ASPECTS OF THE REPRODUCTIVE BIOLOGY OF CUCUMARIA FRONDOSA (GUNNERUS, 1770) AND PSOLUS FABRICII (DUBEN AND KOREN, 1846) (ECHINODERMATA:HOLOTHUROIDEA) IN SHALLOW WATERS OF THE AVALON PENINSULA, NEWFOUNDLAND

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ASPECTS OF THE REPRODUCTIVE BIOLOGY OF *CUCUMARIA FRONDOSA* (GUNNERUS, 1770) AND *PSOLUS FABRICII* (DÜBEN AND KOREN, 1846) (ECHINODERMATA:HOLOTHUROIDEA) IN SHALLOW WATERS OF THE AVALON PENINSULA, NEWFOUNDLAND

by

© Lawrence William Coady, B.Sc.

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the Requirements for the Degree of
Master of Science

Marine Sciences Research Laboratory
Department of Biology
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ASPECTS OF THE REPRODUCTIVE BIOLOGY OF *CUCUMARIA FRONDOSA* (GUNNERUS,1770) AND *PSOLUS FABRICII* (DÜBEN AND KOREN,1846) (ECHINODERMATA : HOLOTHUROIDEA) IN SHALLOW WATERS OF THE AVALON PENINSULA, NEWFOUNDLAND.

by Lawrence William Coady

ABSTRACT

Populations of the holothurian species *Cucumaria frondosa* and *Psolus fabricii* were sampled twice monthly from shallow waters of the Avalon Peninsula, Newfoundland, from August,1971 through August, 1972. Large numbers of both species were also collected and maintained in a free-flowing laboratory seawater system for purposes of general observation and comparative investigation.

Both species exhibit an annual reproductive cycle.

The gonads of male and female individuals of both species consist of the same histological elements throughout the year, varying only in width. From outside to inside, these are: visceral peritoneum, nerves, muscles, haemal fluid (lined on either side by an extrahaemal and intrahaemal basal lamina), and germinal epithelium. The cellular components of the gonads of immature individuals are similar.

The timing and duration of separate histological developments definable as activation, development, maturity, spawning and spent phases of the reproductive cycle, were clearly evident in males of the two species. Active spermatogenesis commenced in early June following the spawning period and continued for approximately six months until the end of November when spermatogenesis slowed as the animals attained maturity. The first indications of spawning occurred in February and spawnings continued until the beginning of May. Spawnings are largely

intermittent and completely spent individuals were not evident in any of the histological preparations examined until the latter part of the spawning period.

Although gametogenic developments through progressive stages of maturation were less clearly evident in females, the two sexes appear to develop at different rates. Whereas the majority of males attained the mature reproductive condition several months prior to spawning, the final maturation divisions of developing oocytes which give rise to fertilizable ova, did not occur until the time of spawning itself.

There is a marked seasonal variation in the number of accessory cells (nutritive phagocytes) within the lumen of the gonads of both sexes.

The timing of spawning appears to be more closely related to nutritive factors associated with the spring phytoplankton "bloom" rather than to demonstrable physical parameters of the environment.

Histological aspects of the reproductive cycle of individuals maintained in the laboratory during the same time period were similar up to the time of spawning. Subsequent to spawning there was a marked depreciation in the rate at which gametogenic activities were renewed. It is felt that the observed difference in recovery rate was due to nutrient deficiences created by the concentrated feeding action of fouling organisms in the piping system of the laboratory's seawater supply.

Natural populations of *Cucumaria frondosa* and *Psolus fabricii* are composed of approximately equal numbers of both sexes. Sexes may

be distinguished externally by the appearance of the genital papilla. In females, the opening of the gonoduct to the exterior appears as a single tubular opening whereas in males sperm are released through several openings, each of which appear as minute projections of the genital papilla.

Gonad index values (ratio gonad volume/total wet weight) in these species do not reflect variations in gonad size. Neither do changes in gonad volume reflect the variable size and/or number of sex cells occurring in the gonad at different times of the reproductive cycle. Pronounced seasonal fluctuations in gonad volume and total wet weight associated with variable food levels at different times of the year give rise to a situation where reproductive developments are best defined histologically.

- nec iuxta intuitum ego indico ; homo enim

videt ea quae parent .

-I Samuel 16.7 (St.Jerome).

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- Lawrence William Coady

INTRODUCTION

When the Marine Sciences Research Laboratory (MSRL) at Logy Bay in Newfoundland opened in 1967, an expert advisory group met to discuss marine research needs in Newfoundland waters. Elisabeth Deichmann of Harvard University, in a document prepared for this meeting on holothurian research potentialities in the northwest Atlantic, pointed out that during the past fifty years the holothurian fauna of Newfoundland shallow waters have been completely ignored.

When this study was undertaken in May of 1971, SCUBA surveys were made along the coast of Newfoundland to determine the availability of holothurian forms. Throughout the study additional information on the occurrence of different holothurian species was gathered from divers operating out of the MSRL on various other projects around Newfoundland and along the Labrador coast.

Five species of holothurians (ECHINODERMATA; HOLOTHUROIDEA) are available to divers in depths of less than 100 feet, with the degree of availability depending largely on the substrate preference of the species concerned. The dendrochirotes *Cucumaria frondosa* (Gunnerus) and *Psolus fabricii* (Duben and Koren), which attach to open rock surfaces, were the two species most frequently observed and were recorded in the majority of areas surveyed. A third species of dendrochirote, *Psolus phantapus* (Strussenfeldt), which buries itself in loose gravel, is only noticeable when it extends its feeding appendages and is therefore difficult to find during months of the year when feeding activities are infrequent in this species. The

small apodan, *Chirodota laevis* (Fabricius), a true Arctic species (Ekman, 1953) found in Newfoundland waters and *Leptosynapta tenuis* (Ayres, 1851) are forms which burrow and are collected only infrequently on the chance occurrence of finding individuals under rocks or boulders.

A chart of the island of Newfoundland, showing the areas in which the different holothurian species were recorded during these surveys, is given in Figure 1. Considerably more diving was carried out on the Avalon Peninsula of Newfoundland and more information is therefore available from this area. Whereas *Cucumaria frondosa* and *Psolus fabricii* are readily evident in areas where they occur, *Psolus phantapus*, *Chirodota laevis* and *Leptosynapta tenuis* require a considerable amount of careful search before they are found, and for this reason alone, were not recorded as frequently.

In Figure 2 are shown the locations of four diving sites examined by SCUBA divers along the Labrador coast of Newfoundland. The dive at Saglek Bay was made in August of 1972 and the remaining three dives were made in the fall of 1971. Large numbers of *C. frondosa* and *P. fabricii* were reported from each of the four locations.

Although these records provide a far from complete picture of the occurrence and distribution of holothurians in shallow waters of Newfoundland and Labrador, *C. frondosa* and *P. fabricii* do appear to be widely distributed in both areas. An accurate estimate of the distribution of *P. phantapus*, *C. laevis* and *L. tenuis* will require more careful study.

Figure 1 - Map of the island of Newfoundland showing locations in which holothurian species were found by scuba divers in depths of less than 100 feet of water.

Figure 2 - Map of the Labrador coast showing the location of four sites examined by scuba divers. Large numbers of *Cucumaria frondosa* and *Psolus fabricii* were found at each site.

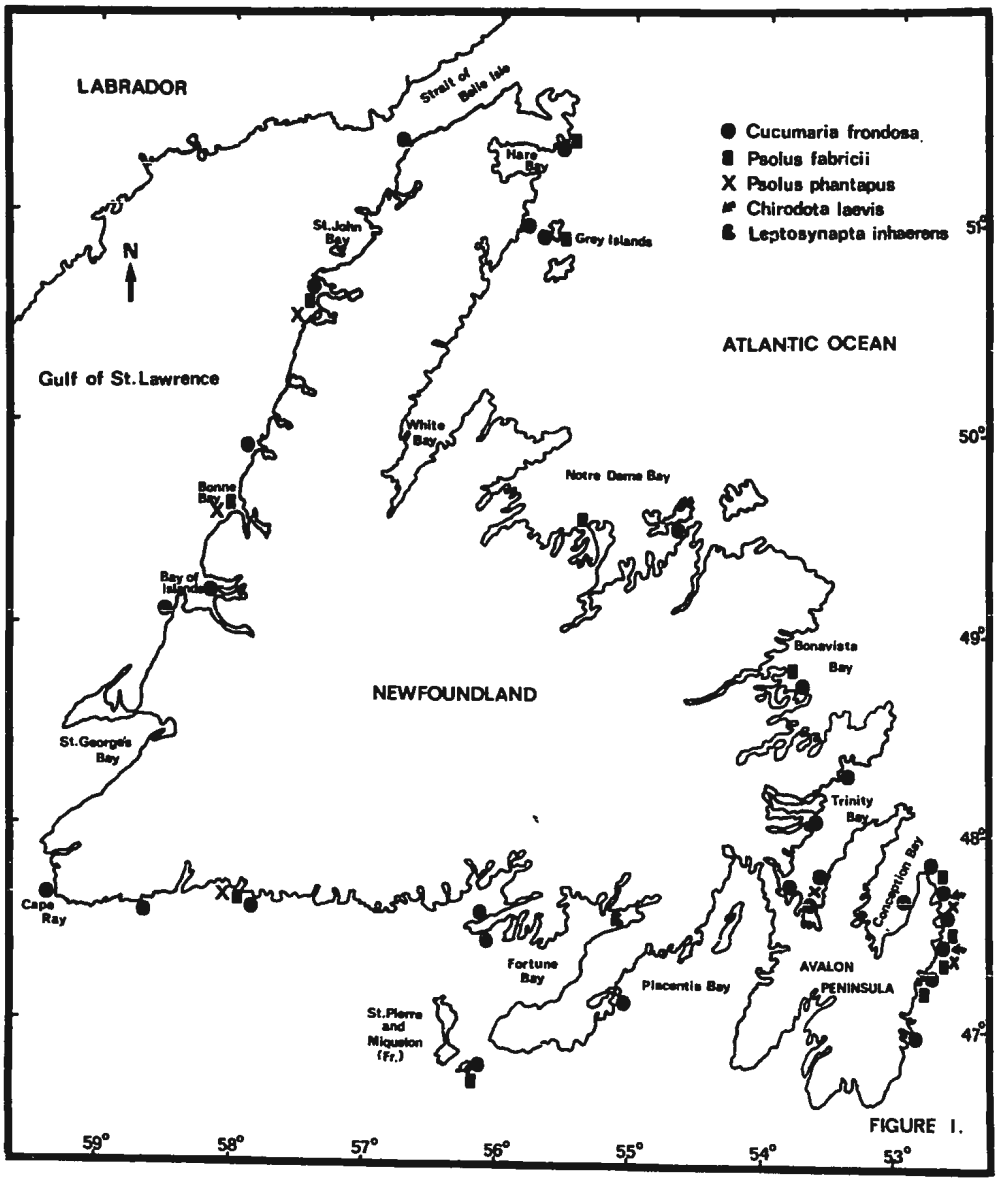
LABRADOR
Strait of Belle Isle
Hare Bay
St. John Bay
Grey Islands
N
Cucumaria frondosa
Psolus fabricii
Psolus phantapus
Chirodota laevis
Leptosynapta inhaerens
ATLANTIC OCEAN
Gulf of St. Lawrence
White Bay
Notre Dame Bay
Bonne Bay
Bay of Islands
Bonavista Bay
NEWFOUNDLAND
St. George's Bay
Trinity Bay
Cape Ray
Fortune Bay
Placentia Bay
Conception Bay
AVALON PENINSULA
St. Pierre and Miquelon (Fr.)
59°
58°
57°
56°
55°
54°
53°
50°
49°
48°
47°

FIGURE 1.

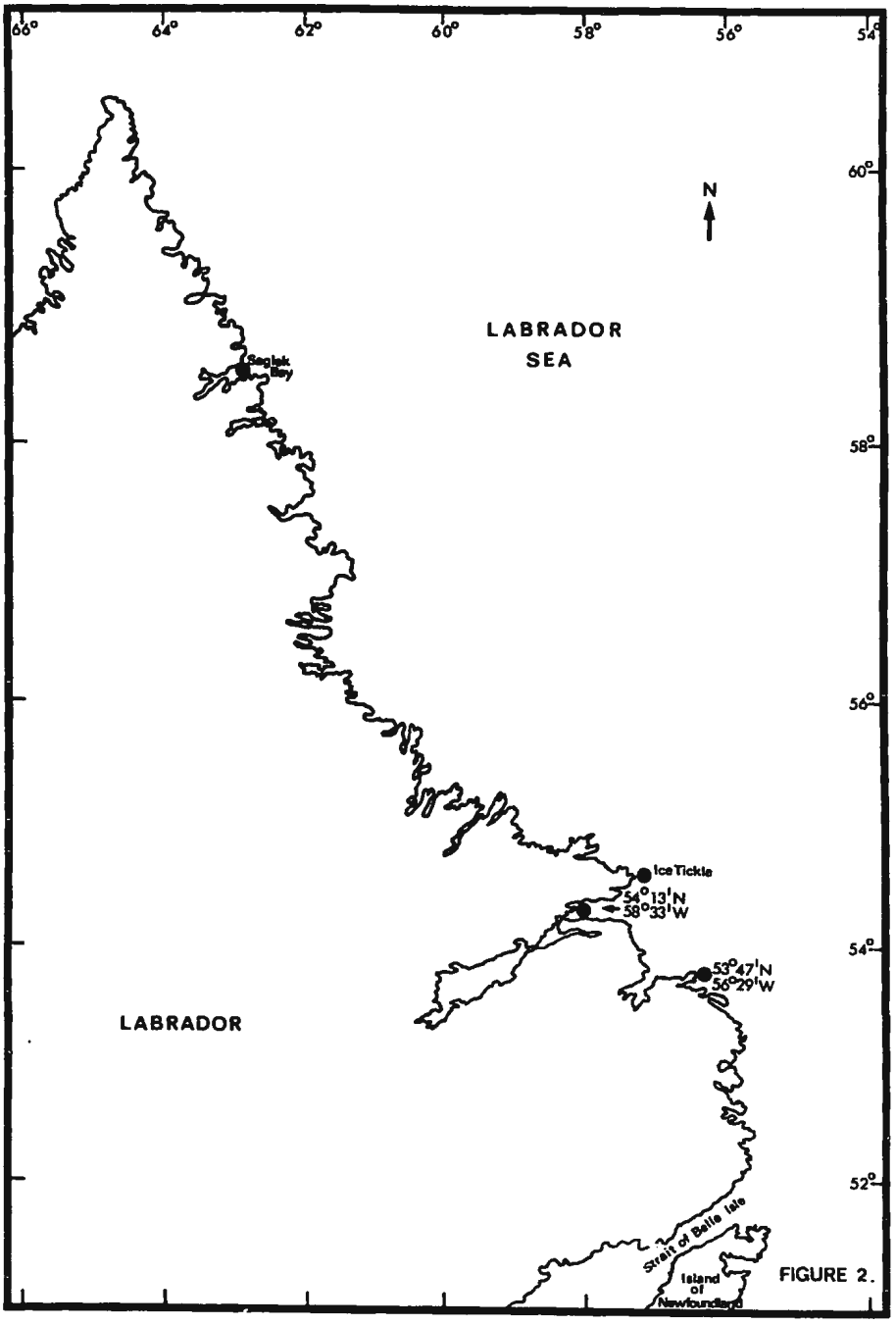
66°
64°
62°
60°
58°
56°
54°
60°
58°
56°
54°
52°
N
LABRADOR
SEA
Seglek Bay
Ice Tickle
54°13'N
58°33'W
53°47'N
56°29'W
LABRADOR
Strait of Belle Isle
Island of Newfoundland

FIGURE 2.

A year-round evaluation of the events which constitute the reproductive cycle of any invertebrate species requires a ready supply of large number of individuals. For this reason the study was confined to the two most available forms--*C. frondosa* and *P. fabricii*.

Cucumaria frondosa has an Arctic and sub-Arctic distribution in the northern Atlantic, ranging from the shores of Scandinavia and Great Britain across to the Faeroes, southern Iceland, Greenland and down to the coast of New England (Deichmann, 1930).

A closely related species of similar appearance, *Cucumaria japonica* Semper[1,2] originally recorded from the north Pacific, was found in west Greenland and in Jones Sound by Mortensen (1932). A re-examination by Mortensen of Greenland forms originally recorded as *C. frondosa* showed them to be *C. japonica*. It now seems as if the former species *C. frondosa* occupies the northeast Atlantic region and extends northward as far as Davis Strait where it appears to be replaced largely by *C. japonica* (Mortensen, 1932).

Mortensen seemed to feel that the occurrence of *C. japonica* in Greenland seas indicated that this species is distributed also over the Arctic Sea to the north of America and in the Bering Sea. This may mean that many of the northern Canadian records (a list is given by Grainger, 1955) of the occurrence of *C. frondosa* are incorrect.

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[1]the visible differences between the two species are limited to the spicules (see Grainger, 1955).

[2]also referred to as *Cucumaria frondosa japonica;* Mortensen (1932) remained uncertain as to whether it should be considered as being a subspecific form of *Cucumaria frondosa* or as a separate species; Grainger (1955) tentatively considers it as being a separate species.

Grieg[1], re-examining specimens he collected in 1907 and 1909 from southern Ellesmere and northern Devon Island and recorded as *C. frondosa*, found that they agreed with Mortensen's interpretation and were *C. japonica*. The species is listed as *C. japonica* in the "Calanus" collections (between 1947 and 1952) from the eastern Canadian Arctic (Grainger, 1955). No other work has been carried out on the problem in the north Atlantic since 1955 (pers. comm. with Grainger, 1972).

The range of *Psolus fabricii* extends as far south as the coast of Massachusetts. It is common along the coast of Labrador (Fig. 2), the west coast of Greenland and the west and south coast of Iceland. Along the European coast it does not extend very far south on the coast of Norway and it is not known from the British Isles (Deichmann, 1930; Mortensen, 1927). Grainger (1955) listed the northern Canadian records of the species and concluded that the species is probably circumpolar.

C. frondosa occurs just below low tidemark and has been taken in waters as deep as 200 fathoms. Although it is found intertidally in some areas (Jordan, 1972), it was not observed intertidally along the coasts of Newfoundland or Labrador during this study. *P. fabricii* is also found just below tidemark and the species is known to be quite common at depths up to several hundred fathoms (Deichmann, 1930).

Deichmann's (1930) review of holothurians of the western Atlantic Ocean includes complete taxonomic descriptions of both species.

---

[1]personal communication quoted by Mortensen (1932).

C. frondosa is described as a soft-skinned animal with a maximum length of about 50 cm., usually barrel-shaped and dark brown in color[1] with ten feeding tentacles of equal size. Although the tube feet are arranged in five ambulacral rows, they are more or less non-functional in the two dorsal ambulacra. The orange-red *P. fabricii*, on the other hand, is covered, except for the ventral sole and introvert, with imbricating scales. The ventral sole has a maximum length of approximately 10 cm. Tube feet are developed exclusively on the ventral sole and except for a few on the soft-skinned introvert and in the form of anal papillae around the anal opening, the entire dorsal side is devoid of podia. *P. fabricii* also has ten feeding tentacles of equal size.

The reproductive systems of the two species are similar. The single gonad[2] attached to the dorsal mesentery consists of numerous long unbranched tubules lying in the perivisceral coelom and united basally into a single gonoduct. The external opening of the gonoduct, the gonopore, is mounted on a genital papilla situated at the base of the tentacles in the middorsal line of the right dorsal interradius. Sexes are separate. The gonad is readily sexed during most of the year by close macroscopic examination of its contents. Sex cells are

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[1]although yellowish, grey and white individuals of *C. frondosa* appear to be quite common in the Maritime region of Canada and along the New England coast, these color variations were extremely rare in most of the populations observed around the island of Newfoundland; they were more common along the Labrador coast.

[2]holothurians lack the pentamerous symmetry typical of the reproductive system of other echinoderm classes.

shed into seawater and fertilization and development are external. Both species have free-swimming lecithotrophic larvae.

Interest in the reproductive biology of echinoderms is a recent and expanding one and considerably less is known of their breeding habits than of their other physiological functions. Many of the earlier studies in the Echinodermata were carried out by embryologists, finding species of the phylum particularly suitable for their research. In addition to the information on breeding activity gathered from these developmental studies, much of the early information on the reproductive cycles of echinoderms was provided indirectly by systematists studying world-wide collections gathered by various scientific expeditions.

Early research dealing specifically with the reproductive biology of echinoderms was largely descriptive and often failed to provide a complete picture of the reproductive cycle of the species being studied. The reproductive cycle in Giese's definition comprises the "series of events from the time of activation, growth and gametogenesis in the gonad to spawning of the gametes and recession of gonadal activity to a relatively sustained resting level and including the duration of the rest period" (Giese, 1959). An evaluation of the reproductive cycle, therefore. necessitates detailed monitoring of the morphological and histological features of the gonad throughout the reproductive cycle. Supplementary identifications of the physical, chemical and ecological influences to which the species is exposed throughout its reproductive cycle are useful in providing indications of the environmental factors

influencing the reproductive cycle.

The methods most useful and advantageous for studies of reproductive cycles in marine invertebrates have been outlined by Geise (1959) who covered the literature "as far back as seemed feasible to go and including citations up to August, 1958."

Hyman's (1955) treatise on the Invertebrates-Volume IV, The Echinodermata, provides a wealth of information on aspects of the reproductive biology of the different echinoderm groups.

Boolootian (1966) affords a further review of literature pertaining to the reproductive biology of echinoderms. Included in the article is a resume of information on environmental factors affecting reproductive cycles and information on the biochemical changes which accompany these events.

Davis (1971) provides a review of previous light-microscopic and electron-microscopic descriptions of echinoderm gonads and a comparative study of the fine structure of the gonadal walls of Echinodermata based on electron microscopy.

Gonad index, a measurement of the ratio of gonad size to the total wet weight of the animal, is a widely accepted technique of determining the reproductive condition of echinoderms and other invertebrate groups (Farmanfarmaian *et al.*, 1958; Giese, 1959; Choe, 1963; Moore *et al.*, 1963). The gonad index is generally calculated as the volume or weight of the gonad divided by the total wet weight of the animal expressed as a percentage by multiplication by 100. Variations in gonad index are generally interpreted as an expression of the relative increase in gonad size which accompanies the

maturation process. Although a high gonad index generally indicates pre-spawning animals and a low gonad index post-spawning or spent individuals, such are not always the case. In echinoids, for example, there may be a build-up of stored nutrients in the gonad prior to the initiation of gametogenesis and for this reason the gonad may be largest just after spawning (Hilts and Giese, 1949; Greenfield *et al.*, 1958; Giese *et al.*, 1964).

Various researchers have seen the need to provide modified or alternate methods of monitoring variation in gonad size. Fuji (1960), working in echinoids, proposed a gonad coefficient, or the ratio of the wet weight of the gonadal tissue to the total test volume, as a unit of measurement of the degree of maturity of the gonad. The close correlation of gonad coefficient to gametogenic development makes it a reliable unit in determining relative gonad weight in echinoids. Jordan (1972), in calculating gonad indices for *Cucumaria frondosa* from the New England coast, found it convenient to use a size index as a substitute for total wet weight. This size index, which was designed for intraspecific comparisons of size in holothurians, uses linear measurements as a substitute for volume and weight.

The use of laboratory animals in reproductive studies is reliable only if laboratory conditions approximate those of the natural state. An accurate evaluation of the similarity of gametogenic processes between lab-maintained individuals and individuals sampled under field conditions necessitates regular histological comparison of the two groups throughout the reproductive cycle. Such a comparison should indicate which, if any, laboratory parameters

enhance or retard normal development of sex cells in experimental animals and, at the same time, may provide an indication of possible environmental influences affecting the reproductive cycle.

The histological features of the male and female gonad throughout the reproductive cycle are frequently divided into five distinct phases, namely: activation, growth, maturity, spawning and resting. These phases are distinguished on the basis of the cellular activities which occur in the germinal epithelium of the gonad wall and which relate to the production, development, maturation and release of sex cells. References which are particularly useful in outlining pertinent features of the histological changes accompanying gametogenesis in echinoderms are Tennent *et al.* (1931), Tanaka (1958), Fuji (1960) and Pearse (1964).

More is known of the reproductive biology of the Echinoidea than of any other group of echinoderms (Boolootian, 1965). The list of reproductive studies on the Holothuroidea is not extensive. Several of the more important works have been carried out by the Japanese, who utilize many of the western Pacific species as a source of food[1] (Inaba, 1930, 1937; Sibuya, 1936a, 1936b; Tanaka, 1958; Choe and Ohshima, 1961; Choe, 1963). It is an interesting fact that *Cucumaria frondosa* itself is an extremely palatable species (Agassiz, 1865) and the closely related species *Cucumaria japonica*, mentioned earlier, is widely used as a source of food in the Indo-Pacific area. In view of the large concentrations of *C. frondosa* in shallow

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1. bêche-de-mer or trepang.

Newfoundland waters, the species may possibly merit commercial exploitation.

General reproductive works on holothurian species include those of Mortensen (1937, 1938) in the Red Sea, Johnson and Johnson (1950) in Puget Sound, Newth (1916) and Fish (1967) in the British Isles and Pearse (1968) in the Indo-Pacific region.

Asexual reproduction by transverse fission has been described in several holothurian species (Crozier, 1915; Frizzell and Exline, 1955; Bonham and Held, 1963). There are also species of echinoderms in which hermaphroditism is the prevalent sexual condition (Ackermann, 1902; Clark, 1907; Runnstrom, 1927). Direct development and a brooding habit appear to be quite common in holothurian species of polar waters, especially in the Antarctic Ocean (Clark, 1901; Vaney, 1907; Ekman, 1925; Hyman, 1955).

Reproductive studies of holothurians along the New England coast by Pearse (1908); Ohshima (1925); Just (1929); Colwin (1948) and Costello *et al.* (1957), bear greater relevance to the present work. An ecological study of *Cucumaria frondosa* at Lamoine Beach, Maine by Jordan (1972) includes information on the reproductive biology of the species which affords a useful comparison with the Newfoundland situation. Other studies on *C. frondosa* are those by Edwards (1910); Newth (1916); Runnstrom and Runnstrom (1918-19, 1921) and Mortensen (1932).

The spawning period of *C. japonica* has been studied by Kinosita and Sibuya (1941).

The free-swimming stages of *C. frondosa* and *P. fabricii* were

observed by Agassiz and Agassiz in 1865 and an 1882 paper by Bell mentions the resemblance of the young of *P. fabricii* to those of *P. phantapus*. Other than these latter two works I know of no others which deal with aspects of the reproductive biology of *P. fabricii*.

The present work attempts to provide a detailed account of the reproductive cycle of *Cucumaria frondosa* and *Psolus fabricii* from shallow waters of the Avalon Peninsula of Newfoundland. Populations of both species were sampled twice monthly from August, 1971 through August, 1972. Determinations of gonad index, spawning observations and histological preparations of reproductive tissue were used to define the annual reproductive cycle of both species. A comparative investigation was undertaken of differences in the reproductive cycles of individuals maintained in the laboratory and individuals sampled in the field. Experimental attempts were made to induce spawning in mature individuals of *C. frondosa* and a brief histological examination was made of the immature gonad of the same species.

METHODS AND MATERIALS

Sampling Procedure

SCUBA collections of *Cucumaria frondosa* and *Psolus fabricii* were made at regular intervals twice monthly between August, 1971 and August, 1972. Several sampling sessions were omitted when storm or inshore ice conditions made it unsuitable for diving. In Figure 3 are shown the areas and the localities in which these collections were made.

Specimens of *C. frondosa* were collected at Outer Cove, in Tor Bay, in depths of between 10 and 30 feet. Outer Cove (exposed to the open Atlantic) is a relatively shallow cove with a maximum depth of approximately 35 feet. Large numbers of *C. frondosa* of all sizes occupy the mixed sand, gravel, boulder and bedrock substratum (Fig. 4). Collections were made of 15 individuals during each sampling session. All individuals collected exceeded 70.0 grams in total wet weight.

In July of 1971, one month before regular sampling began, 150 individuals of *C. frondosa* from the Outer Cove study area were transferred to the university's Marine Sciences Research Laboratory (MSRL) at Logy Bay. These animals were placed in a series of 3 vertically-arranged trays attached to an A-frame support as shown in Figure 6. Seawater enters the uppermost tray from a horizontal pipe connected to the Laboratory's main seawater system.[1] The

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[1] seawater at the MSRL is pumped from a man-made cave 30 feet below tide level in Logy Bay to a reservoir above the laboratory from which the water is gravity-fed, on demand, through a polyvinylchloride (PVC) piping system to each laboratory.

Figure 3 - Map showing the location of study areas referred to in the text.

A..... island of Newfoundland

B..... Avalon Peninsula of Newfoundland.

C..... section of the eastern coast of the Avalon Peninsula.

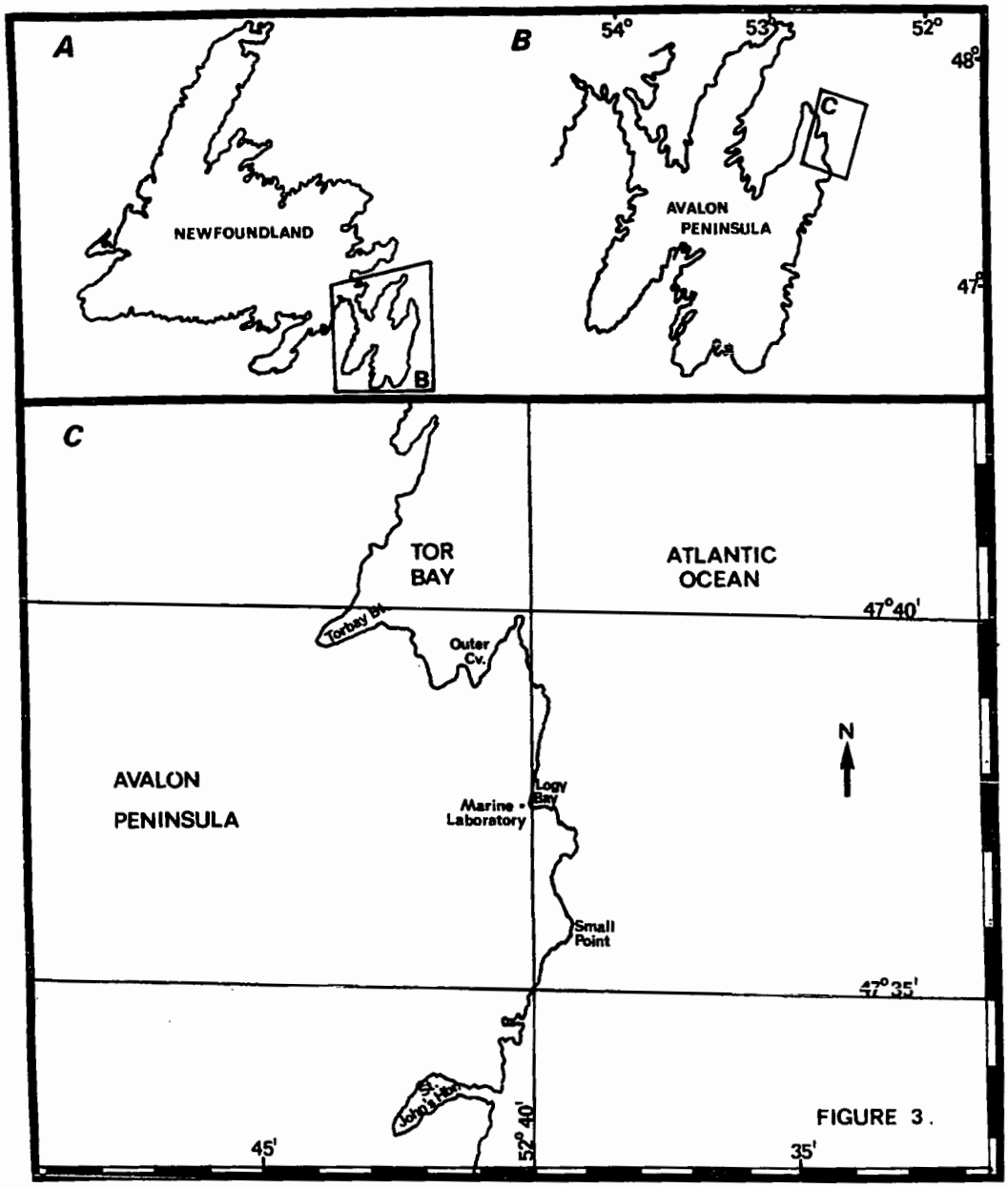

A
B
54°
53°
52°
48°
47°
NEWFOUNDLAND
AVALON
PENINSULA
C
C
TOR
BAY
ATLANTIC
OCEAN
47°40'
Torbay
Outer
Cv.
N
AVALON
PENINSULA
Logy
Bay
Marine
Laboratory
Small
Point
47°35'
St.
John's
52°40'
45'
35'

FIGURE 3.

Figure 5 - Underwater photograph of the Logy Bay study area illustrating the near-vertical substrate on which *Psolus fabricii* was sampled.

Figure 4 - Underwater photograph of the Outer Cove study area illustrating the general rock-boulder substrate on which *Cucumaria frondosa* was sampled.

FIGURE 5.

FIGURE 4.

Figure 6 - The A-frame holding trays in which individuals of *Cucumaria frondosa* were maintained in the laboratory.

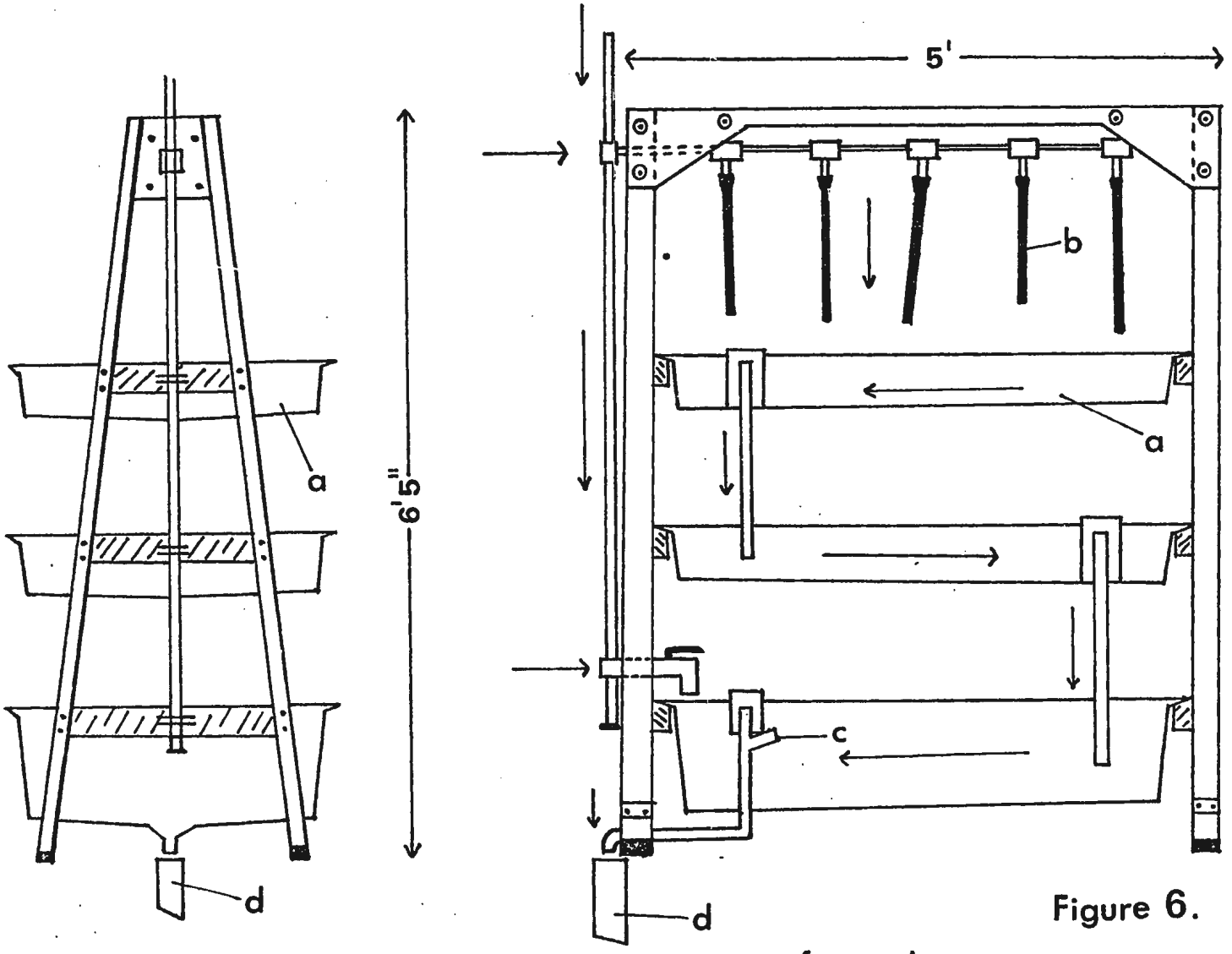
a .....fibreglass tray

b .....rubber tubing used to direct seawater into the uppermost tray

c .....position of the net trap used in the collection of shed ova

d .....drain

Arrows indicate the direction of water flow.



side view

front view

Figure 6.

overflow of seawater entering each tray passes to the tray below it and is finally discharged through a drain at the bottom.

Five individuals from this group were sampled bimonthly from August, 1971 until August, 1972 in a comparative investigation of reproductive features between lab-maintained individuals and individuals collected and subsequently examined on a regular basis from Outer Cove.

The more sessile *P. fabricii* which seems to prefer vertical or near-vertical bedrock surfaces for attachment was sampled from Logy Bay (Fig. 5). Ten individuals were collected twice monthly from depths between 35 and 50 feet. All individuals collected exceeded 70.0 grams in total wet weight.

The sampling dates for each study group are listed in Appendices I to III.

Seawater temperatures were recorded at the sampling sites during each sampling session.

Gonad Index

All individuals were left out of seawater for 10-15 minutes before total wet weight (to 0.01 gram) was determined. This procedure is essential as varying amounts of seawater adding to the total wet weight may be present in the respiratory trees of the organism. Leaving the animals out of water permits the release of this seawater through the cloacal opening (vent) before weighing. Each animal was placed on absorbent paper towelling to remove excess water from the surface of the animal prior to weighing. All weighings were carried out on a Mettler-top loading balance.

After weighing the animals were dissected and the sex of each specimen was determined and recorded. The sex of the gonad was generally evident macroscopically by the presence of oocytes in females or milt in the males. On rare occasions, however, and particularly in spent individuals, it became necessary to examine portions of gonad material by use of a compound microscope before the sex could be determined reliably.

The volume of the gonad was defined as the volume of seawater (to 0.5 milliliter) displaced by the gonad when placed in a graduated cylinder partially filled with seawater. Each gonad was placed on absorbent paper towelling to remove excess moisture from the surface of the tubules before volume measurements were made.

Gonad index was calculated for each individual as the volume of the gonad divided by the total wet weight of the animal. All figures were expressed as a percentage by multiplication by 100.

Histology

After the gonads had been examined macroscopically they were fixed in Bouin's Fluid (picric-acetic-formol solution) for a minimum period of 24 hours. Fixation generally resulted in slight shrinkage of the reproductive tissue. The tissues were then dehydrated in a series of 70%, 90%, 95% and 100% ethyl alcohol solutions, cleared in toluene and finally mounted in paraffin (Paraplast-melting point 56°-57°C).

An AO Spencer No. 820 rotary microtome was used for sectioning. Histological sections were cut 8-10 microns thick from medial portions

of the gonad tubules and stained with Delafield's hematoxylin followed by eosin using the regressive method of hematoxylin staining-Delafield's Hematoxylin II as outlined in Humason (1967).

Microscopic examination of reproductive tissue prepared in this manner over a 12 month period provided a detailed description of the seasonal progression of histological events in the gonad leading from gametogenesis through growth and maturation to the eventual release of sex cells by both species.

The histological features of the gonad wall are described through this thesis on the basis of the scheme outlined by Davis (1971). The gonad wall elements are the same in both male and female individuals; from outside to inside these are: visceral peritoneum, nerves, muscles, haemal fluid (lined on either side by the extrahaemal and intrahaemal basal lamina) and the germinal epithelium.

Variations in the thickness of the visceral peritoneum throughout the study period were measured from the histological preparations of reproductive tissue of each study group. The average monthly thickness of the visceral peritoneum was determined from a minimum of 40 measurements in each case and all measurements were restricted to cross-sections of gonad tubules.

In February of 1972, at a time when the majority of adult *C. frondosa* were in a predominantly mature reproductive condition, approximately 200 of the smallest individuals available from Outer Cove were collected with SCUBA equipment, returned to the laboratory, dissected and observed for the presence of sex cells. A total of

23 gonads representative of various size groups from 1.5 grams to 70.0 grams total wet weight were fixed in Bouin's Fluid, mounted in Paraplast, sectioned and stained for histological examination and comparison.

Immature *P. fabricii*, on the other hand, are so few in number in areas where they are known to occur that a similar examination in this species was not carried out.

Spawning

Cucumaria frondosa and *P. fabricii* adjusted well to seawater conditions at the MSRL and large numbers of individuals of both species were maintained at the Laboratory for purposes of general observation and experimentation.

Spawning Period. Although histological preparations of reproductive tissue provide the best indication of the timing and duration of the spawning period, records of individual spawnings by lab-maintained individuals offer worthwhile supplementary information.

A small fine-meshed net was placed over the drain of the holding trays used for *C. frondosa* in the laboratory (Fig. 6). This net was checked regularly for the presence of shed ova.

Additional records were kept of observed laboratory spawnings for other individuals of *C. frondosa* and *P. fabricii* which had been collected from different locations at different times during the study and placed in a Laboratory wet bench.[1]

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[1]Five specimens of *Psolus phantapus* which had been collected in early March during a SCUBA dive at Torbay Bight (Fig. 3) were also kept under spawning observation.

As the flushing rate of water through the Laboratory's seawater system is quite rapid and as the chance occurrence of large numbers of ova entering with incoming seawater is very slight, the presence of ova in the holding trays or in the wet bench was taken as an indication of a recent spawning, even though the actual spawning may not have been observed.

Records of spawning males, on the other hand, were restricted entirely to observed incidents as sperm emitted by spawning males dissipates quickly in seawater after shedding and are too small to be found in the drain net used for trapping ova.

Spawning Behavior. Observations and records were made in the laboratory of the time of day of each observed spawning, the behavior of the spawning individual, the duration of the spawning process and the incidence of repetitive spawnings.

Spawning Induction. There is, at present, no reliable method of inducing spawning in *C. frondosa*. A study was undertaken of several methods which have been used with some success by various authors on other species of holothurians. Due to the general scarcity of adult *P. fabricii* in the field, experiments were restricted to *C. frondosa*.

On February 22, 1972, with the first indications from regular samplings of the commencement of the spawning period, approximately 275 individuals of *C. frondosa* were collected with SCUBA equipment from Torbay Bight (Fig. 3) and returned to the MSRL. These animals were placed in a large 250 gallon circular

holding tank supplied with running seawater. Dissection of several of these individuals revealed that they were in a predominantly mature reproductive condition at the time of collection.

Experimental individuals were chosen randomly from the holding tank and none were used more than once in an experimental procedure. Individuals observed spawning in the holding tank were removed and discarded. A ten-gallon aquarium filled with aerated seawater was used for each experimental animal. All experiments, except when stated otherwise, were carried out in a controlled-environment chamber equipped with light and temperature control systems. Ten animals were used in each of these tests. Five additional animals, placed in aerated aquaria under natural day light and laboratory temperature conditions served as "controls". The duration of each experiment was 72 hours. The reproductive condition of each animal was determined by dissection at the end of the experimental period.

Several methods, outlined below, were examined as potential means of inducing spawning. These experiments were carried out from February 23, 1972 until April 8, 1972.

Temperature. Seven experiments, employing varying degrees of sudden or gradual increase of temperature were carried out under normal day-length conditions.

Sudden temperature increases of 3°C, 4°C, 5°C and 10°C were achieved by direct transfer of experimental individuals from the holding tank to seawater of the higher temperature level.

Gradual temperature increases from -0.5°C to 5°C, 10°C and

15°C were accomplished by transferring the test animals to the controlled environment chamber in individual aquaria containing seawater from the holding tank and allowing this seawater to warm slowly in the chamber to the desired temperature. Two and one half hours were generally required for a temperature increase of 5°C.

Light. Light conditions were modified from the normal day-length, day-light cycles in several ways. Experimental animals were exposed to constant conditions of subdued (dimmed) light, complete darkness, or perpetual light during test periods of 3 days in each case. Additional experiments were carried out using 16 hour light--8 hour dark and 8 hour light--16 hour dark photoperiod regimes. Temperature levels during these studies remained at the normal seawater level of -0.5°C.

Presence of Male Sex Cells. Specimens of *C. frondosa* were placed in aquaria with seawater containing milt obtained directly from spawning males.

The sex of the individual being tested was determined by dissection at the end of the experimental period, and a total of 13 males and 17 females were tested. All experiments were performed in a laboratory wet bench under natural day-length and normal seawater temperature conditions.

RESULTS

The values used in the preparation of graphs which appear in the Results are listed in Appendices I-III.

Temperature

Seawater temperatures recorded during the study period are given in Figure 7. The waters of the Avalon Peninsula of Newfoundland are under the direct influence of the cold Labrador Current and temperatures during the study period from August, 1971 to August, 1972 ranged from <1.25°C in early March to 12.5°C in early September. Laboratory temperatures were generally 0.25°C to 1.0°C higher than those in the ocean.

Gonad Index

Average monthly gonad index values for *C. frondosa* collected at Outer Cove during the period August, 1971 to August, 1972 are given in Figure 8, wherein arrows indicate the duration of the spawning period. The bands on either side of the mean values indicate the computed 95% confidence limits. Gonad index remained relatively steady from August, 1971 until December. There was a slight increase during January just prior to the spawning period. The values decreased during the spawning period, rose to a maximum level in June following spawning and finally in August returned to a level comparable to that recorded during the same period a year earlier.

Several variations from the above pattern are apparent from

Figure 7 - Ocean temperatures recorded at the study sites, August, 1971 through August, 1972.

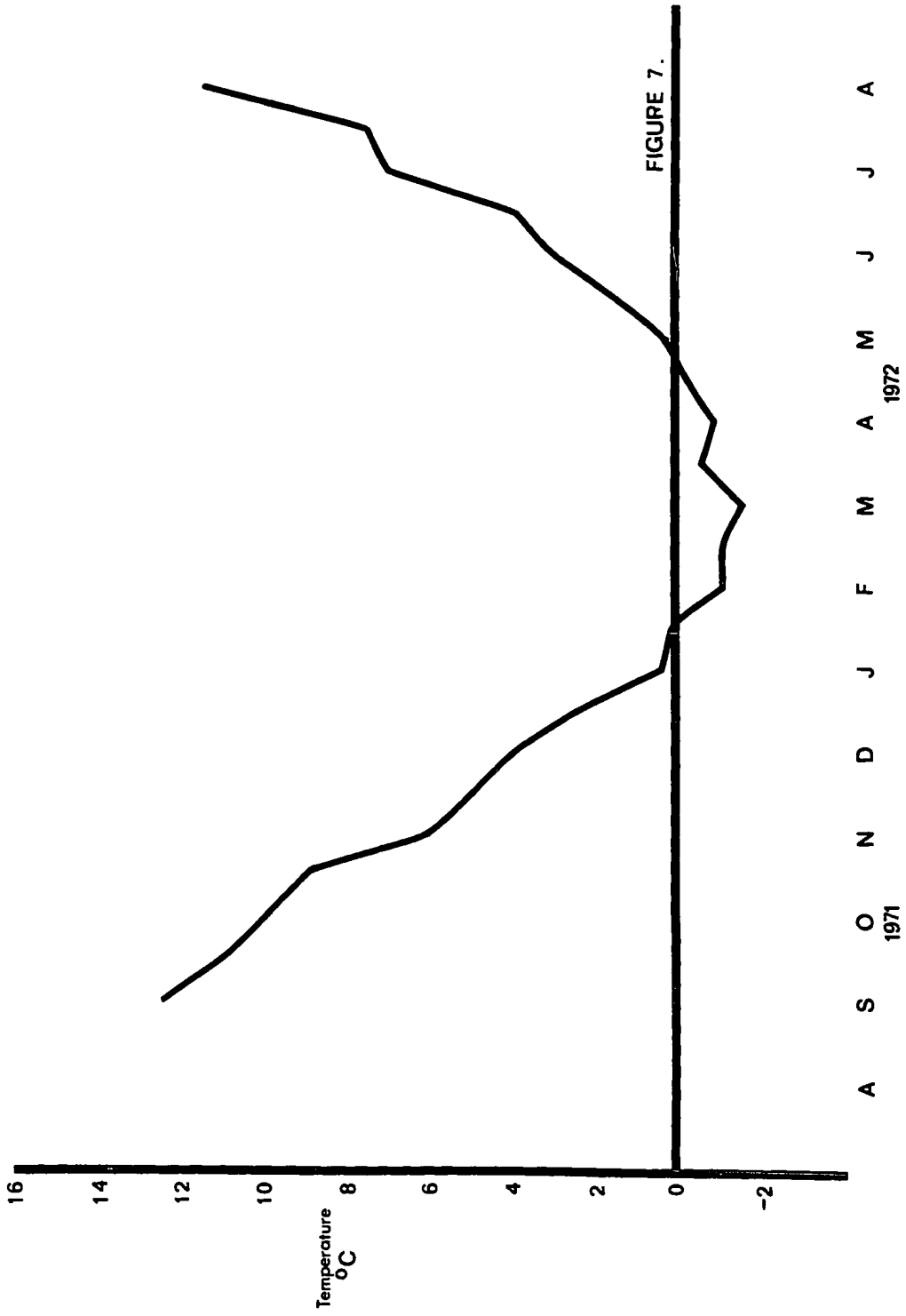

FIGURE 7.

Figure 9, which depicts monthly gonad index values for *C. frondosa* maintained at the MSRL. The index value in lab-maintained individuals fluctuated from the beginning of the study in August, 1971 until March and the greater variability in values when compared to the field situation is undoubtedly a result of the lower number of individuals sampled in the laboratory. The sudden drop in index values with spawning was more extreme and the increase in values after spawning was less pronounced than they were at Outer Cove.

As can be seen in Figure 10, average monthly gonad index for *P. fabricii* sampled in Logy Bay undergoes progressive increase to maximum values just prior to spawning. The index drops with spawning and resumes a pattern of steady increase after the spawning period.

Total Wet Weight

Mean monthly total wet weights for *C. frondosa* sampled at Outer Cove are given in Figure 11. A decline of approximately 80 grams in average wet weight from August, 1971 until February was followed by a rapid increase of 70-75 grams over the two month period February through March. The fluctuation in wet weight which followed this was slight, with values remaining relatively high until August 1972.

Monthly variations in average total wet weight for *C. frondosa* maintained in the laboratory, as shown in Figure 12, are quite similar. Minimal wet weight values were reached in the laboratory at the same time as in Outer Cove (i.e. February). Although the degree of wet weight increase during the spawning period is similar (i.e. 70-75

GONAD INDEX :

Figure 8 - Gonad index of *Cucumaria frondosa* collected at Outer Cove.

Figure 9 - Gonad index of *Cucumaria frondosa* maintained in the laboratory.

Figure 10- Gonad index of *Psolus fabricii* collected at Logy Bay.

All values are average monthly gonad indices for the population sampled. 95% confidence limits are shown as solid lines on either side of the mean values. Arrows indicate the duration of the spawning period for each population.

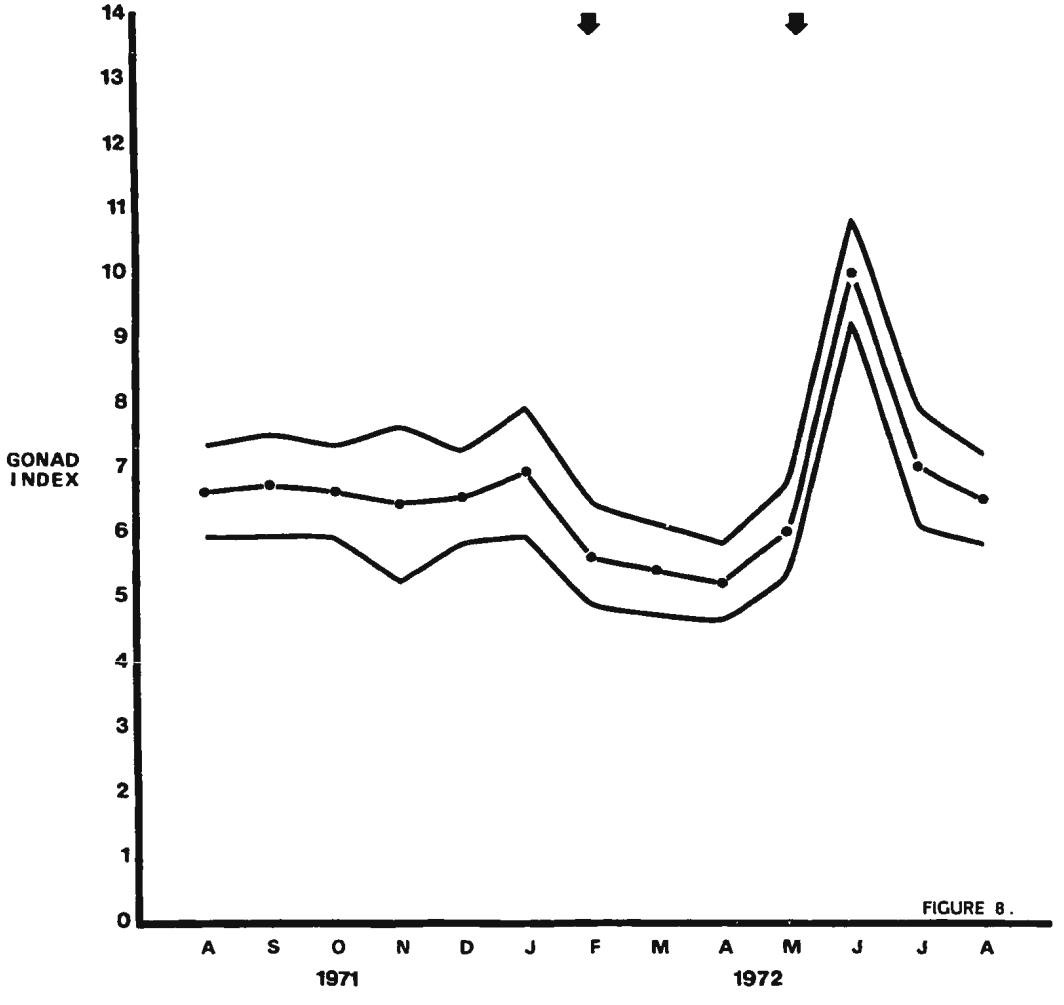
GONAD INDEX
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
A
S
O
N
D
J
F
M
A
M
J
J
A
1971
1972

FIGURE 8.

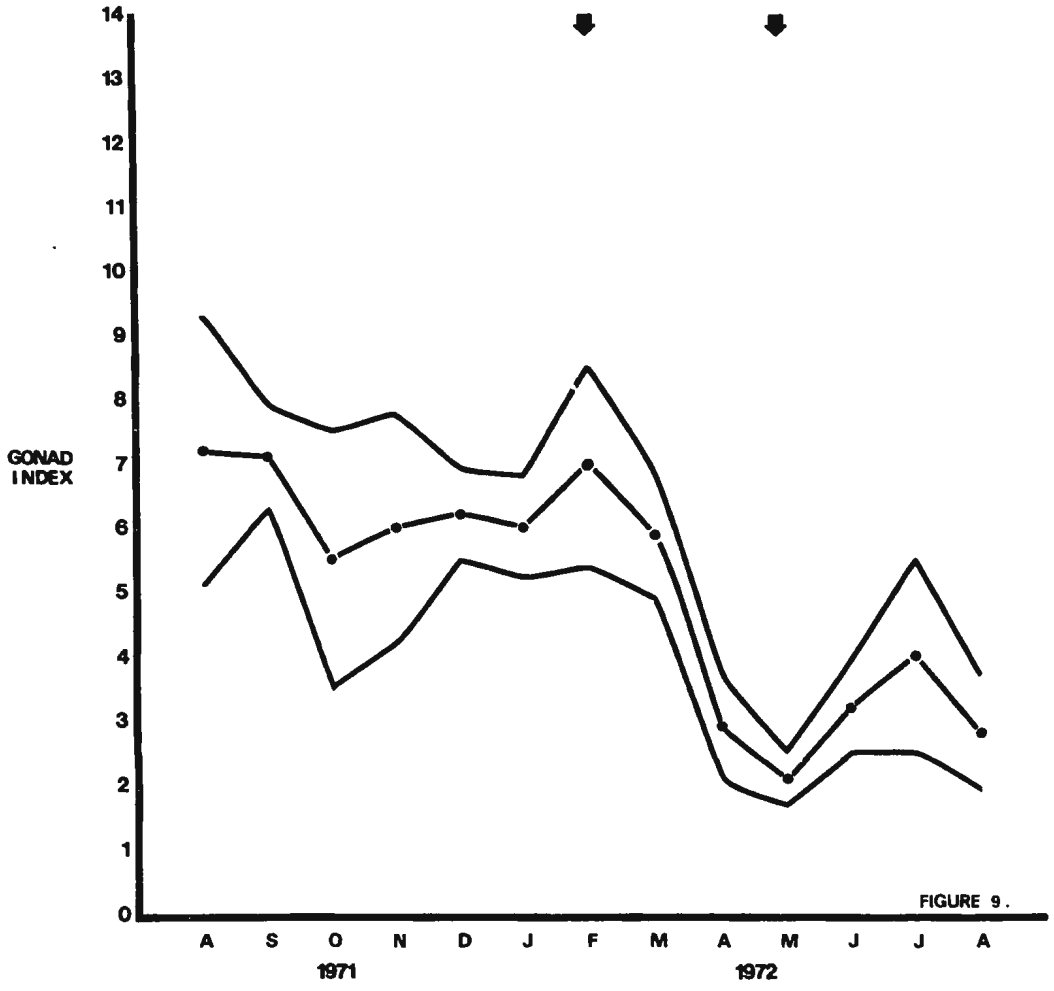
GONAD INDEX
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
A
S
O
N
D
J
F
M
A
M
J
J
A
1971
1972

FIGURE 9.

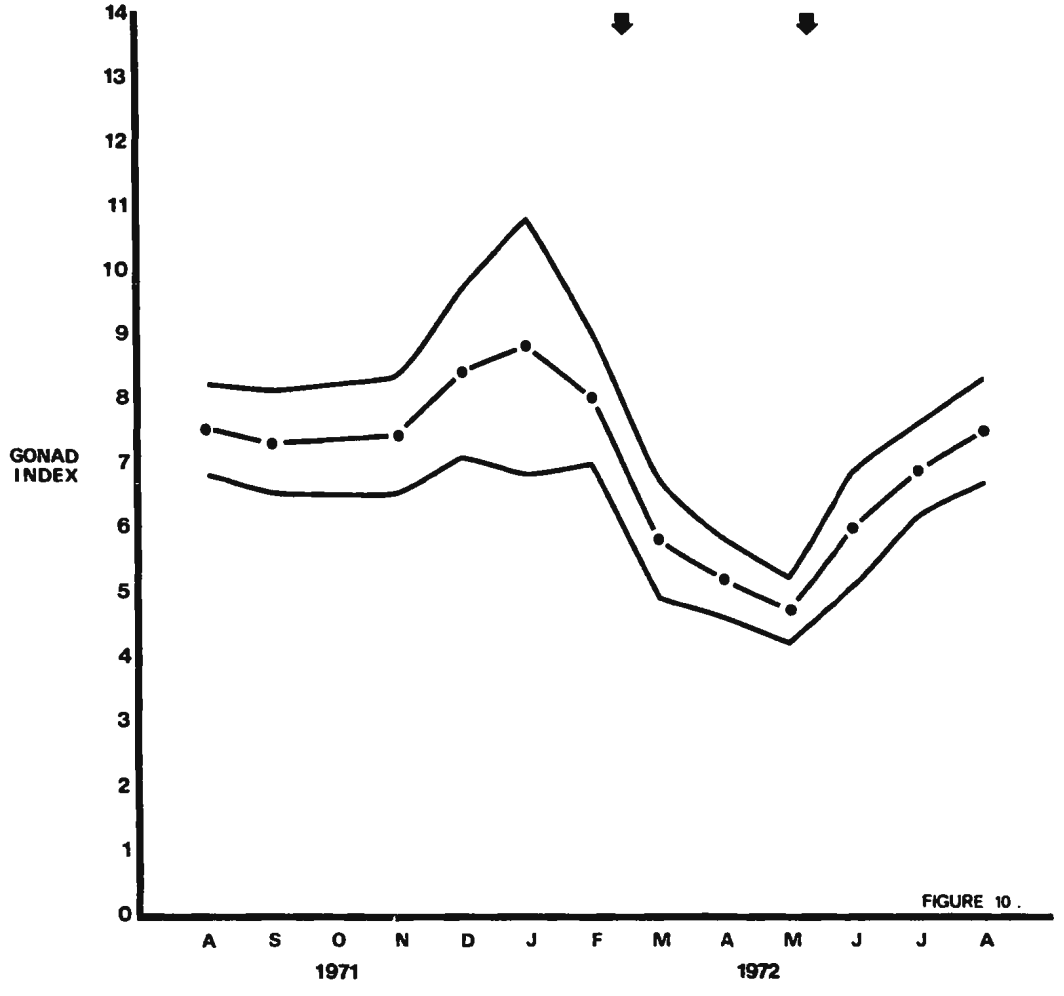
GONAD INDEX
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
A
S
O
N
D
J
F
M
A
M
J
J
A
1971
1972

FIGURE 10.

grams), the peak value is reached in May, a month later than at Outer Cove. The reduction in wet weight following the peak recorded in March continued until the end of the study.

Continuous decrease in total wet weight during the first six months of the study followed by a steady increase in total wet weight between February and the beginning of May is again evident in Figure 13 depicting total wet weight values for *P. fabricii* collected in Logy Bay. There was a decrease of approximately 37 grams between maximum and minimum values recorded for the population.

Cucumaria frondosa and *P. fabricii* are both typical collision feeders and the bulk of food ingested is phytoplankton. Regular plankton tows of waters approximately one mile offshore from Small Point (Fig. 3), near the sampling sites, were carried out by Moskovits and Burke (unpublished data) of the MSRL during the study period. Only preliminary quantitative information is available from these studies at the present time and this information is presented in each of the three figures (Fig. 11,12,13) depicting monthly variations in total wet weight for the groups studied. Phytoplankton levels, recorded as "low" during the February 10th collection, were noted to be increasing in the March 7th tow. The gut tracts of animals which had been sampled at the collection sites and in the laboratory become rather abruptly distended with food material[1] around the middle of

---

[1] primarily diatoms, dinoflagellates and filamentous green algae.

TOTAL WET WEIGHT :

Figure 11 - Total wet weight of *Cucumaria frondosa* collected at Outer Cove.

Figure 12 - Total wet weight of *Cucumaria frondosa* maintained in the laboratory.

Figure 13 - Total wet weight of *Psolus fabricii* collected at Logy Bay.

All values are average monthly total wet weights for the population sampled. The relative concentrations of phytoplankton shown are for dates on which plankton tows were made at Small Point during the same time period.

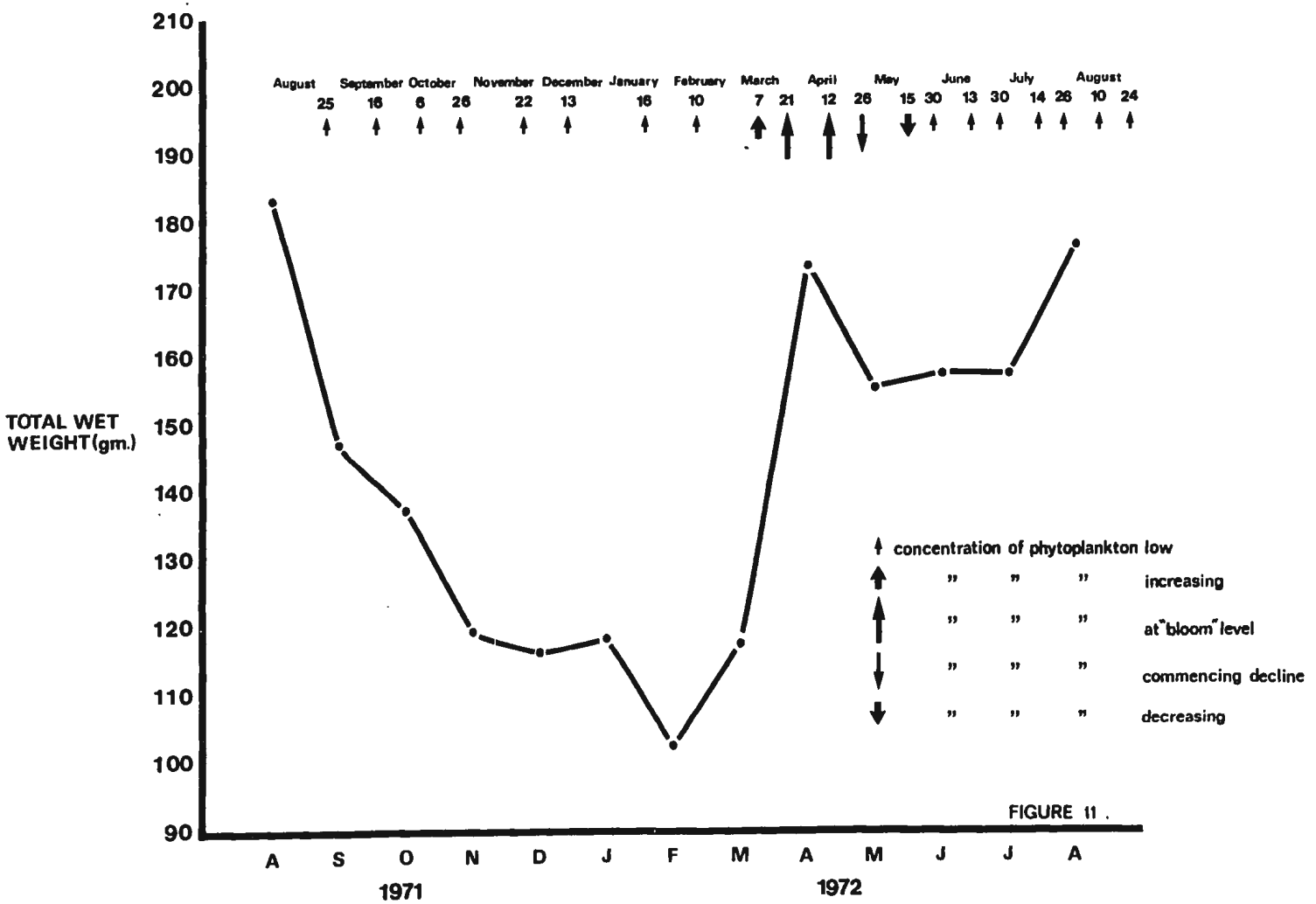
TOTAL WET WEIGHT(gm.)
210
200
190
180
170
160
150
140
130
120
110
100
90
August 25
September 16
October 6 26
November 22
December 13
January 16
February 10
March 7 21
April 12 26
May 15
June 30 13
July 30 14
August 26 10 24
concentration of phytoplankton low
" " " increasing
" " " at "bloom" level
" " " commencing decline
" " " decreasing
A S O N D J F M A M J J A
1971
1972

FIGURE 11.

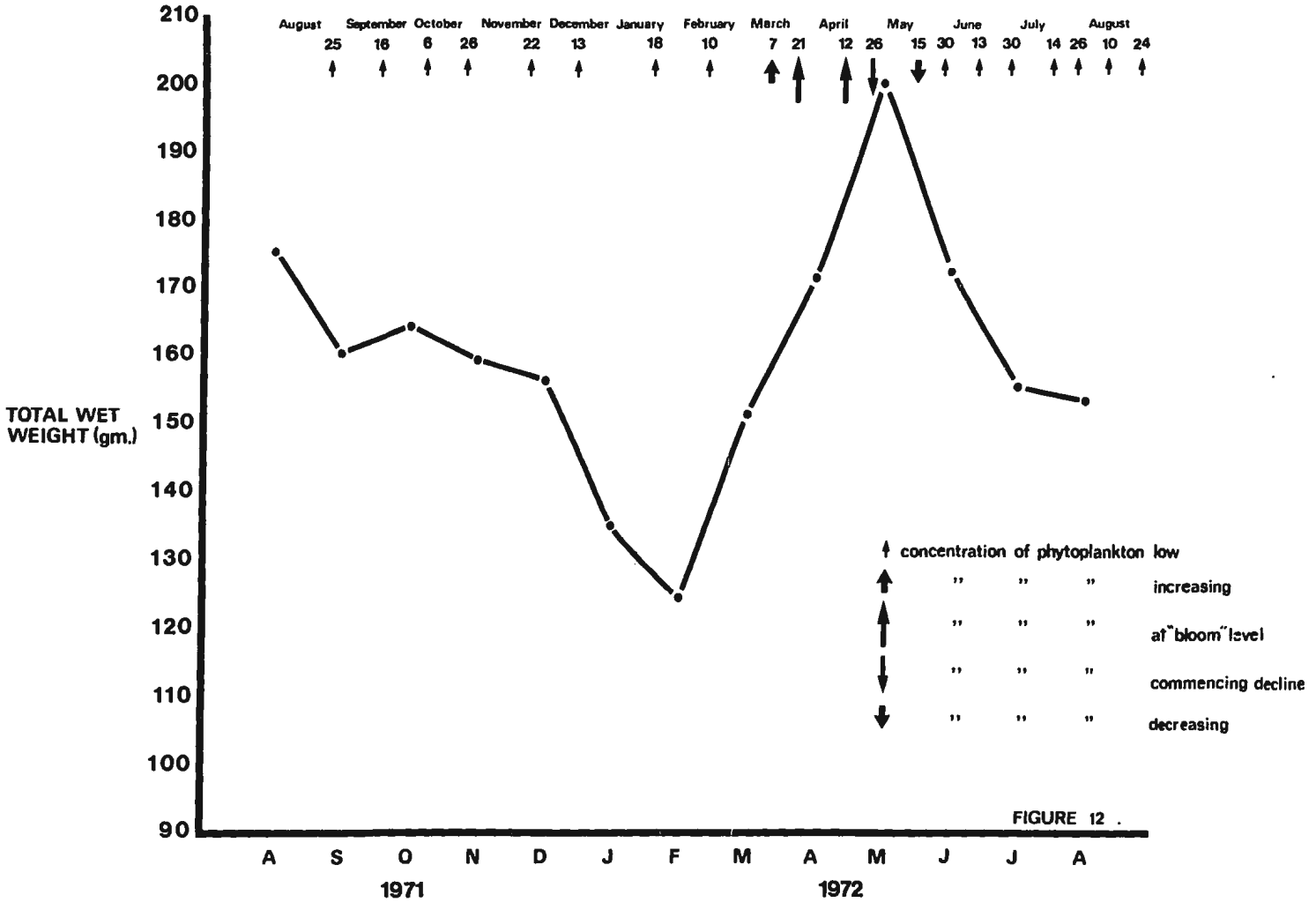
August 25
September 16
October 6 26
November 22
December 13
January 18
February 10
March 7 21
April 12
May 26 15
June 30 13
July 30 14 26
August 10 24
210
200
190
180
170
160
150
140
130
120
110
100
90
TOTAL WET WEIGHT (gm.)
A S O N D J F M A M J J A
1971
1972
concentration of phytoplankton low
" " " increasing
" " " at "bloom" level
" " " commencing decline
" " " decreasing

FIGURE 12 .

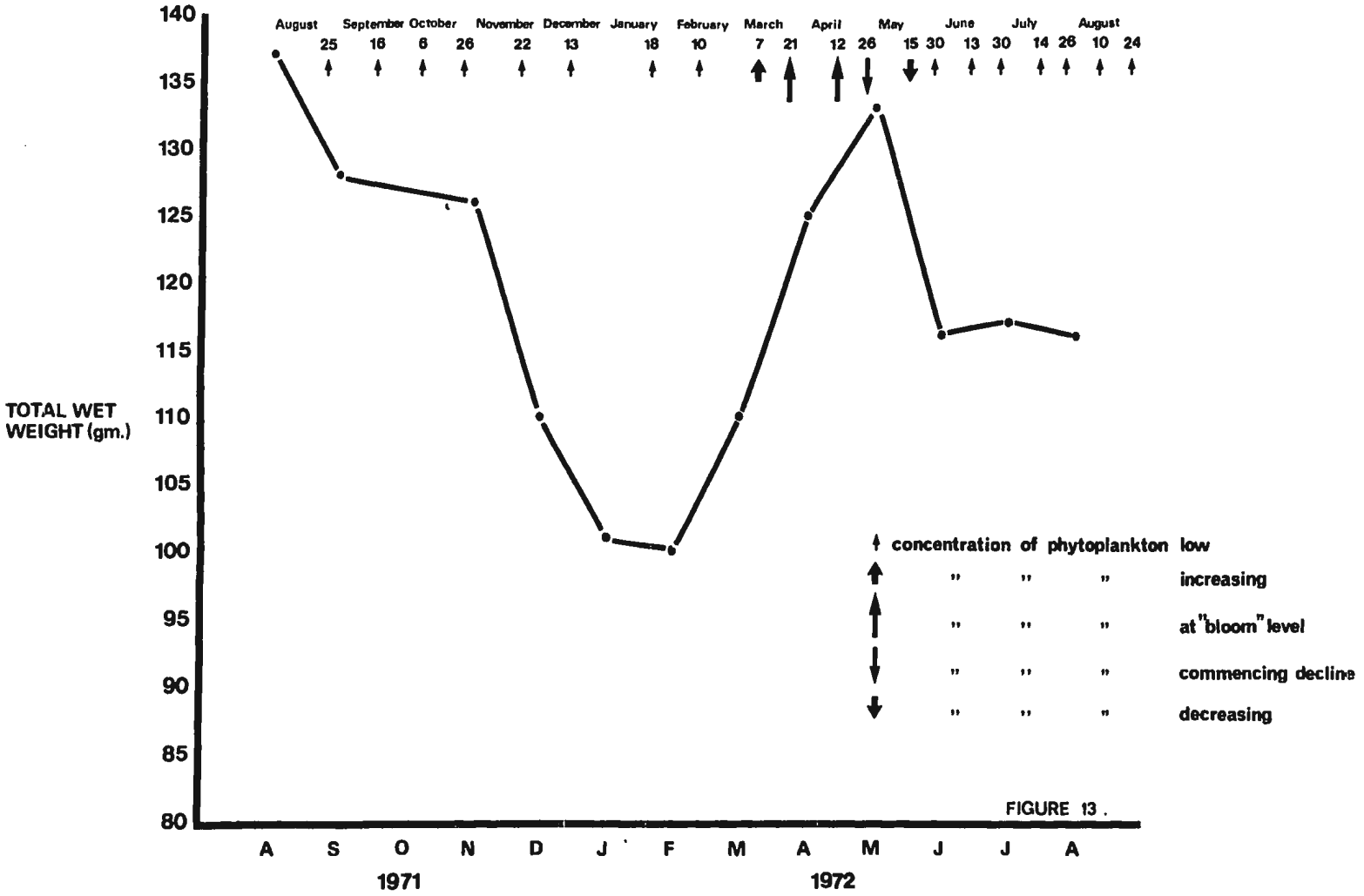
TOTAL WET WEIGHT (gm.)
140
135
130
125
120
115
110
105
100
95
90
85
80
August 25
September 16
October 6
26
November 22
December 13
January 18
February 10
March 7
21
April 12
26
May 15
June 30
13
July 30
14
August 26
10
24
A S O N D J F M A M J J A
1971
1972
concentration of phytoplankton low
" " " increasing
" " " at "bloom" level
" " " commencing decline
" " " decreasing

FIGURE 13.

February. Although the presented data of Moscovits and Burke give no information on phytoplankton levels between February 10th and March 7th the increased feeding success of both lab and field populations shortly after the February 10th tow suggests that the levels of phytoplankton increased markedly in this interval.

The marked increase in total wet weights observed in Figures 11, 12 and 13 occur during the spring plankton "bloom" in each case. The peak weight values are reached during or slightly after the spring plankton "bloom" and decrease gradually throughout the year after the "bloom" disappears.

Gonad Volume

Average monthly gonad volumes of *C. frondosa* taken at Outer Cove are shown in Figure 14. After several months of steady reduction in size, totaling approximately 6 milliliters, minimal values were recorded in February at the beginning of the spawning period. Maximum gonad size was reached just after the spawning period following a 9.6 milliliter increase in average gonad volume over the four month period from February to the beginning of June.

Values for laboratory specimens are given in Figure 15. The progressive reduction in gonad volume which occurs from the beginning of the study is most abrupt in March with spawning. The subsequent recovery in gonad size during and after the spawning period is slight when compared to the sudden increase in gonad volume recorded for the species at Outer Cove during the same time period.

Gonad volumes for *P. fabricii* are shown in Figure 16. Gonad

GONAD VOLUME :

Figure 14 - Gonad volume of *Cucumaria frondosa* collected at Outer Cove.

Figure 15 - Gonad volume of *Cucumaria frondosa* maintained in the laboratory.

Figure 16 - Gonad volume of *Psolus fabricii* collected at Logy Bay.

All values are average monthly gonad volumes for the population sampled. Arrows indicate the duration of the spawning period.

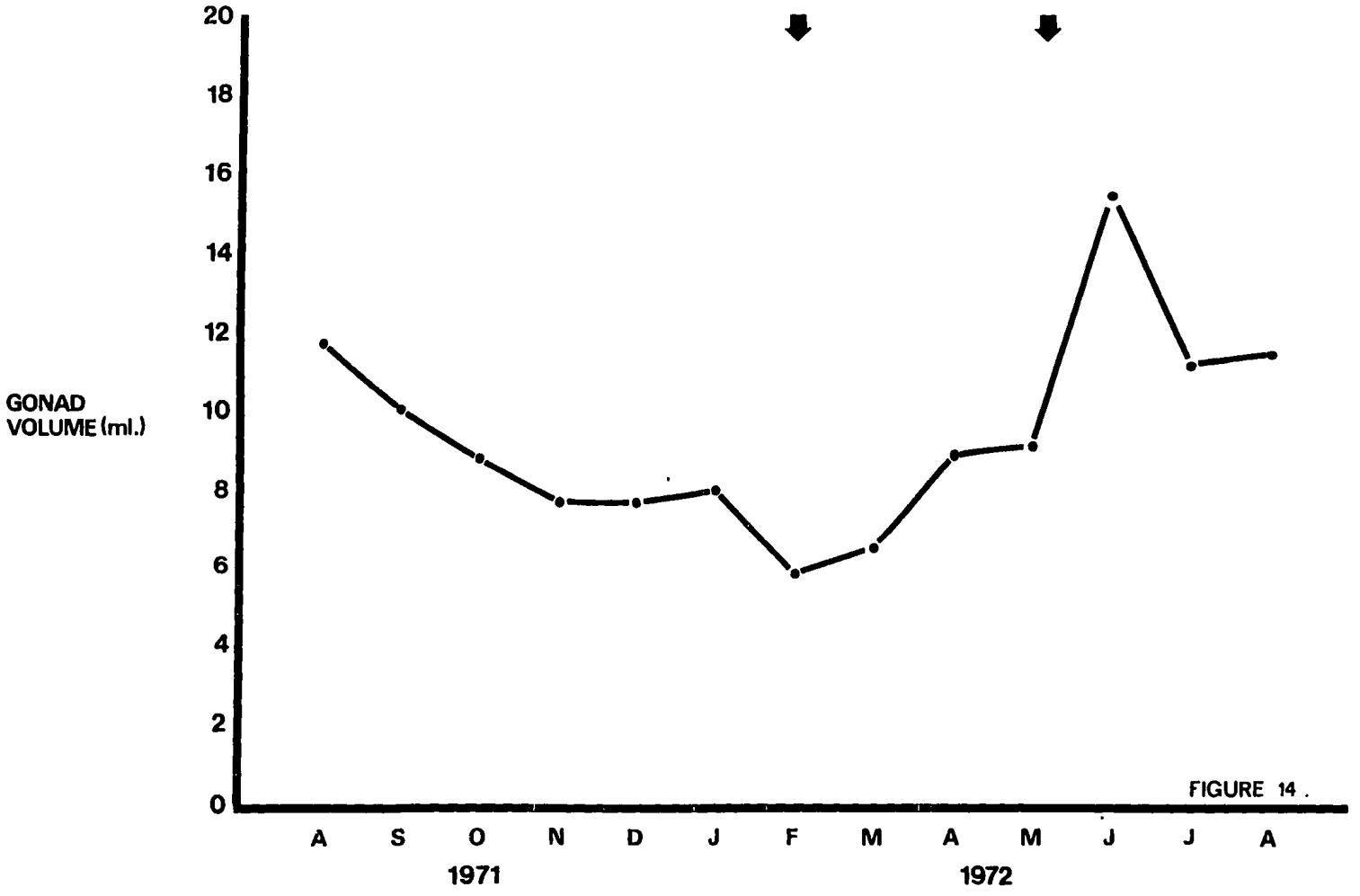
GONAD
VOLUME (ml.)
20
18
16
14
12
10
8
6
4
2
0
A
S
O
N
D
J
F
M
A
M
J
J
A
1971
1972

FIGURE 14 .

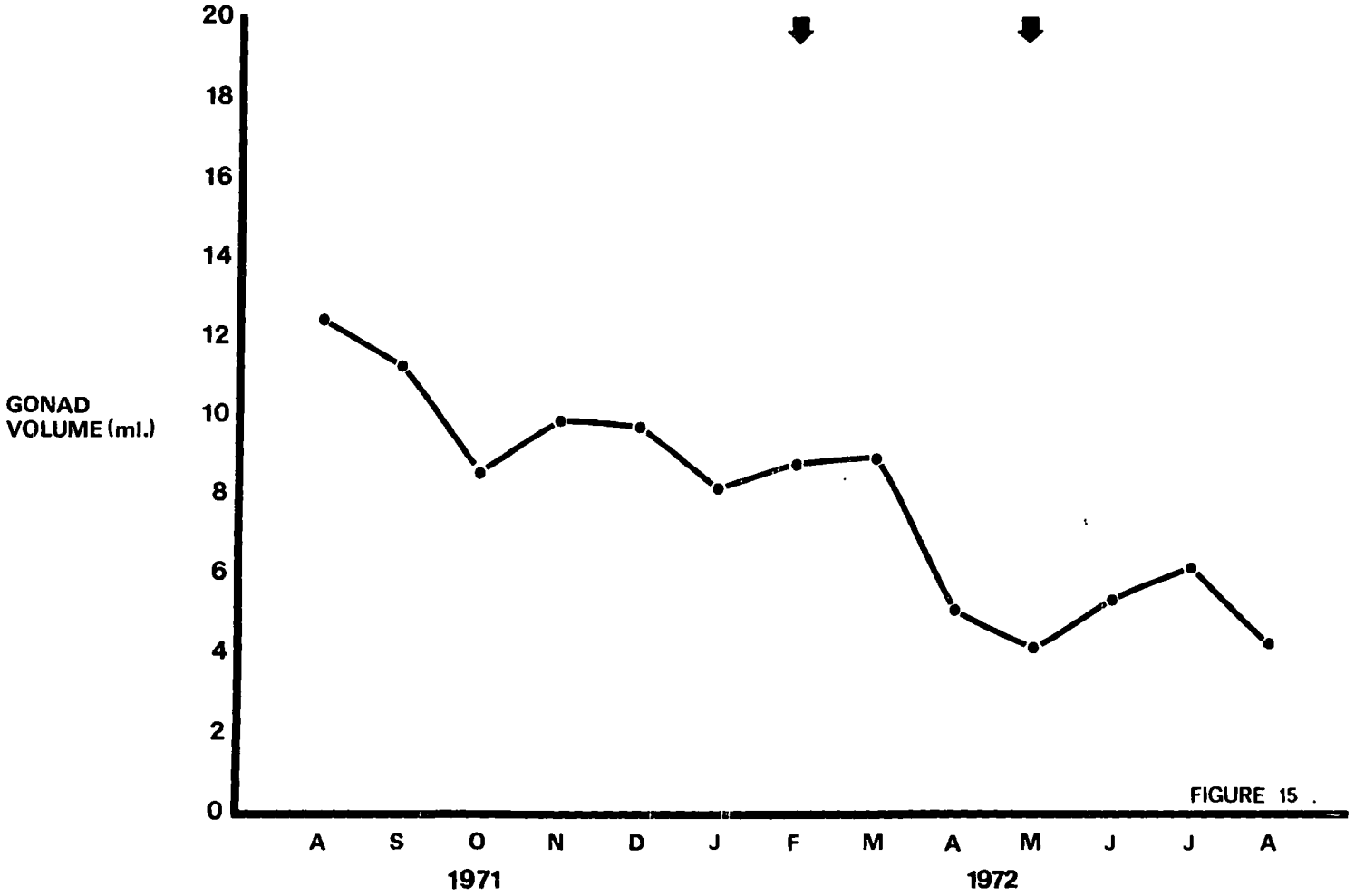
GONAD
VOLUME (ml.)
20
18
16
14
12
10
8
6
4
2
0
A
S
O
N
D
J
F
M
A
M
J
J
A
1971
1972

FIGURE 15 .

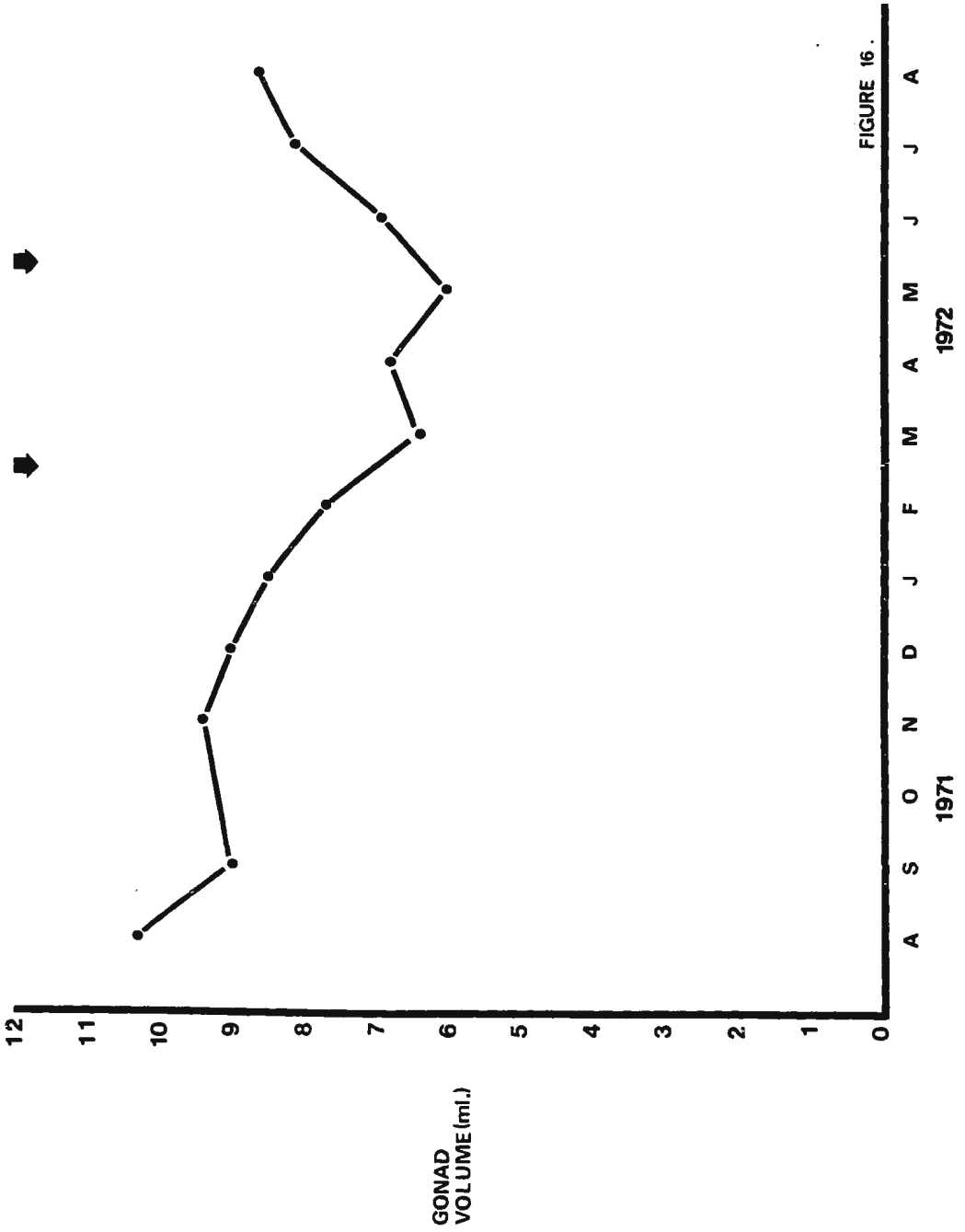
GONAD VOLUME (ml.)
0
1
2
3
4
5
6
7
8
9
10
11
12
A S O N D J F M A M J J A
1971
1972

FIGURE 16.

volumes decreased approximately 4.3 milliliters over the nine month period from August, 1971 to May. Following the minimal value reached in May, there was an increase of around 2.6 milliliters in average gonad volume during the remainder of the study period.

Thickness of the Visceral Peritoneum

Figures 17, 18 and 19 demonstrate the monthly variation in the thickness of the visceral peritoneum as measured from histological preparations of reproductive tissue for each group of experimental animals. In each case, thickness is greater in female than in male individuals.

The variation in thickness of the visceral peritoneum is most pronounced in Outer Cove *C. frondosa* (Figure 17). A gradual decrease of approximately 59 microns throughout the first seven months of the study is followed by an abrupt increase of some 85 microns over a three month period with maximum values being reached in June, and following June, values decline.

Although a decline in the thickness of the visceral peritoneum during the first half of the study period is also apparent in Figure 18 for *C. frondosa* maintained in the laboratory, the subsequent increase in thickness apparent at Outer Cove does not occur.

In *P. fabricii* (Fig. 19) the visceral peritoneum remains practically uniform in thickness throughout the first eight months of the study period. There is a slight thickening in May and June following the spawning period but the degree of increase (31 microns) is far less than occurred in Outer Cove *C. frondosa*.

THICKNESS OF THE VISCERAL PERITONEUM:

Figure 17 - Thickness of the visceral peritoneum of *Cucumaria frondosa* collected at Outer Cove.

Figure 18 - Thickness of the visceral peritoneum of *Cucumaria frondosa* maintained in the laboratory.

Figure 19 - Thickness of the visceral peritoneum of *Psolus fabricii* collected at Logy Bay.

All values are average monthly thicknesses of the visceral peritoneum for the population sampled. Measurements are from histological preparations of reproductive tissue fixed in Bouin's Fluid. Combined values for both sexes and separate values for each sex are given. Arrows indicate the duration of the spawning period.

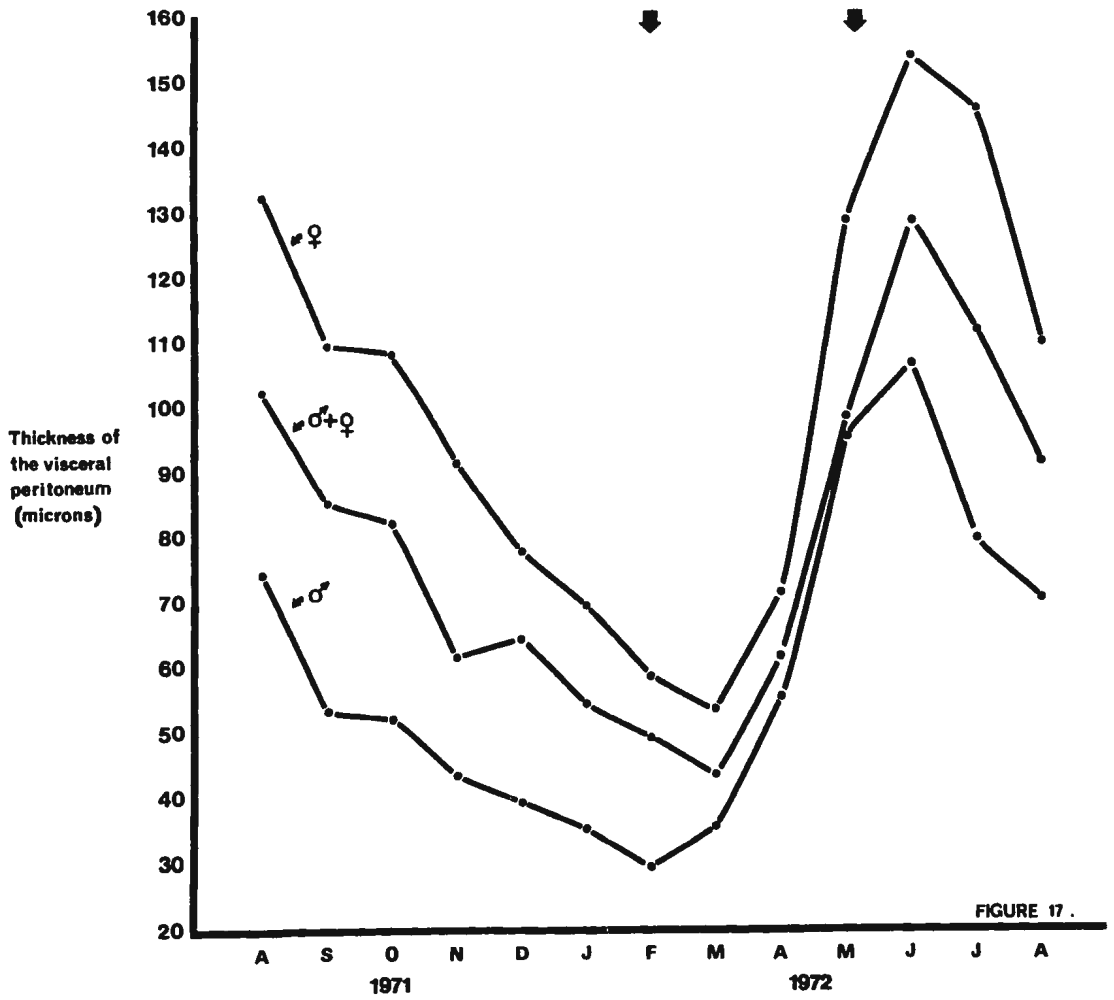
Thickness of the visceral peritoneum (microns)
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
♀
♂+♀
♂
A S O N D J F M A M J J A
1971
1972

FIGURE 17.

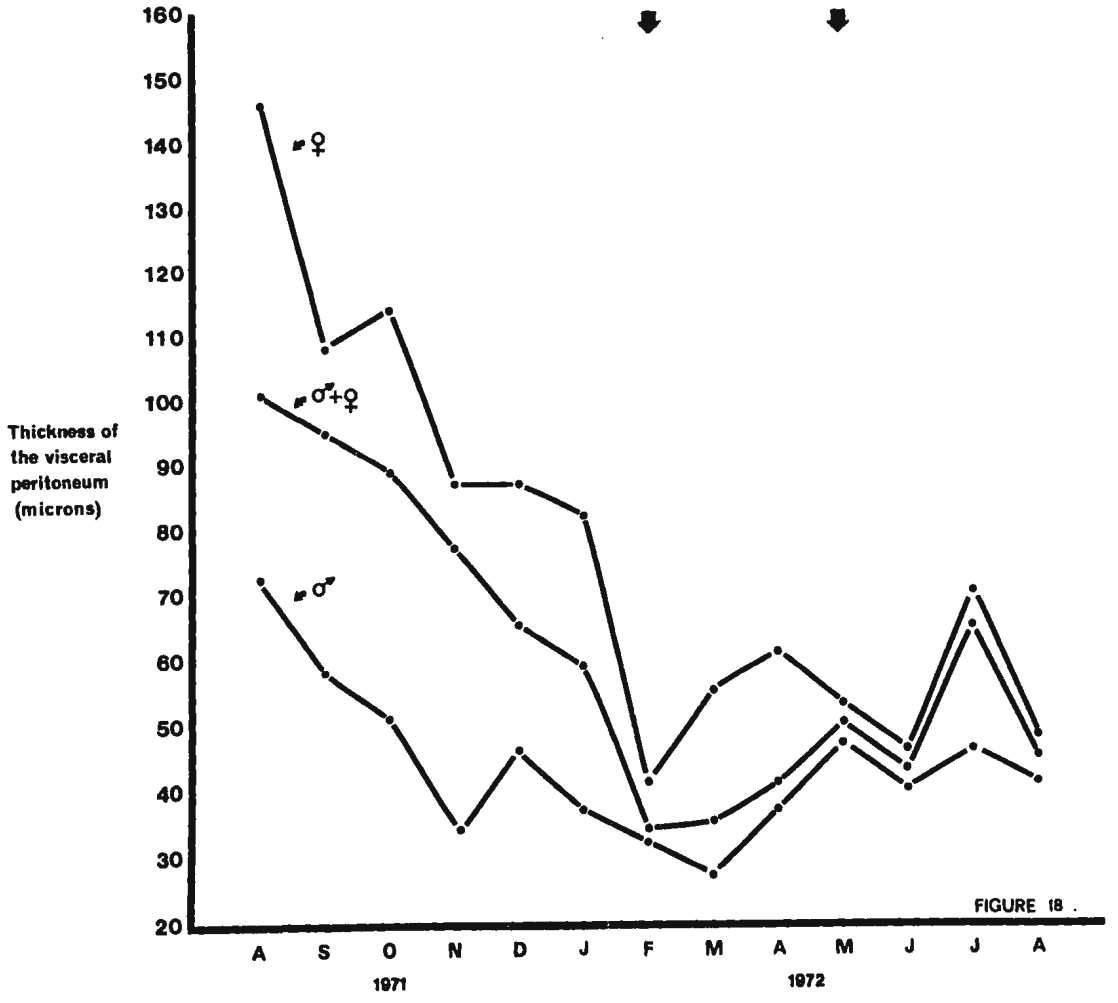
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
♂+♀
♂
Thickness of the visceral peritoneum (microns)
A S O N D J F M A M J J A
1971
1972

FIGURE 18 .

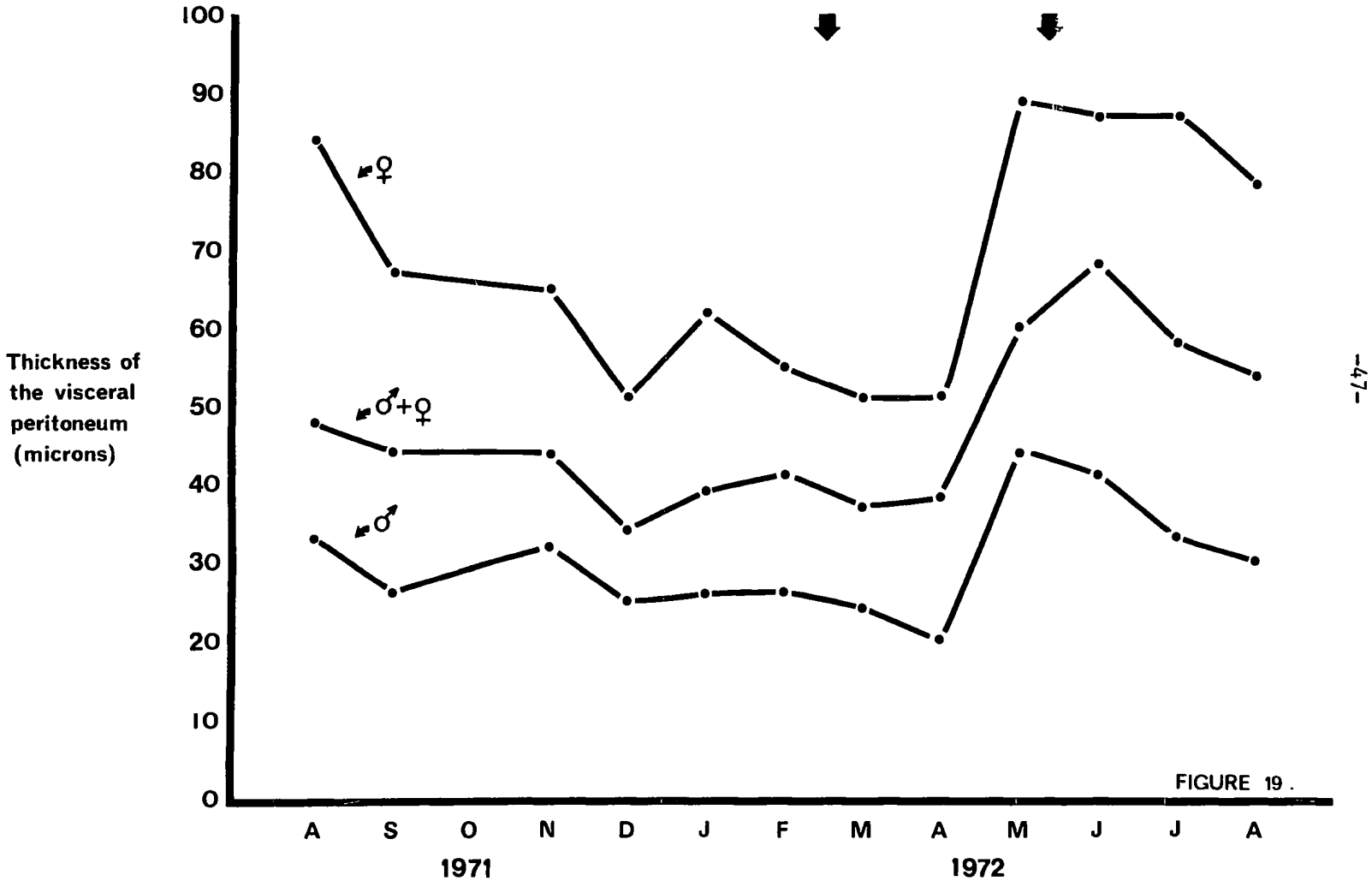
Thickness of the visceral peritoneum (microns)
100
90
80
70
60
50
40
30
20
10
0
♀
♂+♀
♂
A S O N D J F M A M J J A
1971
1972

FIGURE 19.

Sex Ratio

A total of 589 individuals of *C. frondosa* were examined during the study. Of these 290, or 49.23% were males and 299 or 50.76% were females.

A total of 220 individuals of *P. fabricii* were examined during the study. Of these 117 or 53.18% were males and 103 or 46.81% were females.

No incidences of hermaphroditism were recorded in any of the histological examinations carried out.

Spawning Period - Laboratory

Male and female individuals of both *C. frondosa* and *P. fabricii* were observed spawning in the laboratory.

Individuals of *C. frondosa* which had been placed in the Laboratory in July, 1971 for regular sampling and histological examination first spawned on February 25th, with spawnings continuing for just under a two month period or until the 23rd of April. The duration of the spawning period was determined from the occurrence of shed ova in the net trap which had been set up in the drain of the holding trays (Fig. 6) and a total of twelve females and seven males were also observed spawning during this period. Temperatures of the seawater in the holding trays during this period ranged from -1.0°C to 0°C.

Records of spawning males were restricted entirely to observation as the net trap was incapable of catching the minute sperm. Subsequent histological examination of males from this group

indicated that some of the males had spawned unnoticed several weeks before spawned ova first appeared in the trap.

With the end of the study (August, 1972) the majority of the individuals of this group had been sacrificed and dissected through the programme of regular sampling. A spent condition was recorded for all gonads examined after the spawning period.

Thirty individuals of *P. fabricii* had been placed in a laboratory wet bench in September, 1971. Of these, a total of seven males and five females were observed spawning. The first spawning was noted on April 17th and spawnings were recorded at different times until May 10th, in all a total period of just over three weeks. Temperatures of the laboratory seawater during this period ranged from 0°C to 0.5°C. The gonads of individuals which had not been observed spawning were examined on May 21st and each of these were found to be completely spent.[1]

The spawning periods of *C. frondosa* sampled from Outer Cove and *P. fabricii* collected in Logy Bay were determined from regular histological preparations of reproductive tissue and are listed in the section dealing with Histology (p.61).

Spawning Behavior

Aspects of spawning behavior, as described from detailed

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[1]three individuals of *Psolus phantapus* collected in early March and placed in the laboratory spawned on April 26; four individuals of *P. phantapus* were also collected and dissected on April 28 and each of these exhibited partially spent gonads.

observations of individuals found spawning in the laboratory were very similar in *C. frondosa* and *P. fabricii*.

During spawning the introvert and feeding appendages of both species are extended. This feeding-like posture is often accompanied by an exaggerated swelling and extension of the anterior portion of the animal associated with the introvert. Although the feeding appendages remain extended during spawning they do not ordinarily expand to the same degree typical of feeding animals and feeding activities (insertion of the feeding appendages into the mouth) do not occur.

In *P. fabricii* a pronounced bulging of the oral region and a resultant lifting of the circum-oral scales may occur prior to spawning serving as a useful indication that the animal is about to spawn. When it occurs the bulging generally appears several days prior to spawning and subsides shortly afterwards. In one instance, however, a male specimen observed in the laboratory held this posture for sixteen days before spawning was recorded and for four days afterwards. This posture was also noted in several individuals of *P. fabricii* observed during the March 20th sampling dive at Logy Bay.

The orientation of spawning animals to water currents is extremely variable. The most commonly observed orientation is similar to that assumed by feeding animals in that the feeding appendages are oriented directly into or at right angles to the direction of water flow.

Sex cells are released from the gonoduct at a variable rate which may cease momentarily or slow considerably several times during a single spawning. Repetitive spawnings, at two and three day

intervals were not uncommon in either species. In at least one incidence in the laboratory a single female specimen of *P. fabricii* was observed to spawn on three separate occasions over a six day period. The duration of spawning varied amongst individuals, lasting from one half hour to three and occasionally four hours. There does not seem to be a preferred time of day for spawning.

The reddish ova are emitted individually (Fig. 20b) or as clusters (Fig. 20a) held together in a suspension matrix which dissipates quickly in seawater, thereby freeing the unfertilized ova from one another.

Smaller immature oocytes are released with mature ova (Fig. 20b). The majority of ova in both species rise to the surface of the water at the time of release.[1] It was noticed in the laboratory that these ova assume a vertical distribution within the upper several inches of the water column. Certain other ova settled to the bottom of the wet bench. These variations in the buoyancy of shed ova may reflect the state of development or degree of vitellogenesis and therefore the viability or fertilizability of these sex cells.

Sperm are released in streams of concentrated milt from each of several openings of the genital papilla (Fig. 20c,d) and the dense cloud of motile sperm formed as the milt spreads dissipates quickly.

In both sexes of the two species, the genital papilla enlarges

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[1]it is an interesting contrast that ova of the burrowing species *Psolus phantapus* observed being released in the laboratory were demersal and settled quickly to the bottom of the wet bench in small clusters.

Figure 20 - Photographs of the spawning process.

a..... spawning female *Cucumaria frondosa* showing the release of sex cells from the gonoduct.

b..... spawning female *Cucumaria frondosa* showing a string of released sex cells held together by the suspension matrix of the gonad tubule; note the different sizes of sex cells being released.

c..... spawning male *Cucumaria frondosa*.

d..... spawning male *Psolus fabricii*; note the enlarged genital papilla and the streams of milt being released from the several openings of the gonoduct.

abbreviations, in alphabetical order:

FA - feeding appendage
G - gonoduct
GP - gonopore
I - introvert
M - mouth
O - ovum
S - sperm
TF - tube foot

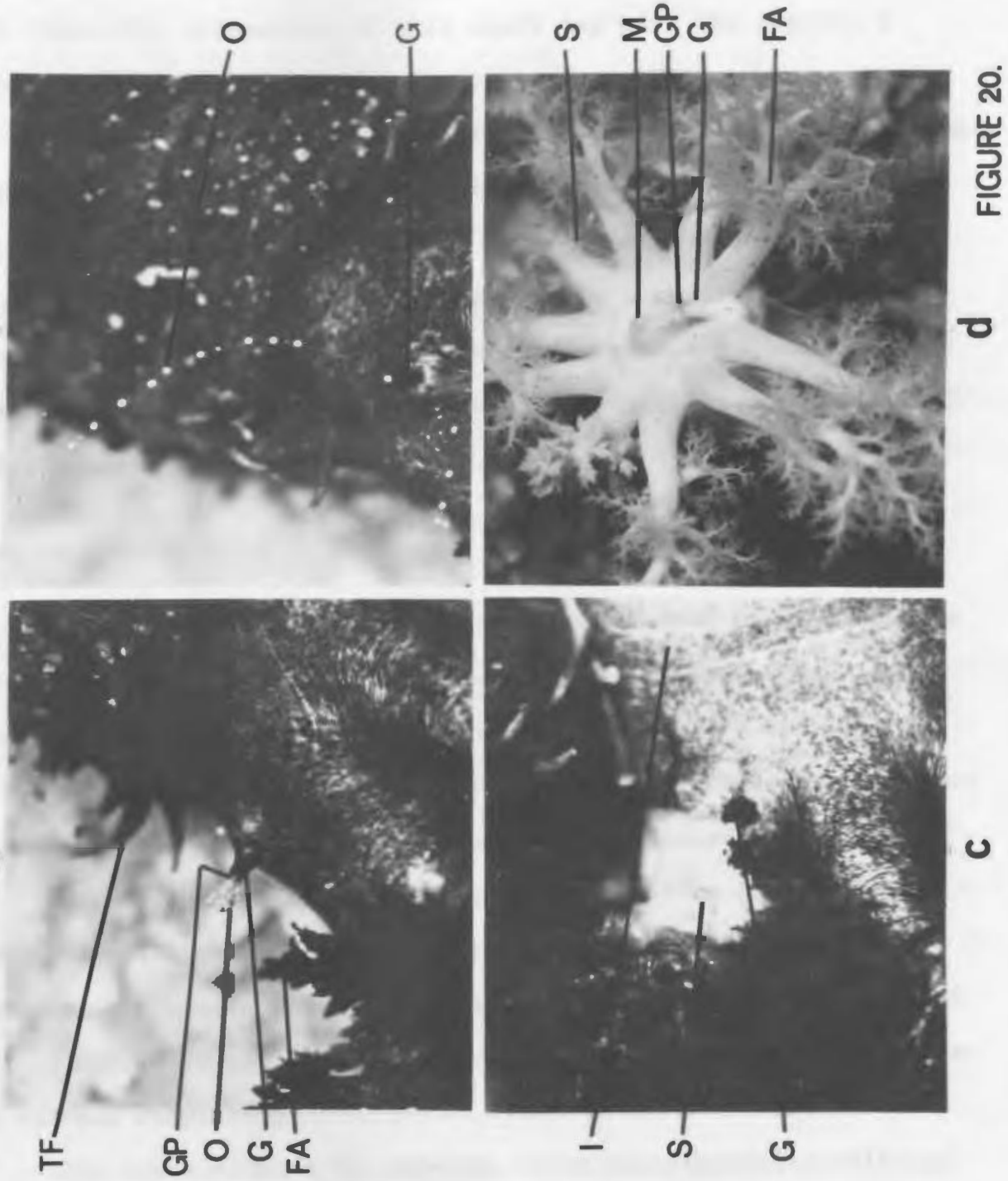

FIGURE 20.

during the spawning period. In males, a short stalk-like extension forms at the base of the genital papilla during spawning. In males of *P. fabricii*, elongation of this stalk may give the papilla a certain flexibility of lateral movement (Fig. 20d).

In females, the terminal portion of the gonoduct bears a single tubular opening whereas in males the gonopore is sub-divided into smaller openings of variable number (compare Fig. 20a with 20d). Although variations in the appearance of the gonopore are most pronounced during the spawning season when the gonoduct increases in size the difference noted is a useful and reliable method of sex differentiation at any time of year.

Spawning Induction

Only three of the more than 150 experimental animals spawned during an attempted induction procedure. A male and a female *C. frondosa* spawned when exposed to a sudden temperature increase of 4°C. A second female spawned under experimental conditions of total darkness. None of the "control" individuals spawned.

Dissection of experimental individuals after each 72 hour induction trial showed the majority of animals tested to be in a predominantly mature reproductive condition. A small percentage of test animals were partially spent but virtually none of the animals had spawned completely.

The low incidence of spawning under experimental conditions indicates the unreliability of any of the methods tested. The spawning which did occur may only reflect the natural tendency

of these animals to spawn at the time of year in which the experiments were carried out.

Macroscopic Features of the Mature Gonad

Variations in the macroscopic appearance of the male and female gonad throughout the reproductive cycle were similar in both *C. frondosa* and *P. fabricii*. There is no essential difference in gross structure between the male and female gonad.

In male individuals the thick concentration of sperm and suspension medium present during the late development and mature phases of the gonad give it a light pink coloration. With spawning and the release of sex cells and during early gametogenesis the gonad appears reddish-brown and is practically indistinguishable in macroscopic appearance from the female gonad at the same time of year.

The female gonad assumes a dark reddish brown coloration throughout the year. Developing oocytes are visible within the tubules of the gonad during late development and mature phases of the reproductive cycle.

The male gonad is generally larger than that of the female and average monthly gonad volumes are higher for males during most of the year.

In individuals sampled directly from the ocean, shrinkage and shortening of the gonad tubules with spawning is very slight and there is a noticeable increase in the size of the gonad during and slightly after the spawning period.

In lab-maintained individuals of both species, shrinkage and

shortening of the gonad tubules with spawning is extreme. The tubules also undergo a loss of coloration and appear as minute, whitish threads. A slight debris of unspent immature (small) oocytes may be evident as reddish particles inside of the tubules. The increase in size after the spawning period is slight in comparison to the increase which occurred in naturally occurring populations.

Histological Features of the Mature Gonad

The structure of the gonad wall is similar in male and female individuals of both *C. frondosa* and *P. fabricii* and consists of the same cellular layers throughout the year varying only in width.

In light microscopic observations of reproductive tissue stained with hematoxylin and eosin the outer visceral peritoneum is seen to consist of tall columnar epithelial cells (Fig. 23a). Numerous globular inclusions are evident in these epithelial cells and in many instances they appear more concentrated in the outer portion of the peritoneum (Fig. 25d).

Inside of the visceral peritoneum there is a thin layer of muscle cell fibers arranged in a circular fashion at right angles to the long axis of the peritoneal epithelial cells. Nerve components which are generally found in this area of the gonad wall (Davis, 1971) were not readily evident with the light microscope.

A haemal fluid, containing various types of cellular inclusions, shows clearly as a lightly stained almost opaque layer of variable thickness. The haemal space assumes an evenly circular or irregular outline of variable thickness depending on the presence

of convolutions in the germinal epithelium. An extrahaemal and intrahaemal basal lamina occur on either side of the haemal space (Fig. 20).

In males, the germinal epithelium appears as a darkly staining layer of variable thickness containing dense concentrations of spermatogonia, primary and secondary spermatocytes and spermatids interspersed with accessory cells (Fig. 21; 22; 23). During active spermatogenesis, spermatozoa may be seen "detaching" from the inside of the germinal epithelium and concentrating in the lumen of the gonad tubule (Fig. 22; 23). In histological preparations of more mature individuals a thin empty space was evident around the edge of the lumen between the germinal epithelium and the central "core" of spermatozoa (Fig. 22d; 23d).

The haemal space of the female gonad is generally more extensive than that of males. It often occupies large portions of the lumen as a result of the heavily convoluted condition of the germinal epithelium in the female animal (Fig. 24a).

Oogonia appear as darkly staining cells along the germinal epithelium (Fig. 25b). These oogonia divide mitotically giving rise to primary oocytes (Fig. 24a; 24d) after the last oogonial division. During the later stages of oogenesis primary oocytes undergo vitellogenesis laying down large amount of yolk material within the cytoplasm. Post-vitellogenic oocytes generally stain less darkly with hematoxylin (Fig. 24).

Primary oocytes give rise to secondary oocytes (Fig. 24) through meiosis. No polar bodies were evident in any of the histological preparations used so that the distinction between large

primary oocytes and newly-formed secondary oocytes was difficult to make.

As the oocyte enlarges it bulges into the lumen of the gonad so that its surface of attachment with the germinal epithelium diminishes. The oocytes contain a large germinal vesicle and nucleolar numbers are generally high (Fig. 24). Occasionally nucleoli may appear extruded into the cytoplasm.

With maturation the secondary oocyte gives rise to the ootid egg or ovum. The nuclei of mature ova are smaller than the germinal vesicle of the secondary oocytes and are often more centrally located, although this latter feature is not often a consistently reliable method by which the two may be distinguished. The breakdown of the germinal vesicle associated with the formation of the ovum was not observed. It seems probable that the final maturation divisions occur just before the ova are shed.

Follicle cells are generally evident around developing oocytes (Fig. 24b; 25c). The developing oocytes or mature ova may appear variably polygonal or rhombohedral in shape (Fig. 24)--an effect which may be due to crowding in the tubule.

The presence of accessory cells in the lumen of the gonadal tubules is a common histological feature of both sexes throughout the reproductive cycle. In males, these cells are evident among the spermatogonia of tubules undergoing recovery after the spawning period (Fig. 21b) or as scattered bundles of phagocytic cells in the lumens of extremely ripe, partially spent (Fig. 27a) or completely spent individuals. In females, accessory cells are abundant throughout

the year in close association with developing oocytes (Fig. 24; 25) and probably serve a dual nutritional and phagocytic function.

Aspects of the Immature Gonad

The gonads of sexually immature *C. frondosa* appear macroscopically as short, thin, thread-like tubules of semi-transparent white or yellow coloration. The histological structure of the gonad wall of the immature gonad is similar in nature to that of adult animals. The haemal space is well developed and the germinal epithelium appears as a thin layer of "inactive" cells convoluted throughout an empty lumen (Fig. 26a, b, c).

In February of 1972, just prior to the commencement of the spawning period, an examination was made of 173 individuals of *C. frondosa* of less than 70.0 grams total wet weight. Table I lists the number of animals examined in various size categories and shows the number in each which were immature or which exhibited the presence of sex cells. Of the total, 113 or 65.3% were immature and 60 or 34.7% were able to be sexed with the aid of a dissection microscope. Females were more readily identifiable in the smaller size categories than were males. The gonads of all animals weighing more than 55.0 grams contained mature sex cells while immature individuals were evident in all size categories of animals less than 55.0 grams total wet weight.

The smallest female identified weighed 19.9 grams. Oocytes and ova present in the tubules of this animal (Fig. 26d) and in tubules of other females in the smaller size categories, were small, few in

TABLE I

The Incidence of Immature and Mature Individuals

of *Cucumaria frondosa* in Various Size

Ranges Less Than 70.0 Grams

Total Wet Weight

Size Category (range of total wet weight in grams)	Number of individuals examined	Number of immature individuals	Number of individuals exhibiting male sex cells	Number of individuals exhibiting female sex cells
0- 5	5	5	0	0
6-10	13	13	0	0
11-15	24	24	0	0
16-20	15	12	1	2
21-25	18	17	0	1
26-30	16	12	0	4
31-35	14	8	1	5
36-40	10	8	0	2
41-45	7	5	0	2
46-50	14	6	4	4
51-55	11	3	3	5
56-60	6	0	3	3
61-65	7	0	2	5
66-70	13	0	8	5
TOTAL	173	113	22	38

number and predominantly previtellogenic in nature.

The smallest male identified weighed 19.7 grams and only one tubule of this individual contained spermatozoa.

The Reproductive Cycle of Males - histological features

The annual reproductive cycle of male individuals of *C. frondosa* and *P. fabricii* may be divided into five stages defined on the basis of histological changes occurring in the germinal epithelium of the gonad wall and by the presence or absence of spermatozoa in the lumen of the gonad tubules.

Activation or recovery. This stage is characterized by an initiation of spermatogenic activities. The germinal epithelium, which appears as a shrunken inactive layer in the spent animal (Fig. 27b), undergoes considerable thickening. A rapid proliferation of spermatogonia and accessory cells in the germinal epithelium results in a thick, often convoluted, darkly staining layer of cells lining the lumen of the tubule. A slight residue of unspent spermatozoa from the previous reproductive cycle may be present in an otherwise empty lumen.

In *C. frondosa* the germinal epithelium generally assumes an even thickness around the lumen (Fig. 21a) whereas in males of *P. fabricii*, the germinal epithelium appears as characteristically lengthy projections containing varying amounts of haemal fluid (Fig. 21c, d).

Accessory cells may be found in the lumen of the gonadal

Figure 21 - Photomicrographs of the activation stage in the male gonad.

a..... *Cucumaria frondosa* ; cross-section of a single gonad tubule showing the thick germinal epithelium. June, 1972 ; 70X .

b..... *Cucumaria frondosa* ; enlargement of the germinal epithelium showing the presence of numerous spermatogonia and accessory cells. June, 1972 ; 200X .

c & d..... *Psolus fabricii* ; cross-sections of single gonad tubules illustrating the lengthy projections of the haemal space and germinal epithelium characteristic of the activation stage in this species. July, 1972 ; 75X .

abbreviations, in alphabetical order:
AC - accessory cells
EHL- extrahaemal basal lamina
GE - germinal epithelium
HF - haemal fluid
HS - haemal space
IHL- intrahaemal basal lamina
L - lumen
SG - spermatogonia
VP - visceral peritoneum

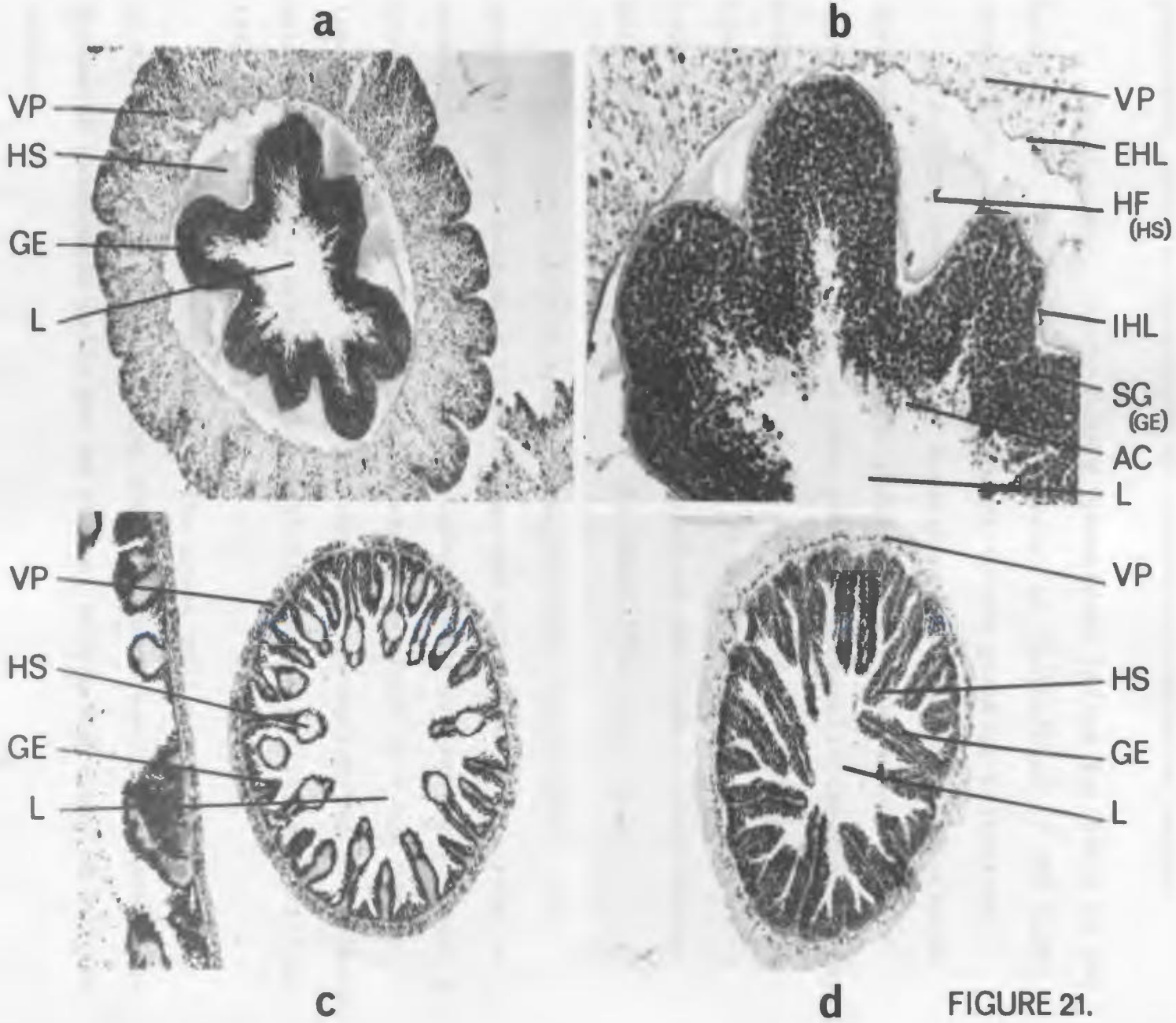

FIGURE 21.

tubule at this stage. These cells are likely remnants of earlier phagocytic activity subsequent to the previous spawning period.

Development. Newly-formed spermatozoa occupy the lumen of the gonad tubule. Progressive concentration of spermatozoa in the lumen occurs with continued spermatogenesis in the germinal epithelium.

As the concentration of spermatozoa increases with continued development and maturation, the germinal epithelium become less convoluted and the number of less advanced germ cells (spermatogonia, primary and secondary spermatocytes and spermatids) surrounding the lumen, decreases (Fig. 22a, b, c). In males of *P. fabricii* the lengthy projections of germinal epithelium and haemal fluid shorten with the continued maturation of the gonad (Fig. 23a, c, d).

Maturity. Active spermatogenesis slows considerably. The germinal layer decreases in thickness and appears as a thin layer of essentially "inactive" germ cells, loosely-knit and arranged around a dense "core" of heavily staining spermatozoa which fill the entire lumen. There are very few spermatocytes or spermatids and in the more mature individuals spermatogenic activity is practically non-existent (Fig. 22d; 23d).

The visceral peritoneum of the gonad wall may be very thin at this stage (Fig. 23d) and the gonad ruptures easily with handling. Masses of phagocytic cells may be present within the lumen of certain tubules.

Spawning. The gonads of spawning males contain a mixture of

Figure 22 - Photomicrographs of the development and mature stages in the male gonad of *Cucumaria frondosa*.

a..... early development; cross-section of a gonad tubule showing the initial formation of spermatozoa. July, 1972 ; 90X .

b & c..... further stages of development illustrating the increasing concentrations of spermatozoa within the lumen. September-October, 1971 ; 75X .

d..... maturity; note the reduced thickness of the germinal epithelium and the dense "core" of spermatozoa within the lumen. January, 1972 ; 75X .

abbreviations, in alphabetical order:
GE - germinal epithelium
HS - haemal space
L - lumen
SZ - spermatozoa
VP - visceral peritoneum

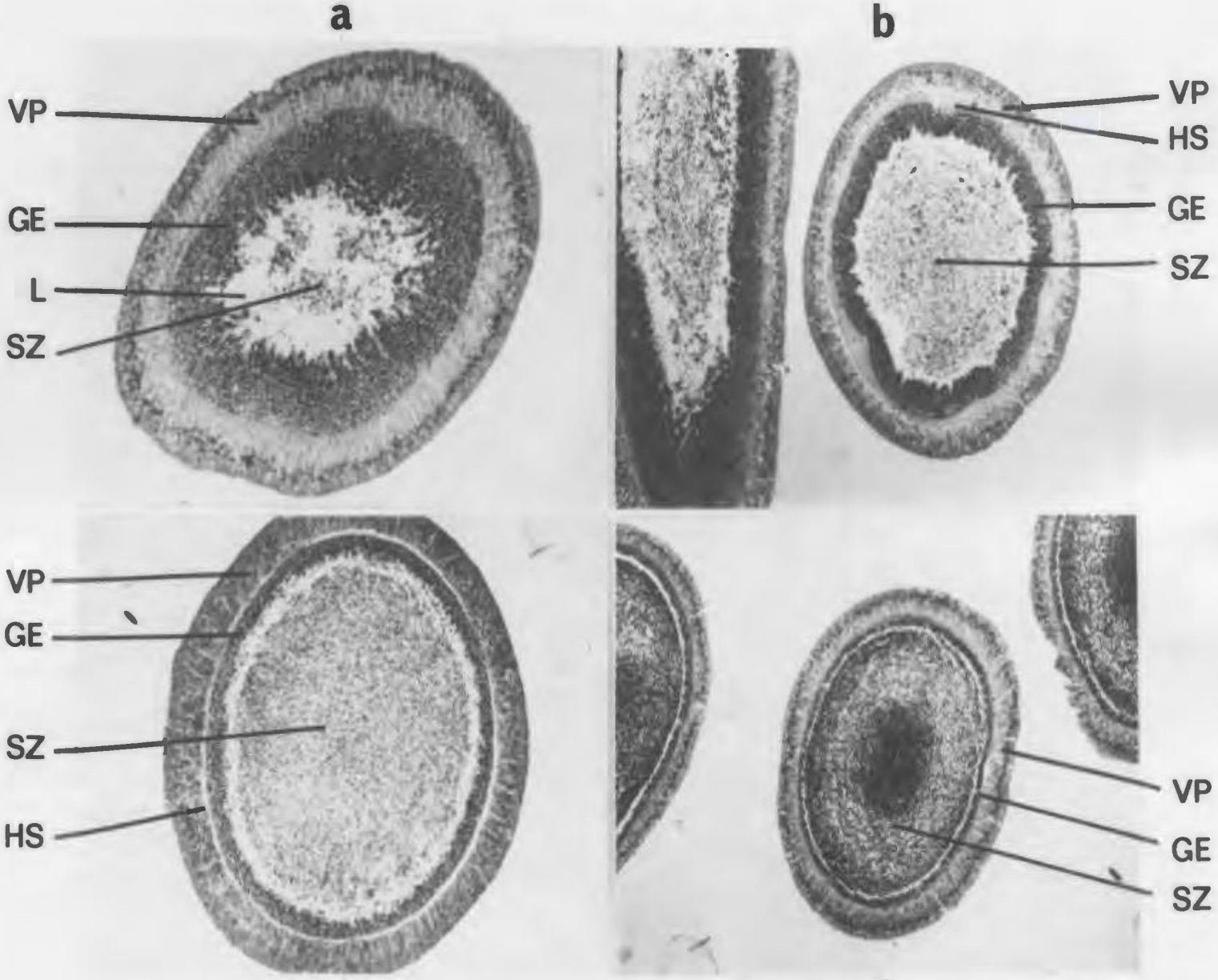

FIGURE 22.

Figure 23 - Photomicrographs of the development and mature stages in the male gonad of *Psolus fabricii*.

a..... early development; cross-section of a gonad tubule showing the initial formation of spermatozoa. August, 1971 ; 75X .

b..... early development; longitudinal section of a gonad tubule illustrating the extent of the haemal space. August, 1971 ; 75X .

c..... increasing concentrations of spermatozoa; the projections of haemal space shorten. December,1971 ; 75X .

d..... maturity; note the reduced thickness of the germinal epithelium and the dense "core" of spermatozoa. January,1972 ; 75X .

abbreviations, in alphabetical order : GE - germinal epithelium
HS - haemal space
L - lumen
SZ - spermatozoa
VP - visceral peritoneum

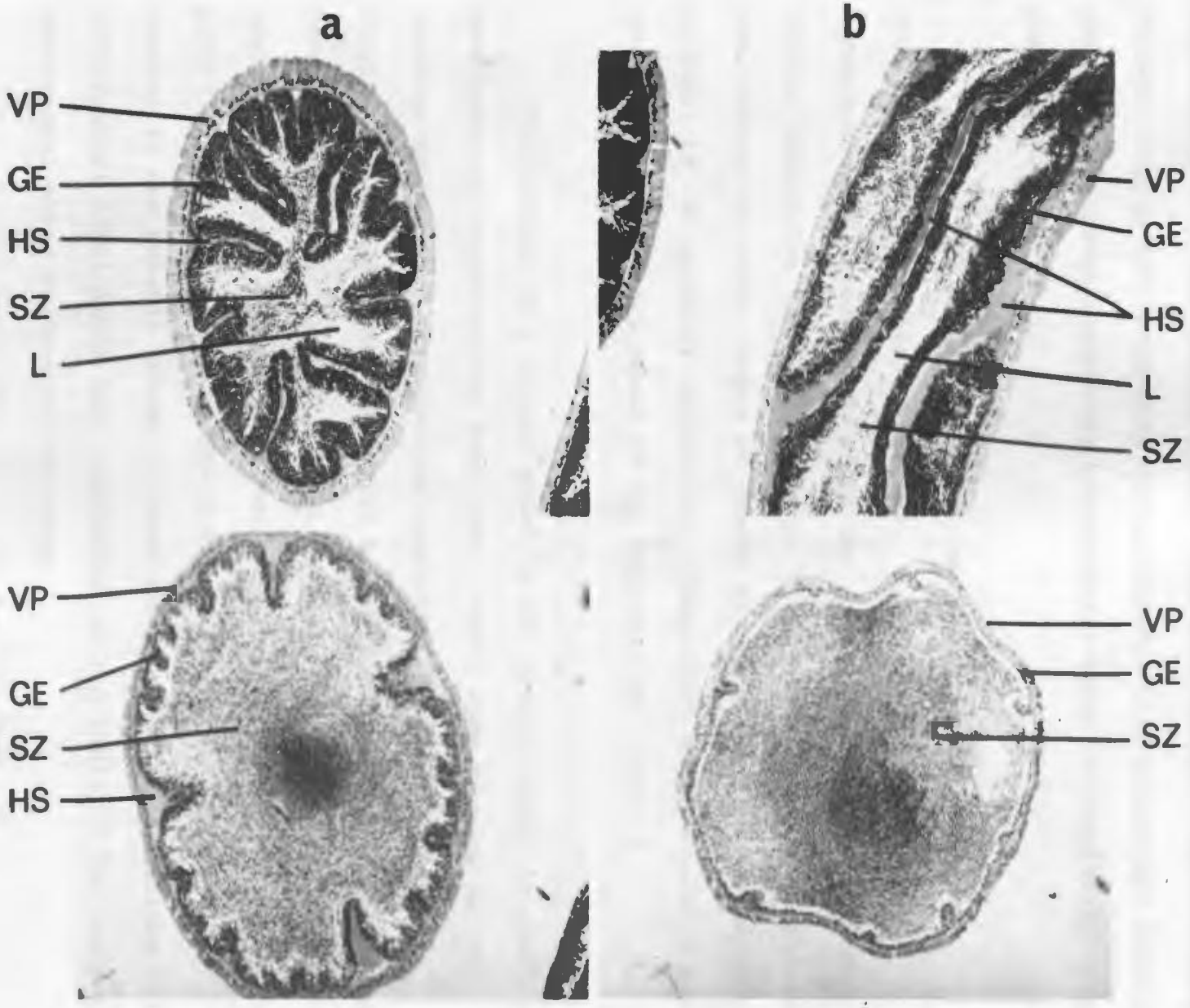

FIGURE 23.

tubules of variable appearance. Some tubules contain the dense concentration of spermatozoa typical of mature animals, while others contain varying concentrations of spermatozoa with occasional gaps or spaces in the lumen indicative of partially spent or completely spent individuals (Fig. 27a).

Spent. The vast majority of tubules in spent males have released their sex cells (Fig. 27b). Small numbers of residual spermatozoa are generally found in the lumen of spent tubules and tubules containing few spermatozoa at this stage may be distinguished from tubules in early stages of growth by the absence of spermatogenic activity in the germinal epithelium. Phagocytic accessory cells are common throughout this phase of the reproductive cycle.

The tubules of a single gonad do not always appear in similar developmental stages at the same time. While some tubules may be observed in a mature reproductive condition, for example, other tubules of the same gonad may still be undergoing active spermatogenesis associated with early developmental phases. Where stages of the reproductive cycle vary in different tubules of the same gonad, generally the represented stages are in close succession to one another in the general scheme of development. Gonads exhibiting this differential development were classified on the basis of the dominant development phase evident in the gonad.

It seems probable that the majority of animals do not spawn completely during the spawning period. In histological preparations,

mature tubules commonly appear among tubules undergoing proliferation of germinal cells associated with a renewed reproductive cycle. Phagocytic activity, characterized by bundles of phagocytic cells is a common feature of these "remnant" mature tubules.

Each of the male *C. frondosa* sampled at Outer Cove were classified into one of the five reproductive stages outlined above. Table II gives a complete summary of the incidence of animals falling into each category during each month of the study period. After a brief resting period subsequent to spawning, the reproductive cycle commenced anew in June with activation of the gonad. Development occurred from July until October. Active spermatogenesis slowed in November with maturation and the gonads appeared mature during November, December and January. The spawning period, commencing just after the beginning of February, lasted until the first week in May--in all, a total period of slightly over three months, during which recorded temperatures at Outer Cove ranged from -1.25°C to 0.25°C. Completely spent individuals were evident during the latter part of the spawning period and were most common during the month of May.

The annual reproductive cycle of male *C. frondosa* maintained in the laboratory during the same period is outlined in a similar fashion in Table III. From the beginning of the study in August, 1971, until May, differences between the lab-maintained and Outer Cove population are negligible and reflect only the greater size of the samples from Outer Cove. Renewed gametogenic activity after May in lab-maintained animals, however, is lacking and the spent condition

lasted until the end of the study period.

The timing and duration of each phase of the reproductive cycle of *P. fabricii* collected from Logy Bay are given in Table IV. The spawning period commenced during the second half of February, and lasted approximately two and one half months until the first week in May. Temperatures recorded at Logy Bay during the spawning period ranged from -1.25°C to -0.25°C. The spent condition lasted for about two months (May and June) and new developments were initiated by the beginning of July. Development occurred from August until the end of December.

The Reproductive Cycle of Females - histological features

The sequence of gametogenic events leading to the formation of fertilizable ova in the female gonad of echinoderms is generally reflected in the variable appearance of the gonad throughout the reproductive cycle. In many echinoderm species these developments have been followed on the basis of the increasing size which accompanies maturation and vitellogenesis of the developing oocytes (Tanaka, 1958; Fuji, 1960; Pearse, 1968; Jordan, 1972). The criteria commonly used in delineating various phases of the reproductive cycle relate to the predominance of arbitrarily-chosen size categories of developing oocytes. The annual reproductive cycles of female *C. frondosa* and *P. fabricii*, however, are extremely difficult ones to interpret in this manner. In histological preparations of ovaries, the sequence of events comprising the reproductive cycle is very similar to males although, for a number of reasons, separate

Figure 24 - Histological features of the development of oocytes in the female gonad.

All sections are longitudinal sections through single gonad tubules.

a..... *Psolus fabricii*; tubule containing predominantly immature oocytes. January,1972 ; 75X .

b..... *Cucumaria frondosa*; tubule containing predominantly mature oocytes. January,1972 ; 90X .

c & d..... *Psolus fabricii*; tubules containing mixtures of oocytes of various sizes; note the presence of accessory cells. December,1971 ; 80X .

abbreviations, in alphabetical order:
AC - accessory cells
FC - follicle cell
GE - germinal epithelium
GV - germinal vesicle
HS - haemal space
L - lumen
N - nucleolus
NAC- nutritive accessory cells
OG - oogonia
PO - primary oocyte
SO - secondary oocyte
VP - visceral peritoneum

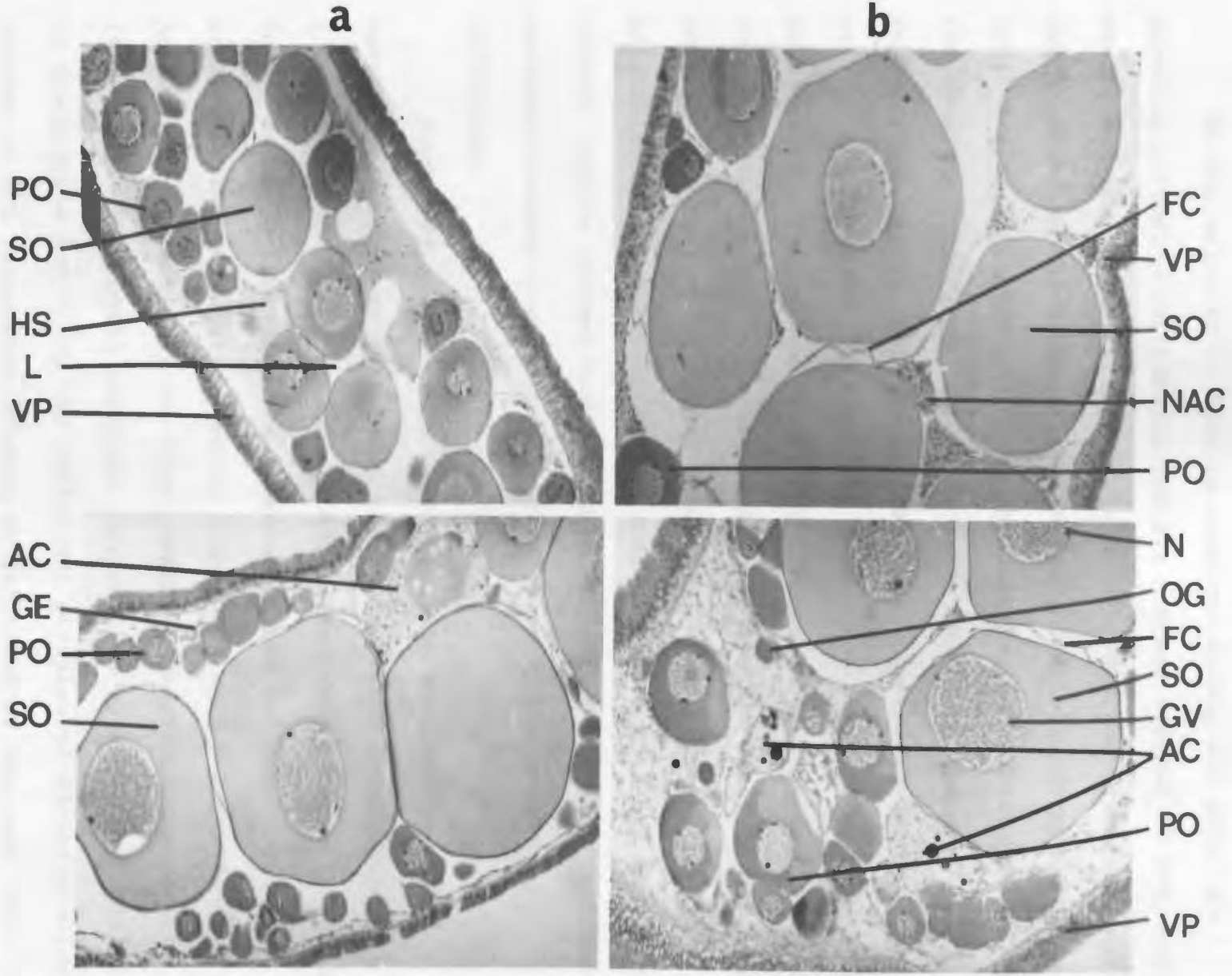

FIGURE 24.

developmental stages are less clearly definable.

In the majority of females examined during and after the spawning period, spent tubules contain a mixed residue of ova, and mature and immature oocytes (Fig. 27c, d). Ova and advanced oocytes which remain in spent tubules may be extruded later or may undergo cytolysis as accessory cells are abundant in the ovaries just after the spawning period and during the early phases of the next reproductive cycle. Oogonia and immature oocytes embedded in the wall of the lumen and hardly loose enough for shedding at the time more mature stages are emitted, may continue to mature into the next reproductive cycle. It becomes difficult at this stage to distinguish tubules in a resting condition from tubules undergoing renewed activation of the germinal epithelium, as the oogonia and immature primary oocytes present may be either "unspent-residuals" from the previous reproductive cycle or newly formed by the germinal epithelium.

Accessory cells may be found in close association with developing oocytes or throughout the lumen of the female gonad during all months of the year. These cells are particularly abundant in May and June following the spawning period and their presence at these times were most commonly associated with phagocytic activity as evidenced by the high number of deteriorating oocytes (Fig. 27d). Phagocytic activity, however, is by no means restricted to these three months as the indistinct profiles of disintegrating oocytes of all sizes were evident at all times of the year (Fig. 25d). This continual phagocytic activity may represent a means of controlling

Figure 25 - Histological features of accessory cells within the lumen of the female gonad.

All sections are cross-sections through gonad tubules.

a,b & c..... accessory cells,containing globular inclusions and fragments of deteriorated oocytes.

a - *Cucumaria frondosa;* August,1971; 25X .

b - *Psolus fabricii;* August,1971; 200X .

c - *Cucumaria frondosa;* December,1971; 200X .

d..... phagocytic accessory cells; note the indistinct border of the disintegrating oocyte.

Psolus fabricii; November,1971; 200X .

abbreviations, in alphabetical order:
AC - accessory cells
FC - follicle cell
GE - germinal epithelium
HS - haemal space
L - lumen
OG - oogonia
O(P)- oocyte undergoing phagocytosis
PO - primary oocyte
SO - secondary oocyte
VP - visceral peritoneum

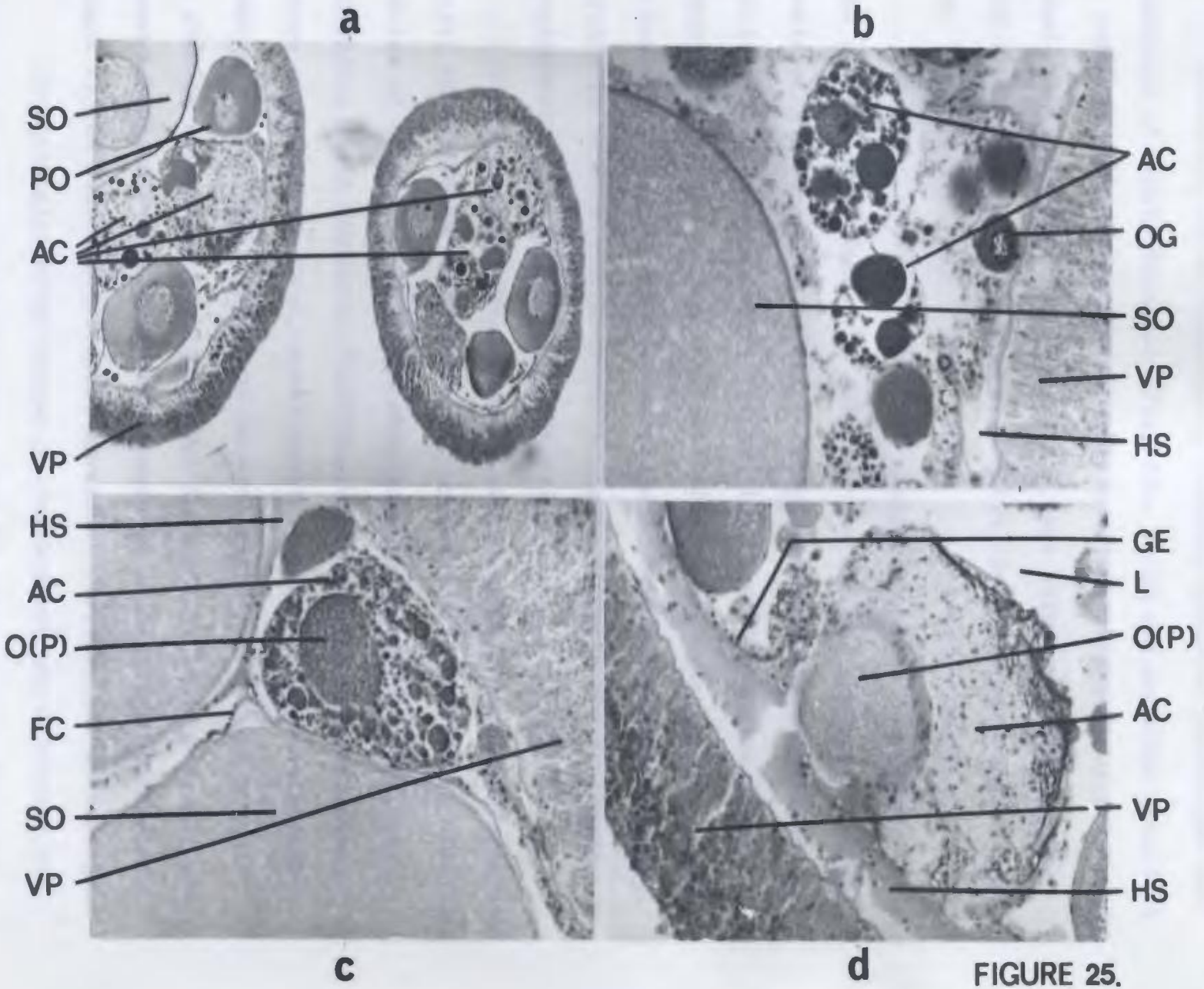

FIGURE 25.

over-production of ova.

Accessory cells, containing numerous globular inclusions and fragments of oocyte material (Fig. 25a, b, c) and probably nutritive in function, are common during early development phases of the female gonad. They are generally larger and more extensive at this time than at later stages of the reproductive cycle (Compare Fig. 25a with Fig. 25b, c).

The distinction between the activation phase and early development phases in the female is difficult to make as there is a marked overlapping of breeding cycles. The presence of phagocytic and nutritional accessory cells, the availability of all size ranges of "unspent" oocytes and the incidence of renewed oogenesis giving rise to rapidly growing oocytes create an overwhelming complex picture whereby it becomes extremely difficult to estimate the degree of development and regression respectively.

Throughout the developmental stages of the female reproductive cycle, the variable development and appearance of oocytes in different tubules of the same gonad pose additional problems. In any one individual it is common to find tubules containing predominantly mature oocytes (Fig. 24b), other tubules containing predominantly immature oocytes (Fig. 24a) and in even further tubules a mixture of growing and mature oocytes of all size ranges (Fig. 24c, d). It becomes difficult under such circumstances to delineate different stages of the reproductive cycle by casual histological inspection.

Increasingly mature stages of development are difficult to determine by measuring the average size of oocytes throughout the

reproductive cycle. Continual production of oogonia by the germinal epithelium throughout much of the cycle, continual phagocytosis of immature and mature oocytes and variable growth rates of oocytes give rise to a situation in which the average size of oocytes is as great in the early stages of development as it is several months later when the gonads are essentially mature.

In spite of these difficulties, certain generalizations relating to the annual reproductive cycle of females of *C. frondosa* and *P. fabricii* can be made:

(i) although the relative concentrations of oogonia, primary and secondary oocytes and mature ova vary somewhat at different stages of the reproductive cycle, large numbers of each are present in the gonad at all times of the year so that quantitative comparisons are difficult to make; separate developments, definable as activation, development and maturity phases in the female gonad are difficult to interpret and become recognizable only as a matter of considerable experience;

(ii) although oogonia are present throughout the entire reproductive cycle they are most common during the early development phases of the gonad;

(iii) mature ova predominate in the lumen of the mature gonad just prior to spawning; these ova are loosely arranged and there seems to be a breakdown of the follicle cells and haemal space within the lumen; oogonial cells and developing oocytes of all sizes may still be present in the lumen of mature gonads but there is a substantial reduction in their numbers;

Figure 26 - Histological features of the immature gonad of *Cucumaria frondosa*.

a..... individual weighing 5-10 grams. February, 1972; 180X .

b & c..... individuals weighing 25-30 grams. February, 1972; 70X .

d..... individual weighing 19.9 grams; note the presence of oocytes. February,1972; 75X .

abbreviations, in alphabetical order:
GE - germinal epithelium
HS - haemal space
L - lumen
PO - primary oocyte
SO - secondary oocyte
VP - visceral peritoneum

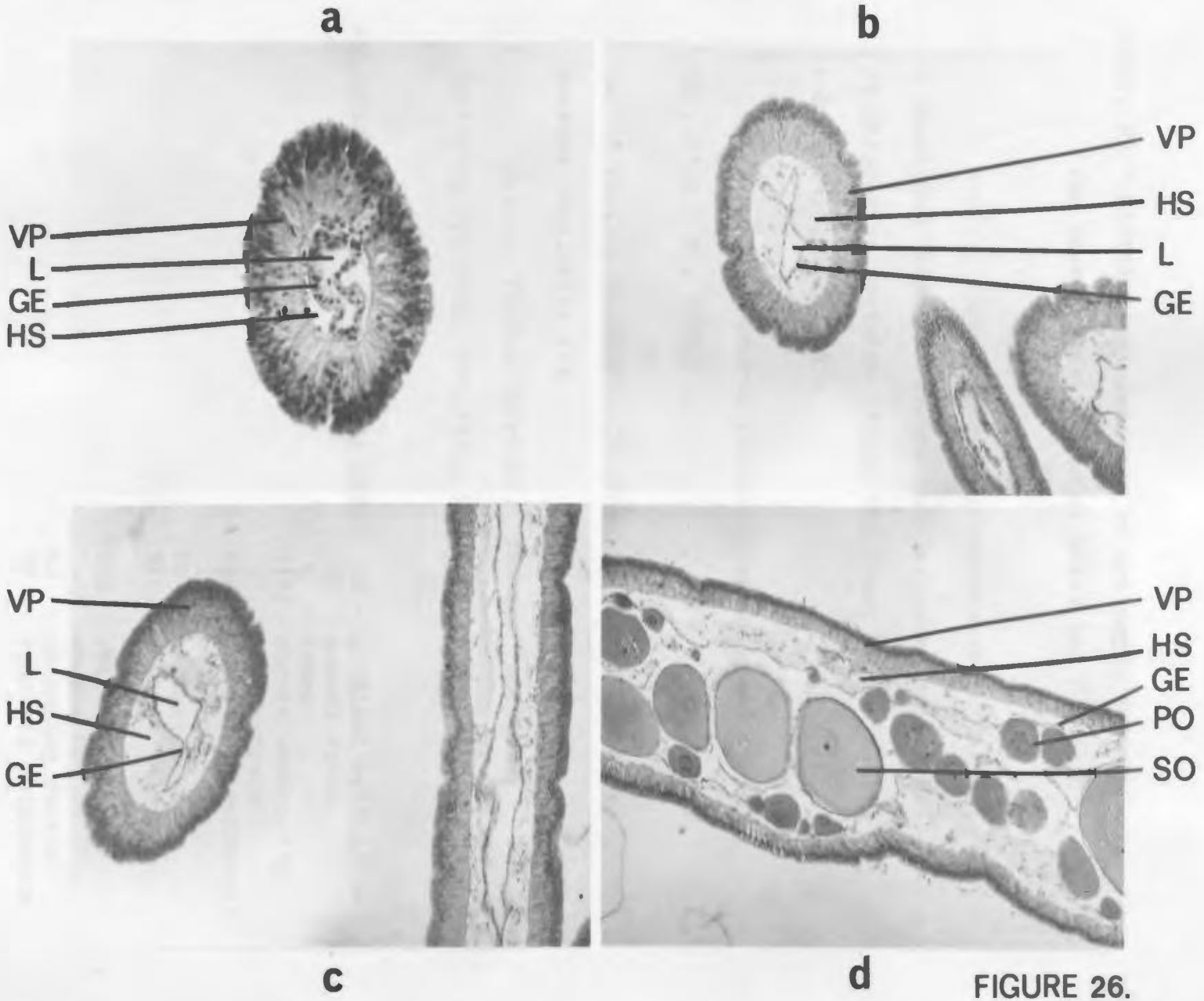

FIGURE 26.

Figure 27 - Histological features of the spent stage of the reproductive cycle of males and females.

a..... *Psolus fabricii;* cross-section through a spent and "remnant" mature tubule; note the presence of phagocytic activity within the lumen of the mature tubule. May,1972; 70X .

Б..... *Cucumaria frondosa;* spent gonad tubules. May,1972; 75X . (male) .

c..... *Cucumaria frondosa;* "remnant" primary oocytes. May,1972; 75X .

d..... *Psolus fabricii;* "remnant" oocytes following spawning. May,1972; 90X .

abbreviations, in alphabetical order:
GE - germinal epithelium
HS - haemal space
L - lumen
O(P)- oocyte undergoing phagocytosis
PAC - phagocytic accessory cells
PO - primary oocyte
RFL - remnant follicle layer
RSZ - residual spermatozoa
SO - secondary oocyte
VP - visceral peritoneum

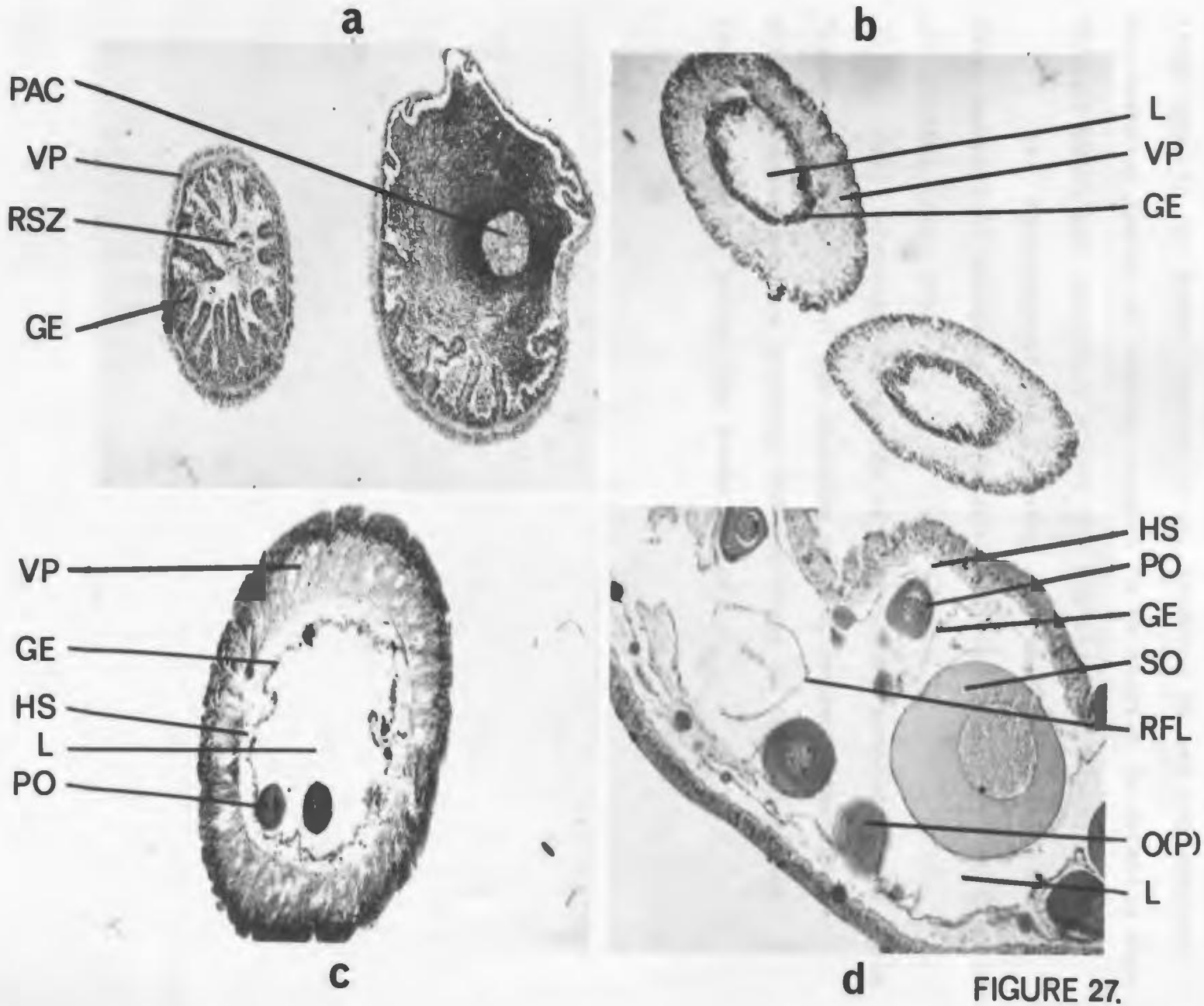

FIGURE 27.

(iv) spawning animals may be characterized by the presence of large gaps in the lumen created by the shedding of ova and oocytes; the spawning period of females appears to be shorter in duration than that of males and commences around the beginning of March;

(v) histological and spawning observations indicate that large numbers of immature oocytes are shed with mature ova at the time of spawning (Fig. 20b);

(vi) although the gonads of individuals sampled in the field "recover" rapidly after the spawning period, renewed gametogenic activity subsequent to spawning did not occur in lab-maintained females up to the end of the study period.

TABLE II

The Monthly Percentages in Five Histological Stages

of Reproduction, of Male *Cucumaria Frondosa*

Sampled at Outer Cove

Month	Total Number of Males Examined	Reproductive Stage				
		Activation	Development	Mature	Spawning	Spent
August 1971	15	-	100%	-	-	-
September 1971	13	-	92%	8%	-	-
October 1971	7	-	86%	14%	-	-
November 1971	9	-	33%	67%	-	-
December 1971	10	-	-	100%	-	-
January 1972	7	-	-	100%	-	-
February 1972	9	-	-	44%	56%	-
March 1972	17	-	-	47%	53%	-
April 1972	19	-	-	17%	66%	17%
May 1972	17	-	-	6%	12%	82%
June 1972	15	60%	13%	-	-	27%
July 1972	14	7%	86%	-	-	7%
August 1972	14	-	100%	-	-	-

TABLE III

The Monthly Percentages in Five Histological Stages of Reproduction, of Male *Cucumaria frondosa* Maintained in the Laboratory

Month	Total Number of Males Examined	Reproductive Stage				
		Activation	Development	Mature	Spawning	Spent
August 1971	6	-	100%	-	-	-
September 1971	5	-	100%	-	-	-
October 1971	2	-	100%	-	-	-
November 1971	3	-	-	100%	-	-
December 1971	5	-	-	100%	-	-
January 1972	5	-	-	100%	-	-
February 1972	7	-	-	57%	43%	-
March 1972	7	-	-	43%	57%	
April 1972	6	-	-	-	67%	33%
May 1972	5	-	-	-	-	100%
June 1972	5	-	-	-	-	100%
July 1972	2	-	-	-	-	100%
August 1972	6	-	-	-	-	100%

TABLE IV

The Monthly Percentages in Five Histological Stages of Reproduction, of Male *Psolus fabricii* Sampled at Logy Bay

Month	Total Number of Males Examined	Reproductive Stage				
		Activation	Development	Mature	Spawning	Spent
August 1971	14	14%	86%	-	-	-
September 1971	12	-	100%	-	-	-
October 1971	-	-	-	-	-	-
November 1971	6	-	33%	67%	-	-
December 1971	7	-	43%	57%	-	-
January 1972	7	-	-	100%	-	-
February 1972	10	-	-	80%	20%	-
March 1972	10	-	-	50%	50%	-
April 1972	7	-	-	29%	71%	-
May 1972	13	-	-	-	8%	92%
June 1972	9	11%	-	-	-	89%
July 1972	10	100%	-	-	-	-
August 1972	9	22%	78%	-	-	-

DISCUSSION

Gonad index, the ratio of gonad volume or gonad weight to the total wet weight of the animal is a widely accepted method of following the variation in gonad size which accompanies reproductive developments in echinoderm species. In situations where the gonad attains maximum size in the mature animal just prior to spawning and is smallest in the spent individual following the spawning period, there is a general increase in gonad index throughout the development and maturation phases of the reproductive cycle. Tanaka (1958), for example, in studies of the holothurian *Stichopus japonicus* through consecutive reproductive cycles found a recurring pattern during each cycle of steadily increasing gonad index from minimal values when the gonads were determined histologically to be spent to maximum values when they were mature, these values falling rapidly during the "shedding" period. Similar patterns of variation in gonad index are common in other species of echinoderms (Mitsukuri, 1903; Lasker and Giese, 1954; Bennett and Giese, 1955; Tanaka, 1958; Giese, 1959; Fuji, 1960; to name a few).

Results from the present study show that gonad index values for *Cucumaria frondosa* collected at Outer Cove (Fig. 8) remain relatively steady during the development and maturation phases of the reproductive cycle, drop slightly with spawning and reach maximal values immediately following the spawning period when few sex cells are present in the gonads. An increase in gonad size does not occur during the maturation and build-up of sex cells in the gonads;

instead, there is a decrease in average monthly gonad volume during this period. Rather than a reduction in gonad size with spawning there is an increase commencing from minimal values at the onset of spawning, continuing to rise, with maximum levels reached just after the spawning period (Fig.14).

Gonad volume appears to be influenced to a very large extent by the thickness of the visceral peritoneum of the gonad wall rather than by variations in gametogenic activity. Measurements of the thickness of the visceral peritoneum throughout the reproductive cycle of *C. frondosa* at Outer Cove (Fig 17) indicate that maximum gonad volumes are reached at the same time as the visceral peritoneum attains maximum thickness, ie. just after the spawning period. Shrinkage and folding of the peritoneum with the release of gametes from the gonadal lumen is minimal and cannot of itself account for the phenomenal increase in gonad volume which occurs during the spawning and post-spawning periods. Macroscopic examination of the gonad tubules also shows that there is neither a reduction in the diameter of the tubules nor a shortening in the length of the tubules during these periods. The steady reduction in gonad volume which occurred during the development and maturation phases of the gonad is accompanied by a steady decrease in the thickness of the visceral peritoneum. The increase in gonad volume during the spawning period is also accompanied by an increase in the thickness of the visceral peritoneum. The presence of numerous globular inclusions throughout the visceral peritoneum suggests that the layer may act as a site of food storage. The layer thickens at the time of the spring phytoplankton "bloom"

when food supplies are greatest and there is a gradual reduction in the thickness of the visceral peritoneum with the development of sex cells.

Systematic variations in the biochemical makeup of the main organs of echinoderm species have been correlated with different stages of the reproductive cycle by various authors. Data on the starfish *Pisaster ochraceus*, *Odontaster validus* and *Leptasterias hexactis* indicate the accumulation of stored nutrient in the pyloric caecae which shrink as the gonads increase in size (Greenfield *et al.*, 1958; Pearse, 1965; Chia, 1969). In sea urchins large amounts of food material, primarily lipids, may be stored in the gonads (Hilts and Giese, 1949; Giese *et al.*, 1958; Giese *et al.*, 1964). In the echinoid *Sterechinus neumayeri*, the nutritive phagocytes of the gonad seem to serve the same function as the pyloric caecae of starfish, as nutrients stored in these cells appear to be utilized during times of low phytoproduction in order to maintain a relatively constant rate of gametogenesis (Pearse and Giese, 1966).

Histochemical studies of echinoid gonads have shown that ovaries generally contain more lipid than do testes (Pearse and Giese, 1966). In the present work, the thickness of the visceral peritoneum was found to be greater in female than in male individuals of the three groups studied and although a thorough histochemical analysis is warranted, this may reflect the greater food requirements of developing oocytes.

Throughout the development phases of the reproductive cycle there is also a rapid decline in the average monthly total wet weight

of the population of *C. frondosa* sampled at Outer Cove (Fig. 11). Minimal wet weight values occur at the same time as minimal gonad volumes (i.e. at the onset of the spawning period) and there is a rapid increase of 70-75 grams in total wet weight during the spawning period. Available information on phytoplankton concentrations in the ocean during the same time period shows that wet weight increases occur during the time of the spring phytoplankton "bloom" and fall steadily throughout the remainder of the year. This wet weight increase may simply indicate an increased amount of food material in the gut tract of these animals. On the other hand, it may indicate a build-up of body tissues as food storage sites at a time of year when food supplies are optimal.

Jordan (1972), in an ecological study of *Cucumaria frondosa* along the New England coast, found that the average size of animals on inshore rocks was smaller in fall and early winter than in the summer. He felt that the variations in average total wet weight were due to an offshore migration of larger animals. It seems unlikely, however, that the variations in total wet weight at Outer Cove during this study are due to migratory habits in the species as the degree and timing of wet weight changes in laboratory-confined animals of *C. frondosa* (Fig. 12) were similar to those experienced at Outer Cove.

Definite increments or reductions in the size of the gonad are not reflected in the gonad index itself. The pronounced seasonal fluctuations in gonad volume and total wet weight experienced during the annual reproductive cycle of *C. frondosa* (Outer Cove) result in

peak gonad indices after the spawning period when few sex cells are present in the gonad. Ripe individuals of *C. frondosa*, then, do not generally exhibit high gonad index values. Neither do animals exhibiting high gonad indices necessarily have ripe gametes.

Variation in gonad index in laboratory maintained *C. frondosa* during the same time period (Fig. 9) offers a worthwhile comparison to the situation at Outer Cove. Initial gonad index levels during the histologically-indicated development phases are very similar in value in the two groups. With spawning, however, the decrease in gonad index in the laboratory is more pronounced and the subsequent recovery in gonad index following spawning does not take place. Furthermore, the increase in gonad volume which followed spawning in the Outer Cove animals (Fig. 14) did not occur in the lab-maintained population (Fig. 15). There was, instead, a continued drop in the gonad volume of laboratory animals following spawning. This reduction in gonad size was accompanied by pronounced shrinkage, shortening and loss of coloration in the gonad tubules--changes which did not occur at Outer Cove. Shrinkage was not accompanied by thickening of the visceral peritoneum, which lends further support to the assumption that the thickening of the visceral peritoneum in Outer Cove animals is caused by the addition of material to the gonad wall and not simply to the folding of the wall with the release of sex cells.

Mean monthly total wet weights in laboratory animals (Fig. 12) increased to the same degree (70-75 grams) during the spring phytoplankton "bloom" as they did at Outer Cove. Whereas wet weight values for Outer

Cove animals remain relatively high after the plankton "bloom", these values drop rapidly in the laboratory population. Sergy (1972), in studies of fouling organisms in the seawater system of the MSRL, noted that much of the fresh phytoplankton material entering the reservoir prior to circulation throughout the laboratory appeared as dead and decaying debris in the wet benches and tables of individual laboratories. He attributed this condition to the concentrated feeding action of plankton feeders present in the piping network and partially to turbulence created locally in portions of the flow system. Starvation has been observed to retard development of the gonad in the starfish *Pisaster ochraceus* with no sex cells being formed (Fe⁄der, 1956). Greenfield *et al.* (1958) also reported that nutrition is definitely correlated with growth of the gonad in the sea urchin, *Strongylocentrotus purpuratus*. If indeed, the observed increases in wet weight in *C. frondosa* are due to increased food supply, it may be assumed that there is sufficient material (quantity) to allow for wet weight increases, yet an insufficient quality to this food to permit the rapid recovery and development of gonad tissue which occurs in the naturally occurring population after the spawning period.

J.W.Evans of the MSRL (pers. comm.),in comparisons of the growth rates of immature scallops (*Placopecten magellanicus* Gmelin.) at the MSRL and in Logy Bay, finds that growth rates are significantly slower in lab-maintained populations. He also concludes that nutrient deficiences in the laboratory seawater system retard normal development. A complete explanation of the degree of depreciation in food quality

occurring in the seawater system of the MSRL and the subsequent effects of this on the general physiology of animals being maintained in the laboratory for research purposes is an exceedingly complex one and warrants further investigation.

Individuals of *C. frondosa* maintained for up to four years at the MSRL do spawn annually. The failure of lab-maintained *C. frondosa* to commence renewed gametogenic activities subsequent to spawning and before August, 1972 during this study points out the need for careful determination of the fecundity, fertilizability and viability of eggs and sperm shed by animals such as these which undergo consecutive reproductive cycles under laboratory conditions.

Variation in gonad index recorded for *Psolus fabricii* at Logy Bay (Fig. 10) assumes the more "typical" pattern of steady increase with progressive stages of development and maturation. Gonad index reaches peak value just prior to the spawning period and decreases rapidly during the spawning period itself. Although index values rise during the development phases of the reproductive cycle, average monthly gonad volumes (Fig. 16) decrease. Gonad volumes remain low during the spawning period and do not show the rapid increase which occurred in the Outer Cove population of *C. frondosa*. Average monthly total wet weights (Fig. 13) and visceral peritoneum thicknesses (Fig. 19) both increase during the time of increased food supply associated with the spring phytoplankton "bloom." The degrees of increase are considerably less than were evident in the two experimental populations of *C. frondosa* and there is less appreciable correlation

between changes in gonad volume and changes in the thickness of the visceral peritoneum.

One of the more notable features of the annual reproductive cycle of both species is the seasonal variation in the number of accessory cells within the gonads. The importance of the dual role played by these "nutritive-phagocytes" (Hilts and Giese, 1965) during gametogenic development is seldom realized and warrants further investigation at the ultrastructural level.

The preponderance of these cells in the gonadal lumen of both sexes after the spawning period and during early development phases is most frequently associated with phagocytic activity. The resorption by phagocytes of residual germ cells is well known and has been extensively studied in pelecypod mollusks (Loosanoff, 1937, 1942; Tateishi and Adachi, 1957; Tranter, 1958; Marson, 1958). Post spawning phagocytic activity in the gonads of echinoids have been described by Holland and Giese (1965) and Fuji (1960) and similar activity in the gonads of holothurians has been outlined by Ohshima (1925) and Tanaka (1958). The large numbers of accessory cells at a time when few sex cells are present in the gonad makes interpretation of the gonad index difficult to make and the increase in gonad volume which occurs during this period of the reproductive cycle in *C. frondosa* and *P. fabricii* may be due, in part, to the increase in the number of these cells.

Although phagocytic activity is most common after the spawning period in both *C. frondosa* and *P. fabricii* it is by no means restricted

to this stage of the reproductive cycle. In males, phagocytic accessory cells may be present in the lumen of mature tubules and in females deteriorating oocytes surrounded by phagocytic cells (Fig. 25d) are common at all stages of the reproductive cycle. Phagocytic activity at these times probably represents a means of controlling the over-production of sex cells and this is especially significant in females where there is a limited attachment area for developing oocytes and where crowding would likely cause a retardation of their growth.

There is a close association of accessory cells with spermatogonia during activation and development phases of the male gonad (Fig. 21b). Phagocytic cells packed with globular inclusions and with what appear to be fragments of deteriorated oocytes may also be found in association with developing oocytes throughout the majority of the reproductive cycle in females of these species (Fig. 25). These cells are larger in the lumen of the female gonad during the early development phases of the reproductive cycle (Fig. 25a), indicating, perhaps, a reduction in material through absorption by developing oocytes as development progresses. Chatlynne (1969), in a histochemical study of oogenesis in the echinoid *Strongylocentrotus purpuratus*, felt that although indistinct borders occur between such phagocytes and growing oocytes, it remained unclear whether the condition represented an engulfment of the oocyte by the phagocyte or active transport of nutrients in the reverse direction. An ultrastructural investigation of the relationship of accessory cells to the surface of the developing oocyte in *Strongylocentrotus drobachiensis* by Bal (1970), however, revealed that

microvilli covering the surface of the oocyte do indeed derive nutrients from the accessory cells, thus demonstrating clearly the nutritive role of these cells.

Cucumaria frondosa and *P. fabricii* both exhibit annual reproductive cycles. Although the series of histological events comprising the reproductive cycle of females of these species is more difficult to follow than those in males (for reasons given earlier) it does seem as if the two sexes develop at different rates. Males attain the mature reproductive condition in the fall of the year several months prior to the commencement of spawning and gametogenic activity slows at this time. The high percentage of immature oocytes evident in females during months prior to the spawning period, on the other hand, implies continued growth and development of oocytes during the same period.

In the North Sea, *C. frondosa* spawns during the months of February and March and in June and July farther north in Arctic waters (Runnstrom and Runnstrom, 1921). Along the New England coast it appears to spawn in mid-April (Jordan, 1972). Histological preparations of reproductive tissue throughout this study indicate that spawning in male individuals of both *C. frondosa* and *P. fabricii* lasted for approximately three months from the beginning of February until the beginning of April with the majority of spawnings occurring during the latter part of this period (Tables II-IV). Completely spent individuals were not evident until April and May so that intermittent or repetitive spawnings, as observed in the laboratory, would seem to be an important aspect of the spawning process. The spawning

period of females appears to be shorter in duration than that of males. Whereas males maintained in the laboratory were observed spawning in the laboratory during February, females were not observed spawning until the second week in March. Histological indications, also, for both laboratory and field populations indicate that the vast majority of spawnings in February are by males.

The recognition of the physical factors regulating the reproductive cycle and which induce spawning is important to an understanding of the reproduction and fundamental ecology of marine invertebrates. Orton (1920) has shown that a broad correlation exists between sea temperature and breeding in marine animals. Thorson (1950) presents a solid basis for a biological rule of the greatest importance, namely, "that among marine invertebrates shedding their eggs and sperm freely in the water, the males are the first to spawn; thus stimulating the females to shed their eggs which, shed directly into a suspension of sperm of their own species have an especially good chance of being fertilized." Fox (1924) found that the reproductive periodicity of the sea urchin *Diadema setosum* is affected by the lunar cycle. Dense accumulations of phytoplankton may be prerequisite to the mass spawning of certain populations of benthic invertebrates (Miyazaki, 1938; Thorson, 1936, 1946; Pearse, 1966; Schoener, 1968; Vernberg and Vernberg, 1970). Where phytoproduction is markedly seasonal the release of sex cells at a time when food supply is optimal is especially important to benthic species having planktotrophic larvae. In certain species migrations or spawning

aggregations occur prior to spawning (Stott, 1931). Inshore species exposed to changes in the salinity and/or pH of seawater by heavy freshwater runoff may spawn at times of the year particularly suited to the survival of sex cells (Thorson, 1950).

Cucumaria frondosa and *P. fabricii*, as already noted, are spring spawners. Temperature levels during the spawning period are at minimal levels for the year and fluctuate between -1.25°C and 0°C. Spawning may also be associated with the period of maximum phyto-production in these waters--the spring phytoplankton "bloom." As the larvae of these species are essentially lecithotrophic, spawning at the time of optimal food levels represents no particular advantage for the free swimming larvae. It seems likely, however, that continued activity and development of the gonad depends on an adequate food supply and fluctuations in nutrient levels may result in variations in the size of the gonads and the numbers of sex cells produced from year to year.

Temperature, photo-period and sex cell experiments aimed at inducing spawning in *C. frondosa* during this study failed to do so and further induction studies are required. Jordan (1972) found that spawning was induced in laboratory female *C. frondosa* exposed to a temperature drop of 2°C and then warmed in a simulated plankton bloom created by the introduction of dense concentrations of *Dunaliella* sp. It seems likely, from the present study, that the annual reproductive cycles of *C. frondosa* and *P. fabricii* are more closely aligned with nutritive factors rather than to demonstrable physical parameters of

the environment so that the spawning period of these species will probably vary slightly in timing and duration from year to year. Reproductive synchrony in any one species, as it relates to the physical or chemical parameters controlling it, are only recognizable with extensive comparisons of different populations from different areas and the final evaluation of whether temperature or phytoproduction or both or neither regulate the timing of spawning of these animals will require further investigation.

Although the age-growth structure of populations of *C. frondosa* and *P. fabricii* in Newfoundland waters requires examination, both species are plankton feeders so that growth rates within various age groups of a single population should be relatively uniform. Data on the presence of sex cells in the gonads of *C. frondosa* of various size categories less than 70.0 grams total wet weight, as given in Table I, imply that first maturity in this species is reached at various ages. The factors which induce first maturity in this species are unknown, and judging from the incidence of immature individuals in all size categories of individuals less than 55.0 grams total wet weight, the recognition of such factors will be a difficult one to make.

Behavior aspects of the spawning process are similar in both *C. frondosa* and *P. fabricii*. The extended body posture, general orientation to water currents, cessation of feeding activity and enlarged dorsal genital papilla undoubtedly serve to assure that sex cells are released in the water column away from both the ocean

floor and the feeding appendages of the parent. In males, the several openings of the gonoduct to the exterior aid in the dispersion of the free-swimming sperm. Repetitive spawnings by both sexes help to increase the chances of successful fertilization of the ova during a prolonged spawning period.

The populations of *C. frondosa* and *P. fabricii* sampled during the study were composed of approximately equal numbers of males and females so that random sampling of adequate numbers of these species generally resulted in a sufficient ratio of both sexes to meet research needs. Although recognition of the difference in the appearance of the genital papilla between sexes (Fig. 20) should make it easier for SCUBA-equipped divers to collect individuals according to sex, such a distinction can only be made when the animals assume a feeding posture and the method may therefore prove inadequate during the late fall and early winter months when feeding activities are infrequent in these species.

SUMMARY AND CONCLUSIONS

1. Populations of *Cucumaria frondosa* and *Psolus fabricii* were sampled with SCUBA equipment twice monthly from August, 1971 through August, 1972. A number of individuals of both species were also collected and maintained in the free-flowing seawater system of the MSRL for purposes of general observation and comparative investigation.

2. The structure of the gonad wall consists of the same cellular elements throughout the year, varying only in width. The gonad wall elements are the same in both male and female individuals and are similar in both species; from outside to inside, these are: visceral peritoneum, nerves, muscles, haemal fluid (lined on either side by the extrahaemal and intrahaemal basal lamina) and the germinal epithelium.

3. The histological elements of the gonad wall of immature individuals are similar to those of mature individuals with the obvious difference of an "inactive" germinal epithelium.

4. *Cucumaria frondosa* and *Psolus fabricii* both exhibit an annual reproductive cycle.

5. Reproductive developments in male individuals of both species were divided into five histological stages defined on the basis of spermatogenic activities occurring in the germinal epithelium of the gonad wall.

6. Active gametogenesis in male *C. frondosa* collected at Outer Cove commenced in early June and continued for approximately six months until the end of November when spermatogenesis slowed as the animals attained maturity. The first indications of spawning occurred in early

February and spawnings continued for three months until the beginning of May. Completely spent individuals were not evident until April so that intermittent spawnings would appear to be a common feature of the spawning process. Gametogenic activity commenced in early June following the spawning period.

7. Spermatogenic activities in *P. fabricii* sampled at Logy Bay were similar in timing and duration to those of *C. frondosa*. Spawnings were not as frequent during the early part of the spawning period and completely spent individuals were not evident until May.

8. Although gametogenic developments in females are more complex and less clearly definable than those of males, it does seem as if the two sexes develop at different rates. Males attain the mature reproductive condition several months prior to spawning whereas the final maturation divisions of developing oocytes which give rise to fertilizable ova do not occur until just before spawning itself. Males also appear to spawn slightly earlier.

9. The chronology of reproductive developments in lab-maintained individuals of both species differed in that the spawning period was shorter (just slightly) and the recovery rate after spawning slower.

10. There is a pronounced variation in the numbers of accessory cells (nutritive phagocytes) within the lumen of the gonads of both sexes throughout the annual reproductive cycles of these species.

11. Variations in gonad size appear to be influenced to a very great extent by the thickness of the visceral peritoneum of the gonad wall rather than by variations in the number of sex cells within the gonadal lumen. The visceral peritoneum may act as a site of food

storage. The layer thickened at the time of the spring phytoplankton "bloom" and diminished in thickness with the development of sex cells. The layer is also generally thicker in female individuals than in male individuals of the same species, which may indicate the greater food requirements of developing oocytes.

12. The failure of lab-maintained animals to commence renewed gametogenic activity subsequent to spawning is a likely result of nutrient deficiences created by the concentrated feeding action of biofouling organisms in the piping system of the laboratory's seawater supply.

13. The mean monthly total wet weight of both species increased considerably during the time of the spring phytoplankton "bloom" and decreased steadily during the remainder of the year. This increase is a reflection of the increased amount of food material in the gut tracts of these animals and may also be due to the build-up of body tissues (gonad, intestine, body wall) as food storage sites at a time of optimal food supply.

14. Variations in gonad index do not reflect the changes in gonad size accompanying gametogenic developments in these species.

15. Males and females of these species may be distinguished externally by the general appearance of the genital papilla. In females, the opening of the gonoduct appears as a single tubular opening. In males, there are several openings of the gonoduct which appear as minute projections of the genital papilla.

16. The populations of *C. frondosa* and *P. fabricii* sampled during the study were composed of approximately equal numbers of both

sexes.

17. Immature individuals of *C. frondosa* were common in all size ranges of individuals less than 55.0 grams total wet weight.

18. Individuals of both species were observed spawning in the laboratory. Behavioral aspects of the spawning process serve to enhance the dispersal, survival and chance of fertilization of released sex cells.

19. Variations in temperature and photoperiod conditions and the introduction of male sex cells were attempted unsuccessfully as methods of inducing spawning in *C. frondosa* .

20. The timing of the spawning process in *C. frondosa* and *P. fabricii* appears to be more closely related to nutritive factors associated with the spring phytoplankton "bloom" rather than to demonstrable physical parameters of the environment.

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APPENDIX

APPENDIX I - Data Summary - *Cucumaria frondosa*
Sampled at Outer Cove

Month	Sampling Dates	Water Temperature (°C)	Sex Ratio ♂	Sex Ratio ♀	Mean Monthly Gonad Volume (ml.)	Mean Monthly Total Wet Weight (gm.)	Mean Monthly Gonad Index	95% Confidence limits for G.I. $\bar{x} \pm 1.96\ \sigma/\sqrt{n}$	Mean Monthly Thickness of the Visceral Peritoneum (μ) ♂	♀	Both Sexes
August	4-8-71	-	10	5	11.7	183	6.6	0.69	74	132	102
	16-8-71	-	5	10							
September	3-9-71	12.50	8	7	10.0	147	6.7	0.84	53	109	85
	17-9-71	11.50	5	10							
October	-	-	-	-	8.8	137	6.6	0.68	52	108	82
	17-10-71	9.00	7	8							
November	2-11-71	6.00	9	6	7.7	119	6.4	1.20	43	91	61
	-	-	-	-							
December	1-12-71	4.00	4	11	7.7	116	6.5	0.70	39	77	64
	16-12-71	2.50	6	9							
January	-	-	-	-	8.0	118	6.9	1.08	35	69	54
	12-1-72	0.25	7	8							
February	7-2-72	-1.00	4	11	5.8	102	5.6	0.80	29	58	49
	16-2-72	-1.00	5	10							

APPENDIX I - (Continued)

Month	Sampling Dates	Water Temperature (°C)	Sex Ratio ♂	Sex Ratio ♀	Mean Monthly Gonad Volume (ml.)	Mean Monthly Total Wet Weight (gm.)	Mean Monthly Gonad Index	95% Confidence limits for G.I. $\bar{x} \pm 1.96\ \sigma/\sqrt{n}$	Mean Monthly Thickness of the Viserceral Peritoneum (μ) ♂	♀	Both Sexes
March	3-3-72	-1.25	7	8	6.5	117	5.4	0.73	35	58	49
	21-3-72	-0.50	10	5							
April	7-4-72	-0.75	9	6	8.9	173	5.2	0.58	55	71	61
	21-4-72	-0.25	10	5							
May	5-5-72	0.25	7	8	9.1	155	6.0	0.74	95	128	98
	16-5-72	0.00	10	5							
June	1-6-72	3.00	7	8	15.4	157	10.0	0.78	106	153	128
	16-6-72	3.75	8	7							
July	4-7-72	7.0[illegible]	9	6	11.1	157	7.0	0.90	79	145	111
	13-7-72	7.50	5	10							
August	4-8-72	11.50	6	9	11.4	176	6.5	0.67	70	109	91
	18-8-72	10.00	8	7							

APPENDIX II - Data Summary - *Cucumaria frondosa* Maintained in the Laboratory

Month	Sampling Dates	Water Temperature (°C)	Sex Ratio ♂	Sex Ratio ♀	Mean Monthly Gonad Volume (ml.)	Mean Monthly Total Wet Weight (gm.)	Mean Monthly Gonad Index	95% Confidence limits for G.I. $\bar{x} \pm 1.96\ \sigma/\sqrt{n}$	Mean Monthly Thickness of the Visceral Peritoneum (μ) ♂	♀	Both Sexes
August	5-8-71	-	4	1	12.4	175	7.2	2.12	72	146	101
	16-8-71	-	2	3							
September	3-9-71	13.00	2	3	11.2	160	7.1	0.81	58	108	95
	16-9-71	12.50	3	2							
October	-	-	-	-	8.5	164	5.5	2.02	51	114	89
	15-10-71	9.00	2	3							
November	3-11-71	6.00	3	2	9.8	159	6.0	1.79	35	87	77
	-	-	-	-							
December	1-12-71	4.25	2	3	9.7	156	6.2	0.73	46	87	65
	16-12-71	2.50	3	2							
January	6-1-72	0.50	2	3	8.1	134	6.0	0.78	37	82	59
	17-1-72	0.25	3	2							
February	4-2-72	-1.00	3	2	8.7	124	7.0	1.45	32	41	34
	16-2-72	0.00	4	1							

APPENDIX II - (Continued)

Month	Sampling Dates	Water Temperature (°C)	Sex Ratio ♂	Sex Ratio ♀	Mean Monthly Gonad Volume (ml.)	Mean Monthly Total Wet Weight (gm.)	Mean Monthly Gonad Index	95% Confidence limits for G.I. $\bar{x} \pm 1.96\ \sigma/\sqrt{n}$	Mean Monthly Thickness of the Visceral Peritoneum (μ) ♂	♀	Both Sexes
March	1-3-72	-1.00	3	2	8.9	151	5.9	1.02	27	55	35
	18-3-72	-0.75	4	1							
April	6-4-72	-0.75	3	2	5.1	171	2.9	0.81	37	61	41
	17-4-72	0.00	3	2							
May	1-5-72	0.00	2	3	4.1	200	2.1	0.35	47	53	50
	16-5-72	0.50	3	2							
June	1-6-72	2.00	3	2	5.3	172	3.2	0.73	40	46	43
	15-6-72	4.25	2	3							
July	4-7-72	8.50	2	3	6.1	155	4.0	1.49	46	70	65
	14-7-72	8.50	0	5							
August	4-8-72	11.50	3	2	4.2	153	2.8	0.90	41	48	45
	18-8-72	10.50	3	2							

APPENDIX III - Data Summary - *Cucumaria frondosa*
Collected at Logy Bay

Month	Sampling Dates	Water Temperature (°C)	Sex Ratio ♂	Sex Ratio ♀	Mean Monthly Gonad Volume (ml.)	Mean Monthly Total Wet Weight (gm.)	Mean Monthly Gonad Index	95% Confidence limits for G.I. $\bar{x} \pm 1.96\ \sigma/\sqrt{n}$	Mean Monthly Thickness of the Visceral Peritoneum (μ) ♂	♀	Both Sexes
August	6-8-71	-	7	3	10.3	137	7.5	0.72	33	84	48
	17-8-71	-	7	3							
September	6-9-71	12.00	6	4	9.0	128	7.3	0.79	26	67	44
	16-9-71	11.50	6	4							
October	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-							
November	3-11-71	6.00	6	4	9.4	126	7.4	0.86	32	65	44
	-	-	-	-							
December	-	-	-	-	9.0	110	8.4	1.32	25	51	34
	17-12-71	2.00	7	3							
January	-	-	-	-	8.5	101	8.8	2.02	26	62	39
	10-1-72	0.25	7	3							
February	1-2-72	-1.00	5	5	7.7	100	8.0	1.02	26	55	41
	14-2-72	-1.00	5	5							

APPENDIX III - (Continued)

Month	Sampling Dates	Water Temperature (°C)	Sex Ratio ♂	Sex Ratio ♀	Mean Monthly Gonad Volume (ml.)	Mean Monthly Total Wet Weight (gm.)	Mean Monthly Gonad Index	95% Confidence limits for G.I. $\bar{x} \pm 1.96\ \sigma/\sqrt{n}$	Mean Monthly Thickness of the Visceral Peritoneum (μ) ♂	♀	Both Sexes
March	1-3-72	-1.25	5	5	6.4	110	5.8	0.89	24	51	37
	20-3-72	-0.75	5	5							
April	7-4-72	-0.75	1	9	6.8	125	5.2	0.56	20	51	38
	21-4-72	-0.25	6	4							
May	1-5-72	-0.25	6	4	6.0	133	4.7	0.48	44	89	60
	15-5-72	0.00	7	3							
June	1-6-72	2.00	4	6	6.9	116	6.0	0.85	41	87	68
	15-6-72	3.50	5	5							
July	4-7-72	7.00	4	6	8.1	117	6.9	0.72	33	87	58
	14-7-72	8.00	6	4							
August	4-8-72	11.00	5	5	8.6	116	7.5	0.81	30	78	54
	18-8-72	10.00	4	6							