Summer 1972

SOME ASPECTS OF THE BIOLOGY OF A BROODING STARFISH, LEPTASTERIAS LITTORALIS (STIMPSON) 1853

FRANCIS XAVIER O'BRIEN
University of New Hampshire, Durham

Recommended Citation
O'BRIEN, FRANCIS XAVIER, "SOME ASPECTS OF THE BIOLOGY OF A BROODING STARFISH, LEPTASTERIAS LITTORALIS (STIMPSON) 1853" (1972). *Doctoral Dissertations*. 2343.
https://scholars.unh.edu/dissertation/2343

INFORMATION TO USERS

This dissertation was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.

2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in "sectioning" the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again — beginning below the first row and continuing on until complete.

4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from "photographs" if essential to the understanding of the dissertation. Silver prints of "photographs" may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.

University Microfilms

300 North Zeeb Road
Ann Arbor, Michigan 48106

A Xerox Education Company

73-4363

O'BRIEN, Francis Xavier, 1935-
SOME ASPECTS OF THE BIOLOGY OF A BROODING STARFISH, LEPTASTERIAS LITTORALIS (STIMPSON) 1853.

University of New Hampshire, Ph.D., 1972
Zoology

University Microfilms, A XEROX Company, Ann Arbor, Michigan

SOME ASPECTS OF THE BIOLOGY
OF A BROODING STARFISH,
LEPTASTERIAS LITTORALIS (STIMPSON) 1853

by

FRANCIS XAVIER O'BRIEN

B. A., Suffolk University, 1963

M. S., University of New Hampshire, 1965

A THESIS

Submitted to the University of New Hampshire

In Partial Fulfillment of

The Requirements for the Degree of

Doctor of Philosophy

Graduate School

Department of Zoology

June, 1972

This thesis has been examined and approved.

Emery F. Swan

Thesis director, Emery F. Swan, Prof. of Zoology

Wilbur L Bullock

Wilbur L. Bullock, Prof. of Zoology

A. E. Teeri

Arthur E. Teeri, Prof. of Biochemistry

John J. Sasner

John J. Sasner, Assoc. Prof. of Zoology

Hugh F Mulligan

Hugh F. Mulligan, Assoc. Prof. of Botany

July 6, 1972

Date

ACKNOWLEDGEMENTS

Dr. Emery F. Swan first made me aware of *Leptasterias littoralis* and has continued to offer suggestions and encouragement to me during the course of this study. I wish to express my gratitude to him for his kind assistance and guidance.

I also wish to thank the other members of my doctoral committee, Dr. Wilbur Bullock, Dr. John Sasner, Dr. Arthur Teeri, and Dr. Hugh Mulligan for their guidance and suggestions in the preparation of the dissertation.

To my wife, Marion, I express special thanks for her continued support and encouragement which made this work possible.

TABLE OF CONTENTS

LIST OF TABLES.. v

LIST OF ILLUSTRATIONS... vi

ABSTRACT.. vii

I. INTRODUCTION... 1

II. MATERIAL AND METHODS.. 9

III. EXTERNAL MORPHOLOGY.. 12

1. Color.. 12

2. Size... 12

3. Skeletal Plates.. 17

4. Dermal Papulae... 19

5. Spines and Pedicellariae..................................... 19

IV. NATURAL HISTORY... 32

1. Habitat.. 33

2. Feeding.. 41

3. Reproduction and Brooding Behavior........................... 52

V. HISTOLOGY OF THE CARDIAC STOMACH............................. 60

1. Normal Histology... 62

2. Brooding Starfish.. 65

3. Starved Starfish... 66

VI. GENERAL DISCUSSION.. 70

LITERATURE CITED.. 74

APPENDIX.. 81

LIST OF TABLES

1. A Summary of the Comparative Dimensions of the Three Species at Comparable Sizes.. 18
2. A Summary of the Taxonomic Characteristics of *Leptasterias littoralis*, *Leptasterias tenera*, and *Asterias rubens*............. 30
3. Species List of the More Common Invertebrates Found at Gove Point... 40
4. A Summary of the Feeding and Brooding Activities of *Leptasterias littoralis* Collected Between August, 1966 and March, 1969......... 42

LIST OF ILLUSTRATIONS

1. Aboral View of Preserved and Dried Specimens of *Leptasterias tenera*, *Leptasterias littoralis*, and *Asterias rubens*......... 16
2. Aboral View of a Starfish Showing Major Radius (R) and Minor Radius (r)... 21
3. Cross Section Through the Arm of a Starfish Showing the Relative Positions of the Skeletal Plates and Spines......... 21
4. Series of Spines Taken From Mid-arm Region of *Leptasterias littoralis* Showing Comparative Sizes and Shapes.............. 25
5. Major Type Pedicellaria From *Leptasterias littoralis*......... 27
6. Minor Type Pedicellaria From *Leptasterias littoralis*......... 27
7. A View of the Lower Intertidal Zone at Gove Point Where *Leptasterias littoralis* may be Collected.................... 35
8. Two Specimens of *Leptasterias littoralis* Attached to the Surface of an Overturned Rock in April, 1967................ 38
9. Section Through Cardiac Stomach Wall Showing Arrangement of Typical Epithelial Cells and Several Mucous Gland Cells...... 64
10. Section Through Lower Region of Stomach Showing Channel and Associated Ridges...................................... 64
11. Section Through Region of the Esophagus of a Fed Starfish.... 68
12. Section Through Region of the Esophagus of a Starfish Starved for 60 Days...................................... 68

ABSTRACT

The external morphology of the brooding starfish *Leptasterias littoralis* (Stimpson) of the Northwest Atlantic coast is described and compared with that of *Leptasterias tenera* (Stimpson) and *Asterias rubens* L., two other species having ranges partially overlapping with that of *L. littoralis*. Seasonal movements, feeding habits, and reproductive behavior were examined; and particular attention was devoted to the question as to whether or not feeding is completely suspended throughout the brooding period.

L. littoralis spends the summer living among the rockweed, *Ascophyllum nodosum*, in rocky areas that are protected from direct wave action. In the fall these starfish move beneath the rocks or into crevices where they remain until spring. This fall migration appears to provide the starfish with protection from ice damage, insures that sufficient numbers of the animals are aggregated for effective reproduction, and brings the brooding females in contact with a suitable substrate for attachment.

In the summer *L. littoralis* feeds actively on a variety of crustaceans and mollusks. Amphipods appear to be eaten most frequently when the starfish were living among the algae. The amphipods are trapped and engulfed by the partially extruded cardiac stomach, and digestion is accomplished without the withdrawal of this stomach and enveloped food through the mouth into the body cavity of the starfish. Feeding activity is reduced in the winter when the animals are living beneaths rocks,

but a number of these animals have been observed with their stomachs extruded and covered with detritus.

L. littoralis reproduces when the water temperature drops below nine degrees centigrade, usually in late October or early November. The males spawn first, soon followed by the females, and evidence is presented that suggests that the spawning is completed during a single tidal cycle. The females carry the embryos through the winter below the mouth in a brood pouch formed by arching the arms.

Throughout the brooding period the presence of the brood prevents the parent from feeding in the usual manner, but some evidence has been found to suggest that adjunct methods of feeding may occur during brooding. Particulate matter is trapped by epidermal mucus secretions which are passed to the region of the tube feet and retained in the ambulacral groove. This is suggestive of the epidermal digestion and absorption described by Pequignat (1966) in Asterias rubens. Another source of nutrients that may be utilized by the brooding starfish is from those eggs in the center of the brood mass which fail to develop. These eggs are in close proximity to the perioral membrane across which ciliary currents are directed toward the mouth. Flagellary currents are also maintained across the surface of the cardiac stomach during brooding which suggests that this organ continues to function.

Histological examinations of the cardiac stomach showed no obvious change in the structure of this organ after sixty days of brooding. However, secretory granules which are normally found in the epithelium in the region of the esophagus were found to be depleted after sixty days of starvation. No change was noted in the rest of the stomach wall.

SECTION I

INTRODUCTION

Leptasterias littoralis (Stimpson) is a five-rayed, brooding seastar found inhabiting the rocky shore from the mid-littoral zone to a depth of about 50 fathoms along the shore of the Western Atlantic between Labrador and Casco Bay, Maine. The species was described in 1853 and names Asteracanthion littoralis by Stimpson who found it among the algae on the shores of Grand Manan Island in the Bay of Fundy. The original description is as follows:

> Body turmid [=tumid], rays very broad. Ambulacral spines in two rows, slender, blunt, or even clavate at their tips. Spines on the sides larger than those on the back, but both short, blunt, and showing great uniformity in size and distribution. Its color is always a dark green above, and it never exceeds an inch and one-half in diameter. It is very common among the fuci in the middle region of the littoral zone, or even near high-water mark - elsewhere I have never found it.

It has since been reported from Labrador by Bush (1884), from the Gulf of St. Lawrence by Prefontaine (1962), and from New Brunswick by Ganong (1888), in the Bay of Fundy by Bousfield (1958), at Eastport, Maine by Verrill (1871) (1895), and in Casco Bay by Verrill (1874) and Kingsley (1901). Whiteaves (1884) reported its presence in a collection taken from Ungava Bay, Northern Quebec, but Grainger (1955) noted that it was not collected from this area during the Calanus expeditions between 1947 and 1952. Grainger (1964) also stated that Leptasterias littoralis was not taken from any of the stations during the Blue Dolphin expeditions along the coast of Labrador from 1949 to 1952.

Apart from the above questionable record of Whiteaves (1884) the reported range for Leptasterias littoralis appears to be from Labrador (Grainger, 1964) to Kennebunk Beach (Dearborn, personal communication) - this latter location being some 25 miles southwest from the Casco Bay records of Verrill (1874) and Kingsley (1901).

The species name of this seastar has undergone several nomenclatural changes since being described by Stimpson as Asteracanthion littoralis in 1853. In 1866, Verrill recognized the priority of the generic name Asterias as established by Linnaeus in 1758 and placed these seastars in the genus Asterias, subgenus Asteracanthion. Thus, the name that Verrill assigned was "Asterias (Asteracanthion) littoralis (Stimp. sp.)". In the same report, Verrill established the genus Leptasterias and included in it, four closely related species, but did not include the species here discussed. It was not until 1879 that Verrill placed it in this genus under the name Leptasterias littoralis (Stimp.) Verrill.

The genus Leptasterias was established to include those small seastars allied to Asterias having comparatively large papulae placed singly or in groups of two or three along the sides or back of the rays. In Asterias, the papulae are small and occur in crowded clusters. Verrill also suggested that members of the genus Leptasterias differ from Asterias in their mode of reproduction, in that parental care of the eggs and embryos is common in Leptasterias.

Clark (1904) suggested that the characteristics used by Verrill were of insufficient importance and constancy to warrent separation from the genus Asterias. In his study of the echinoderms of the Woods Hole region, he placed the locally occurring members of the genus *Leptasterias* in the genus *Asterias*. Ganong (1888), in his survey of the echinoderms of New Brunswick, also referred to *Leptasterias littoralis* as *Asterias littoralis*.

Later, Verrill (1914) stated that "the most important generic feature is the fact that the genital pores are on the actinal side, between the proximal inferomarginal plates, not dorsal, as in *Asterias*." Since that time it has been demonstrated that brooding of the young is characteristic of all members of the genus about which reproductive information is available.

Brood protection may occur simply by protecting the embryos in a temporary pouch between the rays, or, as in the case of *Leptasterias groenlandica* (Lutken), the young are actually held in the cardiac stomach of the parent until the young stars develop. The latter case has been demonstrated by Lieberkind (1920) and Fisher (1930). Brooding behavior has been demonstrated in the type species for the genus, *Leptasterias mulleri* (Sars) by Sars (1846).

In all of the above reports, very little is said about *Leptasterias littoralis* except that it was included in collections made at the various sites. Since it was described, practically no work has been done on it. Verrill (1895) did note in his collection made at Eastport that "This, like other species of *Leptasterias*, carries its large ova and young attached in large clusters around the mouth." He also states that it breeds early in the season around April and May. No other significant published descriptive material on *Leptasterias littoralis* since Stimpson's original description of 1853 has come to the writer's attention.

Verrill's (1895) observations regarding the brooding season for *Leptasterias littoralis* apparently include only the terminal stages of the development of the young. Evidence obtained both in the field and in the laboratory in the present study appear to indicate that the animals begin to brood the young in late fall and carry the brood through the winter (c.f. sec. IV). The young stars leave the care of the mother sometime in April or May. The duration of the brooding period may be up to six months. This brooding period is considerably longer than the brooding period of other closely related species on which such data are available. One reason for this prolonged brooding period may be the effects of very low temperatures on the developing young. The temperatures during the winter where *Leptasterias littoralis* are found are generally below 5° C. in the water and may drop considerably below 0° C. when the animals are exposed at low tide.

Chia (1964) noted that *Leptasterias hexactis* (Stimpson) had a brooding period of two months when kept at a temperature between 9 and 11 degrees centigrade. Kubo (1951) reported that *Leptasterias ochotensis similispinis* Clark from Akkeshi, Japan, begin brooding in the middle of April and young are released from the parent around the middle of June. Kubo gives a temperature of 7.5° C. during the reproductive season.

Fisher (1930) gives the following information relating temperature to reproduction of several arctic seastars. *Leptasterias arctica* (Murdoch) and *Leptasterias polaris* (Muller and Troschel) from Bristol Bay, in the Pribilof Islands, reproduce while the bottom temperatures are around 4°C. A similar critical temperature is given for Leptasterias groenlandica which reproduces between May and August. Fisher states that the young are ready to leave the parent after June 14th. This would seem to indicate a brooding period of only six or seven weeks if fertilization were to occur in early May. However, none of these species is found intertidally, so it is likely that the minimum temperature encountered would be around 4°C.

Gemmill (1914) found that the planktonic larvae of Asterias rubens Linnaeus complete metamorphosis after eight or nine weeks. Gordon (1929) reported that a culture of Asterias forbesi (Desor) completed its larval development in 30 days. Loosanoff (1964) gives the time of metamorphosis for Asterias forbesi as 21 days after fertilization, though this may be prolonged by adverse environmental conditions. These species reproduce during the late spring and early summer months when water temperatures are higher.

Although, the time required for the larval development of these species may be similar, marked differences in the mode of development are noted. The eggs of Asterias are tiny and numerous. They are released into the water where fertilization occurs. A free-swimming, planktotrophic larva develops. No parental care of the young is demonstrated.

The eggs of Leptasterias are large, yolky, and few in number. As they are released from the gonopores, each egg is taken by the tube feet of the mother and passed to the brood pouch. The brood pouch is formed beneath the mouth by arching of the rays. Fertilization occurs here and the eggs adhere to each other in the brood pouch. The developing young are lecithotrophic. They remain in the brood pouch until development is completed. At that time, the young stars are released from the protection of the mother.

It has been suggested by a number of authors (Verrill, 1914; Fisher, 1930; Hyman, 1955; Chia, 1966b) that the brooding female does not feed for the duration of the brooding period. This is presumed to result from the blockage of the mouth by the brooding young. In the case of Leptasterias littoralis, where the brooding period may be six months long, the question is raised - What effect does this prolonged period of starvation have upon the parent?

There are no accounts in the literature concerning the feeding habits of Leptasterias littoralis. The obstruction of the mouth of the parent by the brooding young would obviously prevent feeding by the characteristic method of the Asteriidae. Most members of this family are carnivorous predators. They feed by holding their prey with the aid of the tube feet and extruding their cardiac stomach which engulfs the prey. Digestion is external and the digested food is taken into the body by ciliary currents. Once the prey is digested, the undigested parts are released and the stomach is withdrawn.

If the mouth is obstructed by the brooding young this method of feeding is impossible. However, the possibility of other feeding methods exists. Gemmill (1915) noted that ciliary currents which converged on the mouth on Porania pulvillus were used to feed. He suggested that some mucus-ciliary feeding may occur in other species as an adjunct to the taking of larger prey. Budington (1942) demonstrated the existence of a ciliary current producing mechanism in Asterias forbesi. He states that "all currents on the perioral membrane flow towards the mouth and thus contribute, though in immeasurable degree, to nutritional intake." A similar situation has been demonstrated in Henricia sanguinolenta by Anderson (1960).

The possibility of nutritional intake by other means than the ingestion of food through the mouth has recently been investigated. Ferguson (1963) has demonstrated that large quantities of nutrients are absorbed directly from the external medium by the epidermal cells on the oral surface of Asterias forbesi. More recently, Pequignat (1966) has shown evidence of both digestion and absorption of organic materials placed in contact with the epidermis of Asterias rubens. Either a ciliary current producing mechanism or the direct absorption of nutrients through the epidermis could satisfy the metabolic demands of a brood-carrying seastar without the necessity of dispossessing the young.

The chief objectives of this study are a.) to determine the normal feeding habits of Leptasterias littoralis; b.) to find out if the histology of the cardiac stomach changes during the period while the female is brooding; c.) to determine what are the possible sources of nutrients available to the brooding female. It is also hoped that by including a more complete description of the morphology, natural history and reproductive behavior of Leptasterias littoralis a better understanding of this species may be gained. I have also included, for comparative purposes, some information about Leptasterias tenera (Stimpson) and Asterias rubens Linnaeus (=vulgaris), two closely related species, which are commonly found in the same area as *Leptasterias littoralis* and with which this species may be confused. Most of the American work done on *Asterias rubens* refers to this species as *A. vulgaris*, however Tortonese (1963) says that *A. vulgaris* is conspecific with *A. rubens* and not even separable on a subspecific basis.

SECTION II

METHODS AND MATERIALS

The animals used in this study were collected over a period of three and one-half years at five different locations in the Passamaquoddy Bay Region of Maine and in New Brunswick. Most of the specimens were collected at Gove Point, North Lubec, Maine (Latitude 44° 54' N., Longitude 67° 04' W.). Additional specimens were taken at Crow Neck, North Trescott, Maine; at Cobscook Bay State Park, in Edmunds, Maine; at Starboard Island, Machias Bay, Maine; and at St. Andrews, New Brunswick.* A total of 807 specimens were collected and examined during the course of this study. Additional specimens were examined at the Museum of Comparative Zoology at Harvard University.

Live animals were transported back to the laboratory at the University of New Hampshire in large plastic bags containing seawater. The bags were kept in a cooler packed with ice during transport. In the laboratory the animals were transferred to plastic trays or large fingerbowls containing seawater kept at a constant temperature of 4° C. The seawater was changed every two days.

Animals collected during the summer months were kept at a

---

*Approximate N latitude and W longitude

	N	W
Crow Neck	44° 52'	67° 08'
Edmunds	44° 52'	67° 10'
Starboard Island	44° 36'	67° 23'
St. Andrews	45° 04'	67° 03'

temperature of approximately 10^o in a recirculating seawater system until November when they were transferred to plastic trays at the lower temperature. The feeding of these seastars in the laboratory will be discussed in a later section of this paper.

Studies of the external morphology were done on living specimens and on specimens relaxed in 8% magnesium chloride until flaccid. Observations of the spines, pedicellariae, and skeletal plates were made using a Bausch and Lomb Stereozoom (Model AVB-73) dissecting microscope after the animals had been preserved in 80% ethyl alcohol and placed in commercial brand "Clorox" for varying lengths of time to remove the epidermis.

The cardiac stomachs were removed from a number of seastars to determine if any histological changes occured as a result of the brooding behavior or from starvation. Several animals that were carrying embryos were removed at ten day intervals from the seawater, relaxed in 8% magnesium chloride and sacrificed. The aboral surface was removed by making a longitudinal incision around the margin of the body and separating the two halves by cutting the digestive tract at the junction of the cardiac stomach and the pyloric stomach. The five-lobed cardiac stomach was then excised by severing the retractor harnesses and by cutting around the peristomial membrane. The entire cardiac stomach was then removed from the body and cut into five equal parts.

The tissues were prepared for sectioning in the following manner. 1. Fixed in Bouin's Fixative for 24 hours. 2. Dehydrated in a graded series of alcohols (Appendix). 3. Embedded in "Paraplast" a prepared embedding media manufactured by Biological Research Inc.

Sections were cut at from 5 to 10 µ with an American Optical Company "800" rotary microtome.

Staining procedures, which are given in the Appendix, included the following. 1. Ehrlich's hematoxylin followed by eosin or chromotroph 2R was used for general histological observations. Good results were obtained by using Wu's (1940) method for double-staining in toto with hematoxylin and eosin. 2. Peterson's acid alizarin blue stain was used to demonstrate muscle and connective tissue (Conn, Darrow, and Emmel, (1960). 3. Glycogen and other polysaccharide complexes were demonstrated by the use of a periodic acid-Schiff routine controlled by salivary digestion (Humason, 1962).

The animals used in the starvation experiments were kept in filtered sea water. These animals were rinsed off with fresh water each time the seawater was changed to prevent the accumulation of bacteria and protozoans that might serve as a source of nutrients. Several of these animals were removed after 10, 20, 30, 40, 50 and 60 days of starvation for histological examination of the cardiac stomach to determine if any changes occurred as a result of starvation. Observations were also made on the gonads and pyloric caeca of these animals.

SECTION III

EXTERNAL MORPHOLOGY

Leptasterias littoralis is similar in appearance to the more common members of the Asteriidae which occur in the Northwest Atlantic, typically, having five arms radiating from a central disk. Although many species in this genus commonly have six arms, such individuals are rare in this species. I have seen only one specimen having six arms that could be readily assigned to this species. Individuals having four arms were found occasionally but these usually resulted from the loss of an arm and were, almost without exception, in the process of regenerating a replacement.

COLOR

Leptasterias littoralis is one of the few sea stars in which the color may be used as a character by which it may be distinguished from other species. Deichmann (unpublished manuscript) states that it is always dark green above, without markings of any kind and with but little variation. All of the living specimens that were examined in the present study were green on the top, or aboral side and cream colored on the bottom, or oral side.

SIZE

Stimpson (1853) states that these sea stars never exceed an inch and a half in diameter. Verrill (1866) observed specimens up to three inches in diameter around Eastport, Maine and he also reported specimens six to eight inches in diameter in deeper waters according to Ganong (1888). The largest specimen that I have observed was about four inches in diameter while most of them were between one and three inches.

Starfish size has usually been expressed in terms of some kind of a measurement of arm length but methods used in measuring have seldom been uniform. Some of the ways in which these measurements have been taken include the following. 1. Bull (1934) measured all five arms from the tip of the arm to the center of the disk, and took the average as mean arm length. 2. Galtsoff and Loosanoff (1939) measured the distance between the tip of the arm on the left side of the madreporite and the tip of the fourth arm from it, counting in a clockwise direction. 3. Vevers (1949) used the distance between the anus and the tip of the longest arm as an expression of the starfish size. 4. Quayle (1954) measured the distance from the tip of the single ray opposite the madreporite to the mouth.

Since most of the asteriidae have the ability to extend and contract their arms to some degree none of the above methods gives consistent results. The arms of a starfish may be extended when the animal is moving about actively or slightly contracted when it is inactive. A single measurement taken at one time may vary considerably from the same measurement taken at another time when measuring living animals. More consistent results may be obtained when measuring preserved animals although the specimens may shrink 10 to 20 per cent from their original life size according to Chia (1966b).

In any event, such single measurements provide little useful information to the investigator. No accurate correlation between starfish age and size can be obtained from such measurements according to Mead (1901). He has shown that starfish growth depends primarily upon the abundance and suitability of the food available to them, so it is possible to find a large well-fed individual of the same age as a small poorly-nourished individual.

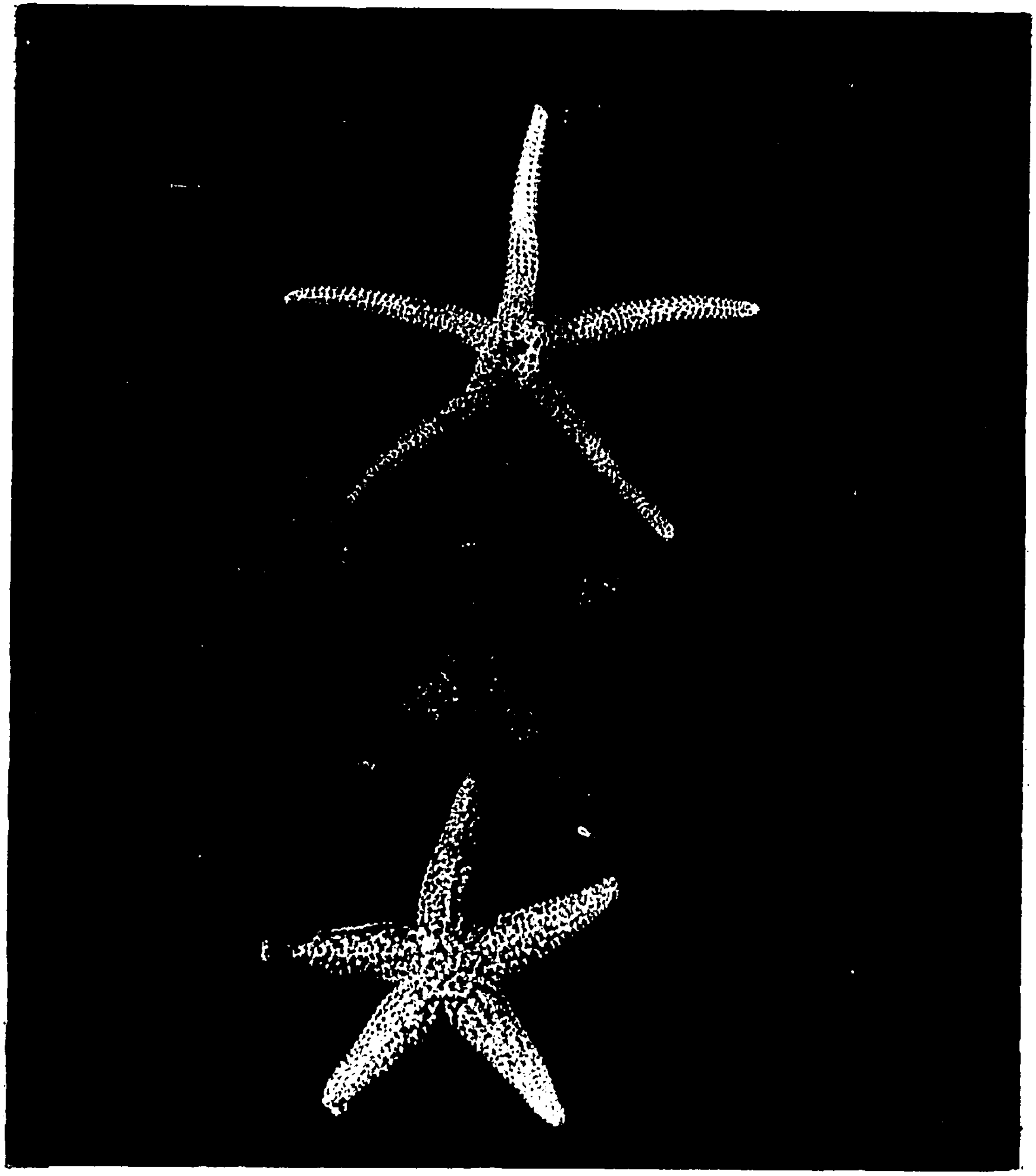
For taxonomic purposes, a more accurate expression of starfish size may be made in terms of some ratio of measurements that indicate the relative proportions of the arms to the disk. Mortensen (1927) cites the following method:

> The relationship between the length of the arms and the diameter of disk is of systematic importance; the distance from centre of disk to the point of the arm is designated R, the distance from the centre to the edge of disk midway between the arms, r. When it is stated, for example, that $R = 5r$, it means that the distance from the centre of the disk to the point of the arms is five times as large as the distance from the centre to the edge of the disk.

In the above method the major measurement is the length of the arm and the minor measurement is the radius of the disk. However, Hyman (1955) states that "in taxonomic descriptions of asteroids it is customary to state the measurement R, from the disk center to the arm tip, and r, the diameter of the disk." The minor measurement in this case is the diameter of the disk as opposed to the radius used in Mortensen's method. Unfortunately some taxonomic descriptions have failed to note which of these methods was used. I have used Mortensen's method in this study because a more accurate measurement of the radius of the disk can be taken from a pentamerous starfish (Figure 2). It should be noted that those measurements were taken from preserved specimens so the animals were slightly larger in life.

Figure 1 shows representative specimens of three species of asteroids that occur in the region of this study. *Leptasterias tenera* can easily be identified by its small disk and long slender arms. The other two species have shorter, stouter arms radiating from a proportionally larger disk. The arms of *Leptasterias littoralis* are

Figure 1. Aboral view of preserved and dried specimens of *Leptasterias tenera* (top), *Leptasterias littoralis* (middle) and *Asterias rubens* (bottom). (actual size).



usually somewhat swollen at the proximal end especially when the gonads are filled or when the female is brooding. Asterias rubens has stout arms but these taper more evenly towards the distal end than do the arms of Leptasterias littoralis.

A summary is given in Table 1 of the comparative dimensions of the three species at comparable sizes. In this comparison most of the seastars were between two and three inches in diameter but it can be seen that the proportion of the arms to the disks are different for each species. The arms of Leptasterias littoralis are about four times the radius of the disk (R/r = 4.0) while the arms of Leptasterias tenera are about six times as long (R/r = 5.9). Asterias rubens has slightly longer arms (R/r = 4.6) than Leptasterias littoralis yet shorter than those of Leptasterias tenera.

SKELETAL PLATES

The endoskeleton of Leptasterias littoralis is comprised of a rather loose network of calcareous plates, or ossicles, imbedded in the body wall. These skeletal plates are arranged in more or less definite rows along the horizontal axis on the sides and oral surfaces of the arms. The names and locations of these plates are shown in Figure 3.

The carinal plates on the aboral surface of the arms and the plates on the disk appear to be randomly scattered without pattern, whereas in Asterias rubens there is almost always a prominent median longitudinal series of plates on the aboral surface of the arms. There are usually two incomplete rows of plates on each side of the carinals but these do not extend all the way to the end of the arm.

The superomarginal plates form a complete straight row from the disk to the tip on either side of the arm. The inferomarginal plates

TABLE 1

A SUMMARY OF THE COMPARATIVE DIMENSIONS OF THE THREE SPECIES AT COMPARABLE SIZES

SPECIES	NUMBER	R* Average	r** Average	R/r
Leptasterias littoralis	50	26.1	6.47	4.0
Leptasterias tenera	62	30.5	5.17	5.9
Asterias rubens	43	25.1	5.40	4.6

*R = distance from the center of the disk to the tip of one arm, in millimeters.

**r = distance from the center of the disk to the edge of the interradii, in millimeters are arranged in a straight row from the disk until about two thirds the length of the arm where they become fused with and almost indistinguishable from the adambulacral plates. Both the adambulacral and ambulacral plates form complete rows from the disk to the distal end of the arm.

All of the plates on the sides and tops of the arms are loosely articulated and may slide up and over adjacent plates to permit some shortening and extending of the arms. This loose articulation of the plates gives a considerable amount of flexibility to the arms and permits the animal to assume the brooding posture as well as grasp a large prey while feeding.

DERMAL PAPULAE

The dermal papulae, thin tubular outgrowths of the body wall which function as respiratory structures, project through the papular spaces between the skeletal plates on the aboral and lateral surfaces of the arms and disk, while the tube feet project between the skeletal plates on the oral surface of the arms. The papulae are usually large and for the most part occur singularly in Leptasterias although occasionally two or three may occur in one papular area. Because of the arrangement of the skeletal plates the papulae on the aboral surface are scattered without pattern while on the sides of the arms they occur in rather straight rows paralleling the skeletal plates from the disk to the tips of the arms. In Asterias, the papulae are much smaller and occur in groups of from three to ten papulae in each papular space.

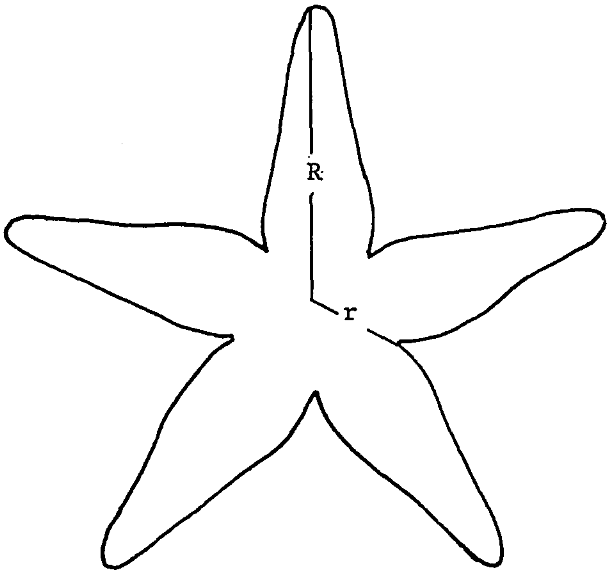
SPINES AND PEDICELLARIAE

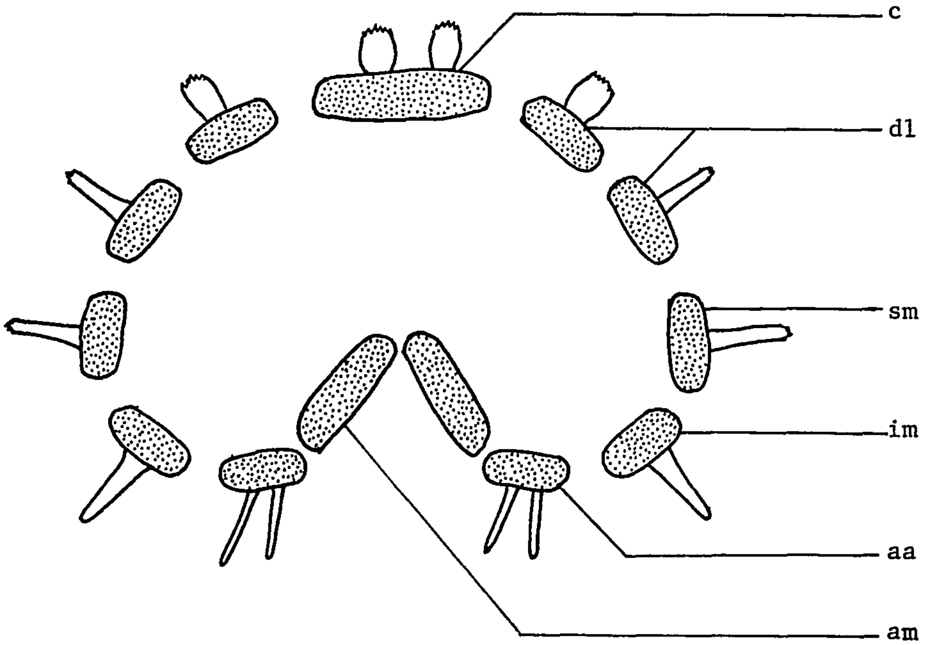
The distribution and shape of the spines and pedicellariae provide useful characteristics that may be used to distinguish between different species of starfish. The spines are calcareous projections,

Figure 2. Aboral view of a starfish showing major radius (R) and minor radius (r).

Figure 3. Cross section through the arm of a starfish showing the relative positions of the skeletal plates and spines.

c.	carinal
dl.	dorsal-lateral
sm.	superomarginal
im.	inferomarginal
aa.	adambulacral
am.	ambulacral


R
r


c
dl
sm
im
aa
am

usually covered with epidermis, that rest upon the skeletal plates but are not fused to them. Spines are usually found associated with each of the plates in the body with the exception of the ambulacrals and they have tiny muscles attached to the base that permits some slight movement over the raised tubercle on the plate with which they articulate.

The pedicellariae are tiny two-jawed, calcareous structures that are scattered over the surface of the body. It is believed that their function is to remove debris and small organisms from the surface of the body although recently Pequignat (1966) has suggested that the pedicellariae may trap and hold organic particles or tiny organisms for epidermal digestion.

There are two types of pedicellariae commonly found on *Leptasterias* and *Asterias*. The major type has straight, forcep-like jaws, and the minor type has crossed, sissors-like jaws (Figures 5 and 6). Both types of pedicellariae are completely covered with epidermis and are attached to the body by a tiny peduncle. The pedicellariae may occur singularly but more commonly they are found in clusters surrounding the bases of the spines.

Since the skeletal plates on the aboral surface of *Leptasterias littoralis* are arranged without pattern the spines appear to be randomly scattered. The carinal spines are short, blunt and minutely thorny at the tip (Figure 4a). There are usually two or three spines on each plate although occasionally there may be but one. Near the distal end of the arm these spines usually occur singularly. When the arms are contracted these spines may be almost obscured by a retractile sheath of epidermis that encircles the spine bases. When the arm is extended this sheath is flattened as the skin is pulled taut and the spines then project to a greater distance from the body. Pedicellariae are almost completely lacking on the aboral surface of the arms and disk of Leptasterias littoralis.

The carinal spines of Leptasterias tenera are very slender and sharply pointed at the tip. These occur singularly for the most part and the base of each spine is surrounded by a wreath of tiny pedicellariae, usually six to ten in number.

In Asterias rubens, the carinal spines are usually singular but occasionally two spines may occur on each plate. As in Leptasterias tenera these spines have from two to ten tiny pedicellariae around the base.

The superomarginal spines of Leptasterias littoralis are longer than the carinals but are still blunt and thorny at the tip (Figure 4b). They usually occur singularly but occasionally two spines may occur on a single plate. There are generally two or three minor pedicellariae attached to the bases of these spines.

Leptasterias tenera has sharp slender superomarginal spines that are similar to the carinal spines yet having more pedicellariae, usually from five to fifteen at each base. These spines are usually singular and evenly spaced along the margin of the arm.

Asterias rubens has short blunt superomarginal spines that occur in pairs on most plates. A wreath of from five to ten minor pedicellariae occurs at each base.

The inferomarginal spines of Leptasterias littoralis are longer than either the carinals or superomarginals and they have rather smooth blunt tips (Figure 4c). There are two spines on each of the proximal

Figure 4. Series of spines taken from mid-arm region of *Leptasterias littoralis* showing comparative sizes and shapes.

a. carinal spine
b. superomarginal spine
c. inferomarginal spine
d. adambulacral spine

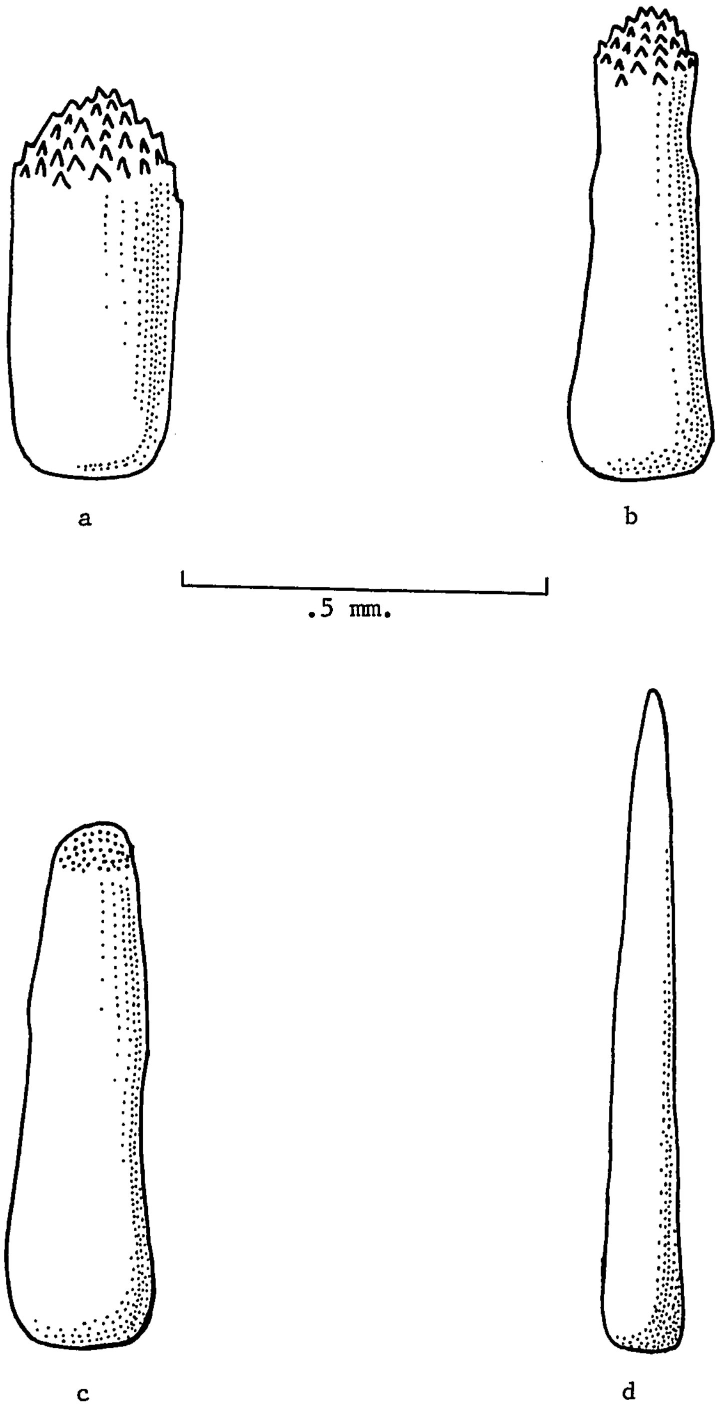
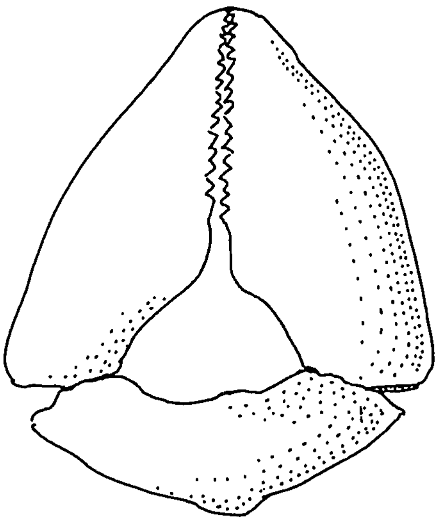
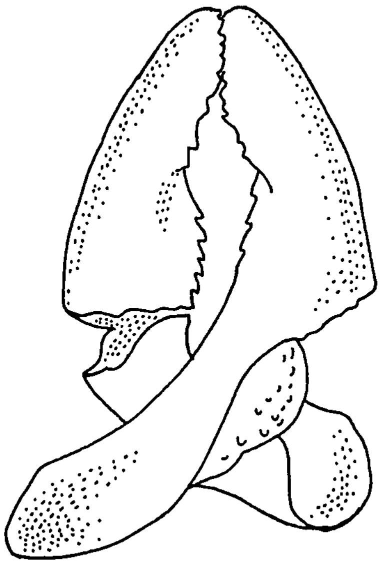

a
b
.5 mm.
c
d

Figure 5. Major type pedicellaria from *Leptasterias littoralis*.

Figure 6. Minor type pedicellaria from *Leptasterias littoralis*.





plates while only one spine occurs on each plate near the distal end of the arm. A partial wreath of from five to ten pedicellariae occurs about one third of the way up from the base of the spine.

In Leptasterias tenera the inferomarginal spines are stouter than the superomarginals yet they still terminate in a sharp point. There are two spines on most plates except near the distal end where there may be but one. Each spine is surrounded by a complete wreath of from fifteen to twenty pedicellariae.

The inferomarginal spines of Asterias rubens are the largest and most prominent of all. There are usually three spines on each plate but occasionally only two. There are fewer pedicellariae around these spines than are found around the carinals and superomarginals. Usually two or three pedicellariae are found about half way up the spine.

Leptasterias littoralis has long, slender, smooth-tipped spines on the adambulacral plates (Figure 4d). There are usually two spines on each plate although toward the distal end single spines alternate with double spines. There are no pedicellariae on any of the adambulacral spines but a single pedicellaria is found on the medial surface of each of these plates in the ambulacral groove.

The adambulacral spines of Leptasterias tenera are small, very slender and sharply pointed. They occur in two longitudinal rows with the lateral row partially wreathed with minor pedicellariae. The more medial row has no pedicellariae. As in Leptasterias littoralis there is a row of rather large pedicellariae on the medial side of the adambulacral plates.

Asterias rubens has adambulacral plates with one or two (usually two) spines which are long, slender and somewhat pointed.

There are almost always one or two major pedicellariae located near the tips of these spines, especially those at the proximal end of the arm.

Major pedicellariae may also be found scattered over the entire surface of *Asterias rubens* but are most common in the interradial areas and on the oral spines around the mouth. Major pedicellariae are extremely rare in both species of *Leptasterias* though occasionally one or two may be found between the arms on the oral surface.

A summary of these characteristics of the three species is given in the following table.

TABLE 2. A SUMMARY OF THE TAXONOMIC CHARACTERISTICS OF LEPTASTERIAS LITTORALIS, LEPTASTERIAS TENERA, AND ASTERIAS RUBENS

CHARACTERISTIC	LEPTASTERIAS LITTORALIS	LEPTASTERIAS TENERA	ASTERIAS RUBENS
color in life	green	purplish-pink to nearly white	yellow, orange, purple, cream, pink
shape	short blunt arms about four times radius of disk	along slender arms about six times radius of disk	stout arms tapered at distal ends about four and a half times radius of disk
papulae	large; single per area sometimes two or three	large; single per area sometimes two or three	small, in clusters of three to ten per area
carinal spines	short, blunt, thorny without pedicellariae one, two or three spines on each plate, do not form obvious rows	slender, sharply pointed, mostly single with six to ten pedicellariae forms straight row	short, blunt, some slightly capitate; one or two on each plate with two to ten pedicellariae; form prominent rows
superomarginal spines	longer than carinals, blunt and thorny, single, sometimes double; two or three pedicellaria at base	not longer than carinals, slender and sharply pointed, mostly single with six to ten pedicellaria at base	short, blunt with smooth tips, two spines on each plate; five to ten pedicellaria at each base

inferomarginal spines	longer than carinals and superomarginals, blunt with smooth tips; two on each plate, some single near distal end, five to ten pedicellariae one-third way up from base	stouter than carinals and superomarginals, sharp tips; two on each plate, single near distal end; fifteen to twenty pedicellariae around each spine	larger than carinals and superomarginals with smooth blunt tips; fewer (two or three) pedicellariae half way up spine; three spines arranged obliquely on each plate
adambulacral	long, slender, smooth tips; two on each plate, alternate one and two at distal end; no pedicellariae	small, slender, sharply pointed; two spines on each plate; partial wreath of pedicellariae on lateral row	long, slender, somewhat pointed; two on each plate; pne pr two major pedicellariae near tip of each spine at proximal end of row
major pedicellariae	occasionally one or two on skin in interradial area	occasionally one or two on skin in interradial area	scattered over entire body, common an adambulacral and oral spines
minor pedicellariae	few on aboral surface, partial wreaths on lateral spines, none on adambulacral spines	form wreaths around most spines on aboral, lateral and adambulacral surfaces	found in dense wreaths around most spines except adambulacrals

SECTION IV

NATURAL HISTORY

Starfish are among the most conspicuous animals found living in the intertidal zone along the coast of the Northwest Atlantic and are commonly found in collections taken from offshore waters. Because of their abundance and the ease with which they may be collected, they have provided a good source of material for embryological and physiological investigations. Since some of them compete with man for food, especially bivalve mollusks, considerable interest has been shown in their feeding and migratory habits, as well as in their control.

The most common species of starfish that occur in the region of this study include *Asterias rubens*, *Leptasterias tenera*, *Henricia sanguinolenta*, as well as *Leptasterias littoralis*. *Asterias* grows to a comparatively large size (up to one foot in diameter) and is an active voracious predator on mollusks. Since they are frequently gregariou and rather brightly colored they are easy to locate in those areas in which they occur.

Leptasterias tenera, a sublittoral species, is almost always purplish-pink or white in color which contrasts with the drab mud-sand bottom on which it lives. It is also quite gregarious and in some areas hundreds may be taken from a single trawl.

The blood star, *Henricia sanguinolenta*, is usually red, purple or bright orange in color. Thus it too is poorly camouflaged as it moves across the bottom. On the other hand, *Leptasterias littoralis* is more cryptic in its behavior, and its color is ideally suited for the habitat in which it lives.

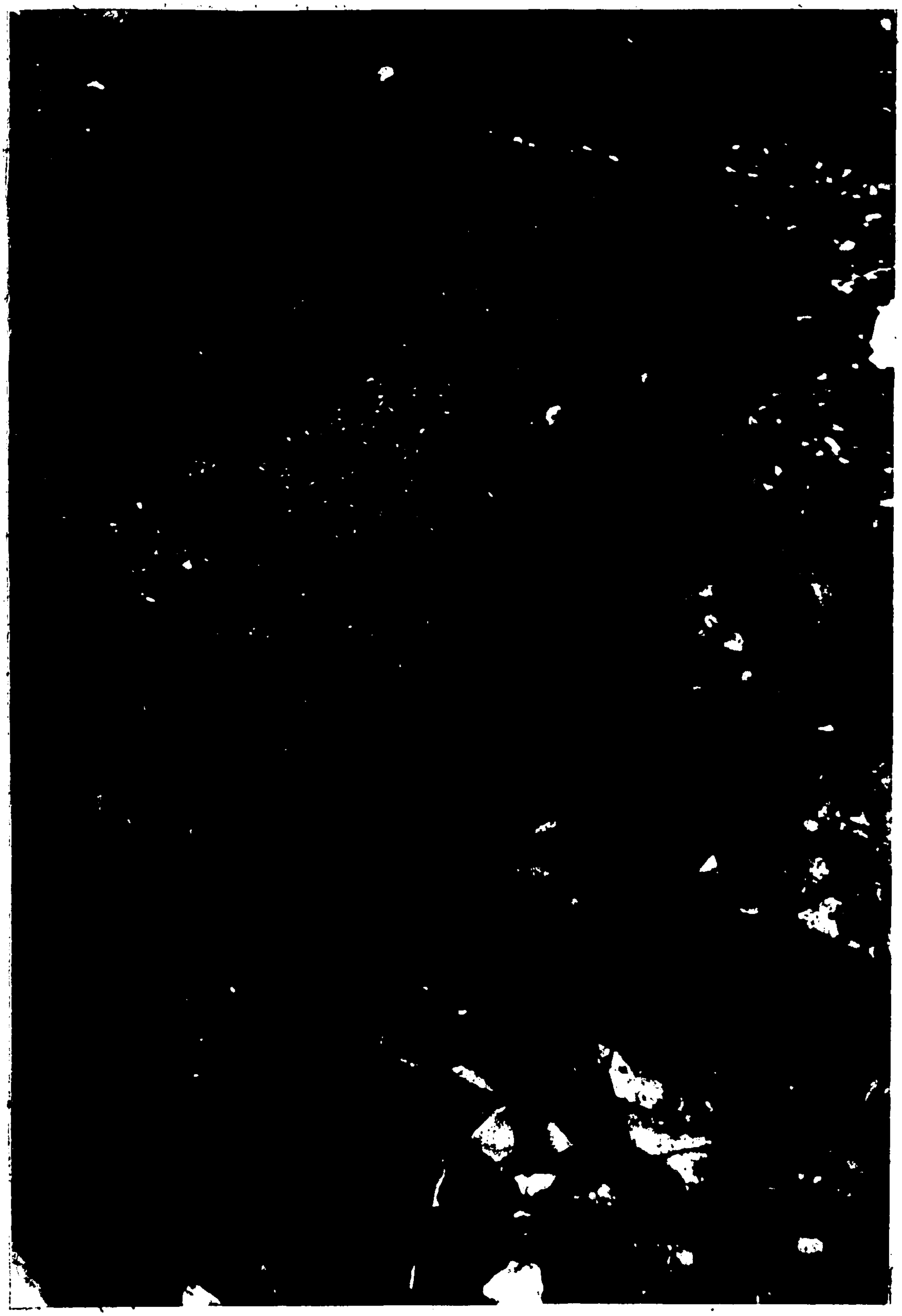
HABITAT

The typical intertidal habitat in which *Leptasterias littoralis* is found is a rocky beach having large granite or slate outcroppings and numerous large boulders. The outcroppings frequently have deep crevices cut into the surface, and undercutting around their bases is common. The rocks, boulders, and outcroppings are usually overgrown with a thick covering of algae, mostly *Ascophyllum nodosum* (Linnaeus) Le Jolis (Figure 7). All of the areas from which specimens were taken during this study were somewhat protected from direct wave action although strong currents run parallel to the beach at several of these locations.

From about April to early October the starfish are almost always found living among the clumps of *Ascophyllum*, which may grow to a length of up to two meters during the summer and cover almost all of the rock surface. Normally the animals remain near the holdfasts of the algae and are not found on the clear surfaces of the rocks. This probably keeps them from being dislodged from the rock surface by the sweeping action of the algae set in motion by waves and currents.

This association between the starfish and the algae is advantageous to the starfish in several ways. In the first place, the green color of the starfish blends with the color of the algae making them quite difficult to see. Secondly, since the algae remain wet during low tide, some protection against desiccation is provided. This is of particular importance to those starfish living in the middle of the intertidal zone where they may be exposed for several hours. Lastly

Figure 7. A view of the lower intertidal zone at Gove Point where *Leptasterias littoralis* may be collected. This picture was taken at low tide in April, 1967. Note that the *Ascophyllum nodosum* in the foreground has been closely cropped, probably as a result of winter icing.



on hot summer days the temperature on the surface of the rocks in the intertidal zone may exceed the tolerance limits of the starfish. Considerably lower temperatures are found beneath the algae, as evaporation of the residual water occurs enabling the starfish to survive until covered again by the incoming tide.

During the fall the starfish leave the algae and move into crevices or beneath the rocks where they remain until the following spring. After the first week of November it is almost impossible to find any of these starfish among the algae or on the exposed surfaces of the rocks. However it is not unusual to find two or three or even more starfish clinging to the undersurface of a boulder one or two feet in diameter (Figure 8).

The starfish benefit in several ways from this fall migration to the undersides of the rocks. At this time the animals are about to begin the reproductive season when a concentration of several starfish in close proximity to one another is beneficial. This type of aggregation is assured since there are fewer available habitats beneath the rocks than are available among the algae or on the surface of the rocks. Secondly, since the female will protect her brood throughout the winter with little, if any movement, she requires a solid substrate for attachment. The underside of a rock or the sides of a crevice provide such a substrate. These points are discussed further in the section on reproduction.

In addition to the requirements that insure reproductive success, a third advantage to wintering beneath the rocks is that it provides some protection from icing over, that occurs in the intertidal zone during the winter months, and from the scouring of the rock surfaces

Figure 8. Two specimens of Leptasterias littoralis attached to the surface of an overturned rock in April, 1967. Rock is about 15 inches in diameter.

by ice floes during the late winter and early spring. Several inches of ice may form on the algae during a low tide and cropping of the algae occurs later as the ice breaks up and drifts away. Any animal that remains on the algae through the winter would have a reduced chance for survival. Undoubtedly some mortality does occur among those starfish beneath the rocks as the ice floes grind over the intertidal zone but since the larger rocks are not moved, the probability of survival beneath them is good.

Attempts to find Leptasterias littoralis on a number of beaches that were exposed to direct wave action from the open sea were unsuccessful. I suspect that the rolling of the smaller rocks, and the scouring of the large boulders and outcroppings, by sand and gravel during storms make such areas uninhabitable by these starfish.

A partial listing of other invertebrate species which are commonly found in the same habitat as Leptasterias littoralis is given in Table 3. The most abundant groups of animals found living in, or attached to, the algae include hydroids, crustaceans, ectoprocts, and gastropods, all of which may serve as food for the starfish. Nemerteans, chitons, polychaets, crustaceans, and brittle stars are among the most abundant animals found living beneath the rocks. The rock gunnel, Pholis gunnellus, is the only species of vertebrate that is commonly found in the intertidal zone during the low tide.

I've found no evidence of predation on Leptasterias in my field studies but at least two of its cohabitants in the intertidal zone may occasionally prey on it. On two different occasions a large specimen (8 inch diameter) of Asterias rubens was observed while feeding on smaller specimens of Leptasterias in an aquarium. Blegvad

TABLE 3. SPECIES LIST OF THE MORE COMMON INVERTEBRATES FOUND AT GOVE POINT

PORIFERA
- *Halichondria panicea*
- *Halisarca* sp.
- *Leucosolenia* sp.

CNIDARIA
- *Clava leptostyla*
- *Sertularia pumila*
- *Campanularia flexuosa*
- *Obelia geniculata*
- *Metridium senile*
- *Tealia crassicornis*

PLATYHELMINTHES
- *Notoplana atomata*

RHYNCHOCOELA
- *Lineus ruber*
- *Amphiporus bioculatus*
- *Amphiporus angulatus*
- *Tetrastemma candidum*

ANNELIDA
- *Amphitrite ornata*
- *Amphitrite johnstoni*
- *Nereis virens*
- *Lepidonotus squamatus*
- *Harmathoe imbricata*
- *Spirobis borealis*
- *Spirobis spirillum*
- *Clitellio arenarius*

MOLLUSCA
- *Ischnochiton ruber*
- *Ischnochiton albus*
- *Tonicella marmorea*
- *Acmaea testudinalis*
- *Thais lapillus*
- *Littorina littorea*
- *Littorina obtusata*
- *Dendronotus frondosa*
- *Onchidoris fusca*
- *Onchidoris aspersa*
- *Coryphella rufibranchialis*
- *Cuthona concinna*

MOLLUSCA
- *Aeolidea papillosa*
- *Mytilus edulis*
- *Anomia simplex*
- *Hiatella arctica*

ARTHROPODA
- *Balanus balanus*
- *Balanus balanoides*
- *Spirontocaris groenlandica*
- *Paleomonetes vulgaris*
- *Idotea baltica*
- *Idotea phosphorea*
- *Edotea* sp.
- *Caprella geometrica*
- *Caprella grahami*
- *Gammarus oceanicus*
- *Marinogrammarus* sp.

ECTOPROCTA
- *Flustrellidra hispida*
- *Electra pilosa*
- *Electra hispida*
- *Crisia* sp.

BRACHIOPODA
- *Terebratulina septentrionalis*

ECHINODERMATA
- *Asterias rubens*
- *Henricia sanguinolenta*
- *Solaster endeca*
- *Ophiopholis aculeata*
- *Amphipholis squamata*
- *Strongylocentrotus drobachiensis*
- *Cucumaria frondosa*

UROCHORDATA
- *Halocynthia pyriformis*
- *Mogula* sp.
- *Botryllus schlosseri*

(1914) reported that large Asterias rubens were seen feeding on smaller members of the same species so it would seem probable that they would prey on Leptasterias occasionally. Solaster endeca L. has also been shown to actively prey on asteroids (Mortensen, 1927; Bull, 1934) but its occurrence in the intertidal zone is rare, and since these species are seldom found in the algae where a chance encounter with Leptasterias might occur it seems unlikely that this type of predation is of much significance.

FEEDING

The feeding behavior of starfish has been the subject of considerable interest at least since the time of Aristotle who speculated on the manner by which starfish induce their bivalve prey to open. Although mollusks are generally considered to be the main food item taken in by starfish (Forbes, 1841; Cuenot, 1887; Mead, 1901; Galtsoff and Loosanoff, 1939) it is known that a wide variety of animals, as well as some plants, and even detritus and dissolved organics are used as food. A review of this topic is given by Feder and Christensen in Boolootian's Physiology of Echinodermata published in 1966.

To find out what the feeding habits of Leptasterias littoralis are, evidence was obtained in both the field and in the laboratory. Field data were obtained by examining each animal at the time of collection for evidence of feeding activity. Either the presence of food in or near the mouth, or the partial extrusion of the cardiac stomach was considered to be evidence of feeding activity. These examinations were conducted for a period of two and half years during which time 651 animals were examined. These results are summarized in Table 4.

Feeding activity was observed in at least one specimen in every

TABLE 4. A SUMMARY OF THE FEEDING AND BROODING ACTIVITIES OF *LEPTASTERIAS LITTORALIS* COLLECTED BETWEEN AUGUST, 1966 AND MARCH, 1969.

DATE	NUMBER EXAMINED	NUMBER BROODING	FEEDING ACTIVITY	% FEEDING	LOCATION	TYPE OF FOOD
8/66	45	0	8	17.7	in algae	amphipods, isopods
11/66	103	0*	6	5.8	under rocks	detritus, chiton
4/67	110	36	1	0.9	under rocks	mussel
8/67	118	0	21	17.8	in algae	small gastropods, barnacles, isopods, amphipods
11/67	137	0**	5	3.7	under rocks	detritus
3/68	35	18	1	2.9	under rocks	*Ulva*
8/68	37	0	12	32.4	in algae	*Littorina obtusata* euphausid, caprellids, amphipods
3/69	66	14	0	0.0	under rocks	none

* Synchronous shedding of gametes occurred overnight during transport.

** Shedding occurred in laboratory within 72 hours after collection.

collection except for the last one made in March, 1969. The greatest amount of feeding activity was found among those animals collected during the summer months when they were living in the algae, and the most common food noted during the summer were crustaceans, gastropods and barnacles. It should be noted that these types of prey have hard body coverings and the time required for digesting the soft parts would be lengthy. Caution must be used in interpreting these findings, since all of the starfish examined were collected during the low tide. Most feeding activity is probably initiated during the high tide when the starfish are covered with water, so it is possible that soft-bodied prey captured at high tide may be ingested and not be noticed at the time of collection.

It can also be seen that feeding activity is at a minimum when the animals are under the rocks during the winter months. Some starfish were found to have their stomachs partially extruded and covered with detritus during these times. A single specimen was found under a rock in November 1966 with a partially digested chiton in the folds of its extruded stomach but at the time it was found the starfish had not yet begun to reproduce.

In only one instance was feeding observed in a starfish carrying young in the brood pouch. Brood-carrying animals are handicapped in that the mouth is blocked by the brood and, since the brood is held in place by the tube feet, it appears unlikely that any normal feeding activity could be carried on during this period. In March, 1968 a starfish was found that was carrying a brood of almost completely developed young. At the time of collection the brood was partially displaced and one lobe of the cardiac stomach was extruded beyond the brood. A small fragment (1.5 cm in length) of sea lettuce was partially enveloped by the stomach tissues. After teasing the fragment out with forceps it was found that about four-fifths of the total length was completely surrounded by the stomach. I suspect that the starfish was using the detritus on the sea lettuce for food since it is doubtful that the enzyme necessary for digesting cellulose is present in this species. In their survey of the distribution of cellulase in invertebrates, Yokoe and Yasumasu (1963) were unable to detect this enzyme in any of the asteroids which they tested. At no other time during this study was Leptasterias found to be feeding on algae.

The stomach contents of a number of preserved starfish were examined from collections taken during both winter and summer. Unidentified particulate matter was occasionally found in the stomachs of both winter and summer specimens, while identifiable fragments of crustacean exoskeletons were commonly found in those specimens collected during the summer.

These field observations suggest that the starfish feed more actively, and on a wider variety of prey during the summer months. Coincident with the lowering of the water temperature and the onset of the reproductive season feeding activity is reduced. That feeding stops in some starfish at low temperatures had been demonstrated by Hancock (1955) who found that Asterias rubens stops feeding at temperatures below 3° C., and Zinn (1937) who showed that 5.2° C. is the minimum temperature at which Asterias forbesi in Narragansett Bay will feed. However, the presence of detritus at the mouth and the partial extrusion of the cardiac stomach even during the wintertime suggests that a complete cessasion of feeding does not occur. More likely, feeding activity is reduced to a minimum.

It is possible that the metabolic needs may be met during the winter by a reabsorption of body tissues. Ebert (1967) has demonstrated that negative growth occurs in the purple sea urchin, _Strongylocentrotus purpuratus_ (Stimpson) when food is scarce, and Mead (1901) has shown a similar occurrence in _Asterias forbesi_. It would be difficult to demonstrate negative growth in _Leptasterias_ because of the problem in making accurate measurements of the animal's size and to date no other evidence of negative growth in starfish has come to my attention.

Starfish kept at 10° in the laboratory tended to feed sporadically. These animals have been fed small gastropods, small mussels, brine shrimp larvae, isopods and amphipods. Animals kept at 4° were never seen to feed on whole organisms except when force fed. At the lower temperature the starfish showed very little movement unless disturbed and would usually not move even if a suitable prey was placed beside them.

A simple test for food preference was designed in which three _Leptasterias_ were placed in each of five large finger bowls containing filtered seawater and several strands of _Ascophyllum_. A number of small gammarid amphipods, several small chitons, small mussels and small gastropods were placed in each of the finger bowls. The starfish soon began climbing on the _Ascophyllum_ and remained active for several hours. The first evidence of feeding occurred after about five hours when it was noted that one _Leptasterias_ had trapped an amphipod and was engulfing it with its partially extruded stomach. Shortly afterwards another amphipod was trapped by the same starfish. Both amphipods were partially enveloped by the cardiac stomach. The folds of the stomach were wrapped around the posterior end of one amphipod while the head end remained free. The other amphipod was held securely by part of the stomach which was wrapped around its midsection leaving both the head and telson free. The antennae of both amphipods were seen to twitch periodically for several hours. No attempt was made by the starfish to draw the food into the mouth. After about 20 hours the empty exoskeletons of the amphipods were released by the starfish. In both cases the exoskeletons were completely intact except for a break in the exoskeleton in the region just posterior to the dorsal surface of the head. All of the appendages remained intact even though the entire skeleton was empty. It appears that the starfish had secreted digestive enzymes into the amphipod and then drawn out the digested flesh.

After one week it was noted that more than 20 amphipods had been eaten in a similar fashion by the fifteen starfish. During this time a total of two mussels and one chiton had also been eaten.

Previous attempts at feeding the starfish in the lab had given inconclusive results. Small gastropods and mussels had been placed in the finger bowls and trays with the starfish, but these often remained untouched for several weeks. Obviously the starfish would have no difficulty capturing these prey since they move very slowly if at all. It was not until the Ascophyllum was put into the water that feeding activity became intensive.

The method by which the slow moving starfish are able to capture the rapidly swimming amphipods appears to be as follows. The starfish usually crawl along, straddling several strands of algae, with a number of the tube feet extended. As the amphipod swims between the algal strands, apparently in search of food, it may come in contact with the extended tube feet of the starfish. After a single foot makes contact with the prey, the adjacent feet are directed toward it. If the amphipod does not move away, it may become ensnared by a number of tube feet and will then be passed by the tube feet toward the mouth. Frequently the amphipod is able to break free from the tube feet by rapidly flexing its body several times in quick succession. I observed one amphipod free itself twice, in a period of ten minutes, from the grasp of a starfish only to be captured a third time and eaten.

In several instances feeding was successfully initiated in starfish held in 4^{o} water by turning the starfish over and holding a small mussel over its mouth. The tube feet would then grasp the prey and hold it until the stomach was extruded and could enclose the mussel. After the mussel had been digested, the stomach would be retracted and the empty shell released. The animal would then remain inactive and even refuse to be force-fed for several weeks. This suggests that the animals are capable of feeding on whole organisms even at the low temperatures during the winter months, but such feeding is not required.

Unfed starfish placed in unfiltered seawater frequently secrete a mucus from the skin of the top and sides of the arms and disk, forming long strands which are slowly moved by ciliary action toward the oral surface. Whether these mucus secretions function solely in the removal of debris that falls on the surface of the starfish, or play some role in the process of epidermal digestion has not yet been satisfactorily explained. To see if these secretions may be used as an adjunct method of feeding I placed three Leptasterias in a small finger bowl containing seawater to which was added several milliliters of concentrated brine shrimp larvae. Within 24 hours each of the starfish had secreted an abundance of mucus in which a considerable number of brine shrimp larvae were trapped. Three starfish that had been kept in a separate finger bowl containing filtered seawater showed no evidence of mucus secretions during this time.

Upon reaching the oral surface of the starfish the mucus strands were held between the tube feet in the ambulacral groove but were not passed toward the mouth. Whether any digestion occurred here was not determined, but it is suggestive of Pequignat's (1966) demonstration of epidermal digestion and absorption with Asterias rubens. He found that when stained pieces of organic material were placed in the ambulacral groove, digestion occurred and that the tube feet in the area of the food were subsequently stained as absorption occurred through the skin.

Other evidence for this type of nutrient procurement through the epidermis has been provided by Ferguson (1963) who fed radioactive clams to Asterias forbesi. He showed that large quantities of nutrients are absorbed directly from the external medium by epidermal cells, especially those of the tube feet and the ambulacral groove. In subsequent studies, Ferguson (1967a, 1967b, 1968, 1969) has explored in greater detail the uptake from dilute solutions of free amino acids and sugars by starfish through the epidermis. He has concluded that these substances may significantly contribute to the maintenance of the superficial tissues of the body but are not transported to the internal organs.

Either the direct absorption of dissolved organics or the trapping and digestion of particulate matter by mucus secretions could be an important alternate method of nutrient procurement for an inactive starfish living beneath the rocks when oral feeding is restricted as is the case with Leptasterias littoralis during the winter months.

Nonbrooding starfish have been found in the winter with their cardiac stomachs extruded and covered with detritus. It is possible that the detritus is digested and taken in by the ciliated surfaces of the cardiac stomach for storage or distribution to the internal organs Since the brood mass blocks the mouth, it is unlikely that a brooding starfish would feed in this manner.

Brood-carrying females have been kept from mid-November until early April without showing any evidence of feeding activity, although small mussels and gastropods were available to them. Occasionally mucus strands were secreted by these animals and these may have functioned in the procurement of nutrients for these animals. But this source of nutrients would appear only to serve to maintain the external tissues of the body wall.

The question still remains unanswered as to whether or not brooding females stop feeding completely, or whether they simply obtain their nutrients by a method other than oral feeding. Chia (1964) has shown that in Leptasterias hexactis stored food materials and zymogen granules in the pyloric caeca disappear after about forty days of brooding. He has also demonstrated that a brooding female will resume feeding after two days, if the brood is removed from the brood pouch. He suggests that a constant stimulation by the embryos is apparently necessary to prevent feeding.

Although direct evidence is lacking there is some circumstantial evidence that suggests that, at least in Leptasterias littoralis the ability to digest food is not seriously impaired by the presence of the embryos in the brood pouch. The brood mass is continually being washed by water currents produced by the movement of cilia and the tube feet. Chia (1964) suggests that in L. hexactis these currents remove dirt and debris which become attached to the embryos and also insure that a uniform environment is maintained around the embryos. These currents may also carry suspended particles which upon coming in contact with the peristomial membrane would be transported by ciliary currents toward the mouth. They may then be transported across the flagellated (Anderson, 1954) surface of the cardiac stomach for digestion and internal transport. That these flagellary currents are active even in a brooding starfish has been demonstrated by excising the stomach and sprinkling carmine particles on the luminal surface. Since the function of these flagella on the stomach surface is to transport nutrients to the pyloric caeca, it would seem to be an inefficient expenditure of energy to maintain these currents in the absence of any food source.

Another possible source of nutrients that may be available to the brooding female would be from those eggs and embryos that fail to develop to maturity. An average sized female may begin to brood several hundred to a thousand eggs, but many of these fail to develop to the young adult stage. Most of the peripheral eggs in the brood complete development, but a high percentage of the innermost eggs fail to survive until the end of the brooding period. Some of these eggs may not be fertilized but may be retained for weeks in apparently good condition.

In one instance a female collected in November had been isolated and kept in filtered seawater as part of an experiment to determine the effects of starvation. During the fifth week of starvation she released approximately 300 eggs in a period of about one and a half hours. The eggs were bright yellow and appeared to be in good condition except for about 5%, which were colorless. Since no fertilization occurred, the female had difficulty in holding the eggs in the brood pouch but finally assumed the brooding position with about fifty of the eggs held in place by the tube feet. Several days later only about a dozen eggs remained in the brood pouch, and these still appeared to be in good condition. The rest of the eggs had been ingested as indicated by the presence of green, granular fecal material at the anus. The starfish remained in the brooding position for about two more days until the remaining eggs had been ingested. This suggests that in spite of the fact that the starfish was carrying a brood the ability to feed was not inhibited.

The fate of unfertilized eggs or embryos that fail to complete development is not known for certain except that they are not ejected from the brood pouch. Again the possibility exists that they break down and since they are in close proximity to the mouth they may serve as a source of nutrients for the brooding parent.

REPRODUCTION AND BROODING BEHAVIOR

The reproductive season for Leptasterias littoralis begins sometime between the middle of October and the middle of November in the region of this study, after the animals have moved beneath the rocks. At this time the arms of the starfish are swollen by the paired gonads filled with gametes. Copulation does not occur in this species, although it has been noted in Leptasterias ochotensis similispinis by Kubo (1951). However some type of sexual aggregation is essential to insure that successful fertilization of the eggs occurs. Thus several starfish can usually be found in close proximity to one another beneath the same rock or in the same crevice. The rise and fall of the tide and the swift currents that characterize this region would tend to disperse the sperm quite rapidly if they were released when the animals were living in the algae but these tidal effects are moderated in the crevices and under the rocks.

The males spawn prior to the females by extruding the sperm from the paired genital papillae located on the oral surface in each of the interradial areas. The sperm are released from all ten gonads simultaneously in long strings that resemble toothpaste being squeezed from a tube. The sperm strings settle to the bottom, and after several minutes of contact with the water they begin to disintegrate as the sperm cells become active. Spawning in the males may last for one or two hours but several more hours may elapse before all of the sperm are liberated from the strings. This rather prolonged release of sperm probably insures that viable sperm will be present in the water for the duration of the female spawning period.

The females, possibly as a result of a stimulus from the presence of sperm in the water, then begin to spawn. The eggs are released slowly through the genital papillae, usually at a rate of one egg per minute from a single papilla. Eggs may be released from all ten papillae at the same time, or from just a few at a time, depending upon the position of the starfish. The eggs are yellow, measure about .8 to .9 mm. in diameter and are quite pliable when released. As each egg is squeezed through the gonopore at the tip of the papilla it is constricted and appears dumbbell-shaped when half way through. Once it has passed through the gonopore it regains its normal spherical shape and is fertilized if sperm are present.

The eggs are then taken by the tube feet and passed toward the mouth where the brood pouch is formed by arching of the arms. The eggs are held in the brood pouch by the tube feet until the fertilization membrane develops. The stickiness of this membrane causes the eggs to adhere to each other in the brood pouch. Occasionally eggs are dropped by the tube feet and are lost before they reach the brood pouch. This entire process may take up to five hours with an average sized female (R = 25 mm.) releasing six hundred to seven hundred eggs. If the eggs are not fertilized, they fail to adhere to each other, and most will be lost from the brood pouch. The female will still attempt to hold them in place with her tube feet, but after several hours she will abandon the brooding position and may even eat the eggs as has been described in the preceding section.

Since some Leptasterias littoralis live in the middle of the intertidal zone and may be immersed for only half of a tidal cycle or approximately six hours, it is interesting to note the amount of time required for the female to release a full brood of eggs. The sperm must have water in which to swim to the eggs. Thus one suspects that spawning must be completed before the tide goes out. In order for the female to complete her spawning, she must begin to release her eggs shortly after the animals are covered by the incoming tide. To determine if spawning could be interrupted when the animals were exposed and then resumed when immersed again a simple test was designed.

Two males and two females that had not yet spawned in November were placed in a small aquarium. One male was induced to spawn by injecting a .5 M isotonic KCl solution (Tyler, 1949) into its coelom. About ten minutes after injection it began to release sperm causing the water to become quite cloudy. Within an hour the second male began to spawn and both females began to spawn shortly afterwards. The first male appeared to be completely spent before the others began to spawn. Its spawning was probably accelerated by the effects of the KCl. The second male continued to spawn for almost two hours. Before he had stopped spawning he was removed from the aquarium, rinsed briefly in fresh water to kill any sperm that may have remained on him, and was placed in a second aquarium containing only a wet rock.

The two females were removed from the water after another half hour while they were still in the process of releasing eggs. Each was rinsed briefly in fresh water, and they too were placed on the surface of a wet rock. The spawning had been successfully interrupted although eggs could still be seen in the genital papillae.

Two and a half hours later fresh seawater was poured into the aquarium to immerse the three starfish. Both females began to release eggs again but the male failed to release any more sperm. Those eggs released after the initial spawn was interrupted were not fertilized, and most of them fell out of the brood pouches.

This experiment suggests that the females may successfully spawn over a period of two or perhaps more tidal cycles if sperm is present in the water, but a male probably releases all of his sperm during a single tidal cycle. It appears that in order to insure reproductive success the starfish would begin to spawn shortly after the incoming tide covers them. This would increase the probability of viable sperm being present during the time required for the female to complete the formation of her brood.

That the presence of sperm in the water will induce spawning in the female starfish has been demonstrated, but it is not known for certain what environmental cues induce the males to spawn. I have found some evidence that suggest that decreasing water temperature may in part be responsible.

In early November, 1966, a number of starfish that had been collected prior to the beginning of the natural spawning season, were being held in plastic bags filled with seawater in an ice-filled styrofoam cooler. The water temperature at the time the collections were made was between eight and ten degrees. During the night most of the starfish in each bag released their gametes and by morning the water was cloudy with sperm. Some of the females were carrying broods of fertilized eggs and a large number of unattended eggs were found at the bottom of the bags. The water temperature in the bags had dropped to near 0^{o} during the night because of the ice in the cooler.

A similar situation occurred during the following night when starfish that had been collected during that day were stored in the ice-filled cooler. All of these broods failed to develop and were abandoned by the parents during the following weeks. Development, as far as it went, was irregular, probably as a result of polyspermy caused by the overabundance of sperm in the water at the time of fertilization.

The following November I collected 137 starfish at a time when the water temperature was nine degrees centigrade. None of these was brooding at the time of collection. Twenty-five of these starfish were kept alive and returned to the laboratory in a plastic bag without ice. In the laboratory fifteen starfish were placed into an aquarium which was then slowly cooled to four degrees. Synchronous spawning occurred within forty-eight hours, beginning sometime during the early morning and was apparently completed shortly before noon. During this time the remaining starfish had been kept in an aquarium provided with recirculating seawater at a temperature of twelve degrees and no spawning occurred in this group. These findings suggest that at some temperature below nine degrees spawning is induced.

It would be advantageous for *Leptasterias littoralis* to respond to decreasing water temperatures as an environmental cue for at least two reasons. Water temperatures do not normally drop below ten degrees in this region until late in the fall at which time most, if not all, of the starfish are beneath the rocks and at a time when the gonads are filled with mature gametes. If spawning were induced when the starfish were still living in the algae, the dispersal of the sperm and the inability of the female to assume the brooding position would greatly reduce the chances for successful reproduction.

It has also been noted in the laboratory that when brooding females are kept in water at a temperature of twelve degrees they will soon abandon their broods. Although the reason for this is not known, it is apparent that they become quite active and will feed at this temperature while at lower temperatures the brooding females seldom show any movement other than to reestablish their position if disturbed. If spawning in the field is not initiated until the temperature drops considerably below this temperature, it is unlikely that those higher temperatures that may cause the females to abandon their broods will be encountered until the following spring.

Once the eggs are fertilized the female will remain in the brooding position until the embryos complete their development into young stars which are capable of leaving the parent and taking up an independent existence. The shortest time required for complete development noted in this study was three and a half months. This occurred in a group of brooding females kept in an aquarium at a temperature of eight degrees. At four degrees the brooding period was normally between four and five months and in one instance a female carried her brood for six months before the young were released.

Observations made during the field studies would seem to indicate that such a prolonged brooding period is not unusual for this species. Of two hundred and eleven specimens that were collected in either March or April during three successive years, slightly fewer than one third of them were carrying almost completely developed young. If it can be assumed that approximately fifty percent of the two hundred and eleven were males, or juvenile females that did not reproduce, then the percentage of brood-carrying females is quite high. It has been noted earlier that the conditions in the intertidal zone are quite harsh during the winter months. Thus it is reasonable to expect that the young stars would be protected until the return of more favorable conditions in the spring.

I have found no evidence either in the laboratory or from field studies that would indicate that a female reproduces more than once each year. On the contrary, post-spawning examinations of the gonads reveal them to be empty of mature eggs and considerably shrunken in length. Ripe ovaries extend about four-fifths of the length of the arm with within ten days after spawning the ovaries are reduced to about a quarter of their original length.

None of the young <u>Leptasterias</u> that were raised in the laboratory survived for more than a week or two after being released from the brood pouch, and I believe that this failure to survive resulted from a lack of suitable food. At the time of their release the young are slightly larger than one millimeter in diameter. They lack any epidermal pigmentation except for the eyespot found on the tip of each arm. There are from six to eight pairs of tube feet on each arm and numerous scattered spines and few dermal papulae are found on the aboral and lateral surfaces of the body. Although the opening of the mouth is formed, occasionally some yolk material may still be seen within the body of a young star. At this stage I have not been able to distinguish them on morphological bases from the young of *Leptasterias tenera* or from the young of *Asterias*.

SECTION V

HISTOLOGY OF THE CARDIAC STOMACH

The digestive tract of *Leptasterias littoralis* is similar to that of most members of the family Asteriidae. It consists of a mouth and a short esophagus leading into a voluminous extrusible cardiac stomach which is much folded and fills most of the interior of the disk when retracted. The structure of the cardiac stomach in *Leptasterias* closely resembles that of *Asterias forbesi* which has been described in detail by Anderson (1954). The lobes of the stomach are anchored to the ambulacral ossicles in the arms by a retractor mechanism made up of collagenous and muscular fibers. According to Anderson, when feeding is initiated the muscle fibers in the retractor mechanism and in the stomach wall relax. The muscles in the body wall contract causing an increased intracoelomic pressure that forces the stomach out through the mouth. After feeding has stopped the body wall muscles relax while the stomach and retractor muscles contract forcing the coelomic fluid back into the arms and causing the stomach to be withdrawn through the mouth.

Fibers from the retractor mechanism radiate over the surface of the cardiac stomach and most of them terminate near the mouth. There is a series of channels on the luminal surface of the stomach which is in close association with the retractor fibers. The system of channels begins at the oral end of the cardiac stomach and they converge as they pass upwards over the surface. Anderson (1954) has shown that the channels are the only places on the surface of the epithelium where currents do not flow upward toward the pyloric stomach and caeca. The function of these channels is not yet well understood.

The cardiac stomach is joined at the top by a narrow constriction to the small flattened pyloric stomach which is connected by five short ducts to the paired pyloric caeca located in each of the arms. A short intestine ascends from the pyloric stomach to the anus which is located on the aboral surface of the disk.

The pyloric caeca function primarily in the production of digestive enzymes and the absorption and storage of nutrients. Chia (1969) studied histological changes in the pyloric caeca in _Leptasterias_ _hexactis_ during brooding and starvation. He found that food reserves disappear and zymogen cells are inactivated after four weeks of brooding. However, even after ten weeks of starvation in a nonbrooding starfish the food reserves are still plentiful in the pyloric caeca and there is no detectable change in the zymogen cells. He suggests that the utilization of food reserves is much greater during brooding than during starvation.

The function of the cardiac stomach is to procure and hold food during the initial stages of digestion. After contact is made with a prey organism, the cardiac stomach is extruded through the mouth of the starfish and may completely engulf the prey. It is not believed that any digestive enzymes are produced by the cardiac stomach itself but enzymes are transported over its surface from the pyloric caeca to the site of digestion. Partially digested material is then passed back over the surface of the stomach, through the mouth and into the pyloric stomach.

After feeding has stopped the stomach is retracted through the mouth but some partially digested particles may remain in the stomach for several days. It would appear that if feeding stopped completely in a brooding starfish the cardiac stomach would be nonfunctional and histological changes may occur. Wintzell (1918) has shown that such change (stomach wall becomes thin and reduced) does occur in the brittle star, _Ophiothrix fragilis_, during a post-spawning period when they do not feed. To find out if any changes did occur in _Leptasterias_ during the brooding period, or as a result of starvation, histological examinations were periodically made on the cardiac stomachs of both brooding and starved starfish for comparison with the stomach of a normal well-fed starfish.

NORMAL HISTOLOGY

A cross section through the wall of the stomach reveals three more of less distinct regions. The innermost layer, adjacent to the lumen of the stomach, is mostly comprised of long flagellated columnar epithelial cells occasionally interspersed with mucous gland cells. The typical epithelial cells have ovoid nuclei located near the middle region of the cells and have a well developed brush border at the distal ends (Figure 9). There are also specialized epithelial cells located on the ridges that border the branching system of channels on the stomach wall. These specialized epithelial cells have long, cigar-shaped nuclei occupying about one-half the length of the cells (Figure 10). In addition to the specialized cells the ridges are characterized by the presence of many mucous gland cells.

In the region of the esophagus the epithelial layer takes on a somewhat corrugated appearance (Figure 11).

Figure 9. Section through cardiac stomach wall showing arrangement of typical epithelial cells and several mucous gland cells. Lumen at center. Wu's hematoxylin and eosin.

Figure 10. Section through lower region of the stomach showing channel and associated ridges. Note dense elongate nuclei on the ridges bordering the channel. Ehrlich's hematoxylin and eosin.


50µ


50µ

The cells in this region are of the specialized type with cigar-shaped nuclei and there are a large number of mucous gland cells. At the distal ends of these cells are found what Anderson (1954) describes as granular secretions which are probably produced by a type of secretory cell different from the mucous gland cells. The nature of these secretions is not yet understood but their occurrence is restricted to the region near the mouth. Figure 11 shows these secretory granules as they appear in normal tissue. This inner layer of epithelial cells accounts for about four-fifths of the total thickness of the stomach wall.

Nerve fibers, connective tissue, and muscle fibers are found in the very thin middle layer of the wall. The connective tissue appears to form a basement membrane of the epithelium and sends short branching fibers into the nerve and muscle layers.

The outermost layer of the cardiac stomach is a single sheet of simple cuboidal epithelial cells which appear to be continuous with the peritoneum lining the body cavity. These cells are small and each contains a single oval nucleus which occupies most of the cell. These cells are also flagellated and they are uniform in appearance over the entire outer surface of the stomach wall.

BROODING STARFISH

Brooding starfish were kept in seawater that was changed every two days. Specimens were removed for histological examination every ten days for a period of sixty days. No discernible change was noted in the histology of the stomach wall during this period. The epithelial cells remained unaffected as did the mucous gland cells. These findings appear to be consistent with Chia's (1969) observations of pyloric caeca tissue in _Leptasterias hexactis_. He noted that although zymogen cells became inactive, the mucous cells and current producing cells were unaffected after four weeks of brooding.

STARVED STARFISH

The starfish used in the starvation experiment were kept in filtered natural seawater that was changed every two days for sixty days. These specimens showed little activity during this period other than to right themselves if overturned. They remained attached to the bottom of the aquarium by their tube feet and showed no obvious ill effects as a result of starvation. There were several occasions during this period when one or more of the starfish showed evidence of epidermal secretions. In contrast to these observations several starfish which had been kept in a fresh preparation of artificial seawater failed to survive beyond six weeks. After the third week these animals became extremely flaccid and were unable to maintain attachment to the aquarium with their tube feet.

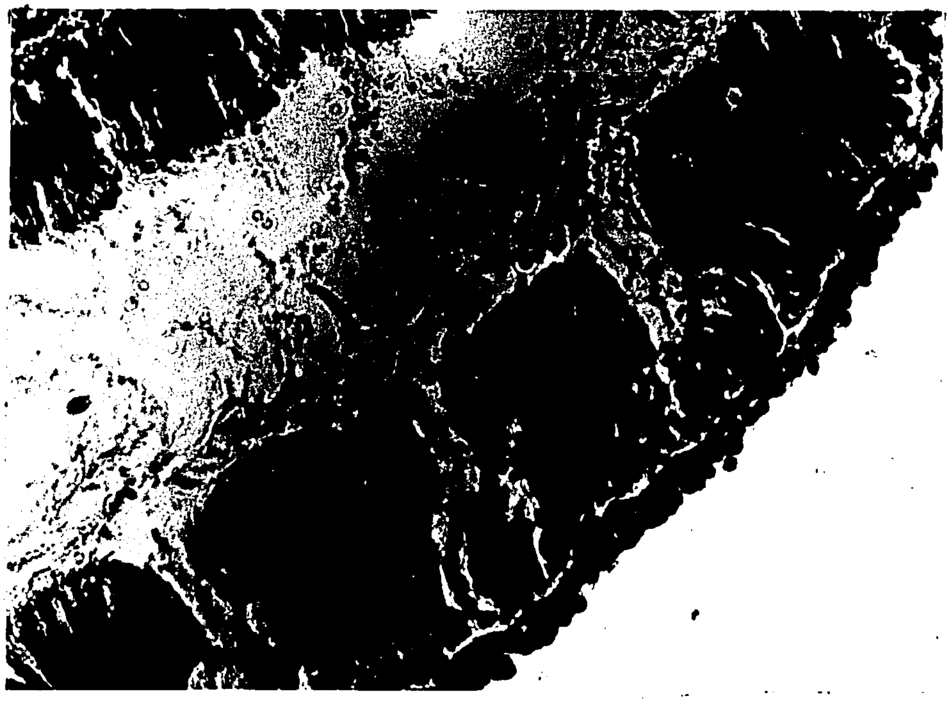
The most noticeable histological change occurred after sixty days of starvation in the region of the esophagus where the granular secretions characteristic of the epithelial layer were depleted (Figure 12). In addition to the depletion of the granules, the mucous gland cells in this region appear to be larger and more abundant than in normal tissue. The rest of the stomach wall appears to be unchanged. Since the nature of the secretions is not understood it is difficult to determine the cause or the significance of their disappearance at this time.

Figure 11. Section through the region of the esophagus of a fed starfish. Note secretory granules in the epithelial layer. Peterson's acid alizarin blue.

Figure 12. Section through the region of the esophagus of a starfish starved for 60 days. Note the absense of the secretory granules and increase in the abundance of mucous cells in the epithelial layer. Peterson's acid alizarin blue.

50μ

50μ



What may be of significance here is the difference noted between the starfish kept in natural filtered seawater and those kept in artificial seawater. It is known that a variety of aquatic invertebrates are capable of utilizing dissolved organics that are commonly found in the environment. Stephens and Schinske (1961) demonstrated that representatives of ten phyla of invertebrates could remove glycine from seawater. In later papers, Stephens (1962, 1963) and Stephens and Virkar (1966) showed that other amino acids and glucose could be removed from dilute solutions by annelids and by an ophiuroid. Ferguson (1969) has shown that various dissolved amino acids, glucose, and possibly proteins are taken up continually by the exposed superficial tissues of the starfish, Echinaster echinophorus. The filtering process used in this study removed only the suspended particulate matter from the seawater. No attempt was made to remove dissolved organic matter. If dissolved organics were present in this water it could provide a source of nutrients, however slight, to the starfish. The artificial seawater prepared with INSTANT OCEAN synthetic sea salts dissolved in distilled water provided no such dissolved nutrients and the starfish kept in this water failed to survive. Unfortunately there is no way to determine with certainty that their death resulted from starvation but continued studies along this line may prove to be fruitful.

SECTION VI

GENERAL DISCUSSION

At the time that this study was begun it was hoped that answers might be found to a number of puzzling questions about the biology of a little known starfish, Leptasterias littoralis. Some aspects of its biology are described in this paper but some new questions have arisen and are yet to be answered.

The habitat of those members of this species which live in the intertidal zone has been described and it has been shown that they spend about half of the year living in close association with the rockweed, Ascophyllum nodosum, while the rest of the year is spent beneath the rocks or in crevices. This species seems to be well adapted to life in the intertidal zone in spite of the harshness of this environment. Its association with the algae during the summer provides the starfish with protection from predators, desiccation, and excessive temperatures, and food is abundant on or among the thalli of the algae.

The animals move beneath the rocks when the temperature begins to drop in the fall. This movement is coincident with the onset of the reproductive season, and most of the starfish will have spawned by the end of November. It is also at this time that feeding activity is reduced. This may result from a decrease in the metabolic needs of the animals, or perhaps, simply because a more restricted diet is available to them beneath the rocks. Since the females will brood their young during the winter and their movement is curtailed, normal feeding is not possible. In any case they no longer move about actively searching for prey.

There is a paucity of information about the subtidal members of this species. It would be interesting to know if a seasonal movement is characteristic of them, and also, if they are associated with other species of algae since *Ascophyllum* is generally found only in the shallow near-shore waters. These starfish would not be exposed to all of the same environmental hazards that affect the intertidal starfish but they would probably be more susceptible to predation, especially by *Asterias rubens* and *Solaster endeca*. Their reproductive season may be later than that of the intertidal animals if it is induced by decreasing temperatures. According to Lauzier and Hull (1969) the bottom temperatures in the region of the Bay of Fundy are highest (maximum average = 8.9^{o}) in November and begin to drop in December. Along the coast the temperatures are highest in August and have dropped to 7.8^{o} by November. Evidence found in this study suggests that the intertidal starfish reproduce after the temperature drops below nine degrees but this probably does not hold true for the subtidal starfish. In any event little is known about their biology in contrast to the intertidal populations and further study would be useful.

This species has a range of only about eight hundred statute miles along the Northwest Atlantic coast and its bathymetric range extends from the mid-tide level down to about forty-two meters. There are at least two other species of *Leptasterias* whose ranges overlap that of *Leptasterias littoralis*. *Leptasterias tenera* is found from the Bay of Fundy south to Cape Hattaras and it is widely distributed over the continental shelf (Wigley, personal communication). However, it is not known to occur in the intertidal zone. Leptasterias polaris (Muller and Troschel) is found in the Gulf of St. Lawrence and its range extends northward into the Arctic Ocean and over into the North Pacific Ocean as far south as Washington (Fisher, 1930). Its bathymetric range is from the low water mark to about 110 meters being most common between 18 and 55 meters (Brunel, personal communication). Although both of these species have a wider distribution than Leptasterias littoralis neither has successfully adapted to life in the intertidal zone.

The question of whether feeding stops completely in brooding Leptasterias has not yet been satisfactorily answered. Chia (1964) has demonstrated that there is an inactivation of the secretory activity by the zymogen cells during brooding in Leptasterias hexactis and he suggests that a constant stimulation by the embryos is necessary to prevent feeding. However, Osterud (1918) found small gastropods in the stomachs of the same species during late brooding stages which suggests that they are capable of some type of feeding. I have found some evidence that Leptasterias littoralis does not lose the ability to feed during brooding. The ciliary currents across the cardiac stomach are maintained during brooding, and no histological changes were noted in this organ after sixty days of brooding. No decrease in the size of the pyloric caeca was noted after four months of brooding although one may suspect that these organs might be a source of nutrients if reabsorption of body tissues did occur. The gonads are reduced in size after the first few days following spawning but this is probably just a shrinkage resulting from the loss of the gametes rather than tissue reabsorption.

One area that might profitably be investigated is the uptake and transport of dissolved nutrients by this species. This type of nutrient procurement has been reported in asteroids by Ferguson (1967a, 1967b, 1968, 1969); in a holothuroid and an ophiuroid by Fontaine and Chia (1968); and in echinoids by Pequignat (1966), Sawbridge and Bell (1969), and others. The usefulness of this type of an adjunct feeding mechanism to an organism whose oral feeding may be interrupted for up to six months is obvious. Epidermal absorption probably occurs in *Leptasterias littoralis* but its total contribution to the nutritional requirements of this starfish is not known. It would seem that where detritus has accumulated, especially in its winter habitat beneath the rocks, that dissolved nutrients may be concentrated to a level higher than that found in open seawater. Measurements of the amounts and kinds of dissolved nutrients in these microhabitats, and studies of the ability of *Leptasterias littoralis* to utilize them would be useful in gaining a better understanding of the biology of this starfish.

LITERATURE CITED

Anderson, J. M., 1954. Studies on the cardiac stomach of the starfish, Asterias forbesi. Biol. Bull., mar. biol. Lab., Woods Hole, 107, 157-173.

Anderson, J. M., 1960. Histological studies on the digestive system of a starfish, Henricia, with notes on Tiedemann's pouches in starfishes. Biol. Bull., mar. biol. Lab., Woods Hole, 119, 371-398.

Blegvad, H., 1914. Food and conditions of nourishment among the communities of invertebrate animals found on or in the sea bottom in Danish waters. Rep. Danish biol. Sta., 22, 41-78.

Bousfield, E. L., and A. H. Leim, 1958. The fauna of Minas Basin and Minas Channel. Nat. Mus. Canada, 166, 1-29.

Budington, R. A., 1942. The ciliary transport-system of Asterias forbesi. Biol. Bull., mar. biol. Lab., Woods Hole, 83, 438-450.

Bull, H. O., 1934. Aquarium observations on the rate of growth and enemies of the common starfish Asterias rubens L. Rep. Dove Mar. Lab., Ser. 3, 2, 60-65.

Bush, K. J., 1884. Catalogue of Mollusca and Echinodermata dredged on the coast of Labrador by the expedition under the direction of Mr. W. A. Stearns, in 1882. Proc. U.S. nat. Mus., 6, 236-247.

Chia, F., 1964. The development and reproductive biology of a brooding starfish, Leptasterias hexactis (Stimpson). Doctoral

Dissertation, University of Washington.

Chia, F., 1966a. Systematics of the six-rayed sea star, Leptasterias, in the vicinity of San Juan Island, Washington. Systematic Zool., 15, 300-306.

Chia, F., 1966b. Brooding behavior of a six-rayed starfish, Leptasterias hexactis. Biol. Bull., mar. biol. Lab., Woods Hole, 130, 304-315.

Chia, F., 1969. Histology of the pyloric caeca and its changes during brooding and starvation in a starfish, Leptasterias hexactis. Biol. Bull., mar. biol. Lab., 136, 185-192.

Clark, H. L., 1904. The Echinoderms of the Woods Hole Region. Bull. U.S. Fish. Comm., 22, 547-576.

Conn, H. J., M. A. Darrow, and V. M. Emmel, 1960. Staining Procedures. Baltimore, Williams and Wilkins, 289 pp.

Cuenot, L., 1887. Contribution a l'etude anatomique des asterides. Arch. Zool. exp. gen. ser. 2, 5 suppl., 144 pp.

Ebert, T. A., 1967. Negative growth and longevity in the purple sea urchin Strongylocentrotus purpuratus (Stimpson). Science, 157 (3788), 557-558.

Feder, H., and A. M. Christensen, 1966. Aspects of Asteroid Biology, p. 87-127. In R. A. Boolootian ed., Physiology of Echinodermata. Interscience, New York.

Ferguson, J. C., 1963. An autoradiographic study of the distribution of ingested nutrients in the starfish, Asterias forbesi. Amer. Zool., 3, 524.

Ferguson, J. C., 1967a. Utilization of dissolved exogenous nutrients by the starfishes, Asterias forbesi and Henricia sanguinolenta.

Biol. Bull., mar. biol. Lab., Woods Hole, 132, 161-173.

Ferguson, J. C., 1967b. An autoradiographic study of the utilization of free exogenous amino acids by starfishes. Biol. Bull., mar. biol. Lab., Woods Hole, 133, 317-329.

Ferguson, J. C., 1968. An autoradiographic analysis of the uptake of exogenous glucose by three species of starfishes. Amer. Zool., 8, 805.

Ferguson, J. C., 1969. Feeding activity in Echinaster and its induction with dissolved nutrients. Biol. Bull., mar. biol. Lab., Woods Hole, 136, 374-384.

Fisher, W. K., 1930. Asteroidea of the North Pacific and adjacent waters. Bull. U.S. nat. Mus., 76, Part III, 356 pp.

Fontaine, A. R., and F. Chia, 1968. Echinoderms: An autoradiographic study of assimilation of dissolved organic molecules. Science, 161 (3846), 1153-1155.

Forbes, E., 1841. A history of British starfishes and other animals of the class Echinodermata. London, J. Van Voorst, 270 pp.

Galtsoff, P.S., and V. L. Loosanoff, 1939. Natural history and method of controlling the starfish (Asterias forbesi). Bull. U.S. Bur. Fish., 49, 73-132.

Ganong, W. F., 1888. The Echinodermata of New Brunswick. Bull. Nat. Hist. Soc., New Brunswick, 7, 12-69.

Gemmill, J. F., 1914. The development and certain points in the adult structure of Asterias rubens. Phil. Trans., 205 B, 213-294.

Gemmill, J. F., 1915. On the ciliation of asterids, and on the question of ciliary nutrition in certain species. Proc. zool.

Soc. Lond., 1915 (1), 1-19.

Gordon, I., 1929. Skeletal development in *Arbacia*, *Echinarachnius*, and *Leptasterias*. Phil. Trans. Ser. B., *217*, 289-334.

Grainger, E. H., 1955. Echinoderms of Ungava Bay, Hudson Strait, Frobisher Bay and Cumberland Sound. J. Fish. Res. Bd. Can., *12*, 899-915.

Grainger, E. H., 1964. Asteroidea of the Blue Dolphin Expeditions to Labrador. Proc. U.S. nat. Mus., *115*, 31-46.

Hancock, D. A., 1955. The feeding behaviour of starfish on Essex oyster beds. J. mar. biol. Ass. U.K., *34*, 313-331.

Humason, G. L., 1962. Animal Tissue Techniques. San Francisco, Freeman, 468 pp.

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

Kingsley, J. S., 1901. Preliminary catalogue of the marine invertebrata of Casco Bay, Maine. Proc. Portland Soc. nat. Hist., *2*, 159-183.

Kubo, K., 1951. Some observations on the development of the seastar *Leptasterias* *ochotensis* *similispinus* (Clark). J. Fac. Sci. Hokkaido Univ. ser. 6 Zool., 10, 97-105.

Lauzier, L. M., and J. H. Hull, 1969. Coastal Station Data. Temperatures along the Canadian Atlantic coast. 1921-1969. Fish. Res. Bd. Can., Tech. Rep. 150, 25 pp.

Lieberkind, I., 1920. On a starfish (*Asterias* *groenlandica*) which hatches its young in its stomach. Vidensk. Meddr. dansk. naturh. Foren. ser. 8, *72*, 121-126.

Loosanoff, V. L., 1964. Variations in time and intensity of setting of the starfish, Asterias forbesi, in Long Island Sound during a twenty-five year period. Biol. Bull. mar. biol. Lab., Woods Hole, 126, 423-429.

Mead, A. D., 1901. The natural history of the starfish. Bull. U.S. Fish. Comm., 19 (1899), 203-224.

Mortensen, Th., 1927. Handbook of the echinoderms of the British Isles. Oxford Univ. Press, 471 pp.

Osterud, H. L., 1918. Preliminary observations on the development of Leptasterias hexactis. Publ. Puget Sd. mar. (biol.) Sta., 2 (32), 1-16.

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

Prefontaine, G., and P. Brunel, 1962. Liste d'invertebres marins recueillis dan l'estuaire du Saint-Laurent de 1929 a 1934. Can. Nat., 89, 237-263.

Quayle, D. B., 1954. Growth of the purple seastar. Oyster Bull., 5, 11-13.

Sars, M., 1846. Beobachtungen uber dei Entwicklung der Seesterne. Fauna Littoralis Norvegiae, 1, 47-62.

Sawbridge, D. F., and M. Bell, 1969. Pacific shores. Science, 164 (3883), 1089.

Stephens, G. C., 1962. Uptake of organic material by aquatic invertebrates. I. Uptake of glucose by the solitary coral, Fungia scutaria. Biol. Bull. mar. biol. Lab., Woods Hole, 123, 648-659.

Stephens, G. C., 1963. Uptake of organic material by aquatic invertebrates. II. Accumulation of amino acids by the bamboo worm, Clymenella torquata. Comp. Biochem. Physiol., 10, 191-209.

Stephens, G. C., and R. A. Schinske, 1961. Uptake of amino acids by marine invertebrates. Limnol. and Oceanog., 6, 175-181.

Stephens, G. C., and R. A. Virkar, 1966. Uptake of organic material by aquatic invertebrates. IV. The influence of salinity on the uptake of amino acids by the brittle star, Ophiactis arenosa. Biol. Bull. mar. biol. Lab., Woods Hole, 131, 172-185.

Stimpson, W., 1853. Synopsis of the marine invertebrates of Grand Manan. Smith. Contrib., 6, 14.

Thorson, G., 1957. Bottom communities (sublittoral or shallow shelf). Hedgpeth, J. W. (ed.). Treatise on marine ecology and paleoecology. Vol. 1, Ecology. Mem. 67 geol. Soc. Amer., 461-584.

Tortonese, E., 1963. Differenziazone geografica e superspecie nel genere Asterias (Echinodermata). Monit. zool. ital., 70 - 71, 212-221.

Tyler, A., 1949. A simple non-injurious method for inducing repeated spawning of sea urchins and sand dollars. Collect. Net, 19, 19-20.

Verrill, A. E., 1866. On the polyps and echinoderms of New England, with descriptions of new species. Proc. Boston Soc. nat. Hist., 16, 333-351.

Verrill, A. E., 1971. Marine fauna of Eastport, Me. Bull. Essex Inst., 3, 2-6.

Verrill, A. E., 1874. Explorations of Casco Bay by the U.S. Fish Commission, in 1873. Proc. Amer. Assoc. Adv. Sci., 22, 340-395.

Verrill, A. E., 1879. Preliminary check list of the marine Invertebrata of the Atlantic Coast, from Cape Cod to the Gulf of St. Lawrence. 32 pp. Published by author.

Verrill, A. E., 1895. Distribution of the echinoderms of North America. Amer. J. Sci., 49, 127-141, 197-212.

Verrill, A. E., 1914. Monograph of the shallow-water starfishes of the North Pacific from the Arctic Ocean to California. Harriman Alaska Ser., Smithsonian Inst., 14, 201 pp.

Vevers, H. G., 1949. The biology of Asterias rubens L. I. Growth and reproduction. J. mar. biol. Ass. U.K., 28, 165-187.

Wintzell, J., 1918. Bidrag till de skandinavska ophiuridernas biologi och fysiologi. Dissertation, Uppsala. (Not seen, cited from Thorson, 1947).

Whiteaves, J. F., 1884. List of the marine invertebrates from Hudson's Strait. Rep. Prog. Geol. Surv. Canada, 1882 - 83 -84.

Wu, F. W., 1940. Double-staining in toto with hematoxylin and eosin. Science, 92 (2396), 515-516.

Yokoe, Y., and I. Yasumasu, 1964. The distribution of cellulase in invertebrates. Comp. Biochem. Physiol., 13, 323-338.

Zinn, D. J., 1937. The growth and development of starfish in Narragansett Bay in relation to temperature and food supply. Masters thesis. Univ. Rhode Island.

APPENDIX

STAIN PREPARATION AND PROCEDURES

Fixation in Bouin's Fixative

Fixative preparation

Picric acid, saturated solution	75.0 ml
Formalin, concentrated	25.0 ml
Glacial acetic acid	5.0 ml

Fixing procedure

Fix for twenty-four hours or longer
Wash tissue overnight in running water before embedding

Dehydration in Ethyl Alcohol and Embedding in Paraplast

30% alcohol solution	1 hour
50% alcohol solution	1 hour
70% alcohol solution	1 hour
95% alcohol solution	1 hour
Absolute alcohol #1	½ hour
Absolute alcohol #2	1 hour
Xylene #1	½ hour
Xylene #2	1 hour
Melted paraplast #1	1 hour
Melted paraplast #2	1 hour

Stain Preparation and Staining Procedure

Ehrlich's Hematoxylin

Hematoxylin crystals	4 g.
Ammonium alum	6 g.
Ethyl alcohol	200 ml.
Glycerol	200 ml.
Distilled	200 ml.
Glacial acetic acid	20 ml.

Dissolve hematoxylin crystals in alcohol and add other ingredients. Add 0.6 grams of sodium iodate for immediate maturing.

Eosin Y

Eosin Y	1 g.
70% ethyl alcohol	1000 ml.
Glacial acetic acid	5 ml.

Dilute with equal volumes of 70% ethyl alcohol for use.

Staining Procedure

Remove paraplast in xylene, two changes	10 min.
Hydrate in ethyl alcohol	
Absolute	5 min.
90%	3 min.
70%	3 min.
50%	3 min.
Running water	5 min.
Hematoxylin solution	10 to 20 min.
Rinse in tap water	2 min.
0.1% HCl in distilled water	10 to 20 sec.
Running water	20 min.
Eosin Y solution	2 min.
Clear in xylene	
Mount	

Double staining _in toto_ with Hematoxylin and Eosin (Wu, 1940)

Ehrlich's Hematoxylin (modified)

Ehrlich's hematoxylin, stock solution	8 ml.
50% ethyl alcohol	30 ml.
Glacial acetic acid	2 ml.

Procedure

Fix in Bouin's for 1 to 3 days according to size. Wash and preserve in 70% alcohol.

Remove yellow color in tissue by soaking in several changes of 70% alcohol saturated with $NaHCO_3$ or $KHCO_3$ (1 hr./mm.). Wash out bicarbonate in 50% alcohol for one or more hours.

Stain in 20 volumes of modified Ehrlich's hematoxylin for two to five days (1 day/2mm.). Shake from time to time to insure uniform penetration.

Rinse in water. Soak in several changes of 30% alcohol containing 0.5% acetic acid to extract the excess stain (1 hr./mm.). Last change should remain colorless after the tissue remains in it for ½ hour.

Slow running tap water overnight.

Dehydrate through alcohol of 30, 50, 70, 85, and 95% for six to 24 hours each.

95% alcohol with 0.3% eosin (alcohol-soluble), 12 to 24 hours.

Absolute alcohol with 0.3% eosin, 12 to 24 hours.

Clear in chloroform by sinking as follows: In a small vial pour a layer of chloroform on the bottom and then a layer of absolute alcohol with eosin. Place tissue in the alcohol and allow to sink slowly to the bottom. Leave for 2 to 6 hours or until tissue has sunk to the bottom.

Chloroform saturated with paraplast overnight.

Melted paraplast, two or three baths (1 to 3 hrs. each). Embed in the usual way.

Cut sections and attach to slides by the albumen-water method and dry for 24 hours or longer.

Put sections in xylene to remove paraplast, bring to absolute alcohol to differentiate the eosin, then back to xylene. Mount in balsam.

Stain Preparation and Procedure for Peterson's Acid Alizarin Blue Stain

Sorenson's Citrate Solution

Citric acid	21 g.
1 N sodium hydroxide solution	200 ml.
Distilled water to make	1000 ml.

Acid Alizarin Blue Solution

Acid alizarin blue	0.25 g.
Aluminum sulfate	5.0 g.
Distilled water	50.0 g.

Boil for five minutes, cool and filter. Restore to original volume. Buffer to pH 2.25 by adding 20 ml. of Sorenson's solution. Add 30 ml. 10N HCl solution.

5% Phosphotungstic Acid Solution

Phosphotungstic acid	5.0 g.
Distilled water	100.0 g.

Aniline Blue-Orange G Solution

Anilin blue, water soluble	0.5 g.
Orange G	2.0 g.
Distilled water	100.0 ml.

Bring to a boil, cool and filter. Add 8.0 ml. glacial acetic acid.

Remove paraplast in usual manner.

Stain for three minutes in acid alizarin blue solution.

Rinse in distilled water by quickly immersing and withdrawing slide two or three times.

Differentiate in 5% phosphotungstic acid for two or three minutes.

Rinse in distilled water.

Stain for two or three minutes in undiluted anilin blue-orange G solution. If stains proves to be too intense it may be diluted in one, two or three volumes distilled water.

Rinse briefly in distilled water.

Dehydrate in 95% alcohol followed by absolute alcohol.

Clear with xylene and mount.