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DEVELOPMENT AND REPRODUCTIVE BIOLOGY OF THE FEATHER STAR *FLOROMETRA SERRATISSIMA* (ECHINODERMATA: CRINOIDEA)

BY

PHILIP V. MLADENOV

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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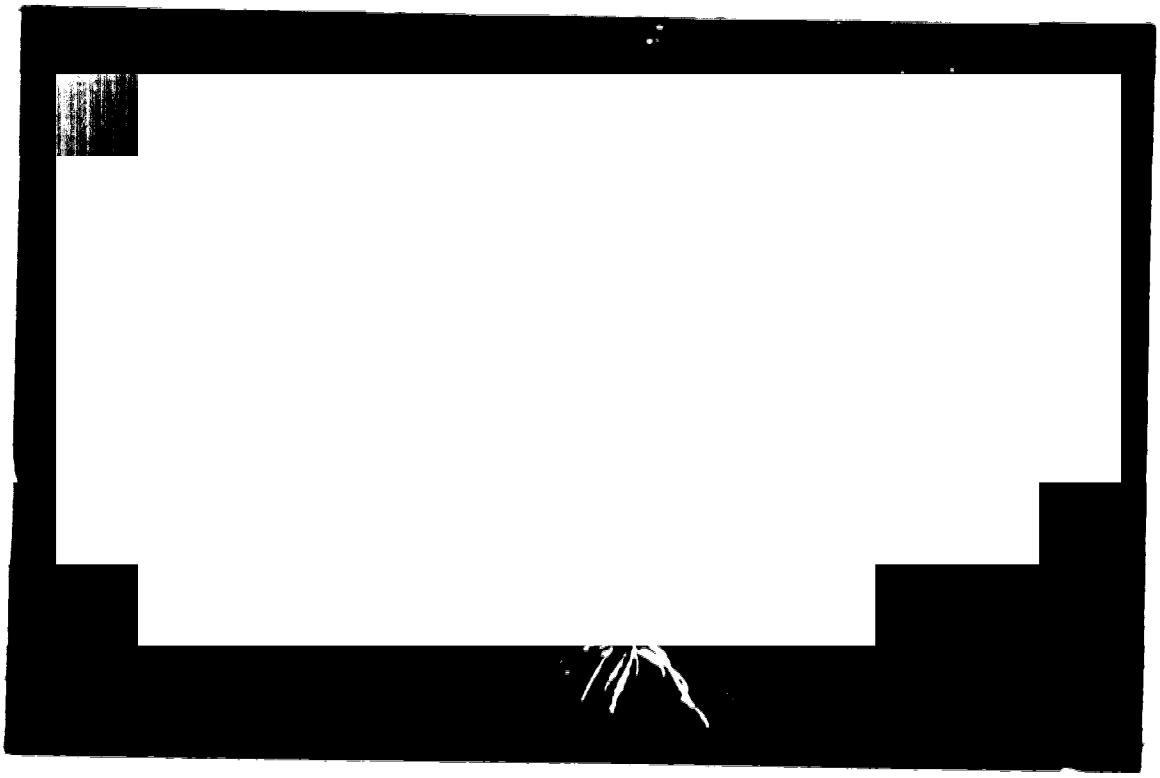

Florometra serratissima (A.H. Clark)

ABSTRACT

Research on the development and reproductive biology of the 10-armed comatulid crinoid Florometra serratissima (A.H. Clark) was carried out at the Bamfield Marine Station, Bamfield, British Columbia from November 1977 to December 1979. All specimens were collected using S.C.U.B.A. from a population of roughly 12,800 individuals found at depths of from 17 m to 34 m in waters adjacent to the Marine Station.

F. serratissima is normally dioecious, but anomalous hermaphrodites do exist. The gonads, which are located within the pinnules on the lower two-thirds of each arm, consist of an outer layer, an inner layer enclosing a gonadal lumen, and an intermediate haemal space. Oogonia originate on the luminal surface of the inner layer and differentiate into oocytes which bulge into the haemal space within a fibrous outpocketing (germinal lamina) derived from a basal lamina underlying the inner layer. Each oocyte remains attached to the inner layer by a stalk. Oocytes stay intimately associated with the haemal space throughout growth, which suggests that nutrients reach the oocytes via the haemal space. Prior to spawning, oocytes squeeze through a small opening in the inner layer (ovulation) and enter the ovarian lumen where maturation is completed. Evidence indicating that the germinal lamina is actively involved in this process is presented. Gametes are shed from nipples which form on the genital pinnules just before spawning. Unspawned ova and defective oocytes are phagocytized by the inner layer.

All of the genital pinnules on an individual are in reproductive synchrony. The population exhibits continuous reproduction, the first

time that such a breeding pattern has been demonstrated in a crinoid. About one-fifth of the female population is preparing to spawn at any one time. In most individuals, gametogenesis takes place continuously. In some, however, a lowering of reproductive activity occurs during the winter, but this phenomenon is not evident at the level of the population. A female spawns at least nine times per year, and it is estimated that a large specimen would release roughly 214,000 ova during this interval. The hypothesis that continuous reproduction in F. serratissima is a response to a low fecundity imposed by body structure, combined with a pelagic mode of development, is discussed.

F. serratissima undergoes embyronic development within a ridged fertilization membrane. A uniformly ciliated (UC) larva hatches from the fertilization membrane commencing 35 h after fertilization (9.5° to 11.5°C), and by four days there is a doliolaria. Doliolaria begin to settle as early as 4.6 days, but settlement can be delayed for up to nine more days. Settlement occurs gregariously in culture. The possible significance of gregarious settlement in the formation of adult aggregations is considered. Following settlement, there is a rapid metamorphosis into a stalked cystidean. After sixteen days, five oral plates on the cystidean open and 15 papillate tube feet, which are utilized in food capture, are extended into the surrounding water. This marks the beginning of the pentacrinoid stage using the terminology of this report. Usage of the term pentacrinoid by previous workers is discussed. Rudiments of all 10 arms of the adult are present in the four-month-old pentacrinoid. By six months, pentacrinoids have an arm span of 6.5 mm, but cirri and pinnules are not yet present.

The growth rate of F. serratissima increases exponentially in pentacrinoids, juveniles and young adults. Individuals become sexually mature at about four years of age, and attain maximum size at about eight years of age. Growth is likely determinate. Just under 80% of all adults have at least one regenerating arm. Arm loss followed by regeneration is likely a result of attacks by the sea star Pycnopodia helianthoides and the crab Oregonia gracilis. There is some evidence that the rate of regeneration per arm in F. serratissima decreases slightly as the total number of regenerating arms on an individual increases.

INTRODUCTION

The major theme of this research is development and reproduction in the feather star Florometra serratissima (A.H. Clark). I believe that a study of this kind is useful for three reasons. Firstly, virtually nothing is known about any aspect of the biology of F. serratissima, the lone representative of the Crinoidea on the west coast of North America, and a species which is of considerable interest in its own right. Secondly, and perhaps more importantly, the Crinoidea are the least understood class of the Echinodermata, and any information gathered on the biology of F. serratissima will contribute to a better understanding of the Crinoidea as a whole. Thirdly, and most generally, an increased knowledge of the Crinoidea will ultimately serve to clarify the relationship between the crinoids and the other echinoderm classes.

Development and reproduction is a broad subject, and I have therefore found it convenient to deal with various aspects of the topic separately, with the result that this thesis comprises four independent papers. Taken together, however, I feel that these papers provide a coherent account of reproduction and development in F. serratissima. In each paper, I have attempted a thorough review of the literature, much of which is quite old. Although this would be a monumental task with regards to the other echinoderm classes, one of the satisfactions provided by working with the Crinoidea is that it is still possible for an individual to become familiar with much of the pertinent literature.

The opening paper deals with the gross structure and microanatomy of the reproductive organs which, in turn, leads to a discussion of the

problems of nutrient transport to developing oocytes and the mechanism of oocyte ovulation. The second paper examines the breeding pattern of F. serratissima at both the level of the population and in individuals in some detail. The third paper, the longest of the four, provides a description of development in F. serratissima from the fertilized egg to the late pentacrinoid, introduces the fascinating topic of larval behaviour in this species, and discusses problems associated with the terminology of crinoid developmental stages. The final paper deals with a variety of subjects including growth rates, age at sexual maturity, life span, regeneration and predation, all of which are important, either directly or indirectly, to an overall understanding of reproductive processes in F. serratissima.

This work is by no means complete, and I fear that it raises more questions than it provides answers. This is the nature of science, however, and I will be satisfied if this study stimulates just a few readers to attempt to answer some of the questions.

ACKNOWLEDGEMENTS

My decision to work with this crinoid was an outcome of several discussions carried out with my supervisor Dr. Fu-Shiang Chia at the outset of my field work. I am grateful for those discussions, and for Dr. Chia's subsequent advice, encouragement, and understanding offered throughout this study.

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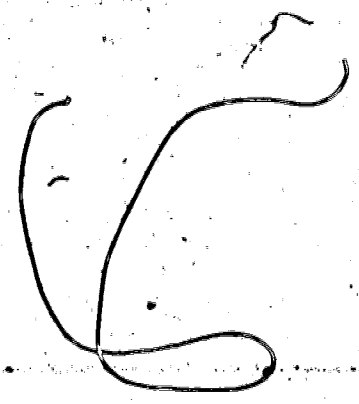

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Gonadal Structure, Oocyte Development, Ovulation and Spawning in the Feather Star *Florometra serratissima* (Echinodermata: Crinoidea)

ABSTRACT

Florometra serratissima (A. H. Clark) is normally dioecious, although anomalous hermaphrodites are occasionally encountered. The gonads, which are located within the genital pinnules on the lower two-thirds of each of the 10 arms, consist of an outer layer, an inner layer enclosing a gonadal lumen, and an intermediate haemal space. The outer layer and inner layer are derived from the genital tube and genital cord, respectively, which are located in the genital coelomic canal of the arms.

The inner layer of the ovary consists of a squamous epithelium. Oogonia originate individually on the luminal surface of the inner layer, whereas oocytes bulge into the haemal space. Each oocyte is contained within a fibrous outpocketing (germinal lamina) of a basal lamina underlying the inner layer, and each is attached to the inner layer by a short stalk. As an oocyte grows, the inner layer becomes folded in such a way that a narrow channel from the haemal space is created that nearly completely surrounds the oocyte. Such close association between an oocyte and the haemal space suggests that nutrients reach the oocyte by way of the haemal space.

Prior to spawning, each large oocyte becomes markedly deformed while passing through a small hole in the inner layer to enter the ovarian lumen where maturation is completed. This process is called ovulation. Evidence suggesting that the mechanism of ovulation lies with the germinal lamina is presented.

Gametes are shed from the tips of nipples which form on the distal side of each genital pinnule just before spawning. Unspawned ova and

defective oocytes undergo cytolysis in the ovarian lumen and the resulting fragments are phagocytized by the inner layer.

INTRODUCTION

Much of the early information relating to the structure of the reproductive organs in crinoids was derived from observations made on species of Antedon from British and Mediterranean waters. Dujardin (1835, cited by Perrier, 1886) and Thompson (1836) were the first to note that the ovaries in feather stars are located in the pinnules, but they were unsure of the position of the testes. Johannes Müller (1841, cited by Perrier, 1886) showed that the sexes are separate and that both the ovaries and testes are contained in certain of the pinnules, known as genital pinnules. Müller also noted that a cord, which he believed to be a nerve, runs the length of each arm. Semper (1875, cited by Ludwig and Hamann, 1907) and Carpenter (1876) determined that this was not a nerve but a genital cord which interconnects all of the gonads in the pinnules of the same arm. A detailed description of the position and structure of the genital cord was given by Ludwig (1877) and Hamann (1889), and their findings are clearly summarized in Ludwig and Hamann (1907), and briefly in Hyman (1955). In this report, the results of a light microscopic investigation of the reproductive elements in both the arms and pinnules of the feather star Florometra serratissima (A.H. Clark) are presented and compared to earlier work on other crinoid species. In addition, the distribution of the reproductive organs of two anomalous hermaphroditic individuals is described.

The differentiation and growth of oocytes of the feather star Antedon bifida were investigated by Chubb (1906) and Harvey (1930). A conspicuous basophilic organelle of uncertain origin and function called the yolk nucleus (= Balbiani body) is present in the cytoplasm of small

to medium-sized oocytes of this species. Holland (1976) studied the fine structure of the yolk nucleus of A. bifida and also reviewed previous work on the subject of the yolk nucleus which includes light microscopic observations by early researchers, and recent cytochemical and autoradiographic studies by Italian workers. According to Holland's unpublished observations (cited by Holland, 1976), a yolk nucleus is lacking in Florometra serratissima. In contrast, however, all specimens of F. serratissima examined over the course of this study were found to have yolk nuclei. In this paper, the relationship between the occurrence of the yolk nucleus in F. serratissima and oocyte size is investigated.

Holland (1971) studied the fine structure of immature ovaries of the West Indian feather star Nemaster rubiginosa. To date, this is the only published fine structural investigation of crinoid ovaries. Holland divided the ovary into an outer layer or visceral peritoneum, an intermediate layer containing the developing oocytes surrounded by haemal fluid, and an inner layer of squamous epithelium lining a tubular cavity called the ovarian lumen. The cytology of the inner layer of the ovary of the Japanese feather star Comanthus japonica was described using light microscopy by Holland et al. (1975). It was discovered that the oocytes do not lie free in the intermediate layer of the ovary as originally thought; instead, the inner edge of each oocyte is closely associated with the non-germinal cells of the inner layer. An extracellular coat, referred to as either an external lamina or a chorion, surrounds each developing oocyte. It was suggested, but not convincingly demonstrated, that this structure is continuous with a basal lamina underlying the inner layer. During the present

investigation, the inner layer of the ovary of Florometra serratissima was studied with light, scanning electron, and transmission electron microscopy. The nature of the association between the developing oocytes and the inner layer of the ovary is elucidated, and the functional consequences of this relationship discussed.

Holland and Dan (1975) have shown in Comanthus japonica that at the start of oocyte maturation each oocyte passes through the epithelial cells of the inner layer of the ovary and enters the ovarian lumen where maturation is completed. The dynamic cause of this process, termed ovulation, was not understood. Observations reported here on ovulation in F. serratissima strongly suggest that the extracellular coat surrounding the oocyte is dynamically involved in this process.

Maturation division processes in C. japonica were followed by J.C. Dan and K. Dan (1941) and Dan (1952). Behaviour at spawning has been observed in C. japonica (K. Dan and J.C. Dan, 1941) and Lamprometra klunzingeri (Fishelson, 1968). Gametes are released through one or more small holes which form on the side of each genital pinnule (Marshall, 1902; Chadwick, 1907; Ludwig and Hamann, 1907; K. Dan and J.C. Dan, 1941). This report describes observations made on F. serratissima concerning oocyte maturation, spawning in males and females, fecundity, and phagocytosis of unspawned and defective eggs.

Testicular structure and spermatogenesis in F. serratissima is dealt with only briefly in this paper. This subject has been carefully studied and reviewed by Bickell et al. (in press). However, a comparison of testicular and ovarian structure in F. serratissima in the light of new information gathered in this study is made.

MATERIALS AND METHODS

Specimens of Florometra serratissima (A.H. Clark) were obtained from a population living at a depth of from 17m to 34m at the mouth of Bamfield Inlet, Barkley Sound, British Columbia (48°50'07" N, 125°08'09" W). Collections were made on frequent occasions throughout the period from November 1977 to September 1979 using S.C.U.B.A. The animals were maintained in large tanks of running seawater at the nearby Bamfield Marine Station, Bamfield, British Columbia.

The gross anatomy of the genital pinnules of living animals was studied with a dissecting microscope. Genital pinnules were counted by placing a specimen in a shallow tray of water thereby forcing all 10 arms to be held in a stationary horizontal position. The number of ova present in the genital pinnules of four females just prior to spawning was calculated. To do this, genital pinnules were removed from along the length of the arms, dissected with fine forceps, and the ova present in each genital pinnule counted with the aid of a dissecting microscope and a tally.

Genital pinnules and short pieces of arm were fixed in seawater-Bouin's fluid for at least 24 h. The tissue was then dehydrated in isopropanol, embedded in Paraplast (Sherwood medical), serially sectioned at 7 µm, and stained with haematoxylin and eosin. In order to determine the relationship between oocyte size and the presence of a yolk nucleus, a female was taken from the population at roughly monthly intervals and a genital pinnule removed and prepared for histology in the manner just described. The diameter of the first 50 oocytes seen in nucleolar cross section in the histological sections of each genital pinnule was measured and the presence or absence of a yolk nucleus noted. Since

oocytes were usually oval in outline, the diameter was taken as the average of measurements made along both the long and short axis of each. These data were then grouped into eight size classes at 20 µm invervals from 1 µm to 180 µm, and the percentage of oocytes with and without a yolk nucleus in each size class plotted.

Several genital pinnules were prepared for scanning electron microscopy (SEM). Fixation was for 1 h at room temperature in 2.5% glutaraldehyde buffered in seawater. This was followed by a rinse in seawater, and postfixation for 2 h at room temperature in 2% OsO_4 in seawater. After a rinse in distilled water, the tissue was dehydrated in 30% and 50% ethanol and stored in 70% ethanol in the refrigerator until the material could be transferred to the University of Victoria where a critical point drier was available. Here, dehydration was completed, the tissue run through a 1:3, 1:1, 3:1 amyl acetate-ethanol series, transferred to 100% amyl acetate and dried by the critical point method. The genital pinnules were then fractured with a needle, coated with gold and palladium (80:20) and viewed in a JEOL JSM-35 scanning electron microscope.

Several genital pinnules were also prepared for transmission electron microscopy (TEM). Fixation was for 1 h at room temperature in 2.5% glutaraldehyde in 0.2M Millonig's phosphate buffer and 0.14M NaCl. After a rinse in a 1:1 mixture of 0.4M Millonig's phosphate buffer and 0.6M NaCl, the material was post-fixed for 1 h in 2% OsO_4 in 1.25% $NaHCO_3$ buffer. This was followed by a rinse in 1.25% $NaHCO_3$, and decalcification for 24 h in a 1:1 mixture of 2% ascorbic acid and 0.3M NaCl (Dietrich and Fontaine, 1975). The tissue was then dehydrated in ethanol and embedded in Epon (Luft, 1961). Thin sections were cut on a

Porter-Blum MT-1 ultramicrotome with glass knives, stained sequentially with uranyl acetate and lead citrate, and photographed on a Philips 300 transmission electron microscope.

RESULTS

Gross Anatomy of the Reproductive Organs

The genital pinnules in Florometra serratissima are located on about the lower two-thirds of each of the 10 arms, although several of the most basal pinnules on each arm are non-reproductive. Each genital pinnule is tapered, being thickest at the base and narrowing towards the tip. The total number of genital pinnules increases linearly with the size of the animal as measured by the length of the longest arm, L (Fig. 1). A few of the most distal genital pinnules on each arm are always very small and they were not included in the counts; such small genital pinnules have been newly formed during growth of the arms. Fig. 1. shows that a large specimen (L = 280 mm) would have approximately 440 genital pinnules, whereas a small individual (L = 130 mm) would have slightly over 100 genital pinnules.

F. serratissima is normally dioecious, with all of the genital pinnules on an individual bearing either a single testis or a single ovary. The genital pinnules of males are creamy-white, while those of females are pink or orange. On this basis, animals can be quickly sexed from a visual examination alone. Of the 398 specimens closely examined over the two-year study period, 210 were males, 184 were females, 2 were hermaphrodites and 2 were unsexable. The proportion of males, P, does not differ significantly from 0.5 ($p > 0.05$) as determined by computing confidence limits for P (Freund, 1967, p. 275). One of the hermaphrodites, collected on 8 March 1978, had a single hermaphroditic genital pinnule on an otherwise all-male body. The basal portion of this pinnule contained many closely-packed orangish oocytes; the thinner

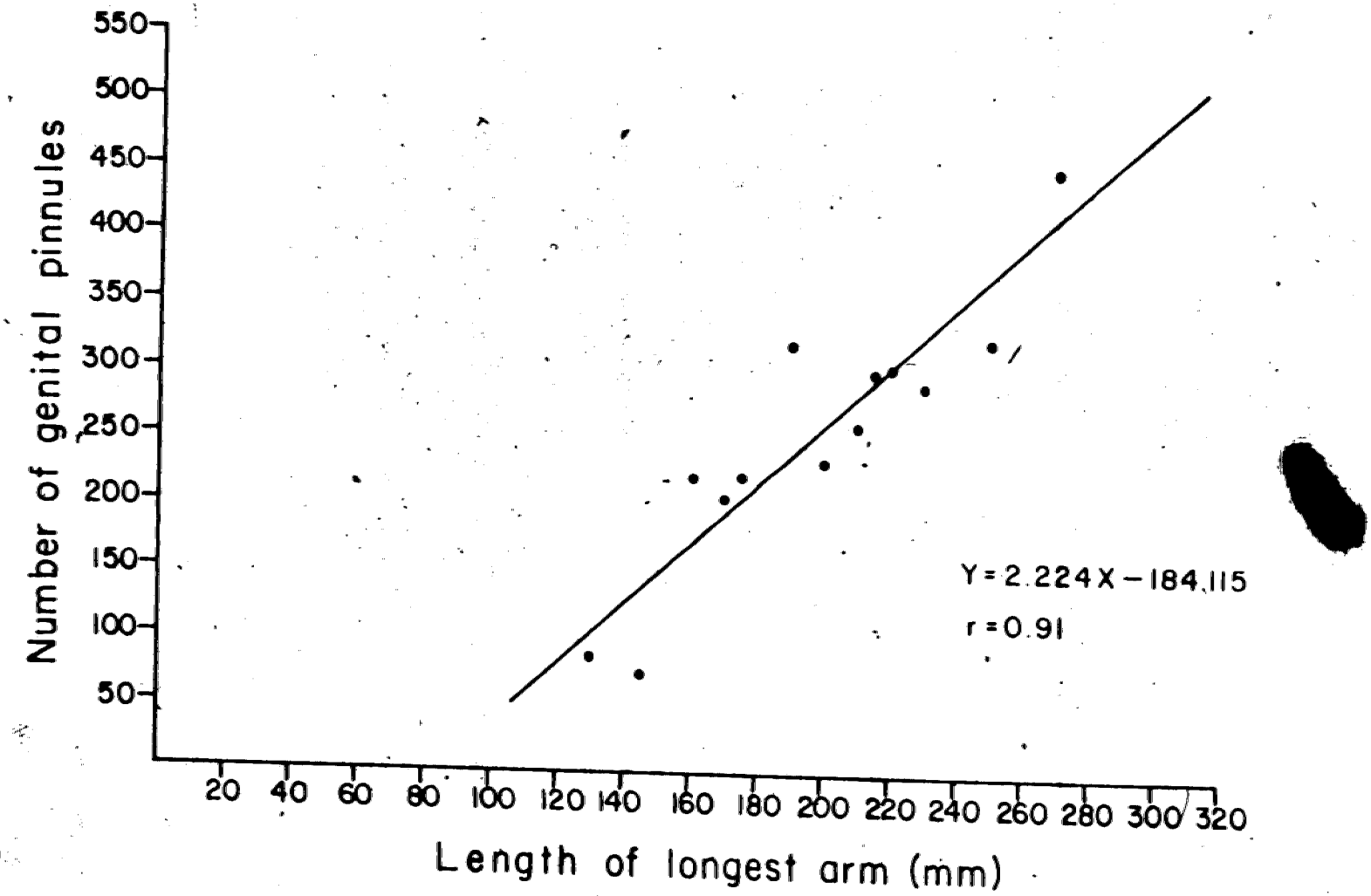


Fig. 1. Florometra serratissima. Relationship between number of genital pinnules and size of an individual as measured by the length of the longest arm. The regression line is significant ($P < 0.01$)

distal portion contained many sperm which were motile when added to seawater. In the second hermaphrodite, found on 15 August 1979, male and female genital pinnules were intermixed along the length of many of the arms, while some of the individual genital pinnules were part male and part female. The male genital pinnules contained motile sperm. A more detailed description of the arrangement of male and female elements on the 10 arms of this animal is found in Table 1.

Microanatomy of the Arms and Genital Pinnules

It is first necessary to describe the structure of the oral part of the arm in order to understand the structure of the genital pinnules. Fig. 2A, which is a cross section through the oral portion of an arm of a female specimen, shows the major arm canals. These consist of a water vascular (= tentacular) canal, a pair of subtentacular coelomic canals, an aboral (= coeliac) coelomic canal, and, between the aboral and subtentacular coelomic canals, a small genital coelomic canal. In F. serratissima, as shown in Fig. 2A, the genital canal freely interconnects with either of the two subtentacular canals throughout the length of the arm. Within the genital canal can be seen the hollow genital tube, which is a haemal vessel (Ludwig and Hamann, 1907), while a solid strand of cells, the genital cord, is suspended within the genital tube (Fig. 2B). At the light microscopic level, the structure of the genital canal of a male is identical to that of a female. The arrangement of the arm canals is illustrated diagrammatically in Fig. 3.

Each arm canal gives off a branch into each genital pinnule, although a branch from only one of the two subtentacular coelomic canals enters the pinnule. Fig. 2C shows a cross section through the thick basal portion of a female genital pinnule. The genital coelomic

Table 1. Florometra serratissima. Arrangement of the genital pinnules on the arms of an anomalous hermaphrodite collected 15 August 1979

Ray[1]	Description
A	(i) Mainly male towards arm base and female towards arm tip, but with some intermixing throughout. (ii) Intermixture of male and female
B	(i) Only male (ii) Intermixture of male and female; a few individual genital pinnules both male and female
C	(i) Like B(ii) (ii) Male towards arm base and female towards arm tip; in middle of arm individual genital pinnules both male and female
D	(i) Only male (ii) Only male
E	(i) Only male (ii) Intermixture of male and female; a few individual genital pinnules both male and female

[1] Orientation according to Carpenter (1884, p.89). "A" is the arm pair opposite the anus when the oral surface of the specimen faces the observer; the remaining four arm pairs are lettered in a clockwise direction.

Fig. 2. Florometra serratissima. Microanatomy of the arms and genital pinnules, and structure of the inner layer of the ovary. (A) Cross section through oral portion of the arm of a female showing major canals and connection (arrowhead) between genital coelomic canal and subtentacular coelomic canal; the latter canal is collapsed. (B) Enlargement of genital coelom of (A) showing genital tube and genital cord. (C) Cross section of a female genital pinnule showing ovary within genital coelom, and oocytes in haemal space between the inner and outer layer of the ovary. (D) Cross section of a male genital pinnule showing testis within genital coelom, the spermatogenic layer just inside the folded inner layer of the testis, and a mass of spermatozoa in the testicular lumen. (E) Oocytes dissected from an ovary (living material) demonstrating that each oocyte develops within a smooth translucent outpocketing of the inner layer of the ovary, and that each is attached to the inner layer by a stalk. (f) SEM of developing oocytes attached to the inner layer of the ovary which consists of a single layer of squamous epithelial cells.
a: Aboral coelomic canal; am: ambulacral groove; c: genital cord; g: genital coelomic canal; h: haemal space; i: inner layer of gonad; o: outer layer of gonad; ol: ovarian lumen; s: spermatogenic layer; sp: spermatozoa; st: stalk of oocyte; stc: subtentacular coelomic canal; t: genital tube; w: water vascular canal


stc
g
.1mm
g
h
ol
i
o
.25 mm
i
ol
i
F

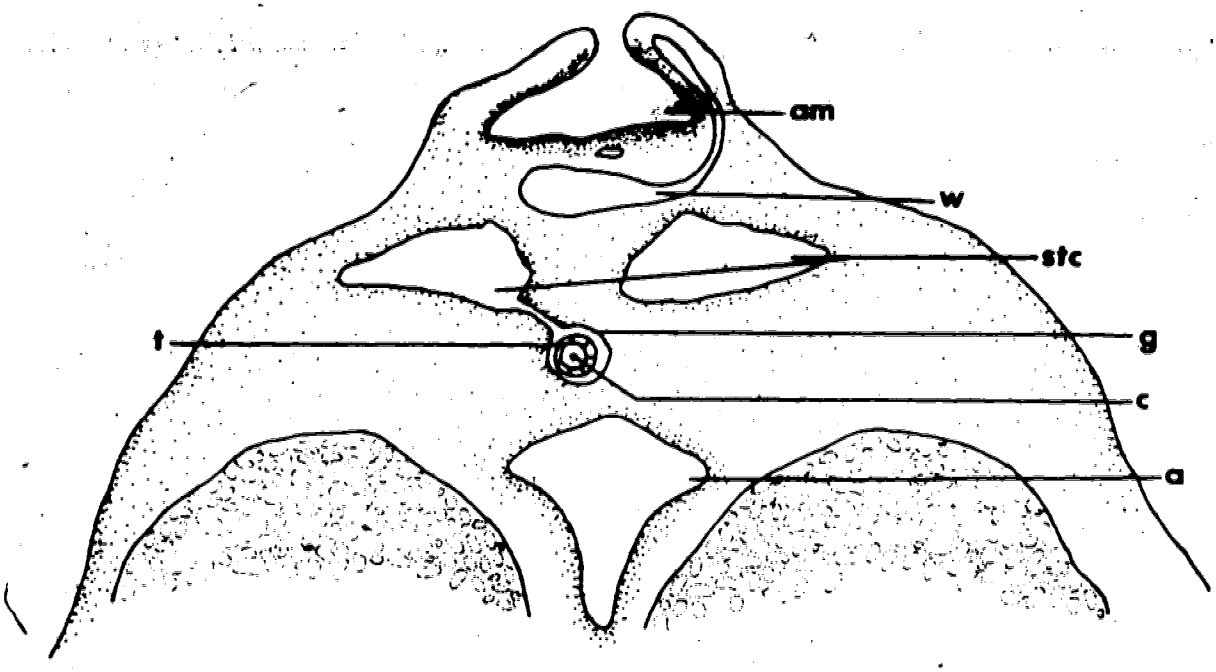


Fig. 3. Florometra serratissima. Diagrammatic cross section through the oral part of an arm. a: Aboral coelomic canal; am: ambulacral groove; c: genital cord; g: genital coelomic canal; stc: sub-tentacular coelomic canal; t: genital tube; w: water vascular canal

canal and the genital tube have expanded to occupy most of the interior of the pinnule. The genital coelom is in open communication with the aboral coelomic canal and interconnects frequently with the single subtentacular coelomic canal. The genital tube now forms the outer, or perivisceral, layer of the ovary. The genital cord has expanded to form the inner or germinal layer of the ovary which encloses the tubular ovarian lumen. Numerous oocytes of various sizes are located in the haemal space between the outer layer and the inner layer of the ovary. The structure of a female genital pinnule is shown diagrammatically in Fig. 4. As will be demonstrated shortly, the oocytes, despite appearances, are actually part of the inner wall of the ovary, and they communicate with the ovarian lumen. The structure of a male genital pinnule is shown in Fig. 2D, and diagrammatically in Fig. 5. In a fashion homologous to that of the ovary, the testis is located within a spacious genital coelom and consists of an outer layer, an inner layer enclosing a testicular lumen, and an intermediate haemal space. The inner layer of the testis is thrown into numerous folds which project into the testicular lumen; each fold encloses a channel of the haemal space. A spermatogenic layer lines the luminal surface of the inner layer, while spermatozoa fill the testicular lumen.

Structure of the Inner Layer of the Ovary

The oocytes do not fall free of one another when an ovary is dissected open, but tend to stay together in clusters. Light microscopic observations on unfixed material (Fig. 2E) demonstrate that the oocytes remain clustered, not because they adhere to one another, but because each oocyte lies within a smooth translucent envelope formed by an outpocketing from the inner layer of the ovary; each oocyte is

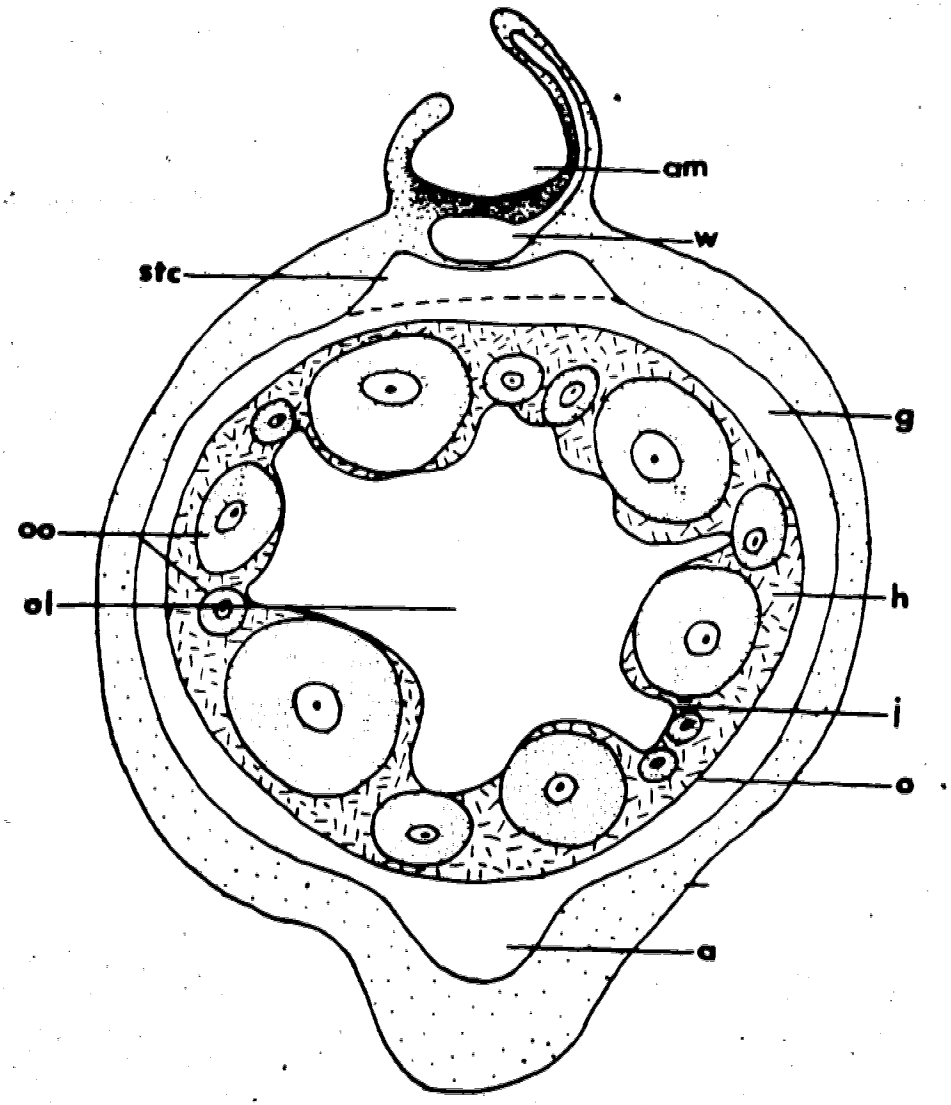


Fig. 4. Florometra serratissima. Diagrammatic cross section of a female genital pinnule. a: Aboral coelomic canal; am: ambulacral groove; g: genital coelomic canal; h: haemal space; i: inner layer of ovary; o: outer layer of ovary; ol: ovarian lumen; oo: oocytes; stc: subtentacular coelomic canal; w: water vascular canal

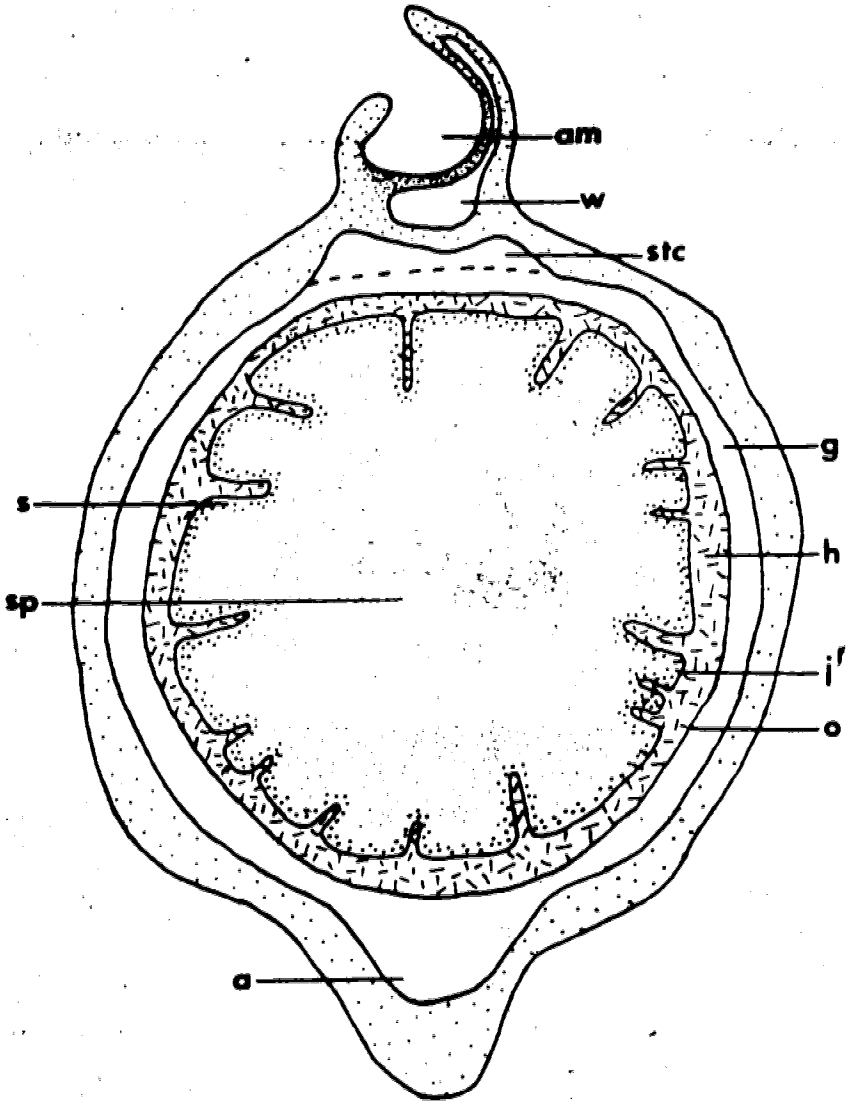


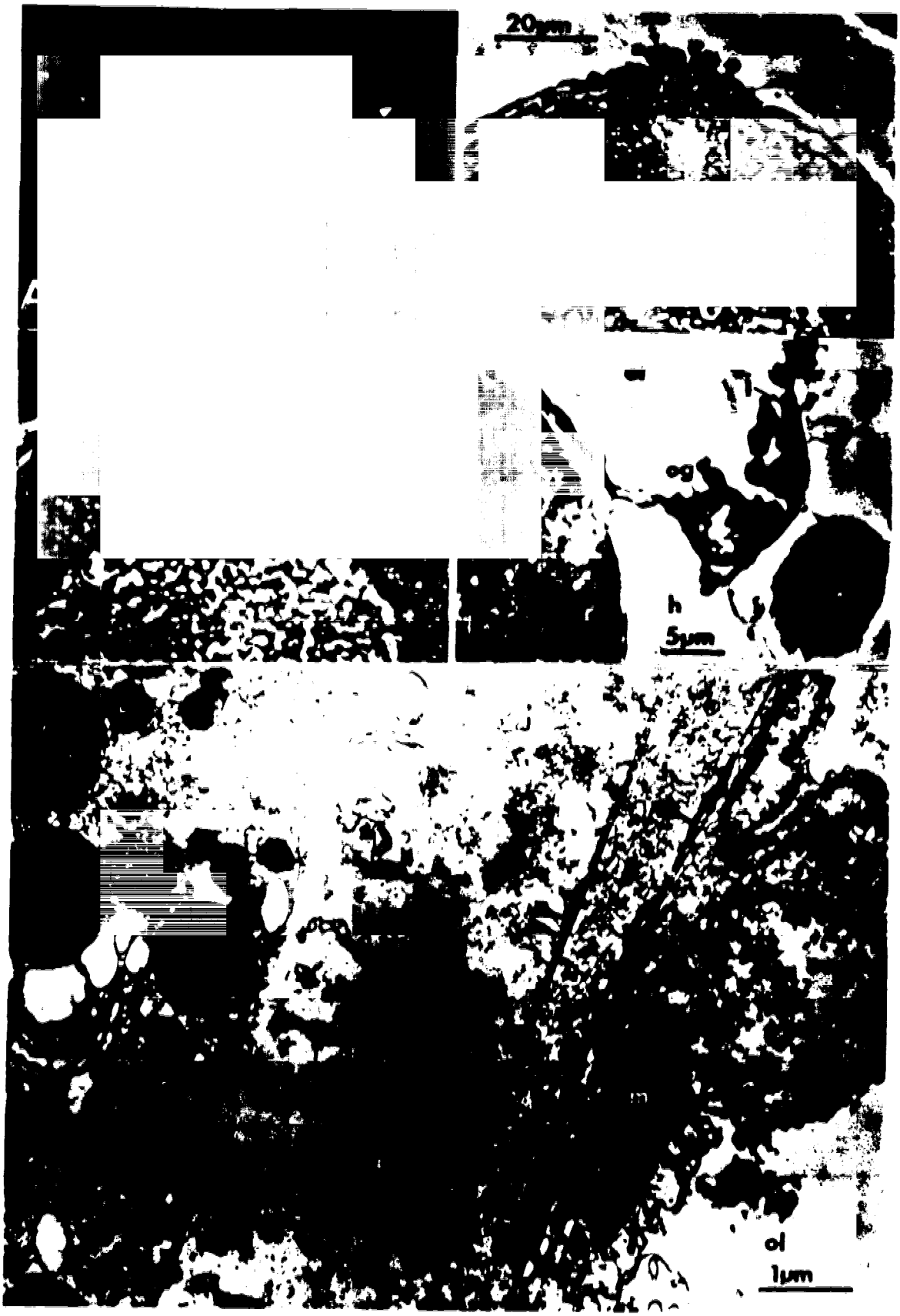
Fig. 5. Florometra serratissima. Diagrammatic cross section of a male genital pinnule. a: Aboral coelomic canal; am: ambulacral groove; g: genital coelomic canal; h: haemal space; i: inner layer of testis; o: outer layer of testis; s: spermatogenic layer; sp: spermatozoa in testicular lumen; stc: subtentacular coelomic canal; w: water vascular canal

attached to the inner layer by a short stalk. A scanning electron micrograph of part of a fractured ovary shows a cluster of developing oocytes (Fig. 2F); each oocyte is surrounded by a smooth envelope and is attached to the inner layer of the ovary. SEM also shows that the inner layer is composed of a squamous epithelium (Fig. 2F) which gives rise to numerous long thin processes (Fig. 6A).

Several spherical bodies are invariably present at the junction of the oocyte stalk with the inner layer (Fig. 6B). These are the nuclei of squamous epithelial cells situated at the periphery of the stalk. The nuclei of these cells project into the ovarian lumen more prominently in comparison to that of adjacent epithelial cells whose nuclei are more flattened. The very tip of the stalk is not covered by epithelial cells, and it is thus in open communication with the ovarian lumen (Fig. 6C).

Fig. 6E is a transmission electron micrograph of part of the inner layer of the ovary and a portion of an underlying oocyte. The surface of the oocyte gives rise to numerous microvilli; each microvillus is about 1 μm in length and projects into a space about 3 μm wide containing a flocculent material. This space will be called the jelly layer after Holland (1971). The outer edge of the jelly layer is bounded by a thin (900 Å) layer of tightly packed fibres which corresponds to the envelop which surrounds each oocyte as shown in Figs. 2E and 2F. The inner layer of the ovary is sparsely ciliated. Such cilia are not present in Fig. 6E, but can be seen in Fig. 11D. Two nuclei of squamous epithelial cells of the inner layer are visible in Fig. 6E, along with muscle fibres. Underlying the inner layer is a thin layer of fibres, more variable in thickness compared to

Fig. 6. Florometra serratissima. Structure of the inner layer of the ovary. (A) SEM of several squamous epithelial cells showing long thin processes (arrowheads). (B) Section through part of an oocyte showing peripheral region of stalk and prominent nuclei of epithelial cells of inner layer of ovary associated with stalk. (C) Section through middle of stalk of oocyte demonstrating that stalk is in open communication with ovarian lumen; cortical granules are evident just beneath the plasma membrane of the oocyte but these do not extend into the stalk. (D) Section of an oogonium located on luminal surface of the inner wall of ovary. (E) TEM of part of inner wall of ovary (right side of micrograph), part of the peripheral region of a large (180 µm in diameter) oocyte (left side of micrograph), and the intervening haemal space; a thin fibrous layer (single arrowhead) underlies the inner layer, while another fibrous layer (double arrowhead) is associated with the oocyte. cg: cortical granules; h: haemal space; i: inner layer of ovary; j: jelly layer; L: lipid droplet; m: muscle fibres; mv: microvilli; nu: nucleus of squamous epithelial cell of inner layer of ovary; og: oogonium; ol: ovarian lumen; st: stalk; Y: yolk granule


20μm
h
5μm
ol
1μm

the layer surrounding the oocyte, but otherwise identical in appearance. Intervening between the two fibrous layers is the haemal space characterized by granular remnants of haemal fluid and scattered vesicular bodies.

These light and electron microscopic observations demonstrate that the envelope enclosing each oocyte is a continuation of a fibrous basal lamina which underlies the squamous epithelium of the inner layer of the ovary. That part of the basal lamina associated with an oocyte will here be called the germinal lamina although it has been called an external lamina in the feather star Nemaster rubiginosa (Holland, 1971), and a chorion in the feather star Comanthus japonica (Holland and Dan, 1975; Holland et al. 1975).

Differentiation and Growth of Oocytes

The smallest germ cells that could be discerned by light microscopy were oogonia, about 8μm in diameter, which occur individually on the luminal side of the inner layer of the ovary (Fig. 6D). These cells contain a single spherical nucleus surrounded by a thin shell of cytoplasm. The nucleus contains a single nucleolus and scattered granules of chromatin material. As each oogonium differentiates into an oocyte and enters the stage of growth, an outpocketing of the basal lamina forms and the oocyte sinks below the squamous epithelium of the inner layer and bulges into the haemal space (Fig. 7A). Smaller oocytes, except for the stalk region, are totally contained within the haemal space, but larger oocytes bulge partly into the haemal space and partly into the ovarian lumen (Fib. 7B). Light microscopy suggests that a fold of the inner layer of the ovary is present in the region between two large oocytes (Fig. 7[illegible]). An examination of this region with TEM

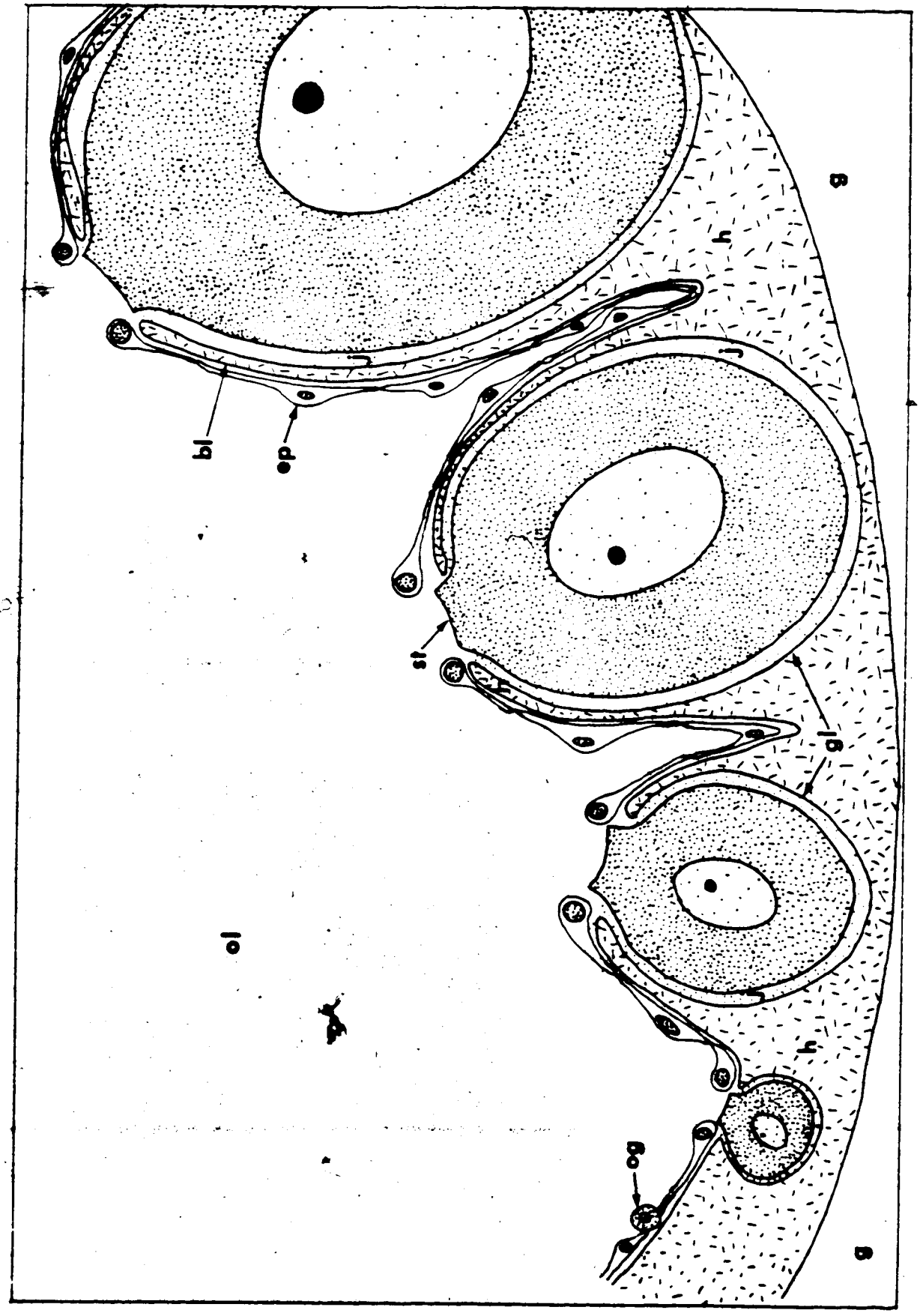
Fig. 7. Florometra serratissima. Structure of the inner layer of the ovary. (cont'd) (A) Cross section of part of ovary showing a small oocyte bulging into haemal space (arrowhead), and a yolk nucleus in the cortical region of a larger oocyte. (B) Thick Epon cross section of ovary showing apparent folds of inner layer of ovary in region between two large oocytes (arrowheads); also evident is the stalk of an oocyte (double arrowhead) and spherical material in the ovarian lumen resulting from cytolysis of unspawned ova and defective oocytes (see Fig. 12B and text). (C) TEM of peripheral region of two large oocytes (upper left and lower right of micrograph) showing intervening fold of inner layer of ovary; the single arrowheads point to the basal lamina; the double arrowheads point to the germinal lamina associated with each oocyte. (D) Oocyte in wrinkled condition; arrows point to furrows in plasma membrane. (E) oocyte in smooth condition.
g: Genital coelom; h: haemal space; i: inner wall of ovary; j: jelly layer; ol: ovarian lumen; Ooc: oocyte; yn: yolk nucleus


70µm
B
Ooc
Ooc

(Fig. 7C) shows this to be indeed the case. A part of the inner wall of the ovary is interposed between the germinal laminae of the two oocytes and is bounded on both sides by a basal lamina and a narrow haemal channel. Thus, even though only a portion of a large oocyte may bulge into the available haemal space, the inner layer becomes folded in a manner that allows the germinal lamina of the oocyte to be almost entirely surrounded by a thin channel of the haemal space. The relationship between a growing oocyte and both the inner layer and haemal space of an ovary is depicted diagrammatically in Fig. 8.

Growing oocytes contain a large germinal vesicle within which is a single, spherical, eccentrically located nucleolus. The cortex of the nucleolus stains more intensely with haematoxylin than the core, and the nucleolus sometimes contains vacuoles in both the core and cortical regions. The ooplasm is basophilic until the oocyte attains a diameter of about 40 µm; the ooplasm then becomes eosinophilic, and yolk granules and lipid droplets begin to appear, first at the periphery of the oocyte, then spreading inwards, as the oocyte grows, to completely fill the ooplasm. A single yolk granule and numerous lipid droplets are visible in the periphery of the oocyte of Fig. 6E. Numerous cortical granules are present in a thin layer just beneath the oolemma of oocytes greater than 120 µm in diameter, but they do not extend into the stalk (Fig. 6C). With TEM (Fig. 11D) cortical granules appear as spherical bodies 1 µm to 1.5 µm in diameter in close association with the plasma membrane of the oocyte. Each cortical granule consists of a moderately electron-transparent ground substance in which are embedded irregular clumps of electron-opaque material. Oocytes greater than 140 µm in diameter are in either a wrinkled or a smooth state. In the

Fig. 8. Florometra serratissima. A diagrammatic illustration of the relationship between a growing oocyte and both the inner layer and haemal space of an ovary. An oogonium is shown at the far left; successive stages in the growth of an oocyte proceed from left to right in the figure. bl: Basal lamina; ep: squamous epithelial cell of inner layer of ovary; g: genital coelom; gl: germinal lamina; h: haemal space; j: jelly layer; og: oogonium; ol: ovarian lumen; st: stalk of oocyte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former, the plasma membrane has a furrowed appearance (Fig. 7D), whereas in the latter, the plasma membrane is smooth (Fig. 7E). Both smooth and wrinkled oocytes are found together within the same ovary.

Near the periphery of many oocytes is a single, deeply basophilic, crescentic yolk nucleus surrounded by a clear patch of ooplasm (Fig. 7A). Within oocytes of comparable size, the yolk nucleus varies in shape from short and thick, and confined to a small part of the ooplasm, to long and very thin, and spread over much of the periphery of the oocyte. Yolk nuclei also vary in staining intensity.

The relationship between the presence of a yolk nucleus and the diameter of an oocyte is shown graphically in Fig. 9. Oocytes less than 21 µm in diameter do not contain a yolk nucleus. A yolk nucleus first appears in oocytes that are between 21 µm and 40 µm in diameter, while many oocytes between 41 µm and 120 µm in diameter contain a yolk nucleus. Oocytes larger than 121 µm in diameter begin to lose their yolk nucleus, and by the time the oocytes are large enough to be ovulated, most have lost the yolk nucleus entirely. In oocytes greater than 161 µm in diameter the yolk nucleus, if present, is very small and almost imperceptible. The yolk nucleus is thus present during the active growth phase of an oocyte, and disappears by the time growth has neared completion.

Ovulation and Oocyte Maturation

All of the genital pinnules of a female ovulate oocytes simultaneously. During ovulation, each of the largest oocytes present in the ovary (diameter greater than 170 µm in histological cross section) becomes markedly deformed as it squeezes through a small circular opening in the squamous epithelium of the inner layer of the ovary at

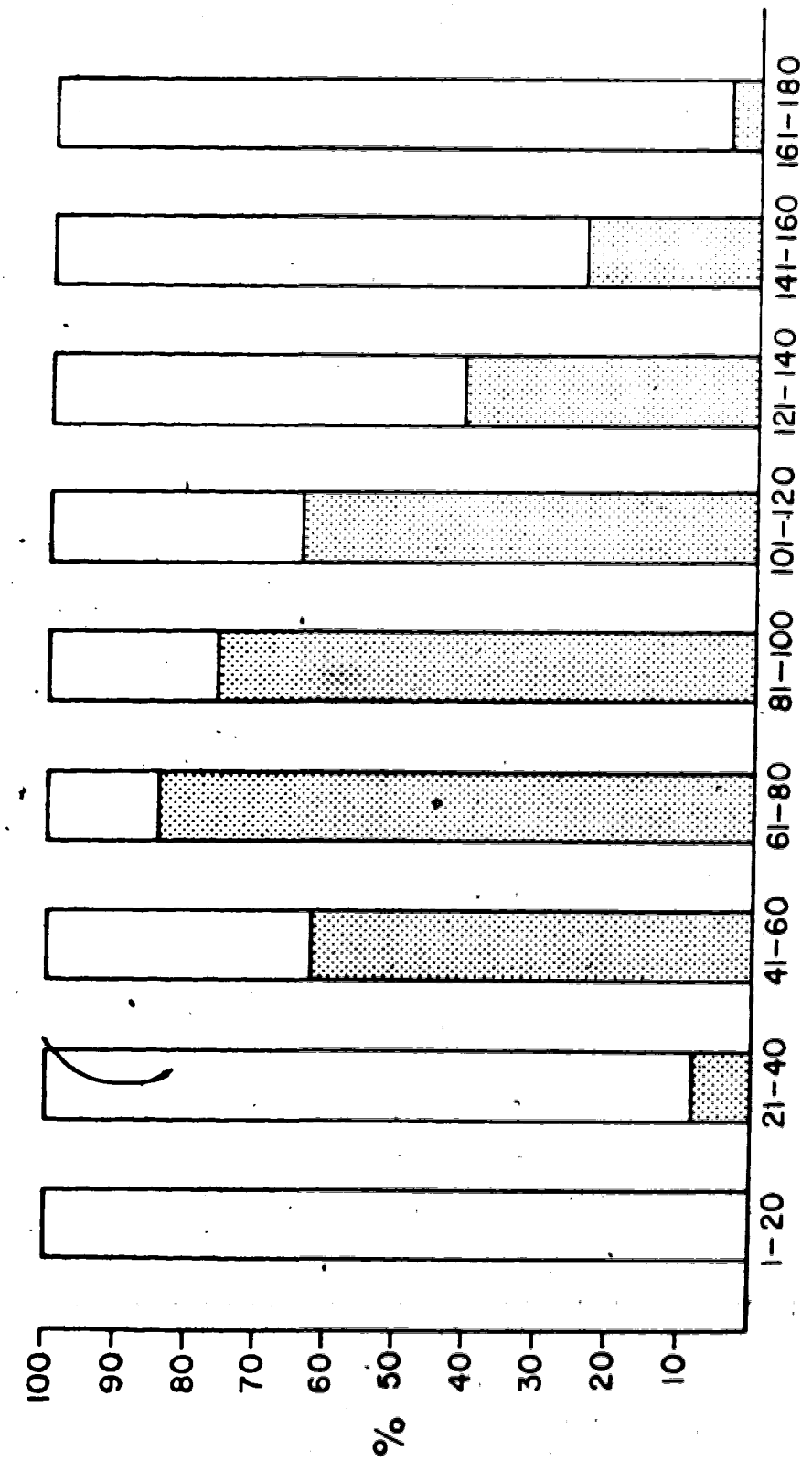


Fig. 9. Floronetra serratisaima. The percentage of oocytes with a yolk nucleus (closed bars) and without a yolk nucleus (open bars) in each size class examined

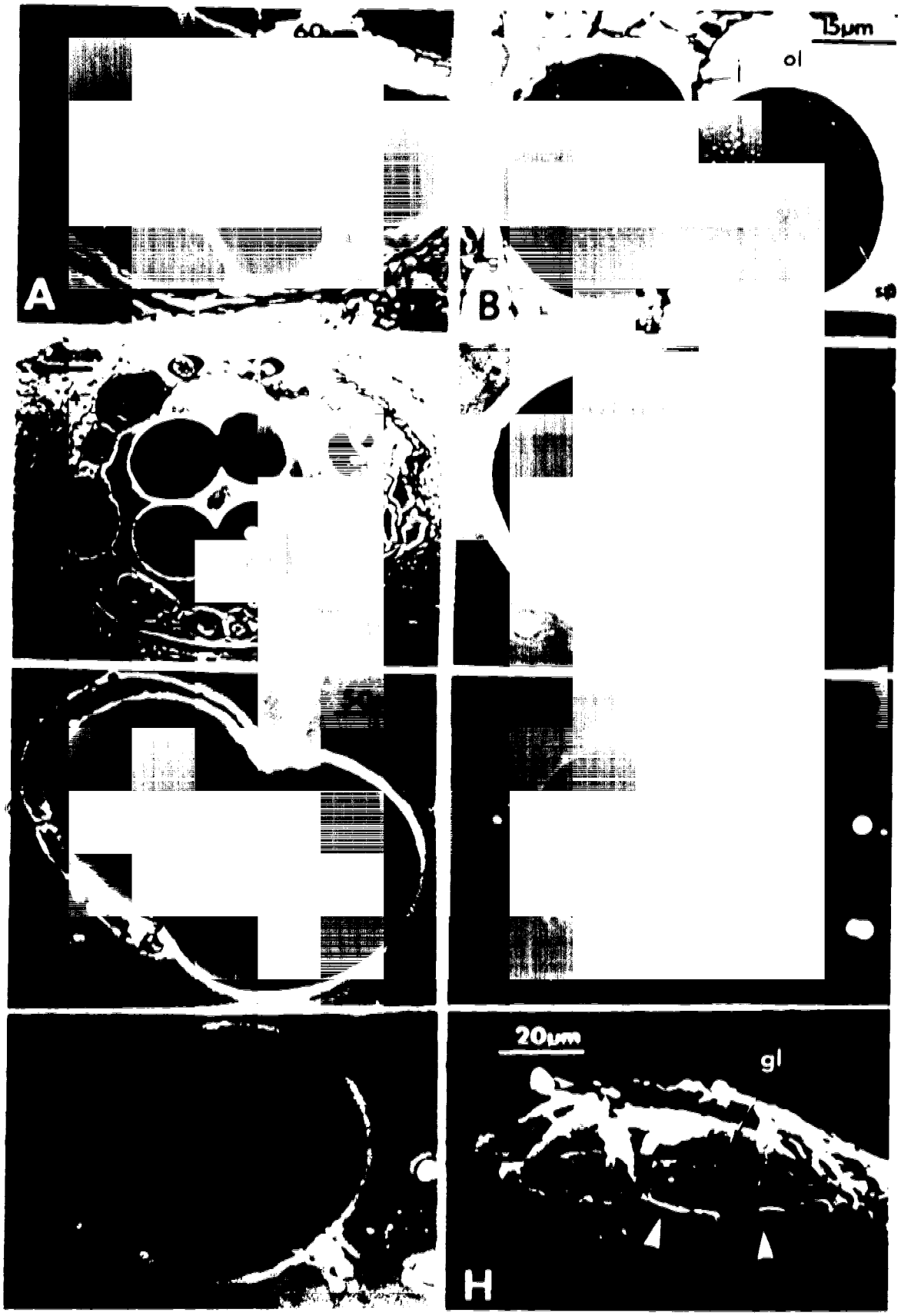
the point where the stalk was located. At the beginning of ovulation, the protruding tip of the oocyte acquires a characteristic arrowhead appearance (Fig. 10A). As the process continues, the oocyte becomes bilobed, with a constriction at the position of the circular opening in the ovarian inner layer (Fig. 10B). Soon, the entire oocyte has passed through the opening and lies free within the ovarian lumen (Fig. 10C). The germinal lamina is left behind in the haemal space in a collapsed state, still attached to the inner ovarian wall.

Oocyte maturation begins either during, or just after, the completion of ovulation. At this time, the germinal vesicle and nucleolus disappear and the ooplasm becomes homogeneous in appearance. When maturation occurs during ovulation, the spindle apparatus of the first meiotic division always appears at the periphery of the oocyte at the former position of the stalk (Fig. 10B). This indicates that the oocyte has a definite polarity during growth, with the animal pole positioned nearest the ovarian lumen, and the vegetal pole bulging into the haemal space.

Once the oocyte has entered the ovarian lumen, it becomes perfectly spherical, the maturation divisions are completed, and two polar bodies are given off at the animal pole (Fig. 10C). The resulting ova collect in the ovarian lumen prior to spawning. The ovarian lumen is often so narrow, especially towards the tip of the genital pinnule, that the ovulated oocytes cannot assume a spherical shape. Such oocytes mature normally, becoming spherical as soon as they are spawned.

A living oocyte, still within its germinal lamina, but freed from its attachment to the inner layer of the ovary by dissection, is still

Fig. 10. Florometra serratissima. Ovulation. (A) Cross section through part of an ovary showing an oocyte in an early stage of ovulation; the tip of the oocyte protruding into the ovarian lumen has a characteristic arrowhead appearance (arrow). (B) A section of an ovulating oocyte in the process of maturation; note the spindle apparatus at the animal pole. (C) Cross section of a female genital pinnule showing ova collecting in ovarian lumen; a polar body is visible on the surface of one of the ova. (D) - (G) Stages in the ovulation of an isolated oocyte photographed from living material; the arrowheads point to regions at the periphery of the opening of the germinal lamina where rolling-up of the germinal lamina takes place. (H) SEM of vegetal pole of an oocyte at the completion of ovulation showing remains of germinal lamina with distinctly rolled-up edges (arrowheads). g: Genital coelom; gl: germinal lamina; h: haemal space; i: inner layer of ovary; ol: ovarian lumen; pb: polar body; sp: spindle apparatus


A
B
ol
gl
H
20µm

capable of ovulation. Stages in this process are shown in Figs. 10D through 10G. The oocyte squeezes through the circular opening in the germinal lamina just as it would if still attached to the inner layer of the ovary. During ovulation the germinal lamina at the rim of the opening rolls up to form a ring of crumpled material (Figs. 10D and 10E). Towards the end of the process the germinal lamina has markedly decreased in surface area and is confined to the slightly crinkled vegetal pole of the oocyte (Fig. 10F). The oocyte has become spherical, and the germinal lamina is reduced to a crumpled mass adhering to the vegetal pole, at the completion of ovulation (Fig. 10G). A scanning electron micrograph of the vegetal pole of an isolated oocyte at this time (Fig. 10H) reveals a ring-like ridge of rolled-up germinal lamina and the collapsed remnants of the same within this ring. The germinal lamina would have been left behind, still attached to the inner layer of the ovary, if this oocyte had not been isolated from the ovary. An isolated oocyte takes approximately 15 min to complete ovulation. Such observations were made at high temperatures, however, since the specimen was on the stage of a compound microscope; ovulation under natural circumstances may take considerably longer.

The preceding observations on ovulation in isolated oocytes, particularly the formation of a ring of rolled-up germinal lamina at the vegetal pole of the oocyte accompanied by a decrease in the total surface area of the germinal lamina, suggest that the germinal lamina is dynamically involved in ovulation. Further consideration of this problem will follow in the discussion section.

Spawning and Phagocytic Stage

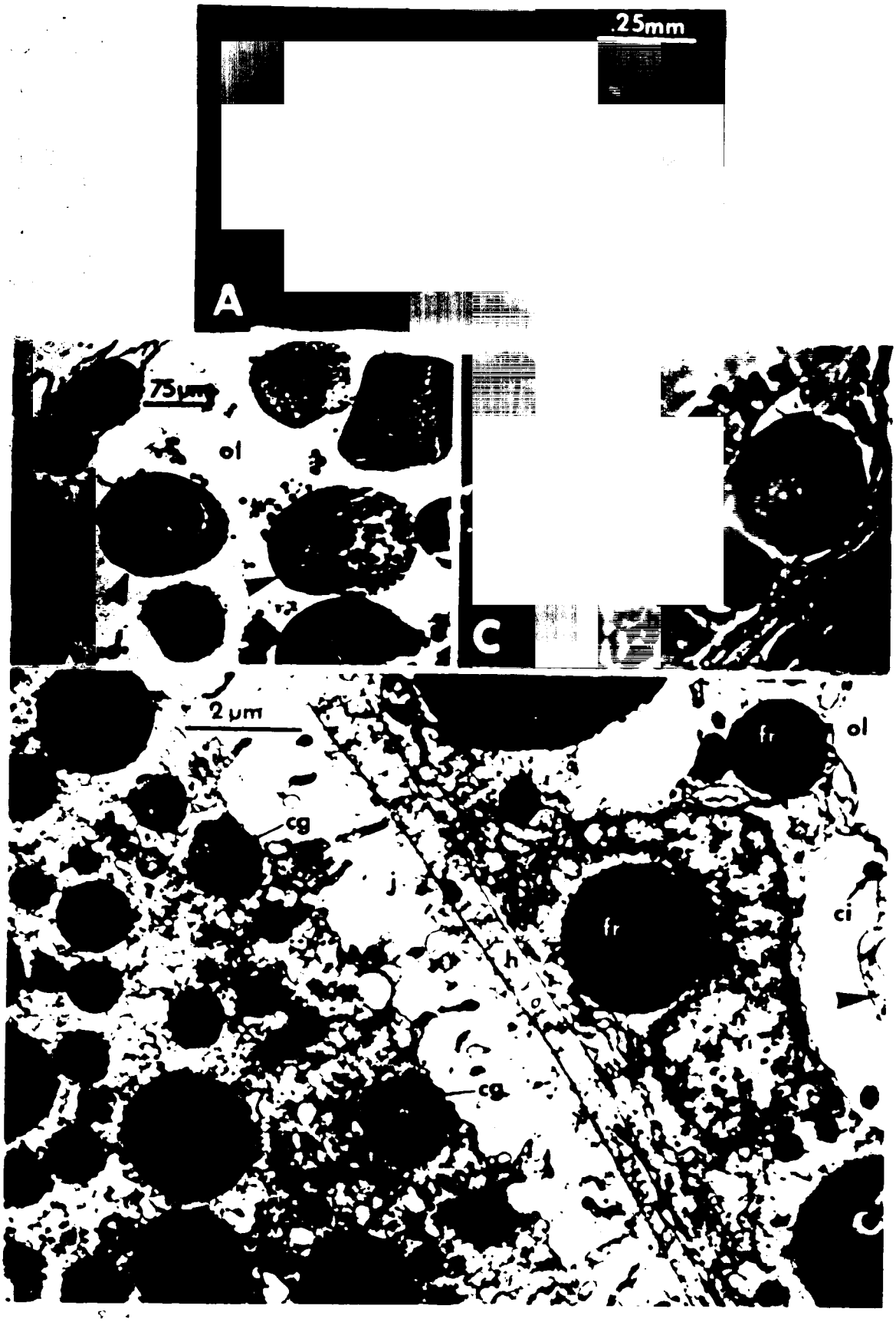
Sequential removal of genital pinnules from females with ovulated

eggs in the laboratory indicated that ova can be retained in the genital pinnules for about three days. There are no permanent structures in the genital pinnules for the exit of ova. If, however, the surface of a genital pinnule containing ovulated eggs is examined with a dissecting microscope, anywhere from one to four colourless circular spots, approximately 0.2 mm in diameter, and visibly different from the adjoining reddish-brown tissue of the genital pinnule, are evident; these will be called germinal spots. They always occur on the more heavily pigmented distal side of the genital pinnule (the side which faces the arm tip) and they represent places in the wall of the genital pinnule through which ova can be extruded. Germinal spots are less evident, though still visible, in females which do not have ovulated eggs. The number of germinal spots present on the genital pinnules is variable. A single spot is always present near the base of the genital pinnule; as more appear, they are added sequentially in line towards the tip of the genital pinnule. The germinal spots result from a localized outward bulging of underlying non-pigmented tissue into the overlying pigmented tissue, causing the latter to be stretched so thinly that the underlying tissue shows through.

Germinal spots are also present on the distal side of male genital pinnules. They are similar in appearance to those of females, but they have a smaller diameter (0.1 mm), and are always multiple.

Just prior to spawning, some of the genital spots and their surrounding tissue bulge outwards to form a protuberance which will be called a genital nipple. Not all of the genital spots present on a pinnule necessarily form a genital nipple. Female genital nipples are shown in Fig. 11A. In this case, there is only one genital nipple on

Fig. 11. Florometra serratissima. Spawning and phagocytic stage. (A) Part of two female genital pinnules just prior to spawning (photographed from living material) showing a single genital nipple at the base of each; a genital spot is visible at the tip of one of the genital nipples. (B) Cross section of part of an ovary showing cytolytic ova (arrowheads) in ovarian lumen forming numerous spherical fragments. (C) Cross section of part of ovary showing columnar, phagocytic epithelial cells of inner layer of ovary. (D) TEM of part of peripheral region of an oocyte (left side of micrograph), and inner layer of ovary in phagocytic stage (right side of micrograph); a sheetlike extension from an epithelial cell of the inner layer is visible (arrowhead at extreme right); engulfed spherical fragments derived from the cytolysis of ova and oocytes in the ovarian lumen are present within the epithelial cell; cortical granules attached to the plasma membrane of the oocyte are visible. cg: Cortical granule; ci: cilium; fr: cellular fragment; gn: genital nipple; gs: genital spot; h: haemal space; i: inner layer of ovary; j: jelly layer; n: nucleus of epithelial oell; ol: ovarian lumen


.25mm
A
75μm
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C

each pinnule, although two, sometimes three nipples can be present. Note that the colourless genital spot is present at the tip of each nipple. Male genital nipples tend to be smaller in size and more numerous than those shown in Fig. 11A. If gentle pressure is applied to a genital pinnule when genital nipples are present, the genital spots rupture and a stream of gametes is emitted from the tip of each nipple. This process must involve simultaneous rupture of both the inner and outer gonadal layers, as well as the pinnule wall.

Florometra serratissima was never observed in the act of spawning, either in the laboratory or in the field. A characteristic whipping motion of the arms has been observed during spawning in other crinoid species (K. Dan and J.C. Dan, 1941b; Fishelson, 1968). If this is the case in F. serratissima as well, then the gametes would presumably be ejected from the distal side of each genital pinnule with some vigour, perhaps aiding in the initial dispersal of eggs and sperm.

Counts were made of the number of ova present in the genital pinnules of four females (L greater than 180 mm), two collected on 27 June 1979 and two on 20 August 1979, in order to gain an estimate of the number of ova that can be discharged during a spawning. The results are presented in Table 2. There is individual variation in the average number of ova present in a genital pinnule. If it is assumed that the figures in Table 2 represent the extremes that are likely to occur, then the overall average of 54 ova (± 24, n = 34), can be used as an estimate of the average number of ova present in the genital pinnules of a female just prior to spawning. Using this estimate in conjunction with Fig. 1, it is possible to estimate the number of ova that a female of a given size can emit at a single spawning. Thus, a

Table 2. *Florometra serratissima*. Counts of ova in genital pinnules.

Date	Average number of ova	S.D.	n
27 June 1979	30	6	10
	68	15	11
20 August 1979	36	7	6
	81	18	7

large female (L = 280 mm) with 440 genital pinnules can spawn roughly 23,800 ova. On the other hand, a small female, (L = 128 mm) with 100 genital pinnules can spawn roughly 5,400 ova. Such predictions assume, first of all, that all ova are spawned; as will be shown below, this is not strictly true. In addition, since the average figure of 54 ova is derived from fairly large animals, predictions of the number of ova spawned by smaller animals is likely overestimated because smaller animals have smaller genital pinnules. Despite such obvious limitations, it is still possible to estimate the fecundity of a female F. serratissima during a spawning more accurately than for most marine invertebrate broadcasters.

In some histological sections of ovaries, both oocytes and ova are found in various stages of disintegration leading to the formation of numerous spherical cell fragments (Figs. 7B and 11B). Such fragments tend to collect in a thick layer against the inner layer of the ovary. The ovary then enters a brief phagocytic stage in which the normally squamous epithelium of the inner layer becomes cuboidal to low columnar (Fig. 11C). TEM of the inner layer at this time shows that the ciliated epithelial cells give off long thin sheetlike extensions into the ovarian lumen (Fig. 11D). The latter probably help to engulf the spherical fragments, many of which are present as inclusions in the cytoplasm of the epithelial cells. In this manner, nutriment present in unspawned and defective eggs is reabsorbed by the ovaries.

DISCUSSION

The light microscopic structure of the genital canal of the arm of Florometra serratissima is comparable to that of other crinoid species as described in Ludwig and Hamann (1907). There is, however, variation of a minor nature between species. In some, the genital cord is attached to the wall of the genital tube, while in others it is suspended within the genital tube, as is the case in F. serratissima. Chadwick (1907) indicated that a genital tube in Antedon bifida is absent, and that the genital cord is suspended directly within the genital canal. If this were true, then the outer layer of the gonad in the genital pinnules of this species should be missing, which is not the case. It is more likely that the genital tube closely enwraps the genital cord thereby giving the impression of a single structure. The amount of communication between the genital canal and the other coelomic canals in the arm appears to vary between species. In F. serratissima the genital canal frequently communicates with the two subtentacular canals while in other crinoids such communication is lacking.

The coelomic canals in the genital pinnules have been described as separate structures in the past (Ludwig and Hamann, 1907, Plate 7, Fig. 2). In F. serratissima the genital and aboral canals merge in the genital pinnule and both interconnect frequently with the subtentacular canal. Communication between the three coelomic canals also occurs in the pinnules of Nemaster rubiginosa (Holland, 1971).

It is very difficult to trace the genital tubes much beyond the base of the arms in F. serratissima, and their ultimate place of origin

is unknown. Carpenter (1876), Perrier (1886), and Russo (1902, cited by Mortensen, 1920, p. 68) claimed that the genital tubes arose from a circumoesophageal plexus that connects with the axial organ; this relationship was more apparent in younger animals than in older ones. Hyman (1955) rejected this idea. It has also been stated (Ludwig and Hamann, 1907) that the primordial germ cells are amoeboid and migrate along the genital cord into the genital pinnules where gametogenesis occurs. Again, this proposal has yet to be substantiated.

All crinoid species which have been studied to date are dioecious. F. serratissima is no exception, but anomalous hermaphroditic specimens are occasionally encountered. Only a very small part of an individual may be hermaphroditic, and this part could be easily overlooked during examination. Hermaphroditism may thus be a fairly frequent phenomenon in F. serratissima, but difficult to observe. Only one other anomalous hermaphroditic crinoid has been reported: K. Dan and J.C. Dan (1941) discovered an individual of Comanthus japonica that was mainly male, but one arm spawned eggs which were infertile. This species passes through a stage in which the gonads are unsexable and it has been suggested that a sex reversal is a possibility at this time (Holland et al., 1975). Hermaphroditism could be a result of an incomplete sex reversal in C. japonica. Individuals of F. serratissima reproduce continuously (Mladenov, 1980) so sequential hermaphroditism is not a possibility.

It has been shown that oogonia originate on the luminal side of the inner layer of the ovary, but as they differentiate into oocytes, they sink below the squamous epithelium of the inner layer within an outpocketing of the basal lamina, and bulge into the haemal space.

Furthermore, as the oocytes outgrow the haemal space and begin to bulge into the ovarian lumen, the inner layer becomes folded in a manner that allows narrow haemal channels to protrude into the ovarian lumen and nearly completely surround the germinal laminae of the developing oocytes. This close association between the haemal space and the developing oocytes supports the hypothesis of Walker (1979) and Bickell et al. (in press) that nutrients are transported to echinoderm germinal cells via the haemal space. In the case of F. serratissima, nutrients in the haemal space would only have to pass through the germinal lamina surrounding each oocyte and enter the jelly layer in order to reach oocytes. It is unlikely that oocytes absorb nutrients from the ovarian lumen via the stalk for two reasons: first, the stalk presents a very tiny surface for the absorption of nutrients in comparison to the area of the oocyte which is in close association with the haemal space; second, the ovarian lumen represents an expansion of the genital cord which, in the arms, is a solid strand of cells, thus making it difficult to see how nutrients could be transported to the ovarian lumen by this route.

In the testis of F. serratissima (Bickell et al., in press) the spermatocytes do not bulge into the haemal space. However, the inner layer of the testis forms numerous invaginations into the testicular lumen; each invagination encloses a narrow haemal channel. The germinal cells line the sides and apices of the invaginations and are therefore in close association with the haemal space. Thus, for both the ovaries and the testes, the structural relationship between the inner gonadal layer, the haemal space, and the germinal cells is consistent with the hypothesis that nutrients reach the germinal cells via the haemal space.

Holland and Kubota (1975) found numerous gonadal accessory cells with intracellular inclusions in the haemal space of the gonads of Comanthus japonica. Fluctuations in the volume of these cells were inversely correlated with volume fluctuations of the germinal cells, and the authors concluded that nutrient reserves in the gonadal accessory cells were being transferred to the germinal cells during gametogenesis. Gonadal accessory cells were not present at any time of the year in the gonadal haemal space of F. serratissima; Bickell et al. (in press) noted that few cells were present in the haemal space of the testis of this species collected in June. As stated earlier, individuals of F. serratissima breed continuously (Mladenov, 1980). As a result, the germinal cells are likely absorbing nutrients on a continuous basis, presumably directly from the haemal fluid, thereby eliminating the necessity for intervening storage cells.

According to Holland (1976) a yolk nucleus is probably present in oocytes of the feather stars Antedon mediterranea and Leptometra phalangium, in addition to those of Antedon bifida, but is lacking in Nemaster rubiginosa, Florometra serratissima, and about 10 other crinoid species that have been examined by light microscopy. He thus concluded that the presence of a yolk nucleus is the exception rather than the rule in crinoids. This study has shown, however, that oocytes of F. serratissima from Barkley Sound, British Columbia, possesses a yolk nucleus. It is also known that oocytes of the Arctic feather star Heliometra glacialis have a yolk nucleus (personal observation).

Chubb (1906) deduced from his histological study of oogenesis in Antedon bifida that the yolk nucleus was an accumulation of "basophile spherules" discharged from the nucleolus during oocyte growth. Harvey

(1930) believed that Chubb's spherules were artifacts caused by shrinkage of the nucleolus during fixation, and he rejected the idea that they contributed to the yolk nucleus. Holland's (1976) fine structural study has shown that the yolk nucleus of A. bifida consists of hundreds of dense clumps; in addition, perinuclear dense granules, each about the size of such a clump, are present in the ooplasm. This author suggests that the perinuclear dense granules are equivalent to the basophile spherules of Chubb and are precursors of the clumps of the yolk nucleus. Basophile spherules were not evident in the material from F. serratissima examined in this study by light microscopy.

The function of the yolk nucleus is a puzzle. Chubb (1906) viewed it as a waste product of cell metabolism. Harvey (1930) implicated it in yolk formation, but as Holland (1976) points out, there is little evidence in support of this. Holland believes that the function of the yolk nucleus is related to the unsettled question of the function of the perinuclear dense granules. Results from the present study suggest that the yolk nucleus is an accumulation of a cell product, perhaps consisting of perinuclear dense granules, which is being produced at a rate faster than it can be used. The yolk nucleus could thus be viewed as a storage site which provides for the future requirements of the growing oocyte. This would explain why the yolk nucleus appears in small to medium-sized oocytes and then gradually disappears as the oocyte approaches its final size. It is conceivable that a population of F. serratissima from a different geographical location, and therefore exposed to different environmental conditions, uses the precursor of the yolk nucleus at a rate high enough that it does not accumulate in the ooplasm as a yolk nucleus. This might account for

the absence of oocyte yolk nuclei in specimens of F. serratissima examined by Holland (1976).

About a week before the spawning date of the feather star Comanthus japonica, the surface of the oocytes becomes covered with hundreds of pits; each pit contains a jelly clump (Holland et al. 1975; Holland, 1977). The wrinkles observed in the plasma membrane of some large oocytes of F. serratissima during this study may be equivalent to these pits, although jelly clumps were not demonstrated. Holland (1977) has shown that such jelly clumps are necessary for formation of the ridged fertilization membrane present in fertilized eggs of C. japonica.

In the echinoderms, ovulation is presently known for the crinoids Comanthus japonica (Holland and Dan, 1975) and Florometra serratissima, while a similar process occurs in the ophiuroid Ophiura lutkeni, and in the holothuroid Parastichopus californicus (personal observations, Friday Harbor Laboratories). Observations made on ovulation in isolated oocytes demonstrates that the mechanism of ovulation must lie with the oocyte and its surrounding germinal lamina. This rules out such possibilities as pressure changes in the haemal space, participation by the epithelial cells of the inner layer of the ovary, or contraction of the basal lamina underlying the epithelial cells. If oocyte motility is involved, it would be expected that the germinal lamina would maintain its overall shape and surface area as the oocyte squeezed through the opening of the germinal lamina to reach the exterior. In reality, however, the germinal lamina rolls up at the periphery of the opening during ovulation, while simultaneously decreasing in overall surface area. This strongly suggests that the germinal lamina forces the oocyte through the opening. This force would be mediated via the jelly layer

surrounding the oocyte.

It is possible that the germinal lamina is an elastic structure. As the oocyte grows, the germinal lamina becomes greatly stretched and thus furnished with elastic potential energy. At the appropriate time, the mechanism holding the oocyte within the germinal lamina, perhaps provided by the epithelial cells surrounding the stalk, is removed, and the germinal lamina forces the oocyte through the opening. This hypothesis may explain why large oocytes, when isolated from the rest of the ovary, often begin to ovulate automatically.

Interestingly enough, in F. serratissima, only large oocytes participate in ovulation, while small oocytes remain in the haemal space within their germinal laminae. The stimulus causing ovulation is thus selective, and likely originates from the oocyte itself, perhaps in response to a more general stimulus mediated via the haemal space or the ovarian lumen. Hormones are presumably involved, but their nature in crinoids is unknown.

It is likely that the genital spots in F. serratissima represent preformed areas of weakness in the wall of the genital pinnule, although such regions could not be identified with assurance in histological sections. The mechanism responsible for the formation of the genital nipples is not known for certain. K. Dan and J. C. Dan (1941) observed genital nipples in C. japonica and attributed this to an increase in the internal pressure of the pinnule. This could be brought about by contraction of muscles present in the gonads. Muscle fibers are known to be present in the outer layer of the ovary of Nemaster rubiginosa (Holland, 1971), in the inner layer of the ovary of F. serratissima (this study), and in the outer layer of the testis of

F. serratissima (Bickell et al., in press).

This study demonstrates that both unspawned ova and defective oocytes undergo cytolysis in the ovarian lumen and that the resulting spherical fragments are actively phagocytized by the epithelial cells of the inner layer. If, as postulated, the germinal cells absorb nutrients from the haemal space, then it is likely that nutriment in the epithelial cells traverses the basal lamina and enters the haemal space where it can then be utilized by the germinal cells.

It was estimated in this account that a large female specimen of F. serratissima releases roughly 23,800 ova during a spawning. This is considerably less than the approximately 2 million ova Holland et al. (1975) calculated that a large female specimen of the 40-armed C. japonica emits on the day of spawning each year. Information of this kind for other crinoid species is not available.

LITERATURE CITED

The periodicals have been abbreviated in accordance with "World list of scientific periodicals, 4th ed. London 1964" or, for more recent journals, "Bibliographic guide for editors and authors, Washington 1974".

Bickell, L.R., F.S. Chia and B.J. Crawford: A fine structural study of the testicular wall and spermatogenesis in the crinoid, Florometra serratissima (Echinodermata). J. Morph. (in press)

Carpenter, P.H.: Report on the Crinoidea collected during the voyage of H.M.S. Challenger during the years 1873-1876. - The stalked crinoids. Rept. Scient. Results Voyage Challenger, Zool. 11, 1-442 + 62 plates (1884)

Carpenter, W.B.: On the structure, physiology and development of Antedon (Comatula, Lamk.) rosaceus. Proc. R. Soc. 24, 211-231 + plates 8 and 9 (1876)

Chadwick, H.C.: Antedon. L.M.B.C. Mem. typ. Br. mar. Pl. Anim. 15, 1-47 + 7 plates (1907)

Chubb, G.C.: The growth of the oocyte in Antedon: a morphological study in the cell-metabolism. Phil. Trans. R. Soc. (B.) 198, 447-505 + plates 29-31 (1906)

Cloney, R.A.: Cytoplasmic filaments and morphogenesis: effects of cytochalasin B on contractile epidermal cells. Z. Zellforsch. mikrosk. Anat. 132, 167-192 (1972)

Crawford, B., R.A. Cloney, and R.D. Cahn. Cloned pigmented retinal cells: the effects of cytochalasin B on ultrastructure and behaviour. Z. Zellforsch. mikrosk. Anat. 130, 135-151 (1972)

Dan, K.: Meiosis in the eggs of the crinoid, Comanthus japonica. Annotnes. zool. jap. 25, 258-264 (1952)

Dan, J.C. and K. Dan: Early development of Comanthus japonicus. Jap. J. Zool. 9, 565-574 + plates 15-17 (1941)

Dan, K. and J.C. Dan: Spawning habit of the crinoid, Comanthus japonicus. Jap. J. Zool. 9, 555-564 + plate 14 (1941)

-- and H. Kubota: Data on the spawning of Comanthus japonica between 1937 and 1955. Embryologia 5, 21-37 (1960)

Dietrich, H.F. and A.R. Fontaine: A decalcification method for ultrastructure of echinoderm tissues. Stain Technol. 50, 351-354 (1975)

Dujardin, M.: L'Institut 3, No. 119, 268 (1835). (Unable to obtain this article; relying on information in Perrier [1886].)

Fishelson, L.: Gamete shedding behaviour of the feather star Lamprometra klunzingeri in its natural habitat. Nature, Lond. 219, 1063 (1968)

Freund, J.E.: Modern elementary statistics, 432 pp. Englewood Cliffs, New Jersey: Prentice Hall 1967

Hamann, O.: Anatomie und Histologie der Ophiuren und Crinoiden. Jena. Z. Naturw. 23, 233-388 + 12 plates (1889)

Harvey, L.A.: Studies on echinoderm oogenesis. - I. Antedon bifida (Pennant). Proc. R. Soc. (B.) 107, 417-441 (1930)

Holland, N.D.: The fine structure of the ovary of the feather star Nemaster rubiginosa (Echinodermata: Crinoidea). Tissue and Cell 3, 161-175 (1971)

-- The fine structure of the yolk nucleus in oocytes of Antedon bifida (Pennant) (Echinodermata: Crinoidea). J. mar. biol. Ass. U.K. 56, 59-63 (1976)

-- The shaping of the ridged fertilization membrane of Comanthus japonica (Echinodermata: Crinoidea). Biol. Bull. mar. biol. Lab., Woods Hole 153, 299-311 (1977)

-- and K. Dan: Ovulation in an echinoderm (Comanthus japonica). Experientia 31, 1078-1079 (1975)

-- and H. Kubota: Fluctuations in the volume of non-germinal cell populations during the annual reproductive cycle of Comanthus japonica (Echinodermata, Crinoidea). Annotnes. zool. jap. 48, 83-89 (1975)

--, J.C. Grimmer and H. Kubota: Gonadal development during the annual reproductive cycle of Comanthus japonica (Echinodermata: Crinoidea). Biol. Bull. mar. biol. Lab., Woods Hole 148, 219-242 (1975)

Hyman, L.H.: The invertebrates. Vol. 4. Echinodermata, 763 pp. New York: McGraw-Hill 1955

Ludwig, H.: Beiträge zur Anatomie der Crinoideen. Z. wiss. Zool. 28, 255-353 + 8 plates (1877)

-- and O. Hamann: Echinodermen (Stachelhäuter). V. Die Seelilien. Bronn's Kl. Ordn. Tierreichs 2, 1415-1602 + plates 9-13 (1907)

Luft, J.H.: Improvements in Epoxy resin embedding methods. J. biophys. biochem. Cytol. 9, 409-414 (1961)

Marshall, W.S.: The genitalpores of the male Antedon rosacea. Zool. Anz. 25, 209-211 (1902)

Mladenov, P.V.: Continuous reproduction in a population and in individuals of the feather star Florometra serratissima (Echinodermata: Crinoidea) at Barkley Sound, British Columbia. In: Development and reproductive biology of the feather star Florometra serratissima (Echinodermata: Crinoidea). Ph.D. thesis, University of Alberta 1980

Mortensen, T.: Studies in the development of crinoids. Pap. Tortugas Lab. 16, 1-94 + plates 1-28 (1920)

Müller, J.: Ueber den Bau von Pentacrinus caput Medusae. Abh. K. Akad. Wiss. Berl. Phys. Kl., pp 177-248 + 6 plates (1841). (Unable to obtain this article; relying on information in Perrier [1886].)

Perrier, E.: Mémoire sur l'organisation et le développment de la Comatule de la Mediterranée (Antedon rosacea Linck). Nouv. Archs Mus. Hist. nat., Paris 9, 53-348 + plates 11-20 (1886)

Russo, A.: Studii su gli Echinodermi. Atti Accad. gioenia Sci. nat. 12, 1-93 (1902). (Unable to obtain this article; relying on information in Mortensen [1920].)

Schroeder, T.E.: The contractile ring: I. Fine structure of dividing mammalian (HeLa) cells and the effects of cytochalasin B. Z. Zellforsch. mikrosk. Anat. 109, 431-449 (1970)

Semper, C.: Brief observations on the anatomy of Comatula. With an addendum by W.B. Carpenter. Ann. Mag. nat. Hist. 16, 202-209 (1875). (Unable to obtain this article; relying on information in Ludgwig and Hamann [1907].)

Thompson, J.V.: Memoir on the star-fish of the genus Comatula, demonstrative of the Pentacrinus europaeus being the young of our indigenous species. Edinburgh New Philosophical Journal 20, 295-300 + 1 plate (1836)

Walker, C.W.: Ultrastructure of the somatic portion of the gonads in asteroids, with emphasis on flagellated collar cells and nutrient transport. J. Morph. 162, 127-162 (1979)

Continuous Reproduction in a Population and in Individuals of the Feather Star *Florometra serratissima* (Echinodermata: Crinoidea) at Barkley Sound, British Columbia

ABSTRACT

The breeding pattern of a population of the comatulid crinoid Florometra serratissima (A.H. Clark) from Barkley Sound, British Columbia was studied at both the populational and individual levels using histological techniques. All of the genital pinnules on a male or female specimen are in reproductive synchrony. The population as a whole exhibits a continuous breeding pattern, and this is the first time that continuous reproduction has been demonstrated in a crinoid. More than 80% of the oocytes present in females are less than 90 µm in diameter. This large pool of small oocytes rapidly and continuously gives rise to a small number of large oocytes which are ovulated and spawned. It is estimated that one-fifth of the female population is preparing to spawn at any one time.

An analysis of breeding patterns in individual animals using specimens maintained in cages under natural conditions showed that gametogenesis in most individuals takes place continuously. In some, a lowering of reproductive activity occurs during the winter, but this phenomenon is not evident at the populational level. A female spawns at least nine times per year, and it is calculated that a large specimen would release about 214,000 ova during this period. The hypothesis that continuous reproduction in F. serratissima is a response to a low fecundity imposed by body structure, combined with a pelagic mode of development, is discussed.

INTRODUCTION

The reproductive cycle of only one crinoid, the feather star Comanthus japonica, has been studied in detail (Dan and Dan, 1941; Dan and Kubota, 1960; Holland et al., 1975). A population of this species found in shallow water near Misaki, Japan, has a well-defined annual reproductive cycle with spawning typically occurring on one afternoon in the first half of October, although spawning occasionally takes place on several afternoons in October.

Information relating to crinoid breeding seasons has been reviewed by A.H. Clark (1921), Hyman (1955) and Boolootian (1966). These summaries suggest that crinoids generally have short breeding periods of one or two months duration that occur at a definite season of the year. Unfortunately, all of the information available to these reviewers, with the exception of that dealing with C. japonica, was limited to single or, at best, sporadic observations concerning spawnings, the presence of large oocytes in ovaries, or the discovery of pentacrinoids on the cirri of adults. Definite conclusions concerning breeding patterns cannot be drawn from these kinds of data, and it is likely that the duration of the breeding season for many crinoid species has been underestimated.

Florometra serratissima (A.H. Clark) is a comatulid crinoid or feather star. It is the only crinoid species found on the west coast of North America, where it ranges from lower California northwards to Alaska; it has been collected from depths of 11 m to 1252 m (Clark and Clark, 1967).

In this study, the reproductive activity of a population of F. serratissima from Barkley Sound, British Columbia, was monitored for

over two years using histological techniques. In addition, reproductive changes within single animals were followed for periods of 10 months or more under natural conditions. Crinoids are admirably suited to a study of individual breeding patterns because they have numerous gonads which can be easily removed and examined at regular intervals without harming the animal. The study of individual reproductive patterns under natural circumstances in other marine invertebrate species has been rarely accomplished in the past (Giese and Pearse, 1974, pp. 12-13). It will be demonstrated that the population of F. serratissima exhibited continuous reproduction, while individuals in the population also showed continuous breeding, with only a slight lowering of reproductive activity in some animals for a brief period during the winter.

MATERIALS AND METHODS

From 15 to 20 large (length of longest arm greater than 180 mm) specimens of Florometra serratissima (A.H. Clark) were collected with S.C.U.B.A. at 4- to 6-week (rarely 8-week) intervals from a population living at a depth of from 17 m to 34 m at the mouth of Bamfield Inlet, Barkley Sound, British Columbia (48° 50' 07" N, 125° 08' 09" W).

Several genital pinnules were removed from the basal fourth of an arm of each female on the day of collection and fixed immediately in seawater-Bouin's fluid. After fixation for at least 24 h, the tissue was dehydrated in isopropanol, cleared in a mixture of α-terpineol and toluene (1:3), and embedded in Paraplast (Sherwood medical). Serial sections at 7 µm over a length of at least 0.6 mm were made through the thick basal portion of one genital pinnule from each female and stained with haematoxylin and eosin. All of the histological sections of each ovary were closely examined to determine if any oocytes were in the process of ovulation, or if the ovarian lumen contained ova. The size-frequency distribution of the oocyte population for each female was then analyzed using the frequency polygon method (Pearse, 1965). The diameter of the first 50 oocytes seen in the histological sections was measured with an ocular micrometer. The oocytes were measured in nucleolar section, and since they were usually oval in outline, the diameter was taken as the average of measurements made along both the long and short axis. The diameter of sectioned oocytes was generally 10% less than that of living oocytes due to shrinkage. These data were grouped into 20 size classes at 10 µm intervals from 0 to 200 µm. The data

from all females for each sampling date were pooled, and the percentage of oocytes in each size class was plotted. The reproductive state of the males at each sampling date was evaluated by removing a genital pinnule from each, and testing for the presence or absence of active sperm in smears.

A histological comparison of reproductive activity in the genital pinnules from a single female was made to determine if the ovaries within an individual were in reproductive synchrony. Five genital pinnules were removed from along the length of each of two arms of a female, fixed in seawater-Bouin's fluid, and their oocyte size-frequency distribution analyzed using the methods just described. The testes of a male were checked for reproductive synchrony by examining smears made from many genital pinnules taken from along the length of all 10 arms.

The reproductive activity of individual animals was monitored over long periods of time. Commencing February 1978, five females and three males were maintained in holding cages placed on the bottom at the mouth of Bamfield Inlet in the centre of the naturally occurring population. These animals were exposed to environmental conditions virtually identical to uncaged animals, but they could be identified and collected on a regular basis.

Each holding cage consisted of a frame constructed of 2.5 cm diameter PVC tubing covered with nylon netting of 2.5 cm mesh size. The cages measured 1m x 1m x 0.5m and were divided into four labelled compartments. Entrance was gained to each compartment through a hole in the netting which could be closed with a purse-string arrangement. The PVC tubing was drilled with numerous holes, allowing the frame to

fill with water, thus allowing the cage to sink. As a precaution, each cage was held in place with a 4.5 kg weight.

At roughly monthly intervals, the eight animals in the holding cages were retrieved in pre-labelled plastic bags by S.C.U.B.A., and brought to the laboratory. A few genital pinnules from the basal fourth of an arm of each feather star were removed and fixed in seawater-Bouin's fluid. The animals were returned to the holding cages on the day after retrieval using S.C.U.B.A. If the specimens were handled only by the calyx, and if diving mitts were not used, this procedure caused the feather stars little harm, and they remained healthy for at least 10 months, normally longer. Monitoring of an individual was terminated as soon as it exhibited excessive damage to the arms. The genital pinnules were prepared for histology in the previously described manner. Histological sections of each ovary were examined for ovulating oocytes and ova, and the size-frequency distribution of the oocyte population was determined for each female. A cross section of a testis from each male at each sampling date was photographed, and variations in the amount of tailed sperm noted qualitatively. In addition, the thickness of the spermatogenic layer (spermatogonia, spermatocytes, and spermatids), and the diameter of the spermatozoal layer of each testis was measured.

To supplement such long-term monitoring of individual reproductive activity, reproduction in two females was monitored on a frequent basis during a one-month period. These females were placed in a 0.5m x 0.5m x 0.5m cage, similar in construction to the larger holding cages, which was lowered to the bottom at the study site on a line. Every three days the cage was raised to the surface, a genital

pinnule carefully removed from each animal and fixed in seawater-Bouin's fluid, and the females then returned immediately to the bottom in the cage. Histological sections were prepared, examined for ovulating oocytes and ova, and the size-frequency structure of the oocytes analyzed.

RESULTS

The size-frequency structure of oocytes in each of 10 genital pinnules removed from along the length of two of the arms of a female on 20 July 1979 is shown in Fig. 12. Most of the genital pinnules contained numerous oocytes extending over a wide range of sizes; a few of the smaller, most distal genital pinnules, however, contained small and medium-sized oocytes, but lacked larger oocytes. Each frequency polygon is polymodal, indicating that several generations (cohorts) of oocytes were present in each genital pinnule; there is no evident correspondence of the modes from one genital pinnule to the next.

This analysis demonstrates that all of the genital pinnules of a female, except for the smaller distal ones, are in reproductive synchrony. Further support for this conclusion comes from the observation that ovulated eggs, when discovered in a female, were present in all of the genital pinnules with the exception of those near the arm tips. These distal genital pinnules, which had been newly acquired during arm growth, had apparently not had time to produce large oocytes suitable for ovulation and spawning. The lack of correspondence of the modes in the frequency polygons suggests that new oocytes are produced frequently but not simultaneously in the genital pinnules.

Smears made from numerous testes taken from a single male on 20 July 1979 showed that all of the genital pinnules contained active sperm, even the small distal ones. The genital pinnules of a male are thus in reproductive synchrony as well. Therefore, the examination

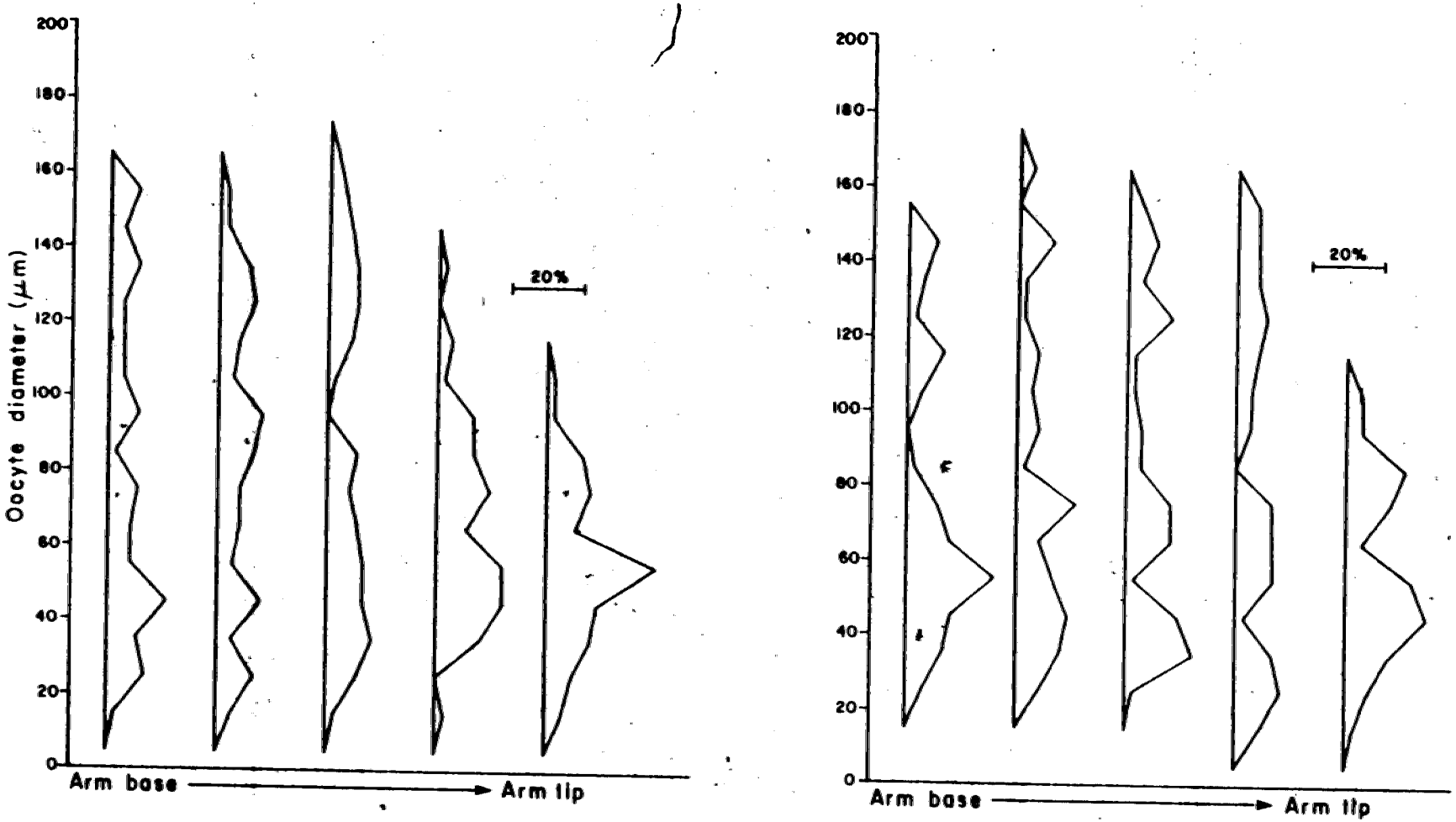


Fig. 12. Florometra serratissima. The size-frequency structure of oocytes in each of 10 genital pinnules removed from along the length of two of the arms of a female on 20 July 1979

of a single genital pinnule from the basal portion of an arm of a male or female specimen of F. serratissima will give a good indication of the overall reproductive activity of the animal.

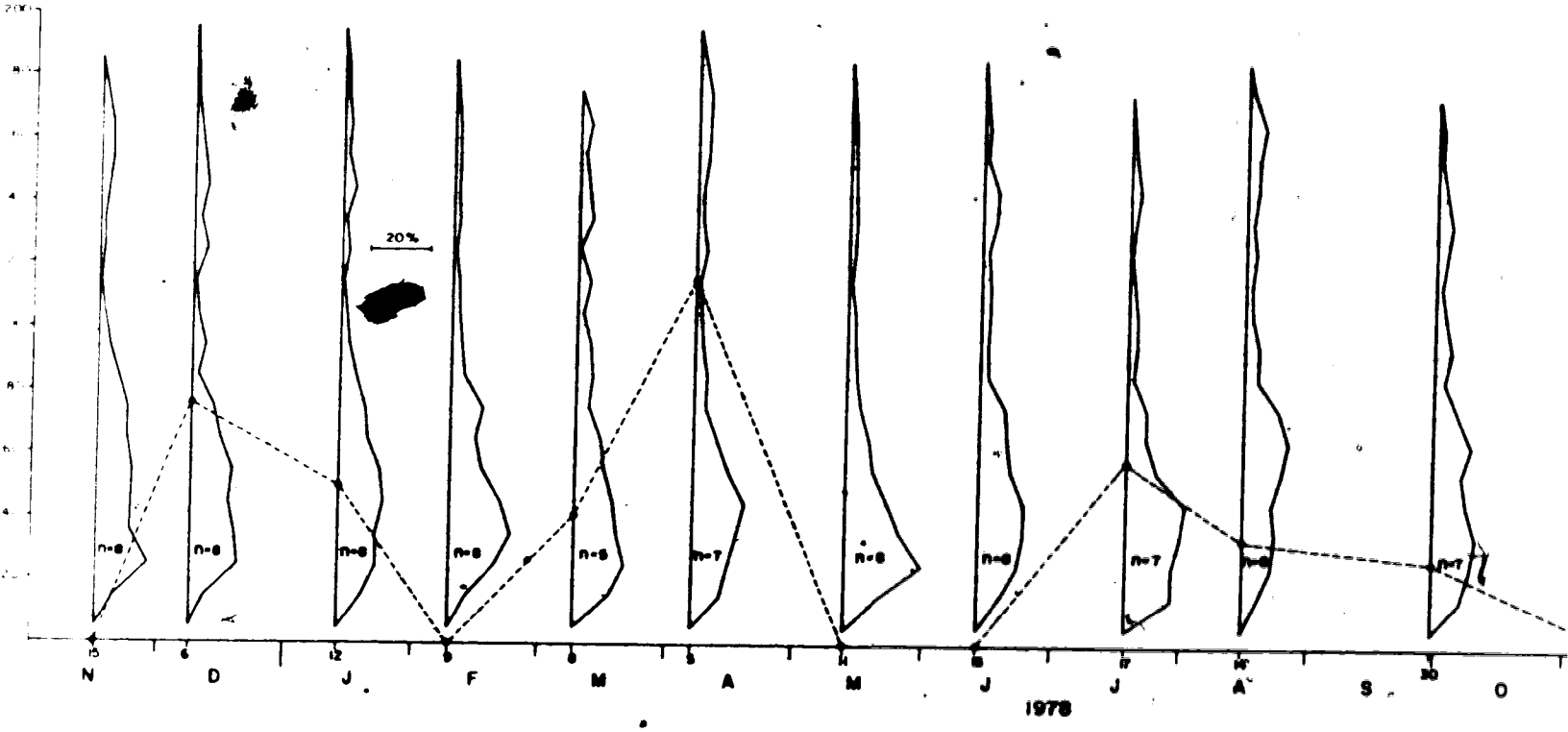
The oocyte size-frequency structure for the female population through the two-year sampling period is given in Fig. 13. In addition, this figure shows the percentage of females at each sampling date with evidence of ovulation (ova in the ovarian lumen or oocytes squeezing into the ovarian lumen) and which were thus preparing to spawn (Mladenov, 1980a).

There is remarkable similarity in the frequency polygons from one sampling date to the next: each is polymodal, with the bulk of the oocytes falling into the lower size classes, but with a few oocytes always present in the higher classes. The smallest oocytes were just under 10 µm in diameter, while the largest were always greater than 160 µm in diameter except for 5 April 1979 when the largest oocytes were between 150 µm and 160 µm in diameter.

The frequency polygon for each individual female at each collection was normally congruent with the average polygon. This is demonstrated for the 3 July 1979 collection in Fig. 14. Some females collected in the winter, however, lacked oocytes in the higher size classes as shown for the 9 February 1978 and 26 January 1979 collections (Fig. 14); note that two females in the former collection and one in the latter collection did not have large oocytes. There is thus some indication of a slight seasonality in reproductive activity at the individual level. The only hint of such seasonality in the average polygons of Fig. 13 is the slightly smaller numbers of oocytes in the higher size classes (greater than 90 µm in diameter) in the

Fig. 13. Florometra serratissima. The oocyte size-frequency structure of the female population over the two-year sampling period (frequency polygons), and the percentage of females with ovulated oocytes or ova (dashed line). Each frequency polygon is an average of the cytometric data from the number of females, n, indicated within the polygon. The sampling date is shown beneath each polygon

14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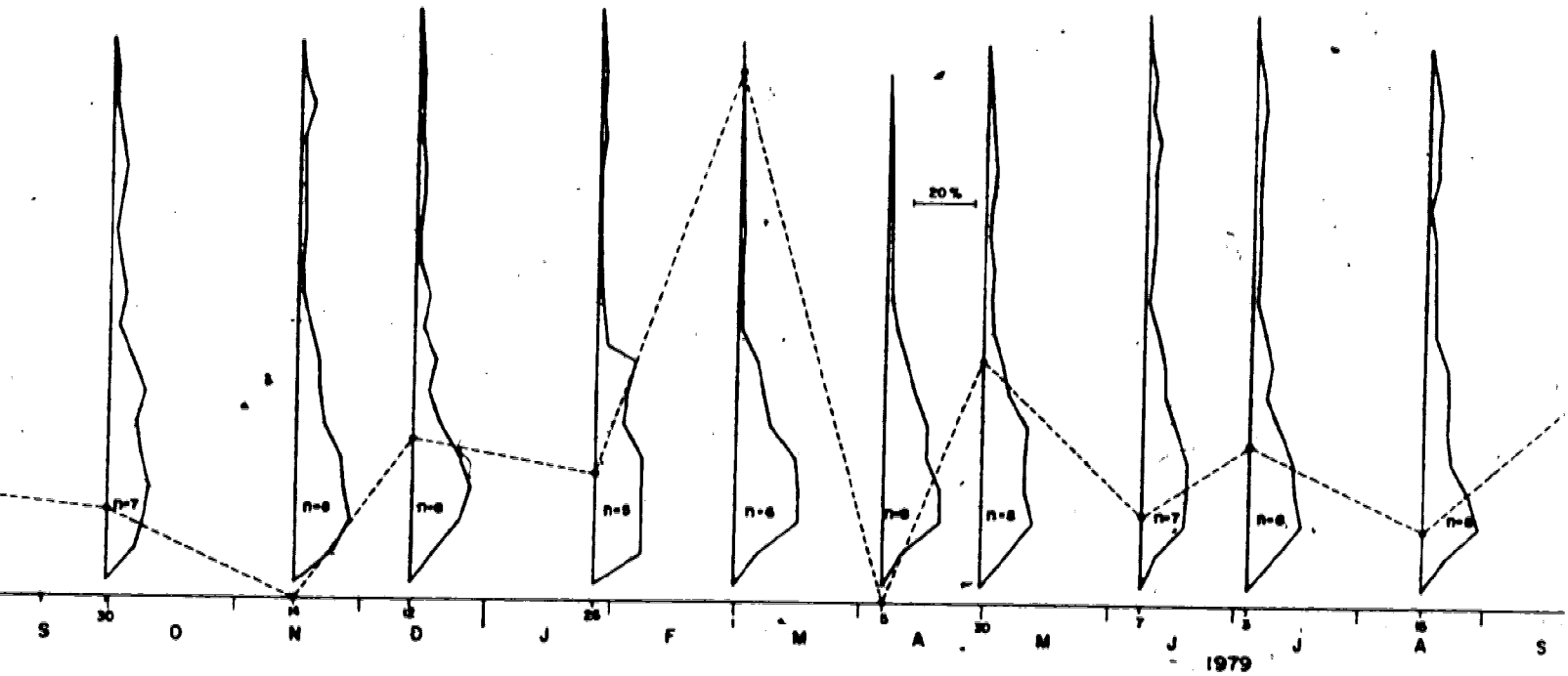
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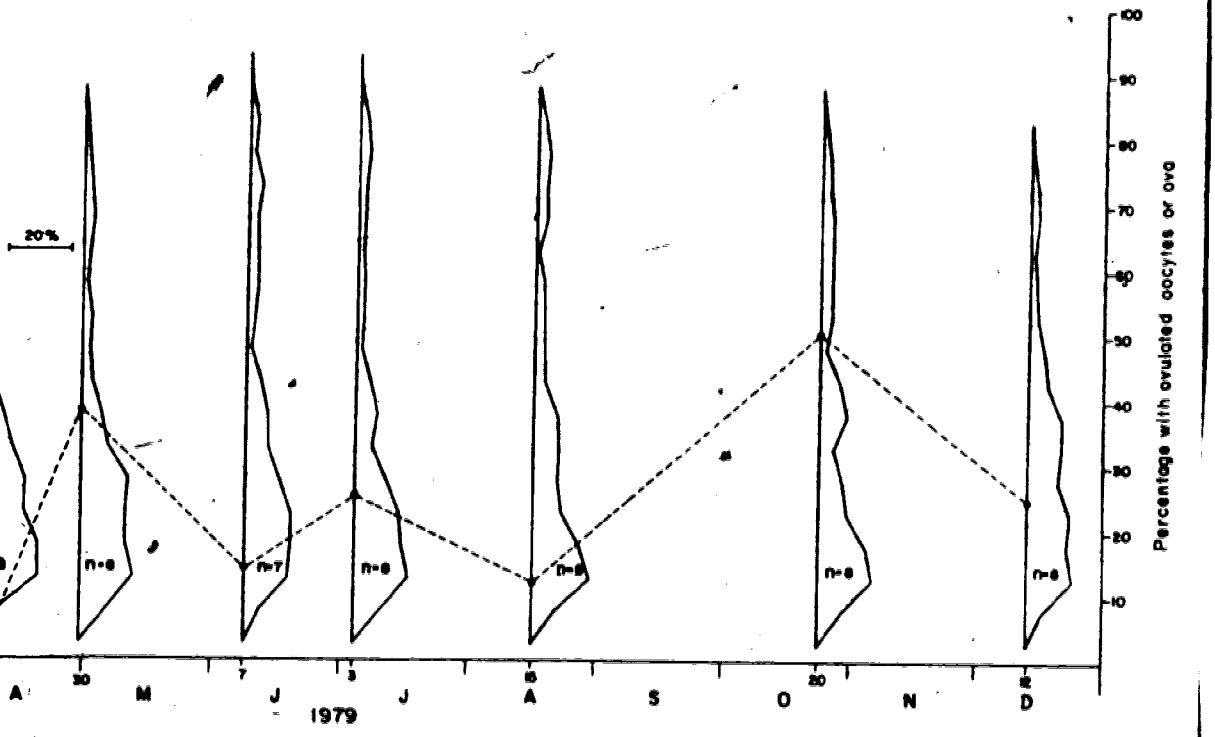
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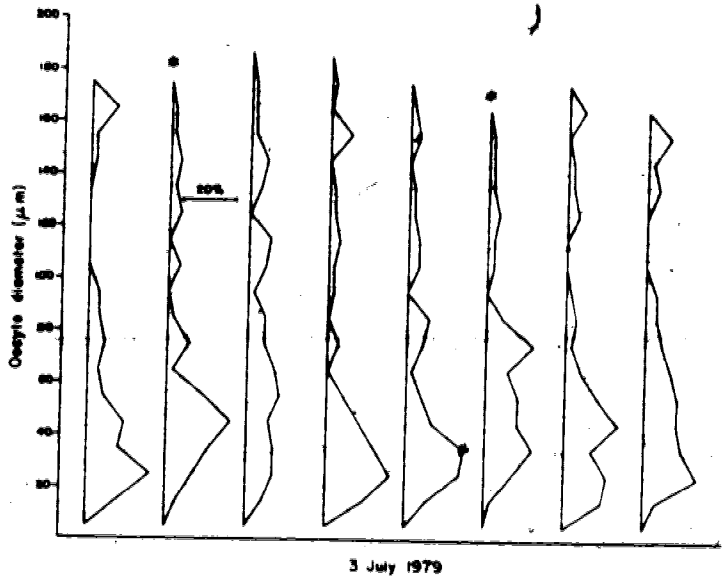
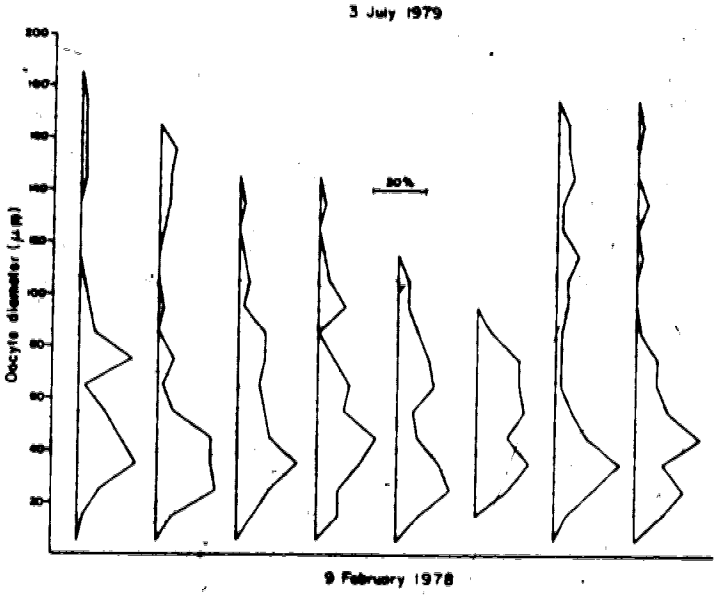
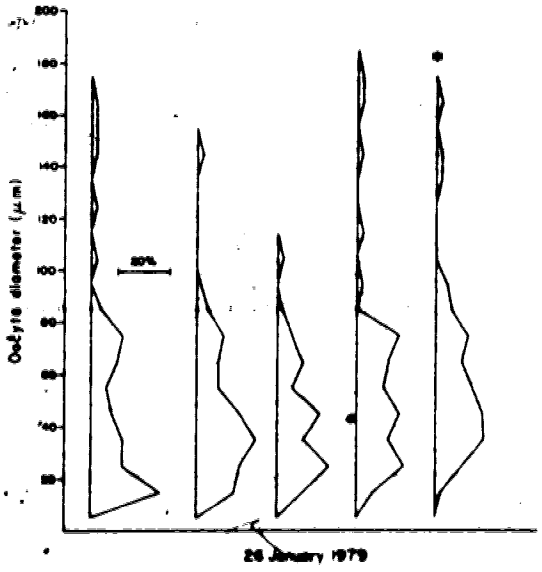
90
80
70
60
50
40
30
20
10
Percentage with ovulated oocytes or ova

Fig. 14. Florometra serratissima. The size-frequency structure of the oocytes of each female collected on 9 February 1978, 26 January 1979, and 3 July 1979. An asterisk above a polygon indicates that ovulated oocytes or ova were present in the genital pinnules


Oocyte diameter (µm)
20%
3 July 1979


Oocyte diameter (µm)
20%
9 February 1978


Oocyte diameter (µm)
20%

9 February 1978, and 26 January, 1 March and 5 April 1979 collections. For all purposes, then, the breeding season of the female population can be considered continuous. That is, at all times of the year, the great majority of females contained some large oocytes which were capable of being ovulated and spawned.

Fig. 15 gives the average oocyte size-frequency distribution for all 22 collections made over the two-year sampling period. Such pooling of the data is justified since the frequency polygons are so similar from one date to the next (Fig. 13). Also presented in Fig. 15 is the ogive of the cumulative frequency distribution for these data. Slightly more than 80% of the oocytes present in the female population were less than 90 µm in diameter, while slightly more than 50% were below 50 µm in diameter. This indicates that the small number of large oocytes present in the population was derived form a large pool of small oocytes which was maintained throughout the year.

Returning to Fig. 13, it can be seen that the percentage of ovulating females fluctuated greatly over the two-year sampling period (from 0% to a high of 83%). The fluctuations exhibit no evidence of seasonality, and when the data from both sampling years are compared, it is evident that some females were preparing to spawn at any time of the year. Further support for this conclusion is given in Table 3 which lists the dates on which ova were observed in the genital pinnules of living females collected and examined for the purpose of obtaining fertilizable eggs for developmental studies. Ova were found in all months except October and December when observations on living animals were not made. Fig. 13, however,

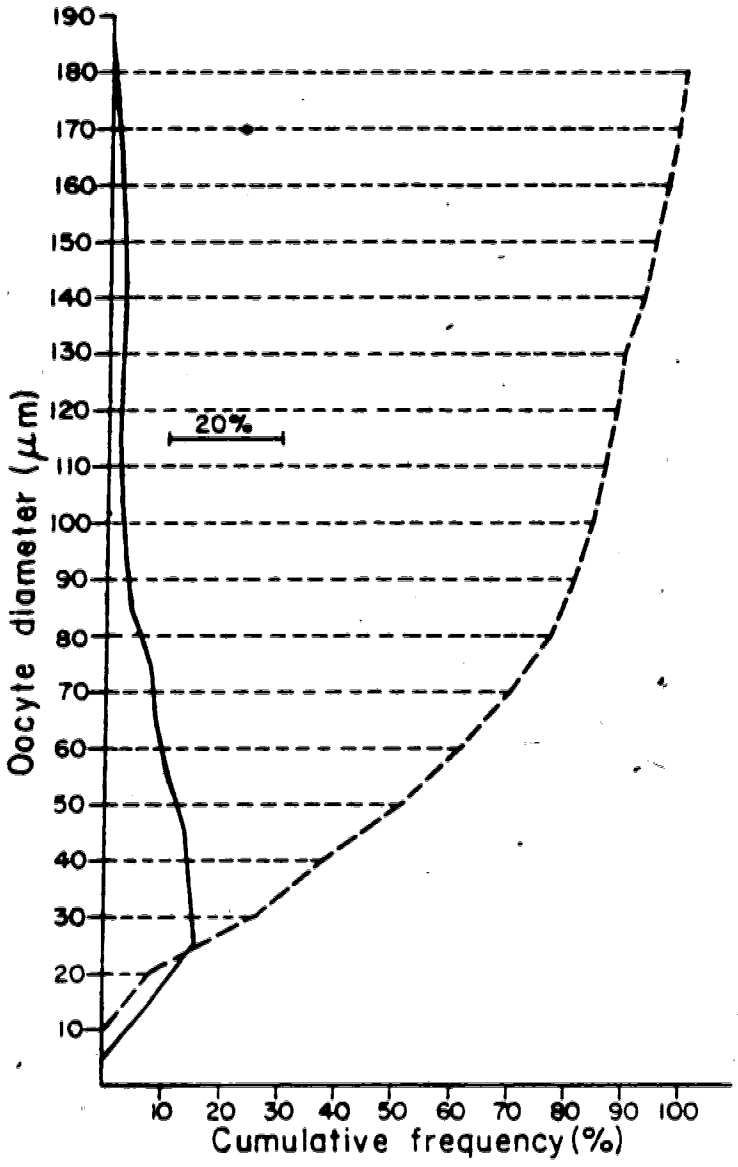


Fig. 15. Florometra serratissima. The average size-frequency distribution of oocytes in the female population (solid line), and the ogive of the cumulative frequency distribution for these data (dashed line). The frequency polygon is an average of the cytometric data for all females from all 22 collections made over the two-year sampling period

Table 3. Florometra serratissima. Dates when ovulated eggs were observed in the genital pinnules of living females

8 November 1977	16 November 1978
12	17
13	
24	Steady observations not made
25	from 1 December 1978 to
29	15 May 1979
14 January 1978	28 May 1979
15	
	11 June 1979
20 February 1978	12
	26
1 April 1978	27
6	28
7	
19	11 July 1979
20	20
	22
13 June 1978	24
16	31
Regular observations not	20 August 1979
made from 20 July 1978 to	21
28 September 1978	
30 September 1978	

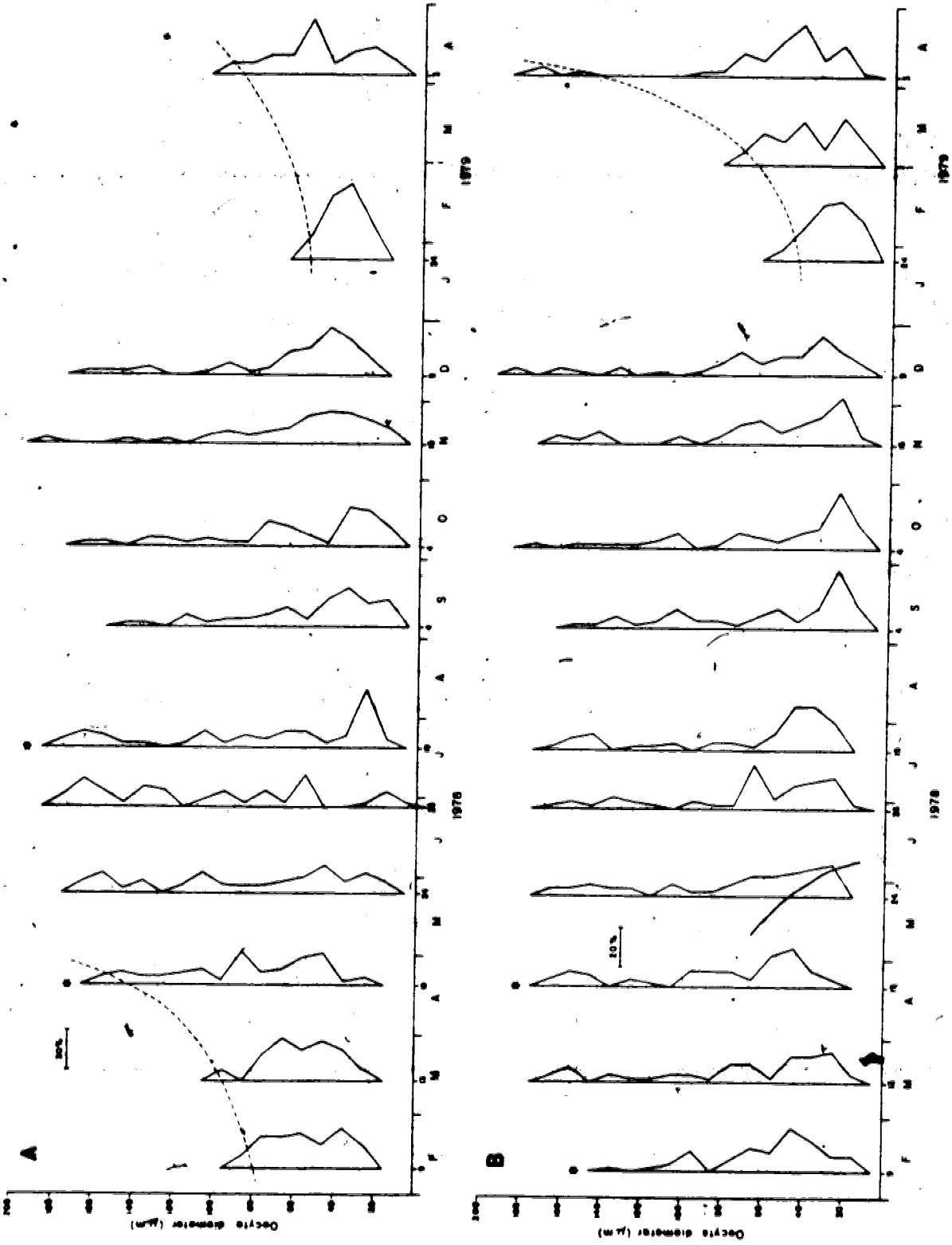
confirms that ovulating females were present in these months as well. The fluctuations in percentage of females ovulating may be partly attributed to random sampling error, although some of the higher peaks may represent times when a greater proportion of the female population than usual was preparing to spawn. The average percentage of females showing ovulation over the two-year period was 22% (± 21%, n = 22) suggesting that, on the whole, roughly one-fifth of the female population was about to spawn at any one time.

Males could be easily identified by their creamy-white genital pinnules. Testis smears demonstrated that all males collected at all times of the year contained active sperm. The breeding season of the male population, like that of the female population, is thus continuous.

Changes in the size-frequency structure of the oocytes in each of five females over a period of 10 or more months are presented in Figs. 16 and 17. Also included in these figures are the dates on which ovulation was noted in each animal and, wherever possible, the course of growth of oocyte generations. The results for each female will be presented in turn.

The first female (Fig. 16A) did not contain large (greater than 100 µm in diameter) oocytes when it was first collected on 9 February 1978, but it did have an abundant supply of smaller oocytes. Over the next two months large oocytes were produced, and by 19 April ovulated eggs were present in the genital pinnules and the animal likely spawned. This female maintained a supply of large oocytes during the spring, summer and fall. Throughout this period five or six modes could usually be distinguished in the polygons suggesting that five

Fig. 16. Florometra serratissima. Changes in the size-frequency structure of oocytes in individual females. The sampling date is given below each polygon. An asterisk above a polygon indicates that ovulated oocytes or ova were present. The dashed lines follow the likely course of growth of an oocyte generation. (A) First female. (B) Second female


A
B
1979
1978
J F M A M J J A S O N D
Oocyte diameter (μm)
20%

or six generations of oocytes were present. Evidence of a second spawning was noted in this female on 19 July 1978, and it is likely that additional spawnings occurred in the intervals between sampling. On 24 January 1979 only a pool of small oocytes was present, but by early April oocyte growth was underway once again. The breeding season for this female thus extended from mid-April to mid-December, during which time it spawned at least twice; there was a period of lower reproductive activity during the winter of 1978 and 1979.

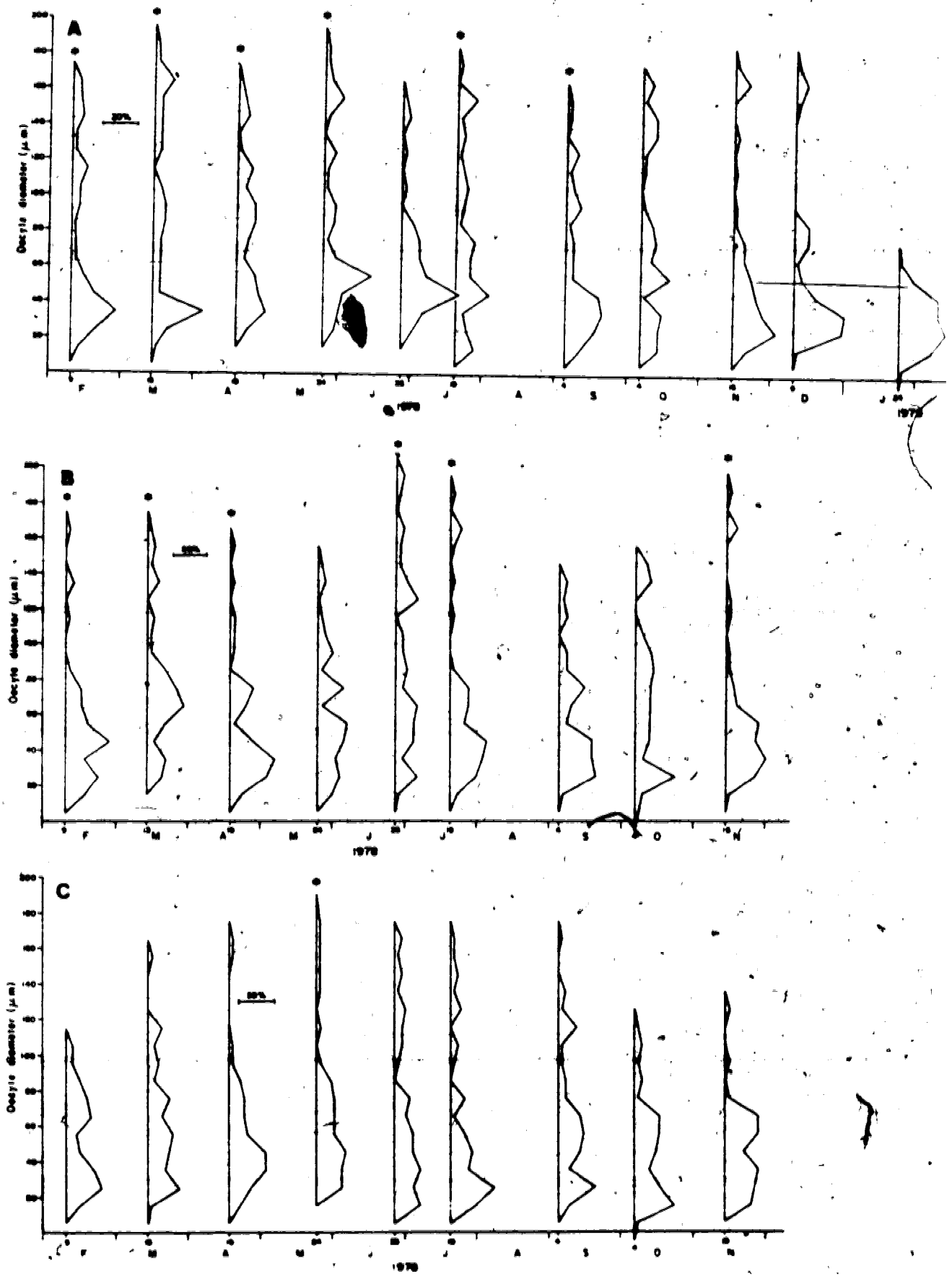
The second female (Fig. 16B) did not show low reproductive activity when it was first collected on 9 February 1978, and it spawned on or near that date. Large oocytes were present over the remainder of the year, with a second spawning noted on 19 April 1978. By January 1979, however, large oocytes had disappeared, but by early April they were present once again, showing that large oocytes can be produced from the pool of small oocytes in as little as two months.

The third female (Fig. 17A) exhibited high reproductive activity from February through to November 1978 during which time ovulation was noted on six occasions. Starting in December, spawned oocytes were not replaced from the pool of small oocytes, so that by January 1979, only small oocytes were present in the genital pinnules. This animal was not monitored after January because several arms had become damaged.

The fourth female (Fig. 17B) showed high reproductive activity throughout the February to November 1978 period over which it was followed, and it spawned at least six times during this interval. This animal was lost after the November collection.

The fifth female (Fig. 17C) showed signs of slightly lowered

Fig. 17. Florometra serratissima. Changes in the size-frequency structure of oocytes in individual females (cont'd). The sampling date is given below each polygon. An asterisk above a polygon indicates that ovulated oocytes or ova were present. (A) Third female. (B) Fourth female. (C) Fifth female


A
B
C
Oocyte diameter (µm)
1978

reproductive activity in February 1978, but large oocytes were present from March until September, during which time ovulation was noted once. In October and November oocytes were lacking in the largest size classes suggesting a lowered level of reproductive activity once again. Monitoring of this animal was stopped after November because it showed arm damage.

In summary, all five females showed prolonged periods of high reproductive activity during which multiple spawnings could be inferred for all except the fifth female in which ovulation was noted only once. Such variability in spawning frequency may be due, in part, to random sampling error, although it is possible that some females spawn more frequently than others. All three females that were monitored during the winter months of 1979 exhibited lowered reproductive activity. This cannot be attributed solely to the culminative effects of handling since some females (Figs. 16A and 17C) had lowered reproductive activity when first collected in February 1978. However, Figs. 13 and 14 demonstrate that this phenomenon only involved a part of the undisturbed female population, so under natural circumstances, many females must have produced gametes without pause through the winter months.

Two females were monitored on a frequent basis from 14 March until 14 April 1979 in order to obtain a better estimate of female spawning frequencies. The oocyte size-frequency structure, and the dates on which ovulated eggs were present, are shown in Fig. 18. As expected, reproductive activity was high throughout the sampling period. Each animal showed ovulation on one occasion, though on different days; females can thus spawn at least once in a monthly

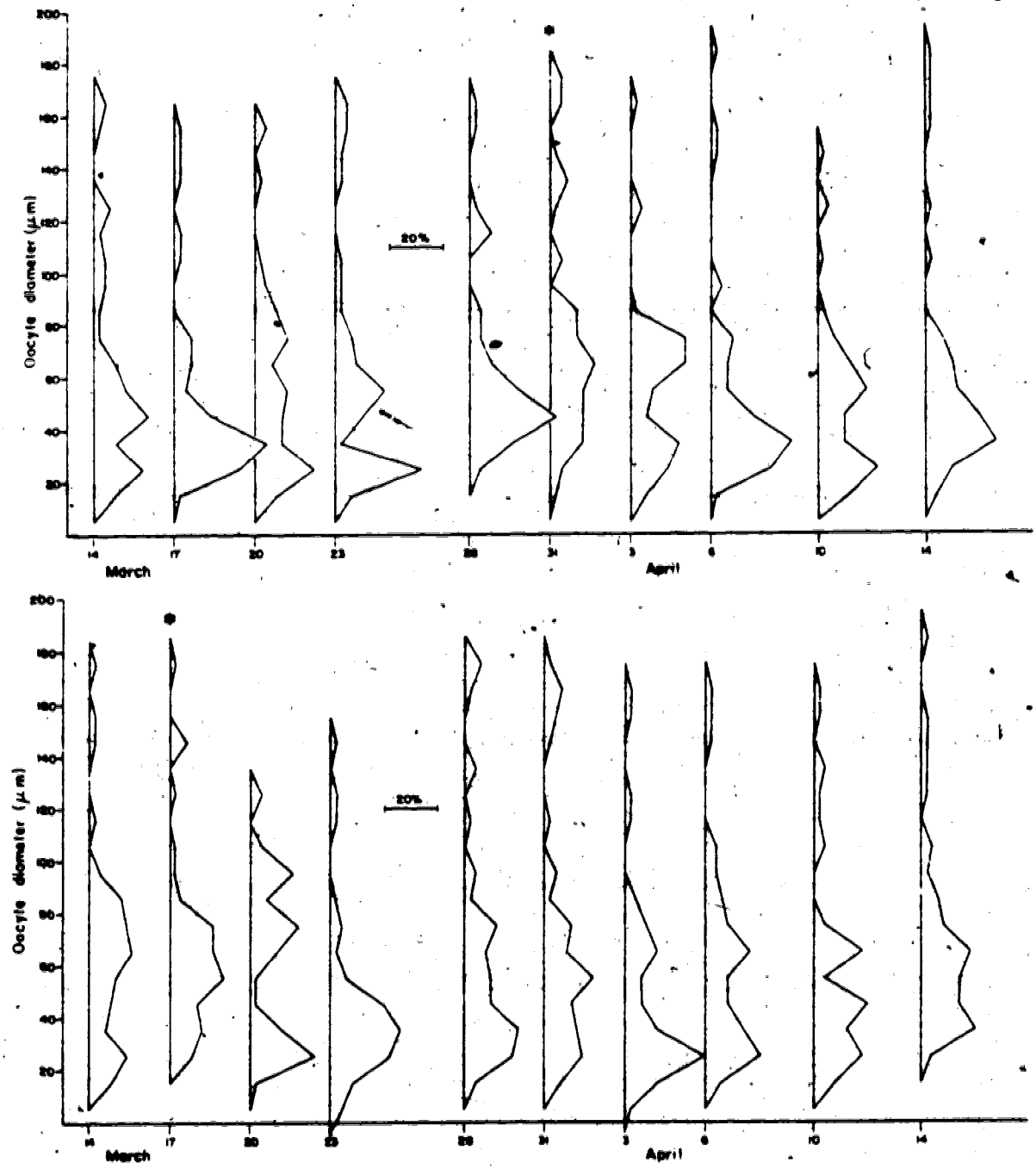


Fig. 18. Florometra serratissima. The size-frequency distribution of oocytes in two females monitored on a frequent basis. The sampling date is given below each polygon. An asterisk above a polygon indicates that ovulated oocytes or ova were present

period. It is therefore not unreasonable to suggest that a female spawns at least nine times in a year, taking into account a period of low winter reproductive activity. Those females not showing a winter pause in reproduction likely spawn at least 12 times in a year.

Changes in the amount of sperm in the testes of an individual male monitored from February 1978 to July 1979 are shown photographically in Fig. 19. Large quantities of sperm were present in the testicular lumen from February until December 1978, while in January and March 1979, sperm was existent, but in small quantities; sperm was again present in large quantities starting in April. Changes in the thickness of the spermatogenic layer and in the diameter of the spermatozoal layer for this male are presented graphically in Fig. 20A. Note that the thickness of the spermatogenic layer remained fairly constant, whereas the amount of sperm fluctuated from one sampling date to the next. Such fluctuations are likely a result of frequent partial spawnings of sperm. The winter decline in sperm production is clearly evident.

Two additional males were monitored from February 1978 until January 1979 (Fig. 20B and C). Again, the thickness of the spermatogenic layer remained fairly constant, but the amount of sperm often fluctuated. In these two males, however, the winter decline in sperm production was not as drastic compared to the first male.

These observations indicate that lowered reproductive activity in the winter is more evident in some males than in others, but sperm production does not usually cease altogether. It was not possible to estimate spawning frequencies in males since they gave no physical indication of incipient spawning equivalent to ovulation in females.

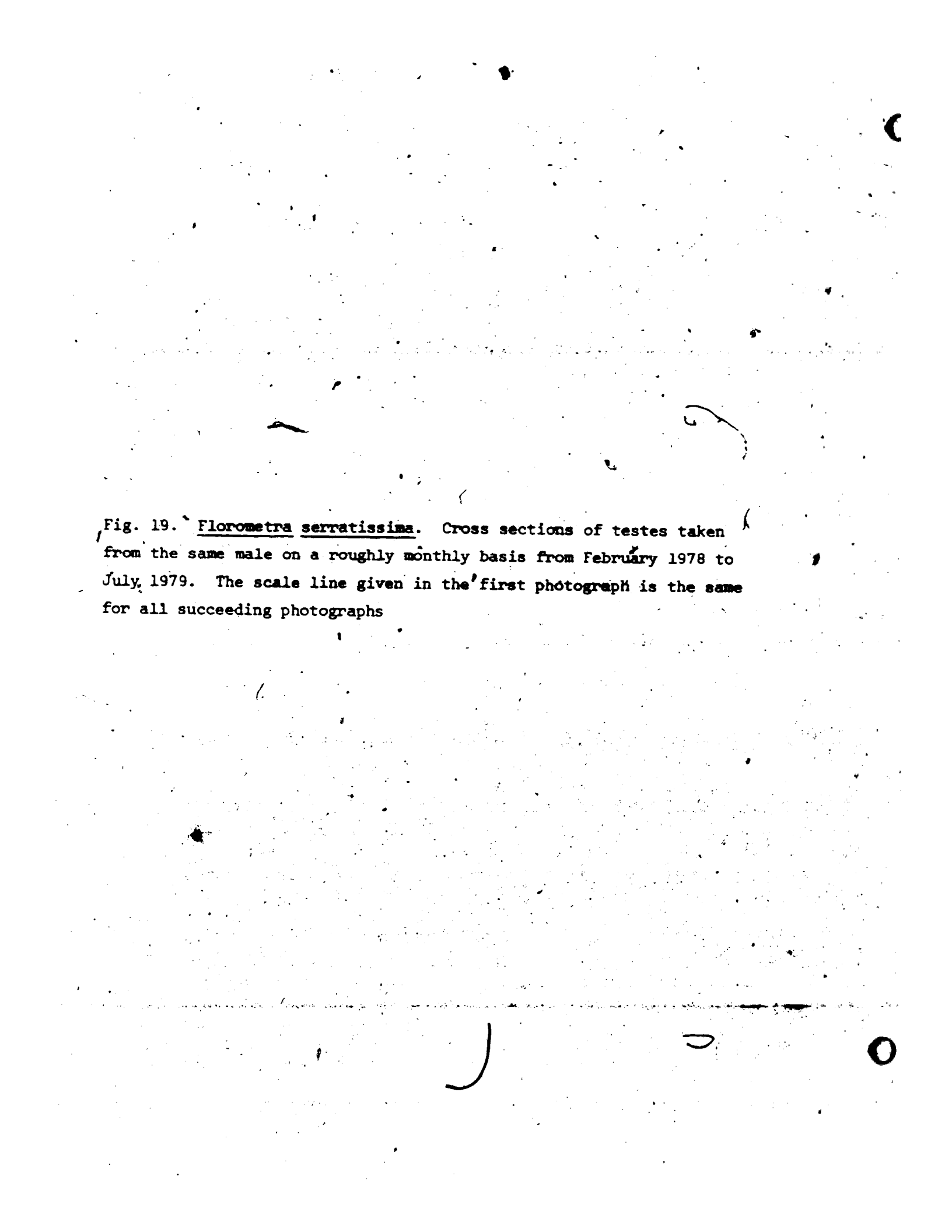


Fig. 19. Florometra serratissima. Cross sections of testes taken from the same male on a roughly monthly basis from February 1978 to July 1979. The scale line given in the first photograph is the same for all succeeding photographs

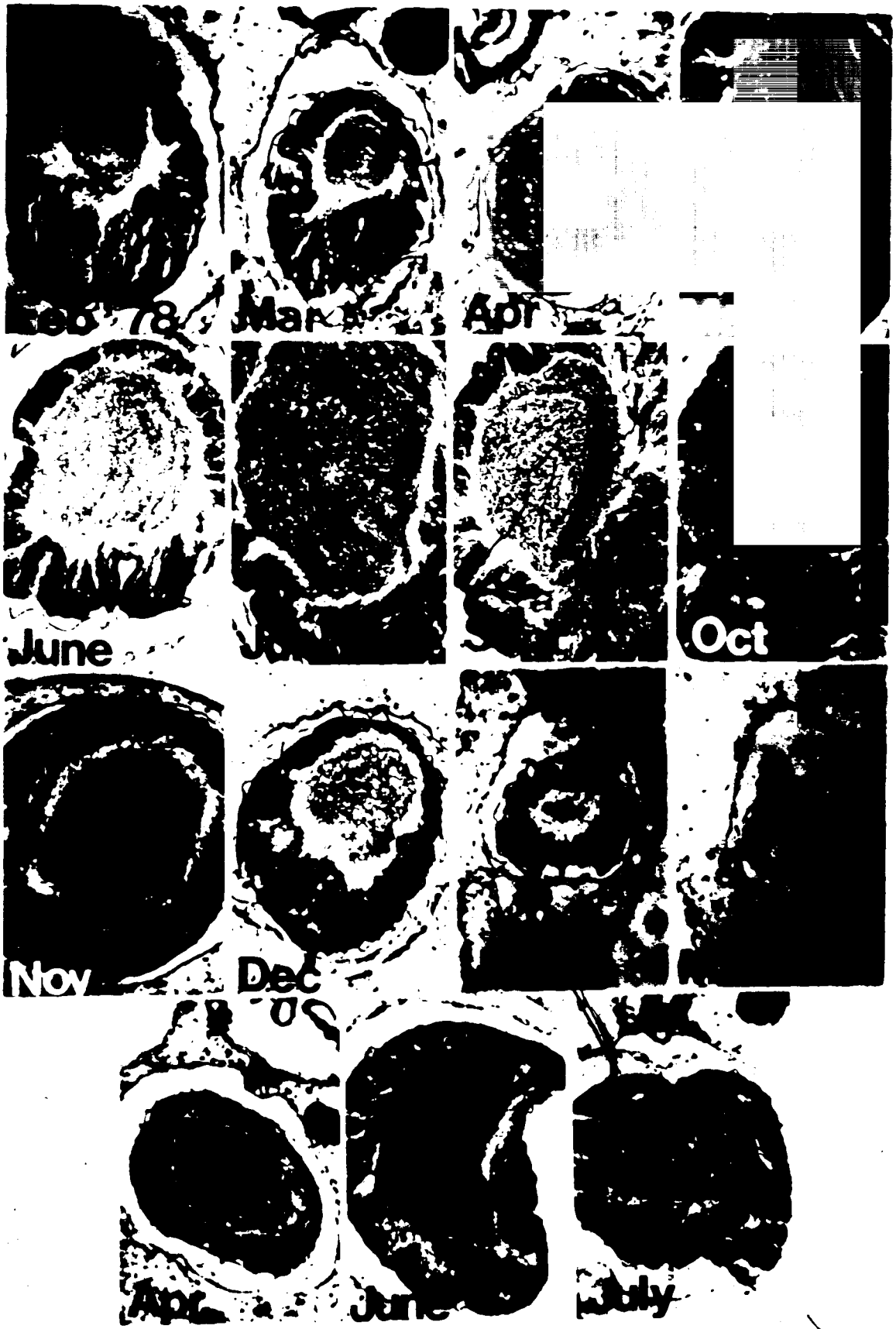
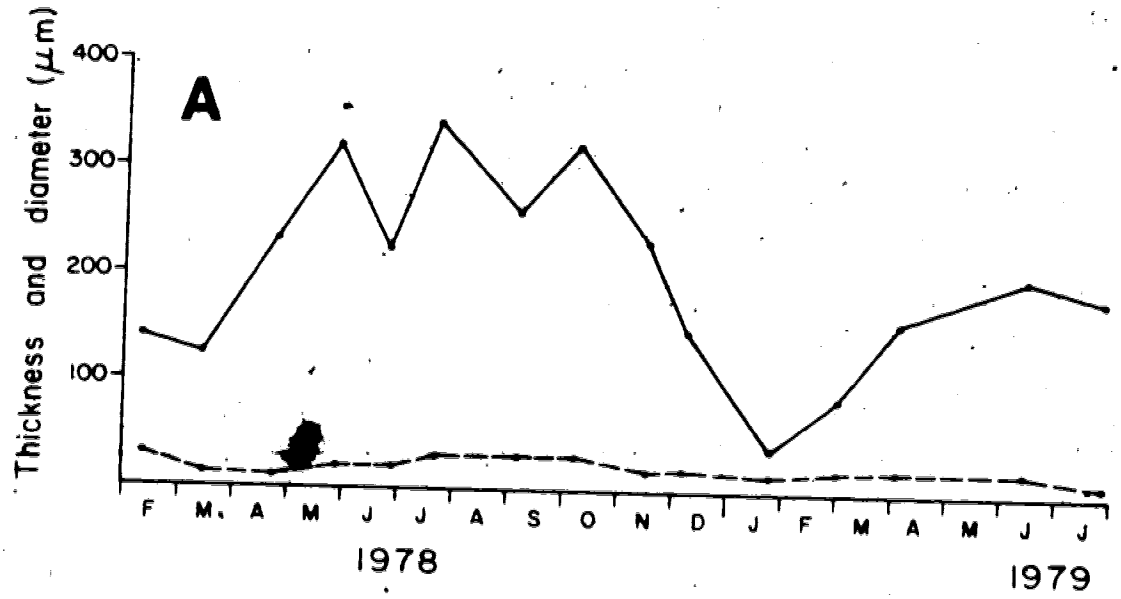
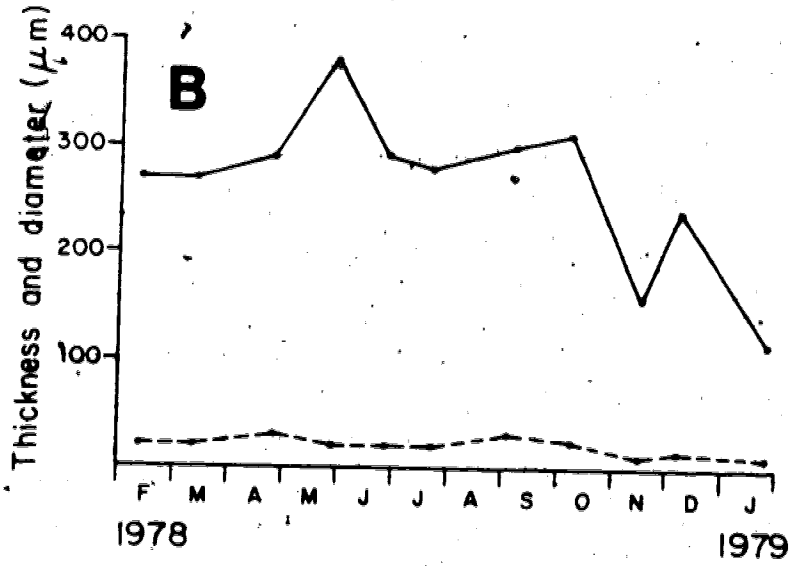
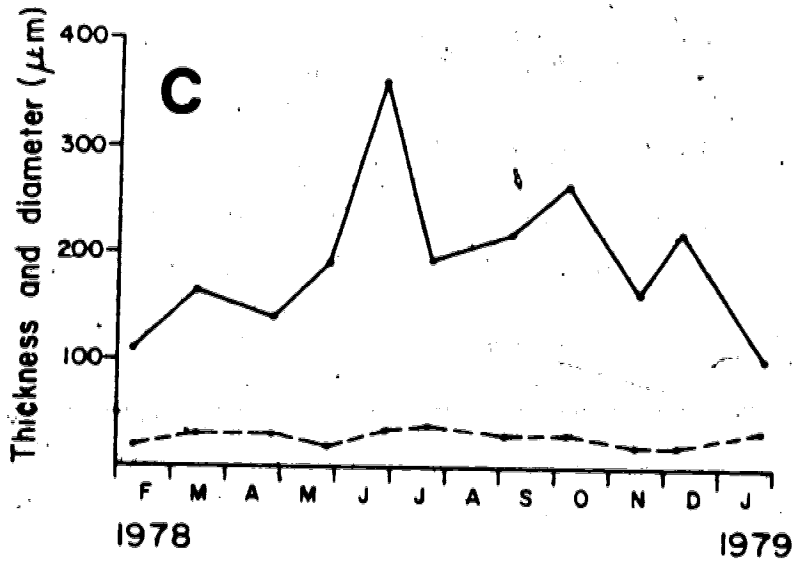

Feb '78
Mar
Apr
June
Oct
Nov
Dec
Apr
June
July

Fig. 20. Florometra serratissima. Changes in the thickness of the spermatogenic layer (spermatogonia, spermatocytes, and spermatids) (dashed lines), and in the diameter of the spermatozoal layer (solid lines), in three male individuals monitored on a roughly monthly basis. (A) First male (same male as shown photographically in Fig. 19). (B) Second male. (C) Third male


A
Thickness and diameter (μm)
400
300
200
100
F M A M J J A S O N D J F M A M J J
1978
1979


B
Thickness and diameter (μm)
400
300
200
100
F M A M J J A S O N D J
1978
1979


C
Thickness and diameter (μm)
400
300
200
100
F M A M J J A S O N D J
1978
1979

DISCUSSION

This is the first time that continuous reproduction has been demonstrated in a crinoid. One worker has suggested that the comatulid Antedon mediterranea reproduces throughout the year, with reproductive activity highest in November, December, February, and April, although others have stated that breeding occurs in the spring in this species (in A.H. Clark, 1921, p.373). It would be necessary to study the reproductive cycle of A. mediterranea over the course of a year in order to resolve this problem. In all other crinoid species for which data are available (A.H. Clark, 1921; Hyman, 1955; Boolootian, 1966) there is, apparently, an annual reproductive cycle with a short breeding season, as exemplified by Comanthus japonica (Holland et al., 1975). Most of the information is based on incomplete observations, however, and it is likely that detailed studies will show that other species have prolonged or continuous breeding seasons. Continuous reproduction is also known for several species of ophiuroids (Mladenov, 1976; Hendler, 1979), asteroids, and echinoids, and may occur in some holothurians (Boolootian, 1966); it is not necessarily confined to tropical species.

In Giese and Pearse (1974, p. 14) the term continuous reproduction is used to describe a population in which individuals produce gametes cyclically but not in synchrony with each other with the result that the population as a whole appears to reproduce continuously. They suggest that the continuous production of gametes throughout the year is rare in an individual, and that successive gametogenic cycles have at least some pause between them. A continuous reproductive pattern

of this kind is found in the sea-urchin Echinometra mathaei (Pearse and Phillips, 1968). In contrast, in the population of Florometra serratissima from Barkley Sound, most males and females produce gametes without pause and spawn on many occasions throughout the year. This, then, is a case where reproduction is continuous at the individual level as well as in the population as a whole.

There is evidence of a slight lowering of reproductive activity in part of the F. serratissima population in January and February, with some males showing reduced rates of sperm production, and with some females failing to produce large oocytes. It is only possible to speculate on the cause of this phenomenon at present. The difference between winter and summer temperatures at the bottom of Bamfield Inlet is not more than 3°C (personal observations) so temperature stress is probably minimal. However, the composition and abundance of suspended organic material on which shallow-water comatulid crinoids feed (Magnus, 1967; Rutman and Fishelson, 1969) undoubtedly varies seasonally in Barkley Sound. Mladenov (1980b) has shown that growth in F. serratissima is slightly slower in winter compared to other times of the year. If food availability is marginal for F. serratissima in the winter, then energy for gamete production may be at a minimum in some individuals at this time. Interestingly enough, the large pool of small oocytes in a female never disappears, so those females with lowered winter reproductive activity are able to respond quickly to more favorable circumstances by producing large oocytes within two months.

It has been shown elsewhere (Mladenov, 1980a) that a large female (length of longest arm = 280 mm) ovulates and spawns about

23,800 ova at one time. This is considerably less than the 2 million ova Holland et al. (1975) have calculated that a large female specimen of the 40-armed Comanthus japonica emits on a single occasion each year. Fecundity in the 10-armed F. serratissima is physically limited by the smaller number of genital pinnules which appear to be filled to capacity when containing 100 ova at most. Continuous gamete production in females of this species may be necessary to ensure that enough ova are spawned to allow for adequate recruitment of young. This is especially true for a broadcaster like F. serratissima which would suffer considerable loss of eggs and larvae in the plankton due to predation and transport to unsuitable substrates. Continuous gamete production in males would then be a necessary characteristic to ensure that sperm are always available to fertilize the ova.

If, as suggested, a female spawns at least nine times in a year, then a large specimen would emit a minimum of 214,000 ova yearly. Other 10-armed feather stars such as Antedon bifida, A. mediterranea, and A. adriatica are smaller than F. serratissima (Clark and Clark, 1967), have slightly larger eggs (A.H. Clark, 1921, p. 410) and, as mentioned earlier, are said to have annual reproductive cycles. As a result, the fecundity of these species must be even lower than that of F. serratissima. All three of these Antedon species, however, retain the extruded ova on the genital pinnules, and the embryos hatch as doliolaria, spending only a short time in the plankton (Thomson, 1865; Bury, 1888; Seeliger, 1892); larval loss due to planktonic predators and to dispersal to unsuitable substrates would thus be minimal.

Continuous reproduction in F. serratissima may therefore be a

response to a low fecundity imposed by body structure combined with a pelagic mode of development. This hypothesis could be tested by studying the reproductive cycle and obtaining fecundity estimates in other 10-armed comatulids known to have a pelagic egg such as Antedon petasus (Mortensen, 1920). It is predicted that such species will have prolonged or continuous breeding patterns.

In a crinoid species like Comanthus japonica, where the entire adult population usually participates in a spawning, gamete densities in the surrounding water would be extremely high, with consequently high rates of fertilization success. Since individuals of F. serratissima release only small numbers of gametes on frequent occasions, gamete densities would be low, and fertilization success might be a problem. The occurrence of this species in fairly dense, discrete aggregations (Mladenov, 1980b) would, however, help to create high gamete densities during a spawning, thereby alleviating this problem somewhat. A natural spawning was never observed during the course of this study so it is not known how a spawning is initiated or how many individuals participate. Observations on other species (Dan and Dan, 1941; Fishelson, 1968) indicate that males spawn first. If this is the case in F. serratissima as well, then females may spawn only in response to the presence of sperm, or some other substance released with sperm, thereby ensuring high rates of fertilization success. It is possible that a spawning is a very localized phenomenon involving only a small number of males and nearby females with ovulated eggs.

At present, it is not known if other populations of F. serratissima reproduce continuously. This species is eurybathic, with a

wide geographic range (Clark and Clark, 1967), and it is quite possible that some populations are influenced by factors which alter the breeding pattern. For instance, pronounced seasonal fluctuations in the food supply would preclude reproduction at certain times of the year. In addition, such factors as reduced predation pressure on eggs and larvae, or increased survival after settlement, might lead to a shortening of the breeding season with allocation of energy thus saved towards other purposes. A study of a population in deeper water, or in a different geographical location, would be of great interest.

LITERATURE CITED

The periodicals have been abbreviated in accordance with "World list of scientific periodicals, 4th ed. London 1964" or, for more recent journals, "Bibliographic guide for editors and authors, Washington 1974".

Boolootian, R.A.: Reproductive cycles. In: Physiology of Echinodermata, pp 561-613. Ed. by R.A. Boolootian. New York: —Interscience Publishers 1966

Bury, H.: The early stages in the development of Antedon rosacea. Phil. Trans. R. Soc. (B.) 179, 257-301 + plates 43-47 (1888)

Clark, A.H.: A monograph of the existing crinoids. Bull. U.S. natn. Mus. 82, vol. 1, pt. 2, 1-795 + 57 plates (1921)

-- and A.M. Clark: A monograph of the existing crinoids. Bull. U.S. natn. Mus. 82, vol. 1, pt. 5, 1-860 (1967)

Dan, K. and J.C. Dan: Spawning habit of the crinoid, Comanthus japonicus. Jap. J. Zool. 9, 555-564 + plate 14 (1941)

-- and H. Kubota: Data on the spawning of Comanthus japonica between 1937 and 1935. Embryologia 5, 21-37 (1960)

Fishelson, L.: Gamete shedding behaviour of the feather star Lamprometra klunzingeri in its natural habitat. Nature, Lond. 219, 1063 (1968)

Giese, A.C. and J. S. Pearse (Eds.): Reproduction of marine invertebrates. Vol. 1. Acoelomate and pseudocoelomate metazoans, 546 pp. New York: Academic Press 1974

Hendler, G.: Reproductive periodicity of ophiuroids (Echinodermata: Ophiuroidea) on the Atlantic and Pacific coasts of Panamá. In: Reproductive ecology of marine invertebrates, pp 145-156. Ed. by S.E. Stancyk. Columbia, South Carolina: University of South Carolina Press 1979

Holland, N.D., J.C. Grimmer and H. Kubota: Gonadal development during the annual reproductive cycle of Comanthus japonica (Echinodermata: Crinoidea). Biol. Bull. mar. biol. Lab., Woods Hole 148, 219-242 (1975)

Hyman, L.H.: The invertebrates. Vol. 4. Echinodermata, 763 pp. New York: McGraw-Hill 1955

Magnus, D.B.E.: Ecological and ethological studies and experiments on the echinoderms of the Red Sea: II. Heterometra savignyi. Stud. Trop. Oceanogr. 5, 644-650 (1967)

Mladenov, P.V.: Reproduction and larval development in ophiuroids (Echinodermata) of Barbados, West Indies, 136 pp. M.Sc. thesis, McGill University 1976

-- Gonadal structure, oocyte development, ovulation and spawning in the feather star Florometra serratissima (Echinodermata: Crinoidea). In: Development and reproductive biology of the feather Florometra serratissima (Echinodermata: Crinoidea). Ph.D. thesis, University of Alberta 1980a

-- Aggregation, growth, regeneration and predation in the feather star Florometra serratissima (Echinodermata: Crinoidea). In: Development and reproductive biology of the feather star Florometra serratissima (Echinodermata: Crinoidea). Ph.D. thesis, University of Alberta 1980b

Mortensen, T.: Studies in the development of crinoids. Pap, Tortugas Lab. 16, 1-94 + plates 1-28 (1920)

Pearse, J.S.: Reproductive periodicities in several contrasting populations of Odontaster validus Koehler, a common Antarctic asteroid. Antarct. Res. Ser. 5, 39-85 (1965)

-- and Phillips: Continuous reproduction in the Indo-Pacific sea urchin Echinometra mathaei at Rottnest Island, Western Australia. Aust. J. mar. Freshwater Res. 19, 161-172 (1968)

Rutman, J. and L. Fishelson: Food composition and feeding behaviour of shallow-water crinoids at Eilat (Red Sea). Mar. Biol. 3, 46-57 (1969)

Seeliger, O.: Studien zur Entwicklungsgeschichte der Crinoiden. (Antedon rosacea.). Zool. Jb. Abtheilung für Anatomie und Ontogenie der Thiere 6, 161-444 + plates 12-22 (1892)

Thomson, W.: On the embryogeny of Antedon rosaceus, Linck (Comatula rosacea of Lamarck). Phil. Trans. R. Soc. 155, pt. 2, 513-545 + plates 23-27 (1865)

Development and Larval Behaviour in the Feather Star *Florometra serratissima* (Echinodermata: Crinoidea)

ABSTRACT

The development of the feather star Florometra serratissima (A.H. Clark) from fertilization to the six-month-old pentacrinoid is described for the first time using light and scanning electron microscopy. Embryonic development takes place within a ridged fertilization membrane. The first two cleavages begin unilaterally; subsequent cleavages are unequal with a tendency towards asynchrony. Gastrulation is by blastular invagination without previous primary mesenchyme formation. Cilia are swollen terminally during ciliogenesis.

Commencing 35 h after fertilization (9.5° to 11.5°C), a uniformly ciliated (UC) larva with an apical tuft of longer cilia hatches from the fertilization membrane; by four days there is a doliolaria with four ciliated bands and a vestibular invagination. The surface of the UC larva and doliolaria is covered with a delicate glycocalyx supported by ectodermal microvilli. The fully grown cilia have several swellings along the length of their shafts. Larvae swim in a vertical sinusoidal path while rotating about the long axis of the body.

Settlement of the doliolaria, by means of a cement secreted from an antero-ventral adhesive pit, begins as early as 4.6 days, but can be delayed for up to nine more days, at the end of which time abnormal pelagic metamorphosis takes place. Settlement occurs gregariously in culture. The possible significance of gregarious settlement following a period of pelagic dispersal in the formation of adult aggregations of F. serratissima is discussed.

There is a rapid metamorphosis following settlement characterized by: the loss of external cilia; a widening of the space beneath the

glycocalyx; the closure of the vestibular invagination to form a hollow vestibule; and the rotation of the vestibule to the former posterior, now oral, end of the settled form. The result is a stalked cystidean. Tube feet develop within the vestibule which, at this time, is roofed by five oral plates. Sixteen days after settlement, the oral plates open and 15 papillate tube feet, which are used in food capture, are extended into the surrounding water. This marks the beginning of the pentacrinoid stage using the terminology adopted in this report. Previous usage of the term pentacrinoid is discussed.

Rudiments of all 10 arms of the adult crinoid are present in the four-month-old pentacrinoid. By six months, pentacrinoids have an arm span of 6.5 mm, but cirri and pinnules are not yet evident.

INTRODUCTION

The study of crinoid development was initiated by J.V. Thompson (1827, cited by Carpenter, 1866, p. 685) with his description of a new species of stalked crinoid, Pentacrinus europaeus, which he had discovered in the Cove of Cork, Ireland. Subsequent work by Thompson (1836) showed that this small sea lily was, in reality, a stage in the development of the feather star, Antedon bifida, which was then known as Comatula rosacea. Busch, (1849, cited by Carpenter, 1866, p. 690), while studying this species in Scotland, discovered the existence of a free-swimming larval stage, but was unable to trace its development further. Wyville Thomson (1865) published a more complete account of the development of Antedon bifida (which he called Antedon rosaceus) from the fertilized egg through to the early pentacrinoid stage, and Carpenter (1866, 1876) described the late pentacrinoid stage of this species.

The work of these pioneers stimulated further investigations, all dealing with closely related species of Antedon from the Mediterranean Sea, known collectively at that time as Antedon rosacea. Perrier (1886) described the development of Antedon bifida from north Africa; Bury (1888) and Barrois (1888) studied the development of Antedon mediterranea, the former at Naples, Italy, the latter on material from Toulon and Villefranche, France; Seeliger (1892) published an account of the development of Antedon adriatica from Trieste; and Chadwick (1907) described development in a feather star, ostensibly Antedon bifida, but which, according to A.H. Clark, (1921, p. 526) and Clark and Clark (1967, p. 258), is actually based upon material

of Antedon mediterranea sent to him from Naples. Of these works, Seeliger's monograph on A. adriatica is considered the classic older study, and forms the basis of Ludwig and Hamann's (1907), Clark's (1921) and Hyman's (1955) reviews of the development of crinoids.

Theodor Mortensen (1920a) was the first to study the development of other genera of crinoids in accordance with his comparative approach to echinoderm development. He gave a good account of development in the West Indian feather star, Tropiometra carinata (= T. picta); a less complete description of development in the Japanese feather star, Compsometra serrata; and, most notably, he contributed information on the development of the Antarctic feather stars, Isometra vivipara, Notocrinus virilis and Thaumatometra nutrix, all of which possess marsupia and show varying degrees of brood protection. In addition, in the same report (pp. 54-55, and Plate 27), Mortensen gave brief mention of some pentacrinoids of Florometra serratissima found on the cirri of adults dredged during a visit to the Biological Station at Nanaimo, British Columbia, in June and July, 1915. This is the only mention of development in this species prior to the present study. Mortensen also gave an incomplete description of development in the Scandinavian feather star, Antedon petasus (1920b), and in the Red Sea feather stars, Tropiometra audouini, Lamprometra klunzingeri and Heterometra savignyi (1937, 1938).

All of the recent work on crinoid development has been concerned with the Japanese feather star, Comanthus japonica. Dan and Dan (1941) first published a description of development in this species, followed by Dan (1968) and Kubota (1969, 1970). Lately, scanning and transmission electron microscopic techniques have been used to study the

structure and formation of the fertilization membrane (Holland and Jespersen, 1973; Holland, 1977), embryonic development (Holland, 1976; 1978), and larval development (Holland and Kubota, 1975) in C. japonica.

In summary, present understanding of developmental pattern in crinoids is derived entirely from feather stars, for which good information is available for three closely related species of Antedon, for Tropiometra carinata, and for Comanthus japonica. In addition, there is scattered information on perhaps eight other species. Knowledge thus extends to about 2% of the approximately 615 species of living crinoids, about 75 of which are stalked (A.M. Clark, 1977). Clearly, detailed information on the development of additional species from a variety of geographical locations is necessary before crinoid development can be viewed on a truly comparative basis.

In this paper, the development of Florometra serratissima (A.H. Clark) from the fertilized egg to the six-month-old pentacrinoid is described for the first time and compared with other crinoid species for which development is known. In addition, new information is presented on larval swimming and settlement behaviour and on pentacrinoid feeding behaviour.

The different stages in the life cycle of a typical feather star are depicted diagrammatically in Fig. 21. Note that the term pentacrinoid, as adopted throughout this report, applies to the period of development from the time the cystidean puts tube feet into the surrounding water, until the moment the developing juvenile breaks free of the stalk. This usage differs from that of previous students of crinoid development. It will be shown in the last section of this

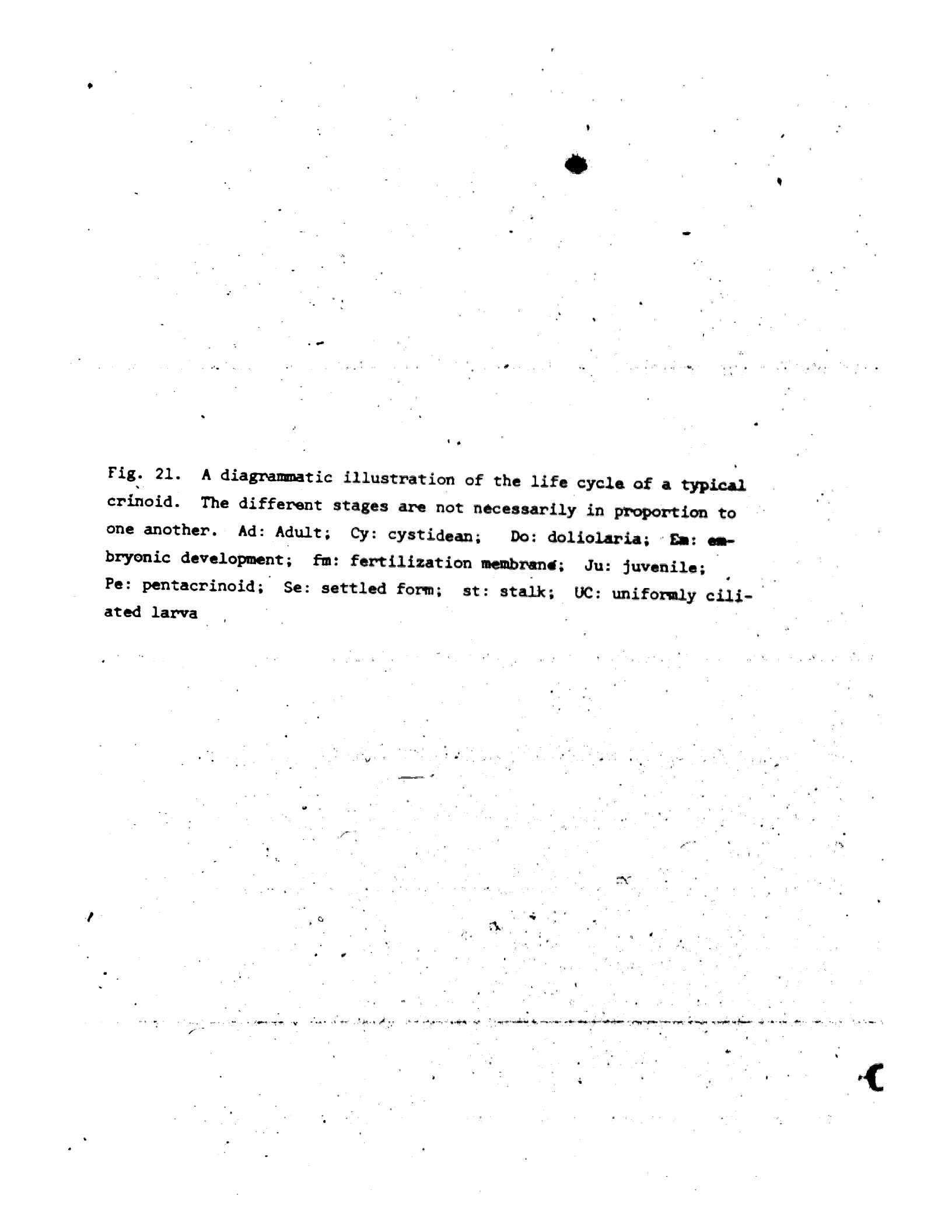
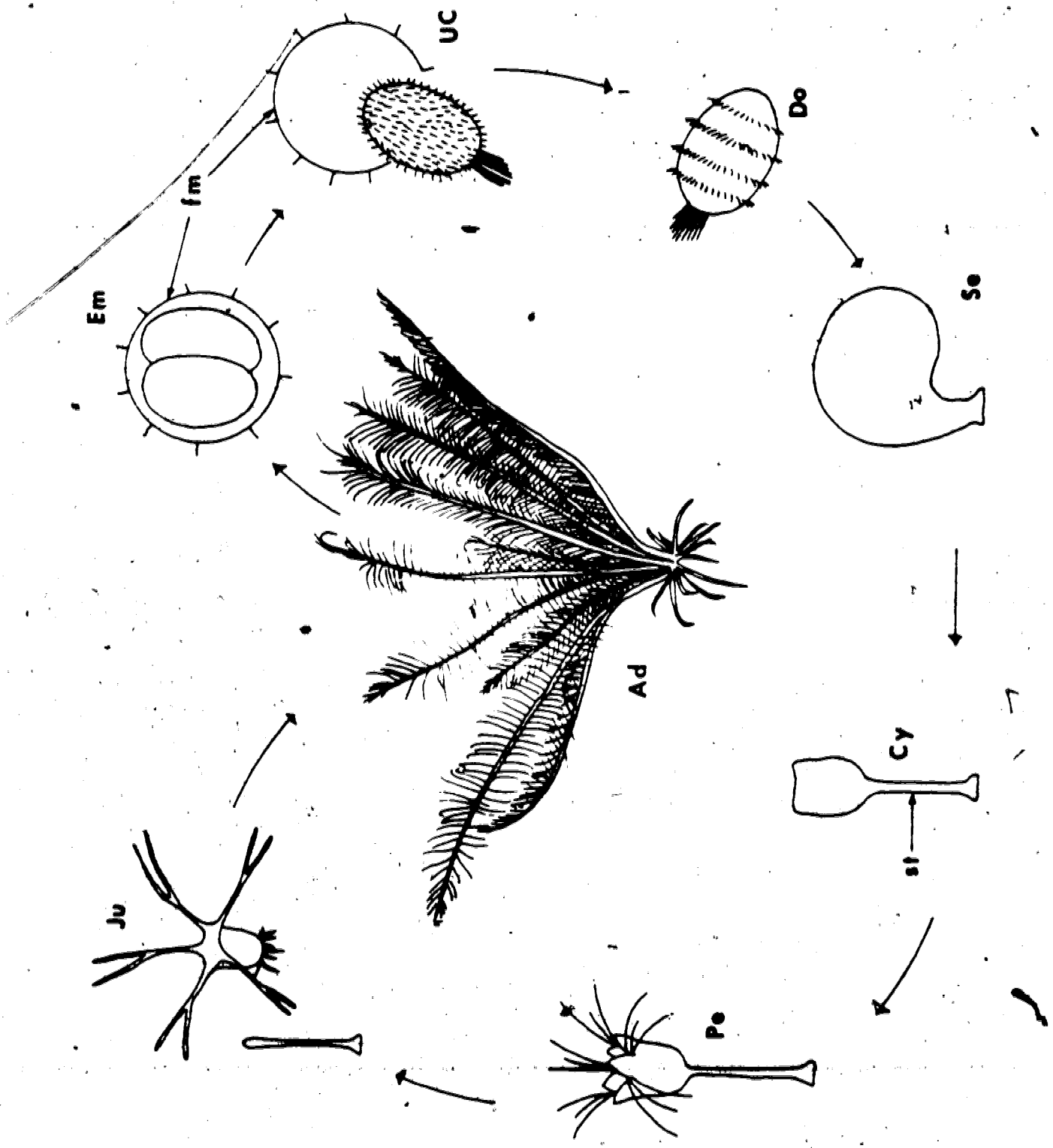


Fig. 21. A diagrammatic illustration of the life cycle of a typical crinoid. The different stages are not necessarily in proportion to one another. Ad: Adult; Cy: cystidean; Do: doliolaria; Em: embryonic development; fm: fertilization membrane; Ju: juvenile; Pe: pentacrinoid; Se: settled form; st: stalk; UC: uniformly ciliated larva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paper, however, that there is considerable confusion in the literature concerning the word pentacrinoid. Justification for the present usage of the term will then be given.

MATERIALS AND METHODS

Specimens of Florometra serratissima (A.H. Clark) were collected by S.C.U.B.A. at depths of from 17 m to 34 m at the mouth of Bamfield Inlet in Barkley Sound, British Columbia. The ovaries of this species contain primary oocytes which cannot be successfully fertilized until after they have ovulated into the ovarian lumen and matured into ova (Mladenov, 1980a). Unfortunately, a female is rarely encountered whose ovaries contain ova, and it is not yet possible to induce ovulation artificially; in addition, natural spawnings in the laboratory are very rare. As a result, the following method was devised to obtain fertilizable eggs; this method allowed small cultures to be started on a fairly frequent basis throughout the year. During S.C.U.B.A. dives, a search was made for females with dark orange, swollen genital pinnules. With practice, such individuals could be easily recognized, especially with the aid of a magnifying glass secured to the diver's wrist. These females were collected in plastic bags and returned to the laboratory where several genital pinnules from each female were removed, carefully dissected open with fine forceps, and their contents examined with a dissecting microscope for ova. Ova can be easily distinguished from large oocytes; they lack the capsular germinal lamina and germinal vesicle of oocytes; they are perfectly spherical; and they do not stick together in clumps as do oocytes. The same females were examined for several days in succession until, eventually, a female was found whose ovaries contained ova.

The ova from many of the ovaries of this female were then amassed, rinsed several times with millepore-filtered (0.45 μm pore size) seawater, and inseminated with one or two drops of concentrated sperm

solution obtained by dissection of a genital pinnule of a male. The genital pinnules of all large males contained active sperm at all times of the year (Mladenov, 1980b). The fertilized eggs were rinsed several times with seawater and then transferred to covered polystyrene dishes and cultured in millepore-filtered seawater. The culture water was changed daily during embryonic and early larval development and every other day after attachment had taken place. The culture dishes were kept partially submerged in a water table where the water temperature fluctuated seasonally between 9.5° and 11.5°C. The cultures were provided with overhead illumination simulating a natural photoperiod.

Developing embryos and larvae were removed from the cultures at frequent intervals for observation. Measurements were made with an ocular micrometer, and photographs were taken with a Zeiss compound photomicroscope. Skeletal elements were examined with polarized light (Fell, 1941).

Larval swimming behaviour, larval pre-settling exploratory behaviour, the distribution of settled larvae, and the feeding behaviour of pentacrinoids was observed in vitro with a dissecting microscope. On one occasion, 200 doliolaria were added to a culture dish in which a grid of 36 1 cm^2 quadrats had been previously drawn on the bottom away from the sides. After the majority of the larvae had settled, the number of animals in each quadrat was recorded. To test for non-random settlement, the frequency distribution of settled forms was compared to a Poisson distribution using chi-square (Crisp, 1961; Young and Braithwaite, 1980).

Specimens at various stages of development were fixed in seawater-Bouin's fluid, dehydrated in isopropanol, cleared in a mixture of

α- terpineol and toluene (1:3), and embedded in Paraplast (Sherwood medical). The tissue was then sectioned at 5 μm to 7 μm and stained with haematoxylin and eosin.

A range of developmental stages was also fixed for scanning electron microscopy (SEM). Pentacrinoids were first relaxed in 7.5% $MgCl_2$ diluted 1:1 with seawater (Pantin, 1969); all other stages were fixed without prior relaxation. Two different fixation procedures were followed. In the first, specimens were fixed for 1 h in 2.5% glutaraldehyde in Millonig's phosphate buffer (0.4M) and then rinsed for 15 min in Millonig's phosphate buffer. Postfixation was for 2 h at room temperature in 2% OsO_4 in 1.25% bicarbonate buffer followed by a 15 min rinse in 1.25% bicarbonate buffer. In the second procedure, fixation was for 1 h in 2.5% glutaraldehyde buffered in seawater followed by a 15 min rinse in seawater. Postfixation was for 2 h at room temperature in 2% OsO_4 in seawater followed by a 15 min rinse in distilled water. After either fixation procedure, the specimens were dehydrated in 30% and 50% ethanol (15 min in each) and stored in 70% ethanol in the refrigerator for up to two months until they could be transferred to Edmonton or Victoria where a critical point dryer was available. Here, dehydration was completed in 85% ethanol (15 min), 95% ethanol (15 min) and 100% ethanol (two changes of 15 min each). The dehydrated specimens were run through a 1:3, 1:1, 3:1 amyl acetate-ethanol series (15 min in each), transferred to 100% amyl acetate for 15 min, and then critical-point dried. On occasion, the specimens were critical-point dried directly from 100% ethanol. After drying, the specimens were mounted on stubs with Scotch double-stick tape, sputter coated with a mixture of gold and palladium (90:10)

and viewed in a Cambridge S150 or Jeol JSM-35 scanning electron microscope. Optimum results were obtained using seawater as a buffer during fixation and postfixation, and by critical point drying directly from 100% ethanol. This procedure gave excellent preservation of the thin glycocalyx which was otherwise badly torn.

An attempt was made to feed pentacrinoids in the laboratory. The diatom, Phaeodactylum bicornutum, and the micro-flagellate, Isochrysis galbana, were cultured in enriched seawater (Provasoli et al., 1957) for this purpose. Although pentacrinoids could be sustained for several months on this diet, little, if any, growth occurred. It was decided that more natural conditions must be provided if older pentacrinoid stages were to be reared. Culture dishes with attached pentacrinoids were thus placed on the bottom of Bamfield Inlet, at a depth of 25 m, in the centre of the naturally occurring adult population. The dishes were set in holes of the appropriate diameter cut in a plastic tray. This tray was secured to two concrete blocks, and the cultures protected by a cage constructed of PVC tubing and nylon netting of 2.5 cm mesh size. The culture dishes were retrieved from the rearing tray by diving three months later. The pentacrinoids were drawn, photographed, and measured with the aid of a dissecting microscope. The cultures were then returned to the rearing tray by diving. This retrieval procedure was repeated two months later.

RESULTS

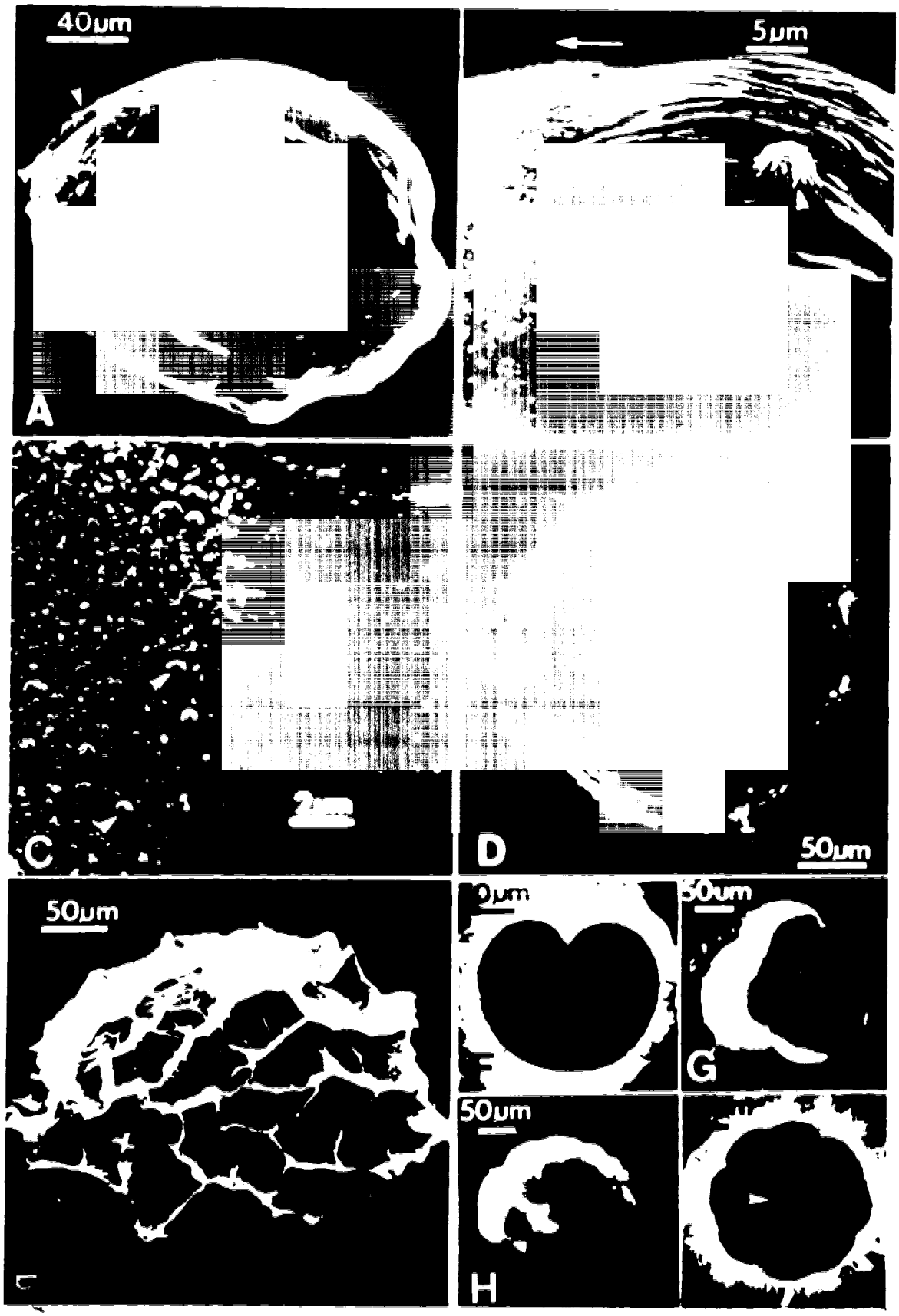
Fertilization

Mature unfertilized eggs of Florometra serratissima are perfectly spherical, pale pink in colour, and oligolecithal. They are slightly denser than seawater and tend to settle slowly to the bottom of a culture dish.

Fig. 22A shows the surface of an egg fixed 40 sec after insemination. The spread of the cortical reaction across the surface of the egg is clearly visible. The membranous material adhering to one pole of the egg is a remnant of the germinal lamina which surrounds the growing oocytes in the ovary, but which is normally left behind during ovulation (Mladenov, 1980a). An emerging polar body is present beneath the rising fertilization membrane (Fig. 22B), indicating that the polar body was still attached to the egg surface at the time of fertilization. This suggests that the egg of F. serratissima can be fertilized shortly after the first meiotic metaphase which occurs during, or just after, ovulation. The egg shown in Figs. 22A, B, and C, was, however, artificially freed from the ovarian lumen and artificially fertilized; under natural circumstances, sperm penetration does not take place until after spawning, when both meiotic divisions are completed. The surface of the egg not yet obscured by the rising fertilization membrane (Fig. 22C) is covered with numerous microvilli and scattered spherical bodies.

The fertilization membrane is fully formed 3 to 4 min after the addition of sperm. It has a very beautiful ornamented appearance. When viewed with the light microscope, the fertilization membrane looks

Fig. 22. Florometra serratissima. Fertilization and cleavage. (A) SEM of the surface of an egg fixed 40 sec after insemination; the spread of the cortical reaction in the direction of the arrows is clearly visible; the arrowhead points to remnants of the germinal lamina. (B) Higher magnification SEM of surface of same egg showing a polar body (arrowhead) beneath the rising fertilization membrane; the arrow indicates the direction of spread of the cortical reaction. (C) High magnification SEM of leading edge of cortical reaction spreading in the direction of the arrow; numerous microvilli and scattered spherical bodies (arrowheads) are visible on the part of the egg surface not yet covered by the rising fertilization membrane (left side of micrograph). (D) A living egg 5 min after insemination; the fertilization membrane appears spiny. (E) SEM of egg 5 min after insemination demonstrating that the fertilization membrane is ridged. (F) Living zygote 2 h after fertilization showing unilateral division furrow of first cleavage. (G) Living eight-cell stage 5 h after fertilization. (H) Living 16-cell stage 6 h after fertilization; the arrowhead points to a lateral pore. (I) View of vegetal pole of living 16-cell stage showing vegetal pore (arrowhead)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spiny (Fig. 22D), but scanning electron microscopy shows that it is actually folded into a series of thin ridges which interconnect with one another in an irregular manner; the tops of the ridges, and the flat areas between the bases of the ridges, are wrinkled (Fig. 22E).

Measurements made on fresh material show that the average diameter of the uncleaved fertilized egg is 207 µm (± 6 µm, n = 24), while the average diameter of the fertilization membrane to the base of the ridges is 241 µm (± 8 µm, n = 25); this leaves a perivitelline space averaging 17 µm in width. The overall diameter of the fertilization membrane, including the ridges, averages 320 µm (± 19 µm, n = 25); the ridges thus average just under 40 µm in height (Fig. 23).

Cleavage, Blastulation and Gastrulation

A schedule of early development for Florometra serratissima at 9.5° to 11.5°C is presented graphically in Fig. 24. It is based on observations of 25 broods reared in the laboratory, and it shows the duration of each developmental stage and the amount of overlap between successive developmental stages. The timetable given in the following description of development is based on the average of observations from all 25 broods.

A unilateral division furrow appears about 2 h after fertilization, giving the embryo a heart-shaped appearance in side view (Fig. 22F). It is assumed that the furrow commences at the animal pole, but this is not known for certain since polar bodies, which normally serve to mark the animal pole, are not persistent in F. serratissima. When the furrow has progressed to a position approximately halfway across the zygote, a second furrow appears at the opposite pole and proceeds

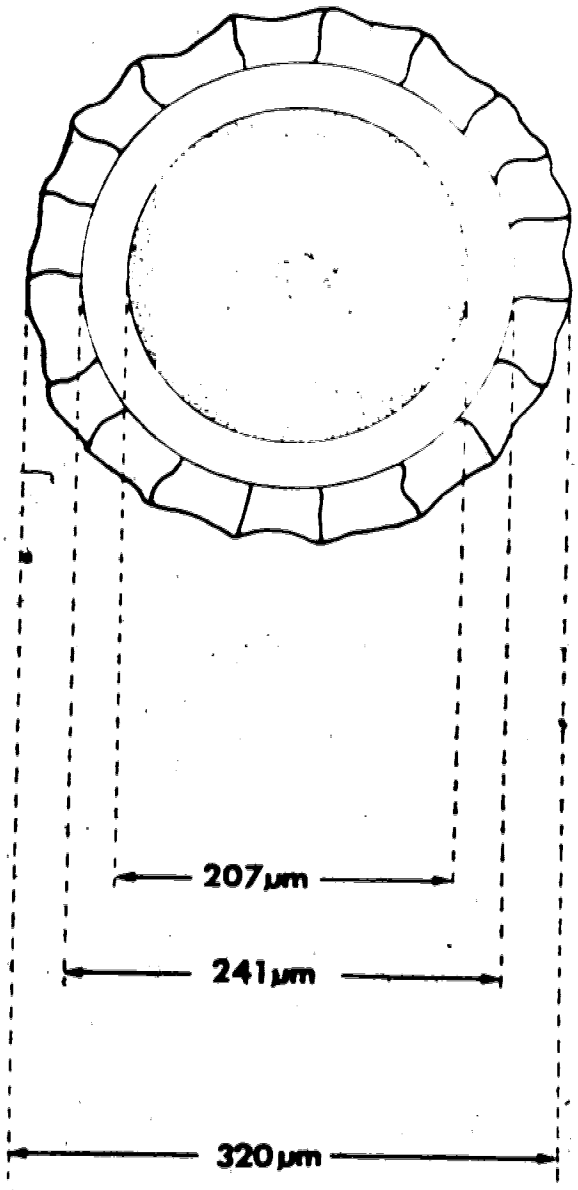


Fig. 23. Florometra serratissima. Average dimensions of the fertilized egg

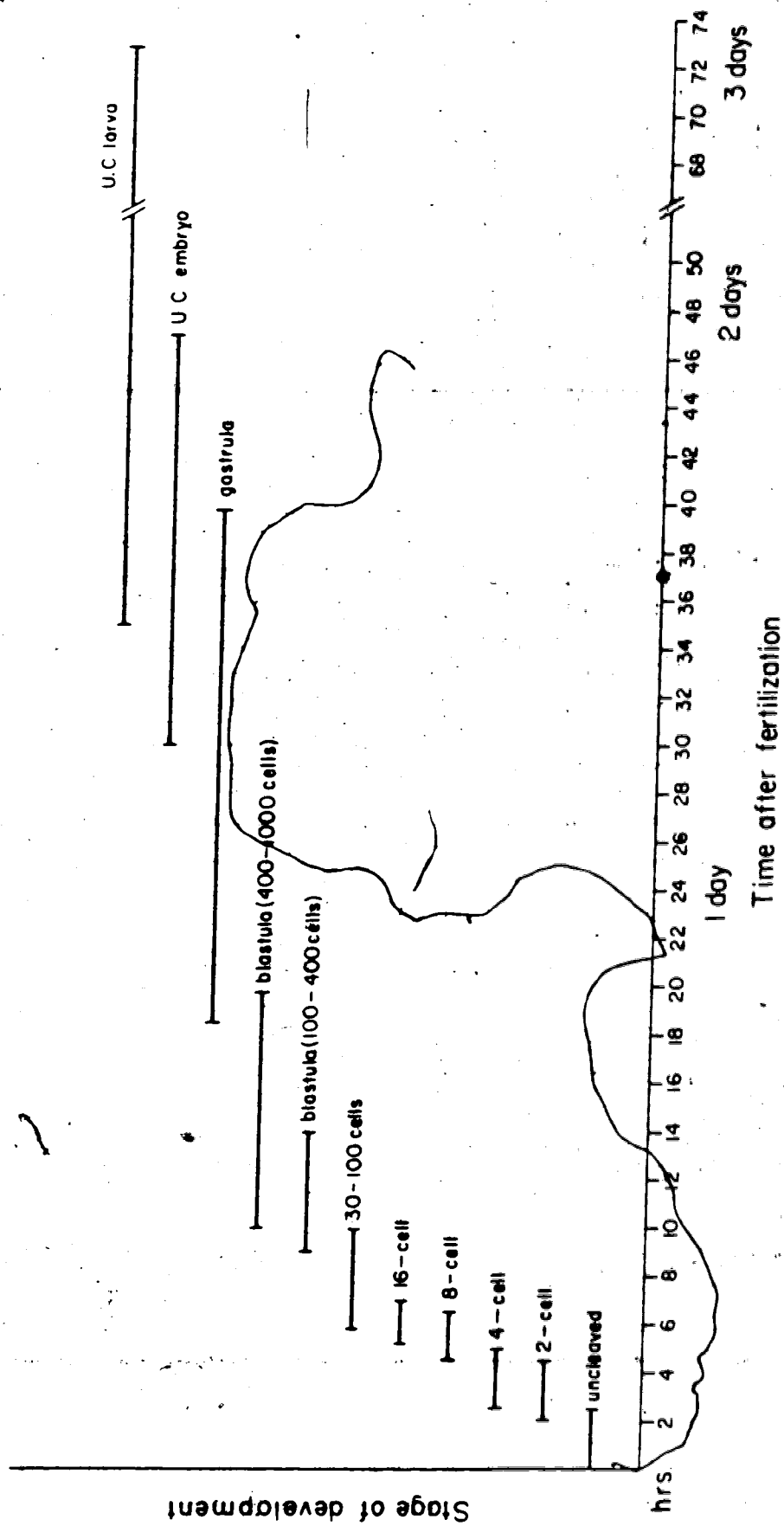


Fig. 24. Florometra serratissima. Duration of early developmental stages (9.5° to 11.5°C)

across to meet the first at a point about two-thirds of the way across the zygote. In this manner a two-cell stage is produced about 2.25 h after fertilization.

The second cleavage is meridional and at right angles to the first, and it too is unilateral, beginning first at the assumed animal pole. It produces a four-cell stage about 3.5 h after fertilization. The third cleavage is equatorial, radial and slightly unequal, giving rise, at about 4.75 h, to an eight-cell stage with two tiers of four cells each; the four vegetal cells are larger than the four animal cells (Fig. 22G).

The fourth cleavage produces an unusual 16-cell stage at about 6 h. Each of the four larger vegetal cells divides with an equal meridional cleavage, producing a tier of eight cells lying in a plane; each of the four smaller animals cells, however, divides with an equal but slightly oblique cleavage, giving rise to eight cells in the form of a hemisphere (Fig. 22H). Three pores are present in the embryo at this time: a large pore surrounded by the eight vegetal blastomeres (Fig. 22I), and designated the vegetal pore; and two small pores positioned opposite one another on the lateral surfaces of the embryo. One of these lateral pores is visible in Fig. 22H. The embryo is thus bilaterally symmetrical at this time.

It should be noted that there is some tendency towards both inequality and asynchrony of cleavage in F. serratissima during the first four cleavage stages. As early as the two-cell stage, therefore, one blastomere can be slightly larger than the other, and embryos with an irregular cell number are sometimes observed. This is not an abnormal feature of development, and healthy larvae result from such

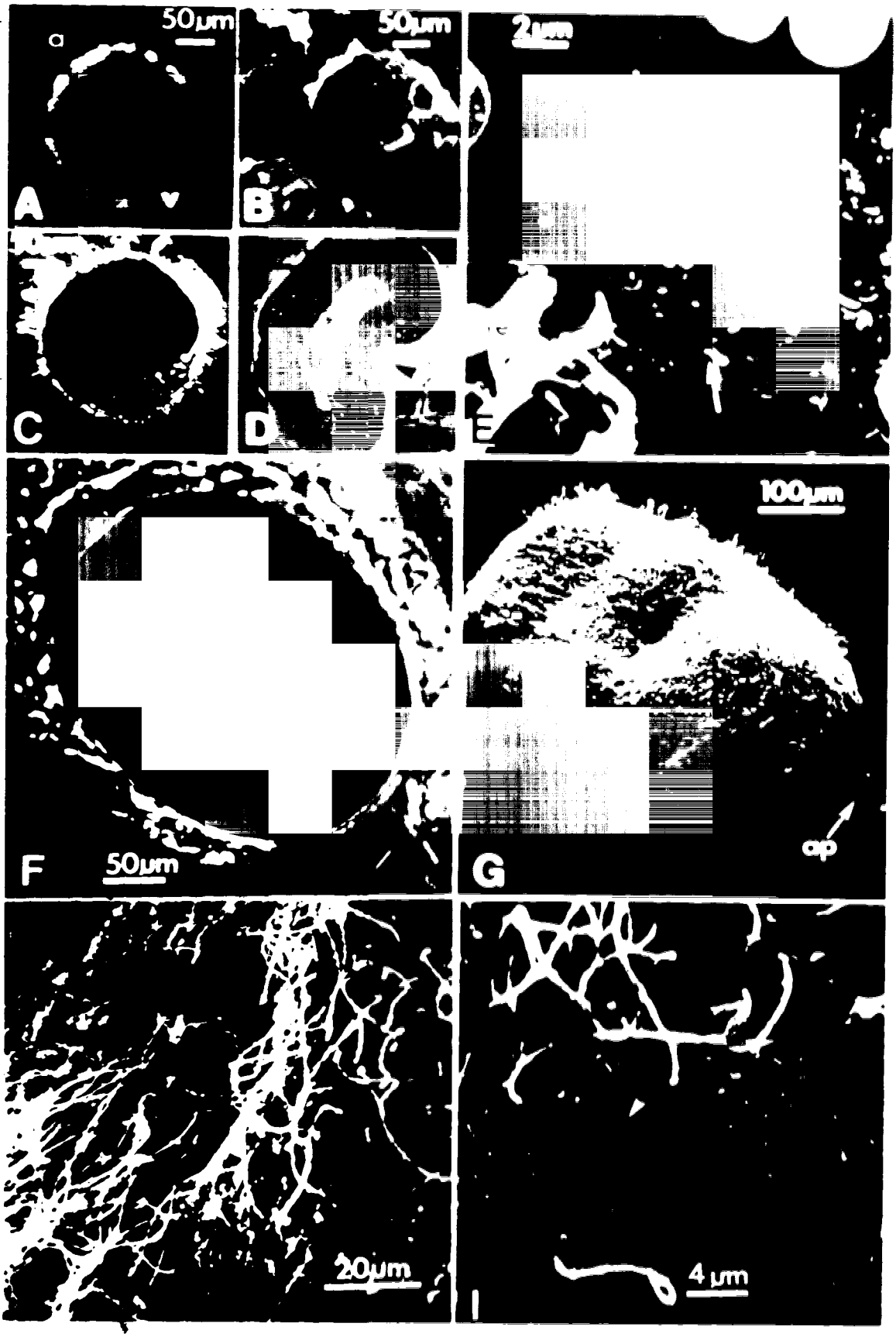
embryos. Cleavage asynchrony becomes very pronounced after the 16-cell stage.

There is a spherical embryo with about 60 cells at 9 h (Fig. 25A). By this time, the two lateral pores have disappeared, but the vegetal pore remains, still surrounded by eight blastomeres. By 12.5 h there is a coeloblastula with about 200 cells; the vegetal pore has disappeared, and the embryo has expanded so that the perivitelline space is very narrow or non-existent (Fig. 25B). The cells at this time have distinctly flattened surfaces where they abut with neighbouring cells. The onset of gastrulation is marked by the formation of a distinctly flattened vegetal plate at about 19 h (Fig. 25C). The embryo consists of over 1000 cells at this time. The central part of the vegetal plate soon begins to invaginate, (Fig. 25D) accompanied by the opening of a small pore, rounded or oval in outline, at the bottom of this deepening depression. Primary mesenchyme is not produced prior to gastrulation, as can be seen in Fig. 25D.

Light microscopic observations show that cilia appear first at the animal pole at approximately 27 h. They have not yet attained their full length, but they are motile, and they will form an apical tuft of cilia. SEM shows that ciliogenesis has just begun elsewhere on the surface of the embryo at this time (Fig. 25E). There is one developing cilium per ectodermal cell, each with a terminal swelling. Each ectodermal cell also gives off numerous filopodia and scattered spherical bodies, especially at the boundary regions between adjacent cells (Fig. 25E).

The embryo is uniformly ciliated at 35 h, with a longer tuft of apical cilia at the animal pole. It is now barrel-shaped and twirls

Fig. 25. Florometra serratissima. Blastula, gastrula, and uniformly ciliated (UC) stages. (A) A living embryo at 9 h with approximately 60 cells. (B) SEM of fractured coeloblastula with about 200 cells at 12.5 h after fertilization. (C) Living early gastrula at 19 h with flattened vegetal plate. (D) Section of a gastrula at 22 h showing invaginating vegetal plate (arrowhead). (E) SEM of ectoderm of 27 h gastrula showing developing cilia, each with a terminal swelling (arrow); numerous filopodia and scattered spherical bodies (arrowhead) are also visible. (F) Light micrograph of a living UC embryo at 40 h in process of hatching; the apical tuft is emerging first. (G) SEM of UC larva 2.5 days after fertilization showing body cilia and longer cilia of apical tuft. (H) SEM of blastopore of 2.5-day-old UC larva. (I) SEM of surface of 3.0-day-old UC larva showing a pore in the glycocalyx (arrowhead) and numerous spherical bodies dotting the surface of the glycocalyx. a: Animal pole; ap: apical tuft; bl: blastocoel; fe: fertilization membrane; v: vegetal pole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rapidly about its longitudinal axis while still within the fertilization membrane. The embryo then hatches through a small hole in the fertilization membrane and becomes a free-living larva; hatching commences at 35 h, with the last embryo emerging at about 47 h. The hole in the fertilization membrane is formed at a point where the apical tuft of the twirling embryo makes contact with the inside of the fertilization membrane, and the embryo emerges with the apical tuft preceding (Fig. 25F). It appears, then, that mechanical action produces the hole. Dan and Dan (1941) and Holland and Kubota (1975) have suggested that the embryo of Comanthus japonica releases an enzyme which dissolves away the fertilization membrane and facilitates hatching. If a hatching enzyme is present in Florometra serratissima, it works in conjunction with the mechanical action of the twirling embryo.

Uniformly Ciliated Larva and Doliolaria

The apical-tuft cilia at the anterior end of the uniformly ciliated (UC) larva (Fig. 25G) measure just over 100 µm in length, while the body cilia are about 19 µm long. The UC larvae are active swimmers and collect near the water surface shortly after hatching. This behaviour occurs in the dark as well as in the light, suggesting that a geonegative response is involved. During swimming, the body cilia beat vigorously, but the apical-tuft cilia are held erect with the tips curling outwards slightly, and they move as a unit in lateral sweeps. The apical tuft always precedes, and the larval body always rotates about the longitudinal axis in a clockwise direction when the anterior end faces the observer. The larvae swim in a vertical

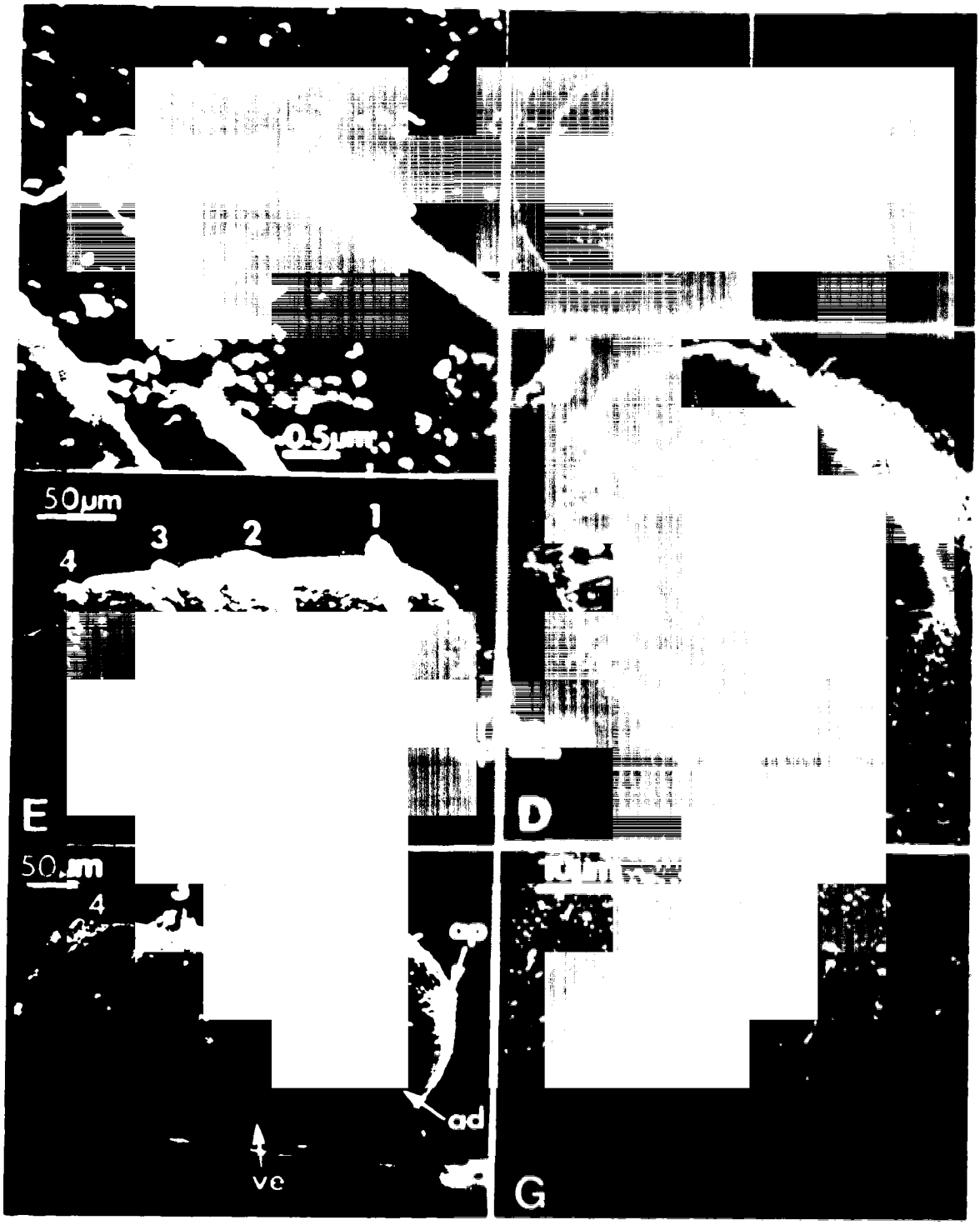
sinusoidal path just below the water surface.

When larvae are observed under a coverslip with the light microscope, spherical swellings are seen along the length of each cilium. Each apical-tuft cilium has four or more swellings, whereas the shorter body cilia have two or three swellings. Such structures are not as evident on the cilia when the larva is not beneath a coverslip, suggesting that the pressure of the coverslip is flattening soft distensible regions along each cilium.

A circular blastopore is present at the posterior end of the UC larva at the time of hatching (Fig. 25H), but soon closes over. The larval surface is covered with a smooth coat, the glycocalyx (after Holland and Kubota, 1975), through which the cilia project (Fig. 25I). The glycocalyx is dotted with numerous spherical bodies; in addition, there are scattered pores with a diameter of about 3 μm in the glycocalyx, one of which can be seen in Fig. 25I. Fig. 26A shows part of the larval surface where the glycocalyx has been disrupted during SEM preparation. Note that the glycocalyx is not in direct contact with the underlying ectoderm but is supported by numerous ectodermal microvilli approximately 0.5 μm in length whose tips protrude through the glycocalyx. The spherical bodies on the surface of the glycocalyx are thus microvillar tips. The space beneath the glycocalyx communicates with the external environment via the pores in the glycocalyx.

A cross section midway through the UC larva (Fig. 26B) shows the columnar ectodermal and entodermal cells, the archenteron, and scattered mesenchyme cells in the blastocoel. A cross section near the anterior end (Fig. 26C) shows a mass of mesenchyme cells at the tip of the archenteron; thus, mesenchyme cells, which were absent at

Fig. 26. Florometra serratissima. Uniformly ciliated (UC) larva and doliolaria. (A) SEM of surface of 3.0-day-old UC larva showing the basal portions of several cilia, remnants of the glycocalyx, and numerous ectodermal microvilli (arrowhead) whose tips penetrate the glycocalyx. (B) Cross section through middle of 3.0-day-old UC larva; scattered mesenchyme cells are present in blastocoel. (C) Cross section through anterior tip of 3.0-day-old UC larva showing mass of spherical mesenchyme cells. (D) SEM of fractured anterior tip of 3.0-day-old UC larva showing a few filopodia stretching from the ectodermal layer to the mesenchyme layer (arrowheads), and the remains of scattered filopodia on the mesenchyme layer. (E) SEM of dorsal side of a doliolaria 4.0 days after fertilization showing four ciliated bands. (F) SEM of 4.6-day-old doliolaria showing the vestibular invagination on ventral surface, and the antero-ventral adhesive pit. (G) SEM of part of a ciliated band of the doliolaria revealing several spherical swellings along the length of each cilium. ad: Adhesive pit; ap: apical tuft; ar: archenteral space; bl: blastocoel; ec: ectoderm; en: entoderm; m: mesenchyme; ve: vestibular invagination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the start of gastrulation, have proliferated from the tip of the archenteron during the later stages of gastrulation. SEM of the fractured anterior tip of the larva (Fig. 26D) shows the columnar ectodermal cells and the more rounded tightly packed mesenchyme cells. Filopodia can be seen stretching from the basal tip of certain ectoderm cells to the mesenchyme layer; in addition, the remains of filopodia cover the mesenchyme layer. It is possible that such filopodia, through contraction, assisted in the elongation of the archenteron in the later stages of gastrulation.

By 3.0 days after fertilization, although the larva is still uniformly ciliated, four more-densely ciliated bands, which encircle the larva transversely, are evident. By 4.0 days the body cilia between these bands have largely disappeared, and the larva is now a doliolaria (Fig. 26E). The ciliated bands of the doliolaria are here numbered one through four from anterior to posterior. A deep depression, called the vestibular invagination, is evident on the ventral surface of the doliolaria (Fig. 26F). It is deep and wide anteriorly, becoming narrower and shallower at its posterior end. A small rounded depression appears on the antero-ventral surface of the doliolaria late on the fourth day of development (Fig. 26F). This structure has been called, among other things, the praeoral pit (Bury, 1888), the suctorial disc (Mortensen, 1920a), and the adhesive pit (Chadwick, 1907; Holland and Kubota, 1975); the latter term will be used here. Ventrally, the first ciliated band curves forward of the vestibular invagination (Fig. 26F) where it is interrupted in the region between the vestibular invagination and the adhesive pit. The second ciliated band curves behind the vestibular invagination and merges with the third band ventrally

(Fig. 26F).

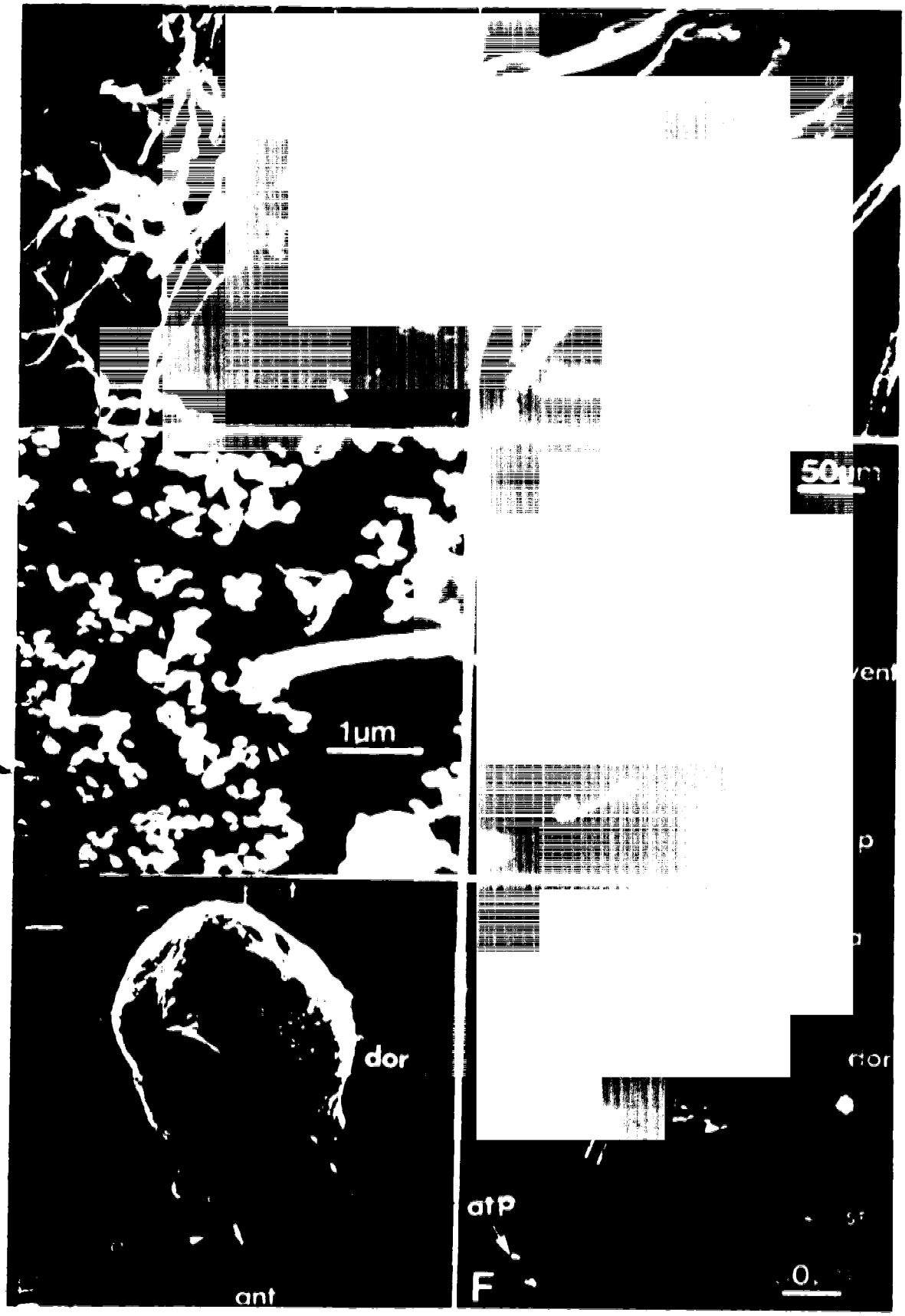
The body and apical-tuft cilia of the doliolaria, like those of the UC stage, have several spherical swellings along their lengths (Figs. 26G and 27A). Higher magnification (Fig. 27B) shows that the swellings, which are about 1 μm in diameter, are creased in a manner suggesting that they can exist in either a collapsed or distended condition. Short cilia with sub-terminal swellings are present near the apical tuft (Fig. 27A). Such cilia may be in the process of lengthening, to eventually add to the numbers of the apical-tuft cilia; alternatively, they may be fully developed cilia.

The doliolaria, like the UC larva, swims in a vertical sinusoidal path just below the water surface while rotating about the longitudinal axis in a clockwise direction when viewed anteriorly. In addition, some of the doliolariae were observed to swing back and forth about a point near the posterior end. The swimming behaviour of the doliolaria is summarized in Fig. 28.

It is known from light microscopic observations of living material that the ectoderm of the doliolaria, especially in the regions between the ciliated bands, contains numerous yellow dots, each about 4 μm in diameter. These dots, called oil-cells or yellow cells (the latter term will be used here), have been observed in the doliolariae of other species by a variety of workers (Thomson, 1865; Bury, 1888; Mortensen, 1920a).

The surface of the doliolaria, like that of the UC larva, is covered with a smooth glycocalyx supported by ectodermal microvilli about 0.5 μm long. The microvilli are branched and have spherical tips; a cluster of microvilli is associated with the base of each cilium

Fig. 27. Florometra serratissima. Doliolaria and settled form. (A) SEM of cilia forming the apical tuft of a 4.0-day-old doliolaria; numerous swellings along the length of each cilium are evident; several shorter cilia with subterminal swellings are visible at the edge of the apical tuft (arrowheads). (B) Higher magnification SEM of cilia from a ciliated band of a 4.0-day-old doliolaria showing creases (arrowheads) on the surface of each spherical swelling. (C) Ectodermal surface of a doliolaria with glycocalyx removed; note the branched microvilli with spherical tips (arrowhead), and the cluster of microvilli associated with the base of a cilium (double arrowhead). (D) A living 4.5-day-old doliolaria photographed with polarized light showing skeletal elements. (E) SEM of a recently settled doliolaria; the vestibular invagination has closed over, a glycocalyx is present, and the adhesive pit has expanded into an attachment disc. (F) A living settled form in the process of rotation photographed with polarized light; the direction of rotation is indicated by the sets of double arrows. ant: Anterior; at d: attachment disc; at p: attachment plate; ca: calyx; dor: dorsal; post: posterior; sk: skeletal plate; st: stalk; vent: ventral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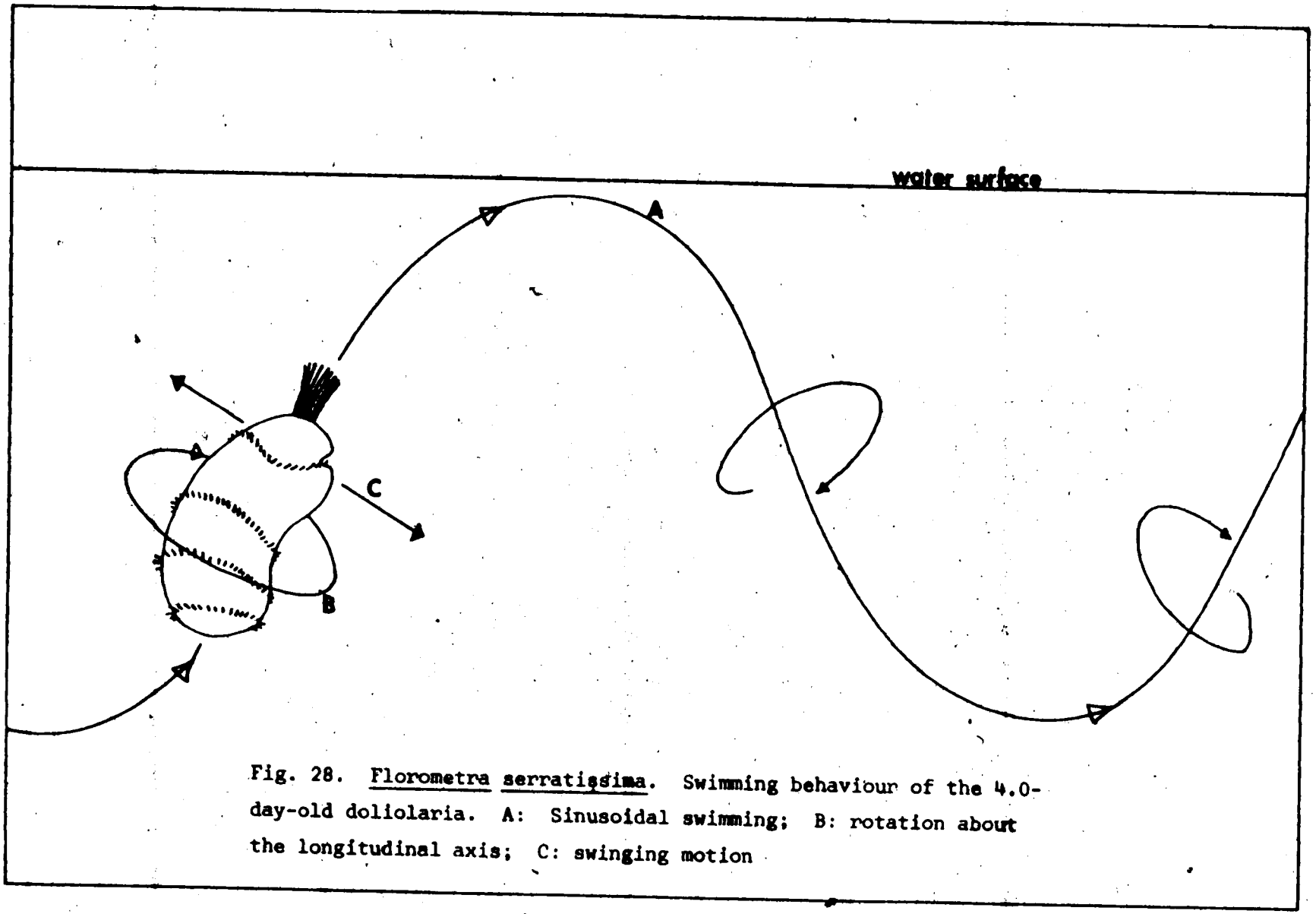


Fig. 28. Florometra serratissima. Swimming behaviour of the 4.0-day-old doliolaria. A: Sinusoidal swimming; B: rotation about the longitudinal axis; C: swinging motion

Fig. 27C).

Polarized light (Fig. 27D) reveals a well-developed larval skeleton. There are 10 skeletal plates (several of which can be seen in Fig. 27D) in the posterior region; these are primordia of the oral and basal plates of the future cystidean. A stalk primordium is positioned anterodorsally, and there is a developing attachment plate at the anterior end of this structure.

Settlement

Doliolariae begin to collect near the bottom of the culture dish midway through the fourth day of development. This behaviour takes place both in the dark and in the light, suggesting that a geopositive response is involved. Each doliolaria follows a sinusoidal path over the bottom. When near the bottom, the apical tuft and adhesive pit region are rubbed along the substrate for a short distance. The doliolaria makes one complete rotation about the longitudinal axis in the interval between successive contacts with the bottom. The overall path, as viewed from above, consists of many random turns. Such swimming behaviour, which always precedes settlement, will be called pre-settling exploratory behaviour.

Just before attachment, the doliolaria rapidly twirls about a particular spot on the bottom; it then attaches by means of a cement secreted from the adhesive pit. Doliolariae begin to settle as early as 4.6 days after fertilization, but many delay settlement for several days. In some instances, 14-day old doliolariae were observed to attach and metamorphose successfully, having thus delayed settlement for nine days. Attachment is temporary at first, and the settled forms can be

detached from the bottom with a jet of water from a Pasteur pipette; attachment becomes permanent after a few hours. At this time, the doliolaria is in a horizontal position with the ventral surface lying almost parallel to the substrate.

Doliolariae formed dense discrete aggregations during settlement in clean polystyrene culture dishes; the doliolariae within an aggregation were normally so close together that they touched one another. Such aggregations were obvious, even during a cursory examination of a culture dish containing settled larvae. A comparison of the distribution of settled forms on the bottom of a culture dish with the expected random (Poisson) distribution verified that settlement was non-random (Fig. 29).

The number of individuals per aggregation, the size of the aggregation (area within which all of the attachment discs of the resulting cystidean were situated), and its position in the culture dish, were noted for nine different cultures, and the results are summarized in Table 4; aggregations with less than five individuals were not included in this table. The maximum number observed in an aggregation was 81. The average size of an aggregation was 22 individuals ($\pm$ 18). Not all of the larvae settled gregariously: scattered individuals and aggregations with less than five animals were always noted. The great majority of the aggregations (79%) formed on the bottom of the culture dish; some (14%) formed just below the waterline; the remainder (7%) formed on the sides of the dish well below the waterline.

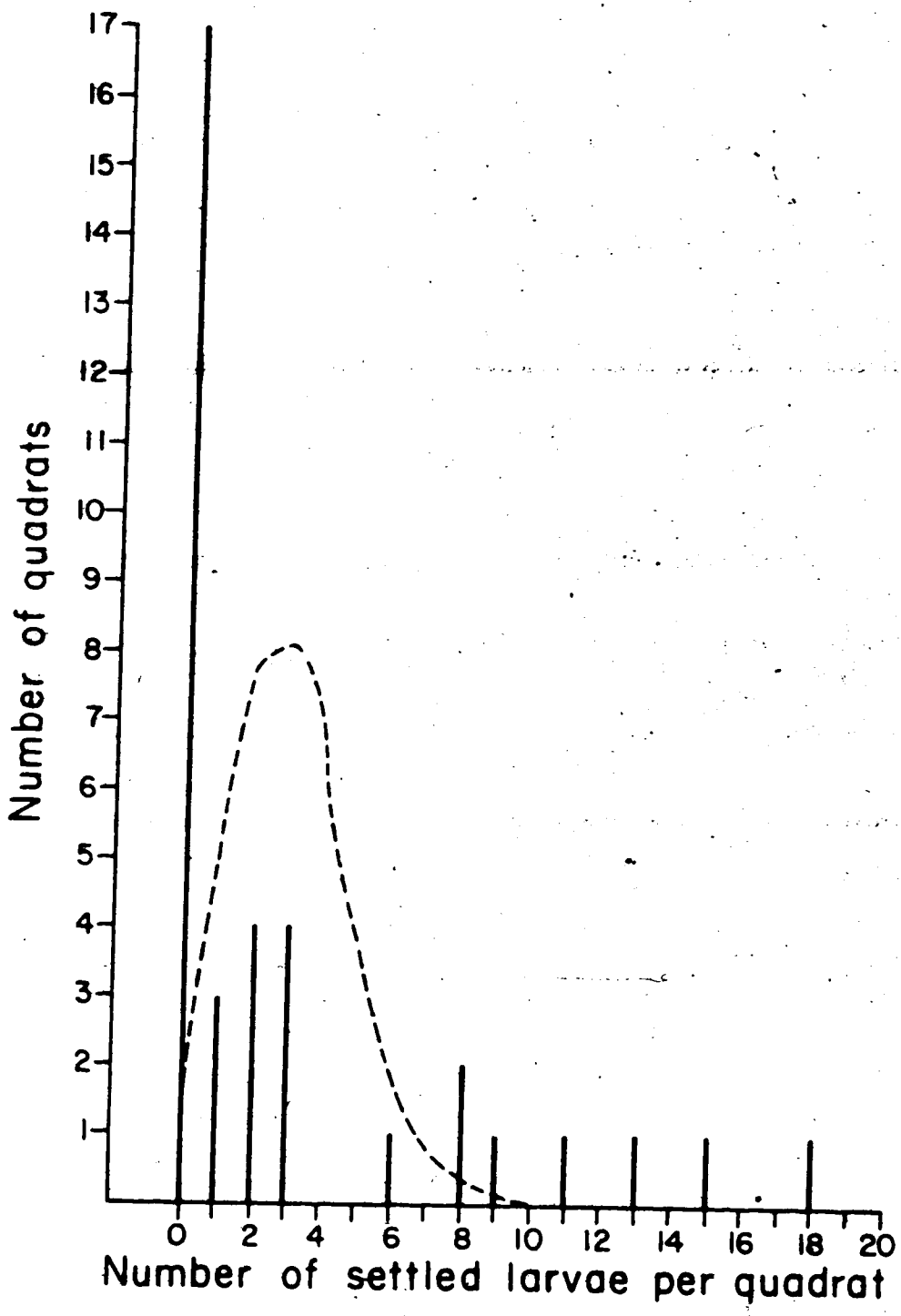


Fig. 29. Florometra serratissima. Distribution of settled doliolaria in a culture dish. The histogram is the actual frequency distribution and the dashed line is the calculated Poisson distribution. The actual frequency distribution departs significantly from random ($P < 0.005$)

Table 4. Florometra serratissima. Observations on larval aggregations

Culture	No. of larvae per aggregation	Size of aggregation[a] (mm^2)	Position of aggregation in culture dish
Feb 20/78	33	7	bottom
April 20/78 (A)	81	8	bottom
	11	-	bottom
	7	-	bottom
April 20/78 (B)	15	-	bottom
	33	4	bottom
	60	7	bottom
	34	3	bottom
	45	-	bottom
April 6/78 (A)	16	-	waterline
	13	-	waterline
April 6/78 (B)	15[b]	0.8	bottom
	17[b]	3	side
	15	3	side
April 7/78 (A)	33	12	waterline
	15	12	bottom edge
	34	12	bottom edge
	15	2	bottom
	6	2	bottom
April 7/78 (B)	28[b]	3	bottom
	32[b]	9	bottom edge
	25[b]	7	bottom edge
Nov 16/78 (A)	23	0.5	bottom edge
Nov 17/78	5	0.8	waterline
	8	0.8	bottom
	5	-	bottom
	6	-	bottom
	6	-	bottom
	6	-	bottom

[a] Area within which all attachment discs found.

[b] Aborted cystideans noted among healthy pentacrinoids.

Metamorphosis

A number of changes occur simultaneously within an hour of permanent attachment: the adhesive pit expands into a large, circular, slightly concave attachment disc; all of the cilia detach from the surface of the doliolaria; the vestibular invagination closes over to form the vestibule; the glycocalyx becomes a distinct sheet-like covering (Fig. 27E). The actual closing of the vestibular invagination was not observed in this study, but previous work with different species (Barrois, 1888; Seeliger, 1892; Mortensen, 1920a) shows that a fold of epidermis spreads posteriorly to anteriorly over the vestibular invagination; this is probably the case in Florometra serratissima as well.

The glycocalyx of the doliolaria, as described earlier, is separated from the ectoderm by a space 0.5 μm wide. Light microscopic observations show that shortly after attachment the width of the space beneath the glycocalyx increases several fold, and scattered yellow cells become apparent in this space.

Over the next 24 h an amazing series of transformations takes place. The settled form first straightens into an upright position so that the posterior end, which was formerly lying close to the substrate, now points away from the substrate. Next, the vestibule, along with the 10 skeletal plates at the postero-ventral region, rotate (Fig. 27F) to the former posterior end of the settled form. Simultaneously, the ectodermal region lying between the attachment disc and the vestibule elongates and is stretched over the side of the lengthening stalk, which is now assuming a straight upright position. At the completion of this process, a globular calyx containing the 10 skeletal plates

and the vestibule at its oral-most end sits on top of an upright stalk secured to the substrate by an attachment plate (Fig. 30A). This is the cystidean stage. Metamorphosis is now completed, and subsequent morphological changes occur much more gradually.

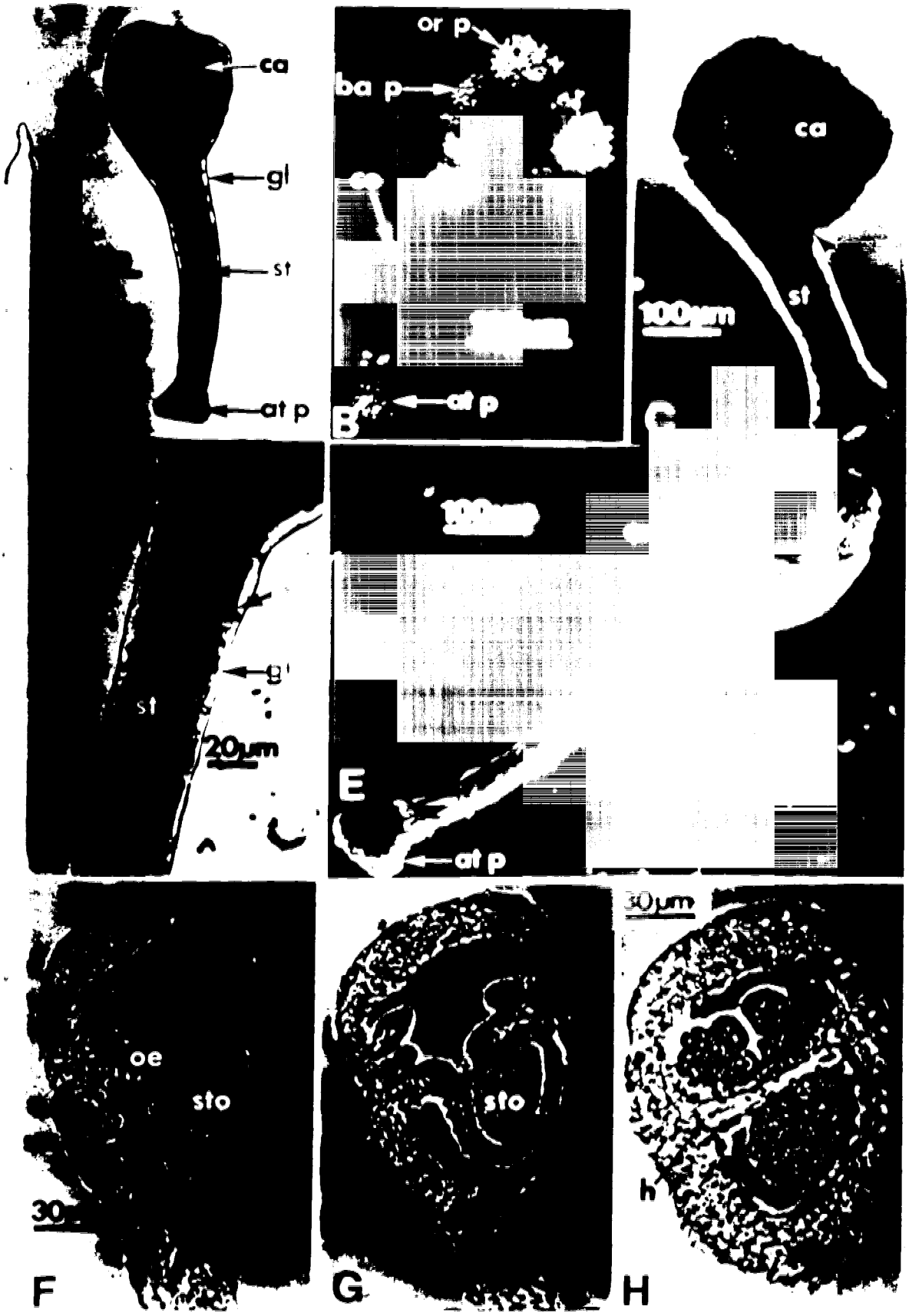
Doliolariae older than 14 days, which have not yet attached, begin to metamorphose pelagically. The rotation of the 10 skeletal plates commences, along with considerable elongation of the stalk, but the cilia are retained, and the glycocalyx remains unchanged. The resulting deformed doliolariae are feeble swimmers and sink to the bottom. Attachment does not occur, and the larvae soon die.

Another kind of abnormal metamorphosis occasionally takes place, this time after the doliolariae have successfully attached. In this case, the elongation of the ectodermal region between the attachment disc and the vestibule does not continue to completion, so the rotation process is hindered, and the calyx remains attached to the lengthening stalk by a fold of tissue (Fig. 30C). Many of these aberrant cystideans survive to become deformed pentacrinoids. This kind of abnormal metamorphosis has also been described for Antedon mediterranea (Barrois, 1888), A. adriatica (Seeliger, 1892), and Tropiometra carinata (Mortensen, 1920a).

Cystidean and Early Pentacrinoid

The cystidean measures 0.3 mm in height at the completion of metamorphosis. It is covered by the glycocalyx (Figs. 30A and 30E), which is now separated from the skeleton by a space 6 µm to 8 µm in width. Scattered yellow cells are located within this space (Fig. 30A). The major portion of each yellow cell is spread over the outer surface of

Fig. 30. Florometra serratissima. Cystidean. (A) Living cystidean two days after settlement; the glycocalyx and underlying yellow cells are evident. (B) Living cystidean two days after settlement photographed with polarized light revealing the various skeletal elements. (C) Living cystidean following abnormal metamorphosis; the calyx is attached to the stalk by a fold of tissue (arrowhead). (D) Part of the stalk of a living cystidean showing glycocalyx and underlying yellow cells. (E) SEM of a cystidean fixed seven days after settlement; the five oral plates of the calyx and the slightly torn glycocalyx are evident. (F) Median longitudinal section through the calyx of a cystidean seven days after settlement showing internal anatomy. (G) Slightly more lateral longitudinal section through the same cystidean; note cells lining the vestibule (double arrowheads), and particles in mouth. (H) Longitudinal section through the periphery of the calyx of the same cystidean; the circum-oesophageal hydrocoel ring is visible. at p: Attachment plate; ba p: basal plate; ca: calyx; co: columnar ossicle; gl: glycocalyx; h: hydrocoel ring; mo: mouth; oe: oesophagus; or p: oral plate; st: stalk; sto: stomach; str: strand; tf: tube foot; ves: vestibule; vf: vestibular floor; vi: visceral cavity; y: yellow cell


ca
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the skeleton, but one end turns upwards and supports the glycocalyx (Fig. 30D). Skeletal elements, as revealed by polarized light (Fig. 30B), consist of an attachment plate, a series of columnar ossicles which support the stalk, a row of five basal plates (= basalia) enclosing the lower portion of the calyx, and a row of five oral plates (= oral valves = oralia = deltoids) at the top of the calyx. Each oral plate lies directly above a basal plate.

Figs. 30F, 30G, and 30H show longitudinal sections through the calyx and upper part of the stalk of a cystidean fixed seven days after settlement. A horizontal layer of tissue, the vestibular floor (Fig. 30F), divides the calyx into an upper and a lower chamber, called the vestibule and the visceral cavity, respectively. The vestibule is roofed over by the five oral plates, as is evident in Fig. 30E. The mouth, a ciliated opening eccentrically placed in the vestibular floor, communicates with the roughly spherical thick-walled stomach through a short oesophagus which is patent at this time (Fig. 30G). The stomach is filled with small orange cells which are probably nutritive in function. Such cells also line the vestibule, being especially prominent just beneath the oral plates. The circum-oesophageal hydrocoel ring is positioned just beneath the vestibular floor and just within the walls of the calyx (Fig. 30H), and it gives off tube feet which project into the vestibule. Transverse sections through the vestibule show that there are 15 tube feet arranged into five groups of three each. The tube feet may dislodge the orange cells from beneath the oral plates and push them to the mouth, and hence into the stomach, where they are utilized; such cells can be seen in the mouth in some sections (Fig. 30G). Fig. 30F shows a strand of cells that originates

near the oesophagus, traverses the length of the stomach, and crosses the gap between the stomach and bottom of the calyx to enter the stalk. This strand continues down the length of the stalk to terminate in the attachment plate. That part of the strand associated with the stomach is the primordium of the axial organ, while the part associated with the stalk is the chambered organ. These terms were used by Mortensen (1920a, p.17, and Plate 6, Fig. 7) in his description of development in the feather star Tropiometra carinata (= T. picta).

The height of the cystidean increases linearly over the two-week period following settlement (Fig. 31). This results from a lengthening of the stalk due to elongation of the existing columnar ossicles and the addition of new ossicles.

The oral plates of the cystidean begin to open about 16 days after settlement. The animal is thus between 21 days (if it attached at the earliest possible time) and 30 days (if it delayed attachment for the maximum period of time) of age. It is 1.8 mm in height at this time, and has 12 ossicles in the stalk. The opening of the oral plates is first indicated by a small perforation at the oral-most tip of the cystidean which soon expands into five cracks which extend aborally between each of the five oral plates. In the early stages of the process, the tube feet can only be extended vertically through the partial opening at the top of the calyx. After a day, however, the oral plates can be opened fully, and the tube feet can be extended into a horizontal position. This marks the beginning of the pentacrinoid stage, and for the first time food can be obtained from an external source.

Fig. 32A shows a pentacrinoid fixed 27 days after attachment and

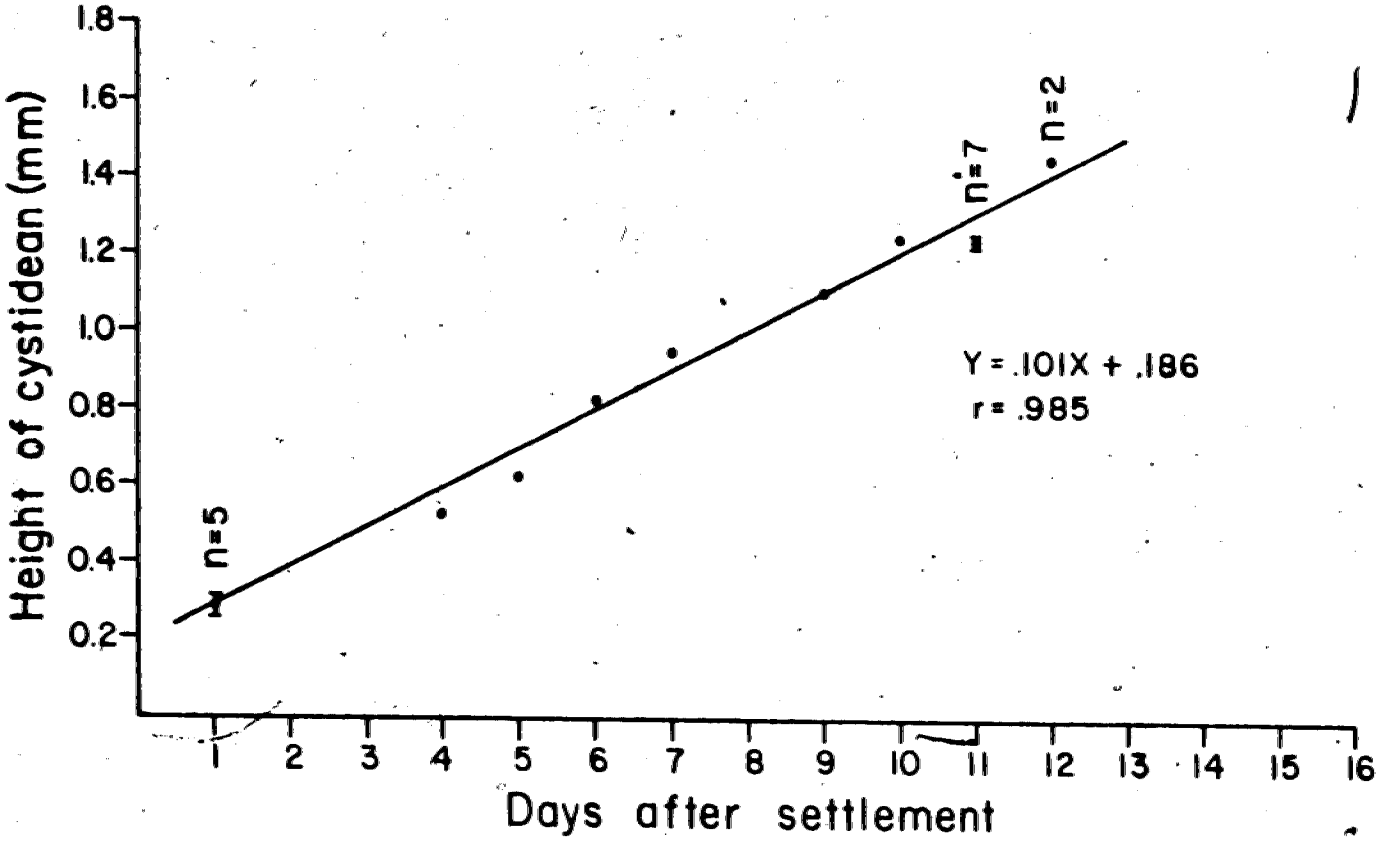


Fig. 31. Florometra serratissima. The relationship between height of cystidean and time after settlement. Where applicable, the number of measurements is shown above each point. The vertical bars show one S.D. on each side of the means. A simple point indicates that only one measurement was made. The regression line is significant ($P < 0.01$)

now measuring 2.7 mm in height. The attachment plate, the stalk, and the calyx are evident; the oral plates of the calyx are partially opened, and several of the tube feet are partially extended. The pentacrinoid is capable of withdrawing the tube feet into the vestibule and closing the oral plates (Fig. 32B). The attachment plate (Fig. 32C) consists of a skeletal plate, the upper surface of which is covered with a glycocalyx, while the underside, which is normally in contact with the substrate, consists of an exposed skeletal meshwork covered with remnants of the attachment cement. The long robust stalk comprises 13 columnar ossicles. The superficial structure of the ossicles is visible beneath the glycocalyx (Figs. 32A and 32D). Each is longitudinally ribbed, with a ring of knobs marking the midpoint. The ribs are connected to one another by short cross-branches. A slight constriction, and a discontinuity in the ribbing, indicates the point of junction of adjacent ossicles. Those ossicles near the midpoint of the stalk are the longest, with the ones towards each end being the shortest. The fenestrated structure of the basal and delicate oral plates of the calyx is visible beneath the glycocalyx (Fig. 32A). Each oral plate is slightly concave with out-turned edges.

Fig. 32E shows a tear in the glycocalyx at the junction of the calyx and stalk. A mat of fibrous strands underlies the glycocalyx at this point. A tear in the glycocalyx overlying part of one of the oral plates exposes the fenestrated skeletal plate below (Fig. 32F). Scattered fibres and a few spherical bodies measuring about 4 µm in diameter are associated with the skeletal plate. At higher magnification (Fig. 32G), it is evident that the fibres penetrate the interstices of the skeleton. In addition, it can be seen that each

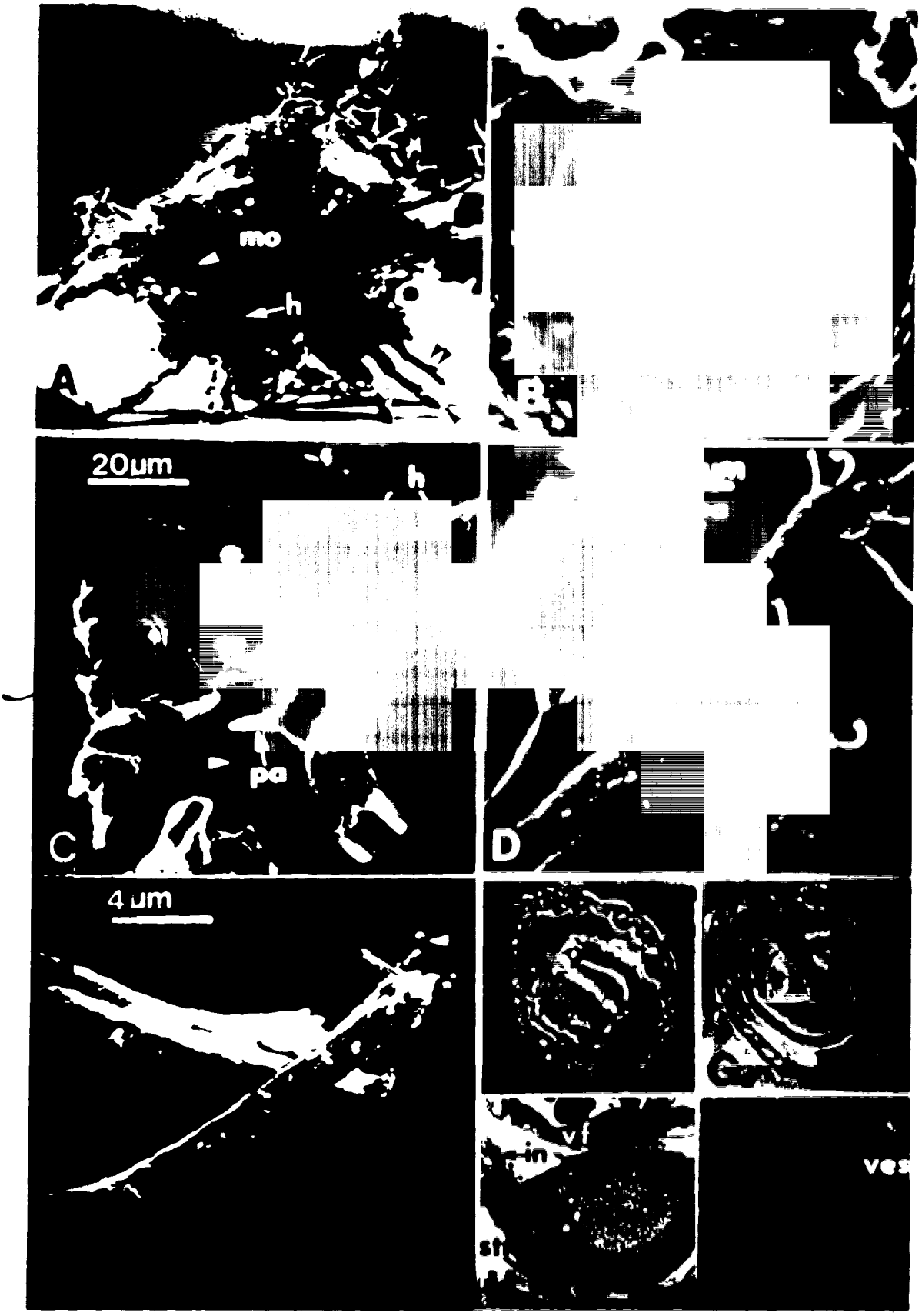
Fig. 32. Florometra serratissima. Pentacrinoid. (A) SEM montage of a pentacrinoid fixed 27 days after attachment. (B) Oral view of the calyx of a pentacrinoid using SEM; the tube feet are withdrawn, and the oral plates are in a nearly closed position; the glycocalyx is torn. (C) SEM of the attachment plate; remnants of cement (arrowhead) are visible on the undersurface. (D) SEM of part of the stalk; the ring of knobs at the midpoint of a columnar ossicle is indicated by double arrowheads; the single arrowhead points to the constriction marking the junction of two ossicles. (E) SEM of a tear in the glycocalyx at the junction between the stalk and calyx; note that fibrous strands underlie the glycocalyx (arrowhead). (F) SEM of a tear in the glycocalyx overlying part of an oral plate; scattered fibres overlie the skeletal plate along with a few spherical bodies (arrowheads). (G) Higher magnification SEM of surface of skeletal plate showing fibres and a single spherical body with a thin process (arrowhead). at p: Attachment plate; ca: calyx; gl: glycocalyx; or p: oral plate; sk: skeletal plate; st: stalk; tf: tube foot; ves: vestibule


A
50μm
rf
PP
orp
ca
B
gl
F
G
5

spherical body gives rise to a thin process. These spherical bodies may correspond to the yellow cells observed in the doliolaria and cystidean stages with light microscopy.

Fig. 33A shows the vestibule ofea pentacrinoid fixed 27 days after attachment. This specimen was relaxed before fixation so that the oral plates are in an open position with the tube feet nearly fully extended. At the bottom of the vestibule is a large circular mouth; in a living unrelaxed pentacrinoid the mouth is a crescentic slit. Higher magnification (Fig. 33B) shows that the mouth opening is surrounded by a prominent ring of tissue. Mucus strands are present at the edge of the mouth and crisscross the opening itself. Numerous spherical bodies, probably associated with mucus production, are present just inside the mouth. The hydrocoel ring is positioned slightly above the level of the mouth and just within the walls of the vestibule as formed by the oral plates (Fig. 33A). This ring gives off 25 tube feet. Fifteen of these originate from the outer edge of the hydrocoel ring and they are quite long when extended. They are arranged into five groups of three tube feet each, and because each group extends in a radial position between two adjacent oral plates, they will be called the radial tube feet. The middle tube foot of each group is the primary (= azygous) tube foot, and it is flanked on either side by a slightly longer secondary tube foot. There are five pairs of much shorter tube feet within the vestibule (Fig. 33A). These arise from the oral surface of the hydrocoel ring in an interradial position, and each pair is adjacent to the vestibular surface of an oral plate. These will be called the interradial tube feet. The tube feet of each interradial pair are connected by a bridge of tissue (Fig. 33C). That part of the

Fig. 33. Florometra serratissima. Pentacrinoid (cont'd). (A) SEM of the vestibule of a pentacrinoid fixed 27 days after attachment; the single black arrowhead points to a primary radial tube foot, while the double black arrowheads show a secondary radial tube foot; the white arrowhead indicates an interradial tube foot. (B) Higher magnification SEM of the mouth showing numerous strands and spherical bodies, likely associated with mucus production, just inside the mouth. (C) SEM of a pair of interradial tube feet; note the bridge of tissue (double arrowheads) connecting the two tube feet; the possible discontinuity in the hydrocoel ring is indicated by an asterisk; a mucus strand extending from a papilla is shown by a single arrowhead. (D) SEM of several radial tube feet showing numerous papillae. (E) Higher magnification SEM of a single papilla showing filaments (arrowhead) extending from its tip. (F) Transverse section through the calyx of a pentacrinoid at a level just below the vestibular floor; this specimen was fixed 30 days after settlement. (G) Transverse section through the calyx of the same pentacrinoid at a slightly more aboral level. (H) Nearly median longitudinal section through the visceral cavity of a pentacrinoid fixed 40 days after settlement; note the strand of tissue between the stomach and floor of the calyx. (I) Slightly more lateral longitudinal section through the visceral cavity of the same pentacrinoid showing the axial organ attached to the stomach and the intestine in cross section. ax: Axial organ; h: hydrocoel ring; in: intestine; mo: mouth; oe: oesophagus; or p: oral plate; pa: papilla; sto: stomach; str: strand; ves: vestibule; vf; vestibular floor; vi: visceral cavity


mo
h
A
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20µm
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pa
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4 µm
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ves

hydrocoel ring between the tube feet of each interradial pair is less prominent than the rest of the ring and may be discontinuous. If this is indeed the case, then the interradial tube feet and their connecting tissue bridges serve as part of the hydrocoel ring.

Each radial tube foot bears 12 to 15 long thin processes or papillae (Fig. 33D). Each papilla (Fig. 32E) is 10 µm to 20 µm long and just under 2 µm in diameter; four or five filaments with slightly bulbous tips, as well as several rounded protuberances, project from the tip of each papilla. The interradial tube feet are also papillate. Each bears four or five small rounded papillae on its sides, a pair of long papillae at its tip, and a single long papilla on its interradial surface; all terminate in filaments. Mucus strands are often seen extending from the tips of these papillae (Fig. 33C).

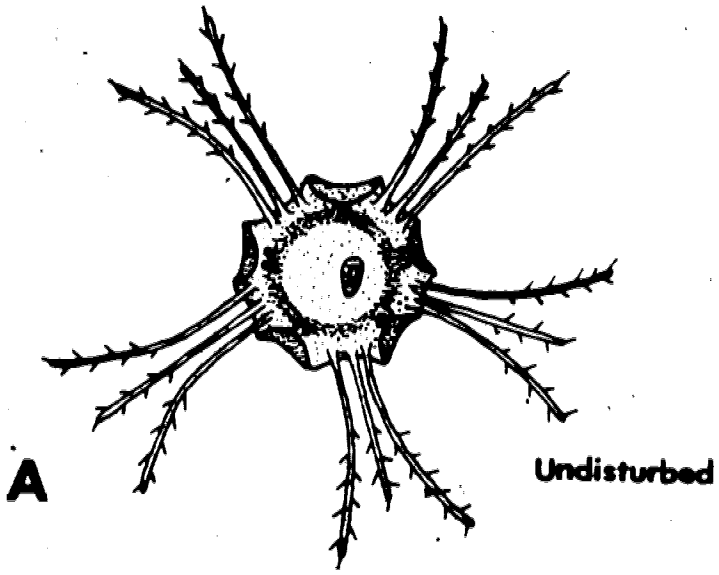
Transverse sections through the calyx of a pentacrinoid unrelaxed prior to fixation show that the mouth is a crescentic slit placed eccentrically in the vestibular floor (Fig. 33F). The mouth is joined to the crescent-shaped stomach by a short oesophagus; the stomach is largely situated on one side of the visceral cavity (Fig. 33G). The tip of the stomach gives off a long thin intestine which curves around the visceral cavity in a clockwise direction (Fig. 33G) and, near its end, turns upwards abruptly to terminate blindly just beneath the vestibular floor (Fig. 33H). The stomach is secured to the wall and floor of the calyx by a horizontal and vertical mesentery. The axial organ, first noted in the cystidean (Fig. 30F), is positioned vertically between the stomach and the intestine and is attached to the outer wall of the stomach (Fig. 32I). It gives off a strand of tissue, which bridges the gap between the bottom of the stomach and the floor

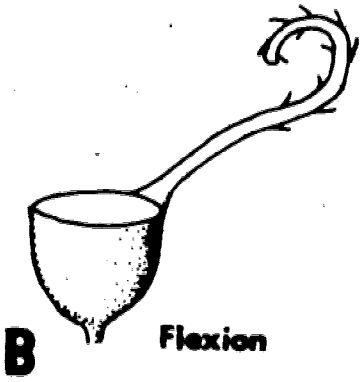
of the calyx (Fig. 33H) and which then continues down the length of the stalk.

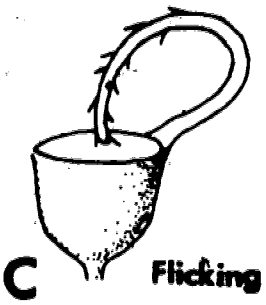
The living undisturbed pentacrinoid holds the 15 fully extended radial tube feet in a plane perpendicular to the oral-aboral axis (Fig. 34A). In this posture, the tube feet span a diameter of just over 1 mm. Feeding responses can be elicited and observed with the dissecting microscope if a suspension of mixed algal cells in seawater (Isochrysis galbana and Phaeodactylum bicornutum) is trickled past the pentacrinoid with a Pasteur pipette. The first noticeable response is a slow sweeping movement of the tube feet in a horizontal plane combined with rapid and successive flexion and extension of the tips of the tube feet (Fig. 34B). Then, one or more of the tube feet quickly flick into the vestibule bringing their tips near the mouth (Fig. 34C). This is followed by a slower re-extension into the horizontal position once again. In addition to such movements, the radial tube feet are often folded into the vestibule in a manner that brings the middle part of each tube foot near the mouth, while the distal portion extends upwards out of the vestibule (Fig. 34D). Each set of three radial tube feet is folded into the vestibule together; several sets can be brought in together; occasionally, all five sets are folded into the vestibule simultaneously, and the oral plates close partially. The short interradial tube feet are very active during feeding: they quickly wipe along the surface of the folded radial tube feet and bend into the mouth; they are likely transferring food particles from the radial tube feet to the mouth.

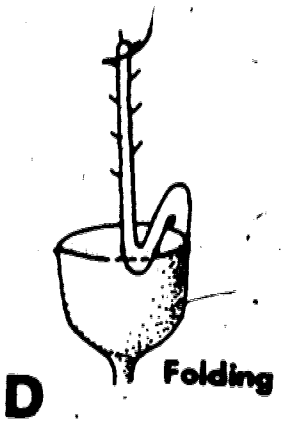
If the calyx of a pentacrinoid is touched with a needle, all of the tube feet quickly fold into the vestibule, and the oral plates

Fig. 34. Florometra serratissima. Diagrammatic illustrations showing feeding movements of the tube feet of a pentacrinoid. (A) Oral view of the calyx of an undisturbed pentacrinoid with oral plates open and 15 radial tube feet in a fully extended position. (B) Flexion of the tip of a tube foot. (C) A tube foot flicking into the vestibule bringing its tip near the mouth. (D) A tube foot folded into the vestibule


A
Undisturbed


B
Flexion


C
Flicking


D
Folding

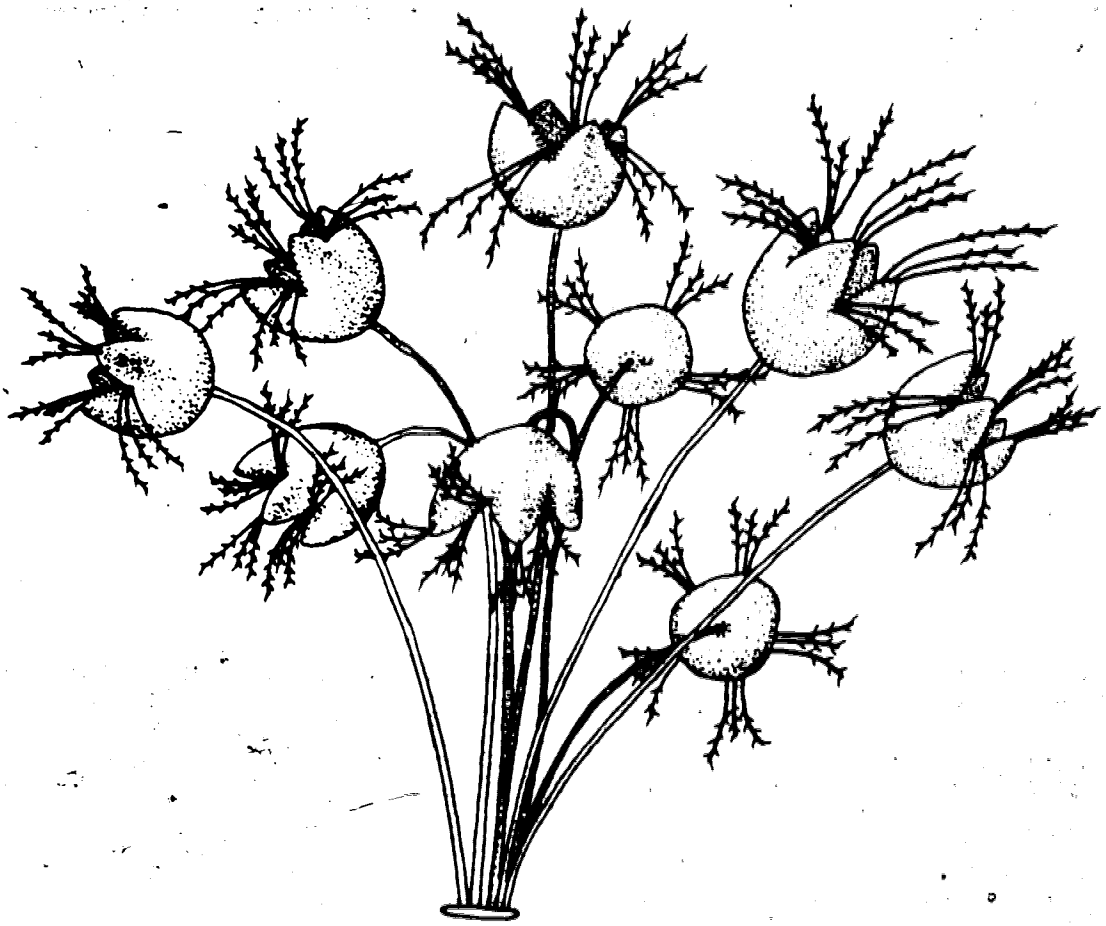


Fig. 35. Florometra serratissima. Sketch of an aggregation of pentacrinoids 30 days after settlement

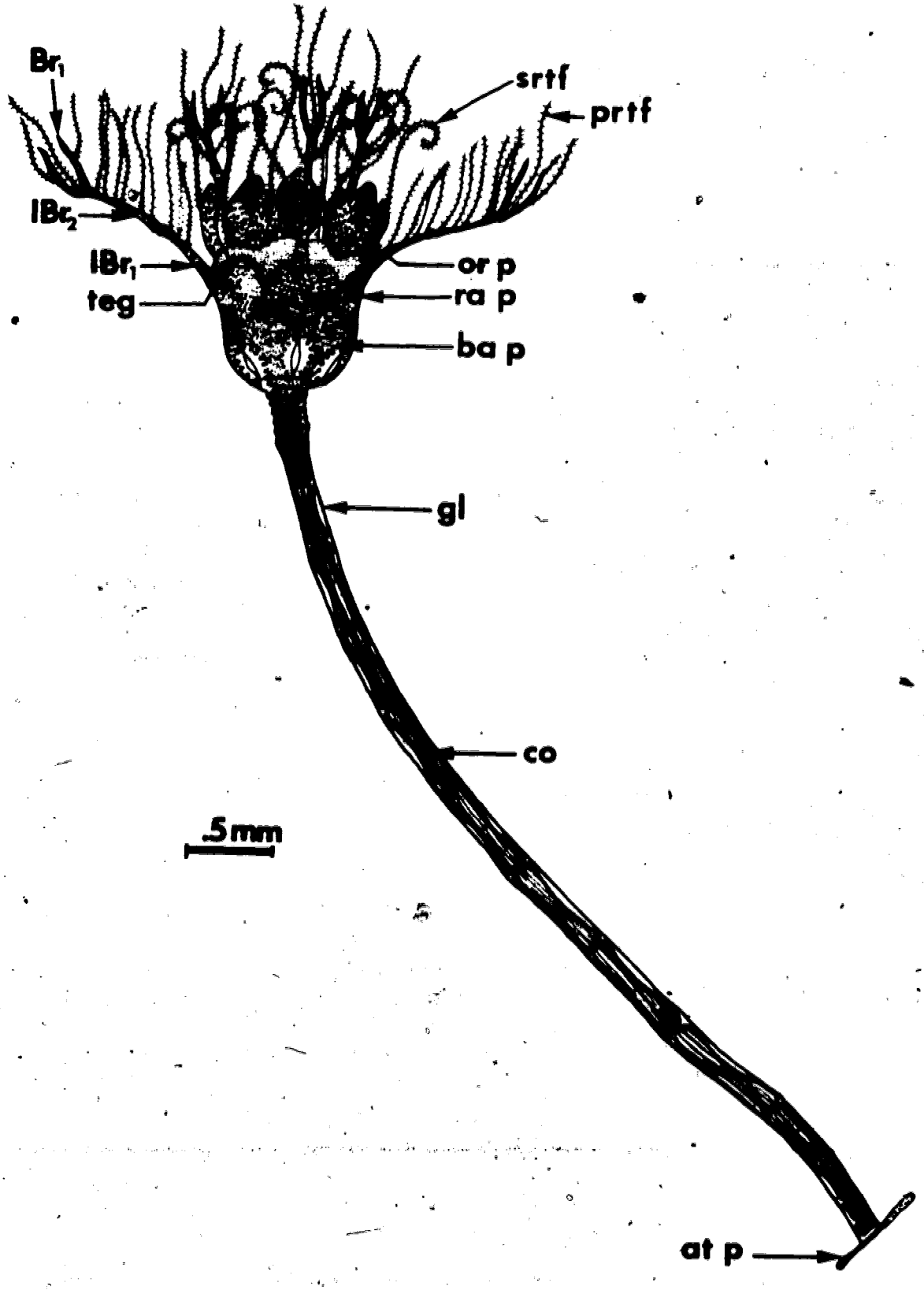
close, thereby protecting the vestibular contents. Recovery is rapid, and the oral plates are opened and the tube feet re-extended within 15 sec. If the stalk of a pentacrinoid is touched gently with a needle, the calyx does not react. The stalk itself is capable of a limited amount of movement: it can shift from a vertical position to a leaning position; in addition, the part near the calyx shows a certain amount of flexibility such that the calyx can be held in either an upright position, or be allowed to droop to one side. Such movements occur imperceptibly over a period of minutes. Even in much older pentacrinoids, the stalk cannot be moved freely or twisted into a spiral as claimed by Thompson (1836) and Chadwick (1907, p. 37) for pentacrinoids of Antedon.

As described earlier, doliolariae settle in aggregations. After metamorphosis, the attachment discs of the resulting cystideans and pentacrinoids become fused together to form a common attachment structure. Crowding in the aggregation is alleviated by the vertical elongation of the stalk. Some of the calyxes of aggregated pentacrinoids are oriented upwards, while others droop to the sides (Fig. 35). Some of the cystideans in an aggregation do not survive to reach the pentacrinoid stage (Table 4). Mortality of this kind was noted in solitary individuals as well and is probably not related to the gregarious habit.

Older Pentacrinoids

Pentacrinoids cultured in the underwater rearing tray showed variability in their rate of development, with individuals of the same age differing in size and stage of development. Fig. 36 shows the most

Fig. 36. Florometra serratissima. Drawing of a four-month-old pentacrinoid cultured in the underwater rearing tray. at p: Attachment plate; ba p: basal plate; Br_1: first ossicle of the undivided arm; co: columnar ossicle; gl: glycocalyx; IBr_1: first post-radial ossicle (= costal); IBr_2: second post-radial ossicle (= axillary); or p: oral plate; prtf: primary radial tube foot; ra p: radial plate; srtf: secondary radial tube foot; teg: tegmen


Br1
srtf
prtf
lBr2
lBr1
teg
or p
ra p
ba p
gl
co
.5mm
at p

advanced four-month-old pentacrinoid examined. It measures just over 8 mm in height and has five small arms which span a distance of 2.4 mm. The stalk consists of 19 ossicles. The six uppermost ossicles are short and disc-like, but the ossicles become progressively longer proceeding towards the base of the stalk; those ossicles near the attachment disc, however, are quite short. This arrangement suggests that the stalk increases in length through the addition and subsequent elongation of new ossicles just beneath the calyx, as proposed by Thomson (1865, p. 528) for the pentacrinoid of Antedon bifida. A glycocalyx is evident over the stalk and aboral part of the calyx.

Another series of five heart-shaped skeletal plates, the radial plates, have appeared in the calyx in a radial position just above the basal plates. Each radial plate gives off a costal ossicle or IBr_1 followed by an axillary or IBr_2, using the terminology of Mortensen (1920a) and A.H. Clark (in A.M. Clark, 1970, pp. 10-12), respectively. In turn, each IBr_2 gives off two brachial ossicles (the Br_1's) which give the arm tips a forked appearance. A narrow gap is apparent between each basal plate; that part of the plate adjacent to the gap is translucent, and lacks the fenestrated appearance typical of the remainder of the plate. The five oral plates, which in the early pentacrinoid were positioned directly above the basal plates, are now located at the top of the calyx, separated from the radial plates by the developing tegmen.

The primary radial tube feet have been incorporated into the developing arms to become the radial canals of the water vascular system; the tip of each primary radial tube foot is still visible in the fork at the arm tips, and corresponds to the terminal tentacle

of asteroids and echinoids. The five pairs of secondary radial tube feet have remained in close proximity to the mouth. These are quite long, with the tips typically curled, and they are probably retained as the oral tube feet which surround the mouth of adult crinoids. The 10 short interradial tube feet have disappeared. New tube feet have been added in alternating fashion along the length of each arm. All of the tube feet are papillate.

The most advanced stage reached was a six-month-old pentacrinoid with a height of 12 mm and with an arm span of 6.5 mm. This individual is shown in Fig. 37 along with two smaller pentacrinoids of the same age; the attachment discs of all three pentacrinoids are touching. Each of the 10 arms of the largest individual consists of 15 brachial ossicles (Br_1 to Br_{15}). The primary radial tube feet have disappeared, but many additional tube feet have been added along the length of each arm. The 10 arms are normally held in a plane perpendicular to the oral-aboral axis, with the tube feet projecting orally. There are at least 250 tube feet, each about 1 mm in length when fully extended and these provide a formidable food-catching array for the pentacrinoid. If the pentacrinoid is disturbed, the arms are flexed into a vertical position, but the tube feet remain exposed.

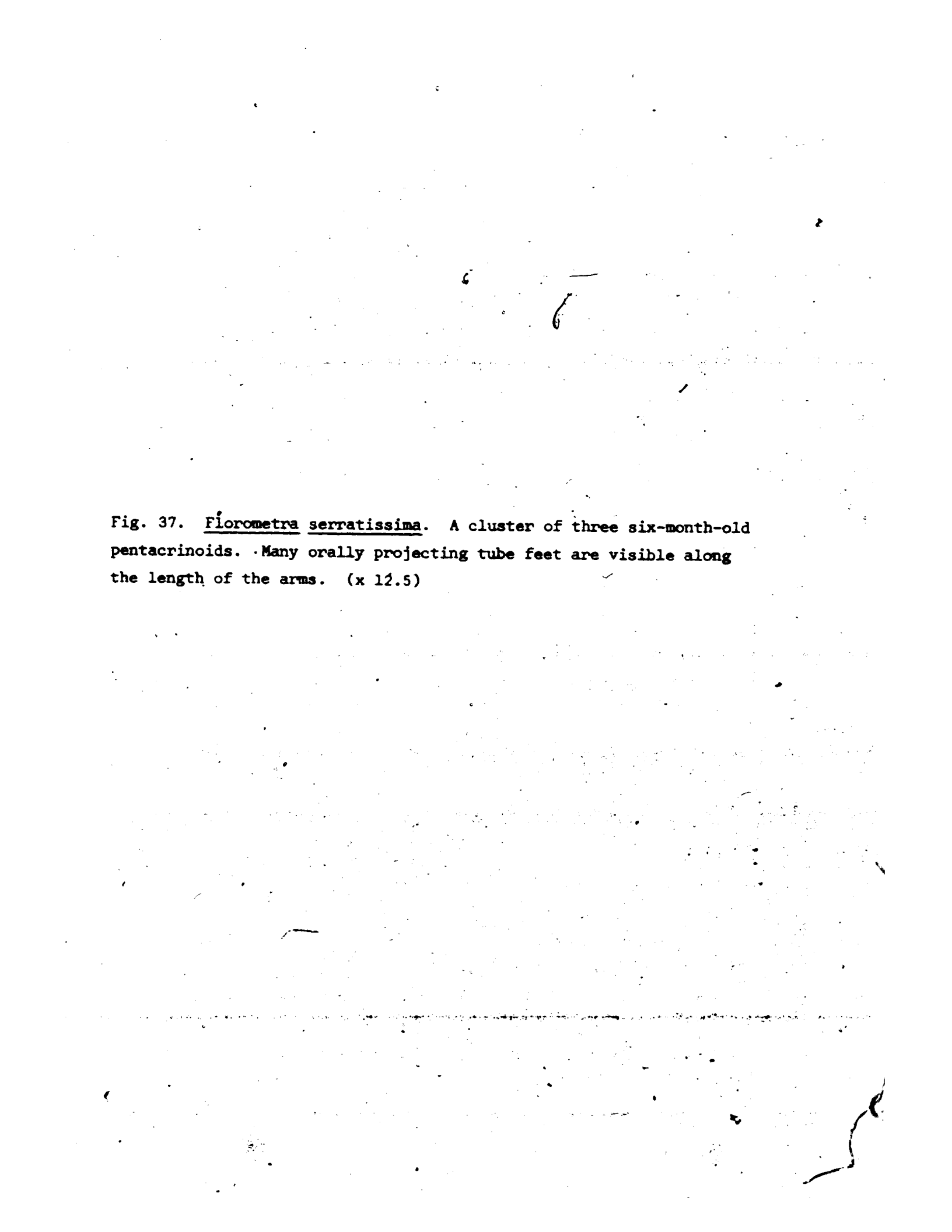
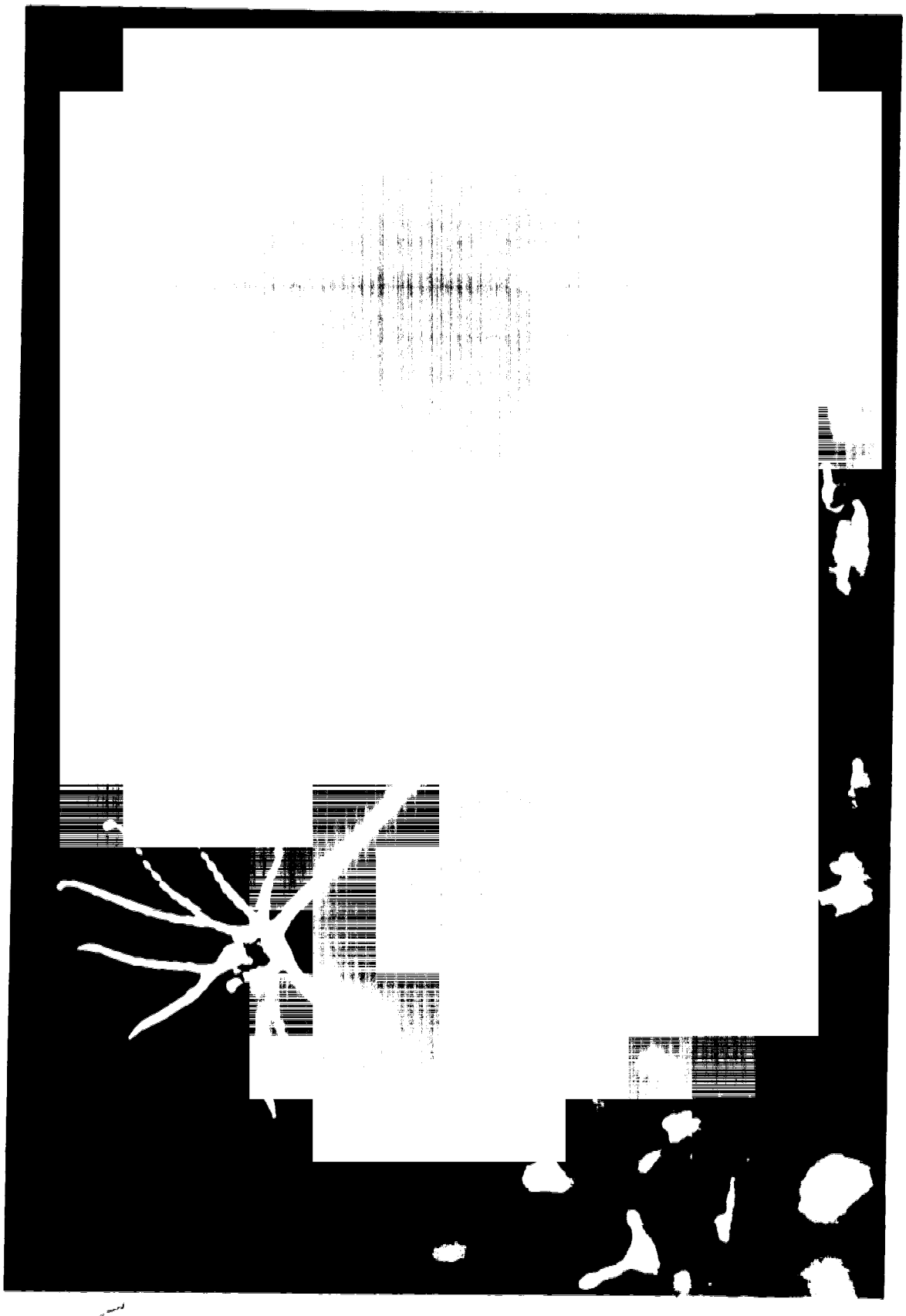


Fig. 37. Florometra serratissima. A cluster of three six-month-old pentacrinoids. Many orally projecting tube feet are visible along the length of the arms. (x 12.5)



DISCUSSION

There is considerable discrepancy in the literature over the usage of the term pentacrinoid. In its original sense, as used by Wyville Thomson (1865), it referred to the entire stalked phase of development from just after attachment and metamorphosis to the time when the young feather star broke away from the stalk. Mortensen (1920a, 1937, 1938) adopted this usage of the term in all of his work with crinoids. Perrier (1886) introduced the term cystid, which referred to the stalked form from immediately after metamorphosis to the time when it developed arms and cirri, after which it was called a phytocrinoid. Bury (1888) advocated this terminology but suggested that the term pentacrinoid be kept to denote any stalked form, either cystid or phytocrinoid. In recent years (Hyman, 1955; Dan, 1968; Holland and Kubota, 1975), the term cystidean has been used to designate the stalked form prior to the development of arms, after which it is called a pentacrinoid.

In this report the terms cystidean and pentacrinoid have been retained, but they have been used in a manner that reflects an obvious functional change in the life-style of the stalked form, namely the transition from a non-feeding to a feeding existence, rather than simply a gradual morphological change. The term cystidean has thus been used to denote the stalked form from just after metamorphosis to the time when the oral plates open allowing the tube-feet to emerge from the vestibule; the stalked form has then been called a pentacrinoid until the time when the young feather star breaks free of the stalk to become a free-living juvenile. The cystidean is therefore a non-feeding stage which survives through the use of stored resources, whereas the pentacrinoid is a feeding stage which is capable

of capturing food with the extended tube feet.

Florometra serratissima is not the only echinoderm with an ornamented fertilization membrane. Such a feature is known for six other crinoids and for three ophiuroids (Table 5); all have pelagic eggs. The fertilization membrane of these species appears spiny with the light microscope; however, SEM observations on F. serratissima in this study, and on Comanthus japonica (Holland and Jespersen, 1973), show that the fertilization membrane is ridged. Such is probably the case for the other eight species in Table 5. Mortensen (1920a) and Holland and Jespersen (1973) suggested that a ridged fertilization membrane is an adaptation for egg dispersal. There is, however, the possibility that dispersal is a fortuitous by-product of another advantage conferred on the pelagic egg by this feature. Perhaps an ornamented fertilization membrane, by increasing the egg diameter considerably, reduces loss due to predation in the water column.

The ridged fertilization membrane of F. serratissima is not identical to that of C. japonica as studied by Holland and Jespersen (1973). Although the diameter of the zygote of both species is virtually identical, the overall diameter of the fertilized egg of F. serratissima is much larger than that of C. japonica (320 µm compared to 260 µm). This is due largely to a difference in the height of the ridges of the fertilization membranes: ridge height is 40 µm in F. serratissima and only 14 µm in C. japonica. In addition to this difference, the ridges in F. serratissima do not outline precise hexagonal facets as they do in C. japonica (compare Fig. 22E from this study with Fig. 2 from Holland and Jespersen, 1973). The material composing the fertilization membrane is thus distributed differently

Table 5. Echinoderms with an ornamented fertilization membrane

Species		Source
Antedon petasus	Crinoidea	Mortensen (1920b)
Comanthus japonica	Crinoidea	Dan and Dan (1941) Holland and Jespersen (1973)
Florometra serratissima	Crinoidea	This study
Heterometra savignyi	Crinoidea	Mortensen (1938)
Lamprometra klunzingeri	Crinoidea	Mortensen (1937)
Tropiometra audouini	Crinoidea	Mortensen (1937)
Tropiometra carinata	Crinoidea	Mortensen (1920a)
Ophiocoma echinata	Ophiuroidea	Grave (1898)
Ophiocoma erinaceus	Ophiuroidea	Mortensen (1937)
Ophiocoma scolopendrina	Ophiuroidea	Mortensen (1937)

in each species; in C. japonica there are many short ridges, while in F. serratissima the ridges are fewer but taller. The ultimate cause of such differences is not known, but may be related to differing dispersal mechanisms or predation pressures on the pelagic eggs.

The first and second cleavages are unilateral in F. serratissima. This is also the case for Comanthus japonica (Dan and Dan, 1941; Holland, 1978), while in the crinoids Antedon adriatica (Seeliger, 1892) and A. bifida (Thomson, 1865, plate 23, Fig. 10) at least the first cleavage is unilateral. The 16-cell stage of F. serratissima is unusual in having the eight animal cells arranged in a hemisphere, and in having a vegetal pore and two lateral pores. The 16-cell stage of C. japonica has a vegetal pore, but the eight animal cells are in a plane and the two lateral pores are absent (Holland, 1978). Cleavage asynchrony is common in F. serratissima, especially after the 16-cell stage, while Holland (1978) noted cleavage asynchrony in C. japonica, especially after the 32-cell stage; the result in both cases is normal larvae. Throughout cleavage in F. serratissima, the cells are tightly pressed against one another along flattened surfaces. This has been called blastomere compaction by Holland (1978), who suggested that it is a general feature of cleavage in crinoid embryos.

In F. serratissima a coeloblastula with about 1000 cells is formed, followed by gastrulation by invagination without previous primary mesenchyme formation. Gastrulation proceeds in this manner in Antedon adriatica (Seeliger, 1892) and A. mediterranea (Bury, 1888), but other crinoids show variable features at gastrulation. In Tropiometra carinata (= T. picta) and Heterometra savignyi (Mortensen 1920a, 1938) there is multi-polar ingression of cells into the blastocoel followed

by invagination; the ingressed cells join, apparently, with the invaginated endodermal cells prior to mesenchyme proliferation. Mortensen (1920b) indicates that in Antedon petasus the blastocoel first fills with cells, and then multiple invagination takes place. In Isometra vivipara, superficial cleavage results in a stereoblastula, and gastrulation is by delamination. In Lamprometra klunzingeri, there is multipolar ingression but no invagination occurs, the gastral cavity being formed instead by schizocoely (Mortensen, 1920a, 1937). Holland (1976, 1978) studied gastrulation in Comanthus japonica in detail. He found that a well-defined blastula was lacking and that gastrulation occurs at an early stage in development (embryo with 50 to 100 cells) through a modified process of invagination referred to as "holoblastic involution". Gastrulation by blastular invagination, as shown by F. serratissima, may be the most widespread and primitive kind of echinoderm gastrulation (Holland, 1978).

The cilia are swollen terminally during growth in the gastrula of Florometra serratissima. Each cilium, once it attains its final length, possesses several swellings along the length of its shaft; this is true for the body and apical-tuft cilia of both the UC larva and doliolaria. Regular swellings of this kind along the shafts of fully grown cilia have not been previously reported. Holland (1976) noted subterminal swellings during ciliogenesis in the gastrula of Comanthus japonica, but found that longer cilia had a constant diameter from base to tip. Holland postulated that the subterminal swellings, since they occur during ciliary growth, contain accumulations of cytoplasm, particularly precursors of microtubules. The significance of the swellings in the fully grown cilia of F. serratissima is not

known. It is conceivable that each swelling flattens into a small paddle during the effective stroke and collapses during the recovery stroke, thereby increasing the efficiency of the ciliary beat; however, the apical-tuft cilia, which do not participate in swimming, have swellings as well. It is possible, then, that the swellings have a sensory function or, alternatively, that they are storage regions for cytoplasmic components essential to the operation and maintainance of the cilia. A future ultrastructural study of the ciliary swellings might provide a definite answer.

The doliolaria of F. serratissima has four ciliated bands. There are either four or five ciliated bands in the doliolaria of other species, the anterior-most band sometimes being absent or incomplete (Table 6).

Ectodermal surface features of F. serratissima before and after metamorphosis are of interest. The glycocalyx of the UC larva and doliolaria is supported by numerous microvilli arising from the apical tips of the ectodermal cells. There is a 0.5 µm space between the glycocalyx and the underlying ectoderm, and numerous pores in the glycocalyx connect this space with the exterior. The ectodermal features of corresponding stages of Comanthus japonica are essentially identical (Holland and Kubota 1975), but pores in the glycocalyx were not noted. Yellow cells are a distinctive feature of the ectoderm of the doliolaria of F. serratissima in the regions between the ciliated bands. During metamorphosis the space beneath the glycocalyx increases in width dramatically (from 0.5 µm to at least 6 µm), but the yellow cells remain in this space and so become situated between the skeleton and the glycocalyx. Seeliger (1892) noted that in the late doliolaria

Table 6. Number of ciliated bands in crinoid doliolariae

Species	Number of bands	Source
Antedon adriatica	5	Seeliger (1892)
Antedon bifida	4	Thomson (1865)
Antedon mediterranea	5	Bury (1888)
Antedon petasus	4	Mortensen (1920b)
Comanthus japonica	5 (first band splits ventrally)	Holland and Kubota (1975)
Compsometra serrata	5 (first band evident on dorsal side only)	Mortensen (1920a)
Florometra serratissima	4	This study
Isometra vivipara	5 (first band evident on dorsal side only)	Mortensen (1920a)
Tropiometra carinata	4	Mortensen (1920a)

of Antedon adriatica the ectoderm was clearly delineated from underlying mesenchyme, but shortly after settlement the ectodermal cells withdrew from the surface to intermingle with the mesenchyme. Cellular movements of this kind may account for the increased space beneath the glycocalyx in F. serratissima after metamorphosis. The glycocalyx and associated yellow cells remain throughout the development of the cystidean and pentacrinoid in F. serratissima. Interestingly enough, yellow cells are present in adult F. serratissima, where they are especially visible in the arm lappets. The significance of the yellow cells is not known, but they may be involved in the support and secretion of the glycocalyx.

Rotation about the longitudinal axis while following a sinusoidal path is a basic feature of swimming behaviour in the UC larva and doliolaria of F. serratissima. The reason for such elaborate motions is not understood, but it may be an orientation mechanism allowing the larva to survey and compare impinging environmental cues. Throughout the UC and early doliolaria stages the larvae swim just beneath the water surface; prior to settling, the doliolaria sink to the bottom. This is common behaviour for invertebrate larvae from coastal waters (Thorson, 1964) and it may be a mechanism that at first enhances dispersal by means of surface currents, and then places the larvae close to the bottom in preparation for settlement. The apical-tuft cilia make contact with the bottom during pre-settling exploratory behaviour which suggests that one of the functions of the apical tuft is substrate selection.

The doliolariae of F. serratissima have the capacity to delay settlement for up to nine days and still attach successfully. This

gives the larvae an extended opportunity to choose a settlement site and increases the dispersal capabilities of the species. Delay of settlement is known or implied for the following additional crinoid species: Antedon adriatica (Seeliger, 1892); Tropiometra carinata (Mortensen, 1920a); Heterometra savignyi (Mortensen, 1938); and Comanthus japonica (Dan, 1957; Holland and Kubota, 1975). In the case of F. serratissima, a portion of all broods reared in the laboratory showed delay of settlement for a variable length of time in clear polystyrene dishes. This suggests that there is a genetic component governing the time at which the larva is likely to settle; otherwise, it would be expected that all of the larvae should settle at about the same time under the conditions of an equally favorable substrate offered by the culture dish. A possible advantage of such an imposed variability in length of pelagic period is increased spread of sibling larvae (Strathmann, 1974).

The doliolar of F. serratissima settle gregariously in culture. Pentacrinoids were never observed in the field during S.C.U.B.A. dives, so it is not possible to support the in vitro evidence with field observations. Carpenter (1866, p. 726), however, found 70 pentacrinoids of Antedon bifida in nearly the same stage of development attached very close together on a patch of Membranipora encrusting a frond of Laminaria. It is possible that gregarious larval settlement in crinoids is common, but not previously looked for. Another form of gregarious settlement, in which larvae settle on adults, has been reported for at least 12 species of crinoids found in dredges (A.H. Clark, 1921, pp. 513-576). In most instances, pentacrinoids are found associated with the cirri of adults, and it is assumed that the pentacrinoids are

of the same species as the adult on which they are found. Mortensen (1920a, p. 54) found a few adult Florometra serratissima with pentacrinoids attached to the cirri (10 pentacrinoids were found on the more than 200 specimens he examined). At least 500 adult F. serratissima were examined over the period of this study (November, 1977 to December, 1979), but pentacrinoids were never found. Pentacrinoids were not found on any substrate, however, so larval recruitment to the area was very low.

At this time, it is only possible to speculate on the significance of gregarious settlement in F. serratissima. The occurrence of adult F. serratissima in discrete aggregations in Barkley Sound, British Columbia, and the general tendency for many crinoid species to be found in aggregations is discussed in Mladenov (1980c). Hyman (1955) attributed the formation of such aggregations to the poor dispersal capabilities of crinoid larvae which results in larvae inevitably settling close to the parents. Although this is probably true for certain antedonids and some other species which show varying degrees of brood protection and which consequently release larvae at a late stage of development (Seeliger, 1892; Bury, 1888; Mortensen, 1920a), it cannot explain the formation of adult aggregations in F. serratissima whose eggs and larvae have good dispersal capabilities. It is proposed that gregarious settlement of doliolariae following a period of pelagic dispersal, either among themselves or on other adults, is an important factor in both the creation and maintenance of adult aggregations of F. serratissima since the resulting juveniles and adults have only limited swimming capabilities and cannot move far from their place of settlement. F. serratissima would thus have

combined the advantages of dispersal with the ability to form adult aggregations. It can be postulated that the ultimate reason for aggregation in F. serratissima, and in crinoids in general, is to allow many individuals to take advantage of a preferred localized habitat. Among other things, type of substrate, salinity fluctuations, local current regime, and the presence of potential predators (Mladenov, 1980c) could conceivably make one localized habitat more suitable than another.

When the oral plates open in the cystidean of F. serratissima, 15 long tube feet are extended into the surrounding water. This is also the case for other crinoid species (Thomson, 1865; Seeliger, 1892; Mortensen, 1920a) with the exception of C. japonica where only 10 tube feet, the secondary radial tube feet, are extended at this time; the primary radial tube feet appear several weeks later in this species (Dan, 1968; Kubota, 1969). It appears, then, that in C. japonica, both gastrulation and the opening of the oral plates occur precociously.

The papillae on the pentacrinoid tube feet of F. serratissima are identical in external appearance to those of C. japonica (Holland and Kubota, 1975). Pentacrinoid papillae have not been studied with TEM, but Holland (1968) cites some of his unpublished TEM observations of papillae of adult F. serratissima which show that the filaments extending from the tip of each papilla are cilia whose basal bodies are in close association with nerve cell processes. The action of the papillae during food capture in both pentacrinoids and adult crinoids is not understood.

In the present study, the early development of the radial plates and of the IBr_1 and IBr_2 arm ossicles of the pentacrinoid of Florometra

serratissima was not observed. By good fortune, Mortensen's (1920a) brief observations on the development of this species were made on pentacrinoids in the early stages of arm formation, and two of his figures are reproduced here (Figs. 38A and 38B) in order to provide an uninterrupted account of development. In Fig. 38A the radial plates have just appeared while the IBr_2's are present as small plates midway on the primary radial tube feet. Mortensen also noted a single anal plate in a radial position beside one of the developing radial plates. The IBr_1's soon appear on the primary tube feet just below the IBr_2's. In the oldest stage observed by Mortensen (Fig. 38B), the radial plates are quite large and separate the basal plates from the oral plates, while the IBr_1's and IBr_2's have enlarged and enclosed the primary tube feet to create five small arms. The anal plate has been displaced by the growing radial plates and now lies in an interradial position between an oral and basal plate (not shown in Fig. 38B). An anal plate was not seen in the four- and six-month old pentacrinoids observed during this study, which suggests that it is resorbed during development.

The transition from a stalked sessile pentacrinoid to a free-living juvenile was not observed in this study. Observations on older pentacrinoids of a variety of species by Carpenter (1866), Mortensen (1920a) and Dan (1957) show that pinnules and cirri are present on the pentacrinoid before it becomes free-living. The cirri are a necessity since they would allow the juvenile to attach to the substrate. Carpenter's (1866, p. 736) observations on the pentacrinoid of Antedon bifida show that the juvenile detaches from the distal tip of the stalk and leaves the stalk behind. The time of separation from the stalk is

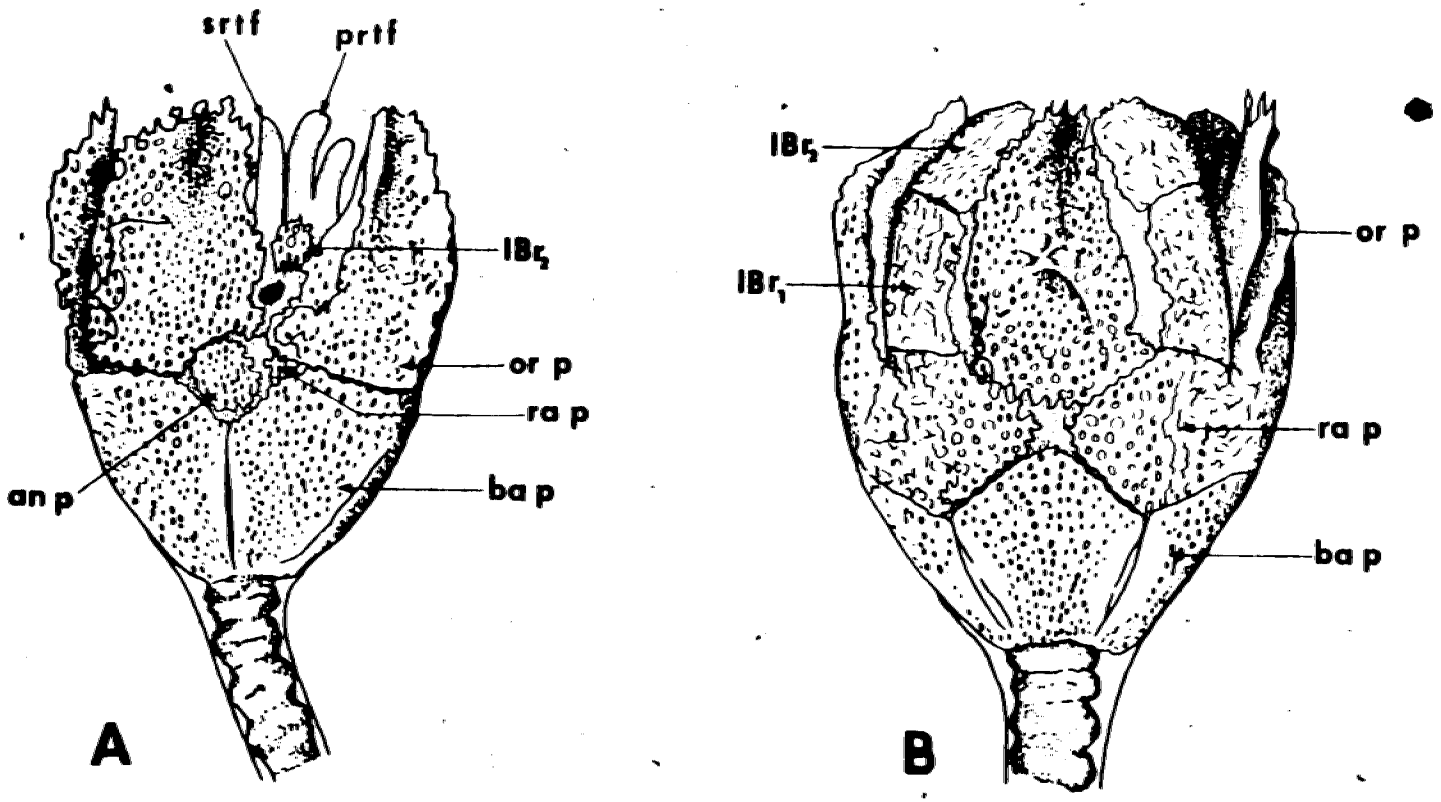


Fig. 38. Florometra serratissima. Early stages of arm formation. (A) A small spicule representing the developing radial plate has just appeared, while an IBr_2 plate is present on the primary radial tube foot; note that an anal plate is present in an interradial position beside a developing radial plate. (B) A later stage showing distinct arms consisting of an IBr_1 and an IBr_2; the radial plates have grown and now separate the basal plates from the oral plates. (After Mortensen, 1920, Plate 27, Figs. 3 and 5.)

variable, with some juveniles detaching at an earlier stage of development than others.

LITERATURE CITED

The periodicals have been abbreviated in accordance with "World list of scientific periodicals, 4th ed. London 1964" or, for more recent journals, "Bibliographic guide for editors and authors, Washington 1974".

Barrois, J.: Recherches sur le développement de la comatule. Recueil Zoologique Suisse 4, 545-651 + plates 25-30 (1888)

Bury, H.: The early stages in the development of Antedon rosacea. Phil. Trans. R. Soc. (B.) 179, 257-301 + plates 43-47 (1888)

Busch, W.: Ueber die Larve der Comatula. Arch. Anat. Physiol., p 400 (1849). (Unable to obtain this article; relying on information in Carpenter [1866].)

Carpenter, W.B.: Researches on the structure, physiology, and development of Antedon (Comatula, Lamk.) rosaceus. - Part 1. Phil. Trans. R. Soc. 156, 671-756 + plates 31-43 (1866)

-- On the structure, physiology and development of Antedon (Comatula, Lamk.) rosaceus. Proc. R. Soc. 24, 211-231 + plates 8 and 9 (1876)

Chadwick, H.C.: Antedon. L.M.B.C. Mem. typ. Br. mar. Pl. Anim. 15, 1-47 + 7 plates (1907)

Clark, A.H.: A monograph of the existing crinoids. Bull. U.S. natn. Mus. 82, vol. 1, pt. 2, 1-795 + 57 plates (1921)

-- and A.M. Clark: A monograph of the existing crinoids. Bull. U.S. natn. Mus. 82, vol. 1, pt. 5, 1-860 (1967)

Clark, A.M.: Echinodermata Crinoidea. Mar. Invertebr. Scand. 3, 1-55 (1970)

-- Starfishes and related echinoderms, 160 pp. British Museum (Natural History): T.F.H. Publications 1977

Crisp, D.J.: Territorial behaviour in barnacle settlement. J. exp. Biol. 38, 429-446 (1961)

Dan, J.C. and K. Dan: Early development of Comanthus japonicus. Jap. J. Zool. 9, 565-574 + plates 15-17 (1941)

Dan, K.: Echinoderma. In: Invertebrate embryology, pp 280-332. Ed. by M. Kumé and K. Dan. Belgrade: Prosveta Press 1968

Fell, H.B.: The direct development of a New Zealand ophiuroid. Q. Jl microsc. Sci. 82, 377-441 (1941)

Grave, C.: Embryology of Ophiocoma echinata, Agassiz. Preliminary note. Johns Hopkins Univ. Circ. 18, 6-7 (1898)

Holland, N.D.: The fine structure of the embryo during the gastrula stage of Comanthus japonica (Echinodermata: Crinoidea). Tissue and Cell 8, 491-510 (1976)

-- The shaping of the ornamented fertilization membrane of Comanthus japonica (Echinodermata: Crinoidea). Biol. Bull. mar. biol. Lab., Woods Hole 153, 299-311 (1977)

-- The fine structure of Comanthus japonica (Echinodermata: Crinoidea) from zygote through early gastrula. Tissue and Cell 10, 93-112 (1978)

-- and Å. Jespersen: The fine structure of the fertilization membrane of the feather star Comanthus japonica (Echinodermata: Crinoidea). Tissue and Cell 5, 209-214 (1973)

-- and H. Kubota: Correlated scanning and transmission electron microscopy of larvae of the feather star Comanthus japonica (Echinodermata: Crinoidea). Trans. Am. Microsc. Soc. 94, 58-70 (1975)

Hyman, L.H.: The invertebrates. Vol. 4. Echinodermata, 763 pp. New York: McGraw-Hill 1955

Kubota, H.: Development of Comanthus japonica I. From spawning to attachment (in Japanese). Jap. J. Develop. Biol. 23, 92-93 (1969)

-- Development of Comanthus japonica II. After setting (in Japanese). Jap. J. Develop. Biol. 24, 40-41 (1970)

Ludwig, H. and O. Hamann: Echinodermen (Stachelhäuter). V. Die Seelilien. Bronn's Kl. Ordn. Tierreichs 2, 1415-1602 + plates 9-13 (1907)

Mladenov, P.V.: Gonadal structure, oocyte development, ovulation and spawning in the feather star Florometra serratissima (Echinodermata: Crinoidea). In: Development and reproductive biology of the feather star Florometra serratissima (Echinodermata: Crinoidea). Ph.D. thesis, University of Alberta 1980a

-- Continuous reproduction in a population and in individuals of the feather star Florometra serratissima (Echinodermata: Crinoidea) at Barkley Sound, British Columbia. In: Development and reproductive biology of the feather star Florometra serratissima (Echinodermata: Crinoidea). Ph.D. thesis, University of Alberta 1980b

-- Aggregation, growth, regeneration and predation in the feather star Florometra serratissima (Echinodermata: Crinoidea). In: Development and reproductive biology of the feather star Florometra serratissima (Echinodermata: Crinoidea). Ph.D. thesis, University of Alberta 1980c

Mortensen, T.: Studies in the development of crinoids. Pap. Tortugas Lab. 16, 1-94 + plates 1-28 (1920a)

-- Notes on the development and the larval forms of some Scandinavian echinoderms. Vidensk. Meddr dansk naturh. Foren. 71, 133-160 (1920b)

-- Contributions to the study of the development and larval forms of echinoderms III. K. danske Vidensk. Selsk. Skr. (Naturv. Math. Afd.), Series 9, Vol. 7, No. 1, 1-65 + plates 1-15 (1937)

-- Contributions to the study of the development and larval forms of echinoderms IV. K. danske Vidensk. Selsk. Skr. (Naturv. Math. Afd.), Series 9, Vol. 7, No. 3, 1-59 + plates 1-12 (1938).

Pantin, C.F.A.: Notes on microscopical technique for zoologists, 77 pp. Cambridge: University Press 1960

Perrier, E.: Mémoire sur l'organisation et le développement de la Comatule de la Mediterranée (Antedon rosacea Linck). Nouv. Archs Mus. Hist. nat., Paris 9, 53-348 + plates 11-20 (1886)

Provasoli, L., J.J.A. McLaughlin and M.R. Droop: The development of artificial media for marine algae. Arch. Mikrobiol. 25, 392-428 (1957)

Seeliger, O.: Studien zur Entwicklungsgeschichte der Crinoiden. (Antedon rosacea). Zool. Jb. Abtheilung für Anatomie und Ontogenie der Thiere 6, 161-444 + plates 12-22 (1892)

Strathmann, R.: The spread of sibling larvae of sedentary marine invertebrates. Am. Nat. 108, 29-44 (1974)

Thompson, J.V.: Memoir on the Pentacrinus europaeus: a recent species discovered in the Cove of Cork, July 1, 1823, 1 p + plates 1-2. 1827. (Unable to obtain this article; relying on information in Carpenter [1866].)

-- Memoir on the star-fish of the genus Comatula, demonstrative of the Pentacrinus europaeus being the young of our indigenous species. Edinburgh New Philosophical Journal 20, 295-300 + 1 plate (1836)

Thomson, W.: On the embryogeny of Antedon rosaceus, Linck (Comatula rosacea of Lamarck). Phil. Trans. R. Soc. 155, pt. 2, 513-545 + plates 23-27 (1865)

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

Young, C.M. and L.F. Braithwaite: Larval behaviour and post-settling morphology in the ascidian, Chelyosoma productum Stimpson. J. Exp. Mar. Biol. Ecol. 42, 157-169 (1980)

Aggregation, Growth, Regeneration and Predation in the Feather Star *Florometra serratissima* (Echinodermata: Crinoidea)

ABSTRACT

It was determined, using S.C.U.B.A., that a population of the feather star Florometra serratissima (A.H. Clark) at the mouth of Bamfield Inlet, Barkley Sound, British Columbia (48° 50' 07" N, 125° 08' 09 W) covers an area of the bottom of roughly 2000 m^2 at a mean density of 1.6/m^2. This localized population, better termed an aggregation, is characterized by a lack of small specimens. The distribution of individuals within this aggregation is contagious.

The growth rate of F. serratissima increases exponentially through the pentacrinoid, juvenile, and young adult stages of development, and then decreases considerably in adults. An individual reaches maximum size in about eight years; it is quite likely that growth is determinate in this species. Individuals become sexually mature at about four years of age.

Just under 80% of the feather stars in the aggregation have at least one regenerating arm, while 18% have five or more regenerating arms. Observations made in the field and on specimens maintained in aquaria suggest that arm loss followed by regeneration in F. serratissima could be due largely to non-fatal attacks by the sea star Pycnopodia helianthoides and the crab Oregonia gracilis. There is some evidence that the rate of regeneration per arm in F. serratissima decreases slightly as the total number of regenerating arms on an individual increases. This is contrary to an earlier proposal concerning rate of crinoid arm regeneration.

INTRODUCTION

Observations made by numerous collectors have shown that in some areas of the world crinoids are found in localized, sometimes very dense, populations (A.H. Clark, 1937; Hyman, 1955; Fell, 1966). The distribution of the 10-armed comatulid crinoid Florometra serratissima (A.H. Clark) in the Barkley Sound region of British Columbia follows such a pattern. In the first part of this paper, the structure of a localized population of this species is described.

Accurate information on rate of growth, age at sexual maturity, and life span of crinoids is not available. These topics were not treated by Hyman (1955), and both Fell (1966) and Swan (1966) emphasized our lack of knowledge concerning them. The following account presents quantitative data on the growth of F. serratissima from the pentacrinoid stage to the full-grown adult; in addition, age at sexual maturity is determined.

Both stalked and unstalked crinoids have the ability to regenerate lost body parts including pinnules, arms, cirri, the tegmen, and the visceral mass (Perrier, 1873; Dendy, 1886; Przibram, 1901, Minckert, 1905). Extant crinoids with regenerating arms are frequently collected, while arm regeneration is occasionally noted in fossil stalked crinoids (Strimple and Beane, 1966). Observations by Minckert (1905) and Reichensperger (1912) indicate that most regenerating arms result from breakage at a syzygy which is a rigid non-muscular union between certain of the arm ossicles (Hyman, 1955). The cause of arm loss in nature is not precisely understood. It might result from wave action, entanglement, or disturbance by other animals (Perrier, 1873), in which case

arm loss may involve simple breakage at points of weakness and not true autotomy; however, arm loss can also occur during exposure to intense sunlight, high temperatures, or lack of oxygen (Hyman, 1955), indicating that mechanical action is not always necessary. A.H. Clark (1921, pp. 119 and 411) suggested that in multibrachiate crinoids (species showing repeated forking of the arms) the original 10 arms are shed at their bases as a result of natural growth changes, thereby leading to the proliferation of two new arms from each stump; if such is the case, then this would be an example of true autotomy.

The histology of crinoid arm regeneration was described by both Perrier (1873) and Reichensperger (1912). Very little information is available on the rate of arm regeneration in crinoids. Casual observations by Perrier (1873) on Antedon bifida led him to suggest that a complete arm could be regenerated in 8 to 10 weeks. Reichensperger (1912) believed that the rate of arm regeneration in individuals of A. mediterranea was directly proportional to the extent of the injury. A regenerating arm with a short stump thus grew more rapidly than one with a long stump; similarly, the more arms removed, the faster the rate of regeneration per arm. During this study, the proportion of individuals in the population of F. serratissima with regenerating arms was quantified. Furthermore, the relationship between the rate of arm regeneration and the extent of arm damage in individual F. serratissima was examined.

Adult crinoids are generally considered to be immune to predation by fishes or invertebrates (A.H. Clark, 1921, p. 687; Mortensen, 1927). This account presents observations suggesting that arm loss followed by regeneration in F. serratissima may be largely attributed to attacks by

MATERIALS AND METHODS

Area of Investigation

This study was carried out on a population of Florometra serratissima (A.H. Clark) located at the mouth of Bamfield Inlet in Barkley Sound, British Columbia (48° 50' 07" N, 125° 08' 09" W) in waters adjacent to the Bamfield Marine Station. The boundaries of the population were established through several surveys conducted with S.C.U.B.A. During such dives, floats were released to the surface from various points along the periphery of the population; the areal extent of the population could then be determined at the surface using the floats along with landmarks present on the shores of Bamfield Inlet.

The density of F. serratissima was estimated by divers in July 1979 in the following manner. At four separate locations within the population a rope transect was laid along the bottom perpendicular to shore from the middle of Bamfield Inlet into shallow water. The number of feather stars visible to a diver within each of a series of 2 m x 2 m contiguous quadrats placed along the length of each transect was then counted.

Growth

The post-cystidean development of F. serratissima was divided into pentacrinoid, juvenile, young adult and adult stages, and the growth rate within each stage was estimated by monitoring changes in the length of the longest arm, L, in individual animals over a period of months. L is a measure of the maximum size of the feather star provided that it shows no signs of regeneration. Other measures of body size such as arm span, total length of all 10 arms, or wet weight

are inconsistent due to variation in the number of regenerating arms between individuals. The diameter of the calyx across the tegmen is also unsuitable as a measure of body size because the calyx rapidly changes size and shape due to muscular contractions and arm movements.

Pentacrinoids were reared in a tray which was placed on the bottom of Bamfield Inlet at a depth of 25 m. Information on larval culturing methods and on the construction of the rearing tray is presented in Mladenov (1980a). Four juvenile specimens of F. serratissima were maintained in a galvanized steel minnow trap secured to the bottom by a 10 kg weight; each juvenile could be distinguished on the basis of size and minor colour variations. One young adult and four adult specimens were held in separate compartments in cages constructed of PVC tubing and covered with nylon netting of 2.5 cm mesh size. Details of the construction of these cages are found in Mladenov (1980a). The rearing tray, the minnow trap, and the holding cages were all placed in the centre of the aggregation of F. serratissima. The pentacrinoids and caged individuals were thus exposed to environmental conditions equivalent to that of uncaged specimens.

The caged individuals were carefully collected with S.C.U.B.A. at intervals of several months and returned to the laboratory in plastic bags. Each specimen was placed, in turn, in a large, shallow (60 cm x 50 cm x 12 cm) tray filled with about 5 cm of seawater. The bottom of this tray was marked with a series of concentric circles radiating from the centre point outwards at 5 mm intervals. The specimen was positioned with the centro-dorsal plate of the calyx at the centre point of the tray; the shallowness of the water forced all 10 arms of the feather star to be held in a horizontal position. The length of

the longest arm, L, from the centre of the centro-dorsal plate to the arm tip, could then be measured quickly and accurately (± 3 mm) when the specimen was viewed from above. The specimens were returned to their cages using S.C.U.B.A. on the day following retrieval. The measurements of an individual ceased when the longest arm became badly damaged. The arms on an individual were distinguished using a modified version of the crinoid orientation proposed by Carpenter (1884, p. 89). With the ventral surface of the specimen upwards, and the anal cone nearest the observer, the left arm of the arm pair opposite the anal cone was designated "A", and the right arm of this pair designated "a"; proceeding in a clockwise direction, each arm of the remaining four arm pairs was designated "B", "b", "C", "c", "D", "d" and "E", "e" (fig. 39). In this way, the particular arm that was being measured on an individual could be identified with confidence from one collection date to the next.

Regeneration

About 20 large (L greater than 180 mm) specimens of F. serratissima were collected on a roughly monthly basis for 15 months, and the number of regenerating arms on each animal noted. An arm was considered to be regenerating even if the break had occurred close to the arm tip. Broken arms lacking any evidence of a new arm rudiment were not counted as regenerating since these were likely broken during collection.

Eighteen specimens with an L of 200 mm (± 10 mm) were collected for the purpose of estimating arm regeneration rates. From each of eight of the specimens, one arm was amputated near its base leaving a stump about 10 mm long; similarly, two arms were amputated from each of two specimens, three arms from each of two specimens, and five arms

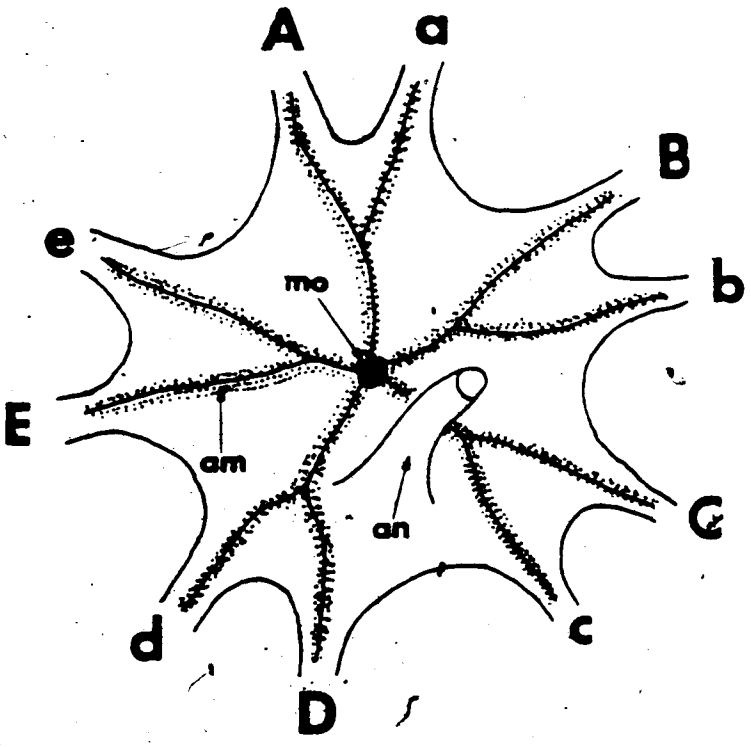


Fig. 39. Florometra serratissima. Diagrammatic illustration of the tegmen and the basal portion of all 10 arms showing the method of arm designation. am: Ambulacral groove; an: anal cone; mo: mouth. (Modified from Carpenter, 1884.)

from one specimen; finally, one arm was amputated midway along its length from each of five specimens. These feather stars were put in cages placed on the bottom of Bamfield Inlet within the aggregation and they were retrieved using S.C.U.B.A. at roughly bimonthly intervals. In the laboratory, the length of the regenerated portion of each arm was determined in the measuring tray, sometimes with the aid of calipers. In those specimens in which more than one arm was amputated, each regenerating arm was distinguished using the previously described orientation. The animals were returned to the cages on the day after their collection. The measurement of a regenerating arm ceased when it became broken, usually as a result of handling during retrieval.

Predation

A few observations of predation on F. serratissima were made during the course of S.C.U.B.A. dives carried out in the study area. In addition, interactions between F. serratissima and possible predators were observed in aquaria.

RESULTS

Density and Overall Size of Population

The boundaries of the F. serratissima population at the mouth of Bamfield Inlet are shown in Fig. 40. Scattered feather stars can be found just outside of these boundaries at very low densities, but as one proceeds a short distance further the feather stars disappear altogether. Individuals of F. serratissima first become abundant at a depth of 17 m (all depths reduced to Lower Normal Tide which is 2.2 m below MWL at Bamfield) and they continue in abundance to the deepest part of the inlet which is 34 m and which is situated roughly in the centre of the population. The bottom of Bamfield Inlet, within the boundaries shown in Fig. 40, is mud throughout, and it slopes gently (10^{o}) to the deepest point. The feather stars are found attached by means of the cirri to any available solid substrate in the area including worm tubes (Phyllochaetopterus prolifica), empty bivalve shells, detached algal fronds, pebbles, and a 30 cm diameter intake pipe for the Bamfield Marine Station which runs along the bottom through the area. The overall extent of the population does not appear to be limited by the presence of suitable solid substrate since such substrate is abundant both within and outside the boundaries of the population as shown in Fig. 40.

The location of the transects is shown in Fig. 40, while the results of the quadrat counts are presented in Table 7A. The mean density of F. serratissima within the boundaries of the population is 6.4/4 m^2. Observations made while S.C.U.B.A. diving suggest that the density of the population is not limited by solid substrate. It can be estimated from Fig. 40, using a planimeter, that the F. serratissima population covers

Fig. 40. Florometra serratissima. Aerial photograph (BC7707:056 from Surveys and Mapping Branch, Ministry of Environment, British Columbia) of the mouth of Bamfield Inlet and adjacent areas showing the outer boundaries (white polygon) of the population and the location of the transects (numbered lines)

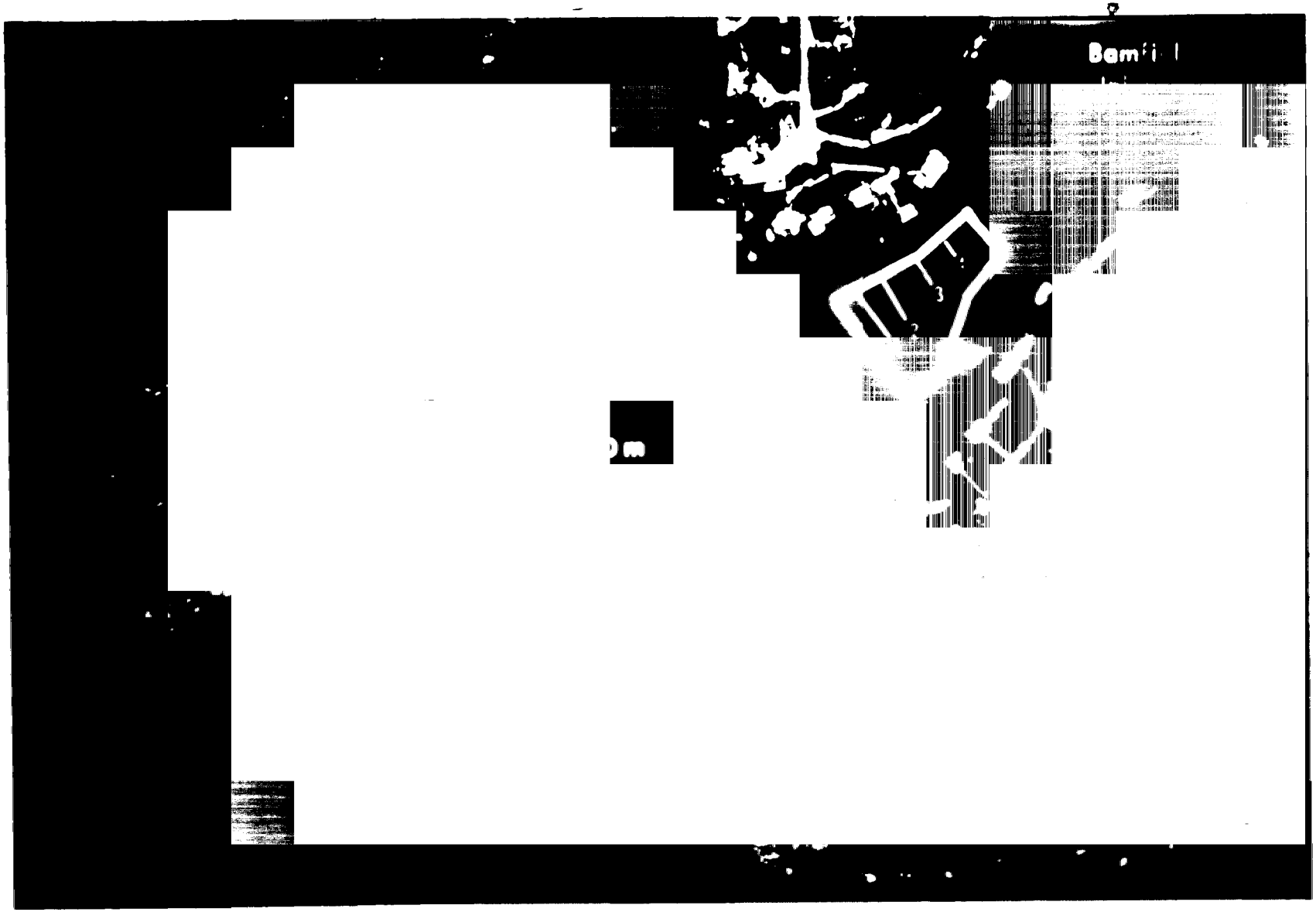


Table 7A. Florometra serratissima. Number of individuals per $4m^2$ contiguous quadrats. Counts are listed in sequence from deep to shallow water. See Fig. 40 for location of transects. $\bar{x} = 6.37$, $s^2 = 7.91$, n=68.

Transect #1:	9, 8, 8, 10, 10, 4, 8, 7, 9, 5, 8, 7, 7, 6, 3, 3, 10, 4, 8, 7, 9, 4, 6, 9, 7.
Transect #2:	11, 8, 7, 3, 3, 8, 3, 8, 4, 2, 4, 11, 1, 10, 3, 9, 4, 7, 11, 9.
Transect #3:	4, 10, 7, 4, 4, 2, 2, 3, 4, 7, 3, 9, 7, 9, 9.
Transect #4:	10, 4, 4, 3, 2, 5, 10, 9.

Table 7B. Florometra serratissima. χ^2 test for goodness-of-fit of a Poisson distribution to data presented in Table 7A. The total x^2 value = 29.39. The actual frequency distribution departs significantly from random ($P < 0.005$).

x	Obs.	Exp.	Obs.-Exp.	χ^2
0 - 2	5	3.22	1.78	0.98
3	9	5.02	3.98	3.16
4	12	7.99	4.01	2.01
5	2	10.18	-8.18	6.57
6	2	10.80	-8.80	7.17
7	10	9.83	0.17	0.00
8	8	7.82	0.18	0.00
9	10	5.53	4.47	3.61
10	7	3.52	3.48	3.44
11	3	2.04	0.96	0.45
12 or more	0	2.00	-2.00	2.00
Total	68	67.95		29.39

an area of the bottom of roughly 8000 m^2. The total number of feather stars in the population is thus about 12,800.

As shown in Table 7A, the variance of the quadrat counts is greater than the mean. A contagious distribution of the feather stars was thus suspected (Elliott, 1977). A χ^2 test for goodness-of-fit of a Poisson distribution to the sample data (Table 7B) confirms that the actual frequency distribution departs significantly from random ($P < 0.005$). This is strong indication that the dispersion of the feather star population is indeed contagious.

An accurate estimate of the size distribution of the population was not accomplished during this study. On a single occasion however (July 15, 1979) the arm length of all individuals found within twelve $4m^2$ quadrats placed haphazardly on the bottom was measured with a meter stick (Fig. 41). These data indicate that the great majority of feather stars have a longest arm greater than 120 mm in length and that small individuals are notably few. Qualitative observations made throughout the year while S.C.U.B.A. diving suggest that small individuals were scarce at all times of the year. Only two very small specimens were encountered over the entire course of this study (November 1977 to December 1979), and they both had a longest arm measuring 35 mm in length.

Growth

Growth data for one pantacrinoid, four juveniles, one young adult, and four adults are presented in Fig. 42. The growth of the pentacrinoid was slow, but growth become more rapid in the juveniles and reached a maximum in the young adult; the growth of adults was comparatively slow. There was little, if any, indication of a seasonal change in the growth

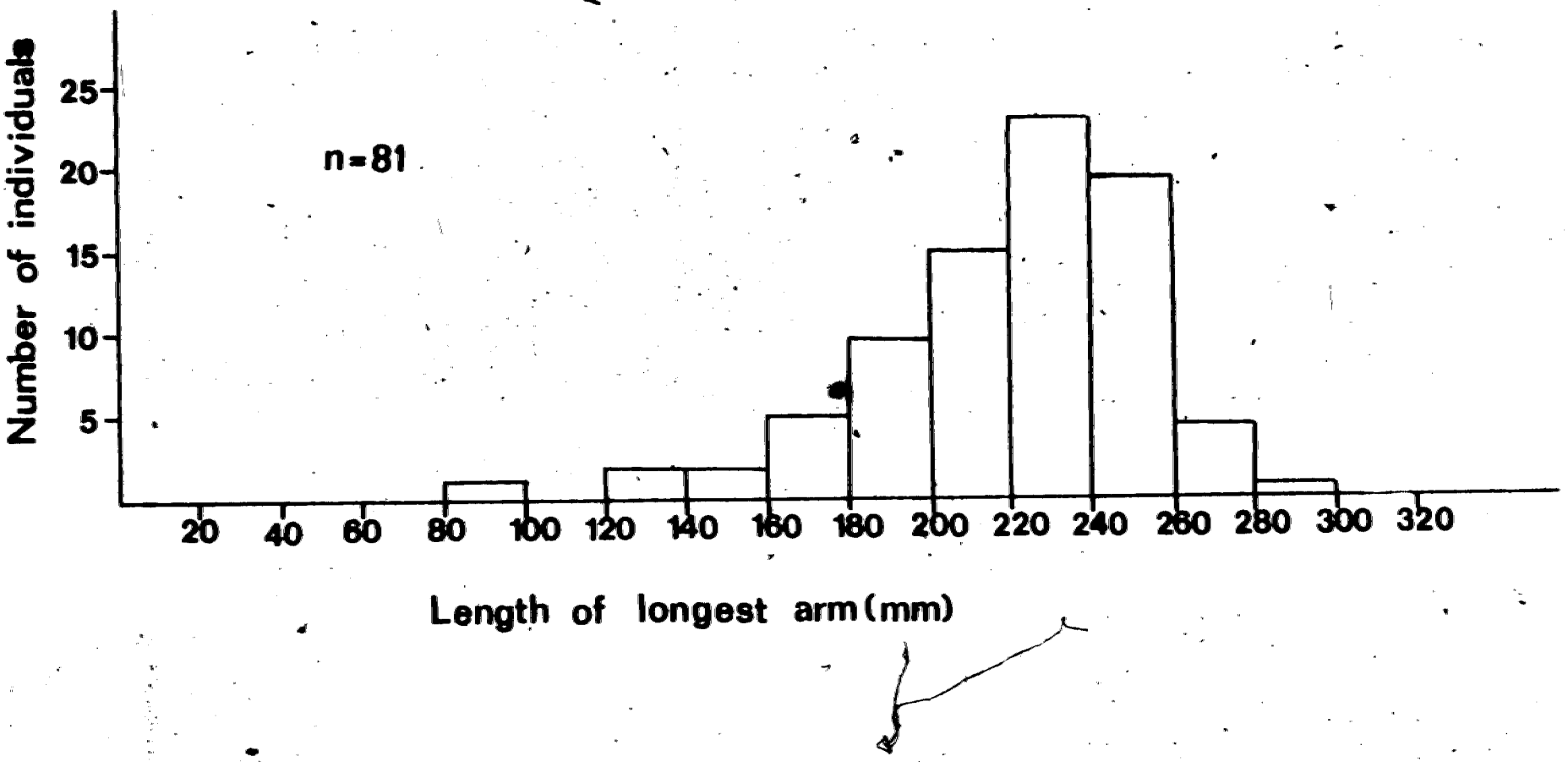


Fig. 41. Florometra serratissima. Size-frequency distribution of all individuals found within twelve $4m^2$ quadrats sampled on July 15, 1979

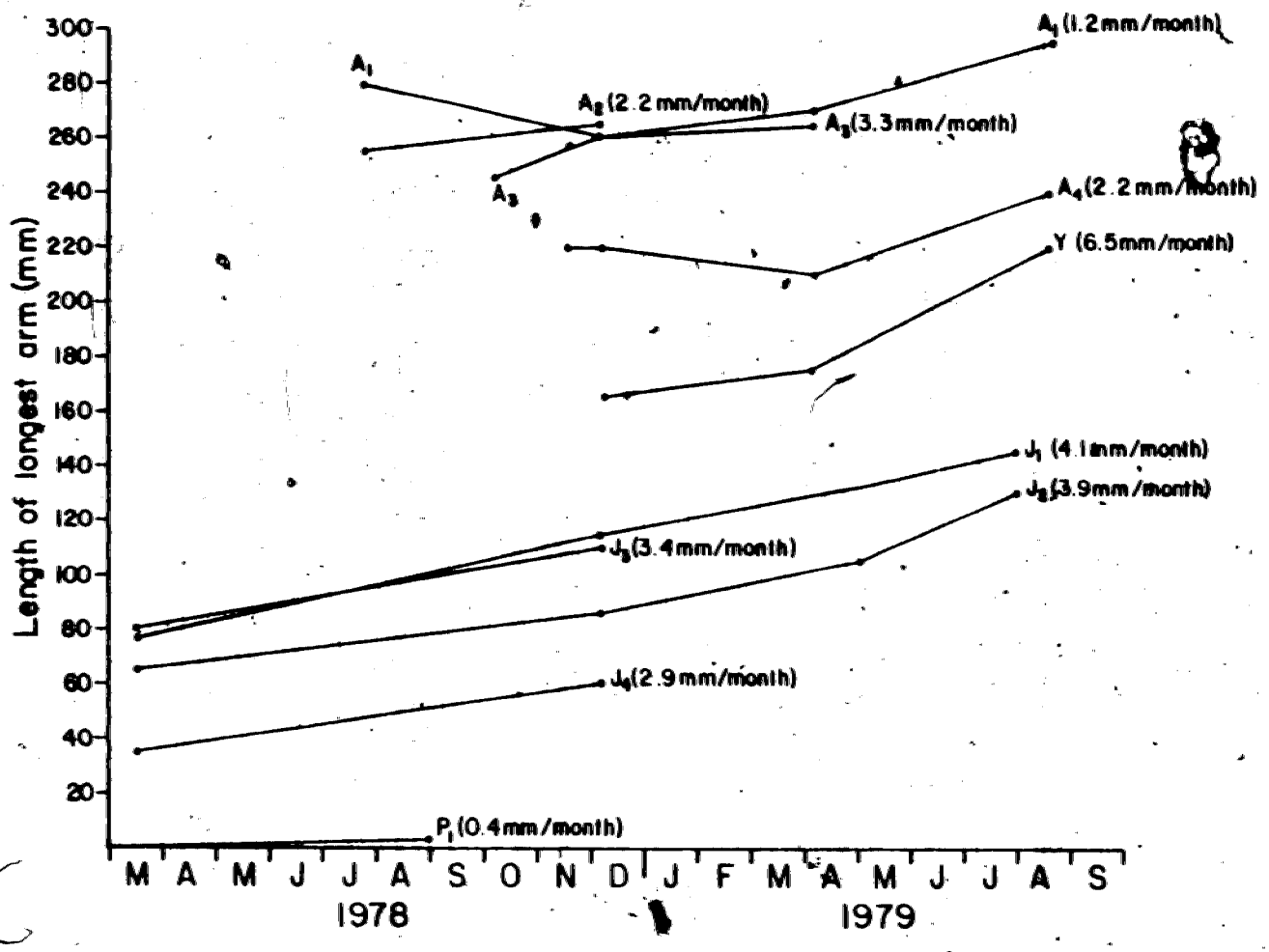


Fig. 42. Florometra serratissima. Growth data for pentacrinoid (P), juvenile (J), young adult (Y) and adult (A) stages as determined from successive measurement of the length of the longest arm, L

rates of the two juveniles (J_1 and J_2) measured on several occasions during the period from March 1978 to August 1979. There was, however, some evidence of faster growth in the spring, summer and fall in the young adult (Y) and in three of the adults (A_1, A_3 and A_4). The average of the available growth data for each developmental stage is given in Table 8. Since the growth of most individuals was followed for a considerable portion of a year, and in some cases for over a year, it is believed that the growth rates listed in Table 8 represent averages which take into account possible seasonal variations in growth. Also given in Table 8 is the assigned range of the longest arm, L, for each growth stage. For the sake of continuity between stages, the lower limit of the juvenile stage was extrapolated to L = 31 mm, although the initial size of the smallest caged juvenile was L = 35 mm. Similarly, the lower limit of the young adult stage was put at L = 146 mm although the initial size of the caged young adult was L = 165 mm. Specimens with L between 2.6 mm and 30 mm could not be cultured, and were not found in the field during S.C.U.B.A. dives. For these reasons, growth data on this stage, termed the late pentacrinoid, are lacking. Since the available data (Fig. 42 and Table 8) show that growth increased steadily from the early pentacrinoid through the juvenile and young adult stages, the growth rate of the late pentacrinoid stage can be reasonably estimated as the average of the growth rates of the preceding and following stages (Table 8). A growth curve for F. serratissima from the early pentacrinoid stage to the large adult can then be constructed using all the data (Fig. 43). This curve has a typical sigmoid shape characterized by exponential growth during the pentacrinoid, juvenile, and young adult

Table 8. Florometra serratissima. Growth Analysis

Stage	Length of longest arm, L (mm)	Average growth rate (mm/month)	Time (months)
Pentacrinoid (P_1)	0 - 2.5	0.4	6
Late Pentacrinoid (P_2)	2.6 - 30	2[1]	14
Juvenile (J)	31 - 145	3.6	32
Young adult (Y)	146 - 220	6.5	11
Adult (A)	221 - 300	2.2	36

[1] Estimated as an average of the growth rates of P and J

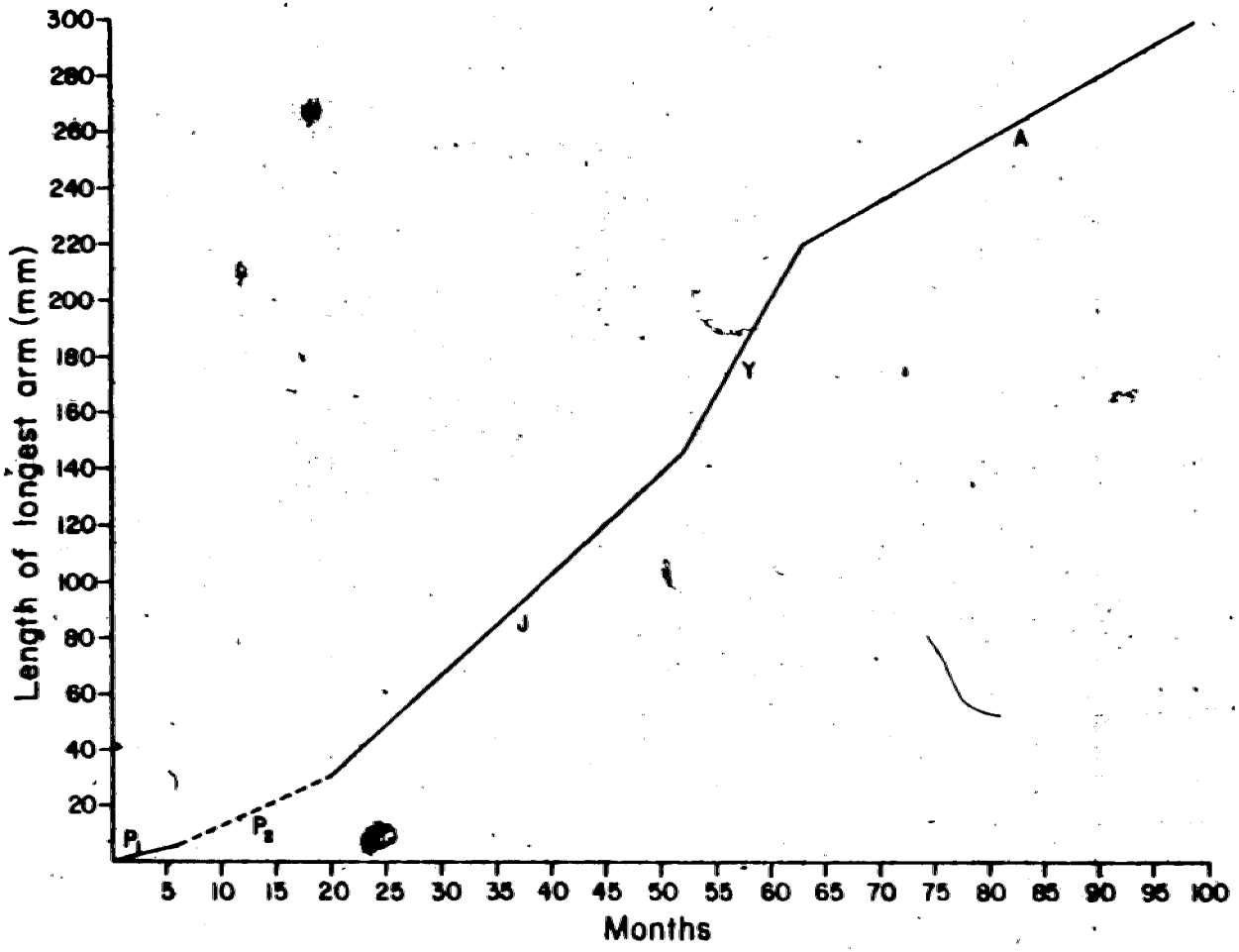


Fig. 43. Florometra serratissima. Growth curve constructed using data from Table 7. The dotted line represents an estimated growth rate. P_1: Pentacrinoid stage; P_2: late pentacrinoid stage; J: juvenile stage; Y: young adult stage; A: adult stage

stages and by a slowing of growth in the adult stage. The relationship between L and the age of a specimen, M, in months can be approximated by $L = 9.84e^{.05M}$ for the exponential growth phase. According to Fig. 42, an individual would reach L = 300 mm, the size of the largest specimens encountered, 8.25 years from the beginning of the pentacrinoid stage. As shown in Mladenov (1980a) the pentacrinoid stage is reached between 21 days and 30 days after fertilization.

The foregoing analysis of growth in F. serratissima can be better evaluated if the available growth data of Fig. 42 are used in an alternative method described by Yamaguchi (1977). During the exponential growth phase of an animal, the time span between any two points on a regression of growth increments on initial sizes can be calculated using the following equation:

$$t = \frac{1}{\ln(1+A)} \ln \frac{S_t + C/A}{S_o + C/A}$$

where t is the time span, S_o and S_t are the size at time 0 and time t respectively, and C and A are the y-intercept and the slope, respectively, of the regression line. Initial size and growth-increment data for the pentacrinoid, juveniles and young adults were extracted from Fig. 42 and plotted in Fig. 44 along with the linear regression. Using the above equation, the time span from L = 0 mm to L = 165 mm is 53.4 months, which is in close agreement with a time span of 55 months for the same size difference predicted using the growth curve of Fig. 43. This suggests that the division of growth in F. serratissima into the five stages listed in Table 8 is a realistic assessment of the growth pattern of this species.

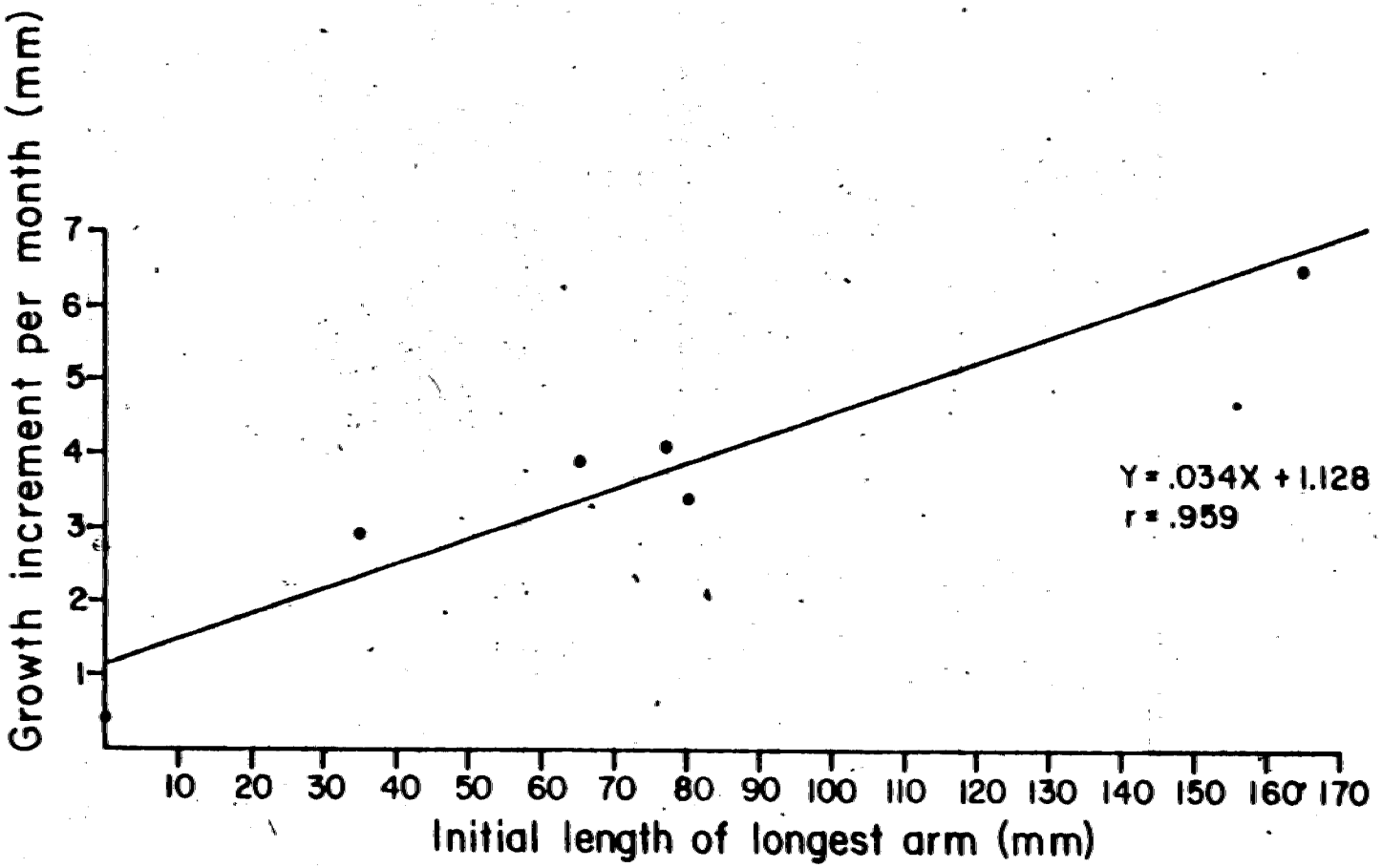


Fig. 44. Florometra serratissima. Growth-increment data for pentacrinoid, juvenile, and young adult stages. The regression line is significant ($P < 0.01$)

Genital pinnules were well developed on two caged juveniles (J_1 and J_2 in Fig. 42) by L of 145 mm and 130 mm respectively. Microscopic observations of the pinnules of uncaged juveniles collected with S.C.U.B.A. indicate that germinal cells first appear at an L of approximately 80 mm, and that by an L of 130 mm tailed sperm and oocytes with a diameter greater than 180 µm are present in the genital pinnules. Individuals of F. serratissima thus become sexually mature during the exponential growth phase at about four years of age.

Regeneration

Florometra serratissima is not a particularly fragile animal, and the arms will remain intact if a specimen is handled gently; if an arm is grasped, however, it immediately breaks off near the base. Specimens kept in a tank with running seawater for periods of longer than a month began gradually to lose their arms until eventually only the calyx and 10 arm stumps remained.

A regenerating arm of F. serratissima can be easily distinguished from the arm stump and adjacent normal arms because it is lighter in colour and more delicate in structure. Individuals with regenerating arms were commonly encountered: just under 80% of the 261 specimens examined had at least one regenerating arm; the number of individuals with numerous regenerating arms was surprisingly high, with 18% having five or more regenerating arms (Fig. 45). The point of breakage of the regenerating arms was not assessed quantitatively, but casual observations showed that most arms had been broken at a syzygy.

The relationship between the average length of the regenerated arm

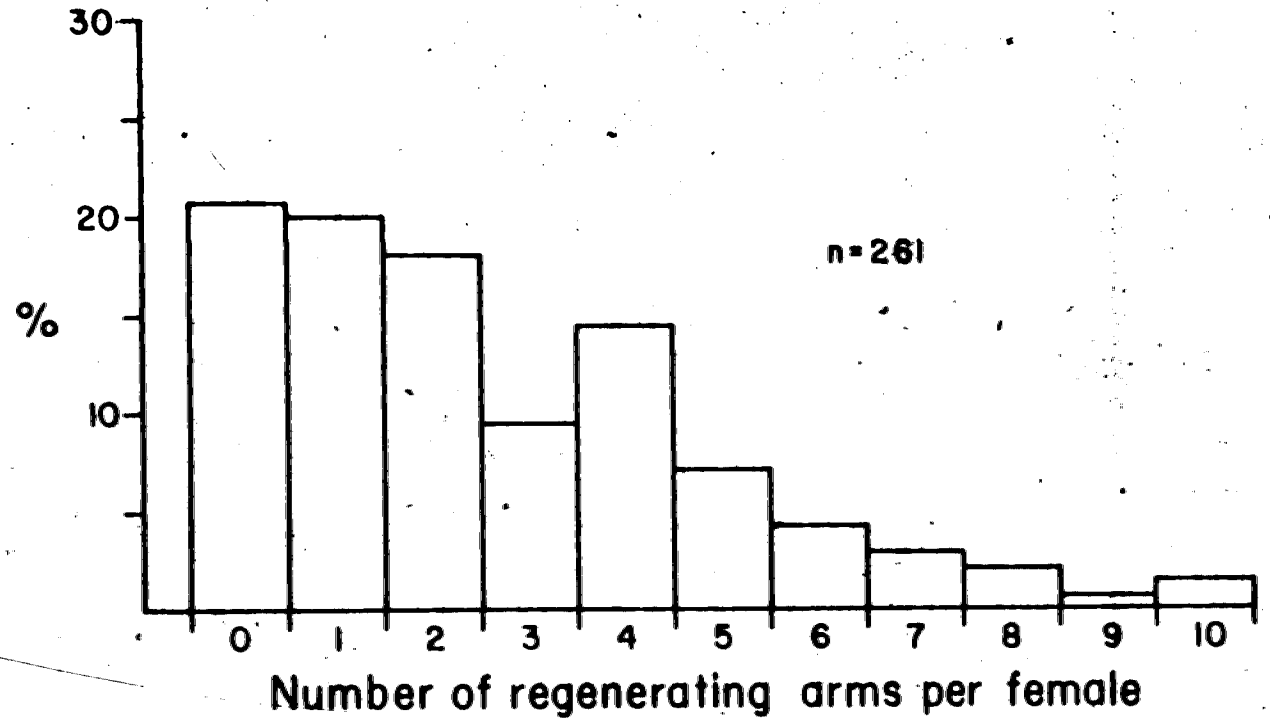


Fig. 45. Florometra serratissima. Distribution of individuals with regenerating arms

portion and the time after amputation for individuals with one, two, three, and five arms amputated near their bases is shown in Figs. 46 and 47. In all cases, after a short recovery period following amputation, the average length of regenerating arm increased at a constant rate, thus allowing a linear regression to be fitted to each set of data. Note that the slope of each regression line, which is equivalent to the rate of regeneration per arm in mm per day, decreases slightly as additional arms are removed. This suggests that the rate of regeneration per arm decreases slightly as the number of regenerating arms on an individual increases. This trend was tested by plotting a regression of rate of arm regeneration on number of arms amputated (Fig. 48). The regression line is significant ($P < 0.05$) suggesting that some correlation is present. In addition, the 95% confidence interval for the slope, β, of the regression line is $-.068 < \beta < -.006$ (Walpole, 1974, p.248), which gives some indication that the slope is indeed negative. There is thus some evidence that the rate of arm regeneration decreases slightly as the number of regenerating arms per individual increases. This trend should be validated by the collection, if possible, of additional data.

The relationship between the average length of regenerated arm and the time after amputation for individuals with one arm amputated midway is shown in Fig. 49. The average rate of regeneration of such arms is roughly equivalent to the average rate of regeneration of a single arm amputated at the base (upper graph of Fig. 46). The difference between the regression slopes of Fig. 49 and upper graph of Fig. 46 is not significant ($p > 0.05$) as tested with an F-test (Sokal and Rohlf, 1973, p.251). This suggests that the rate of arm regeneration is not greatly affected

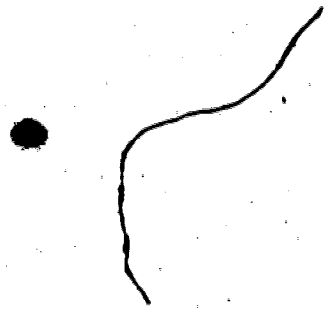
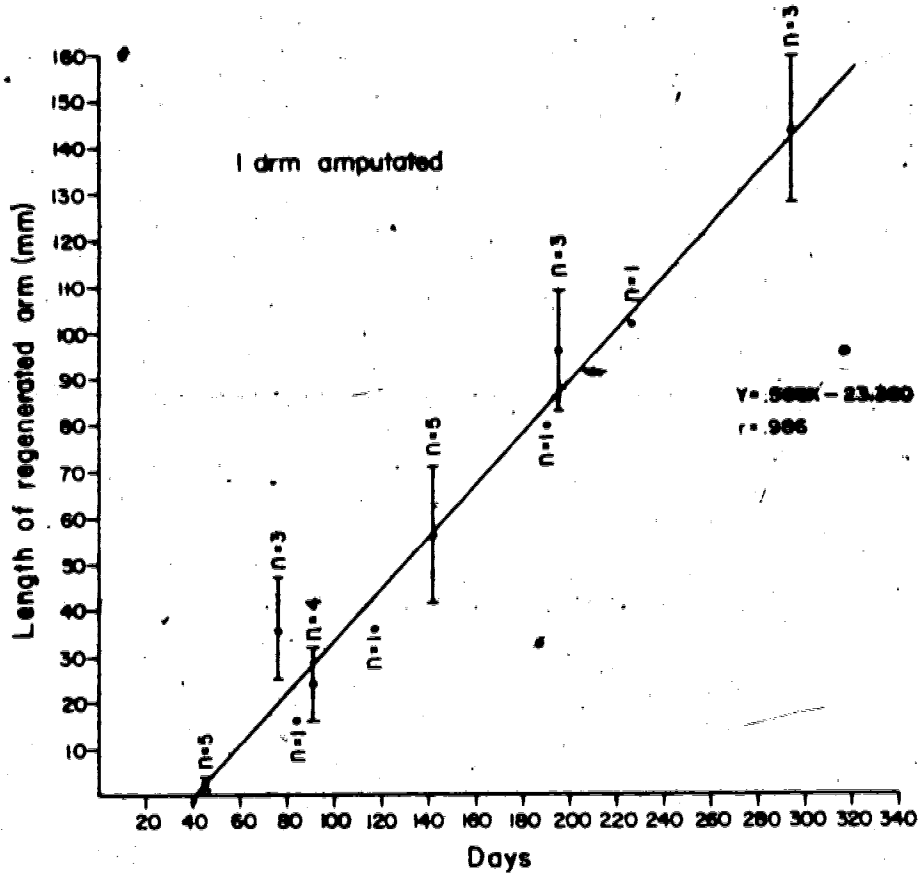


Fig. 46. Florometra serratissima. Relationship between length of regenerated arm and time after amputation for individuals with one and two arms removed. Vertical bars show one S.D. on each side of the means; the number of specimens measured is shown above each point. The regression lines are significant ($P < 0.01$)


1 arm amputated
Length of regenerated arm (mm)
Days
n=5
n=3
n=1
n=4
n=1
n=5
n=1
n=3
n=1
n=3
Y=.588X−23.360
r=.986

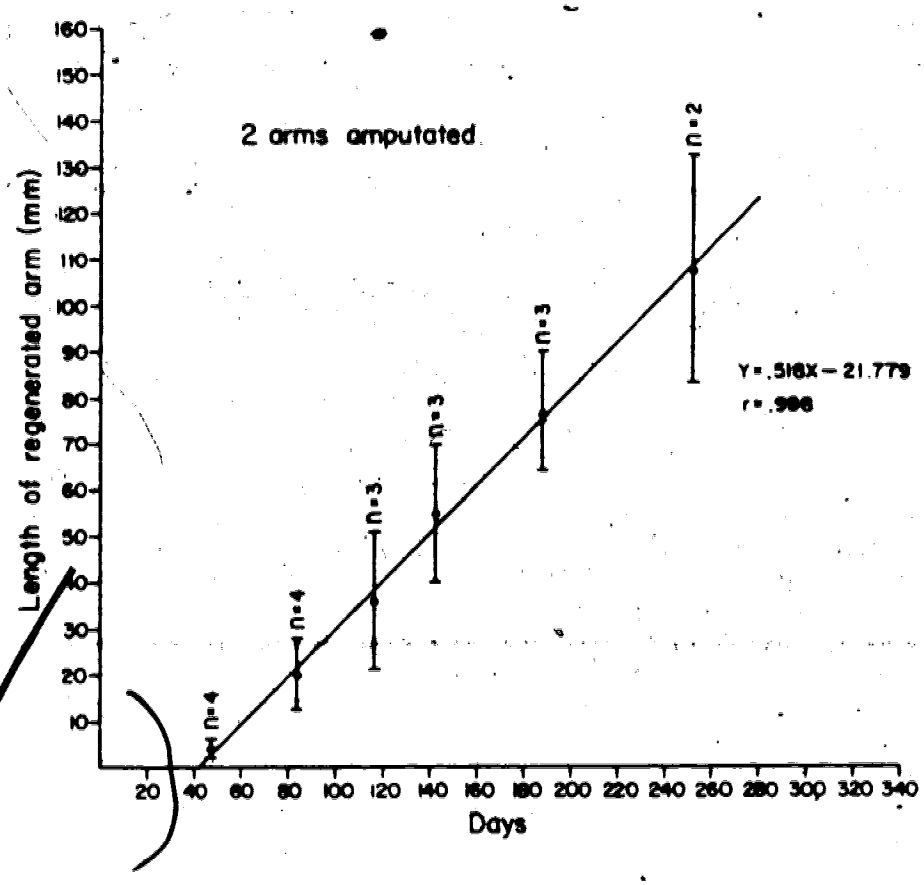
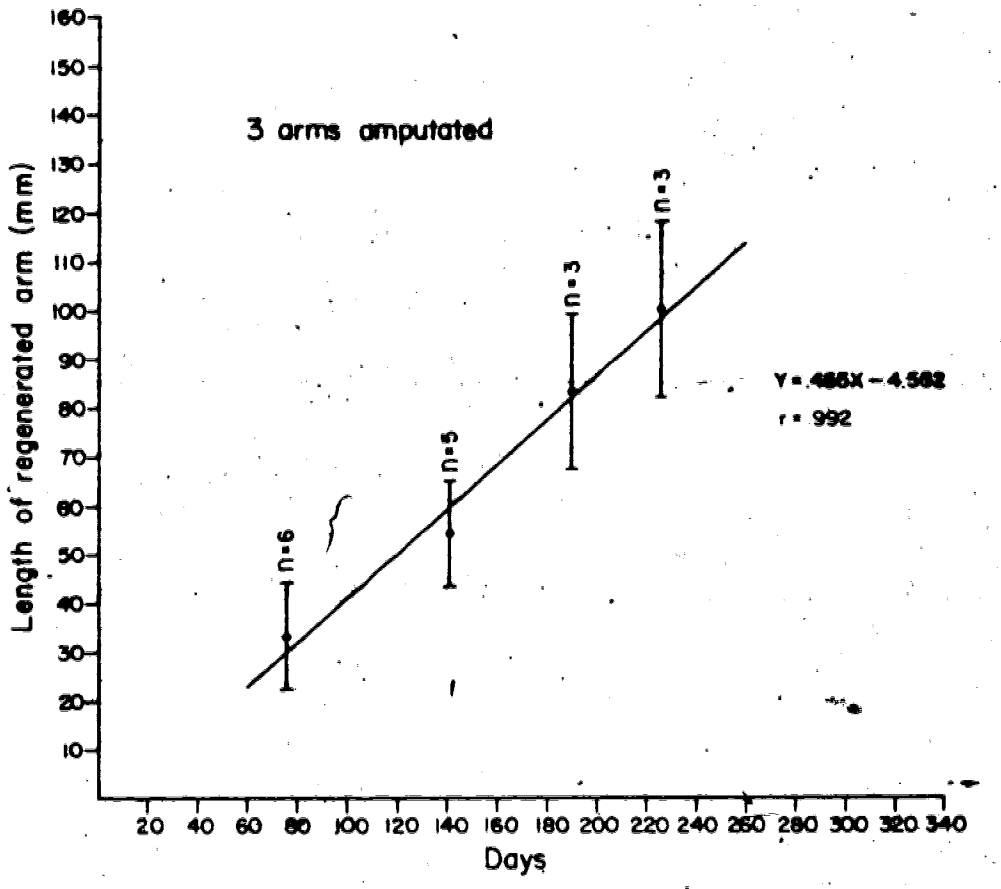
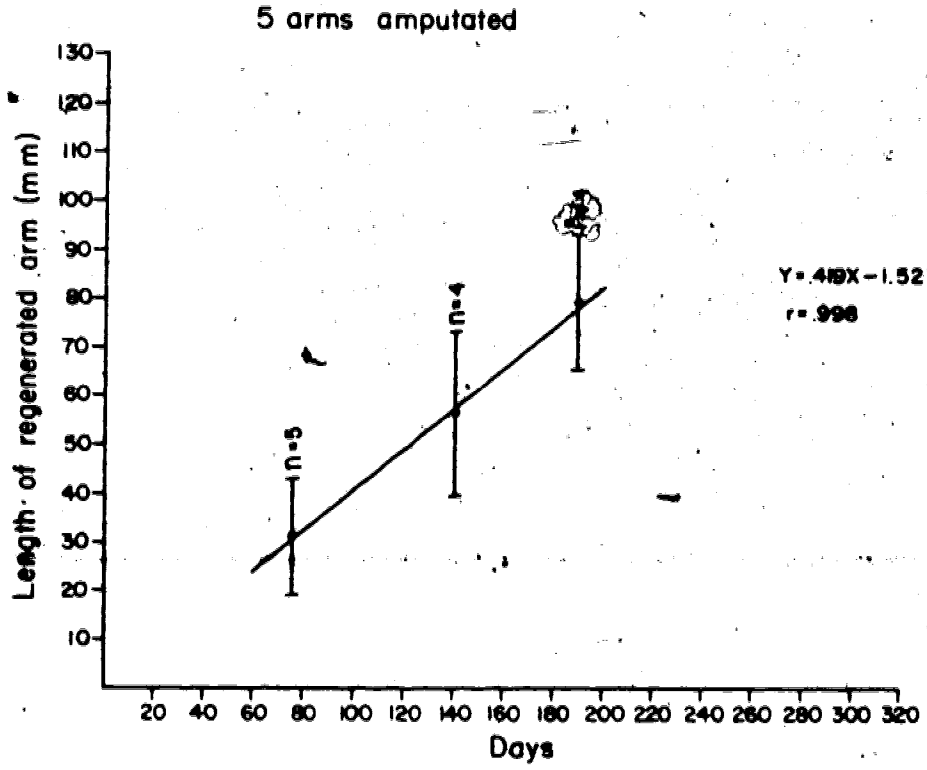

2 arms amputated
Length of regenerated arm (mm)
Days
n=4
n=4
n=3
n=3
n=3
n=2
Y=.518X−21.779
r=.986

Fig. 47. Florometra serratissima. Relationship between length of regenerated arm and time after amputation for individuals with three and five arms removed. Vertical bars show one S.D. on each side of the means; the number of specimens measured is shown above each point. The regression lines are significant ($P < 0.05$)


3 arms amputated
Length of regenerated arm (mm)
Days
n=6
n=5
n=3
n=3
Y=.486X−4.582
r=.992


5 arms amputated
Length of regenerated arm (mm)
Days
n=5
n=4
Y=.419X−1.527
r=.998

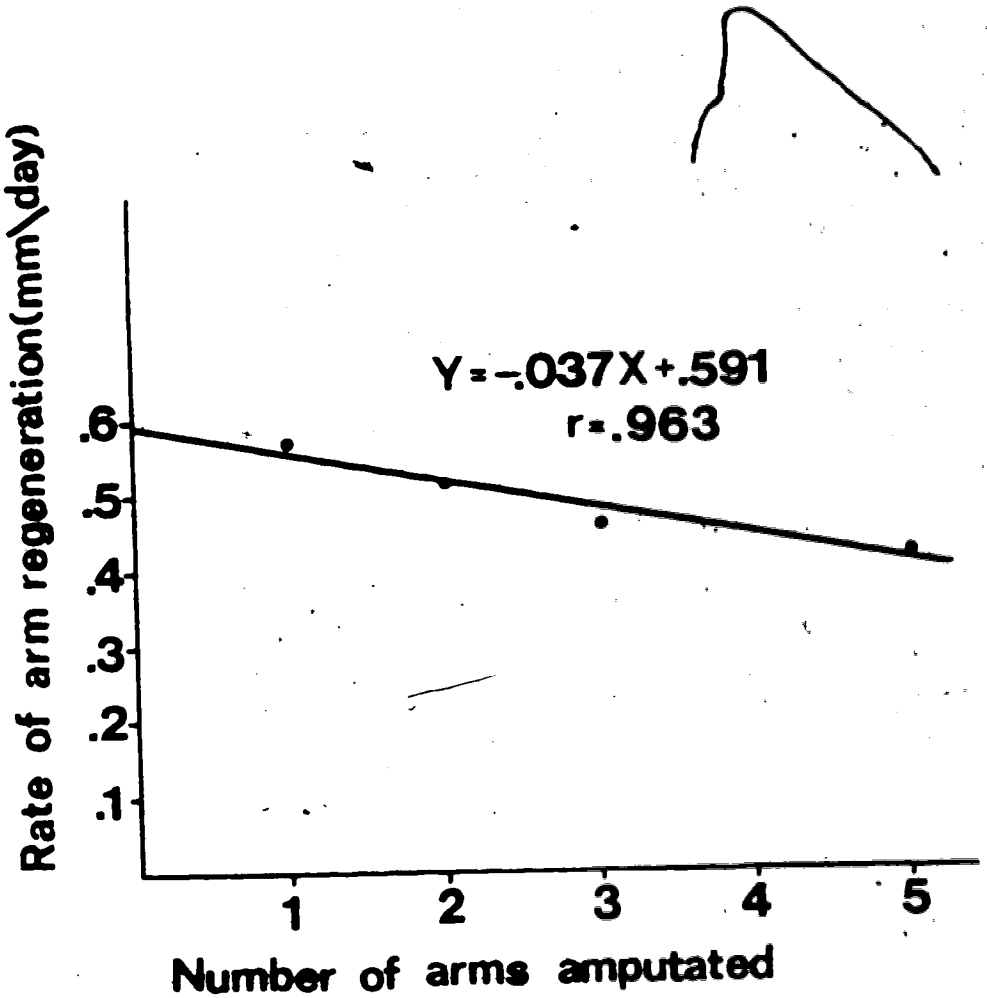


Fig. 48. Florometra serratissima. Relationship between rate of arm regeneration and number of arms amputated. The regression line is significant ($P<0.05$). The 95% confidence interval for the slope, β, of the regression line is $-.068 < \beta < -.006$

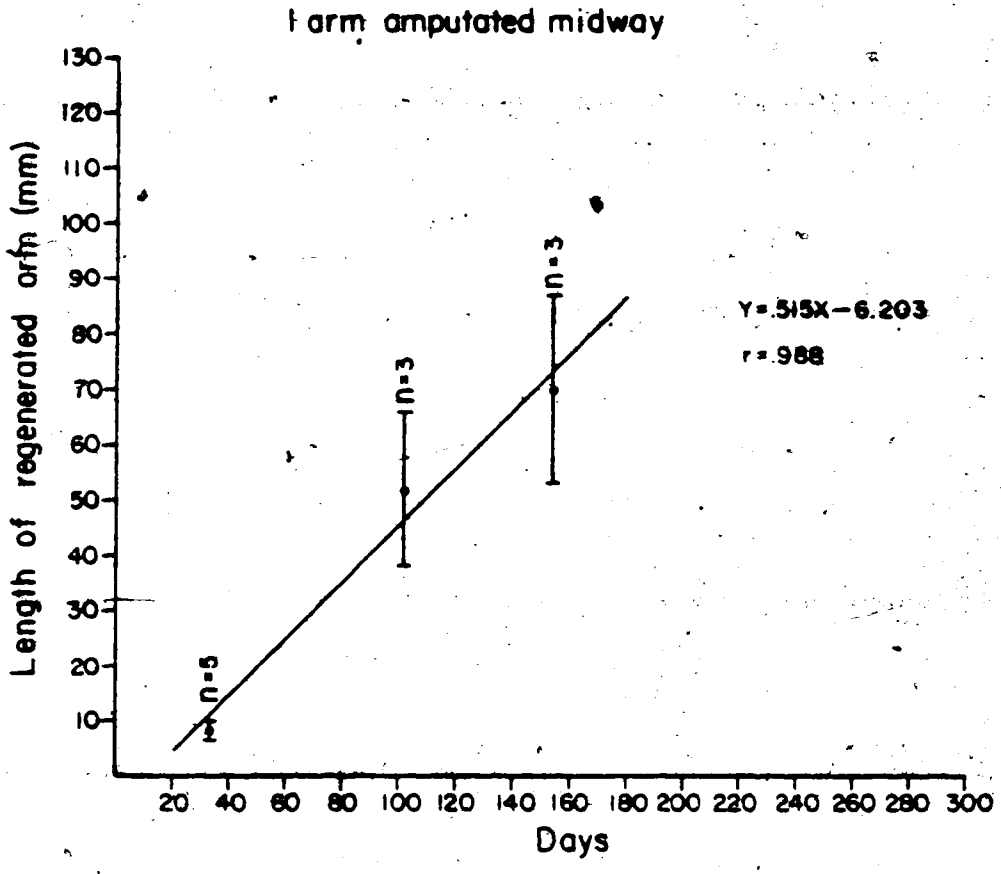


Fig. 49. Florometra serratissima. Relationship between length of regenerated arm and time after amputation for individuals with one arm amputated midway. Vertical bars show one S.D. on each side of the means; the number of specimens measured is shown above each point. The regression line is non-significant ($P > 0.05$)

by the extent of arm amputation. Again, this hypothesis should be tested by the collection, if possible, of additional data.

Genital pinnules containing either tailed sperm or large (diameter greater than 180 µm) oocytes first appear on regenerating arms amputated at the base when the arms reach a total length (including a 10 mm stump) of about 130 mm. The genital pinnules first become evident near the arm base, and then appear sequentially towards the arm tip. A regenerating arm amputated at the base becomes difficult to distinguish from normal arms when it reaches a length, including the stump, of about 140 mm. Due to breakage during handling, it was not possible to follow the course of a regenerating arm to the point where it equalled the length of the normal arms; thus, it is not known when, exactly, the constant rate of arm regeneration begins to slow and then stops.

Predation

An instance of direct predation on F. serratissima was never observed in the field. Indirect evidence suggests, however, that the most likely predator is the sea star Pycnopodia helianthoides, which was commonly noted in the deeper parts of Bamfield Inlet within the feather star population. F. serratissima, which is normally sessile, displays a characteristic escape response when contacted by a wandering P. helianthoides: after about a 5 sec delay, the feather star lifts itself from the bottom through rapid lashing movements of the arms (see Fell, pp. 56 and 57 for a review of swimming in comatulids) and swims to a point 1 or 2 m distant from the sea star. This escape response can also be induced if a P. helianthoides is manually brought into contact with a sessile feather star.

P. helianthoides will eventually eat F. serratissima if both are kept together in an aquarium. The actual capture of a feather star was not observed, but the egested skeletal remains of feather stars were noted. It is likely that the feather stars became exhausted after repeated escape attempts within the confined space of the aquarium, and this led to their eventual capture. Under natural circumstances, instances of successful capture of a feather star by P. helianthoides are probably rare, although the sea star may succeed in retaining an arm of the feather star as a result of the initial contact.

The decorator crab, Oregonia gracilis, is a second potential predator of F. serratissima. On a single occasion during a S.C.U.B.A. dive a decorator crab was observed holding on to the arm of a feather star with one of its chelae. Unfortunately, the crab became disturbed, released the feather star, and moved off, so the extent of damage that can be inflicted by this crab in a natural situation is not known. In an aquarium, O. gracilis was unable to capture and kill a feather star. Under these circumstances, however, some F. serratissima were noted to be missing several arms near the base and this can be attributed, in part at least, to attacks by O. gracilis. It appears, then, that F. serratissima may be subject to attack by P. helianthoides and O. gracilis. It is thought that such encounters would rarely be fatal for the feather star, but there is the possibility that the feather star may lose an arm during escape from the predator.

DISCUSSION

The population of F. serratissima described in this report consists of a discrete collection of individuals at moderately high densities within a small, well-defined area, and it can therefore best be termed an aggregation. Observations made during several trawling expeditions, along with anecdotal information from S.C.U.B.A. divers and other collectors, suggest that similar aggregations are scattered throughout Barkley Sound, separated from one another by distances of at least several kilometers.

The occurrence of crinoids in aggregations is not an unusual phenomenon. Lacaze-Duthiers (1872, cited by Fell, 1966) gave an account of a very dense collection of Antedon bifida at Roscoff in northern France, while Marr (1963) published a photograph taken by A.S. Laughton of an aggregation of an unidentified feather star found at 650 m off northern Spain with densities of $65/m^2$; in the eastern Arctic, Heliometra glacialis occurs in dense aggregations (personal observations). Additional descriptions of crinoid aggregations can be found in Hyman (1955) and Fell (1966).

The density of F. serratissima observed in this study is not nearly as high as densities reported or implied for other crinoid species such as Antedon bifida and the unknown species in Laughton's photograph. These species are very small, however, compared to F. serratissima. For instance, maximum arm length for A. bifida is about 100 mm (Clark and Clark, 1967, p.199), while the arm length for the unknown species as determined from Laughton's photograph, is only 50 to 60 mm. F. serratissima, on the other hand, reaches a maximum arm length of about 300 mm

in Barkley Sound, so the density reported here represents a fair degree of aggregation. The possible role of gregarious larval settlement following a period of pelagic dispersal in the creation and maintenance of adult aggregations of F. serratissima is discussed in Mladenov (1980a).

As shown in this report, individual feather stars are distributed contagiously within the aggregation. In other words, microaggregations are present within the main aggregation. It is unlikely that the distribution of microaggregations is related to the small-scale pattern of gregarious larval settlement since juvenile and adult feather stars are quite capable of shifting their positions within the main aggregation by means of swimming. Furthermore, it is unlikely that it is related to the distribution of hard substrates because such substrates appear not to be limiting. At present, the mode of formation and maintenance of the microaggregations is not known.

It is possible, however, to advance an explanation for the functional significance of the microaggregations. As shown in Mladenov (1980b), individual F. serratissima release only small quantities of gametes on frequent occasions throughout the year; low rate of fertilization success is thus a potential problem. The presence of this species in large aggregations would ensure higher rates of fertilization success than would be possible if individuals were widely scattered. Furthermore, the contagious distribution of feather stars within the main aggregation could serve to further increase the rate of fertilization success. It is quite possible that spawning is a very localized phenomenon which involves only a small number of males and females within a microaggregation or within several adjacent microaggregations. Direct observations

of spawning behavior are necessary to fully substantiate this latter hypothesis.

The F. serratissima aggregation in [redacted]ield Inlet is characterized by a lack of small individuals. As noted in this account, the smallest specimens encountered were two individuals with L = 35 mm, which means they were over two years of age. Mladenov (1980a) did not find pentacrinoids in the aggregation over the period from November 1977 to December 1979. These observations suggest that there was a very low level of larval recruitment to the aggregation over the study period. It is possible that the rate of larval recruitment is very low but steady throughout the year (trickle recruitment) and that pentacrinoids and young juveniles were present in such low numbers that they were simply overlooked. On the other hand, the rate of larval recruitment may indeed be very low and infrequent. An accurate assessment of the size-frequency distribution of the aggregation over a period of several years is required to distinguish between these two alternatives.

Growth in F. serratissima increases exponentially over the first five years of its life, and then the growth rate decreases considerably. It is quite likely that F. serratissima continues to live and reproduce after attaining its maximum size of L = 300 mm at about eight years of age (determinate growth). In this case, regenerative growth to replace lost arms and other body parts would occur, but overall size would increase very slowly or not at all. Determinate growth has been reported for echinoids (Moore and Lopez, 1966; Ebert, 1968; Birkeland and Chia, 1971), and has been proposed for an asteroid (Yamaguchi, 1974).

The endoskeleton of large specimens of F. serratissima was examined for growth zones in an attempt to determine the absolute age of individuals. Arm ossicles, basal plates, and cirral ossicles were studied using techniques outlined in Pearse and Pearse (1975) which have proved successful for elucidating growth zones in the echinoid endoskeleton. Unfortunately, growth zones were not apparent in F. serratissima. The ultimate life span of this species is thus unknown, but it may be very long. The apparent immunity of F. serratissima to mortal attack by predators is supportive of this suggestion.

The number of specimens of F. serratissima with regenerating arms has been shown to be very high. Since F. serratissima is rarely found above a depth of 17 m, wave action cannot be a factor contributing to arm damage; seaweeds, or other objects with which feather stars could become entangled, with resultant arm loss, are not present in that part of Bamfield Inlet occupied by F. serratissima; furthermore, it is not likely that feather stars become entangled amongst themselves because the population density is not high enough. It appears, then, that attacks by the sea star Pycnopodia helianthoides and the crab Oregonia gracilis may be the major cause of arm loss in F. serratissima and thus account for the high proportion of specimens with regenerating arms. Similarly, arm regeneration in amphiurid brittle stars is generally thought to be the result of arm loss due to cropping by bottom feeding fish, sea stars and crabs (Hunt, 1925; Buchanan, 1964; Fenchel, 1965; Salzwedel, 1974).

Since the arms of F. serratissima bear both the feeding and reproductive structures, prompt replacement of lost arms is important to the survival of F. serratissima. Some evidence is presented in this report suggesting that the rate of regeneration per arm decreases slightly as the number of regenerating arms on an individual increases. Presumably, this reflects the effect of the partitioning of an individual's available energy resources for regeneration between an increasing number of regeneration sites. These findings are not in agreement with Reichensperger's (1912) beliefs that the rate of arm regeneration in crinoids following extensive arm damage was more rapid than that following minor damage.

Information on arm regeneration in ophiuroids is considerably more abundant than for crinoids, and it offers some insight into the problems addressed in this paper. Zeleny (1903), working with Ophiura texturata (= Ophioglypha lacertosa) presented quantitative data demonstrating that the greater the number of arms removed from a specimen, the greater the rate of regeneration per arm. This relationship has sometimes been referred to as Zeleny's rule. In a subsequent paper, Zeleny (1905) postulated that this effect was due to an interaction among the arms on an individual such that the presence of unremoved arms somehow retarded the rate at which the amputated arms regenerated. Observations by Morgulis (1909) show that if an arm of the brittle star, Ophiocoma pumila, is cut off at the tip, it regenerates more slowly than an arm cut off near the base; the rate of regeneration of an arm cut off at the middle, however, is similar to that of an arm cut off near the base.

Additional experiments performed by Morgulis did not conform to Zeleny's rule. He found that the rate of arm regeneration on specimens with one to three arms removed is about the same as on specimens with four to five arms removed. Finally, Salzwedel (1974), working with Amphiura filiformis, has demonstrated that as more arms are removed, the total length and total weight of regenerating arm parts becomes greater, but the rate of regeneration per arm slows slightly. These results do not conform to Zeleny's rule, and they are similar to results reported here for F. serratissima. In view of such findings, it is proposed that, contrary to Zeleny's hypothesis, the rate of arm regeneration in crinoids and ophiuroids slows slightly as more arms are amputated.

LITERATURE CITED

The periodicals have been abbreviated in accordance with "World list of scientific periodicals, 4th ed. London 1964" or, for more recent journals, "Bibliographic guide for editors and authors, Washington 1974".

Birkeland, C. and F.S. Chia: Recruitment risk, growth, age and predation in two populations of sand dollars, Dendraster excentricus (Eschscholtz). J. Exp. Mar. Biol. Ecol. 6, 265-278 (1971)

Buchanan, J.B.: A comparative study of some features of the biology of Amphiura filiformis and Amphiura chiajei (Ophiuroidea) considered in relation to their distribution. J. mar. biol. Ass. U.K. 44, 565-576 (1964)

Carpenter, P.H.: Report on the Crinoidea collected during the voyage of H.M.S. Challenger during the years 1873-1876. - The stalked crinoids. Rept. Scient. Results Voyage Challenger, Zool. 11, 1-442 + 62 plates (1884)

Clark, A.H.: A monograph of the existing crinoids. Bull. U.S. natn. Mus. 82, vol. 1, pt. 2, 1-795 + 57 plates (1921)

-- Crinoidea. Scient. Rep. Australas. Antarct. Exped. (ser. C) (Zool. and Bot.) 8, pt. 4, 5-18 (1937)

-- and A.M. Clark: A monograph of the existing crinoids. Bull. U.S. natn. Mus. 82, vol. 1, pt. 5, 1-860 (1967)

Dendy, A.: On the regeneration of the visceral mass in Antedon rosaceus. Owens College. Biological department. Studies in biology 1, 299-312 (1886)

Ebert, T.A.: Growth rates of the sea urchin Strongylocentrotus purpuratus related to food availability and spine abrasion. Ecology 49, 1075-1091 (1968)

Elliot, J.M.: Some methods for the statistical analysis of samples of benthic invertebrates. Freshwater Biological Association Scientific Publication 25, 1-160 (1977)

Fell, H.B.: Ecology of Crinoids. In: Physiology of Echinodermata, pp 49-62. Ed. by R.A. Boolootian. New York: Interscience Publishers 1966

Fenchel, T.: Feeding biology of the sea-star Luidia sarsi Düben and Koren. Ophelia 2, 229-296 (1965)

Hunt, O.D.: The food of the bottom fauna of the Plymouth Fishing Grounds. J. mar. biol. Ass. U.K. 13, 560-599 (1925)

Hyman, L.H.: The invertebrates. Vol. 4. Echinodermata, 762 pp. New York: McGraw-Hill 1955

Lacaze-Duthiers, H.: Station du Pentacrinus europaeus sur les cotes de France. Archs. Zool. exp. gén. ~~et notes et~~ revue, 3, PS 10-13 (1872). (Unable to obtain this article; relying on information in Fell [1966].)

Marn, J.W.S.: Unstalked crinoids of the Antarctic continental shelf. Notes on their natural history and distribution. Phil. Trans. R. Soc. (B.) 246, 327-379 (1963)

Minckert W.: Über Regeneration bei Comatuliden nebst Ausführungen über die Auffassung und Bedeutung der Syzygieen. Arch. Naturgesch. 71, 163-244 + plate 7 (1905)

Mladenov, P.V.: Development and larval behaviour in the feather star Florometra serratissima (Echinodermata: Crinoidea). In: Development and reproductive biology of the feather star Florometra serratissima (Echinodermata: Crinoidea). Ph.D. thesis, University of Alberta 1980a

-- Continuous reproduction in a population and in individuals of the feather star Florometra serratissima (Echinodermata: Crinoidea) at Barkley Sound, British Columbia. In: Development and reproductive biology of the feather star Florometra serratissima (Echinodermata: Crinoidea). Ph.D. thesis, University of Alberta 1980b

Moore, H.B. and N.N. Lopez: The ecology and productivity of Moira atropos (Lamarck). Bull. mar. Sci. Gulf Caribb. 16, 648-667 (1966)

Morgulis, S.: Regeneration in the brittle-star Ophiocoma pumila, with reference to the influence of the nervous system. Daedalus, Boston, Mass. 44, 655-659 + 1 plate (1909)

Mortensen, T.: Handbook of the echinoderms of the British Isles, 471 pp. Oxford University Press 1927

Pearse, J.S. and V.B. Pearse: Growth zones in the echinoid skeleton. Amer. Zool. 15, 731-753 (1975)

Perrier, E.: Recherches sur l'anatomie et la régénération des bras de la Comatula rosacea (Antedon rosaceus, Linck). Arch. Zool. exp. gén. 2, 29-86 + plates 2-4 (1873)

Przibram, H.: Experimentelle Studien über Regeneration. Wilhelm Roux Arch. EntwMech. Org. 11, 321-345 + plates 11-14 (1901)

Reichensperger, A.: Beiträge zur Histologie und zum Verlauf der Regeneration bei Crinoiden. Z. wiss. Zool. 101, 1-69 + plates 1-4 (1912)

Salzwedel, H.: Arm-Regeneration bei Amphiura filiformis (Ophiuroidea). Veroff. Inst. Meeresforsch. Bremerh. 14, 161-167 (1974)

Sokal, R.R. and F.J. Rohlf: Introduction to biostatistics, 368 pp. San Francisco: W.H. Freeman 1973

Strimple, H.L. and B.H. Beane: Reproduction of lost arms on a crinoid from Le Grand, Iowa. Okla. Geol. Notes 26, 35-37 (1966)

Swan, E.F.: Growth, autotomy, and regeneration. In: Physiology of Echinodermata, pp 397-434. Ed. by R.A. Boolootian. New York: Interscience Publishers 1966

Walpole, R.E.: Introduction to statistics, 340 pp. New York: MacMillan 1974

Yamaguchi, M.: Growth of juvenile Acanthaster planci (L.) in the laboratory. Pacif. Sci. 28, 123-138 (1974)

-- Estimating the length of the exponential growth phase: growth increment observations on the coral-reef asteroid Culcita novaeguineae. Mar. Biol. 39, 57-59 (1977)

Zeleny, C.: A study of the rate of regeneration of the arms in the brittle-star, Ophioglypha lacertosa. Biol. Bull. mar. biol. Lab., Woods Hole 6, 12-17 (1903)

-- Compensatory regulation. J. exp. Zool. 2, 1-102 (1905)