SPAWNING AND REPRODUCTIVE MORPHOLOGY

OF SCOLELEPIS SQUAMATA (SPIONIDAE : POLYCHAETA)

by

Susan Leigh Richards

A thesis submitted to the Faculty of Graduate Studies
and Research in partial fulfillment of the require-
ments for the degree of Master of Science in Zoology.

Abstract

The adults of three populations of the spionid polychaete
Scolelepis squamata were studied throughout a year in Barbados.
From quantitative estimates of gametes and numbers of breeding
animals, four statistically reliable outbursts of spawning were
determined, each approximately two months apart, followed by a
cessation of breeding activity from November till February. Sperm-
atophores of this species were described for the first time; they
occurred at each of the four spawning times. Their presence in
this species weakens previous classification of spionids.

General observations on the ecology and morphology of this
species were made. The enlargement of the nephridia during the
breeding season and the morphology of the sperm were examined.
Two varieties of this species were found; their possible relation
to the species in the literature was considered.

Zoology Department
Mc Gill University
Montreal   March 1969

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TABLE OF CONTENTS

LIST OF FIGURES

PREFACE

The study presented here was begun to determine the time of spawning and general aspect of the breeding period of <u>Scolelepis</u> <u>squamata</u>; in the course of the study, many relevant observations on the reproductive morphology were made. The work was carried out at the Mc Gill University tropical marine laboratory, Bellairs Research Institute, in St. James, Barbados. The data was processed at the Mc Gill University Computing Center in Montreal.

Because of the author's training and background, modern American spelling has been used throughout the text, i.e., celom, color, etc. The text-figures have been placed immediately following the text, with the bibliography at the end. Although a departure from tradition, I feel that this arrangement makes it easier for one to refer directly to the figures.

I wish to thank my research supervisor, Dr. Joan Marsden, for her assistance and encouragement; Dr. John B. Lewis, Director of Bellairs Institute; Mrs. Alison Walford, who helped with the collections and records, especially during the September-December 1968 period; Mrs. U. B. Manley of the Mc Gill Computing Center, who helped to design the program for processing the data; Dr. H. Tyson, for his help with the statistical analysis; Mrs. C. Vowinckel, for many patient German translations; and my husband, Mr. Anthony Richards, for much help with the drawings, photography, and the printing of the photographs.

INTRODUCTION

Polychaetes are well known for their diverse modes of reproduction, asexual as well as sexual. Some have more than one means of reproduction; the widespread distribution of polychaetes may be due to their ability to change their mode of reproduction in accordance with ecological conditions (Thorson, 1950). Asexual means of reproduction may take the form of autonomy and regeneration in many species, including some spionids, sabellids, and serpulids, and takes the form of some type of fragmentation (scissiparty or schizometamery) in other serpulids and phyllochaetopterids (Fauvel, 1959).

Most polychaetes are dioecious. In some Errantia and a few Sedentaria, sexual dimorphism is accompanied by epitoky or very apparent modifications, internal and external, of the sexually mature individual. Such highly modified sexual individuals exhibit a swarming behavior, synchronously releasing their gametes at the surface of the sea, at a certain time in sexual maturity.

In other dioecious polychaetes, gametes are released either directly into the sea-water, or some form of sperm transfer takes place, along with internal fertilization; the eggs may be incubated in a protective covering or within the body of the female.

Spionid polychaetes reproduce asexually by regeneration, but the most important means of reproduction is sexual; this has been sparsely studied. Söderström (1920) examined spionids with special regard to reproductive characteristics, on the basis of which, he managed to arrange a classification of the spionids in two subfamilies. One subfamily was characterized by internal

fertilization, and with it, a modified sperm, spermatophores, copulation, and brood protection. The other subfamily had strictly pelagic fertilization, in which both sperm and eggs were released directly into the water. (Scolelepis and its synonymous genera under present classification, were placed in this latter subfamily.)

More recent studies on reproduction in spionids include those on Polydora ciliata (Dorsett, 1961), Scolecolepides viridis (George, 1966), and Simon's (1967) study of Spio setosa. These will be examined more closely below.

There has been only one attempt to study the breeding morphology of Scolelepis squamata -- Joyner's (1962) study of Nerine cirratulus (= S. squamata, Pettibone, 1963). Joyner estimated quantities of gametes in both sexes by examination of the adult worms and their celomic fluid. He did not state at what stage in development a gamete was considered a gamete; both oocytes and sperm develop gradually, and celomic samples might very well reveal gametes at some stage in development, year round. Joyner also measured oocyte sizes; in some species of polychaetes, the developing gametes (particularly oocytes) remain attached to their formative tissue until reaching a certain size, after which they continue their development in the celomic fluid (Fauvel, 1959). It seemed likely that if this were the case in this species, measurement of celomic oocytes might reveal very little.

Thus, to date there are few detailed accounts of breeding morphology in any spionid, and no satisfactory account of the reproduction of S. squamata.

S. squamata has been found to have a wide distribution: England, Denmark, Sweden, France, Italy, Connecticut, New Jersey,

Virginia, Gulf of Mexico, California, South Africa, Cape Verde Islands, and Jamaica. In four of these areas, the presence of larvae in the plankton has been used as a means of indicating the breeding season. These breeding seasons have been indicated as: in England, March till July or later (Joyner, 1962), in Denmark, June till October or possibly January (Hannerz, 1956), in Connecticut, June till August (Dean and Hatfield, 1963), and in California, April to May (Hartman, 1941). Despite the diverse locations, the breeding seasons and their lengths vary tremendously. Although a general picture of the breeding season may be obtained from plankton sampling, the actual details of spawning periods may be difficult to assess.

I found *Scolelepis squamata* to be extremely abundant and easy to collect on the beaches of Barbados. Since the breeding morphology of the adults had never been satisfactorily studied, and since examinations of the adult worms might give an accurate idea of spawning times, I decided to do an intensive study of the adult worms to try and determine how and when they bred.

SAMPLING AND GENERAL OBSERVATIONS

For this study, three areas of beach were chosen in St.
James, along the west coast of Barbados, and called St. James I,
II, and III. St. James I was the northern-most area, separated
by about a mile of continuous beach from St. James II. St. James III
was about a quarter of a mile south of St. James II. (Fig. 1.)
Each area chosen was sampled at regular intervals from the same
10 meter strip of beach.

Scolelepis squamata is found in the intertidal zone of the
beach, living vertically in tubes loosely formed from sand and
mucus. The antennae project out of the individual holes in the
sand as a wave regresses; food is probably picked up from the water
by the sticky, mucus-covered antennae.

The worms appear to be associated with particular sizes of
sand grains; they move up and down the beach with spring and neap
tides, and move along the beach during the winter months (November
to March) when the exceedingly rough seas redistribute the sand.
Possibly the animals also burrow more deeply in the sand during the
winter, since they are then more difficult to collect.

The density of populations along all the beaches is greatest
at mid-tide level (contrary to Mesnil, 1896, who found the density
on the beach of Wimereux greatest near the high tide level). The
smaller worms tend to concentrate towards the lower level of the
beach. The distribution of S. squamata is apparently continuous
around the island wherever beaches occur; the east coast seems to
support a less dense population.

I sampled at the mid-tide level, approximately four hours

before or after low tides. Tentacles of large numbers of animals were observed as a wave regressed; a spadeful of sand from a heavily populated area was placed in a bucket of sea-water. When I stirred the entire mixture, the worms rose to the surface in the vortex, and were picked out with a small tea strainer. I placed the yield of five to ten shovels of sand in a single small jar. This method of sampling resulted in injury to 25 - 30% of the worms, but was the most efficient method of collecting the specimens.

The animals are extremely delicate, often rupturing or breaking with handling. The antennae break off extremely easily, and seem to be very quickly regenerated. Specimens with naturally regenerated heads and tails were common in the collections; laboratory experiments showed animals to be capable of regenerating the anterior 10 - 15 segments, or 20 - 30 posterior segments, or both at once.

I brought the specimens, together with some sand, to the laboratory and placed them in large open plastic trays with fresh sea-water until they were examined. Prior to examination with a binocular dissecting microscope, the worms were anesthetized for three to six minutes in smoke-bubbled sea-water. Worms were preserved from each location at least once a month, in sea-water Bouin's or Carnoy's (6:3:1), after narcotization. They were then embedded, sectioned (7 - 10 µ), and stained with hematoxylin or PAS (used as a general stain, with a light green counterstain).

When raised in the laboratory in sand-filled boxes of plankton netting suspended in fresh running sea-water, the worms burrowed either vertically or horizontally, but could not be induced to feed; they usually died of starvation after four months.

Two "types" of S. squamata are found in Barbados. Dr. Marion Pettibone (personal communication) suggested that they might be considered as varieties of the same species. However, I believe that the distinction may be greater because of the large number of differences that are found between the two: differences in prostomium size and shape, marked coloration patterns, body size and numbers of segments, the level of the beginning of the intestine, size and color of the eggs, size of the sperm, behavior, and distribution both on a single beach and among various beaches.

I worked only with the variety more common on the west coast. It is possible that the earlier authors worked with the other variety, since this less common variety more closely resembles the Nerine cirratulus (= S. squamata, Pettibone, 1963) of earlier descriptions and drawings (Cunningham and Ramage, 1887; Fauvel, 1927). This might account for differences in our descriptions.

DISCOVERY

While examining a collection in June, I found spermatophores attached to the females. This was highly unusual; no one had ever observed spermatophores in this species before, nor expected to find them, in view of reported "evidence" of pelagic fertilization (Claparède and Mecnikow, 1869; Mesnil, 1896; Joyner, 1962), and Söderström's (1920) classification of this species in a subfamily characterized by pelagic fertilization instead of internal fertilization involving spermatophores or copulation.

The spermatophores are visible to the unaided eye, as small white protrusions. Under the microscope, they appear as flexible, leaf-like attachments, very irregular in size and shape (as if an unjelled substance had been squeezed from a pastry tube onto a flat surface, and then solidified), but about the size of a parapodium, pointed or rounded at the tip, and of a milky-white granular appearance. (Fig. 2 A - C.) The attachment to the body of the female is firm; mechanical manipulation does not alter the shape or attachment. The point of attachment varies considerably; one to five spermatophores are placed, apparently randomly, on the gametogenic region, usually in the central portion, and most often on the dorsal, but sometimes on the ventral or lateral, surface. The spermatophore is held together by an unidentified binding matrix; when teased apart in sea-water, the sperm are released and become active.

In many other polychaetes and spionids, the male nephridium is known to form the spermatophore (Fage, 1905, 1906; Söderström, 1920; Goodrich, 1945; Franzén, 1956). The formation of

spermatophores has been described for the archiannelid _Protodrilus_ (Jägersten, 1952). The sperm are pressed out of the sperm duct and at the same time enclosed by a thin film of secretion, resulting in a balloon-shaped structure with a small stalk. The formation of spermatophores in the spionid _Spio martinensis_ has been described somewhat differently (Claparède and Mecnikow, 1869); the mature sperm are drawn into the nephridium, secretory cells in the tube secrete material to enclose the sperm, and several spermatophores are formed and stored in a bend in the nephridial tube.

In this study, although sperm and a mucus-like substance were observed in histological sections in the lower portion of the male nephridium at spawning times, (Fig. 3 C), the actual process of spermatophore formation, the means of transfer or copulation, and the means of attachment, are unknown. But the irregular shapes of the spermatophores (Fig. 2 A - C), the presence of a mucus-like material as a binding matrix (Fig. 2 D), and the presence of a mucus like material associated with the sperm in the male nephridium (Fig. 3 C), all suggest that the sperm, with a binding matrix, are squeezed out of the nephridia directly onto the female epidermis, with the matrix hardening in the sea-water. This implies copulation, although there were no observations to support such an hypothesis.

Isolated sperm, or occasional clumps of two to four sperm, were found in the body cavity of females with spermatophores (Fig. 2 D), which suggests internal fertilization, though the occurrence may have been due to histological aberations, since sperm could not be found actually penetrating the cuticle.

In association with many of the spermatophores, I found a

thin, finger-like papilla of mucus cells (similar to those on the epidermal surface) extending out from the female body (Fig. 2 B). This may have been an empty portion of the spermatophore, or possibly an accretion of the female, related to the attachment or placement of the spermatophore.

Spermatophores are usually found on the females, but they occasionally occur on immature worms or small males. In the capitellid polychaetes, males have been observed to copulate with young males in spermatophore transfer (Eisig, 1887).

Joyner (1962) reported seeing male worms in the laboratory releasing sperm in streams from their nephridiopores; this was observed from time to time in the present study, but is believed to be due to the mechanical stimulation of handling.

STUDY OF SPAWNING

I made collections approximately every two weeks over a period of time extending from November 1967 to December 1968, for St. James I and III. For St. James II, two collections were made per week every other week from November 1967 until the second week of July, when I began sampling once a week, which I continued until December 1968. During the third week of July, no collections were made, due to technical difficulties.

I divided the year into natural weeks, which are referred to by number. Two collections made on different days but in the same week, are thus referred to as being in the same "week-date". A table of the weeks and their corresponding natural times, along with the collections made, is given in Figure 4.

I examined anesthetized specimens using only unbroken, un-injured ones for the data. Each was first classified by sex: male, female, or sex unidentified (simply without visible gametes, not necessarily immature). The gametes are visible during the breeding season: the males appear milky-white; the green eggs of the females can be seen through the body wall. I recorded the number of eyes, number of segments, and position of the anterior end of the intestine for each individual. In addition, the num-ber of segments containing gametes was recorded for every male and female, plus a rough estimate of "condition of fullness". The method of estimation (a modification of Joyner's, 1962) was carried out as follows: in nearly every specimen, the five or six segments preceding the intestine were filled with gametes; if this condition existed alone, or if, in addition, less than

twenty segments contained gametes only in the bases of the para-
podia, I considered the specimen 1/4 full; if the gametes filled
the parapodia of more than twenty segments, the worm was considered
1/2 full; if the gametes filled not only the parapodia but could
also be seen in the celomic space along the gut wall, the specimen
was 3/4 full; when the gametes completely packed the celomic cav-
ity, 4/4 full was recorded. (Fig. 5.)

I recorded data for every specimen until at least 50 animals
of 80 or more segments (adults), and the entire contents of a col-
lection jar, had been examined.

A mature worm presents the following appearance: the intes-
tine begins at segment 36 (mean); the first 30 segments (mean) are
atokous, or without gametes; the 30th - 36th segments are heavily
filled with gametes if any gametes are found in the worm at all.
The worms develop gametes along a posteriorly directed gradient;
when the animals are most full, the gametes extend to segments
60 - 90, with the posterior-most segments remaining atokous. These
observations were confirmed by histological studies.

The worms begin to develop gametes when they have attained
a length of about 80 segments; the segment means of breeding males
and females runs much higher, between 90 - 96 segments (Figs. 6 - 7).
Only rarely is a 75 - 80 segment worm found with gametes, or an
80 - 85 segment worm found without gametes during the breeding
season.

Worms of 80 or more segments are therefore classified in the
breeding population. The number of individuals in each sex (male
and female) has been calculated as a percentage of the total breed-
ing population and plotted against time. Each location has been

illustrated separately (Figs. 8 - 9 A), and a similar plot is presented for the mean of combined values for all three locations (Fig. 9 B). All four graphs show the following trends: a sharp decrease in the relative numbers of males and females in December-January, an initially steep rise in the percentages in February-March, and a tapering off to a more gradual slope, until in one case, by April, and in all cases, by week 43 (18 - 24 August), identifiable males and females comprise nearly 100% of the breeding population.

When examination and recording of data began in November 1967, the males and females were quite full of gametes; the mean number of gametogenic segments ran from 15 to 40; the means of the condition of fullness (hereafter referred to as simply fullness) ran between 1/2 and 3/4. (Figs. 10 - 12.) During November, both measurements decreased sharply, as did the relative numbers of males and females (Figs. 8 - 9). The decreases in all measurements continued until the eighth week (17 - 23 December), when only two or three males or females could be found in a collection of more than 50 adults.

The situation remained appreciably the same through the 12th week (14 - 20 January), after which, the worms appeared to be gradually producing more gametes, as indicated by an increase in the percentage of males and females, and an increase in the numbers of gametogenic segments and their fullness. This trend continued until October, although interrupted in the following manner. The increase in numbers of gametogenic segments and their fullness was stopped at certain intervals; each halt in the progressive increase was followed by a sudden decrease. The decrease resulted in a

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stopped at certain intervals; each halt in the progressive increase
was followed by a sudden decrease. The decrease resulted in a

slight reduction in the percentage of males and females, and a great reduction in the numbers of gametogenic segments and fullness. This pattern was repeated, suggesting that the worms on each occasion, were building up a quantity of gametes and then releasing many from each segment. In most cases, each subsequent increase was greater than the preceding one, resulting in progressively more gametogenic segments and higher fullness estimates. Thus after each periodic discharge, the worms seemed to be replenishing the gametogenic segments and their fullness to a greater degree. These cycles of increase and decrease occurred in the collections for all three locations, at least once, in each of April, June, August, and October. From mid-September until November 1968, the numbers of gametogenic segments and their fullness fluctuated less regularly; a September cycle of increase and decrease was found for St. James II.

In Figures 10 - 12, the means of combined numbers of male and female gametogenic segments are weighted by their corresponding breeding population percentages. (This is necessary for illustrative purposes, to avoid having a few animals during the winter months represent means as high as many animals during the breeding period.) Fullness estimates are expressed in the same way. The resulting graphs show the decrease in both numbers of gametogenic segments and fullness during the December-January period, and the series of sharp increases from February till October.

Since St. James II was the most heavily sampled location, a t-test analysis was carried out on the peaks and corresponding valleys of this graph, to determine which peaks and valleys were due to something other than random sampling. The results of the

t-test analysis are shown in Figure 13. Three of the small peaks
(weeks 13, 15, and 52) are not statistically different from the
small valleys that follow them; the difference between weeks 49 and
50 is not statistically reliable. The five peaks listed below
are shown to be reliably different from the valleys on either side
of them.

Peak	Subsequent Valley	Spermatophores
Week	Week	Week
23 (31 Mar - 6 Apr)	25 (14 - 20 Apr)	25
31 (26 May - 1 Jun)	36 (30 Jun - 6 Jul)	33
41 (4 - 10 Aug)	42 (11 - 17 Aug)	42
45 (1 - 7 Sept)	49 (29 Sept - 5 Oct)	(45 , 50)
50 (6 - 12 Oct)	53 - 57 (27 Oct ...)	51

The occurrence of spermatophores in June coincided with the
beginning of one of the decrease phases, i. e., indicating that the
worms were actually spawning. Spermatophores were found in August
and October on the specimens from St. James II, also coinciding
with decrease phases, and histological evidence reveals spermato-
phores during the April decrease phase. (Fig. 10.)

Spermatophores were found on the worms from St. James II,
but only rarely on the animals from the other locations, probably
because St. James II was much more frequently sampled than the
other beaches. When spermatophores were found on a high percentage
of females on a particular day -- for instance, on 60% of the
females in the collection for week 42 (11 - 17 August) -- on the
following day I found only a small number of females with spermato-
phores (10 - 15% in this case); on the third day none of the worms
had spermatophores.

This indicates that the transfer of spermatophores, fertilization, and the release of eggs, takes place very quickly, within two or three days, so that the chance of hitting one of these days in a bi-monthly sampling pattern is fairly low. This situation could account for the apparent lack of spermatophores on the females from St. James I and III, and for the failure of previous researchers to observe spermatophores.

Referring back to the above table, it will be seen that the four major occurrences of spermatophores coincide with the first, second, third, and fifth peaks or their associated valleys, and that these peaks occur approximately two months apart. There is a record of a few spermatophores for weeks 45 and 50, coinciding with the fourth peak on the graph.

From the observations and the data thus analyzed, it seems that the occurrence of spermatophores is related to spawning, which in turn leads to a decrease in numbers of gametogenic segments and their fullness. It is possible that the fourth and fifth peaks represent more or less continuous spawning activity, perhaps the last and major spawning period of the season. (As previously noted, only daily sampling would reveal every occurrence of spermatophores; it is quite possible that some of these occurrences were missed.)

The peaks and valleys of fullness were not analyzed; they are related to and tend to follow, the line of gametogenic segments. Although the two graphs for the other locations do not correspond exactly to St. James II, it is obvious that they follow the same general pattern of spawning peaks throughout the breeding season. The graphs for St. James I and III suggest that the timing of periodic outbreaks of spawning may differ slightly from beach

to beach.

The fact that the present study showed spawning from April till October, which does not correspond exactly to the findings of others, is not unexpected in view of the difference in locations, and the difference in approach of the studies. Furthermore, tropical specimens often have spawning periods that are more prolonged than those of their northern counterparts (Giese, 1959; Dr. John B. Lewis, personal communication).

The occurrence of four definite spawnings is, however, unusual for this species and for polychaetes in general. For some spionids there is evidence of more than one spawning. A study of Spio setosa showed two spawning periods (one spring and one fall) (Simon, 1967). Two spawning periods (one spring and one fall) have also been recorded for Polydora ciliata (Hannerz, 1956; Dorsett, 1961). Evidence was found for a winter spawning of Pygospio elegans (Söderström, 1920), and later for a summer spawning in the same area (Hannerz, 1956). Four spawnings were noted for the ariciid polychaete Scoloplos armiger in one year, occurring between the end of February and the middle of April (Gibbs, 1968). The four separate spawnings were attributed to different age groups of the polychaete, a spawning pattern found in some decapods and molluscs (Thorson, 1946). Although this is a possible explanation for the findings in this study, there was no evidence to support any such hypothesis; indeed, the increase in numbers of the breeding population, as well as the observed gradual increase in numbers of gametogenic segments and fullness, would suggest that individual specimens are spawning repeatedly.

The average number of segments for males and females

(Figs. 6 - 7) remained about the same throughout the year, with means of 90.795 to 95.694, and low standard deviations (see Fig. 7 B). This is quite different from earlier records of segment means of 150 - 200 for the same species in France (Mesnil, 1896; Fauvel, 1927).

Previously, the sex ratio has been reported as 1 : 1 (Mesnil, 1896). My own findings differ, the ratio ranging from 1 : 1.08 to 1 : 1.24 (female : male) for the three different locations, with an overall ratio of 1 : 1.15 for the combined locations. This overall ratio is shown by the chi-square test to differ from a 1 : 1 ratio because of a factor other than chance. (Fig. 14.)

SMALL WORMS

Very small worms, of 44 segments or less, were found at various times. They differed from adults in two major ways: they had six eyes (one or two pair are usually lost as the worm matures, most adults having only four eyes, or occasionally two), and the level of the beginning of the intestine was slightly less than one half the number of segments. This proportion of pre-intestinal segments to total number of segments continued until the worms reached a size of about 75 segments: i. e., 18 pre-intestinal : 40 total, 21 pre-intestinal : 54 total, 30 pre-intestinal : 65 total, 34 pre-intestinal : 74 total segments. In worms of 75 segments or more, the level of the intestine was always about segment 36. Whether this is due to both pre-intestinal and terminal growth, or whether the intestine itself "recedes" in some fashion, has not been determined.

The class of sex unidentified worms was broken down into four groups: worms of 44 segments or less, those of 54 segments or less, those that were 79 or less segments, and those belonging to the breeding population (having 80 or more segments). The number of individuals in each group has been calculated as a percentage of the entire sex unidentified population for a single date; the data from all three locations was combined. The result is shown in Figure 15. The main interest in this breakdown of sex unidentified animals by size, is to illustrate the presence of very small worms, of 44 segments or less.

According to several authors, the larvae of this species are ready to metamorphose and settle in the sand, when 20 - 36

segments in size (Claparède and Mecnikow, 1869; Hannerz, 1956).
The worms of 44 segments or less (the smallest found was 34 seg-
ments) had therefore probably settled recently. It is interesting
that these very small worms were first found in week 23 (31 March -
6 April), which is also the first known spawning time of the season;
the development of the larvae takes about a month (Hannerz, 1956)
or more (Joyner, 1962). It is possible that a small spawning did
occur at the beginning of February (see Fig. 10, weeks 13 - 15, and
Fig. 13) although the t-test analysis shows a poor probability for
this; it is also possible that some of the larvae produced in the
fall are overwintering. (This latter possibility would have a
biological advantage, since otherwise October-produced larvae would
be settling during November or December, when the sea is apt to be
very rough around Barbados, and the beaches are considerably de-
stroyed.)

The data as presented in Figures 6 - 7 suggests that the
young worms settling after spring and summer periods of spawning
reach adult size before the following winter. Figure 15 suggests
that some of these may even enter the fall breeding population.
A detailed study of the part of the population found at the low
tide levels of the beach is needed to confirm this.

No attempt was made to do any statistical analysis of the
percentage composition of the sex unidentified population, since
observations indicated that the collections were biased towards
larger worms: smaller worms seemed to be found in greater density
at low tide levels, and the sampling was usually carried out at
mid-tide level on the beach, where the larger worms were most dense.

GAMETES

The eggs agreed with previous descriptions of eggs for this species (Cunningham and Ramage, 1887; Mesnil, 1896; Joyner, 1962). They are thick-walled, highly vesicular, and have a large nucleus. When celomic oocytes were extracted by various mechanical means, the range in size did not vary significantly throughout the year (contrary to Joyner, 1962), and it appears that the developing oocytes remain attached to their formative tissue (peritoneal epithelium) until reaching nearly full size; they develop thereafter in the celom. The mean size for the mature elipsoid eggs is about 235 µ by 130 µ.

Histological studies showed an absence of seminal receptacles in the females. Both the male and female nephridia undergo development (enlargement is most evident) during the early part of the breeding season (Fig. 3, Fig. 16) as has been described for many spionids (Fage, 1905; Söderström, 1920; Goodrich, 1945; Simon, 1967). The females with spermatophores attached seem to have the most highly enlarged nephridia; such specimens are also full of bright green eggs; this color seems indicative of oocyte maturity. Since the initial enlargement of the nephridia does not seem to be great enough to allow for the passage of eggs, it seems possible that the nephridia enlarge just before, or while, the spermatophores are attached, to allow for the release of eggs. This may be a type of "sorting mechanism" (as discussed by Clark, 1965) to prevent the release of immature or unfertilized eggs.

It is well to note here, that although both Claparède and Mecnikow (1969) and Mesnil (1896) are often quoted as having seen

<u>Nerine</u> <u>cirratulus</u> release eggs directly into the sea-water, the former workers noticed eggs in their aquaria only after the eggs had been laid and division had begun, and Mesnil observed eggs laid by a single female in his aquarium, an artificial environment which may itself have stimulated artificial release of eggs.

The sperm were observed as fresh material in all stages of development except for the mature stage, which has been studied only in preserved material. Various stages from the secondary spermatocyte to the mature spermatid, are illustrated in Figure 17. The mature sperm (Fig. 17 F) has a small rounded acrosome (about 1.7 μ) with a dictyosome in the shape of a ring. The nucleus is very slightly elongated (about 3.4 μ); the middle-piece is of the same length; the filamentous tail is 34 μ or longer. Whether two partially fused mitochondrial spheres are present in the mid-piece or the mid-piece is composed of diffuse mitochondrial material could not be ascertained because living mature sperm were not available at the time this topic was studied. The axial filament can be seen passing through the middle-piece from the centriole to the tail.

The four mitochondrial spheres can be seen in the young spermatid (Fig. 17 D), and according to Franzén (1956), this characteristic, when retained in the mature sperm, is indicative of a primitive structure, although the spheres may undergo partial fusion in primitive types of sperm associated with pelagic fertilization. Franzén points out that many specialized sperm (especially among polychaetes) retain this primitive feature in the course of genesis but go on to a final condition in which the mitochondrial spheres are fused and the material spread out in a diffuse manner.

Although the mitochondrial mass usually elongates (hence an "elongated middle-piece" in aberrant sperm), it is not the overall length that is the criterion.

The incomplete data available for the spermatozoa of S. squamata is therefore an important piece of the puzzle, and the question remains whether the mature mid-piece represents the same mitochondrial spheres partially fused, or whether the mid-piece consists of diffuse mitochondrial material. (Fig. 17 F.) The sperm of S. squamata agree with the "primitive sperm" described by Franzén, in other respects.

	Primitive Sperm*	Sperm of S. squamata
Nucleus	spherical in young spermatid; spherical, conical, or elongated in mature sperm	spherical in young spermatid; elongated in mature sperm
Acrosome	small cap or cone; may have ring-shaped dictyosome in mature sperm	small cap in spermatid; with ring-shaped dictyosome in mature sperm
Middle-piece	4 - 5 mitochondrial spheres in both spermatid and mature sperm; partial fusion may occur in mature sperm	4 mitochondrial spheres in spermatid; mature sperm ???
Tail	long filament	long filament

_____

*The description here has been simplified to relevant details.

S. squamata does produce spermatophores, and therefore, fertilization is probably not pelagic. If the sperm do have mitochondrial spheres in the middle-piece, then Franzén's theory of a strictly pelagic fertilization associated with a primitive type of sperm, is questionable. However, if the middle-piece is definitely not of the "primitive character", this is further evidence to support Franzén's theory.

CLASSIFICATION

The presence of spermatophores on the worms, has never before been described for <u>S</u>. <u>squamata</u>. Söderström (1920) classified the family Spionidae into two subfamilies on the basis of reproductive morphology: the <u>Spioninae</u> and the <u>Spionidae</u>. The classification, simplified to relevant detail, is given below.

	Subfamily Spioninae (e.g., <u>Spio</u>, <u>Polydora</u>, <u>Pygospio</u>)	Subfamily Spionidae (e.g., <u>Scolelepis</u>, <u>Nerine</u>, <u>Nerinides</u>

Nephridia	great differences be-
	tween atokous and
	epitokous
	1) ♂ : complicated for
	   spermatophore formation
	2) ♀ : vents drawn up
	   to mid-dorsal line
		no marked differences
		between atokous and
		epitokous

Seminal Receptacles	in females	none in females
Eggs	thin-membraned	thick-membraned, highly vesicular
Sperm	long-headed	short-headed
Spermatophores, Copulation	presence	absence

Söderström believed the long-headed sperm to be associated with spermatophores, copulation, and internal fertilization, and the short-headed sperm to be characteristic of pelagic fertilization.

Hannerz (1956) classified the family Spionidae on the basis of larval morphology. His classification agreed with Söderström's, except for one relevant exception: on the basis of larval morphology, Hannerz suggested that the genus <u>Scolelepis</u> fit into neither of the two subfamilies, but shared the characteristics of both, being somewhat closer to the <u>Spionidae</u>. (It must be

remembered that the S. squamata of this study, was referred to as
Nerine cirratulus by both Söderström and Hannerz.)

Franzén (1956) conducted an extensive study of spermiogen-
esis and fertilization in the invertebrates. He pointed to Jäger-
sten's (1952) study of the archiannelid Protodrilus, as evidence
that the placing of spermatophores in the female seminal receptacle
does not necessarily require copulation. Franzén has also indicated:

"It is of importance to point out the occurrence

of spermatophores and of internal fertilization

following from it in the Spionids which possess an

aberrant morphology of the sperm [i.e., other than

the primitive type]. Here the sperms penetrate the

skin, starting from the receptacula, and fertilize

the eggs within the mother animal. Otherwise sperm-

atophores are not essential for internal fertili-

zation. The formation of spermatophores has, however,

the result that the sperms do not come into the free

water, and this is the direct cause of the aberrant

morphology of the sperm.

"Both Söderström and Hannerz have assumed that

an elongated sperm head is connected with the occur-

rence of spermatophores. Here it must be stressed

that the connection between the morphology of the

sperm and the biology of fertilization lies princi-

pally in the composition of the middle piece and

not in the structure of the head ..." (p. 409)

He emphasised the fact that most previous authors had referred
to both the head and middle-piece of the sperm when speaking of

"the head", and that a description of a "long-headed sperm" could refer to a sperm with a long head and a short middle-piece. Franzén proposed the idea that a "primitive type" of sperm was characterized by "a short rounded or oval head, a middle piece containing four mitochondrial spheres, and a tail consisting of a long filament" (p. 461), and suggested that deviations from this primitive type (which he associated with primitive discharge of sperms freely into water), were related to a special biology of fertilization; i.e., modified sperm implied spermatophores with or without copulation, and internal fertilization.

Simon's (1967) work on _Spio setosa_ (belonging to Söderström's subfamily _Spioninae_) supported this theory of Franzén's. _Spio setosa_ agreed with all the _Spioninae_ characteristics, except that the sperm had an elongated head but a short rounded middle-piece; the sperm were deposited in seminal receptacles, but spermatophores were never found.

Franzén's (1956) studies, Jägersten's (1952) findings, and Simon's (1967) work have all implied criticism of the Söderström (1920) and Hannerz (1956) classification. This study invalidates the classification still further.

Even if the _Nerine cirratulus_ of the previous studies were a separate variety of the species used in this study, it is probably very closely related, and it seems unlikely that features peculiar to this variety justify Hannerz's suggestion of classifying _Scolelepis_ as an intermediate between two subfamilies, one of which contained _Nerine_.

Orhage (1962) has disagreed with Söderström's classification, on the basis of adult morphology, and has suggested breaking down

the subfamily <u>Spionidae</u> in a way that would separate <u>Nerine</u> from <u>Scolelepis</u>. Again, the same criticism of Hannerz's suggestion for a modification of classification, applies here.

It appears then, that reproductive characteristics or any single criterion, such as larval or adult morphology, provide a poor basis for classifying spionid polychaetes, and that a great many more studies of individual species at all stages in development need to be made before subdividing the spionids.

CONCLUSION

It is clear that much more detail is needed on spionid reproduction. A study of the mature sperm of _S_. _squamata_ is needed to determine its precise morphology. It would be interesting to correlate a study of the planktonic larvae with the spawning periods. A study of the population of very small, recently settled worms would be interesting in terms of the population structure and the growth of the young into mature, breeding adults. More yearly studies of this sort would show whether the four spawning periods are typical of this species in Barbados. Detailed observation and histological work is necessary to determine the means of formation and transfer of the spermatophores, and the fertilization and release of eggs. Additional work is needed to determine the differences between the two varieties of _S_. _squamata_ found in Barbados, and to relate these to the description of the species in the literature.

This detailed study of breeding in _S_. _squamata_ has contributed a number of new observations on the reproductive morphology of this species, and its spawning behavior in Barbados. Many questions have been raised with respect to spionid reproduction in general. Broad comprehensive studies, although interesting in the detail they do manage to cover, are often misleading in the theories they produce based on scattered detail. It is not until more detailed studies are made that broad generalizations can be formed. Undoubtedly more detail on this and other species will modify still further our understanding of spionid reproduction.

SUMMARY

1. A detailed study of the breeding season in <u>Scolelepis</u> <u>squamata</u> was carried out by examination and records of the adult worms throughout a year, in Barbados.

2. Spermatophores were described for this species for the first time. The presence of spermatophores weakens the classification of spionids that had been made previously on the basis of reproductive characteristics and larval morphology.

3. At least four spawning periods were found by quantitative estimates of numbers of gametogenic segments and numbers of breeding individuals. The reliability of the four spawning peaks was shown by statistical analysis. The presence of spermatophores on the females at each of the four times, was given as evidence that the times were, in fact, spawning outbursts.

4. The four spawning periods occurred each about two months apart, in April, June, August, and October. November to January appeared to be an interphase between breeding seasons.

5. A description of the sperm was made; it has not been ascertained whether the sperm is of the type believed to be associated with internal fertilization. The means of formation and method of transfer of spermatophores are not known.

6. Histological evidence suggests internal fertilization; histological evidence also shows enlargement of both male and female nephridial canals, with a possible secondary enlargement of the female nephridial canals at the time of spawning.

7. It has been suggested that there are two varieties of <u>S</u>. <u>squamata</u> in Barbados, and that the one that was not used in

this study may be more closely related to the species described
in the literature.

8. The sex ratio was found to be 1 : 1.15 (female : male)
and significantly different from a 1 : 1 ratio.

9. It has been suggested that many more detailed studies
are needed on this and other species, before broad generalizations
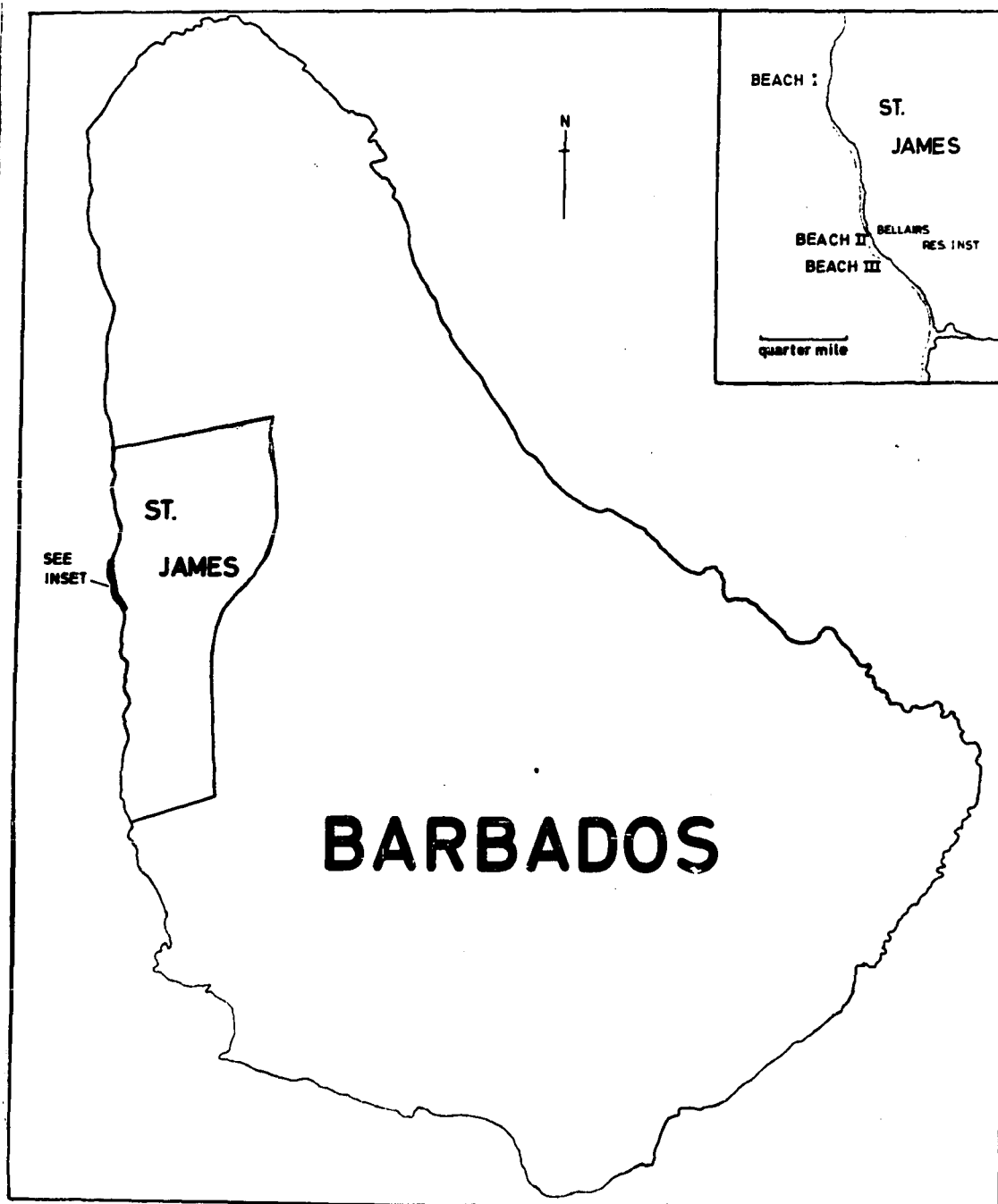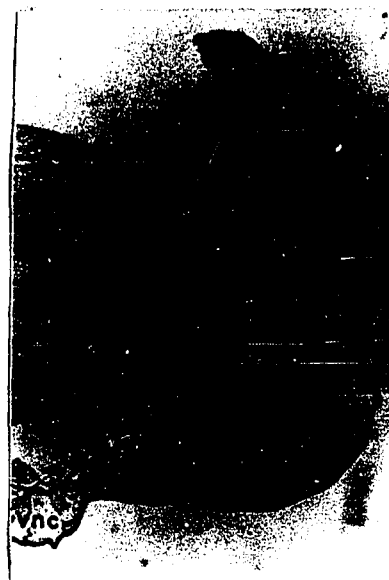concerning spionid reproduction and classification are made.

Figure 1. Map of Barbados with an inset showing the collection
locations on the St. James coast.

A - B. Spermatophores on females from St. James II, 14 June 1968,
        showing various shapes, ventral and dorsal points of
        attachment. (cs) (10X)

C. Spermatophore on female from   D. Detail of C, showing sperm-
    St. James II, 14 June 1968,   atophore binding matrix and
    showing lateral attachment.   sperm in the celom. (cs)
    (cs) (10X)   (10X)

Figure 2. Spermatophores on female worms.

do = developing oocytes; dbv = dorsal blood vessel; i = intestine;
mp = mucus papilla; n = nephridium; nbv = nephridial blood vessel;
o = oocyte; p = parapodium; sp = sperm; spph = spermatophore;
vnc = ventral nerve cord

A - B. Spermatophores on females from St. James II, 14 June 1968,
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C. Spermatophore on female from
St. James II, 14 June 1968,
showing lateral attachment.
(cs) (10X)

D. Detail of C, showing sperm-
atophore binding matrix and
sperm in the celom. (cs)
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A. Small undeveloped male nephridium from St. James I, 26 March 1968. (cs) (10X, 40X)

B. Enlarged male nephridium from St. James II, 14 June 1968, a spawning date. (cs) (10X)
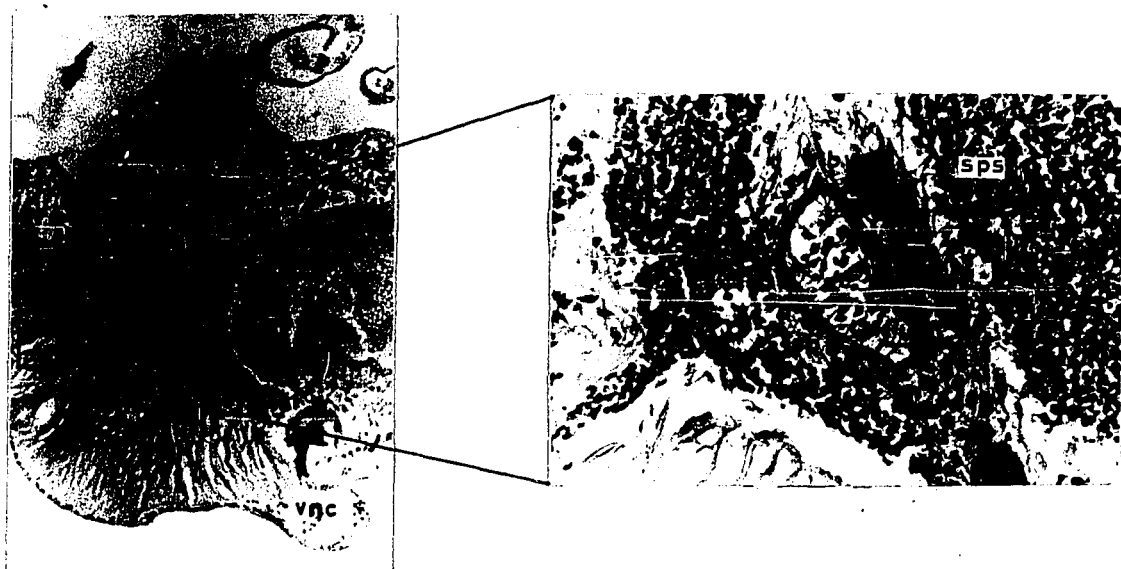
C. Detail of B, showing sperm and a mucus-like material in the nephridium. (40X)

Figure 3. Male nephridia.

dbv = dorsal blood vessel; i = intestine; mm = mucus-like material in nephridium; n = nephridium; nbv = nephridial blood vessel; psp = primary spermatocytes; sp = mature sperm in nephridium; sps = mature and developing sperm in celom; vnc = ventral nerve cord

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Week Number	Inclusive Dates		Beaches		
1	1	– 4 Nov			III
2	5	– 11 Nov		II	
3	12	– 18 Nov			III
4	19	– 25 Nov		II	
5	26 Nov	– 2 Dec	I		III
6	3	– 9 Dec		II	
7	10	– 16 Dec	I		III
8	17	– 23 Dec	I	II	
9	24	– 30 Dec			III
10	31 Dec	– 6 Jan	I		
11	7	– 13 Jan		II	
12	14	– 20 Jan	I		III
13	21	– 27 Jan	I	II	
14	28 Jan	– 3 Feb			III
15	4	– 10 Feb		II	
16	11	– 17 Feb	I		III
17	18	– 24 Feb	I	II	
18	25 Feb	– 2 Mar			III
19	3	– 9 Mar		II	
20	10	– 16 Mar	I		III
21	17	– 23 Mar		II	
22	24	– 30 Mar	I		III
23	31 Mar	– 6 Apr		II	
24	7	– 13 Apr	I		III
25	14	– 20 Apr		II	
26	21	– 27 Apr	I		III
27	28 Apr	– 4 May		II	
28	5	– 11 May	I		III
29	12	– 18 May		II	
30	19	– 25 May	I		III
31	26 May	– 1 Jun		II	

Figure 4. Table of week numbers, corresponding dates, and St. James beaches at which collections were made.

| Week Number	Inclusive Dates			Beaches		
32	2	–	8 Jun	I		III
33	9	–	15 Jun		II	
34	16	–	22 Jun			
35	23	–	29 Jun	I		III
36	30 Jun	–	6 Jul		II	
37	7	–	13 Jul	I		III
38	14	–	20 Jul		II	III
39	21	–	27 Jul	I	II	
40	28 Jul	–	3 Aug		II	III
41	4	–	10 Aug	I	II	
42	11	–	17 Aug		II	III
43	18	–	24 Aug	I	II	
44	25	–	31 Aug		II	III
45	1	–	7 Sept	I	II	
46	8	–	14 Sept		II	III
47	15	–	21 Sept	I	II	
48	22	–	28 Sept		II	III
49	29 Sept	–	5 Oct	I	II	
50	6	–	12 Oct		II	III
51	13	–	19 Oct	I	II	
52	20	–	26 Oct		II	III
53	27 Oct	–	2 Nov		II	
54	3	–	9 Nov	I	II	
55	10	–	16 Nov		II	
56	17	–	23 Nov		II	
57	24	–	30 Nov		II	

Figure 4 (con't). Table of week numbers, corresponding dates, and St. James beaches at which collections were made.

1/4 FULL   1/2 FULL   3/4 FULL   4/4 FULL
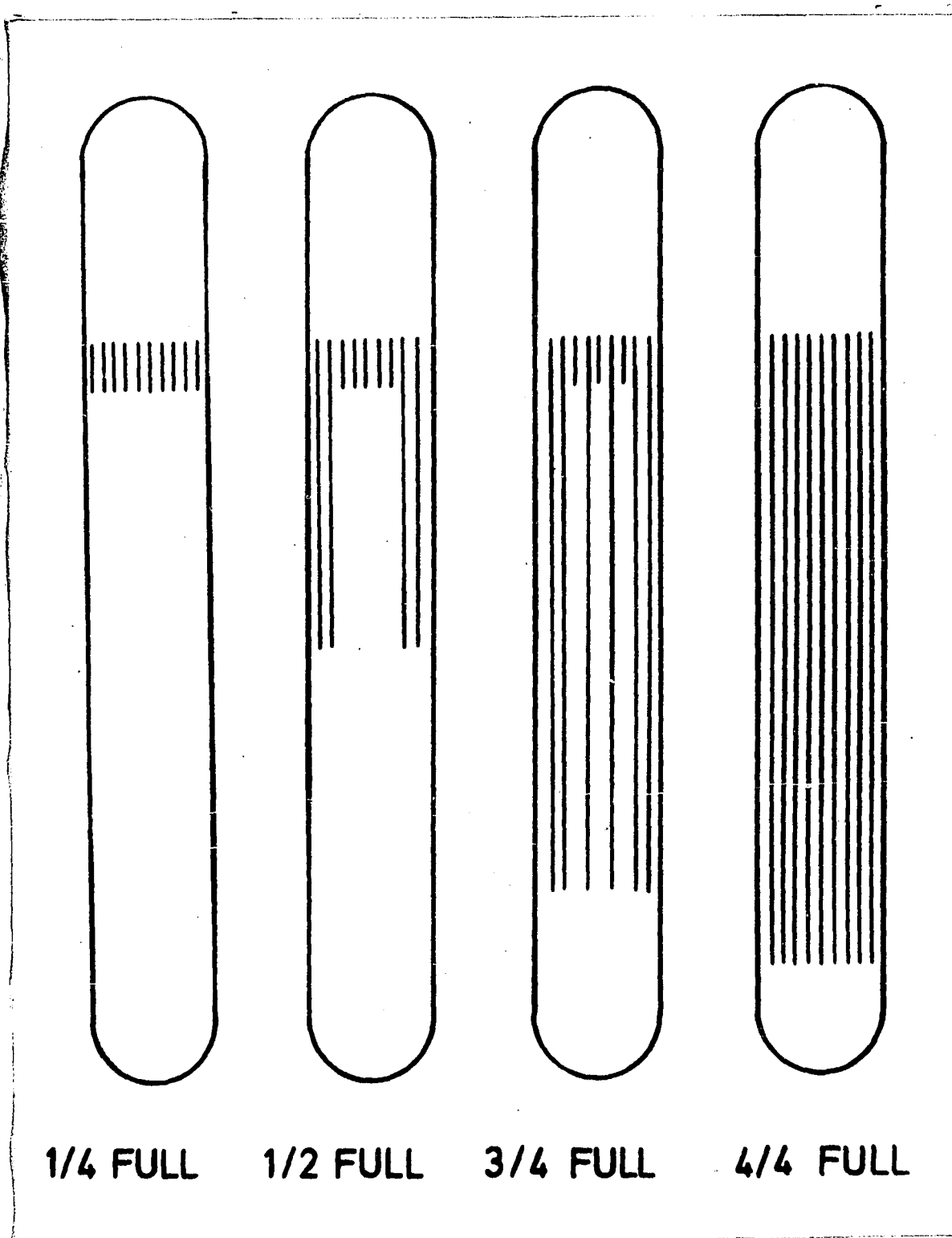
Figure 5.   Criteria for the condition of fullness.
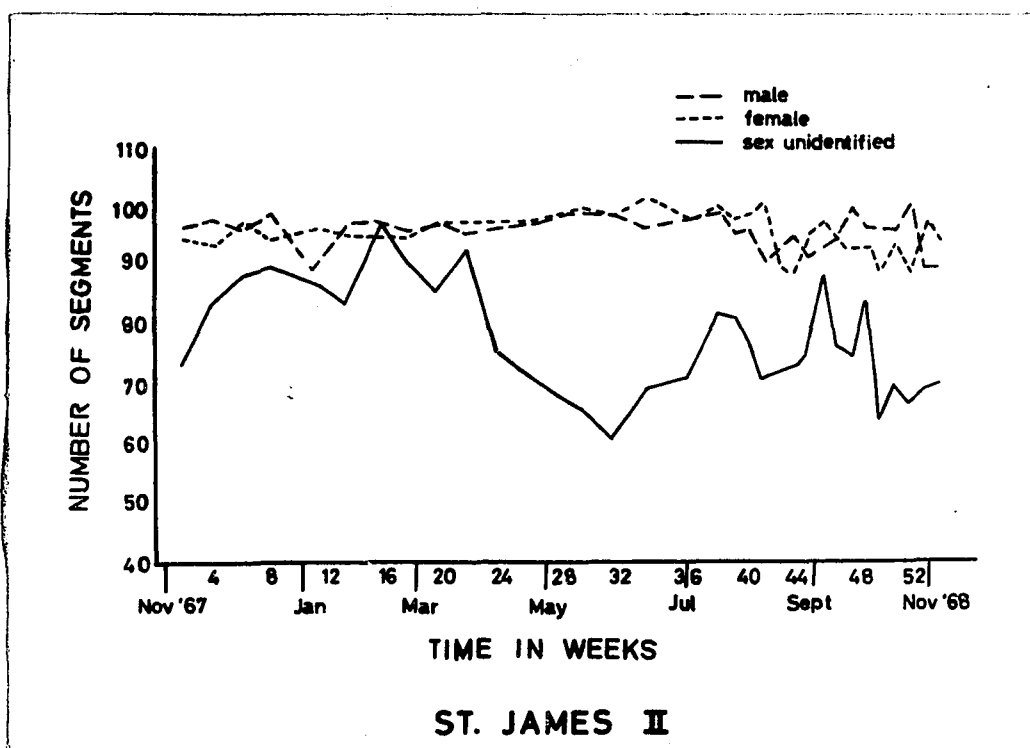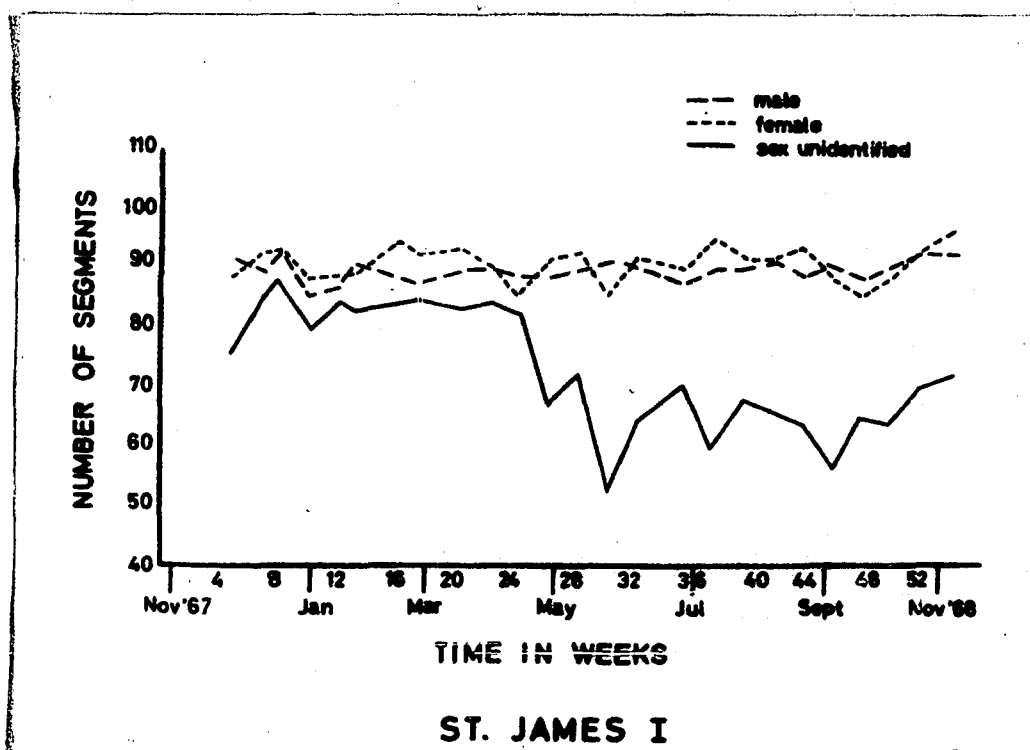
Figure 6. Means of segment numbers plotted against time.

A.
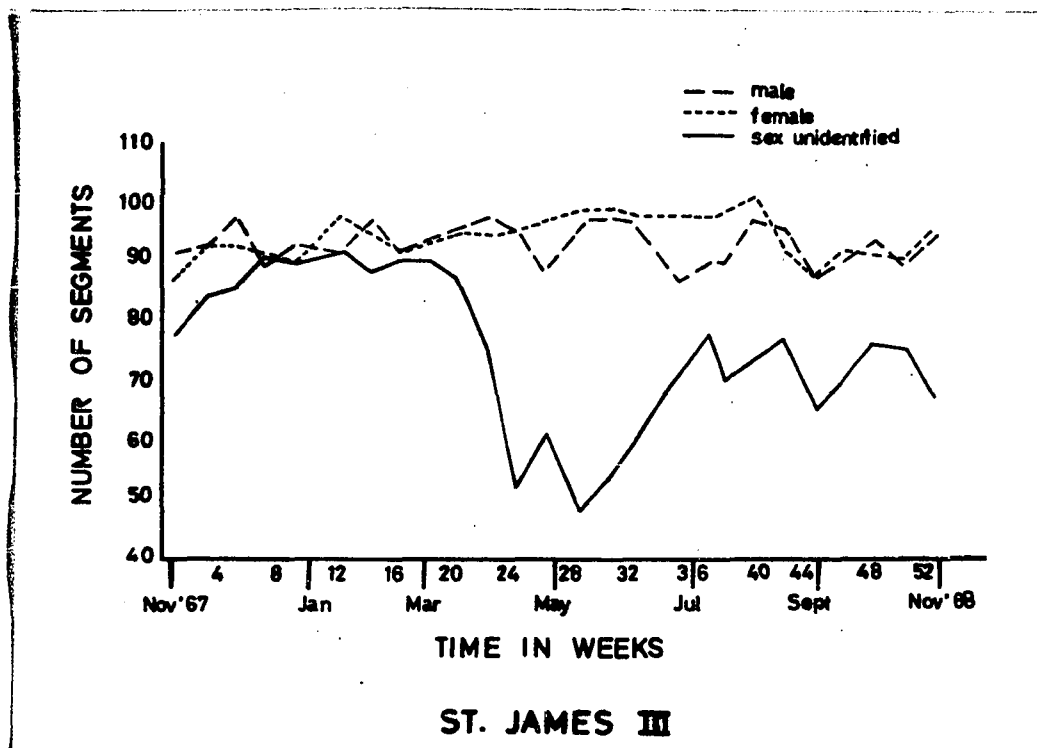
ST. JAMES III

B.

Location	Mean Number of Segments	Standard Deviation
St. James I	90.795	6.1
St. James II	95.694	8.8
St. James III	93.812	9.1

Figure 7. A. Means of segment numbers plotted against time.
B. The standard deviations of mean numbers of segments of adult worms for each beach.
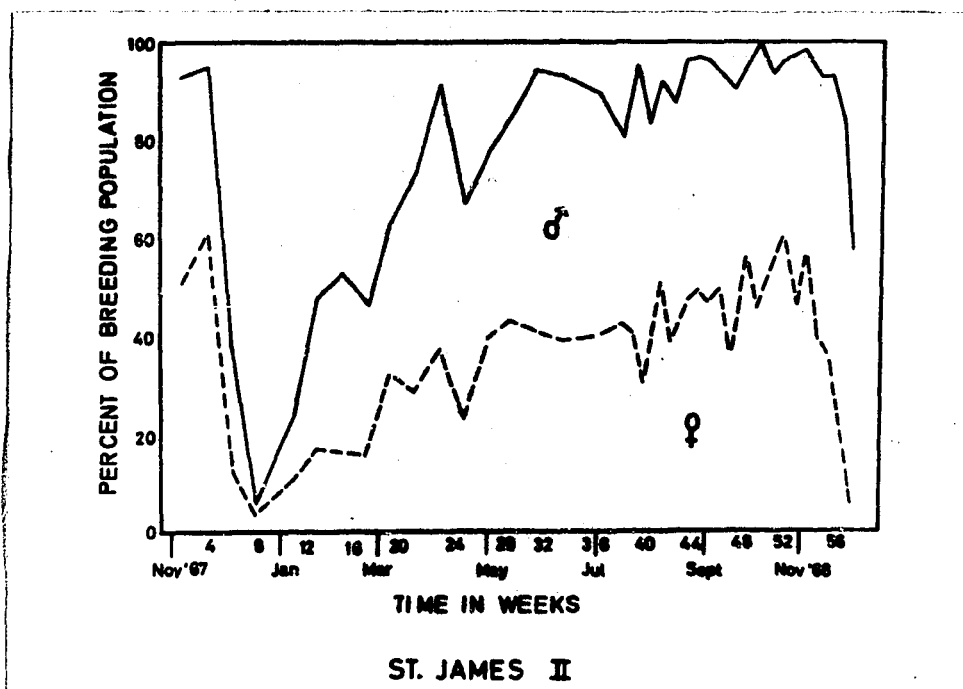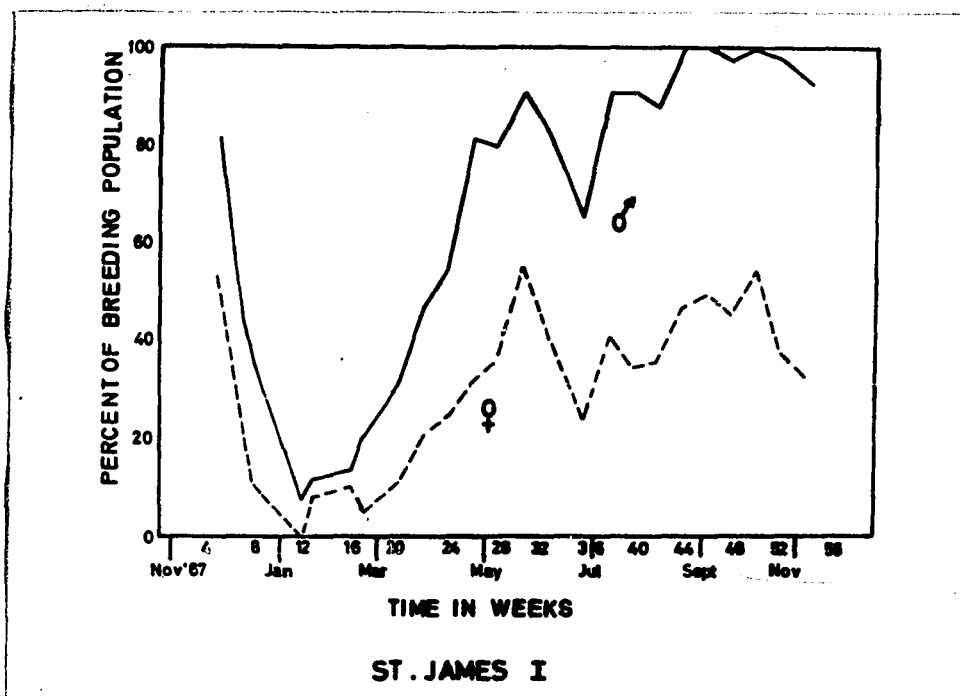
Figure 8. Numbers of each sex expressed as a percentage of the corresponding breeding population, plotted against time. Area below the dotted line includes females; area below the solid line includes males and females.
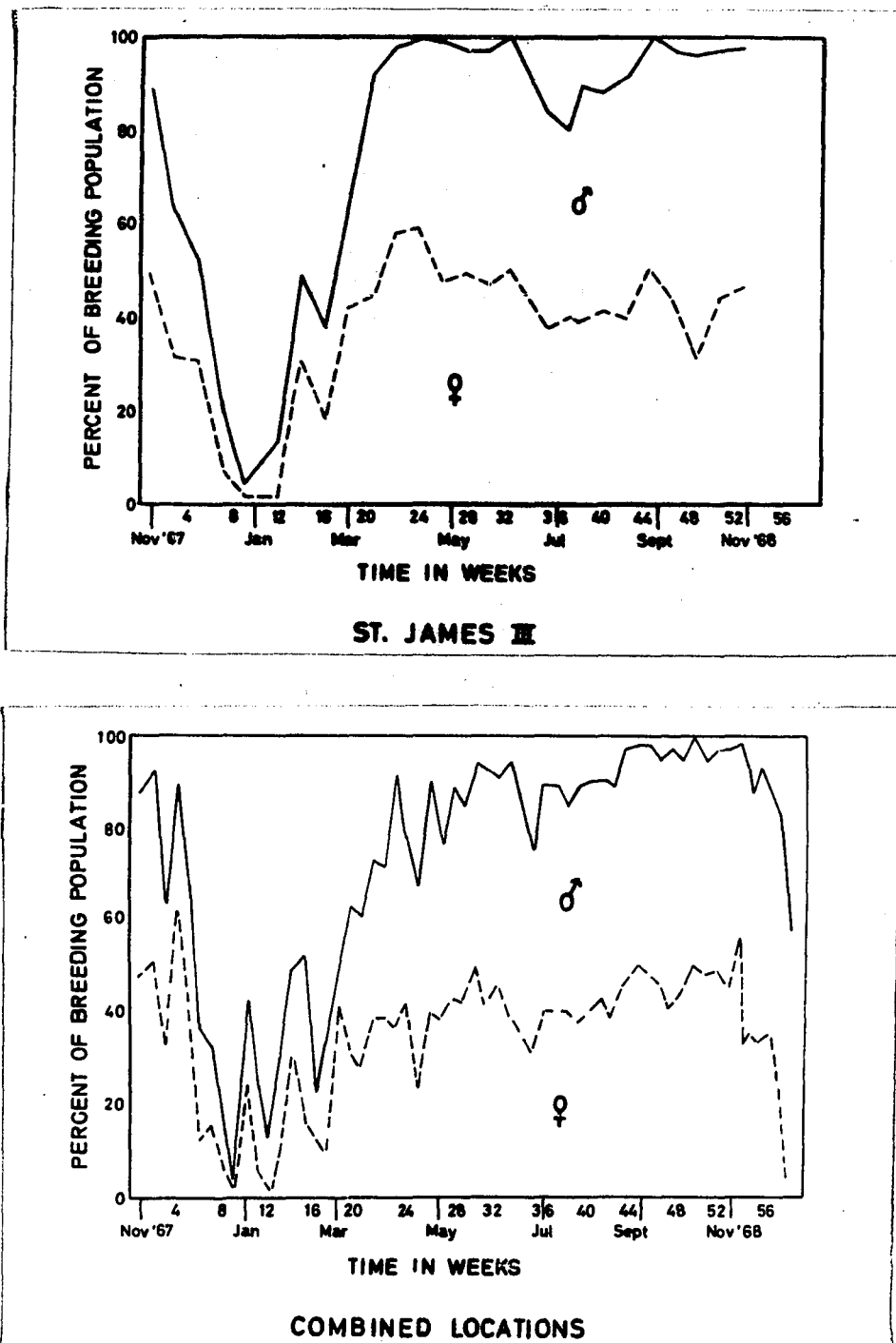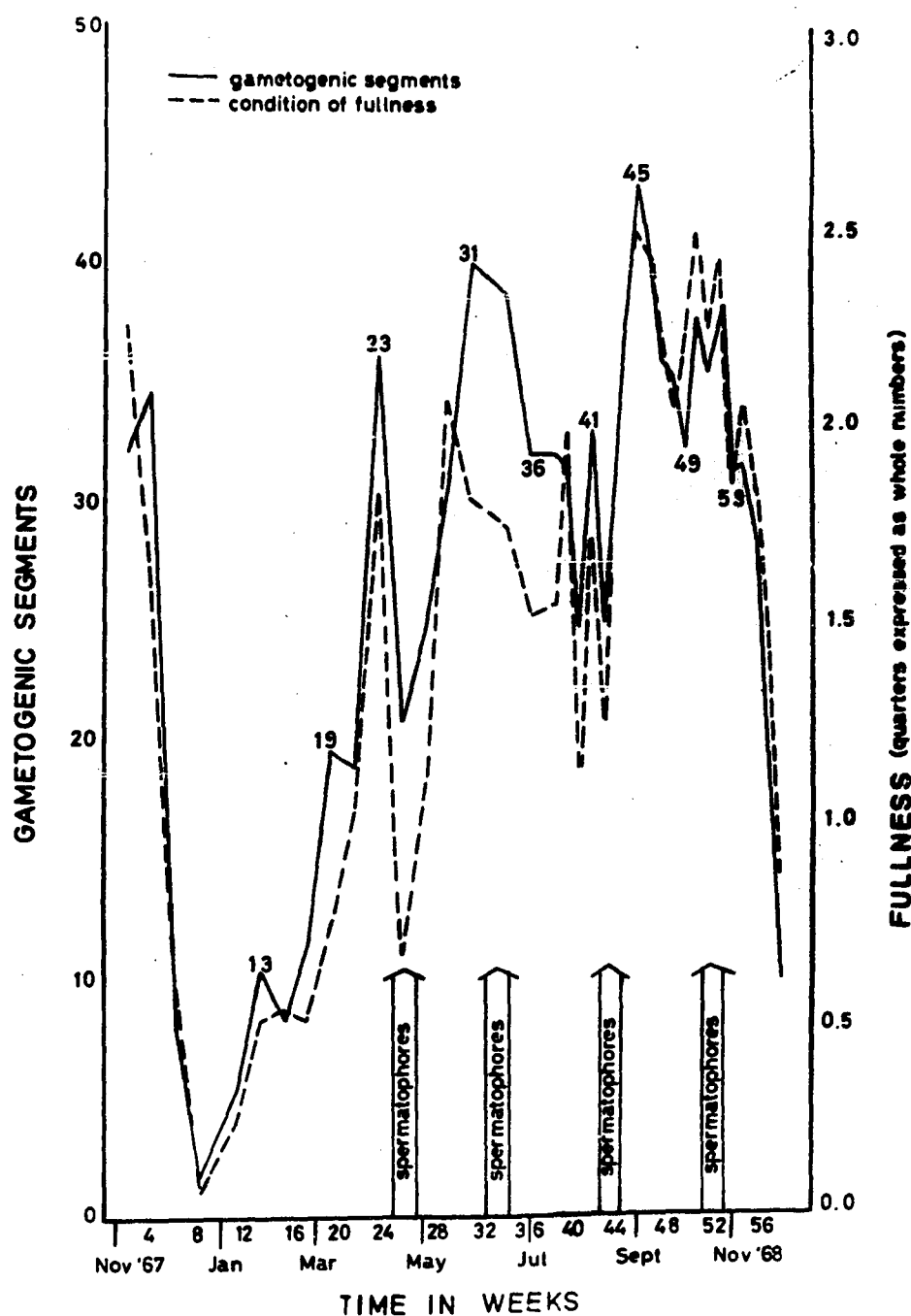
Figure 9. Numbers of each sex expressed as a percentage of the corresponding breeding population, plotted against time. Area below the dotted line includes females; area below the solid line includes males and females.
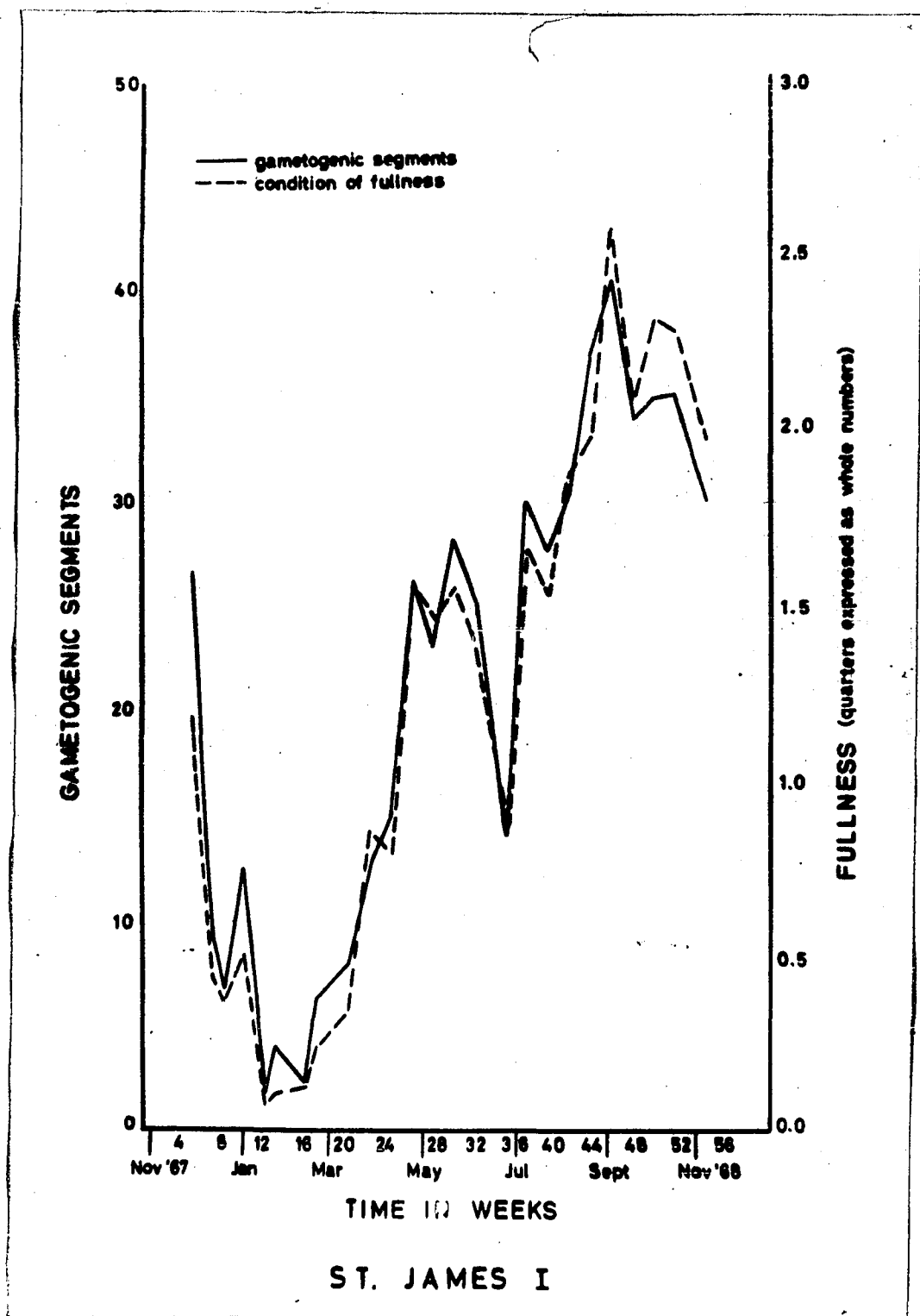
Figure 10. Weighted means of gametogenic segments and fullness, plotted against time. Fullness estimates, although recorded as fractions for the raw data (see Fig. 5), have been converted to whole numbers (1/4 = 1, 1/2 = 2, etc.) for weighting, and are thus expressed as decimals.
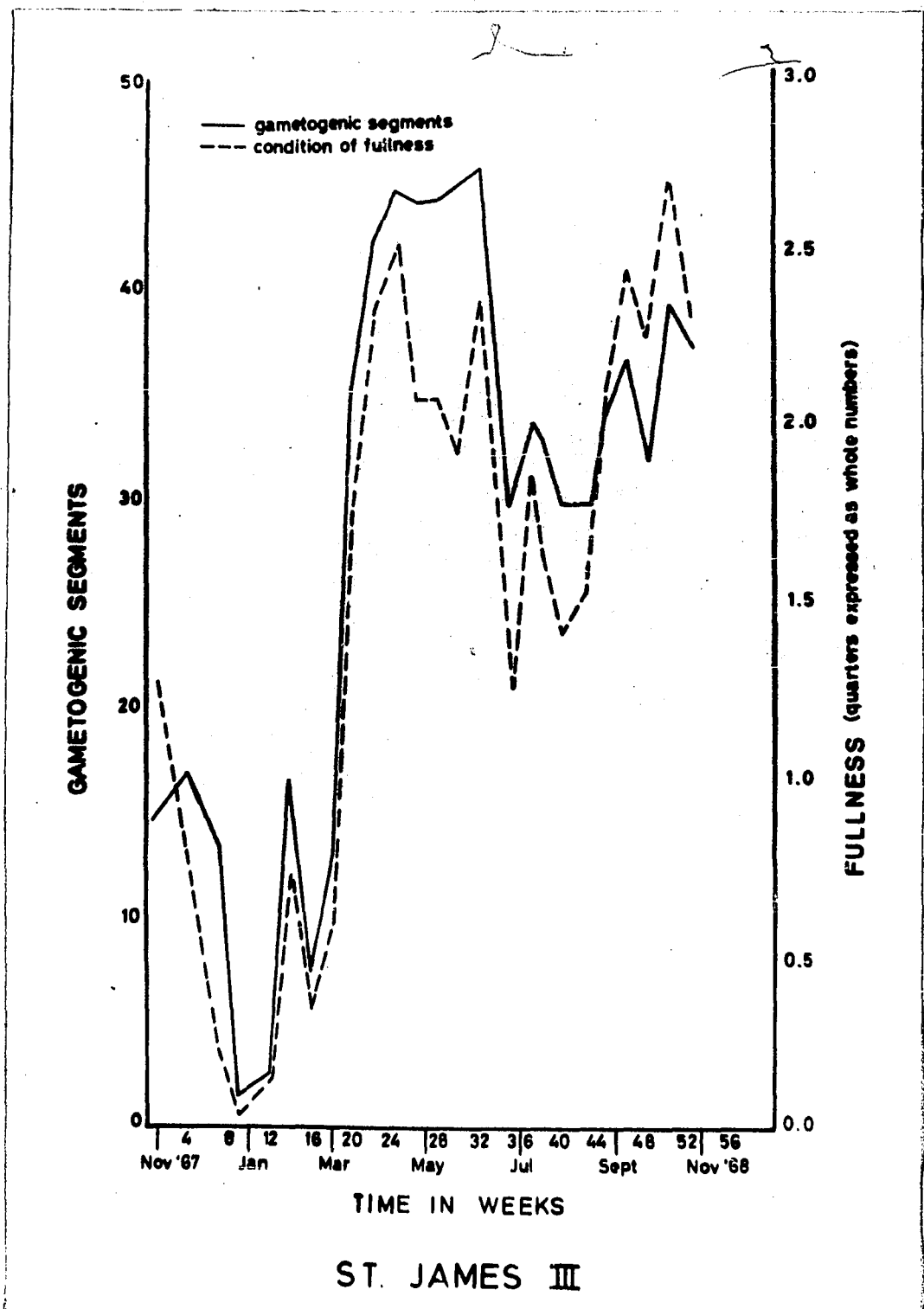
Figure 11. Weighted means of gametogenic segments and fullness plotted against time. Fullness estimates, although recorded as fractions for the raw data (see Fig. 5), have been converted to whole numbers (1/4 = 1, 1/2 = 2, etc.) for weighting, and are thus expressed as decimals.

Figure 12. Weighted means of gametogenic segments and fullness plotted against time. Fullness estimates, although recorded as fractions for the raw data (see Fig. 5), have been converted to whole numbers (1/4 = 1, 1/2 = 2, etc.) for weighting, and are thus expressed as decimals.

T-Test Between Weeks	PROBABILITIES P < 0.001	P ≤ 0.05	Other
8 : 13		X	
13 : 15			0.05 P < 0.1
15 : 19	X		
19 : 21			P < 0.5
21 : 23	X		
23 : 25	X		
25 : 31	X		
31 : 36	X		
36 : 40	X		
40 : 41		X	
41 : 42	X		
42 : 45	X		
45 : 49		X	
49 : 50			0.05 < P < 0.1
49 : 52			P < 0.5
50 : 51		X	
50 : 53	X		
51 : 52			0.10 < P < 0.2
51 : 53			0.10 < P < 0.2
51 : 54			0.10 < P < 0.2
52 : 53			P < 0.5
53 : 57	X		

Figure 13. Probabilities from the t-test analysis of reliability of peaks and valleys of Figure 10, means of gametogenic segments. Since the values used for Figure 10 were weighted with the breeding population percentage, the individual values of gametogenic segments were weighted before using them in the t-test.

Location	Number of Collections	No. ♀	No. ♂	Ratio ♀ : ♂	Chi-Square Test Probabilities
St. James I	25	444	550	1 : 1.24	P < 0.005
St. James II	35	975	1120	1 : 1.15	P < 0.005
St. James III	26	561	608	1 : 1.08	P < 0.100
Combined	86	1980	2278	1 : 1.15	P < 0.005

Figure 14. Table of sex ratios from each location and combined locations, with the resulting probabilities from the chi-square tests. The ratios from the first two locations and the combined data indicate that the sex ratio is probably not a 1 : 1 ratio.
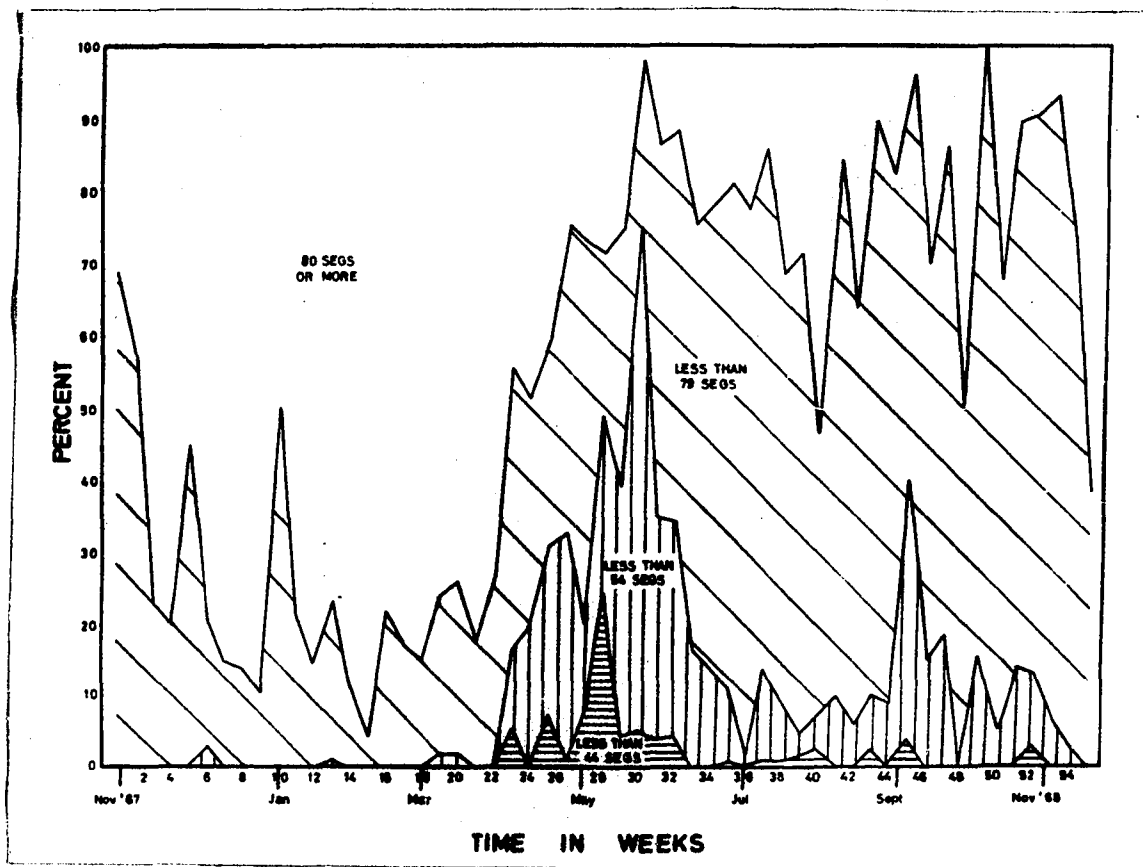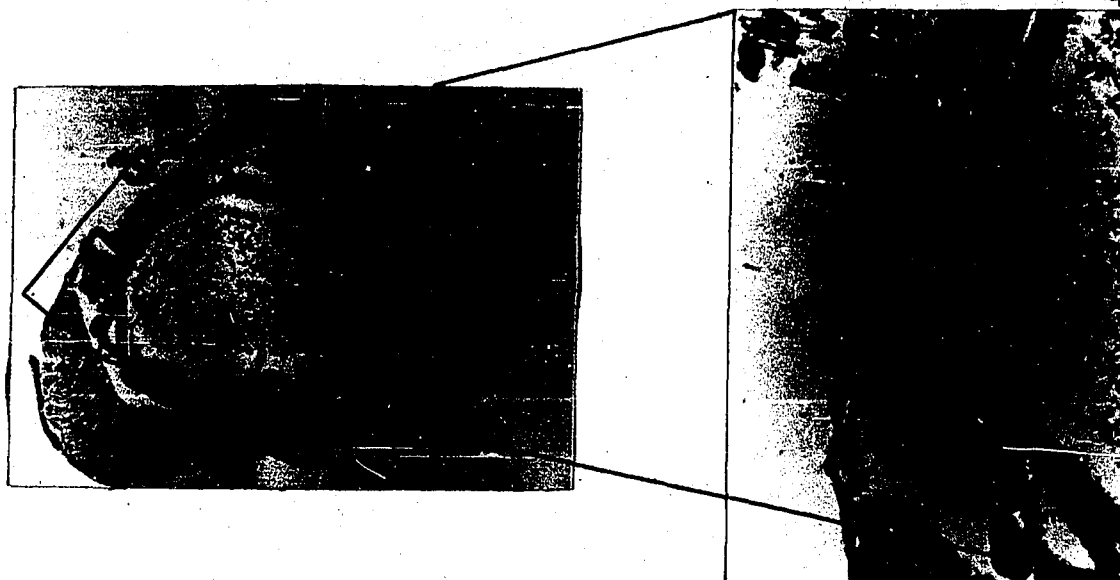
Figure 15. Size groups of sex unidentified worms taken as percentages of the total number of sex unidentified worms for a particular date, and plotted against time.

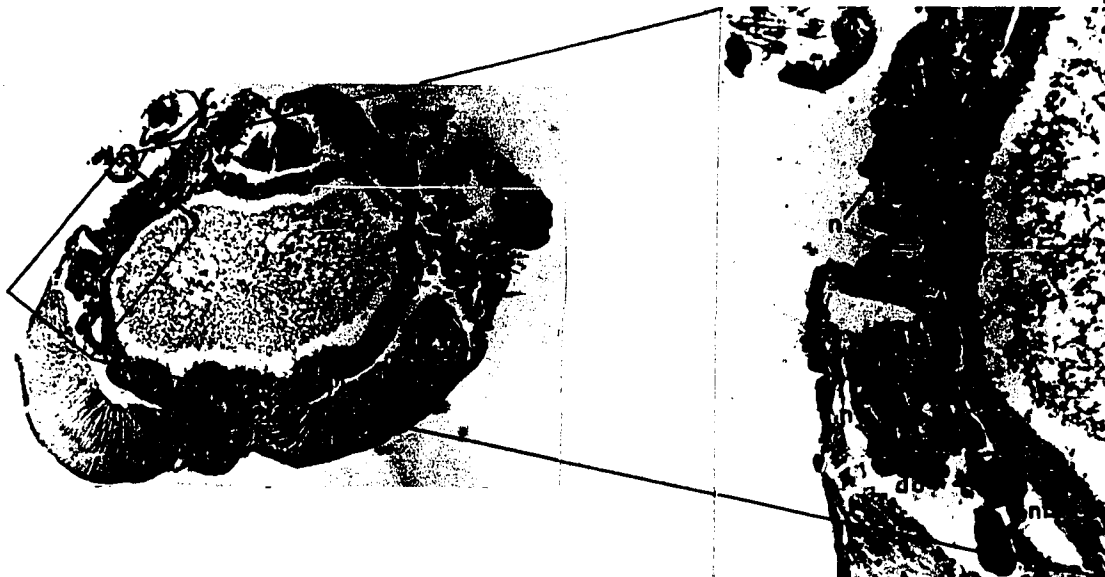A. Small undeveloped female nephridium from St. James I, 26 March 1968. (cs) (10X, 40X)

B. Enlarged nephridium at a later stage in development, from St. James I, 26 March 1968. (cs) (10X)

C. Most highly enlarged nephridium from St. James II, 14 June 1968, a spawning date; spermatophore is attached to another region of the same specimen. (cs) (10X)

Figure 16. Development of female nephridia.

do = developing oocytes; dbv = dorsal blood vessel; i = intestine; n = nephridium; nbv = nephridial blood vessel; o = oocyte; vnc = ventral nerve cord

A. Small undeveloped female nephridium from St. James I, 26 March
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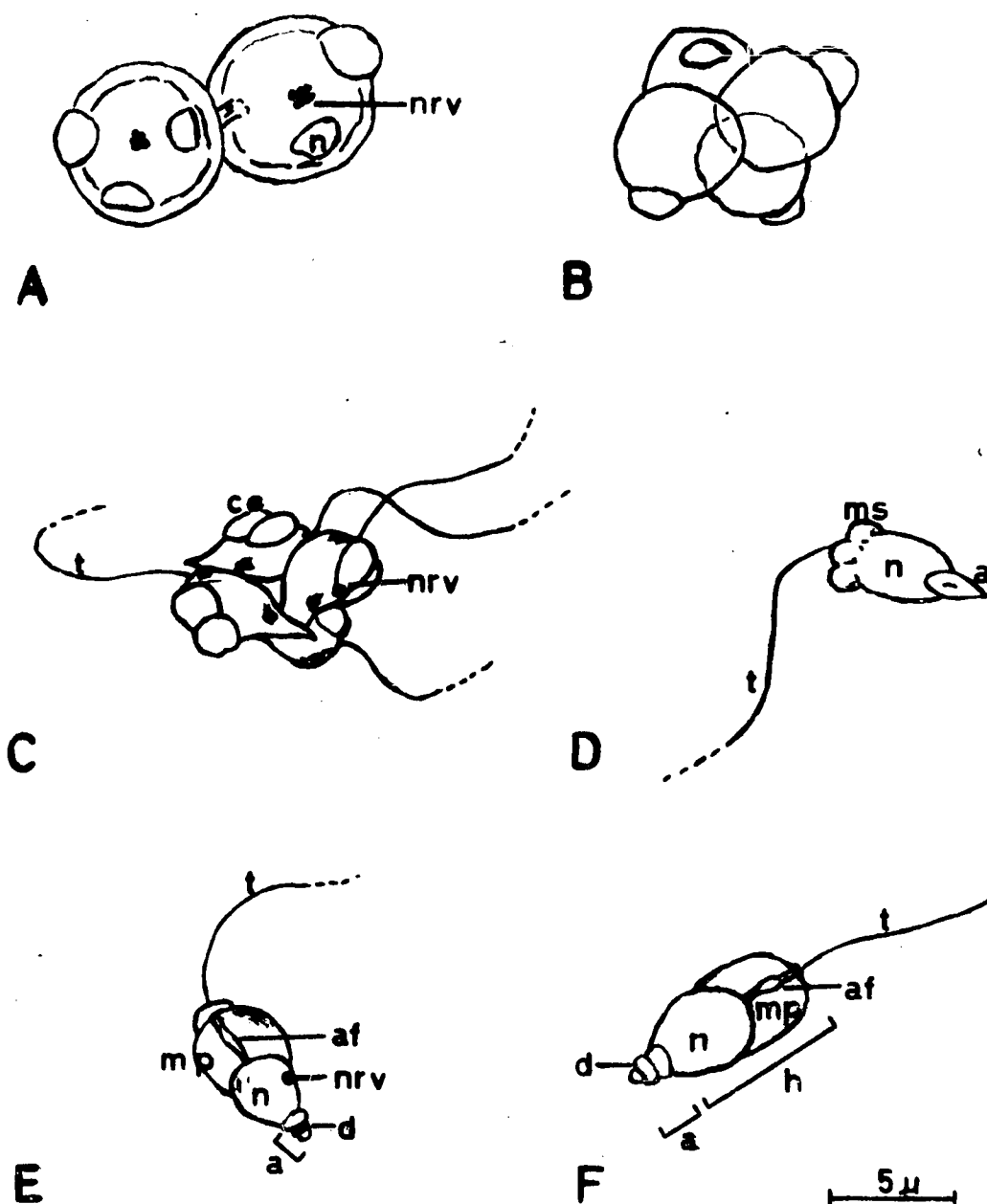n = nephridium; nbv = nephridial blood vessel; o = oocyte; vnc =
ventral nerve cord

Figure 17.   Stages in sperm development. A. Secondary spermato-
cyte. B. Four spermatids, very early stage. C. Four
spermatids, late stage. D. Spermatid with mitochon-
drial spheres. E. Spermatid with well-developed acro-
some. F. Mature sperm. A - E drawn from live sperm,
neutral red staining; F from section of preserved
specimen, PAS.

a = acrosome; af = axial filament; ce = cytoplasmic extrusion;
d = dictyosome; h = head; mp = middle-piece; ms = mitochondrial
spheres; n = nucleus; nrv = neutral red vacuole; t = tail

LITERATURE CITED

Claparède, E., and E. Mecnikow. 1869. Beiträge zur Kenntniss der Entwikelunggeschichte der Chaetopoden. Zeitschr. für Wiss. Zool. 19 (2) : 163 - 205.

Clark, R. B. 1965. Endocrinology and the reproductive biology of polychaetes. Oceanogr. Mar. Biol. Ann. Rev. 3 : 211 - 255.

Cunningham, J. T., and G. A. Ramage. 1887. The Polychaeta Sedentaria of the Firth of Forth. Trans. Roy. Soc. Edinburgh XXXIII : 635 - 684.

Dean, D., and P. Hatfield. 1963. Pelagic larvae of Nerinides agilis (Verrill). Biol. Bull. 124 (2) : 163 - 169.

Dorsett, D. A. 1961. The reproduction and maintenance of Polydora ciliata (Johnst.) at Whitstable. J. Mar. Biol. Ass. U. K. 41 (2) : 383 - 396.

Eisig, H. 1887. Capitelliden des Golfes von Neapel. Fauna und Flora des Golfes von Neapel 16 : 261 - 570.

Fage, L. 1905. Les organes segmentaires des Spionidiens et la maturité sexuelle. Compt. Rend. Soc. Biol. 59 : 452 - 454.

1906. Recherches sur les organes segmentaires des Annélides Polychètes. Ann. Sci. Nat. Zool. 9 (3) : 261 - 410.

Fauvel, P. 1927. Polychetes Sédentaires. Addenda aux Errantes, Archiannélides, Myzostomairs. Faune France 16 : 1 - 494.

1959. Annélides Polychètes: Reproduction. In Traité de Zoologie (P. Grassé, ed.), Masson et Cie., Paris. vol. 5 (1) : 132 - 156.

Franzén, Å. 1956. On spermiogenesis, morphology of the spermatozoon, and biology of fertilization among invertebrates. Zool. Bidrag Från Uppsala 31 : 355 - 482.

George, J. D. 1966. Reproduction and early development of the spionid polychaete, Scolecolepides viridis (Verrill). Biol. Bull. 130 (1) : 76 - 93.

Gibbs, P. E. 1968. Observations on the population of Scoloplos armiger at Whitstable. J. Mar. Biol. Ass. U. K. 48 (1) : 225 - 254.

Giese, A. C. 1959. Comparative physiology: annual reproductive cycles of marine invertebrates. Ann. Rev. Physiol. 21 : 547 - 576.

Goodrich, E. S. 1945. The study of nephridia and genital ducts
since 1895. Quart. J. Micr. Sci. 86 : 113 - 392.

Hannerz, L. 1956. Larval development of the polychaete families
Spionidae Sars, Disomidae Mesnil, and Poecilochaetidae
N. Fam. in the Gullmar Fjord (Sweden). Zool. Bidrag Från
Uppsala 31 : 1 - 204.

Hartman, O. 1941. Some contributions to the biology and life
history of Spionidae from California. Allan Hancock Pacif.
Exped. 7 : 289 - 324.

Jägersten, G. 1952. Studies on the morphology, larval develop-
ment, and biology of Protodrilus. Zool. Bidrag Från
Uppsala 29 : 427 - 511.

Joyner, A. 1962. Reproduction and larval life of Nerine cirratulus
(Delle Chiaje) family Spionidae. Proc. Zool. Soc. London
138 : 655 - 666.

Mesnil, F. 1896. Études de morphologie externe chez les Annélides.
I. Les Spionidiens des côtes de la Manche. Bull. Sci.
Fr. Belg. 29 : 110 - 287.

Orhage, L. 1962. Beiträge zur Kenntnis der Spiomorphen Poly-
chaeten. Acta. Univ. Upsalabstr. Uppsala Diss. Sci. 50 :
5 - 16.

Pettibone, M. 1963. Revision of some genera of polychaete worms
of the family Spionidae, including the description of a
new species of Scolelepis. Proc. Biol. Soc. Wash. 76 :
89 - 104.

Simon, J. L. 1967. Reproduction and larval development of Spio
setosa (Spionidae : Polychaeta). Bull. Mar. Sci. 17 (2)
398 - 431.

Söderström, A. 1920. Studien über die Polychaeten familie Spion-
idae. Inaug. Diss. Uppsala, Almqvist and Wiksell, 286 pp.

Thorson, G. 1946. Reproduction and larval development of Danish
marine bottom invertebrates. Medd. Komm. Dan. Fis. Havund.
Serie: Plankton 4 (1) : 523 pp.

1950. Reproduction and larval ecology of invertebrates.
Biol. Rev. 25 : 1 - 45.