

10. SIPUNCULA

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I. INTRODUCTION

Studies of development in sipunculans were first published in the latter part of the 19th century, but it has been only recently that reproductive cycles have been investigated and that sufficient comparative information has become available for any understanding of the trends and diversity of developmental patterns. In addition to their more usual means of sexual reproduction, a few sipunculans have been found to use different modes of asexual reproduction. Processes of transverse fission and budding have been described by Rajulu and Krishnan (1969), Rice (1970), and Rajulu (1975). Also, an interesting example of parthenogenesis has been discovered and is under current study (Pilger, 1987, 1989).

A number of descriptive studies have been made on breeding cycles of sipunculans; however, there have been no interpretive analyses or experimental considerations of controlling mechanisms. In connection with these studies a few incidental observations have been reported on fecundity. Major references on reproductive cycles are those of Gonse (1956a, b), Rice (1975a), and Gibbs (1975, 1976).

II. ASEXUAL PROPAGATION AND REGENERATION

Although sexual reproduction is the common mode of propagation among sipunculans, asexual reproduction has been reported to occur in two species, *Aspidosiphon brocki* and *Sipunculus robustus* (Rice, 1970 and Rajulu and Krishnan, 1969 respectively). In the former it occurs as a type of transverse fission and in the latter as either budding or transverse fission.

Aspidosiphon brocki is a relatively small tropical species that inhabits borings in coral rock and rubble. It ranges in length from 3 to 10 mm when the anterior extensible introvert is retracted. When extended, the introvert is nearly as long as the trunk and is covered with prominent spines. Asexual reproduction occurs by the constriction of the body and the subsequent detachment of the posterior one-fifth of the animal to form a new individual (Rice, 1970). The juvenile formed from this process incorporates the following tissues from the adult parent: posterior body wall, posterior portion of the intestinal spiral, and some coelomocytes. The remainder of the new individual develops by regeneration. Posterior to the constriction, in the dividing animal, regenerating tissues form an invagination which everts after detachment to become the anterior trunk and introvert of the juvenile. Similarly, an invagination just anterior to the constriction is everted after detachment to form the newly regenerated posterior end of the parent.

Sipunculus robustus, a large sand-burrowing species which attains lengths of 30 cm, has been reported to reproduce asexually by both transverse fission and budding (Rajulu and Krishnan, 1969; Rajulu, 1975). Transverse fission occurs in the posterior one-half of the parent individual, usually as a single constriction resulting in one juvenile. However, as many as five simultaneous constrictions have been observed, producing five new individuals. Lateral buds, occurring as swollen masses of undifferentiated cells posterior to the anus, have been observed on rare occasions to give rise to juvenile worms.

Asexual reproduction in *Sipunculus robustus* was observed only in the laboratory when animals were kept in stale sea water. Thus it may be an anomaly induced by unfavourable environmental conditions. In *Aspidosiphon brocki*, on the other hand, asexual reproduction is a natural phenomenon. Asexually reproducing worms have been found in 15 per cent of animals collected in the field. Although fission may be completed in the laboratory, constrictions have never been initiated in animals maintained in the laboratory.

Whether asexually reproducing populations are capable of sexual reproduction remains to be determined. Mature gametes have not been detected in the coelom of *Aspidosiphon brocki*. However, the presence of gonadal tissue at the base of the ventral retractor muscles indicates the potential for sexual reproduction.

Asexual reproduction is a relatively recent discovery in the Sipuncula, but the regenerative abilities of sipunculans have been known since 1883 when Bülow demonstrated that introverts of sipunculans could be regenerated. Experimenting on *Golfingia vulgaris* and *Aspidosiphon mulleri*, he removed 3 to 7 mm of the extended anterior introverts, including tentacles, brain and parts of the oesophagus, contractile vessel, nerve cord, and retractor muscles. After amputation, the wound was closed by contraction of the circular musculature of the body wall and, within three to five weeks, a functional introvert was fully regenerated. This regenerated introvert was easily distinguished from the rest of the body by its light colour and greater transparency. Peebles and Fox (1933) in studies of *Themiste* (as *Dendrostoma*) *zostericola* reported that tentacles would regenerate as long as

the stumps were retained, but after two to three weeks they found no regeneration when tentacles were severed at the base. Schleip (1934a, b) investigated histological changes during regeneration of the introvert in two species of sipunculans, *Phascolion strombi* (as *Phascolosoma minutum*) and *Golfingia minuta*. He reported that regenerative material had two sources: (1) coelomocytes which differentiated into mesodermal tissue, (2) certain cells of the ventral nerve cord which formed epidermis and nervous tissue. Cells from both sources migrated to the point of injury, forming a mass from which the regenerated tissues differentiated. Schleip (1934a, b) and later Wegener (1938) both reported a "Regenerationsstrang" in the ventral nerve cord. Wegener (1938) looked at regeneration of the introvert in seven species of sipunculans and noted that among these species the regeneration strand varied in its distinctiveness and in its appearance relative to the time of injury. In *Phascolion strombi*, *Aspidosiphon muelleri*, *Sipunculus nudus*, and probably an unidentified species of *Golfingia*, regeneration material was described as a distinct structure in the normal nerve cord. It was most clearly distinguished in *Phascolion strombi*, in which it was separated by a fissure from the ventral nerve cord. In *G. minuta*, *G. vulgaris*, and *Phascolosoma granulosum* the regeneration strand was formed during regeneration from scattered cells lying in the nerve cord. Among the seven species studied by Wegener (1938) there was a striking resemblance in the cytological characters of the regeneration cells originating in the nerve cord. They were generally of elongate form, arranged in strands in the longitudinal axis of the nerve cord, and significantly larger than ganglion cells. Characteristically, the cytoplasm stained darkly with haematoxylin and the nuclei were pale with a distinct nucleolus.

Regeneration of the posterior body of sipunculans has been noted in *Phascolopsis gouldii* (as *Sipunculus gouldii*) (see Andrews, 1890) and in species of *Siphonosoma* (see Spengel, 1912; Fischer, 1925). The only experimental study of regeneration in the posterior body was made by Schleip (1935) on the species *Phascolion strombi* and *Golfingia minuta*. In experiments on *Phascolion strombi*, the intestinal coil extruded through the posterior incision and the animals died within a few days. In *G. minuta* the wound closed by a contraction of the body wall musculature and was further sealed by an aggregated mass of coelomic amoebocytes, believed to give rise to the mesodermal components of regenerative tissue. Cells migrated from the ventral nerve cord to form the ectodermal tissues of the regenerate.

III. PARTHENOGENESIS

One species of sipunculan, *Themiste lageniformis*, from the central east coast of Florida is known to reproduce by parthenogenesis (Pilger, 1987). Populations of *T. lageniformis* occur also in Indo-Pacific and Hawaiian waters (Stephen and Edmonds, 1972; Williams, 1972; Awati and Pradhan, 1936 as *Dendrostoma signifer*). It is not known whether the Indo-Pacific population is parthenogenetic but spec-

imens from the Hawaii population have been documented to produce eggs that activate spontaneously and are assumed to be parthenogenetic (Pilger, 1987).

Studies over a two-year period have shown that parthenogenesis is a normal reproductive process in populations of *Themiste lageniformis* in Florida. Specimens collected at monthly intervals and maintained in isolated compartments in the laboratory spawned eggs that invariably developed into normal embryos in the absence of sperm (Pilger, 1987, 1989). Eggs that were spawned in the presence of sperm also developed normally, but studies of karyotypes to determine whether the sperm made a chromosomal contribution to the developing egg were inconclusive. Microdensitometry of Feulgen-stained embryos and larvae was used to illuminate aspects of the cytogenetics of this reproductive mode (Pilger, 1989). The results of the study suggested that haploid gametes are produced and that fertilization is facultative. If sperm are present eggs will be fertilized and zygogenic development will follow, but in the absence of sperm, development will proceed through parthenogenesis. No significant difference in DNA content was found in those embryos that developed by zygogenesis and those that developed by parthenogenesis. Both lines had the same 4C DNA equivalent that is characteristic of mitotically active diploid cells. Therefore, the diploid chromosome number was restored in the parthenogenetic developmental line to match that of the zygogenic line. Automixis, the fusion of the haploid gamete with a polar-body nucleus or the fusion of daughter-cell nuclei, was the most likely explanation for this observation.

The parthenogenetic populations of *Themiste lageniformis* on the east coast of Florida show an unequal sex ratio, with females outnumbering males 24 to one (See Section V A). Pilger (1987) proposed that sex determination by facultative parthenogenesis could be responsible for the formation and maintenance of the biased sex ratios in these populations. He suggested that parthenogenesis produces all females (i.e. thelytoky) and zygogenesis produces both males and females.

IV. FECUNDITY

Estimates of fecundity are available for two species of sipunculans: *Golfingia minuta*, a small hermaphroditic species from temperate and arctic waters, and *Phascolosoma perlucens*, a widely distributed tropical species (Stephen and Edmonds, 1972). In a population of *G. minuta* from Gullmarfjord on the west coast of Sweden, the usual number of coelomic eggs of maximum size was reported to be 25, although the upper range was 40 to 50 (Akesson, 1958). For a population on the south coast of England at Wembury the fecundity, as determined by counts of mature coelomic oocytes, was 50 with a range of 14 to 119 (Gibbs, 1975). An estimate of the fecundity of *P. perlucens* from the southeast coast of Florida has been obtained by counts of spawned eggs of 12 isolated females over a period of one month at a time of peak spawning activity (Rice, unpublished). The aver-

age number of eggs spawned per individual in one month was 54,400. During the month two females spawned three times, four spawned twice, and the remainder spawned only once.

As a general rule in marine invertebrates, species with high fecundity have small eggs and indirect or planktotrophic development, whereas those with low fecundity have relatively large eggs and direct or lecithotrophic development (Chia, 1974). The two species of sipunculans for which fecundity data are available, *Golfingia minuta* and *Phascolosoma perlucens*, follow this rule. The eggs of *G. minuta* are extraordinarily large, 260 to 280 × 215 to 230 μm, and rich in yolk (Åkesson, 1958). Development is direct, and eggs develop within the burrow of the parent. *Phascolosoma perlucens*, on the other hand, has smaller eggs, measuring 112 × 91 μm, and an indirect development with a free-swimming larval stage (Rice, 1975b).

V. REPRODUCTIVE STRATEGIES

A. Sex Ratios

Males and females occur in equal numbers in most populations of sipunculans, but in a few instances a prevalence of females has been recorded. In 200 specimens of three species of *Golfingia* (as *Phascolosoma*) (*G. elongata*, *G. vulgaris*, *G. minuta*) from the northwest coast of France, Keferstein (1863) found only females. Claparède (1863) examined hundreds of specimens of *G. elongata* from the same area and found only one or two males. More recently, Gibbs (1976) reported that the sexes were about equal in number in a different population of *G. elongata* from the south coast of England. Another *Golfingia* species, *G. pugettensis* from the northwest coast of the United States, was found to have only two males in 100 specimens examined (Cole, 1952). A study of the same population 14 years later revealed a different sex ratio: 50 per cent of the population was male, 37 per cent female, 13 per cent of undetermined sex (Rice, 1966). Unequal sex ratios have also been found in populations of *Themiste lageniformis*: Awati and Pradhan (1936) reported 60 females to one male in a population from the coast of India, whereas the ratio of females to males in the same species, collected in Fort Pierce, Florida, was 24 to one (Pilger, 1987).

Except for the Florida and Hawaiian populations of *Themiste lageniformis* which have been shown to reproduce parthenogenetically (Pilger, 1987; see Section III), no documentation of the reproductive mode has been reported for populations in which females were found to be dominant. Awati and Pradhan (1936), however, proposed but did not test three possible explanations for the unequal sex ratios in the Indian population of *T. lageniformis*: (1) protandry, (2) minute males that were inadvertently overlooked, and (3) parthenogenesis (sic). Pilger (1987) found no evidence for protandry or minute males in the Florida population of this species but determined that they reproduce by parthenogenesis. He further proposed that the

biased sex ratio in the Florida population is produced and maintained by facultative parthenogenesis. That is, parthenogenesis produces only females and zygogenesis produces both males and females. Hawaiian specimens of *T. lageniformis* reproduce by parthenogenesis but the sex ratio has not been determined.

B. Breeding Cycles

Annual breeding cycles have been described for seven species of sipunculans: *Golfingia elongata* (see Gibbs, 1976); *G. minuta* (see Gibbs, 1975); *G. vulgaris* (as *Phascolosoma vulgare*) (see Gonse, 1956b); *G. pugettensis* (see Rice, 1975a); *G. rimicola* (see Gibbs, 1976); two populations of *P. agassizi* (see Towle and Giese, 1967; Rice, 1975a); and *P. arcuatum* (as *lurco*) (see Green, 1975). The first six species are from temperate waters and the seventh is tropical. Methods used in the delineation of breeding cycles have included cytological studies of gametes, observations on coelomic spermatogenesis, and measurements of coelomic oocytes at intervals throughout the year.

The first detailed studies of a sipunculan reproductive cycle were made by Gonse (1956a, b; 1957a, b) on *Golfingia vulgaris* at Roscoff, France. In addition to cytological studies of the ovary and coelomic oocytes (see Rice, Volume I for review), he made monthly measurements of coelomic oocytes for a period of one year (Fig. 1). Small oocytes, less than 61 μm in diameter, were present throughout the year, indicating that the ovary was continually active, releasing young oocytes into the coelom. During the winter, all oocytes in the coelom were small, their growth apparently arrested. In the spring, though small oocytes continued to predominate, oocytes of intermediate size appeared in small numbers, evidence that growth of small oocytes was occurring. During the breeding season, which lasted from June to September, two populations of oocytes were prominent: small, with a mode of 40 μm in diameter, and large, ranging from 146 to 173 μm . Intermediate sizes continued to be present in low frequencies. At the end of the breeding season, as larger oocytes were either spawned or resorbed, the size-frequency distribution was once more unimodal, the single mode consisting of small oocytes.

The annual reproductive cycle of *Golfingia pugettensis*, a sipunculan from the Northwest Pacific Coast of the United States, was also defined by monthly measurements of coelomic oocytes (Rice, 1966, 1975a). The cycle of this species showed many similarities to that of *G. vulgaris*. From October through December, the breeding season for *G. pugettensis*, the size-frequency distribution was bimodal: small oocytes ranged in diameter from 21 to 68 μm and large oocytes from 146 to 172 μm with few cells of intermediate size. In January large oocytes disappeared and small oocytes began to grow as evidenced by an increase in their range to 88 μm . Growth continued during the summer months (June through August) when oocytes of intermediate size (88 to 146 μm in diameter) appeared in low frequencies.

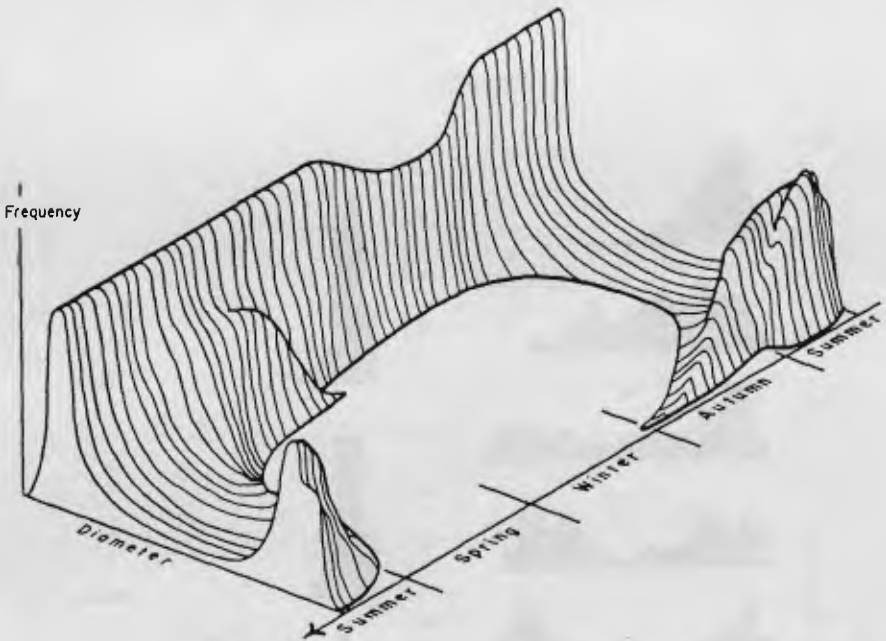


Fig. 1. Three-dimensional diagram of size-frequency curves of coelomic oocytes of *Golfginia vulgaris* over a period of one year. (From Gonse, 1956b, p. 232; reprinted by permission of The Royal Swedish Academy of Sciences.)

For the hermaphroditic species, *Golfginia minuta*, from Wembury, England, Gibbs (1975) characterized the reproductive cycle by making monthly measurements of coelomic oocytes (Fig. 2) and recording stages of coelomic spermatogenesis. The breeding season was ascertained to last from early November to late January, though large oocytes and free spermatozoa were present in a small percentage of the population as late as April. During the breeding season, the coelomic oocytes showed a bimodal distribution with few, if any, intermediate cells. Small oocytes were mostly within the range of 25 to 90 μm in diameter, whereas large oocytes had attained their maximum diameter of 240 to 280 μm . In late spring, following the breeding season, the growth of small oocytes was indicated by the appearance of intermediate cells, which continued to be present through the early fall, lasting until the beginning of the spawning season in November. Gibbs (1975) pointed out that growth of coelomic oocytes followed a sigmoid curve, being most rapid in the intermediate size range (Fig. 3).

Platelets of spermatocytes, the earliest stage in coelomic spermatogenesis, were dominant in most animals from May through September. In October, just prior to the breeding season, the second stage, spermatids equalled the spermatocytes and increased to a marked dominance in December. Free spermatozoa appeared

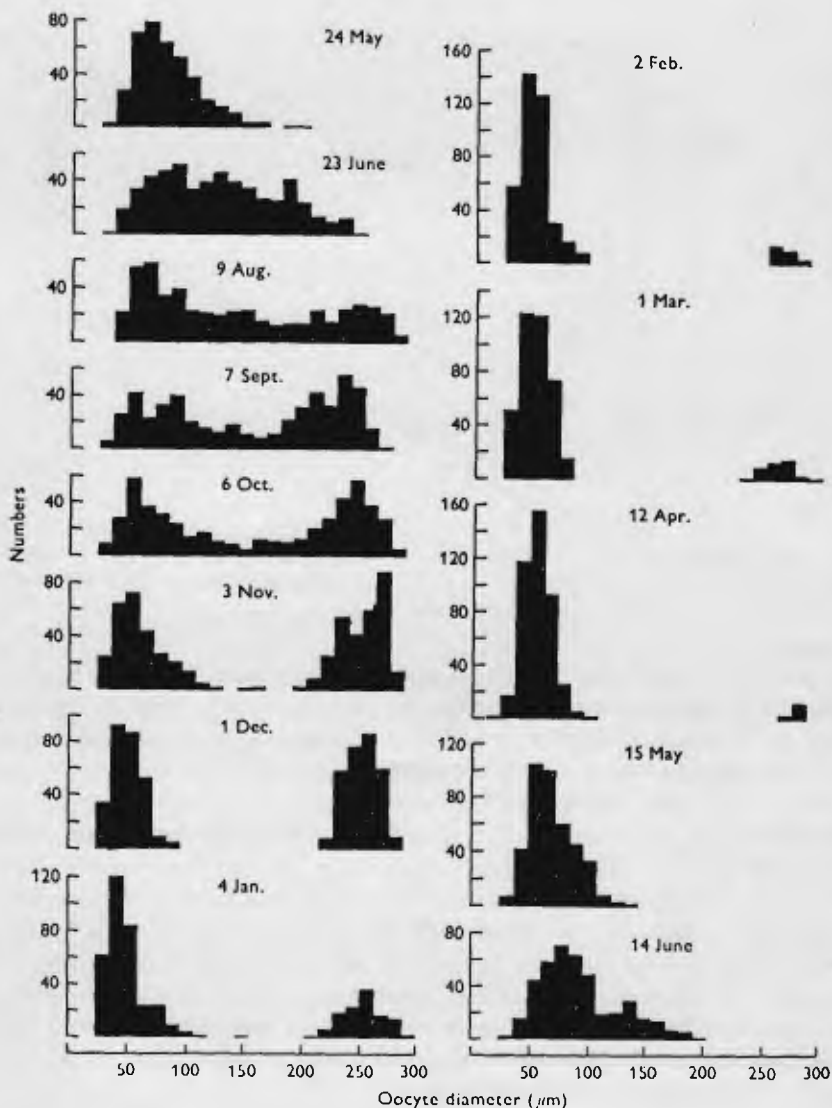


Fig. 2. Size-frequency distributions of coelomic oocytes of *Golfinigia minuta*. Twenty to 30 oocytes were measured from each of 20 worms in monthly samples from May 1971 to June 1972. (From Gibbs, 1975, p. 73; reproduced by permission of Cambridge University Press.)

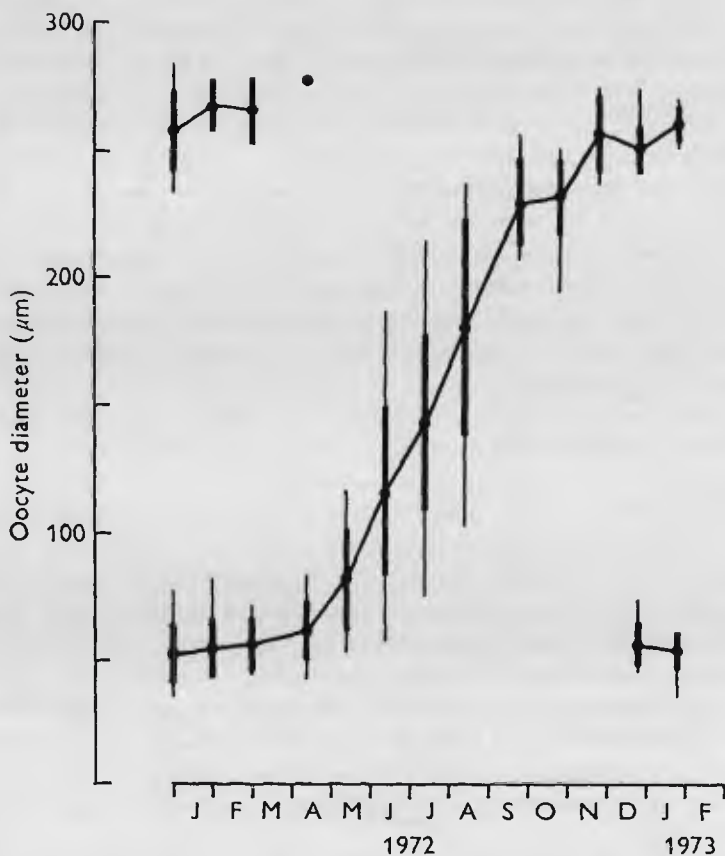


Fig. 3. Annual growth of coelomic oocytes of *Golfingia minuta*. Five of the largest oocytes were measured from each of 20 worms. The mean of each sample is plotted. The range is the thin line and the standard deviation the thick line. (From Gibbs, 1975, p. 74; reproduced by permission of Cambridge University Press.)

in August, increasing in dominance through January and February after the main spawning season, and undergoing a dramatic decrease in March.

Data on reproductive cycles of two other species of *Golfingia*, *G. elongata* and *G. rimicola*, from the southern coast of England near Plymouth were gathered for periods of five to six months before, during, and after the breeding seasons (Gibbs, 1976). The breeding seasons, as determined by measurements of coelomic oocytes, were July and August for *G. elongata* and October and November for *G. rimicola*. As for *G. vulgaris*, *G. pugettensis* and *G. minuta*, described above, size-frequency distributions of coelomic oocytes of both species were bimodal during the breeding

season. The maximum diameters of coelomic eggs of *G. elongata* at this time were 140 to 160 μm and of *G. rimicola* 160 to 180 μm . In both species free spermatozoa were the dominant stage in coelomic spermatogenesis during the breeding period.

In a population of *Phascolosoma agassizi* from the Pacific Northwest Coast of the United States, oocytes of all sizes, including those of intermediate size, were found in the coelom throughout the year (Rice, 1975a). Except for the time immediately preceding the breeding season, there were two major populations of cells, small and large, the population of large cells predominating. Just prior to the breeding season the percentage of small oocytes was reduced, so that they no longer constituted a distinctive mode, and the frequency of intermediate oocytes increased. The breeding season was determined to be June through August. As eggs were spawned during the breeding season, the relative numbers of large eggs decreased, but by October they again formed the dominant mode in a bimodal size-frequency distribution.

A population of *Phascolosoma agassizi* from farther south at Monterey Bay, California was found to breed from March through May (Towle and Giese, 1967). For three months after spawning coelomic gametes were entirely lacking. By July the distribution of oocytes resembled that found most of the year in the population from the Pacific Northwest; i.e. there were oocytes of all sizes with dominant populations of large and small oocytes (Fig. 4). During the fall until the time of spawning the population of large oocytes was dominant, the small and intermediate oocytes forming only a small percentage of the total oocytes.

Differences between the two populations of *Phascolosoma agassizi* are found in the degree and duration of reduction in gonadal activity. In the population from Monterey, gonadal activity is reduced during the breeding season and ceases entirely for three months thereafter. The population from farther north shows continual gonadal activity throughout the year with periods of reduced activity before and during the breeding season.

A tropical species of *Phascolosoma*, *P. arcuatum* (as *lurco*), from Queensland, Australia, was found to breed from December through February (Green, 1975). Similar to the population of *P. agassizi* at Monterey, California, it lacked coelomic oocytes for two months after the breeding season. Male gametes were present in the coelom for only five months of the year, December through April.

In summary, growth of coelomic oocytes in species of *Golfingia* is characterized by phases of arrest and acceleration; the gonad appears to be continually active, producing small oocytes throughout the year. In contrast, in species of *Phascolosoma*, oocyte growth is continual with either reduction or cessation of gonadal production for some part of the year.

Little is known about the factors controlling breeding cycles in sipunculans. Because Carlisle (1959) claims to have found an increase in neurosecretory material in *Sipunculus nudus* before the breeding season (this remains to be confirmed) it has been suggested that neurosecretion may influence the reproductive cycle

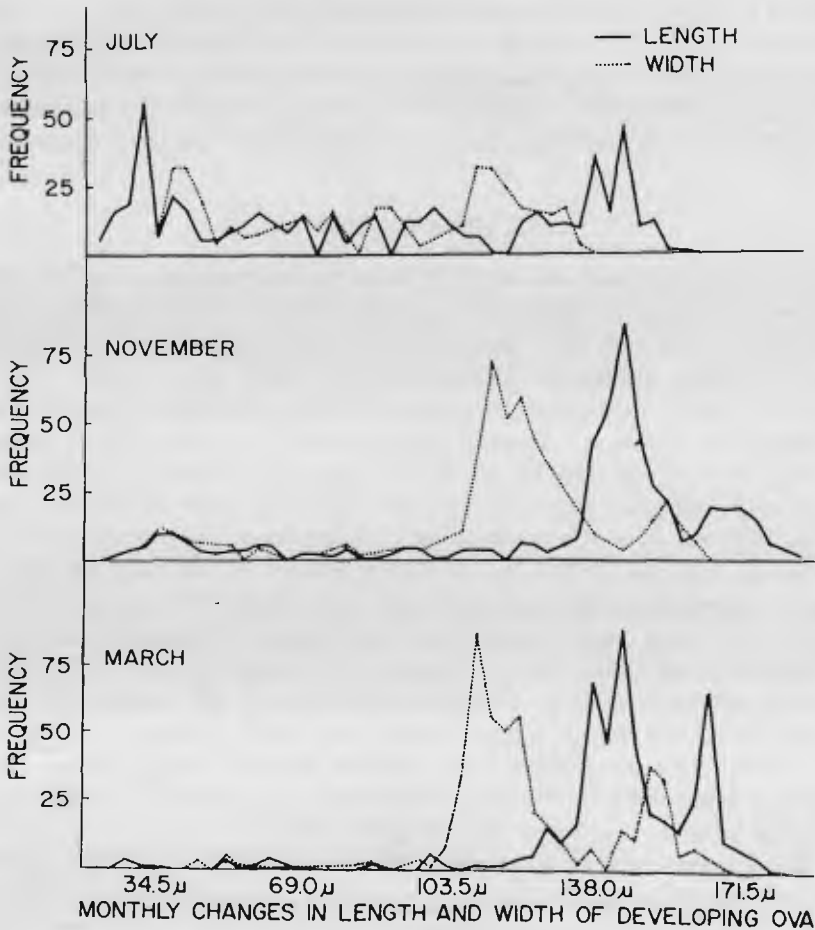


Fig. 4. Size-frequency distribution of coelomic oocytes of *Phascolosoma agassizi* from Monterey Bay, California (1960-1961). Twenty oocytes from each of 25 females were measured each month. (From Towle and Giese, 1967, figure 1 (in part), p. 232; reprinted by permission of The University of Chicago Press.)

(Åkesson, 1961). Other demonstrations of neurosecretion in sipunculans (Gabe, 1953; Åkesson, 1961) have not investigated its relevance to reproduction.

Whether exogenous factors, such as temperature, influence breeding cycles in sipunculans cannot be determined with certainty from the available evidence. No clear pattern emerges from an examination of breeding seasons of populations at different latitudes. A population of *Phascolosoma agassizi* at Monterey, California, on the west coast of the United States spawns before a population 11° farther north (Rice, 1975a). On the other hand, of two populations of *P. arcuatum* (as *lurco*),

separated by 8° latitude on the coast of Australia, the one at higher latitude spawns first (Green, 1975). Observations on spawning of some tropical and subtropical species of sipunculans indicate that spawning may occur throughout the year (Rice, 1975a, b); however the only tropical species for which the breeding cycle is known, *P. arcuatum*, has a restricted breeding season as do species from temperate waters.

VI. CONCLUSIONS

The Sipuncula are a small, taxonomically cohesive, and presumably primitive phylum, yet their modes of reproduction are rather diverse. Sexual reproduction, the usual mode, results in different developmental patterns, ranging from direct development in species having low fecundity and large, yolky eggs to indirect, planktotrophic development in species having higher fecundity and smaller eggs. Asexual reproduction is documented in three species; it may occur by transverse fission, budding, or parthenogenesis. In one species, *Sipunculus robustus*, both transverse fission and budding may occur, but apparently only under abnormal laboratory conditions. However, transverse fission in *Aspidosiphon brocki* and parthenogenesis in *Themiste lageniformis* are known to be normal reproductive processes occurring in naturally reproducing populations in the field. At the present time, populations have been studied in only small segments of the total geographic ranges of these species and over a limited period. Whether the mode of reproduction varies under different environmental or geographic conditions is not known. The disparate sex ratios reported for several species suggest that the phenomenon of asexual reproduction may be more widespread than presently realized. More intraspecific information, as well as comparative information among species, is needed for an analysis of trends and adaptive significance of reproductive traits.

At our present stage of knowledge, information on reproductive cycles in the phylum Sipuncula is largely descriptive. Questions concerning regulatory mechanisms of breeding cycles, including endogenous and exogenous influences, have yet to be explored. But the basis now exists for formulating such questions and devising experimental procedures for finding answers. It is only from experimental evidence and the acquisition of further knowledge that we will be able to uncover the mechanisms controlling reproductive patterns and evaluate the various reproductive traits in terms of their evolutionary significance.

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