break in the collecting area. These translation waves are almost always present, and cause a great deal of water movement back and forth. The depth is about 0.6 to 1.6 m. The bottom is composed of lithified sand dune, as is Kapapa Island (Stearns, 1939). The urchins are located in elongate holes somewhat larger than themselves. The algae are predominantly brown algae of the genera Sargassum, Padina, Dictyopteris, Dictyota, and Pocockiella. The red algae, represented by Jania, Acanthophora,

Hypnea, Laurencia, and Spyridia, are less conspicuous, and the only green alga is Dictyosphaeria.

The Black Point site is located on the east side of Black Point (Kupikipikio Point), on the reef flat inside the major breaking surf but regularly subject to a secondary surf of about 0.3 m. The urchins are located in holes slightly larger than themselves in the sides of low mounds of lithified calcareous material. The depth is about 0.3 m, but the animals are rarely, if ever, exposed for extensive periods. The location is about 60 m from the beach and about 15 m from the reef edge. Fresh-water seeps at a fairly constant rate through the sand of the beach, and probably as a result of this, most of the algae throughout the collecting area are green algae. Ulva reticulata, U. lactuca, and U. fasciata are found in large quantities, and smaller stands of the red algae Acanthophora and Centroceras are also present.

The morphological variation between the two forms was recorded for a large proportion of the urchins collected at Kapapa Island for the reproductive cycle study. Supplemental measurements were made on additional urchins from Kapapa Island, Maili Point, and Makapuu Point.

Finally, the compatibility of the gametes of urchins of the two forms were investigated in specimens from Kapapa Island. Two separate investigations were conducted two weeks apart in the fall of 1969.

HABITAT SPECIALIZATION

Methods and results

Preliminary observations in Hawaii by Dr. T. A. Ebert and myself indicated a possible vertical separation of the habitats of the two color forms. Because of these observations a series of quadrat counts across Echinometra populations were made along transect lines extending out from the shoreline into deeper water. Beginning at the shoreline, a 0.25 m² quadrat (25 x 100 cm) of welded wrought iron was selectively placed over groups of urchins with the long side of the quadrat parallel to the shoreline. The numbers of urchins of each color form were recorded on an underwater slate. The horizontal distance from shore was measured with the quadrat, and the depth was estimated by holding the quadrat vertically. In water deeper than 3 m, the depth was read from a wrist depth gauge with 0.3-0.6 m accuracy. The transects were continued until a depth was reached at which groups of urchins were no longer seen. The transects were generally located in areas where the wave action was not too strong for diving, but which had large populations of urchins. The results of these transects are presented in Figures 7-11.

Discussion

The transects at the Ashihara cabin, Honaunau Bay, Koaie, Kaneilio Point, and the first two at Pupukea are all similarly located on steeply sloping shorelines which rise vertically above the waves. The black color form predominates in the shallow depths, with the greatest numbers occurring from mean sea level to 1 or 2 m

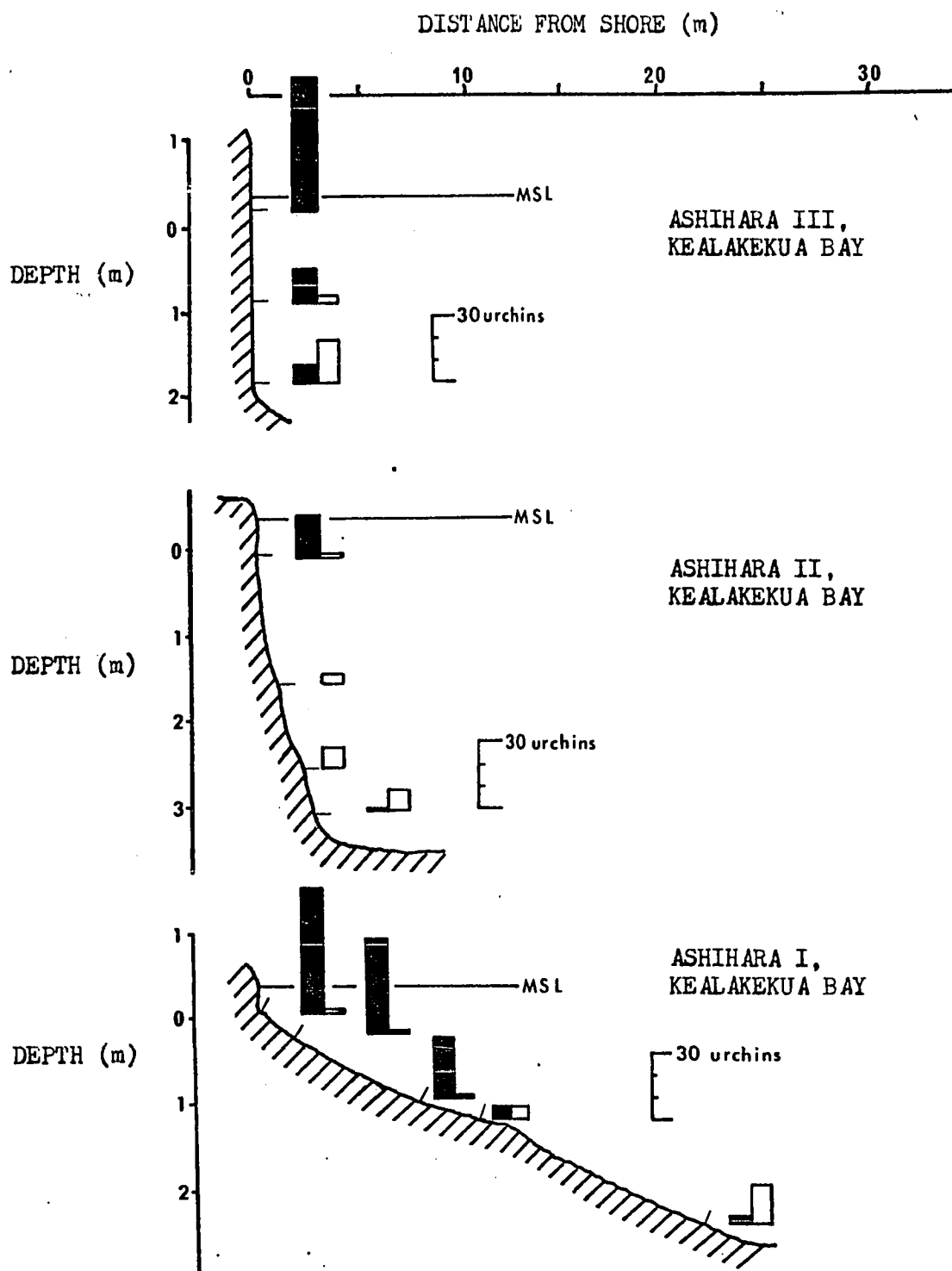


Figure 7. Numbers of Each Color Form of *Echinometra* per 0.25 m^2 Along Transects in Kealakekua Bay, Hawaii. Shaded and Open Bars Indicate Black and Pink/Green Urchins, Respectively.

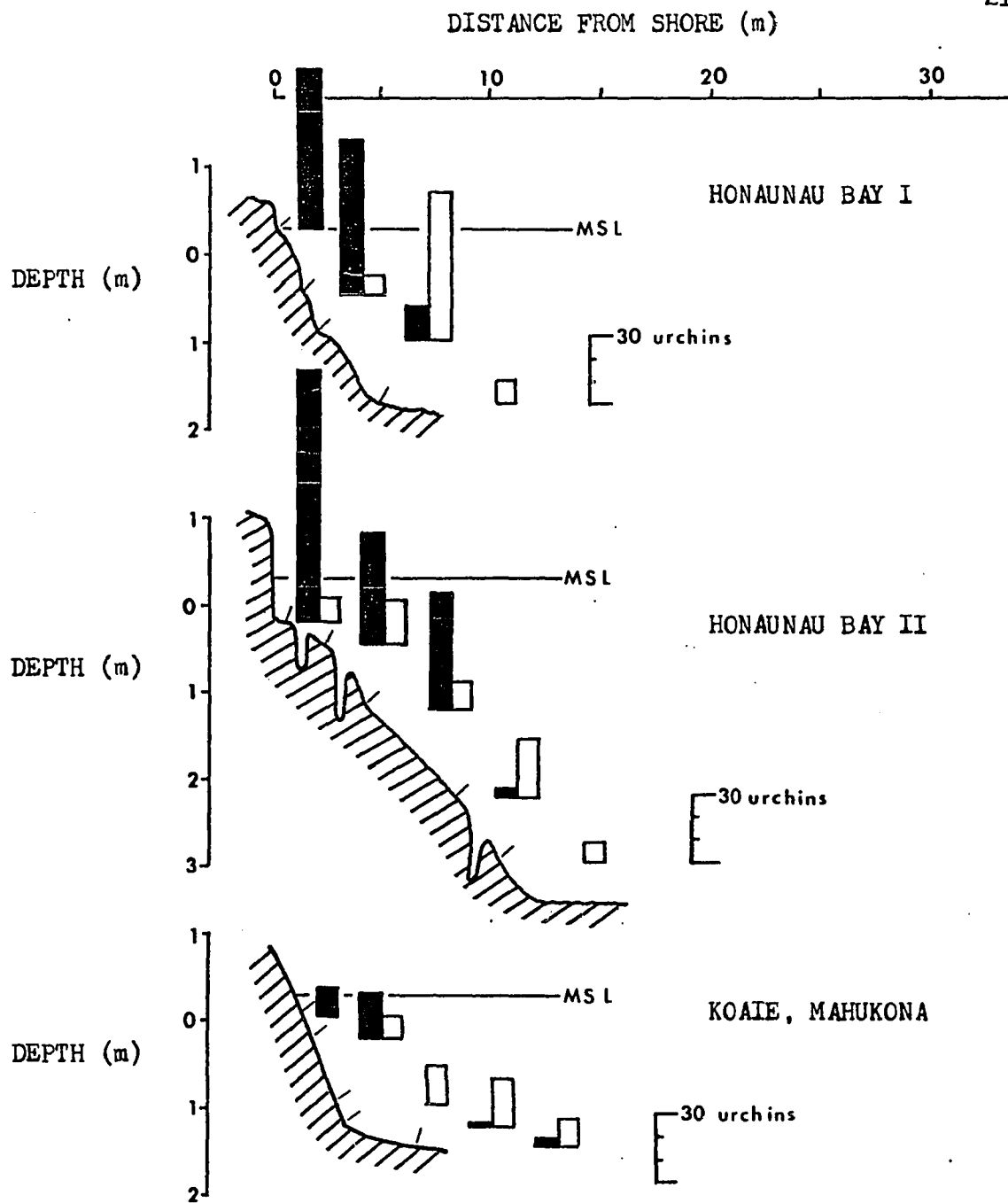


Figure 8. Numbers of Each Color Form of Echinometra per 0.25 m² Along Transects on the Island of Hawaii. Shaded and Open Bars Indicate Black and Pink/Green Urchins, Respectively.

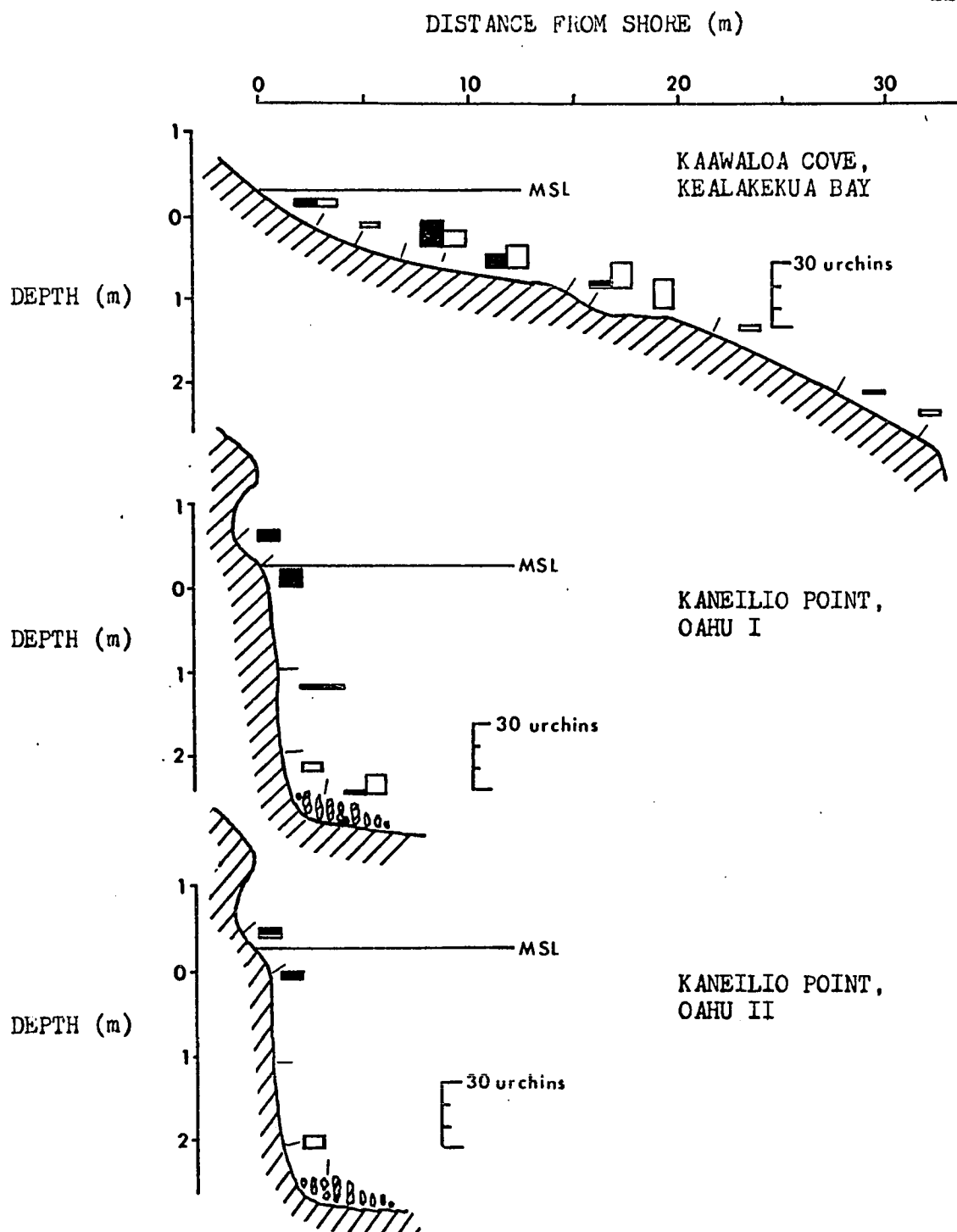


Figure 9. Numbers of Each Color Form of *Echinometra* Per 0.25 m^2 Along Transects on the Islands of Hawaii and Oahu. Shaded and Open Bars Indicate Black and Pink/Green Urchins, Respectively.

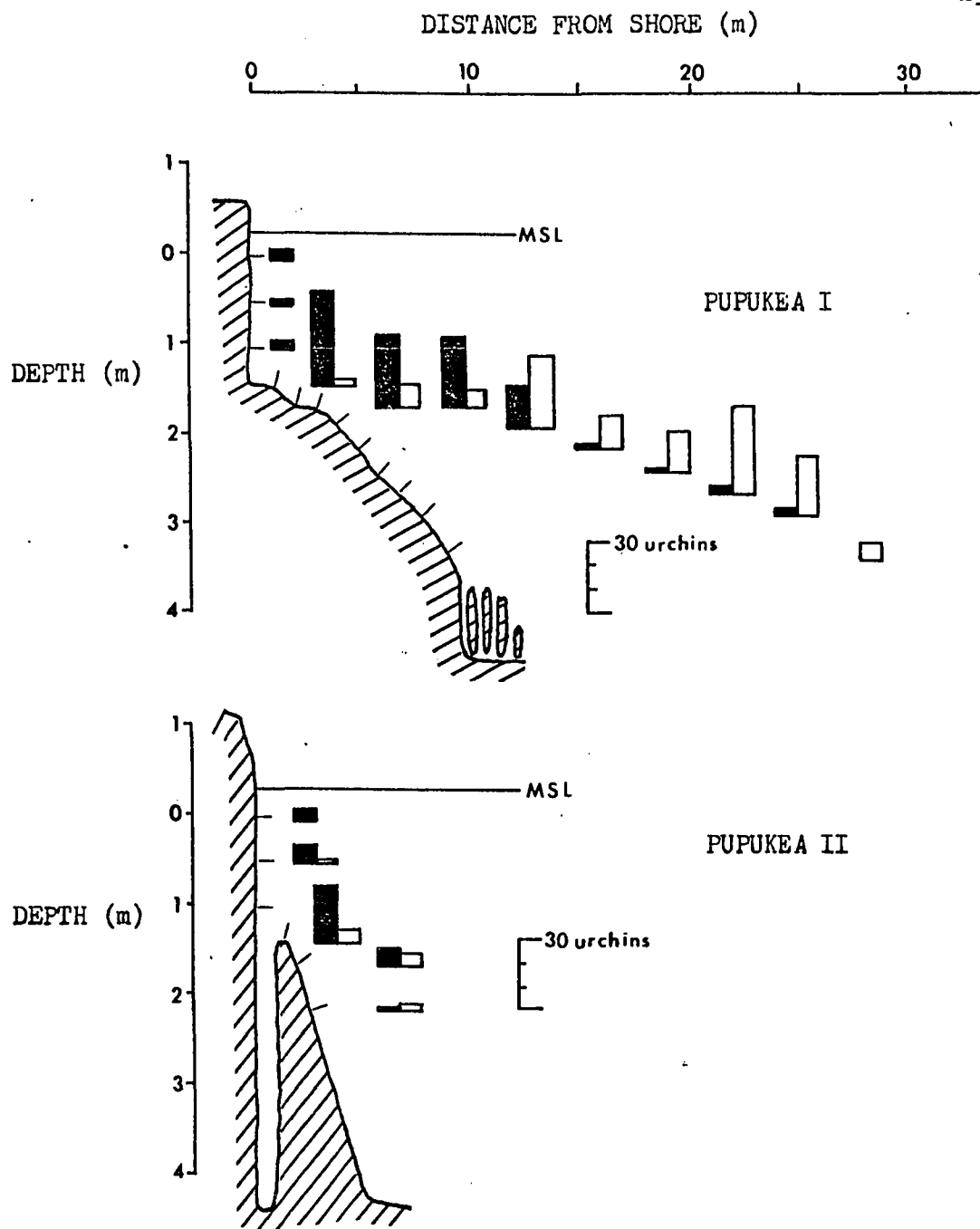


Figure 10. Numbers of Each Color Form of *Echinometra* per 0.25 m² Along Transects at Pupukea, Oahu. Shaded and Open Bars Indicate Black and Pink/Green Urchins, Respectively.

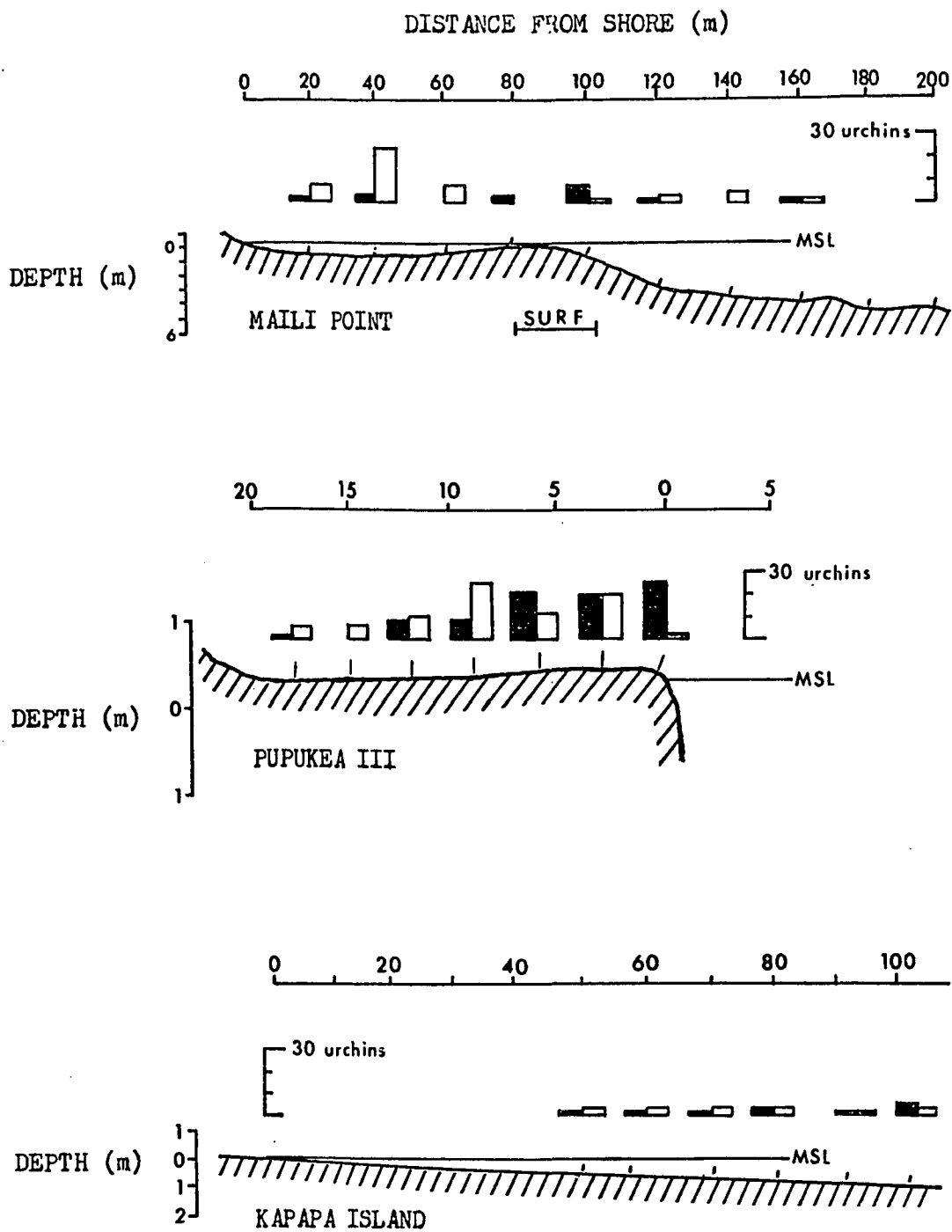


Figure 11. Numbers of Urchins of Each Color Form of Echinometra per 0.25 m^2 Along Transects on Oahu. Shaded and Open Bars Indicate Black and Pink/Green Urchins, Respectively.

below MLLW (datum). The pink/green form predominates at greater depths, with highest numbers occurring from 2 to 3 m below MLLW (datum). Despite the obvious differences, it is difficult to state whether this distribution is correlated with depth or with distance from the zone of greatest water movement, i.e., where the surf breaks. Examination of transects made in areas where the shoreline slopes up very gradually are more instructive. In these situations the surf breaks some distance from the shoreline, and the energy of the wave is dissipated over a wide zone. The transects at Kaawaloa Cove and Kapapa Island are examples of this type. Here a high percentage of pink/green forms can be found even in shallow water. Moreover, the zone where the highest percentage of black urchins is found is some distance from the shoreline -- about where the main surf breaks. This is corroborated to some extent by the distribution along fairly horizontal surfaces, as exemplified by the transects at Maili Point and Pupukea III. Although the difference in elevation is slight, the highest percentages of black forms are found closest to the wave-break.

The correlation between strong surf action and the relative abundance of the black color form is also supported by the predominance of the pink/green form in tidepools. The genus occurs only in tidepools with good circulation, and the black form is found only in those with almost continual circulation. The pink/green form, on the other hand, is frequently found in somewhat quieter pools where the black form is absent. A large tidepool at Pupukea, Oahu and a smaller one in front of the park headquarters at Honaunau, Hawaii

are examples of this situation. I have never seen a tidepool containing only the black form or even a predominant number of the black form.

Additional transects showing the distribution of Echinometra in Hawaii are presented in Doty (1969) and the average of a number of transects is given in Doty (1968). All of these transects utilized a square 1 m² quadrat and were extended down to a depth of 20 to 30 m. The numbers of the black form and the pink/green form dropped sharply below about 2.5 m and 7.5 m respectively, although occasional individuals of the latter were found down to 12.2 m. The deepest black individual I have seen was at 4 m at Maili Point. Mr. James McVey (personal communication) has seen pink/green individuals at 20 m on an artificial reef off Waianae, Oahu.

The distribution of black Echinometra lies in a zone just below that of the shield urchin, Colobocentrotus atratus, although the two may overlap on occasion. The levels of both distributions are higher above MLLW (datum) on extremely exposed coasts, such as the precipitous Blowhole coast of Oahu. Moreover, generally the numbers of both color forms of Echinometra counted in the quadrats were greater on horizontal surfaces than they were on adjacent vertical surfaces.

MORPHOLOGICAL AND PHYSIOLOGICAL VARIATION

Methods and Results

A series of dimensions and morphological features were measured or recorded for a minimum of 29 urchins of each color form. Larger numbers (up to 289) were examined in the case of some of the morphological characters.

The colors of the non-black form were further separated into five arbitrary categories: pink, greenish-pink, pink and green, pinkish-green, and green. The color of the tip of the spine was listed if it varied from that of the base. The color of the test was noted after it had been cleaned in bleach (sodium hypochlorite). The length of the longest spine (from the milled ring to the tip) was measured to the nearest millimeter. The predominant shape of the spines on each urchin was classified into one of three categories of spine shapes. The classification was made by comparison with a selected series of type spines, the measurements for which are given in Table 3. The maximum length and width of the test were measured to the nearest millimeter with a hand-made acrylic plastic sliding caliper. The maximum height of the test was measured with a dial micrometer caliper the jaws of which were held perpendicular to the oral-aboral axis. The same caliper, in conjunction with a dissecting microscope, was used to measure the maximum diameters of the apical system, periproct, and peristome. The number of spine tubercles located on the apical system was counted, and the insertion of any ocular plates was noted. The number of pore-pairs in each of five

Table 3

The Characteristic Spine Shape Categories
Applied to Echinometra in the Present Study

Type	Description	<u>Diameter at base*</u> Length**	<u>Diameter at $\frac{1}{2}$ of length</u> Diameter at base
Slender	Pointed, slender, concave or straight sided	0.12 (0.10 to 0.14)***	0.68 (0.54 to 0.85)
Inter- mediate	Intermediate between slender and heavy	0.16 (0.13 to 0.18)	0.73 (0.64 to 0.83)
Heavy	Heavy, blunt, stout, convex sided	0.18 (0.17 to 0.20)	0.85 (0.83 to 0.92)

* Diameter at base measured just above the milled ring.
(All diameters measured with a drill gauge.)

** Length of spine from milled ring to tip.

*** Mean and range of values.

arcs was counted in each ambulacrum, starting with the arc at the ambitus and continuing aborally. It was noted if the ambulacra were expanded at the edge of the peristome (petaloid). The shape of the auricular tag was classified as low, medium, or high, on the basis of whether its greatest height was less than, equal to, or greater than its width just above the fusion of the auricles. The spicules found in the aboral tubefeet, the gonad, and the gut wall were examined and classified after a portion of the tissue in question had been dissolved in bleach (sodium hypochlorite). The most common types found are shown in Figure 12. Recognition of the spicule types was based on the shape or pattern and size of the spicule. Finally, the ratios of width to length, height to length, apical system diameter to test length, peristome diameter to test length, spine length to test length, and periproct diameter to apical system diameter were calculated.

The taxonomic utility of the various morphological characters is categorized in Table 4. Values obtained from non-Hawaiian urchin measurements were also used in constructing the table.

Significant differences between the two forms in Hawaii were noted in spine color, predominant spine shape, type of gonad spicule, average number of pore-pairs in an arc, and average and maximum test length. The recorded differences are presented in Table 5 and Figure 13.

The predominant spine shape for a total of 224 urchins of each color from Kapapa Island, Oahu, was classified. The classification results were subjected to a 2 x 3 contingency Chi-square test. The

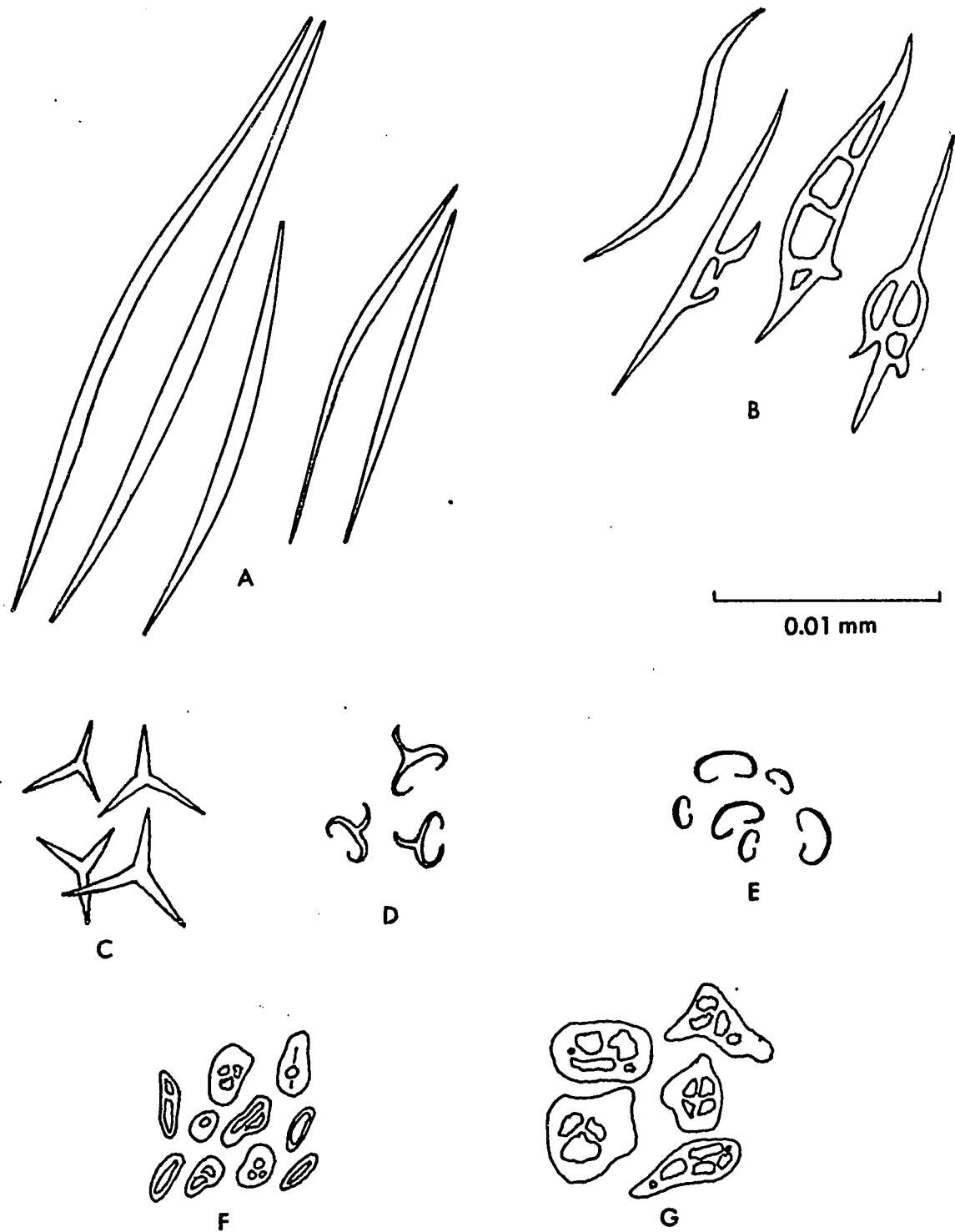


Figure 12. Some types of spicules found in Echinometra in the Indo-Pacific. A. spindle (gonad) B. irregular (gonad) C. tri-radiate (pedicel & gonad) D. curved tri-radiate (pedicel) E. bihamate (all tissues) F. perforate 1 (gut wall) G. perforate 2 (gut wall).

Table 4

Analysis of Morphological Characters of Taxonomic
Value in Echinometra in the Pacific

A. Characters showing differences between the two Hawaiian forms:

spine color;
spine shape;
spindle-shaped gonad spicules;
frequency distribution of pore-pairs per arc;
size frequency distribution.

B. Characters possibly showing differences between urchins from
Hawaii and urchins from other localities:

spine color;
spine tip color relative to spine base color;
average number of pore-pairs per arc.

C. Characters showing allometric variation, but perhaps informative:

spine shape;
frequency of occurrence of insert ocular plates;
number of tubercles on the apical system;
auricular tag shape;
height to length ratio;
apical system length to test length ratio;
periproct diameter to apical system length ratio;
peristome diameter to test length ratio;
maximum spine length to test length ratio.

Table 5

The Frequency Distribution of Morphological Characters in
the Two Color Forms of Echinometra at Kapapa Island, Oahu

Character	Non-black form	Black form
Spine color (289)*:		
pink	52%	
greenish-pink	7%	
pink and green	12%	
pinkish-green	9%	
green	29%	
black		100%
Spine shape (224):		
slender	37%	1%
intermediate	61%	16%
heavy	2%	83%
Spindle-shaped gonad spicules (183):		
present	97%	0.5%
absent	3%	99.5%
Pore-pairs per arc (29):		
three	0.4%	1%
four	84%	68%
five	15%	30%
six		0.2%

* Total number of urchins of each form examined for the character.

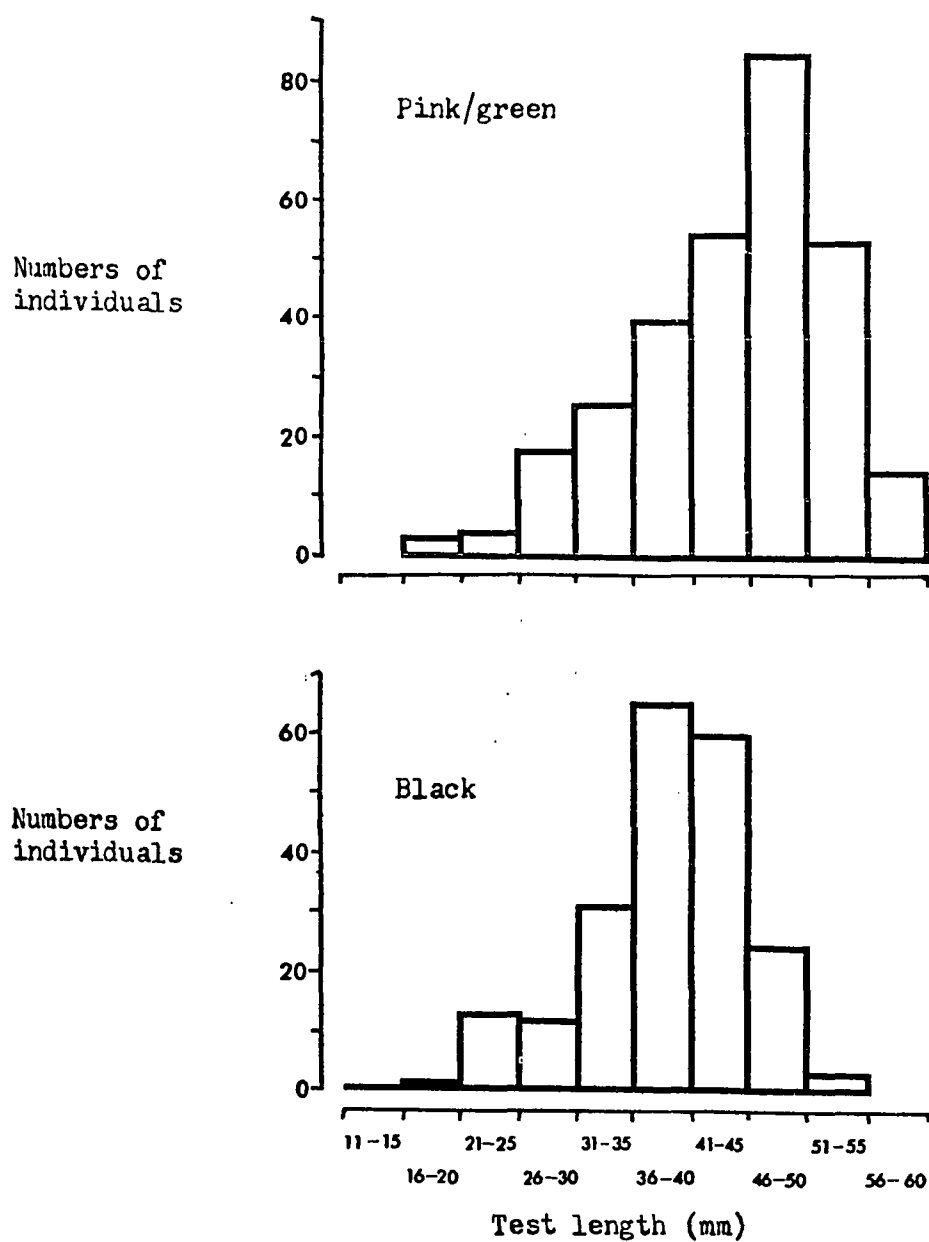


Figure 13. The frequency distribution of test lengths of 389 pink/green and 211 black Echinometra collected at Kapapa Island, Oahu.

null hypothesis tested was that there was no difference between the color forms in the numbers of urchins with each of the three spine types. A value of 309.0 was obtained; this is significant at the 0.001 level. The null hypothesis was therefore rejected.

The presence or absence of spindle-shaped spicules in the gonad was scored for 183 urchins of each color. All were collected at Kapapa Island, and the majority were collected in the course of the reproductive cycle study. In addition to those shown in Table 5, a few individuals from the reef flat at Maili Point, Oahu, and a basalt tidepool at Makapuu Point, Oahu, were examined. Spindle-shaped spicules were found in all of the pink/green individuals and in none of the black ones from these areas.

The numbers of pore-pairs in twenty-five arcs of 29 urchins of each color form from Kapapa Island were counted. Analysis of variance of the results is summarized in Table 6. Significant differences were found both among the urchins of the same color form and between urchins of different color forms. Therefore there is little likelihood that the higher number of pore-pairs per arc found in the black form is the result of chance sampling.

The frequency distributions of the test lengths of 289 pink/green and 211 black urchins from Kapapa Island are plotted in Figure 13. The collections were made for use in the reproductive cycle study and extended over 25 months. As was mentioned, the collections were randomly made for medium to large individuals, with a few small individuals selected purposely. The distributions do not reflect the total population size distributions because of the emphasis on

Table 6

Analysis of Variance of Pore-pairs per Arc Data for
the Two Color Forms of Echinometra in Hawaii

	Degrees of Freedom	Sum of Squares	Mean Square	F Ratio	Level of Significance
Color forms	1	8.34	8.34	8.06	.005
Individuals within forms	56	57.96	1.04	6.91	.001
Arcs within individuals	1392	208.40	0.15		

larger individuals. However, they are valid for showing differences between the two populations. The means and standard deviations of the black and pink/green samples were 38.3 mm and 7.07, and 43.8 mm and 8.40, respectively. A group comparison of the means yielded a t value of 7.71, with 498 degrees of freedom. The probability of the two means being statistically the same is less than 0.001. Moreover, only three individuals of the black form were found that were longer than 50 mm. Many pink/green individuals were longer than 50 mm, and several were longer than 55 mm.

Electrophoretic analysis was attempted on the proteins of five whole gonadal tissue extracts and six coelomic fluid samples for each of the two color forms. Each gram of gonad tissue was homogenized in seven volumes of distilled water and centrifuged for 10 minutes at 7000 rpm to remove particulate material. Cellulose acetate electrophoresis of the extracts produced a single band in the males and two bands in the females of both color forms. No differences were evident between the color forms. The extract procedure did not yield sufficient proteins to permit further analysis.

Solid material was removed from the coelomic fluid samples by means of a clinical centrifuge, and the supernatant was dialyzed for 24 hours. The samples were then frozen and lyophilized. Acrylamide disk electrophoresis of the concentrated samples produced a band from the pink/green form samples that was vague or absent from the black form samples. However, further samples are needed to confirm the difference.

No distinctive differences between the two Hawaiian color forms were detected in the following characters:

- bleached test color,
- auricular tag shape,
- pedicel spicule type,
- number or frequency of insert ocular plates
- gut wall spicule type,
- apical system tuberculation,
- height to length ratio,
- width to length ratio,
- apical system length to test length ratio,
- periproct diameter to apical system length ratio,
- peristome diameter to test length ratio,
- maximum spine length to test length ratio.

Two urchins were found whose color was somewhat intermediate between the two established color forms. One was blackish olive-green and the other was greenish-gray. The tips of the spines of the greenish-gray individual turned light green after it had been in an aquarium for 48 hours. Both had the heavy, blunt spines typical of the black form. Both also had the spindle-shaped spicules in the gonad typical of the pink/green form. All of the morphological information would seem to emphasize the intermediate position of the two specimens.

The habitat differences shown earlier in this paper suggest that there may also be physiological differences between the two forms. Consequently, I have made comparative experimental investigations with respect to resistance to wave force, harmful radiation, desiccation and high temperatures, and salinity changes.

Resistance to wave force by Echinometra is primarily related to spine strength, since they normally live in holes, and the wedged spines prevent movement of the urchin by an external force. The strength of each spine, in turn, is related to its cross-sectional area, its length, and its strength per unit cross-sectional area, among other things (nutritional state, symmetry, etc.).

The strengths of a number of spines of each color form were measured by a simple technique. Each spine was freshly removed from a recently collected urchin and placed in a vertically clamped drill gauge in the smallest hole permitting insertion to the milled ring. An empty plastic cup was suspended from the horizontal spine at a point 10 mm from the drill gauge. (The thickness of the gauge was 1 mm.) Lead shot (No. 7 $\frac{1}{2}$) were then dropped into the cup at a slow, steady rate until the spine broke. The diameter at the break was measured with the drill gauge. From this the cross-sectional area at the break was calculated. The torque was calculated by multiplying the final weight of the cup and shot by the length of spine between the cup and the break. The relation between the cross-sectional area of a spine and the torque required to break it are shown in Figure 14 for both color forms.

The strength per unit cross-sectional area was also calculated for the spine measurements plotted in Figure 14. This value is called the ultimate breaking stress and is computed from the derived equation:

$$s_{\max} = 4Fx / \pi r^3$$

where s_{\max} is the breaking stress, F is the force applied, x is the length of the arm on which the force acts, and r is the radius of the

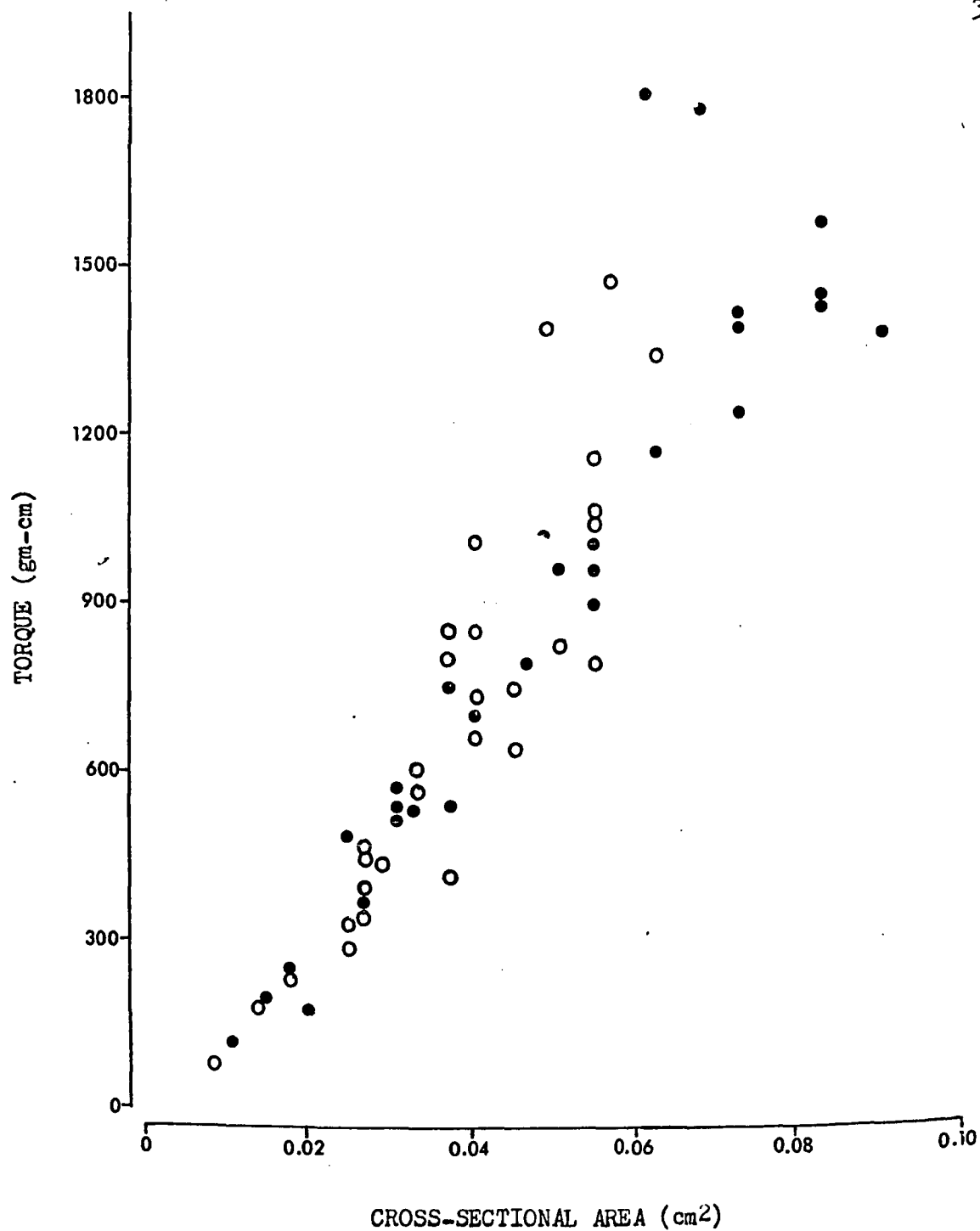


Figure 14. Relation between the cross-sectional area of a spine and the torque required to break it in the two color forms of Echinometra in Hawaii. Black and open circles represent black and pink/green spines, respectively.

spine at the break. The mean breaking stress for all of the spines was 6.25 Kg/mm^2 . This is about the same as the breaking stress of Douglas fir wood. The breaking stress of the larger pink/green spines seemed slightly higher, but it is more than offset by the larger average diameter of the black spines.

Resistance to harmful radiation sometimes appears to be correlated with the presence of black pigment. A simple experiment was designed to determine if there is an obvious difference in the survival of the two color forms when exposed to strong sunlight. Twenty-five black and 25 pink/green urchins were placed in each of two shallow fiberglass tanks. A constant stream of seawater was introduced into each tank, and the outlet located to maintain a depth of about 2 inches. Both tanks were placed out-of-doors. One was left exposed to ambient sunlight, while the other was covered with plywood. The survival of the two color forms in each tank is shown in Figure 15.

Another experiment was designed to test whether the two color forms in Hawaii differ in their resistance to a combination of heating and drying. Three groups of 40 urchins were placed in the sun on a beach mat for periods of one, two, and four hours, respectively. An additional group was left submerged in the water. Each group consisted of equal numbers of both color forms. The size of the urchins of each color form in each group was about the same: 5 small (11-15 mm), 10 medium (25-35 mm), and 5 large individuals (35-51 mm). The urchins were collected on the shallow reef flat at Maili Point, Oahu, and they were first placed on the beach mat one hour after noon. The temperature of the beach mat surface reached 41°C , but averaged

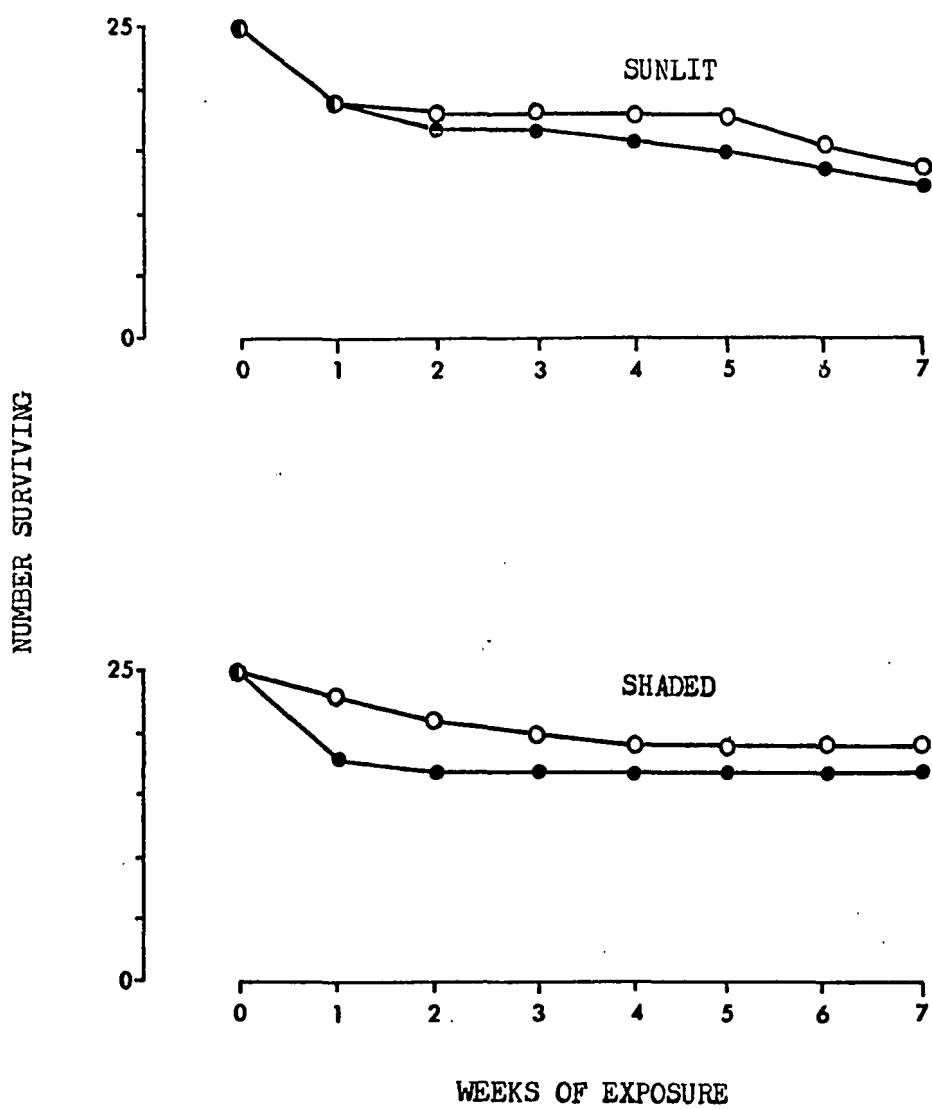


Figure 15. Survival of the two color forms of Echinometra in Hawaii as a function of different exposures to sunlight. Black and open circles represent black and pink/green urchins, respectively.

around 34°C, due to passing clouds obscuring the sun. The air temperature was about 27.5° C in the shade. After the exposure period each group was placed in a shaded aquarium with circulating seawater and an airstone. A daily check was then made of the number of each color in each group surviving. An individual was considered to be alive if it showed spine or tubefoot movement in response to being poked or turned over. The results of the tests are presented in Figure 16.

It is apparent that in most cases mortality was not immediate. Many urchins that appeared moribund at the end of the exposure were not dead, but revived after being returned to seawater. In general, small urchins (11-15 mm) were more sensitive to desiccation, while the large ones either succumbed after longer exposures or survived. The data suggest that the pink/green form is more tolerant to a combination of drying and heating.

Finally, the survival of the two color forms in water of high and low salinity was compared. Five aquariums were filled with water of five different salinities. Hypo-saline water was made from a mixture of fresh tap water and seawater. Hyper-saline water was made by dissolving Instant Ocean Salt in fresh tap water. The actual salinities, as measured with hydrometers were 1 o/oo, 13.5 o/oo, 26 o/oo, 37 o/oo, and 47 o/oo. An airstone was placed in each tank and a strong flow of bubbles was maintained. The tanks were allowed to stand for two days prior to introduction of the urchins. The temperature of all tanks remained at 25° to 26° C. The experimental animals were collected at Kapapa Island. Five urchins of each color

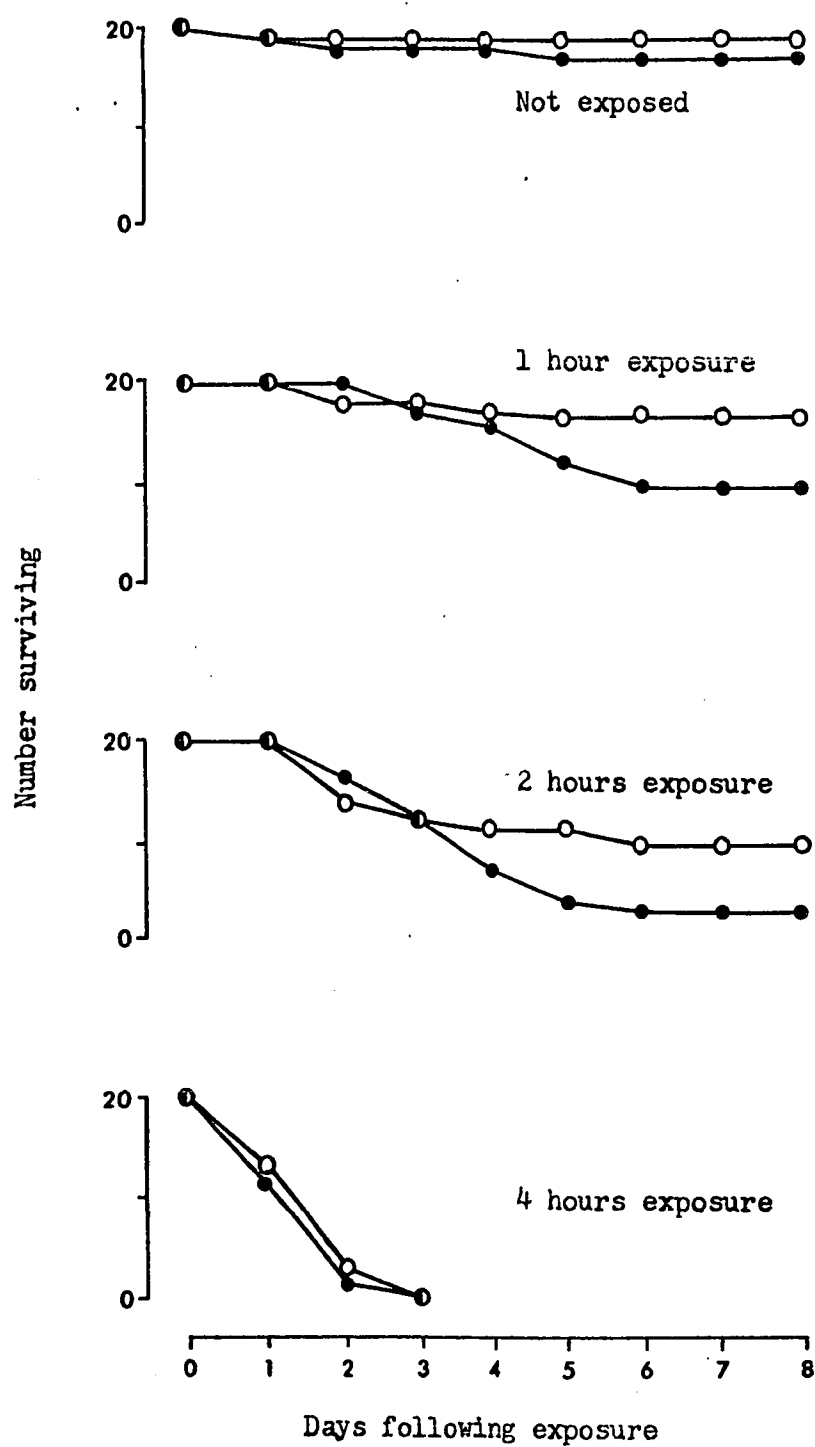


Figure 16. Survival of the two color forms of Echinometra in Hawaii following exposure to drying and heating by sunlight, as a function of time following re-immersion. Black and open circles represent black and pink/green urchins, respectively.

form were placed in each aquarium with no acclimation period. Each group of five consisted of one small (11-15 mm), two or three medium (25-35 mm), and one or two large (35-51 mm) individuals. Survival of the urchins was then checked at irregular intervals. The results of the experiment are shown in Figure 17. Most of the individuals in the hyper- and hypo-saline water exhibited no movement following introduction to the water, and it was necessary to place them in normal seawater to determine if they were still living. An individual was considered to be alive if it showed spine or tubefoot movement in response to being poked or turned upside down. Finally, the large individuals of both color forms seemed less tolerant to salinity change than the small ones.

Discussion

A brief discussion of the morphological variation and the experimental studies will be presented with reference to previous work and the habitat specialization.

The pigment composition of the spines of Echinometra has been investigated in two locations in the Pacific: Hawaii, where Chang, Moore, and Scheuer (1964a and 1964b) have worked with E. oblonga, and Japan, where Dr. K. Nishibori (personal communication) has studied the only form present there, E. mathaei. The results of their studies are summarized in Table 7. Despite the possible absence of spinochrome B in E. oblonga, it is evident that the pigments of the two forms differ primarily in quantity. It is perhaps significant that the percentage yield of spinochrome A is the same in E. oblonga and Colobocentrotus atratus, a related urchin that lives

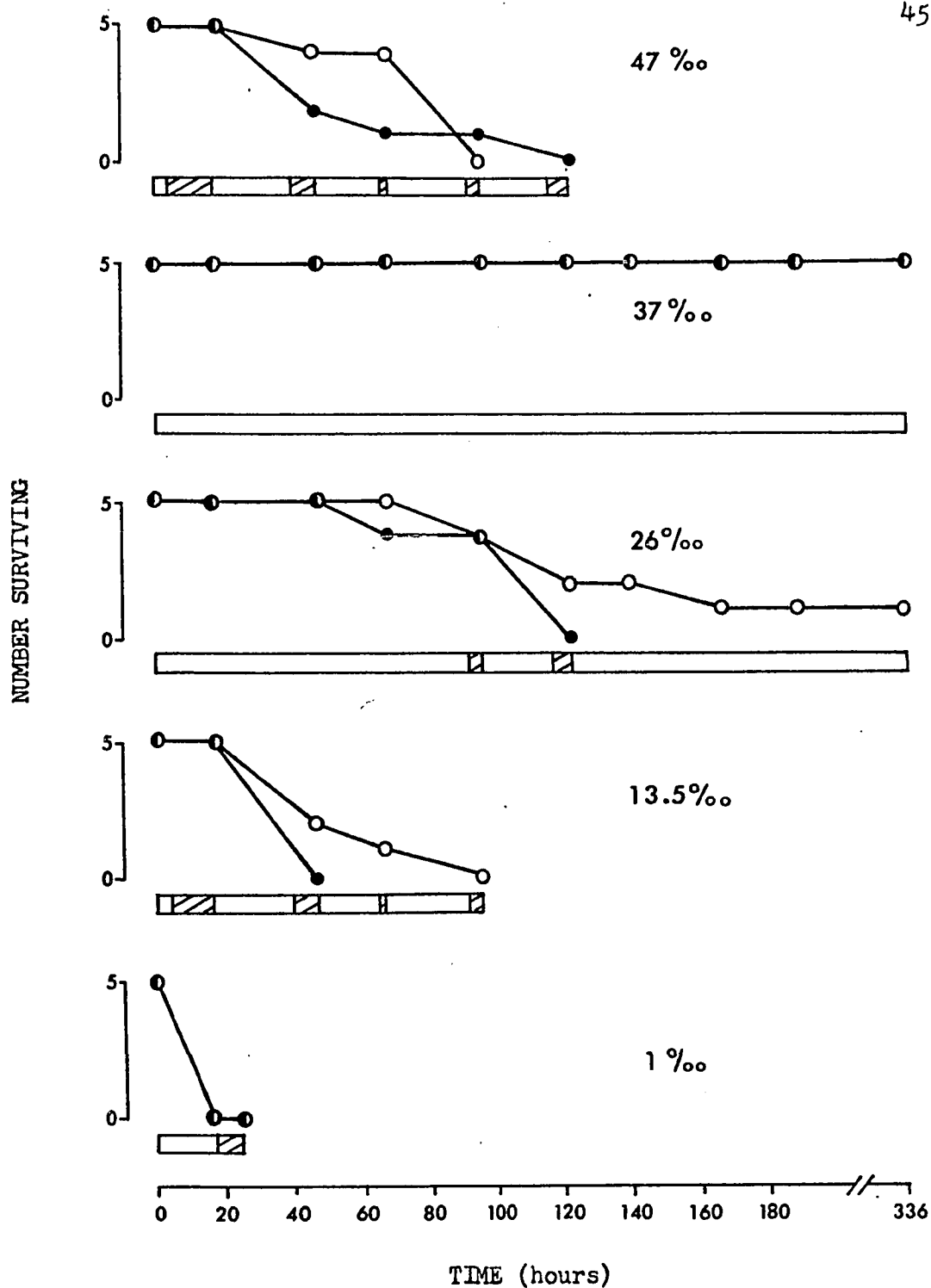


Figure 17. Survival time of the two color forms of *Echinometra* in Hawaii as a function of water salt concentration (‰ salinit.). Black and open circles represent black and pink/green urchins, respectively. The hatched bar under each graph indicates return of the urchins to normal salinity seawater (37 ‰).

Table 7
 Pigment Composition of the Spines of Pacific
Echinometra

<u>Spinochrome</u>	<u>Percentage Yield</u>		
	<u>E. oblonga</u> entire spine	<u>E. mathaei*</u> lower part of spine	<u>E. mathaei*</u> upper part of spine
A	.05**	0.00030	0.01540
B		0.00145	0.01540
C	.01***	0.00055	0.00615

* Dr. K. Mшибori, personal communication.
 Collected in Japan (His identification).

** Chang, Moore, and Scheuer, 1964a
 Collected in Hawaii (Their identification).

*** Chang, Moore, and Scheuer, 1964b
 Collected in Hawaii (Their identification).

even higher on the shore than does E. oblonga.

The detrimental effect of insolation, especially of the ultra-violet rays, on protoplasm is well known. It is generally assumed that the presence of pigment has survival value by screening out harmful radiation. Certainly the individuals of many echinoderm species are darker when found in shallow water. Fontaine (1962) found an increasing frequency of light color phases of the ophiuroid Ophiocomina nigra with increasing depth. A. H. Clark (1921) reported a similar correlation in crinoid color forms. Fox and Hopkins (1966) found a greater proportion of pigmented spines in shallower (intertidal) populations of Arbacia punctulata. The same authors say that Lytechinus variegatus is more darkly pigmented in the northern part of its range, where it occurs in shallower water. Moreover, several species of shallow-water echinoids which lack shielding pigments cover their upper surfaces with various objects (Millott, 1957).

Consequently, the black form of Echinometra in Hawaii might be expected to better resist insolation damage than the pink/green form.

However, no obvious difference in survival was noted in the insolation experiment (Fig. 15). Nevertheless, there is still a possibility of sublethal long-term individual damage. Moreover, the effect of sunlight on the viability of the gametes was not measured.

The possibility that the naphthaquinone pigment in echinoids acts as an algistat which prevents settlement of blue-green algae on the test and spines has been pointed out by Ververs (1966). Although the black form contains more spinochrome than the pink/green form, there

is no evidence pointing to a more serious problem of blue-green algal settling in the surf zone. The only comment I can add is that I have seen an algal film, including both blue-green and coralline algae, on the dull "resorbed" tips of the spines of a black Echino-metra that I transplanted from one area at Kapapa Island to another nearby area in the surf. The tips were still black and opaque, but broke away easily from the glossy base when I touched them.

The surf zone is characterized by strong wave force. The higher frequency of stout spines found in the black form would seem to have survival value in the surf zone especially, since it is reasonable to infer that the stouter the spines, the greater their resistance to breaking, and hence to removal of the animal from this habitat. The data presented in Figure 14 confirm that more torque is necessary to break a larger diameter spine. The average diameter of the base of 50 spines from 50 medium-large urchins of each color collected randomly at Kapapa Island was 2.4 mm for the pink/green form and 3.2 mm for the black form. This represents a large difference in the torque necessary to break the average spine. Moreover, since the black spines remain thick for a greater extent of their length, the difference in torque required is increased if the break occurs anywhere other than at the base.

Moreover, the black form is never seen on flat surfaces, such as under rocks, where the pink/green form is often found. Mortensen (1943) speculates that it is more of a hole-borer than the pink/green form. This tendency enhances the urchin's ability to wedge its spines against the substrate and also lessens the wave forces acting upon it.

Resorption of spines may play a role in nutrition or metabolism as a potential region for storage. It has been observed that spine tip resorption occurs when the animal is undergoing a period of stress. When Echinometra of either color form are collected and transplanted to the laboratory or to a new location in the field, after 2 days the tips of the spines frequently become duller and lighter in color. Pink/green spine tips may become greener, but black spine tips only lose their gloss. This change may occur only at the extreme tip or may extend to the entire distal $3/4$ of the spine. The extent varies from urchin to urchin and also among the spines on the same urchin. Only one end of the urchins may show the change, or it may be visible even on the oral spines. These tips are extremely brittle, and drop off at the slightest pressure. Regeneration often begins soon after they have fallen off. I have seen blue-green algae growing on the dull tips of a black urchin that had been transplanted from one area at Kapapa Island to another area nearby. It seems that either the organic structure, part of the calcite, part of the pigment, or some combination of these is resorbed by the urchin. The similarity in appearance to regenerating tips, together with the irregularity of its distribution and extent both on the same urchin and among urchins implies that the resorption takes place only in a recently regenerated part of a spine. The significance of the resorption is not clear.

The third morphological difference recognized between the two color forms is the presence of spindle-shaped spicules in the gonad of the pink/green form. The function of these spicules is unknown.

Various interpretations are possible: they may serve as muscle attachments, as gonad shape-retainers, or in calcium storage or calcium excretion. Their abundance does not seem to be affected by starvation or loss of spines. A group containing nine pink/green and four black urchins was kept unfed in a recirculating seawater system for $3\frac{1}{2}$ to $7\frac{1}{2}$ months. After $3\frac{1}{2}$ months two black and one pink/green urchins were examined and the rest were examined after $7\frac{1}{2}$ months. Most had lost their primary spines and showed some visible removal of calcite from the test. However, neither the presence nor the abundance of either the gonad spicules or the gut wall spicules appeared to differ from the normal condition.

A final adaptation of apparent advantage in a wave-washed habitat is the greater number of pore-pairs per arc found in the black form, as was shown in Table 5. Although these values were obtained from counts made above the ambitus, it is assumed that a similar difference can be found below the ambitus also. A greater number of tube feet below the ambitus would aid in holding on to the substrate. A greater number above would aid in capturing and retaining drifting algal food materials. Both functions would seem to be more difficult in the strong currents of the surf zone. Other echinoids which live in the surf, such as Heterocentrotus and Colobocentrotus, have widened pore zones in the oral region. However, no difference in this feature was noticed between the two color forms of Echinometra.

No information is available on whether the size frequency distribution variation shown in Figure 13 represents two populations of urchins of the same age and is the result of differences in growth

rate or whether it represents two populations of urchins of different ages and is the result of greater longevity in the pink/green form.

Most marine invertebrates have little tolerance to desiccation, and Echinometra is no exception. Hodgkin (1959) described a mass mortality of intertidal organisms, including Echinometra, on the west coast of Australia following a period of exceptionally low tides and calm seas. A similar case in Puerto Rico is described in Glynn (1968). Again, it might be expected that the black form, living higher on the shore, would be more resistant to desiccation than the pink/green form. However, again no obvious difference in resistance to desiccation and heating was noticed (Fig. 16).

Tokioka (1963) reported a mass mortality of Echinometra at Seto (Wakayama, Japan) due to extremely cold weather in 1963. The air temperature dropped to 0° C; the water temperature dropped to 10°C. It does not seem likely that this has ever occurred in the "high" Hawaiian Islands, however. The lowest air temperature on record near sea level in Honolulu is only 12° C (U.S. Department of Commerce, 1968).

Because fresh-water is less dense than seawater, it tends to form a layer on top of seawater unless disturbed. Thus if fresh-water is introduced into the ocean by rain or from ground water sources, those animals living at higher levels of the intertidal may be subjected to lowered salinities. In addition, on at least some reef flats, ground water near the shore floats on and mixes with intrusive seawater, and there is a resultant onshore-offshore movement of lower salinity water with the tides (Hiatt, 1958). Hiatt

suggests that this gradient is important in coral zonation on reef flats. The relative survival of the two color forms of Echinometra in both high and low salinities is recorded in Figure 17. Although the number of individuals used was small, it is evident that the pink/green form is more tolerant of both low and high salinities. Nevertheless, the degree of tolerance shown by both forms militates against salinity being an important factor in the habitat separation. It is doubtful if salinity changes of even 10 o/oo last for more than a few hours in most areas where Echinometra are found.

Additional vertical gradients might be expected in oxygen concentration and the amount of silt present in the water. However, these factors must be of negligible importance on the wave-washed coasts where Echinometra occurs. On the other hand, it may be noted that the black form has a higher mortality than the pink/green form in aquariums if the inflow is small or no airstones are used.

POLYPHENOTYPIC VARIATION

It is necessary to consider the question of polyphenotypic variation, since both habitat and morphological differences have been shown. The only polyphenotypic variation likely in Echinometra is ecophenotypic variation.

The possibility that the variation in spine color could be environmentally induced will be considered first. The darker color of the black form could be produced through the action of strong sunlight on an individual urchin. This hypothesis was tested by a spine regeneration experiment similar to that reported by Ebert (1967). The spines of 10 small (10-15 mm test length) urchins of each color form were clipped off at one-half their length. Five of each color were then placed in each of two identical resin-coated wooden boxes, through which recirculating seawater flowed. Both boxes were covered with a sheet of clear acrylic plastic. One box was illuminated with a constantly shining light bulb; the other was covered with black plastic so that no light entered. The color of the regenerating spine tips was observed weekly for a month, but no consistent change in coloration was noticed in either box. The black spine bases regenerated dark reddish-black tips in both boxes, and the pink/green bases similarly produced pink/green tips. This is good evidence that the color difference is not the result of different amounts of light shining on the individuals, but is somewhat contrary to the findings of Harvey (1956) with Arbacia lixula, Kristensen (1964) with Diadema antillarum, and Ebert (1967) with Strongylocentrotus purpuratus. The first two reported spine

darkening if the animals were kept or raised in light, but spine lightening if they were kept or raised in the dark. Ebert (loc. cit.) reported the regeneration of purple tips in constant light, but the regeneration of white or green tips in constant darkness.

The fact that pink/green urchins are the most abundant forms found in shallow, intensely-isolated tidepools is one which further argues against the importance of light in black spine color formation.

A second factor that might affect spine color is the type of food ingested. The possibility of selective feeding by the color forms was investigated by three approaches. First, examination of the gut contents of the two forms revealed that they were always full of pieces of macroscopic algae, but that there were no qualitative differences in the types of algae eaten.

Since the urchins normally live in holes which are bare of macroscopic algae, and they are never seen outside of the holes during the daytime, an effort was made to see if they left the holes nocturnally to graze. The locations of a number of urchins of both color forms were carefully plotted in two separate square 4 m^2 quadrats at Kapapa Island. The areas selected were about 90 m from the lee side of the island in about 1 m depth. The quadrats were permanently marked with nails driven into the rock. The locations of all Echinometra inside the quadrats were recorded on underwater slates. The first plotting was done on September 6, 1968, and was repeated that night under a full moon. No change in behavior or location of any of the urchins was noticed. The plotting was repeated on September 20 and 21, 1968, under a new moon. Again no change was

noticed. The plottings were repeated on October 25, 1968, June 18, 1969, and September 20, 1969. So little movement was observed that the results after a full year can be summarized in Table 8. After one year the total net change found was five black urchins lost and one pink/green urchin gained. The new individuals encountered could either be older animals that moved in from outside the quadrat or newly settled juveniles. Both possibilities seem likely, because both of the new black urchins were less than 15 mm long (test length) and three of the new pink/green urchins were less than 25 mm long. However, most of the individuals that were recorded as about 15 to 25 mm long in September, 1968, were still so-classified in September, 1969. This indicates very slow growth and makes it less likely that the pink/green urchins at least were newly settled. The individuals that were missing after 1 year could have either moved outside the quadrats, died, or been killed. Therefore, the number of urchins of the black color form that changed their location noticeably is somewhere between 1.3 and 12.9% of the total number present. The number of pink/green urchins moving lies somewhere between 1.6 and 19.6% of the total number present.

It is concluded that the urchins do not move out of their recesses to feed. Instead, they catch and feed on detached benthic algae that drift by. Ebert (1968) reports that individuals of Strongylocentrotus purpuratus at Sunset Bay, Oregon, also feed in this manner. Such a method presumably permits little selection of food material. Finally, Mr. David A. Olsen (personal communication) has tested the food preference of both color forms of Echinometra,

Table 8

Changes in the Numbers of Echinometra in a Pair of 4 m²
 Quadrats at Kapapa Island, Oahu after 1 Year*

Color	Date	Total	New	Missing	Changed position inside quadrat
Black	6 Sept. 1968	78			
	20 Sept. 1969	78	2 (2.6%)**	7 (9.0%)	1 (1.3%)
Pink/green	6 Sept. 1968	61			
	20 Sept. 1969	62	2 (9.8%)	5 (8.2%)	1 (1.6%)

* Values for the two quadrats have been combined

** The 1968 values were used as a basis for the
 calculation of percentages.

using Leighton's (1966) method, and has found no significant preferences.

The second morphological feature investigated with respect to environmental variation was spine shape. There are indications that spine shape is not wholly genetically determined. The black Echino-metra that live in tidepools were observed to have long, slender spines much more frequently than the ones studied at Kapapa Island, where they are more exposed to surge. The hypothesis tested is that of whether slender spines form in calm water, and the stout spines result from frequent breakage and interrupted regeneration.

A number of black and pink/green urchins were kept in a non-recirculating seawater system for 5 months. A quarter of their aboral spines were clipped near the tips, another quarter were clipped near the base, and the progress of regeneration was observed over a period of 21 weeks. During the experiment the animals were not fed, although there was a growth of filamentous algae on the walls and bottom of the tank on which the animals could browse.

The rate and pattern of regeneration was variable, but did not differ significantly between the color forms. Generally the length increased from 0.5 mm to 1.5 mm per week, with the mode between 0.5 mm and 1.0 mm per week. The rate varied even among the spines on the same urchin. Those tips regenerating on wide bases tended to have slower rates of increase in length. The regenerating tips were very brittle and often broke off during handling and perhaps also during the normal activities of the urchins. The shape of the regenerating tips was also variable. Generally, however, the

tips were more slender and sharper-pointed than the original tips. This did not change even after clipping the spine tips three times each at 3-week intervals. The ability to regenerate decreased in some of the urchins after several weeks, and many of the regenerated tips were still recognizable after 6 weeks. In summary, spine tips regenerating in quiet water tended to be slender and concave-tapered. However, the blunt point could not be experimentally produced by repeated removal of the tip.

It would appear from the fact that more slender spines occur on urchins in calm tidepools and the spine tip regeneration observations made in aquariums that the prime factor regulating spine shape is wave action, although urchin age and/or the abundance of food cannot be ruled out. Nevertheless, despite the environmental relation, the tendency to form heavy spines is far greater in the black form -- as shown in Table 5, which is based on black and pink/green urchins that were subject to the same surf conditions.

Finally, the spindle-shaped spicules are similar to oxea, rhabdus, or diactinic monaxon spicules from sponges. It has been suggested to me that they may actually be ingested sponge spicules. However, two points negate this hypothesis. The urchins evidently feed on drifting algae and do not graze, and the Ca/Mg in the spicules (84/16) is close to that of the calcite in the test, suggesting a similar formation (analysis was done by Dr. Keith E. Chave).

REPRODUCTIVE PERIODICITY

Methods and results

Several indicators of reproductive condition were measured each month for 2 years at two localities (Black Point and Kapapa Island, Oahu) to determine the breeding seasons of the color forms. These indicators included the presence or absence of gametes, maturity of the gametes, and gonad volume. The color, length, width, height, sex, and occurrence of shedding were also recorded for each urchin. Sex was determined by microscopic examination of a seawater smear from part of the gonad immediately above the jaw apparatus. However, in a number of cases, sex could not be determined because of absence of gametes. Maturity of the gametes was noted at the same time. For an approximate determination of maturity, the relative activity of the sperm was estimated for the males, and the number of eggs without germinal vesicles (ova) in a total of 100 eggs counted was used for the females. Gonad volume was measured by seawater displacement. The gonads were preserved in seawater formalin until measurements were made. (The gonad volume of five urchins measured before and after preservation were not significantly different.) The gonad volume was related to animal size by a gonad index ($\text{gonad index} = 100 \times \text{gonad volume} / \text{test volume}$). The total volume of each of 69 black and 111 pink/green whole, live urchins was measured by seawater displacement. The regression equation of $(\text{length} \times \text{width} \times \text{height}) / 1000$ on volume was computed for each color form from these measurements. In each case, the per cent variation in the volume due to the multiple of the three dimensions was 98%, indicating the accuracy of the calculated value. Volumes

for the remaining urchins were therefore calculated from the three dimension measurements and the regression equations.

The variations in three of the indicators of reproductive activity of Hawaiian Echinometra were consistent for both years and are presented in Figures 18-21. The data for the Kapapa pink/green urchins and the Kapapa black urchins are shown in Figures 18 and 19, respectively. Those for the Black Point pink/green are in Figure 20, and for the Black Point black in Figure 21.

The number of individuals which had recognizable gametes was expressed as a percentage of the total number of individuals examined. The maturity of the female gametes was plotted as the mean percentage of ova (mature gametes) for all females in the sample. The mean gonad index does not include urchins of less than 10 cm total animal volume (about 27 mm test length). The gonad indices for the Black Point urchins were not calculated because so few mature black ones were regularly collected at this location.

The values for the ripeness of the male gametes and for the numbers of both sexes which shed were too erratic to be considered as valid. Undoubtedly they were affected by the treatment they received during the collecting trip or in the lab, such as the length of time before processing or the roughness of transport.

Discussion

For purposes of clarity and comparison, a brief introduction of previous work on Echinometra reproductive cycles will be presented before the Hawaiian data are discussed. The results of these studies are summarized in Table 9. The terms dealing with

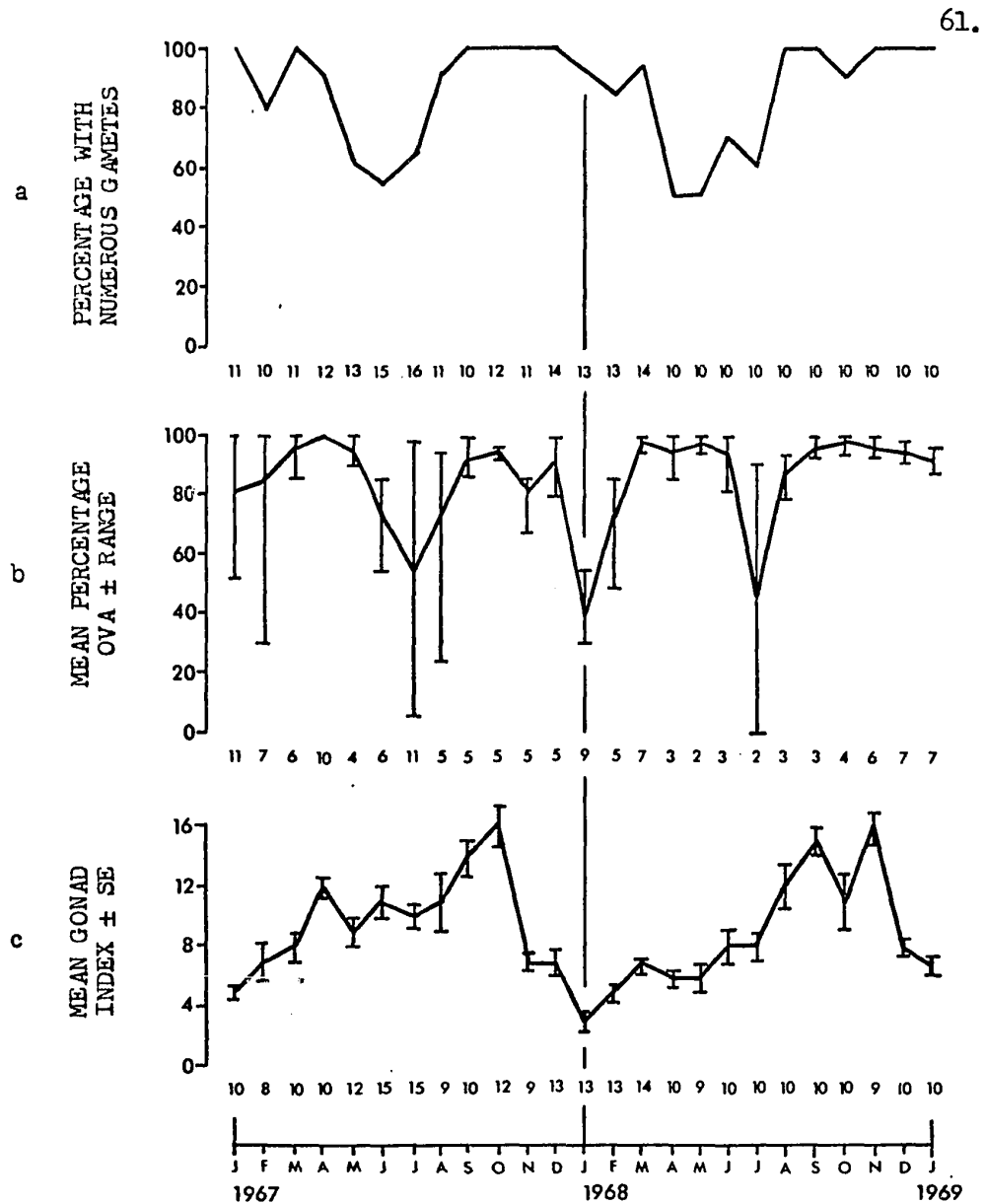


Figure 18. Reproductive condition of pink/green Echinometra from Kapapa Island, Oahu in 1967 and 1968.

- a. Percentage of total containing numerous gametes.
- b. Mean percentage of female gametes that are ova \pm the range of values.
- c. Mean gonad index \pm one standard error.

The numbers below the points of the graphs are the sample sizes.

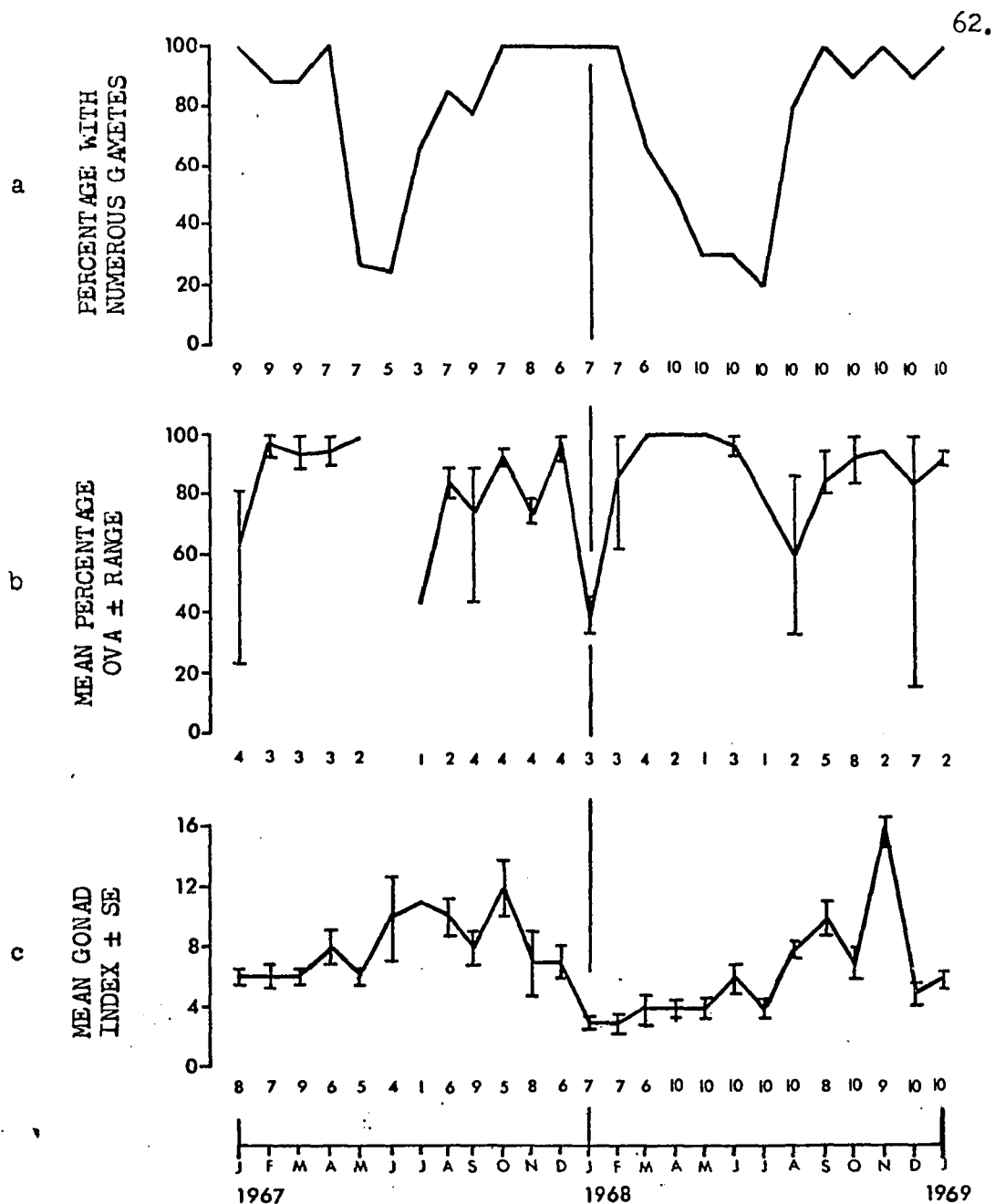


Figure 19. Reproductive condition of black Echinometra from Kapapa Island, Oahu in 1967 and 1968.

- a. Percentage of total containing numerous gametes.
- b. Mean percentage of female gametes that are ova \pm the range of values.
- c. Mean gonad index \pm one standard error

The numbers below the points of the graphs are the sample size.

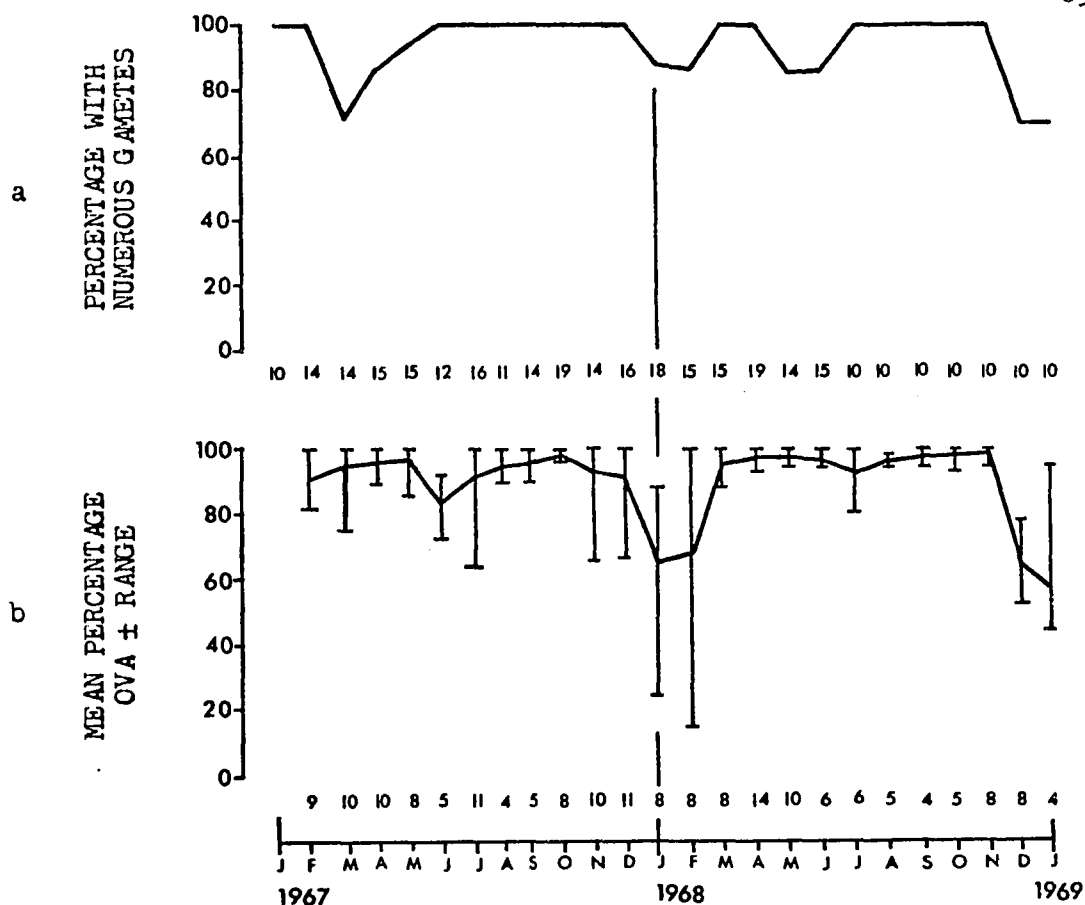


Figure 20. Reproductive condition of pink/green *Echinometra* from Black Point, Oahu in 1967 and 1968.

- Percentage of total containing numerous gametes.
- Mean percentage of female gametes that are ova \pm the range of values.

The numbers below the points of the graphs are the sample sizes.

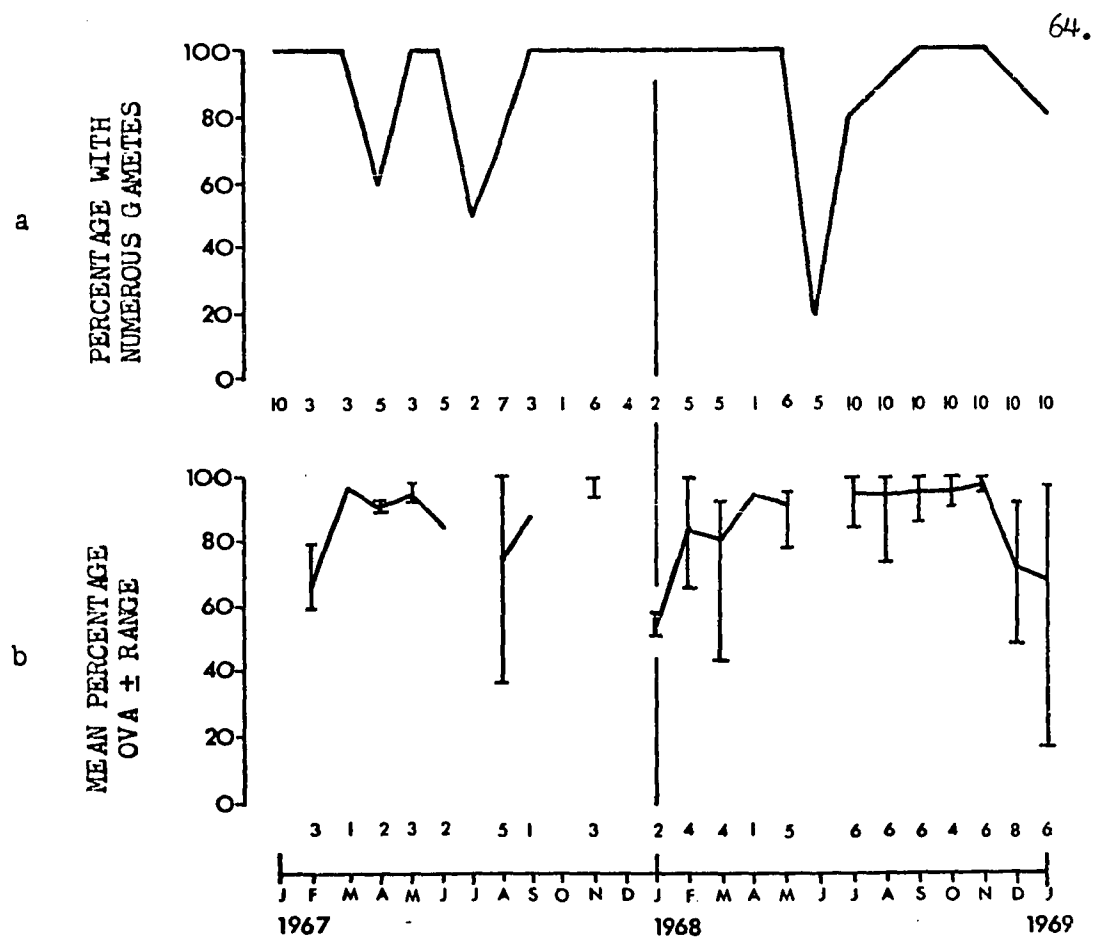


Figure 21. Reproductive condition of black Echinometra from Black Point, Oahu in 1967 and 1968.

- a. Percentage of total containing numerous gametes.
- b. Mean percentage of female gametes that are ova \pm the range of values.

The numbers below the points of the graphs are the sample sizes.

Table 9

Information Reported on Spawning Time
in Species of Echinometra

<u>Species</u>	<u>Location</u>	<u>Annual temp. Range</u>	<u>Breeding periods or notes</u>	<u>Reference</u>
<u>E. mathaei</u>	Rottneest Island West. Aus.	18°-23°C	year-round ?	Pearse & Phillips, 1968
<u>E. mathaei</u>	Wadi El Dom, U.A.R.	18°-28°C	July-Sept.	Pearse, 1969
<u>E. mathaei</u>	Al Ghardaqa, U.A.R.	17°-29°C	July-Sept. and year-round ?	Pearse, 1969
<u>E. mathaei</u>	Shirahama, Japan	14°-28°C	July-August	Onoda, 1936
<u>E. mathaei</u>	Shirahama, Japan		July-Sept.	Tohara & Okada, 1968
<u>E. mathaei</u>	Al Ghardaqa, U.A.R.		Nearly spent in April-May	Mortensen 1937
<u>E. mathaei</u> ?	Equatorial Pacific		Year-round	Pearse 1968
<u>E. mathaei</u> ?	Mer, Murray Islands, North Aust.		Ripe in late September	Tennent, 1929
<u>E. oblonga</u>	Hilo, Hawaii		Ripe in April	Mortensen 1921
<u>E. violacea</u> ?	Mauritius	22°-27°C	Ripe in mid- September	Mortensen 1931
<u>E. vanbrunti</u>	Taboga Island Panama		Ends in Nov.	Mortensen 1921
<u>E. viridis</u>	Margot Fish Shoal, Fla.		July-October	McPherson 1969
<u>E. lucunter</u>	Virginia Key, Pigeon Key, & Margot Fish Shoal, Fla.	22°-31°C	July-Sept.	McPherson 1969
<u>E. lucunter</u>	West Indies		March-April	Mortensen 1921
<u>E. lucunter</u>	Dry Tortugas, Florida		Peak in late July	Tennent, Gardiner & Smith, 1931
<u>E. lucunter</u>	Barbados Island	25°-29°C	May-August	Lewis, 1960

reproduction are defined by Boolootian (1966): reproductive cycle -- the total course of events from nutrient growth and gametogenesis to the resting stage following spawning; reproductive period (breeding season) -- period when mature; fertilizable gametes are present; spawning -- actual shedding of gametes. Extensive studies of the reproductive cycles of Echinometra have been made at Rott-nest Island, Australia (Pearse and Phillips, 1968), the Gulf of Suez area (Pearse, 1969), and off southern Florida (McPherson, 1969).

Rottnest Island (32°00'S) is off Perth, Western Australia, and the population of E. mathaei found there is the most southerly known. Evidently no other West Australian populations are found in the area south of Shark Bay (Clark, 1938); however, this may be due to inadequate sampling. The average annual sea surface temperature range is cool (18° to 23° C, Hodgkin, Marsh, and Smith, 1959), but the animals are numerous and especially robust. Eleven samples were collected in a 26-month period and examined histologically. Results comparable with those made in Hawaii are presented in Fig. 22. Pearse and Phillips (1968) conclude that there was little seasonal change in reproductive activity. The lack of synchrony among individuals and the presence of mature gametes in every sample indicated to Pearse and Phillips that breeding was continuous, with perhaps minor peaks of spawning. Pearse and Phillips suggest that austral winter temperatures on the reef flat must be just high enough to maintain breeding.

Reproductive cycles were studied by Pearse (1969) at two primary locations in the United Arab Republic: Wadi El Dom and Al Ghardaqa. Wadi El Dom (29° 26'N) is in the northern Gulf of Suez,

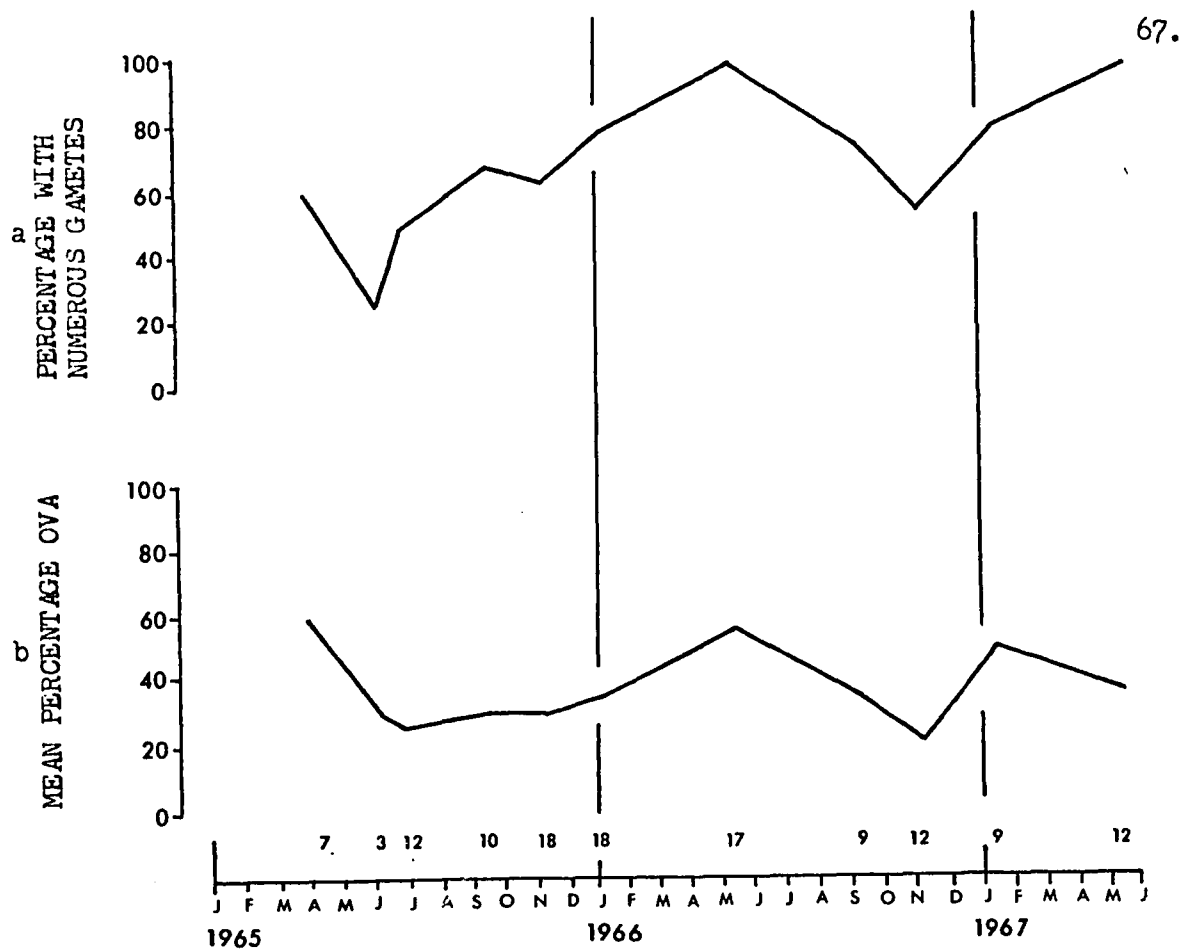


Figure 22. Reproductive condition of *Echinometra* from Rottnest Island, Western Australia. Adapted from Pearse & Phillips (1968).

- a. Percentage of total containing numerous gametes.
- b. Mean percentage of female gametes that are ova.

The numbers below the points of the second graph are the numbers of urchins in the sample.

and Al Ghardaqa ($27^{\circ} 15' N$) is at the junction of the Gulf of Suez and the Red Sea. The mean annual temperature range at Wadi El Dom is about 18° to 28° C; at Al Ghardaqa it is about 17° to 29° C. At Wadi El Dom 23 samples were collected in a 25 month period and examined histologically. A weight-based gonad index was computed for seven of the samples. The results are presented in Figure 23. A well-defined breeding period was evident during July-September. Much individual variation was noted, producing the somewhat prolonged period. Individuals with numerous gametes could not be found during the winter. Annual variation in the gonad index was slight.

At Al Ghardaqa six samples were collected over a 15 month period and examined histologically. The results are presented in Figure 24. A major breeding period occurred in June-September, but mature animals were found at all times of the year.

Two species of Echinometra, E. lucunter and E. viridis, were investigated off southern Florida by McPherson (1969), who used both histological methods and a volume-based gonad index. Populations of E. lucunter were studied at three localities: Virginia Key ($25^{\circ} 44' N$), Margot Fish Shoal ($25^{\circ} 20' N$), and Pigeon Key ($24^{\circ} 30' N$). The mean annual temperature range at Virginia Key is about 22° to 31° C (Moore et al., 1963). Nine samples were collected over 18 months at Virginia Key, 10 samples were collected over 15 months at Margot Fish Shoal, and five samples were collected over 18 months at Pigeon Key. The breeding season extended from July to September at all locations.

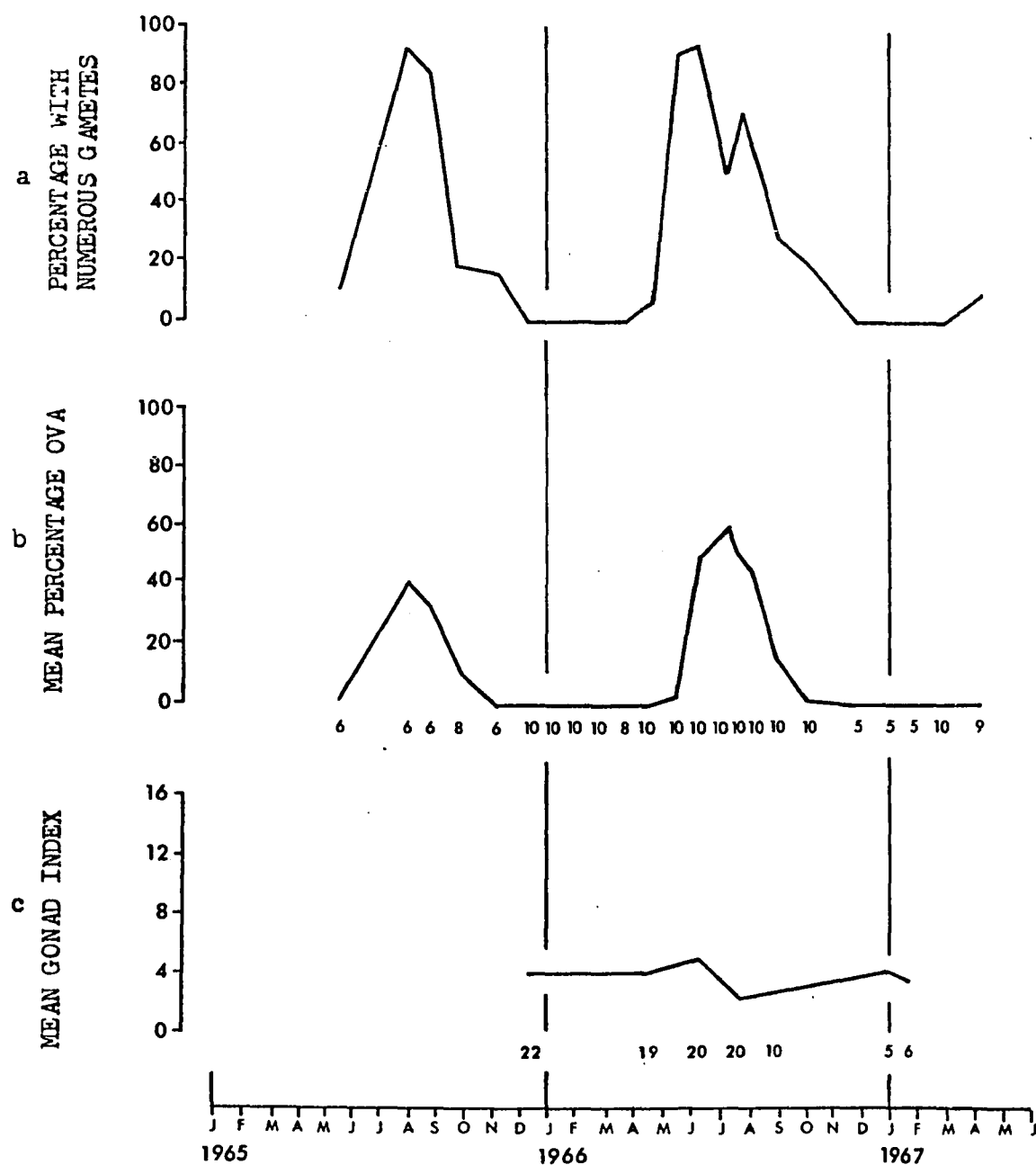


Figure 23. Reproductive condition of Echinometra from Wadi El Dom, U.A.R. Adapted from Pearse (1969)

- Percentage of total containing numerous gametes.
- Mean percentage of female gametes that are ova.
- Mean gonad index.

The numbers below the points of the last two graphs are the numbers of urchins in the sample.

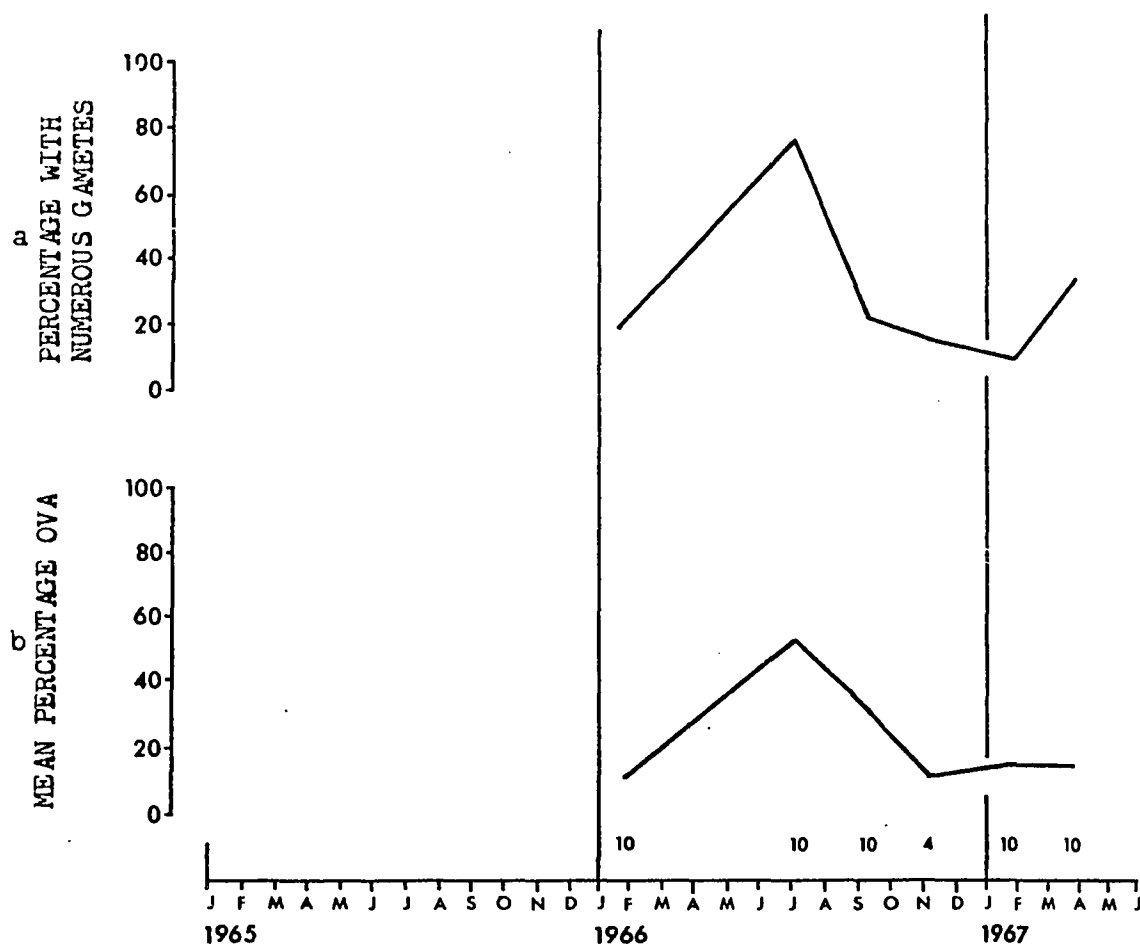


Figure 24. Reproductive condition of Echinometra from Al Ghardaqa, U.A.R. Adapted from Pearse (1969).

- a. Percentage of total containing numerous gametes.
- b. Mean percentage of female gametes that are ova.

The numbers below the points of the second graph are the numbers of sea urchins in the sample.

Echinometra viridis was studied only at Margot Fish Shoal.

Fifteen samples were collected in a 15 month period. It was found that spawning occurred in late October, after a breeding period that started about July or August.

A number of other reports in the literature of the reproductive condition of Echinometra are based on single observations or on methods that are not described. Mortensen (1921) found E. vanbrunti to be at the end of its breeding season in November at Taboga, Panama (8° 48'N). In the same publication he reported that E. lucunter was ripe in March-April in the West Indies. Tennent, Gardiner, and Smith (1931) found the peak of breeding in E. lucunter at Dry Tortugas, Florida (24° 37' N) to occur in the last week of July in 1927. Lewis (1960) said that E. lucunter breeds during May-August in Barbados (13° 10'N, annual mean inshore temperature range about 26°-29°C). He found plutei in the plankton throughout the summer. Mortensen (1921) found E. oblonga to be ripe at Hilo, Hawaii (19° 44'N) in April, 1915, although E. mathaei did not yield very successful fertilizations at that time. Tennent (1929) reared larvae from gametes collected in late September at Mer, Murray Islands, North Australia (about 10° S). Mortensen (1931) reared larvae from gametes obtained in mid-September at Mauritius (20° 20'S, mean annual temperature range 22°-27°C, Hodgkins and Michel, 1960). Onoda (1936) says that ripe specimens of E. mathaei can be found in July-August at Seto Marine Laboratory, Wakayama, Japan (33° 41'N, mean annual temperature about 14°-28°C, Kobayashi, 1969). Tohara and Okada (1968) mention July-early September for the

same locality. Mortensen (1937) reared larvae from gametes obtained from nearly-spent E. mathaei in April-May at Al Ghardaqa, Egypt. Pearse (1968) made single collections at a number of places in the Pacific tropics over the course of a year. Using histological methods, he always found at least a few individuals with ripe gametes. Even though he made only single collections, he concluded from this and from variation in ripeness among the individuals that the breeding season extends throughout the year near the equator.

The results of the investigation of the reproductive cycles of Hawaiian Echinometra are presented in Figures 18-21. Little difference is evident between the color forms, and the present discussion can be applied to both. Because the annual pattern of variation in the reproductive condition measures is similar at both collecting sites and the Kapapa Island site data are more complete, the variation at Kapapa will be considered and discussed as the basic pattern. The eccentricities of the Black Point data will be discussed later.

The variation in the percentage of individuals with numerous gametes indicates that spawning took place in April-June of 1967 and March-June of 1968. The fact that the percentage never dropped to zero, even following spawning, is evidence that the entire population is not synchronized and that a few ripe individuals may be found at all times of the year.

The variation in the mean percentage ova agrees with the above spawning periods, because one would expect that there would be a lag in the percentage ova due to the presence of a few relict ova after spawning had ceased. However, this measure seems to

indicate spawning in early December, 1967. Similarly, there are indications that spawning might have occurred at the same time of year, but somewhat earlier in 1966 and somewhat later in 1968. Evidently these spawns were not severe enough to completely remove all gametes, and hence did not affect the measure of the number of individuals with numerous gametes.

The variation in the gonad index does not seem to reflect these periods of spawning since a decrease in the index is frequently assumed to indicate spawning in sea urchins (Boolootian, 1966). Pearse (1969) comments, however, that the size of the gonads was not indicative of maturity in his study. In addition to its reproductive function, the gonad may serve a nutrient storage function (Anderson, 1966), and changes in the index may reflect either or both functions. Indeed, there may be a specific annual pattern of gonad growth, determined primarily by the uptake of nutrients from the environment, but modified by the transfer of these nutrients into gamete formation and their subsequent removal at spawning. A corollary specific annual pattern of gonad nutrient use was suggested for Strongylocentrotus purpuratus by Lawrence, Lawrence, and Giese (1966). The gonad indices of E. lucunter and E. viridis in Florida (McPherson, 1969), the Echinometra in the Gulf of Suez (Pearse, 1969) and in the present study are all similar in their annual pattern despite their somewhat different spawning periods. The gonad indices of the Wadi El Dom urchins follow much more closely the sum of the gamete volume (estimated by the percentage of individuals with numerous gametes) and the nutrient volume (estimated by the thickness of the nutritive phagocytic layers) than they follow

either of the separate measures alone. If no nutrients were transferred to gamete formation in the spring or lost through spawning during the late summer, the nutrient volume would reach a high peak around August and a low point in January or February. No values for nutrient volumes are available for the Florida or Kapapa populations, but an estimate can perhaps be made. In both cases subtraction of the gamete volume (estimated by the percentage of individuals with mature gametes) from the total gonad volume (represented by the gonad index) indicates that the nutrient volume is maximal during the summer and minimal during the winter. Moreover, Lawrence (1967) found very high levels of nutrients stored in the gut walls of E. lucunter from Costa Rica in July. It is therefore suggested that Echinometra has an annual nutrient uptake and storage cycle that is greater during the summer and less in midwinter. A similar cycle may be present in Strongylocentrotus purpuratus. Ebert (1968) reports that little feeding is done by this urchin during the late winter and early spring.

In the case of the Kapapa urchins, spawning occurs during the spring at the same time that the volume of nutrients taken up from the environment is rapidly increasing. Hence, the total gonad volume does not dramatically drop; it merely levels off or drops slightly. As opposed to the Wadi El Dom and Florida populations (Pearse, 1969; McPherson, 1969), mature gametes are present in most of the Kapapa population throughout the winter, and it is assumed that little of the nutrients taken up during the spring are transferred to gametogenesis. Nor do all individuals spawn in the main spawning season (shown by the fact that the percentage of individuals with numerous gametes never goes below fifty in the pink/green urchins or twenty

in the black urchins). These two factors, together with the rather long period over which spawning occurs (3-4 months), all minimize the drop in the mean gonad index during spawning.

It may be concluded that spawning of Echinometra in Hawaii seems to occur in the spring, with a minor spawning around December. Ripe individuals may be found at all times of the year, but whether these unsynchronized members actually spawn at other times during the year is an unanswered question. During the summer it is almost impossible to induce shedding in Hawaiian Echinometra, but shedding can be induced at most other times of the year (Dr. Robert E. Kane, personal communication). The same lack of synchrony (or year-round presence of mature gametes) has been found by Pearse (1969) at several locations in the tropics, Pearse and Phillips (1968) at Rottneest Island, and Pearse (1969) in the Red Sea. Their interpretation of the data is that year-round spawning activity occurs in populations of Echinometra near the equator. The seasonality reported for Barbados (Lewis, 1960) argues against this being a general rule.

The timing of the reproductive cycle of an organism is set through the process of natural selection so that the greatest number of offspring which grow to maturity are produced. A major part of the total selection may be associated with correlation of a particular stage in the reproductive cycle with a particular environmental variable, such as a planktonic larval life stage with a high concentration of its food. In other cases, the adaptive value of a correlation may be restricted to merely ensuring synchronous gamete maturation. The environmental variables possibly correlated with the reproductive cycles of marine invertebrates in general and echinoderms in particular have been discussed by Giese (1959) and Boolootian (1966),

respectively. Generally the same variables which they list can be suggested for Echinometra: food (either larval or adult), temperature, light, salinity, and lunar phases. Probably one or more of these factors is associated with the timing of the annual reproductive cycle, and one or more is probably responsible for the initiation of spawning. These factors, with three additions, are arranged in tabular form in Table 10.

Unfortunately, the annual variation in the availability of food for either the larvae or the adult Echinometra in Hawaii is not known. During the winter more storm waves strike the windward coast of Oahu, and more algae may be broken off. Nevertheless, no information is available on the seasonal productivity of the algae.

Since the breeding season of Echinometra extends over several months, it is difficult to see how lunar phases could be important in determining the timing of the season. Nor are regular annual salinity changes in the vicinity of populations of Echinometra, in Hawaii at least, probably of any consequence, although significant irregular changes undoubtedly do occur following rainstorms.

Correlation of a reproductive cycle with daylength has selective value only as a reliable measure of less regular temperature changes. Giese (1959) believes that this would be of little value in most marine environments.

Pearse (1969) suggests that the level of nutrient reserves is important in the timing of the reproductive cycles of the Echinometra populations that he has studied.

McPherson (1969) proposed that increasing temperature was

Table 10

Environmental and Internal Factors Possibly
Correlated with the Timing of the Reproductive
Cycle and Spawning in Echinometra

- A. Factors which may be important in reproductive cycle timing:
1. availability of food for adult
 2. availability of food for larvae
 3. temperature
 4. daylength (as an indicator of temperature cycles)
 5. lunar phases (if the breeding season is extremely short)
 6. salinity (if a regular cycle of extreme changes occurs)
 7. abundance of internal nutrient stores
- B. Factors which may be important in the initiation of spawning:
1. sharp temperature changes
 2. sharp salinity changes
 3. lunar phases
 4. abundance of gametes
 5. heavy surf action
 6. critical temperature level

important in the timing of the cycles of Echinometra that he studied off Florida. Pearse (1969) argues against the importance of this factor in Echinometra: 1) The Rottneest Island population experiences a very cool annual temperature range (about 18° to 23°C), but has apparent year-round breeding; 2) Al Ghardaqa is colder (about 17° to 29°C) than Wadi El Dom (about 18° to 28°C), but its urchins have less discrete breeding periods, and 3) Gametogenesis could not be induced in the laboratory with elevated temperatures. All of these points are questionable, as Pearse himself affirms, and I question the first point in particular. It seems to me that the Rottneest population must be non-seasonal in its breeding period for different reasons than those populations located close to the equator. Perhaps the primary synchronizing effect of annual temperature changes is disrupted by "unscheduled" spawning in a significant percentage of the population because of irregular secondary factors, such as extreme salinity changes, heavy surf, or extreme temperature changes. Hodgkin (1959) gives a report of one extreme case of low tides and strong insolation at Rottneest. Furthermore, Moore et al. (1963) suggest that minor temperature fluctuations might have more effect on the spawning of a population of sea urchins living closer to their tolerance limit for temperature than they would on a population living nearer to optimal temperature conditions.

In summary, the reproductive cycle of Echinometra seems most likely to be correlated with nutrient reserve level or temperature. The nutrient level cycle may be considered to be represented by the gonad index cycle if an additional amount is added on to the index to allow for the amount lost during spawning. Comparison of this cycle in the Kapapa urchins with the change in the water temperature

near Kapapa Island (Figure 25) shows a close correlation between the two factors. A similar correlation can be seen in both Pearse's (1969) and McPherson's (1969) data.

Pearse (1969) suggested that the beginning of gametogenesis is the critical stage that is correlated with the cycle of some environmental or internal factor. Once this stage is initiated the gametogenic cycle runs to completion (maturation) in a species-specific, genetically determined manner little influenced by normal environmental changes. The cycle takes at least three months from initiation to maturation. However, the length of time that the mature gametes are held before spawning apparently may vary.

In the Wadi El Dom and Florida populations of Echinometra, the initiation of gametogenesis evidently occurred in the late winter or early spring. This is about the time that the nutrient reserve level and the temperature are increasing. In the Kapapa urchins, gametogenesis began in the summer soon after spawning. I suggest that gametogenesis was begun in both cases when a critical level of nutrient reserves was available. In the Wadi El Dom and Florida urchins, the critical level was not available until the following winter, when the nutrient cycle began again. This is because the spawning occurred so late in the previous nutrient cycle. In the Kapapa urchins, however, spawning occurred early in the cycle, and enough nutrients were available during the summer for initiation of gametogenesis after only a slight delay. Most spawning, nevertheless does not occur until the following spring. The maturation of the new gametes at Kapapa is not complete until after mid-fall when cool temperatures have arrived, and perhaps spawning cannot be

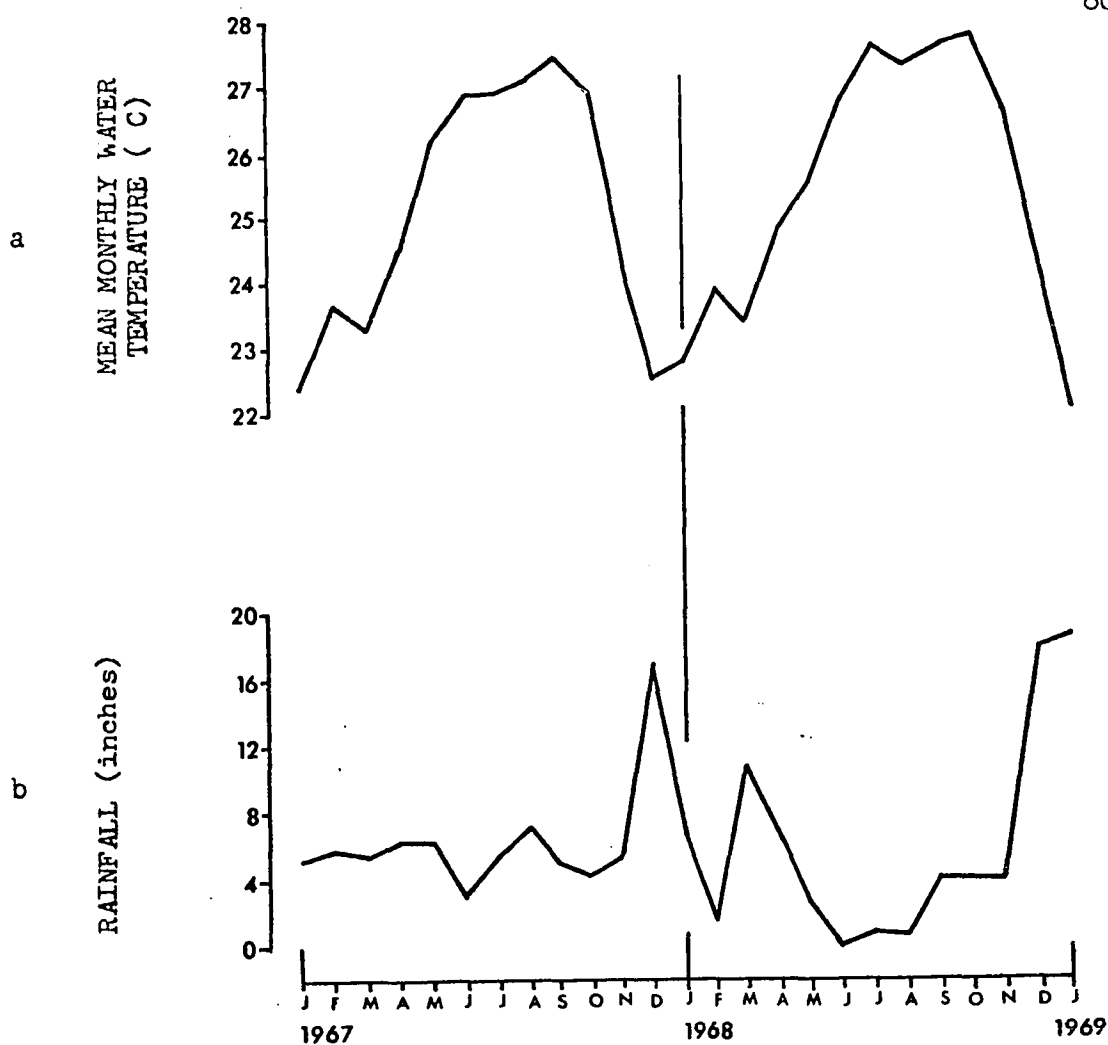


Figure 25. The variation in two environmental factors near Kapapa Island, Oahu, during 1967 and 1968.

- a. Mean monthly water temperatures at Mokuoloe (Coconut) Island, Kaneohe Bay.
- b. Rainfall at Kaneohe Marine Corp Air Station on Mokapu Peninsula in the 30 days prior to each reproductive cycle study collection at Kapapa Island.

triggered in most of the population until some critical level of temperature is again exceeded. Hence mature gametes are held throughout the winter until spawning the next spring.

Finally, it appears that the incomplete December spawning in some urchins at Kapapa is "accidental." Since mature gametes are present, irregular secondary factors may override the temperature control measure and cause "unscheduled" spawning, as was suggested in the case of the Rottnest urchins. Certainly the spawning was closely associated with a period of extremely heavy rainfall in Hawaii (Fig. 25). It seems possible that a heavy rain may lower salinities temporarily even on the open reef flat, and initiate spawning in some of the population of Echinometra. In December of both 1967 and 1968 there were days when more than 5 inches of rain fell in 24 hours.

Populations of animals with specific breeding periods of less than a year may have peaks of spawning associated with phases of the moon. Kobayashi (1969) has recently reported a lunar spawning cycle superimposed on the annual breeding cycle of E. mathaei at Seto, Japan. Peaks of spawning seemed to occur two or three days before and after both the new and the full moon. Such a cycle may occur in all populations of Echinometra, but other studies, including mine in Hawaii, have not made sufficiently frequent collections during the breeding season to detect the cycle.

General agreement of the reproductive activity of the Elack Point urchins with Kapapa Island urchins was detected: spawning periods occurred in the spring of both years and again in December in both

years. However, a higher percentage of both individuals with numerous gametes and mean number of mature gametes present in females was regularly exhibited in the Black Point population. This indicates less synchrony in the population and is similar to the spawning found in the Rottneest Island urchins. The Black Point site is located in shallower water and is closer to shore. It is probable that these urchins are subject to more extreme local changes in temperature or salinity. The same conditions were suggested earlier in this paper as being responsible for the increased asynchrony in the Rottneest urchins.

Although the reproductive cycles of the two color forms are similar, there are minor differences. It is difficult to say whether these differences are the result of chance sampling variation or indicate discrete differences. Both populations of blacks were distinguished by a much more complete spawning of individuals in the group. The percentage of individuals with mature gametes fell regularly to about 20% in the blacks, while the value fell to a minimum of only 50% in the pink/greens. This is a measure of greater synchrony among the blacks. Spawning seemed to start earlier in the spring of both years for the Black Point pink/green urchins than it did for the black urchins. On the other hand, the black urchins at Kapapa seemed to spawn earlier than the Kapapa pink/greens in both the spring and winter of 1968. Moreover, the impression was derived that despite wide monthly variations, the percentage of individuals that shed during handling was correlated within, but not between, color forms.

In summary, the interpretation of these data is that the reproductive cycles and spawning periods of both color forms are similar in Hawaii, although slight differences are suggested. A major period of spawning occurs in the spring and a secondary period around December. Ripe individuals are present in the population throughout the year and may indicate some year-round spawning. The reproductive cycles seem correlated with temperature and nutrient uptake and storage cycles. The December spawning is perhaps caused by an extreme salinity drop. The general contention of Pearse (1968) that tropical populations of Echinometra tend to breed year-round, while those at higher latitudes tend to have more discrete breeding periods is not refuted. However, a year-round study of a truly tropical population is necessary for confirmation.

GAMETE COMPATIBILITY

Methods and results

The degree of reproductive isolations between two closely related populations can be estimated from the compatibility of their gametes, as measured by the frequency of cross-fertilization. The demonstration of fertilization between two such populations does not necessarily prove that they are not two species for as pointed out by Mayr (1963), "Cross-fertility does not prove conspecificity." The formation of hybrids is notoriously easy in echinoids, and Harvey (1956) lists hybrids that have been reported, including several that occurred naturally. However, a lower percentage of fertilizations occurring between groups than occurs within groups may indicate the likelihood of genetic isolation. Accordingly, gamete compatibility of the two color forms was studied in the laboratory.

Adult urchins were collected at Kapapa Island, Oahu, in the morning of October 29 and November 12, 1969. The urchins were transported to the laboratory (University of Hawaii Waikiki Beach Lab) in buckets containing moist paper towels. After each urchin was rinsed with seawater, isotonic KCl was injected into the body cavity, and the urchin was placed upside-down on a beaker of filtered seawater. Sperm and eggs were exuded from most of the urchins and sank to the bottom of the beaker. The amount varied from a drop to several milliliters. The seawater was carefully decanted from the sperm and discarded, and the sperm were placed in a refrigerator until just before the fertilizations. The eggs were washed several

times with filtered seawater to which the chelating agent EDTA had been added. Re-concentration was by hand centrifugation. Finally, a semi-transparent suspension of eggs was placed in a centrifuge tube. After checking the motility and fertilizing capacity of the portion of the sperm to be used, one drop of sperm was added to about 50 ml of filtered seawater. About 1 ml of this dilute mixture was added to the eggs in the centrifuge tube. Examination under a microscope showed that only a few sperm were associated with each egg. In the fertilizations made on October 29, 500 eggs, zygotes, or larvae were examined in a sample from each batch to determine the percentage developing normally. In the November 12 fertilizations, 200 eggs, zygotes, or larvae were examined. The larvae were then placed in Stender dishes for culture. The water was changed daily. No attempt was made to feed the larvae, and resorption of the skeletal elements was obvious on the sixth day, with death following soon.

The crosses using parents of the same color were repeated three times, once on October 29, and twice on November 12, using different individuals as parents. The cross of a black female with a pink/green male was also repeated three times, as was the reciprocal cross. On October 29, two inter-color crosses, one with a black female and the other with a pink/green female, were made using an excessively high concentration of sperm. Numerous sperm surrounded each egg, and polyspermy was probable. Because of the polyspermy, the experiment was terminated after 4 hours. On November 12, an adult urchin was collected which seemed intermediate between the color forms (One of the two intermediates mentioned earlier.). The color of the intermediate urchin was a dark olive green, unlike any

other green seen previously in Hawaii, and its spines were heavy, short, and rounded at the end, as is often typical of the black form. It was a ripe male, and its sperm were used to fertilize eggs from females of both color forms. The results of the cross-fertilizations are summarized in Table 11.

Discussion

The lower percentage of fertilizations and larvae resulting from crosses between color forms is obvious. Similar results from crosses between Strongylocentrotus purpuratus and S. franciscanus (9-18 o/o) have been reported by Chaffee and Mazia (1963). It can be extrapolated from such information that cross-fertilization between the two color forms of Echinometra probably does not occur regularly in the field. The increase in cross-fertilizations found when heavy insemination was used was expected (Lillie, 1921), but it seems unlikely that similar concentrations of sperm occur naturally. The fact that the intermediate-looking urchin produced a high percentage of fertilizations with both color forms is strong evidence that it is indeed a hybrid or intermediate morph.

The plutei from all crosses did not obviously differ in morphology, although the skeletal elements were not examined. All seemed equally healthy. Dr. Ralph Hinegardner (personal communication) has raised larvae from eggs of the black form and sperm of the pink/green form. They were able to feed and grow, but became abnormal after 2 weeks and died. The reverse cross died after gastrulation. Both crosses yielded low percentages of fertilizations. The intermediate adult found at Kapapa shows that full growth of a hybrid may be possible and that active gametes can be produced.

Table 11

The Percentage of Echinometra Eggs Developing to
a Given Time Following Exposure to Various Sperm
as a Function of the Source of Gametes

Cross ♀ ♂		$\frac{1}{2}$ -4 hr. (fertilization membrane- four cell)	18-24 hr. (blastula- early pluteus)	5-6 days (pluteus)
p/g x p/g		94-100	94-100	most living
b x b		100	98-100	most living
p/g x b		2-10	2-10	most living
b x p/g		1-14	1-9	most living
p/g x b*		54		
b x p/g*		19		
p/g x inter- mediate**		64	68	most living
b x inter- mediate**		100	100	most living

* An unusually high concentration of sperm was used.

** An urchin judged intermediate in morphology.

THE ECHINOMETRA MATHAEI/OBLONGA COMPLEX
IN THE INDO-PACIFIC

Morphological variation

A comparison of the forms of Echinometra that occur in other locations in the Pacific with those in Hawaii was made possible by using the collections of Bernice P. Bishop Museum. The collection from Pitcairn Island was the only one that contained sufficient numbers of all reported varieties to permit even preliminary quantitative analysis. All of the morphological characters that were examined in the Hawaiian Echinometra were also investigated in the Pitcairn specimens. Four morphological varieties were recognizable. The morphological differences found between the forms are shown in Table 12. Although the numbers that were examined were very small (4-12 in each variety) it appears that the four varieties can be grouped into two main types on the basis of spine shape and gonad spicules. One has heavy spines and no spindle-shaped spicules in the gonad; the other has slender or intermediate spines and spindle-shaped gonad spicules. The numbers of pore-pairs per arc did not differ greatly between the forms, but only 18 individuals were counted. The forms with heavy spines tended to have more arched tests (larger height to length ratios), but again, more urchins will have to be examined.

Although the urchins from Pitcairn Island could be separated into two forms -- black and non-black -- as those of Hawaii, this distinction cuts across the separation in variation in spine shape and gonad spicule types. A more valid separation seems to be indicated by these last two characters, even though many uniformly gray

Table 12

The Frequency of Morphological Characters in Varieties
of Echinometra from Pitcairn Island

		Spine Color			
		dull-greenish- or pinkish- gray or light green (6)*	brownish-gray, gray, or olive- green, all white- tipped (12)	black, some blue near tips (8)	gray, some blue near tips (4)
Spine shape:	(4)		(12)	(8)	(4)
slender	75%		83%		
intermediate	25%		17%		
heavy				100%	100%
Spindle-shaped gonad spicules:	(6)		(7)	(7)	(4)
present	100%		100%		
absent				100%	100%

* Numbers in parentheses are the total number of
urchins examined for the character.

individuals are included with the black urchins. Thus two forms are still discernible, but they are much less distinct than they are in Hawaii.

The occurrence of spine shape types, spine color types, and spindle-shaped gonad spicules has been surveyed briefly in Echinometra in other areas of the Indo-Pacific. Slender-shaped spines with an abruptly white tip (color change is complete in less than 1 mm of spine length--Fig. 26) are found in many areas. The geographic distributions of abruptly white-tipped spines is shown in Table 13. It is based on literature records or specimens that I have seen. Many other localities should perhaps be included. It seems that all areas having urchins with the white-tipped spines also have urchins with uniformly colored spines of the same shape.

Typical stout, blunt spines are apparently absent in Japan, Rottnest Island, Western Australia (Clark, 1946), Eastern Australia (Clark, 1946), and the Gulf of Suez (Pearse, 1969). The absence of blunt-spined individuals has precluded reports of E. oblonga from these localities.

On the other hand, E. oblonga is the only form reported to occur in the Revillagigedo Islands (Clarion, Socorro) and the Galapagos Islands (Bindloe, South Seymour, Albemarle) (Clark, 1948). It is not certain whether the identification in this case was based on black spine color or stout, blunt spine shape or both.

I have found spindle-shaped spicules in the gonads of typical E. mathaei-like forms (urchins with slender, often white-tipped spines of a variety of non-black colors) in 2 of 2 specimens from

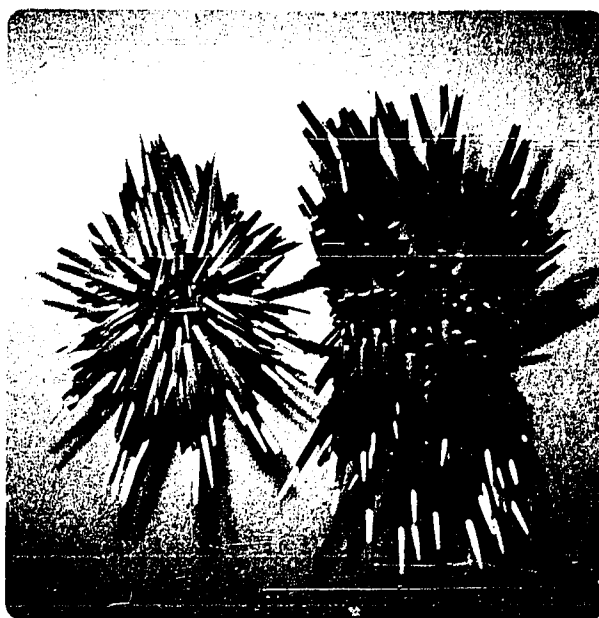


Figure 26. Echinometra from Tahiti showing spines with abruptly white tips.

Table 13

Geographic distribution of Echinometra
with Abruptly White-tipped Spines

Present:

Line Islands (Palmyra, Christmas, Washington)
 Samoa
 Tonga (Tongatabu) (Nuuafoou- Clark, 1931)
 Society Islands (Tahiti)
 Tuamotu Islands (Oeno) (Mangareva-Clark, 1912)
 Northeastern Australia (Clark, 1946)
 Lord Howe Island (Clark, 1938)
 Molucca Islands (Ceram- Döderlein, 1906)
 Philippine Islands (Roxas, 1928)
 Ryukyu Islands (Okinawa)

Apparently absent:

Hawaiian Islands (Oahu, Hawaii, Leeward Islands)
 Wake Island
 Johnston Island
 Gulf of Suez and Northern Red Sea (Pearse, 1969)
 Rottnest Island, Western Australia (Pearse, 1969)
 Marshall Islands (Eniwetok)
 Phoenix Islands (Canton, Howland, Baker)

Oeno Atoll, Tuamotu Islands; 7 of 8 from Tahiti; 26 of 26 from Eniwetok Atoll, Marshall Islands; and 7 of 7 from Okinawa. I found no such spicules in the gonads of 1 black, heavy-spined specimen from Eniwetok, or 13 black and 8 gray, heavy-spined specimens from the Tuamotu Islands (Oeno, Takaroa, Pukapuka).

Finally, the numbers of pore-pairs per arc found in E. mathaei-like urchins from five areas of the Pacific are presented in Table 14.

Therefore, the relationship between the two forms in most of the Pacific seems to be similar to that at Pitcairn. As shown in Table 13, this situation extends from the Line Islands and the Tuamotus to Indonesia and the Philippines. The situation in the Indian Ocean is not clear, and I have been unable to obtain specimens from the area. On the other hand, the relationship between the two forms in the Northern Pacific from the Hawaiian Islands to the Marshall Islands seems to be similar to that at Oahu. As shown in Table 13, no white-tipped spines have been found in this area. No stout, blunt, gray-spined urchins have been seen from this area either. Only five specimens from the Phoenix Islands have been seen, so that the situation there is uncertain. Finally, the occurrence of only one of the forms at some locations shows that monotypic populations can exist.

The variation in the proportion of arcs having three, four, and five pore-pairs in typical E. mathaei from Hawaii and other areas of the Pacific (Table 14) may indicate that the Hawaiian form differs sufficiently to be considered a subspecies. However, more data on the non-Hawaiian populations are necessary before any

Table 14

The Frequency Distribution of the Numbers of Pore-Pairs Per Arc in E. Mathaei-like Urchins (Slender, Non-black Spines, Spindle-Shaped Spicules Usually Present in the Gonad) from Five Areas of the Pacific*

	Locality				
	Pitcairn	Tahiti	Eniwetok	Okinawa	Oahu
Pore-pairs per arc:					
three	6%	3%	3%	1%	0.4%
four	91%	96%	95%	98%	84%
five	2%	1%	2%	1%	15%
Number of urchins examined:	7	8	26	7	29

* Five arcs in each ambulacrum counted, starting at the ambitus and proceeding aborally.

conclusions can be reached.

Habitat specialization

The wide morphological variation in Echinometra in the Indo-Pacific has provoked many observers to seek a correlation with different habitats. Clark (1921) was unable to find such a correlation despite special efforts at Mer, Torres Strait. Mortensen (1943) likewise was unable to discern any correlation, although he repeated an observation reported by Bell in 1902 that the lighter forms occur farther out on the reefs in the Maldives and Laccadive Islands. Contrarily, Tokioka (1953) said that darkly colored individuals are relatively more abundant near the reef edge, while the whitish ones are more abundant away from the reef edge in the Tokara Islands, Ryukyu Islands. Doty and Morrison (1954) also noticed a zonation pattern in a study of Raroia Atoll, Tuamotu Islands. The Echinometra in the Amphiroa zone just landward of the algal ridge were all black while in the Heliopora zone landward of this, the urchins were predominantly pink. Moreover, the pink urchins from more seaward locations had thicker, shorter spines.

Echinometra mathaei is reported by Mortensen (1943) to occur usually in holes in rocks, but also under rocks throughout its range. Pearse (1969) found the same species living in somewhat different conditions at different localities. At Wadi El Dom, they were found completely exposed on coral rubble. Further south, at Al Ghardaqa, they were partially hidden under coral ledges (to whose formation they perhaps contributed). Even further south, at Quseir, they were completely hidden under rocks and in deep crevices. At Rottnest Island, Western Australia the individuals were only partially

hidden, like those at Al Ghardaqa, while in the equatorial areas of the Indo-Pacific, they were completely hidden, as at Quseir. At Mauritius, Hodgkin and Michel (1960) reported the burrows were deeper at higher levels of the shoreline.

It was reported in the Florida Keys by Kier and Grant (1965) that E. lucunter occurs on hard bottom (and occasionally on sand near hard bottom) from the shoreline out to the offshore reefs. It inhabits holes and shallow recesses in rock, clings to the bases of sponges and coral, and works its way under detached blocks. McPherson (1969) mentions that this species occasionally is seen in sea grass (Thalassia) beds.

Kier and Grant (1965) reported no great differences in habitat between E. viridis and E. lucunter, although McPherson (1969) said E. viridis is often absent from inshore areas. Mortensen (1943) said E. vanbrunti is also found in holes and under rocks.

Earlier studies of the habitat of Echinometra have been confined to shorelines and shallow reef flats, and as a result, only the upper limits of populations of Echinometra are usually recorded, frequently in conjunction with a study of intertidal zonation. Thus, Echinometra was reported associated with crustose coralline algae by Sourie (1954) in Senegal, Lewis (1960) in Barbados, Gauld and Buchanan (1959) in Ghana, Hodgkin and Michel (1960) in Mauritius, and Hedgpeth (1969) in the Galapagos Islands. An association with macroscopic algae has been noted by Lawson (1956) in Ghana, Tokioka (1953) in the Ryukyu Islands, and Hedgpeth (1969) in the Galapagos. The upper limit of populations of Echinometra is generally around

mean low water (MLW) or mean low water neap (MLWN), as reported by Sourie (1954), Lewis (1960), Lawson (1956), Kalk (1958, in Mozambique), and Endeian, Kenny and Stephenson (1956) (on the Queensland mainland). As pointed out by Hodgkin (1960), however, the small range of tides at most of these localities makes the zonation a "wave zonation", and the level of a zone of organisms varies greatly in a single locality due to different exposures to waves. Thus, much higher upper limits are reported in more exposed areas (Endeian, Stephenson, and Kenny, 1956; Hodgkin and Michel, 1960). Similar zonation studies in Hawaii (Gosline, 1965; Doty, 1967) mention echinoids only in passing.

Mortensen (1943) has reported the lowest depths from which Echinometra species have been dredged, E. lucunter 45 m, E. vanbrunti 50 m, and E. mathaei 34 m.

Because of the development of inexpensive, efficient diving equipment, investigators have been able to extend their direct observations below the low tide mark in the past 15 years. Two recent studies have made use of diving gear to extend observations of Echinometra below the intertidal zone. Kier and Grant (1965) found E. lucunter from the surface down to 3 m in the Florida Keys. E. viridis ranged from the shoreline down to 12.2 m. Occasional individuals of both species may live deeper. Kier (1966) found many specimens of E. lucunter exposed at low tide at Dominica. They were most abundant from 0.3 to 0.6 m below low tide mark and were rare below 2.4 m deep. Only one individual of E. viridis was found and that one at 15.2 m deep. Interestingly, two color forms of E. lucunter were noticed both in the Florida Keys and at Dominica,

but no separation of habitat was mentioned.

In conclusion, there seems to be less definite habitat specialization in the forms of Echinometra in the rest of the Indo-Pacific than is found in Hawaii. The only positive observations are those of Doty and Morrison (1954) and Tokioka (1953), and the latter observations were made in Japan, where only E. mathaei-like urchins are found.

GENERAL DISCUSSION

Evidence has been given of fairly definite habitat specialization in the two forms of Echinometra in Hawaii. Several differences in morphology have also been shown: spine color, predominant spine shape, average number of pore-pairs per arc, presence or absence of spindle-shaped spicules in the gonad, and mean and maximum test length. Ecophenotypic variation is apparently responsible for very little of the morphological variation. The definite habitat differences and extensive genetically-related morphological differences strongly suggest that the two forms are separate species. Further positive evidence of their reproductive isolation is provided by the relative incompatibility of their gametes. Little divergence in breeding season has occurred, probably because the seasons are determined by the same environment factors in both species.

In contrast with the Hawaiian forms of Echinometra, those in the Indo-South Pacific exhibit apparently fewer morphological differences, and habitat specialization seems less obvious, although it was recognized by Doty and Morrison (1954) at Raroia, Tuamotus. No comparative information on breeding seasons and gamete compatibility are available for these forms.

It is perhaps instructive that in two areas where only the E. mathaei-like form is found (Okinawa and Gulf of Suez) there is a convergence of the normally light color toward a considerable percentage of almost black coloration (Dr. S. J. Townsley, personal communication and Pearse, 1969). Conversely, where both forms are

clearly separate species (Hawaii), there is a complete divergence of color into black and non-black. The sharper distinction in morphology and habitat specialization in Hawaii may be explained in terms of character displacement. The relative lack of divergence in populations in most of the rest of the Pacific implies an absence of reproductive isolation in these populations.

The separation into the species found in Hawaii could have arisen in several ways. It can be speculated that through isolation of populations a single original form could undergo geographic speciation, perhaps diverging under different selective pressures from competition with other urchin species. Tokioka (1963, 1966) gives evidence of competition between intertidal echinoid species in Japan. (The echinoids Anthocidaris and Mespilia expanded their distributions after Echinometra was killed by extremely low temperatures in the winter of 1962-1963.) If the divergence of the isolates is great enough and re-union occurs, they may be reproductively isolated and act as competing species.

However, it seems more likely that in this case a polymorphic stage was involved, with speciation occurring after the development of polymorphism. The adaptive value of polymorphism is in increasing the range of environmental conditions and habitats which can be profitably occupied (Huxley, 1955). For an intertidal-subtidal organism such as Echinometra, living in an environment with a wide range of conditions and habitats in a small area, it would appear that polymorphism would have high selective value. Indeed, fairly distinct sympatric forms have been noticed in E. lucunter (Kier,

1966), E. vanbrunti (Mortensen, 1943), and E. insularis (F. Julian Fell, personal communication). Hence, there is no reason to doubt that the two forms in the Indo-West Pacific did not arise as polymorphs of the same species.

The subsequent speciation could possibly have occurred sympatrically, as Ford (1964) has suggested is the case in two species of butterflies. However, geographic speciation seems much more likely to have occurred. Mayr (1954) has cited the formation of the species of Echinometra recognized by Mortensen (1943) as a classic example of geographic speciation. Monomorphic populations on the periphery of polymorphic populations are common (Mayr, 1963), and, if separated geographically from the main population for sufficient time, they may become reproductively isolated (Huxley, 1955). If the isolates are then re-united with the parent population, competition between them could lead to character divergence and suppression of the morph most similar to the isolate.

SUMMARY AND CONCLUSIONS

Two species or varieties of the sea urchin Echinometra occur in Hawaii. They differ primarily in spine color: one (E. oblonga) is black and the other (E. mathaei) is pink, green or pink-green, but is never black. The relationship between the two forms may be that of polymorphs, polyphenotypes, or separate species. Investigation of the morphological and physiological variation, habitat specialization, reproductive cycles, and gamete compatibility of the two forms was undertaken to elucidate the relation of the two.

1. A series of quadrat counts along vertical transects shows that the abundance of the black form is greatest in areas of surf action, where it numerically dominates the pink/green form. The pink/green form predominates in areas of less turbulence in tidepools, on shallow reef flats, and in deeper water.
2. Significant morphological variation in several characters was found between the two forms. The black form has black spines, usually has stout, blunt spines, rarely has spindle-shaped spicules in the gonad, has a higher average number of pore-pairs per arc, and has a smaller mean and maximum test length. The pink/green form has non-black spines, rarely has heavy spines, almost always has spindle-shaped gonad spicules, has a lower average number of pore-pairs per arc, and has a higher mean and maximum test length. No significant differences were detected in tolerance to insolation, desiccation and heating, extreme salinity changes, or spine strength per unit cross-

sectional area. However, the larger mean diameter of the black spines does make the breaking resistance of the average black spine greater than that of the average pink/green spines.

3. Polyphenotypic variation under the different ecological conditions was investigated with respect to spine color, spine shape, and gonad spicules. The color of regenerating spines was only slightly affected by the amount of illumination received during regeneration. No differences in food seem to be present, as indicated by gut content examination, food preference experiments, and the predominant non-grazing mode of feeding. A correlation of stout spine shape in the black form with water turbulence is suggested by aquarium spine regeneration observations and the higher frequency of occurrence of slender spines on individuals in quiet tidepools. The non-grazing habits of the urchins and the identical Ca/Mg of the test and spicules indicate that the gonad spicules are not derived from ingested sponges.
4. The reproductive condition of both Hawaiian forms was recorded monthly for 2 years. The size of the gonads, the presence of numerous gametes, and the maturity of the eggs were employed in estimating breeding condition. No obvious differences in the spawning seasons of the forms were detected. Major spawning occurred in the spring (March-June), and a second, minor spawning occurred in

December. Mature gametes were found in a few individuals at all times of the year. Initiation of the spring spawning season seemed to be correlated with increasing temperature and internal nutrient supply. The December spawnings appeared correlated with a sharp increase in rainfall, perhaps accompanied by lower salinity, and are possibly not regular every year.

5. Homogametic fertilizations (sperm and eggs from the same color form) were over 94% successful. Heterogametic fertilizations (gametes from different color forms) were about 10% successful. Sperm from an unusual urchin which had immediate morphological characteristics produced 64% fertilizations with one form and 100% fertilizations with the other. Larvae from all crosses survived normally for 6 days, after which they died from starvation. No attempt was made to rear larvae to metamorphosis.
6. Examination of the morphological variation in Echinometra in the rest of the Pacific Ocean shows less distinction between the varieties, with more overlap in morphological features. Stout, blunt spines and an absence of spindle-shaped gonad spicules are correlated with each other, but spine color differences (black versus non-black) are not consistent with this separation. Monomorphic populations of Echinometra occur in the Gulf of Suez, Western Australia, Japan, and the eastern Pacific.
7. The definite habitat specialization, extensive non-ecophenotypic morphological distinctness, and the gamete incompatibility strongly indicate that the two forms in

Hawaii are separate species. The varieties of Echinometra found in the South Pacific are evidently polymorphs of a single species. The distinctness of the two Hawaiian species is probably indicative of character displacement.

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