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REPRODUCTIVE CYCLE OF *PERINEREIS NUNTIA* VAR. *BREVICIRRIS* GRUBE (POLYCHAETA: NEREIDAE)

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ABSTRACT. - Both male and female *Perinereis nuntia* var. *brevicirris* (Grube, 1878) showed epitokal modifications when sexually mature. Such changes included the development of foliaceous parapodial lamellae, the elongation of the dorsal and ventral cirri, the replacement of setae by specialised natatory setae, the vascularisation of the blood vessels, the histolysis of the gut and septa and the enlargement of eyes. Stages in these changes were observed to be synchronised with the stages of gamete ripening.

The worms began to migrate up the tidal zone in June/July for spawning. The spawning cycle was extended, lasting from October to the following April/May. The worms were monotelic and most probably did not survive one spawning cycle. Fertilised eggs must have been transported out by receding tide and the larvae probably, developed and settled in the subtidal seabed, as no juvenile worms could be collected from the tidal zone.

INTRODUCTION

The polychaete worm, *Perinereis nuntia* var. *brevicirris* is common in sandy beaches around Penang Island, Malaysia (Fig. 1). The worms burrow in the sand beneath rocks at the high tide level which is only submerged during extreme high water. They are popular fish bait for anglers. The local villagers collect them by an ingenious method where 'fish slurry' is squeezed into the sand and the worm is swiftly pulled out intact by the head when it popped out of its burrow.

Little information is available on the reproductive cycle of this species in Malaysia. The aim of this study is to document the reproductive cycle of *Perinereis nuntia* var. *brevicirris* on a sandy beach at Bayan Bay, off the east coast of Penang Island.

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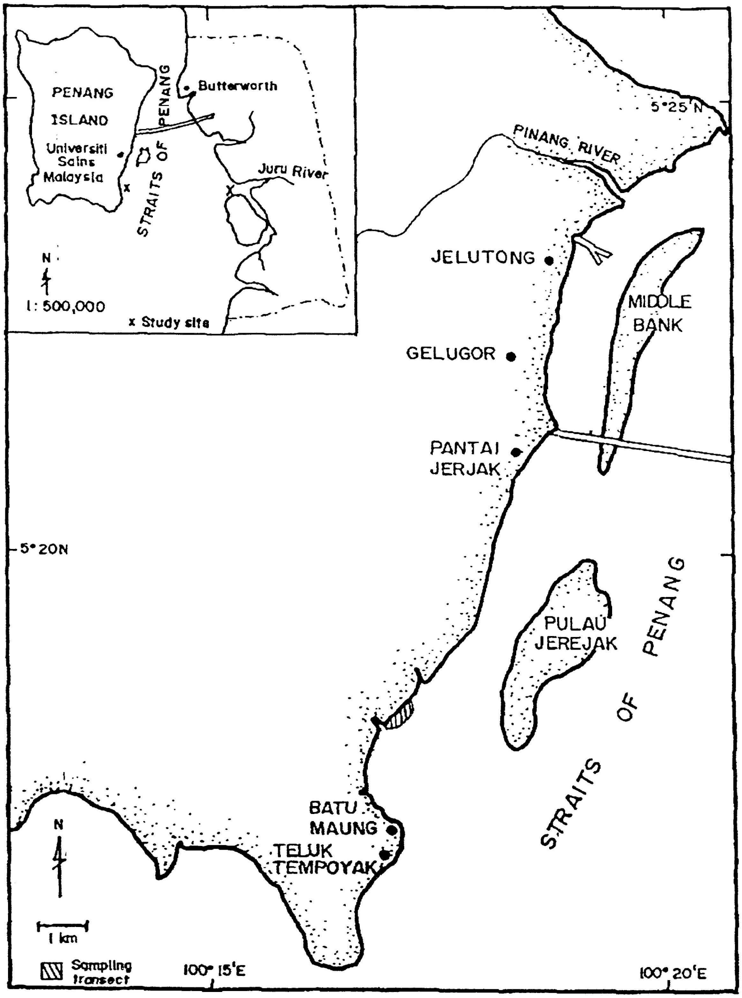

Fig. 1: Map of Penang showing the sampling site.

MATERIALS AND METHODS

The study area is a reclaimed sandy beach at Bayan Baru, off the east coast of Penang Island. The entire stretch of beach is bordered by rocky rip rap beneath which *P. nuntia* var. *brevicirris* make their burrows. The tidal regime is semi-diurnal with a maximum range of 2.5m.

Sampling was done twice per month during extreme low water spring tide. Since the density of the worms was quite low, an extensive stretch up to 60m of the beach below the rip rap was dug up. To ensure the same location was dug during each sampling trip, the rocks were marked with paint. Worms dug up were collected into plastic bags and taken to the laboratory. All worms were relaxed in a solution of 1% MgCl before examination under the microscope.

The external features of every worm was noted. Each worm was slit open and gametes present in the coelom were collected into a petri dish with a little clean seawater. The diameter of 50 oocytes per worm were measured with a micrometer inserted into the eyepiece of a dissecting microscope. Sexual maturity was evaluated by mean oocyte diameter in females and the presence of spermatogonia in males.

Additionally the mean tide and low tide zones of the beach were sampled with a core (diameter 10 cm) to collect juvenile worms. Three cores were collected at each of these zones and the sand sieved through a 0.25 mm mesh. Worms were stained with Rose Bengal and preserved in 5% formalin for examination in the laboratory.

RESULTS

Systematics. - A redescription of *P. nuntia* var. *brevicirris* is made here to avoid confusion with the many other varieties of *P. nuntia* enumerated by Fauvel (1932), and also as a reference for comparison with the epitokous stage.

The prostomium is broadly triangular, unnotched with two antennae, four eyes, and a pair of large two-jointed palps. There are four pairs of tentacular cirri, the longest pair reaching the 7-8 th segment (Fig. 2a). The proboscis bears mainly conical and some bar-shaped paragnaths in the following order, Group I : 1-3; II and IV : cluster in a crescent or triangle; III : a rectangular patch with 2-3 denticles; V : 2 or 3 set in a triangle; VI : on each side a transverse row of 7-10 conical or bar-shaped, more or less mixed together; VII-VIII : 3 or more irregular rows (Fig. 2b & c).

The parapodia are biramous after the first two. Each parapodium bears two conical notopodial lobes and a dorsal cirrus of about half the length of the upper lobe (Fig. 2d). The neuropodium consists of two subequal lobes. The superior lobe is low and somewhat rounded; the other is slender and twice as long. The ventral cirrus is cirriform and half the length of the inferior neuropodial lobe.

Notosetae are homogomph spinigers (Fig.2e) throughout and neurosetae are heterogomph falcigers with coarsely serrated blades (Fig. 2f).

Remark: *P. nuntia* (Savigny, 1818) is characterised by a row of bar-shaped paragnaths across groups V and VI. The diagnostic features of *P. nuntia* var. *brevicirris* are the 3 conical paragnaths arranged in a triangle on group V and the transverse row of mixed conical and bar-shaped ones on group VI of the oral ring.

Sex differentiation. - *P. nuntia* var. *brevicirris* is dioecious and both male and female worms become epitokous when sexually mature. However there is little sexual dimorphism to allow visual separation of the sexes. Differentiation of the sexes, except at a very late stage of maturity, could only be confirmed by the presence of male or female gametes in the coelom. External features in the stages of metamorphosis into an epitoke were observed to be related to the stages of ripening of sexual gametes as described below.

Indeterminate Sex - These comprised of worms which did not contain any gametes in the coelom to allow the confirmation of sex without histological sectioning. The coelom was, however, filled with fat and spindle-shaped muscle fibres, indicating that hydrolysis of the gut and septa had begun.

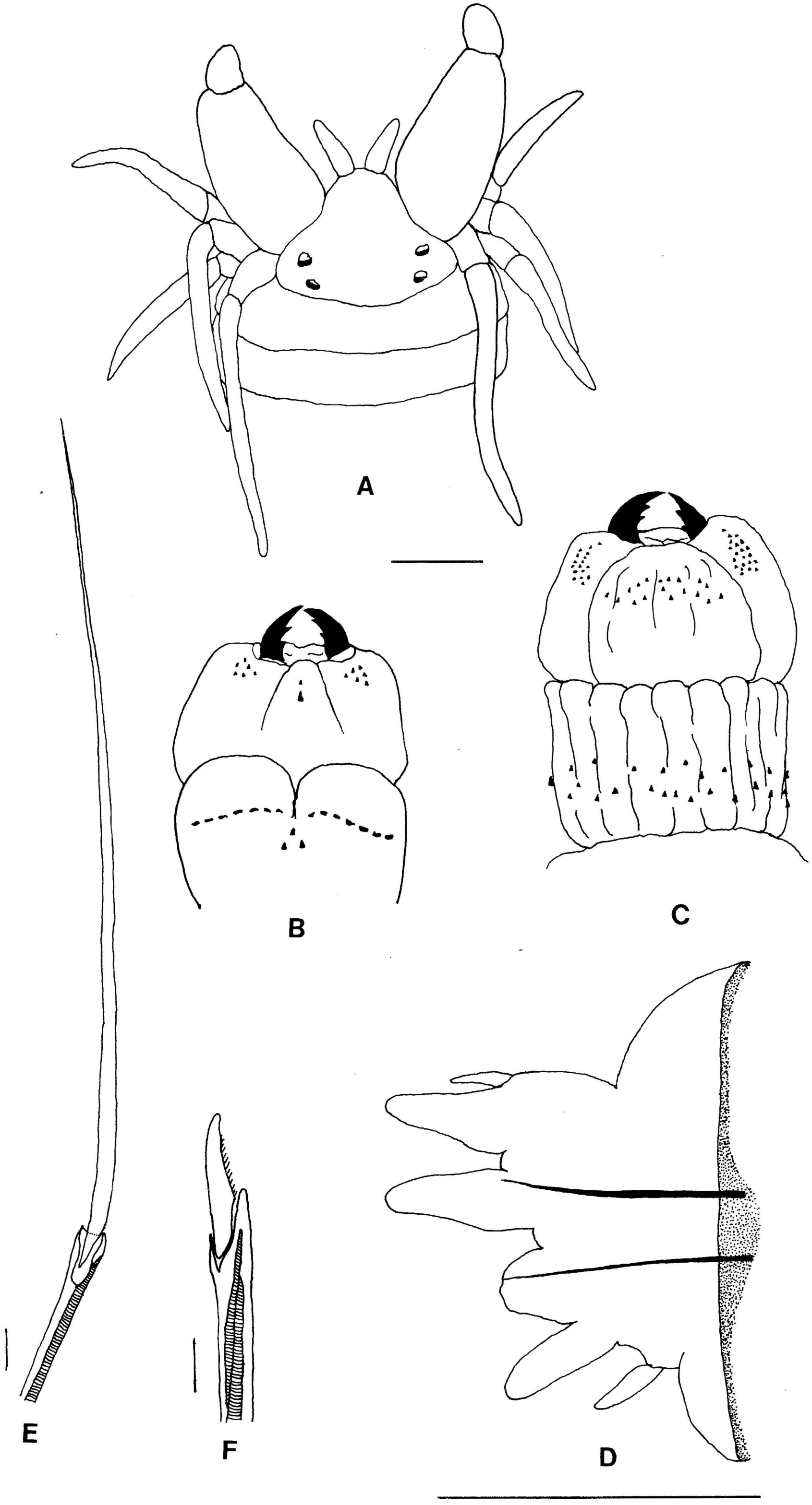

Fig. 2 : *Perinereis nuntia* var. *brevicirris*. a. head; b & c. proboscis, dorsal and ventral views; d. 50 th foot, setae omitted; e. spinigers; f. falcigers. Scale : a,b,c,d = 1mm; e,f = 10 um.

Externally the worm was still 'normal' without any visisble epitokous changes. The mean body width was about 2.5 mm at the widest part and the length was up to 12 cm for about 100 segments. The colour was brownish and devoid of any colour patterns.

Female: Four stages of oogenesis were observed.

Stage I : Very small oocytes in the coelom with a diameter of 50-100 µm. Externally there were no visible changes.

Stage II : Oocyte diameter 100-150 µm. Some external differentiation had begun. The eyes were slightly enlarged. From segment 30-35 the base of the superior notopodial lobe became swollen and vascularised (Fig.3a). Postsetal lamella of the superior lobe was enlarged and flattened, resembling a paddle. The base of the ventral cirrus had enlarged to form a flat, kidney-shaped 'paddle'. The worms were bright red in colour.

Stage III : Oocyte diameter between 150 and 200 µm. The enlargement of the parapodia and 'paddles' continued.

Stage IV : Oocyte diameter of 200-250 µm. All the eyes were so enlarged that they almost coalesced. Palps became short and rounded. The tentacular cirri were shortened and could only reach the second segment. The worms were blue-green, taking on the colour of the mature oocytes, which could be seen through the body wall when stretched. Swimming parapodia were fully developed. Three large flat kidney-shaped 'paddles' were formed, one each at the base of the dorsal and ventral cirri, and one as a postsetal neuropodial lamella. The latter is the largest (Fig. 3b). The postsetal neuropodial lamella formed the largest 'paddle'. A rudimentary 'paddle' was also found at the base between the inferior notopodial lobe and the superior neuropodial lobe. Both the dorsal and ventral cirri have elongated to almost the length of the parapodial lobe. A secondary filament was also formed from the base of the ventral cirrus. The normal setae were beginning to be shedded. Natatory setae had not yet erupted to the surface but were embedded in the parapodia.

Spent female : These contained very small and very few oocytes which were mainly found in the posterior region. The eyes were still slightly enlarged and parapodial lamellae were decreasing to their normal size. All setae were homogomph spinigers and heterogomph falcigers.

Male: Very young males were difficult to identify since spermatogenesis was more difficult to observe. The sex could only be confirmed by the recognition of sperm rosettes in the coelom.

Stage I : Eyes were enlarged and parapodial 'paddles' were beginning to form, similar to those of the stage II females. In the coelom, spermatocytes were grouped together to form small rosettes or sperm plates with a mean diameter of 50 mm.

Stage II : All the 'paddles' were fully formed. Eyes were very enlarged and coalesced. Palps and tentacular cirri have shortened (Fig. 4a). The colour was bright red with white patches at the base of the feet where the spermatogonia were packed. There was little external differentiation from the female except for the dorsal cirrus, which was longer than the parapodial lobe and notched along the ventral side (Fig. 4b). Sperm rosettes increased in size with >100 spermatid cells.

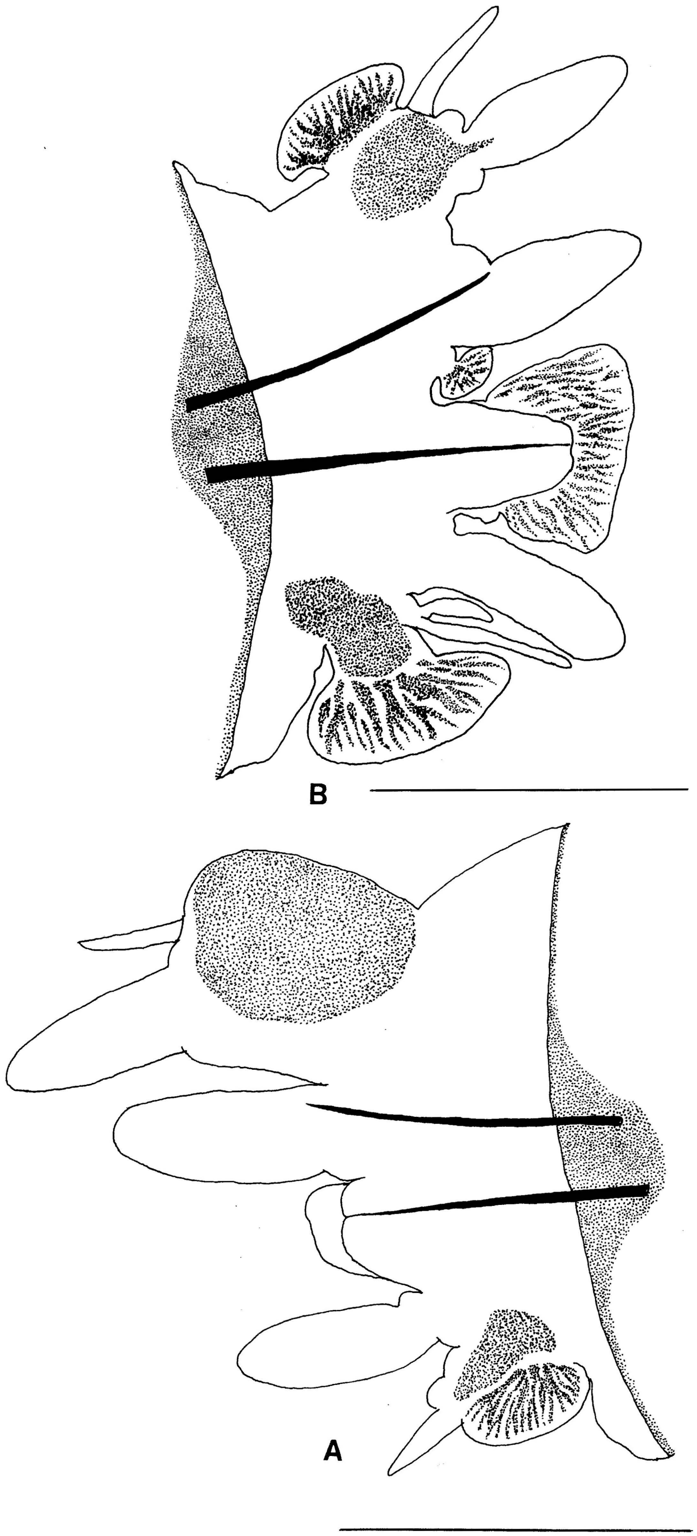

Fig. 3 : Parapodia of female epitokes, setae omitted. a. stage II female; b. stage IV female. Scale : a,b = 1 mm.

Stage III : Spermatogonia with tails were formed. Three spermatozoa per rosette remained attached together by their heads with the tails sticking out. The colour of the worm was bright red.

Stage IV : Heteronereis

A male heteronereis was obtained only on one occasion when one of the worms kept in an aquarium began to swim in the water. Sperms in the coelom were free individuals with tails. All the setae were natatory setae (Fig 4c). The worm was white in colour and a pygidial rosette was present.

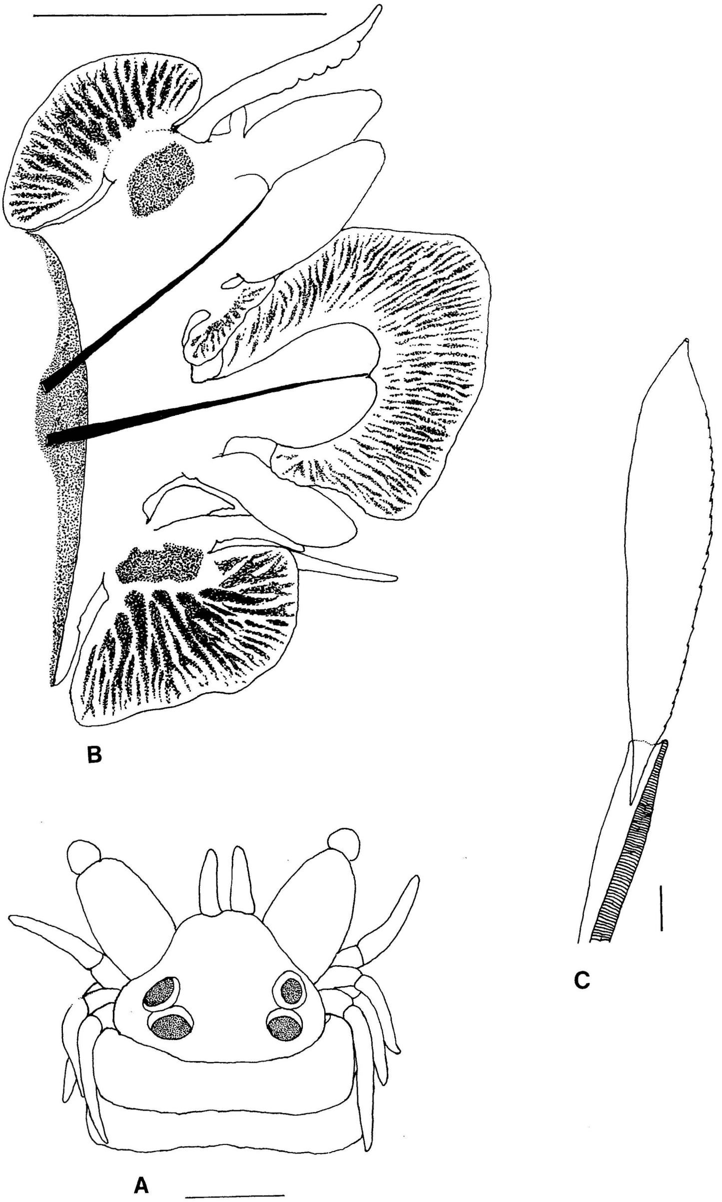

Fig. 4 : Male epitoke. a. head; b. stage IV parapodium, setae omitted; c. natatory seta. Scale : a,b = 1 mm; c = 10 um.

Reproductive cycle. - All core samples at the mean and low tide zones did not yield any *P. nuntia* var *brevicirris* juveniles. This suggested that the larvae might have settled and developed in the subtidal seabed, but migrated up the shore mainly for spawning.

The migration of the worms began when they were at the indeterminate stage. Mass migration, as represented by the large population of indeterminate sex, commenced sometime in June/July and tapered off after December (Fig. 5). Once at the upper tidal zone, the worms burrowed into the sand under rocks and it required from 3 to 4 months for the gametes to ripen. This was shown about four months later by an increased female population which carried oocytes in the coelom. This trend continued up to the following May when the female population began to decline, presumably dying after spawning, although not immediately. The new spawning season each year was thus represented by a different cohort of worms. Female worms could be collected throughout the year but their population peaked from October to April. Male worms were absent in June and July, mainly due to the fact that the early stages of spermatogenesis were difficult to ascertain, unless sperm rosettes were present in the coelom. The population of males was always less than that of females.

The greatest variation in the oocyte diameter was seen during the months of September to the following January. This indicated that the spawning season was rather extended, commencing around October and continuing up to the following April. This was shown by the presence of blue-green oocytes with diameters of 200-250 µm which were ripe for spawning during this period (Fig. 6). Although the oocytes of some females had attained full size by September, they were not shed until October when the first spent females were encountered in the collection. This lapse in time represented the final metamorphosis into the pelagic heteronereis. Spent females did not perish immediately after the release of their gametes as some were found, intact in their burrows. In April and May the majority of the worms were spent with oocytes at various stages of resorption.

DISCUSSION

Both male and female *P. nuntia* var. *brevicirris* were observed to undergo the complete epitokal modifications characteristic of most nereids. Male heteronereis swarmed during spawning. Although swarming female heteronereis were not encountered in this study, the presence of natatory setae is evidence that they do swarm. Several species of nereid which did not swarm at the surface were also observed to develop enlarged parapodial lamellae, but not natatory setae when they were sexually mature (Gravier, 1905; Monro 1931). The elongated and flattened natatory setae were most likely to be responsible for increasing the effective swimming area of the parapodium, the lamellae playing a passive role, though both probably served as floatation devices (Clark, 1961).

Theoretically, gametes can be released from the coelom through the nephridia, the coelomic ducts, through the anus or by rupture of the body wall. Sperm of *P. nuntia* var. *brevicirris* were most likely dispersed through the pygidium as shown by the presence of an anal rosette in the male heteronereis. The mode of shedding of oocytes is more speculative. Many nereids have been known to shed their eggs by dehiscence after rupture of the body wall (Gravier & Dantan, 1928). It was not determined in this study how the eggs of *P. nuntivca* var. *brevicirris* were released, but it is unlikely to have been by rupture of the body wall as spent worms were found to be intact, except for the presence of very small oocytes in the posterior region. Spent females did not die immediately after spawning, but there is

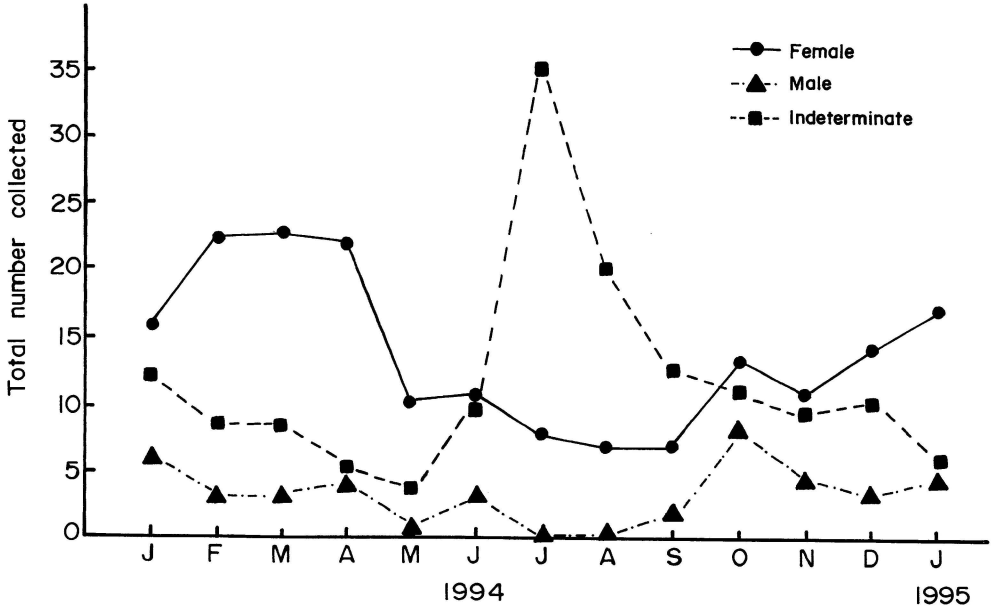

Fig. 5 : Population density distribution of sexually mature *Perinereis nuntia* var. *brevicirris*.

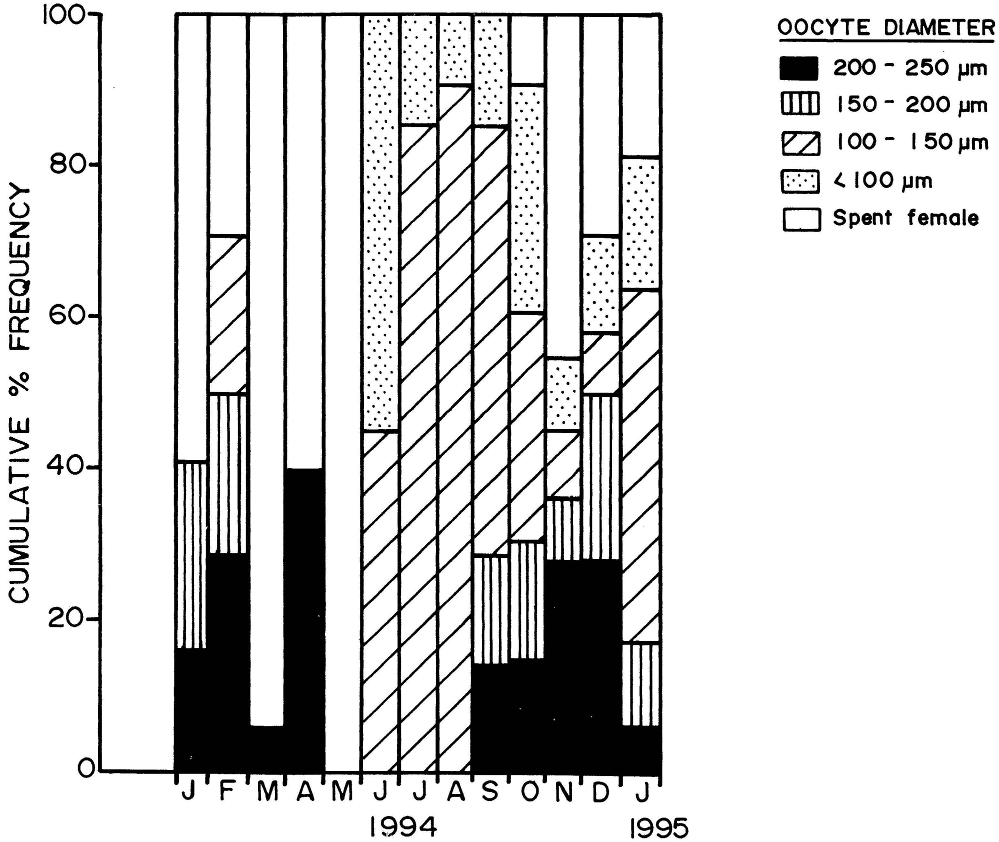

Fig. 6 : Percentage frequency distribution of oocyte diameter.

a high possibility that they did not survive more than one reproductive cycle as shown by the low population of females after the spawning season. This observation is concordant with the fact that most nereids are monotelic, breeding only once in their lifetime and releasing the gametes in one or a few large batches. Often, but not invariably, the spent animals die immediately after spawning (Olive and Clark 1978).

There are several reasons for the initiation of migration of the worms for reproduction. Among others, a rise in sea temperature and/or increase in day length (hence illumination) are thought to trigger off the breeding season in a wide variety of marine organisms. Thorson (1950) proposed that marine invertebrates require very definite and normally higher temperatures to ripen and spawn their gametes than are necessary for them outside this season. Many marine organisms undertake migration during their breeding season, including from deep water to the tidal zone. It has also been observed that an increase in temperature or illumination caused the withdrawal of the inhibitory hormones on epitoke formation secreted by the supra-oesophageal ganglion in Nereidae (Clark, 1961), and also influenced gametogenesis (Olive & Clark, 1978).

In Penang the months of May-July coincide with the equinox in the northern hemisphere, when day lengths are slightly longer and the weather is sunny and hot. In the tidal zone which remained exposed most of the time, temperature averaged about 30 ± 2° C, which is at least 2-3°C higher than on the subtidal seabed. This higher temperature would allow gametogenesis to proceed faster.

The extended breeding season shown by *P. nuntia* var. *brevicirris* is in accordance with Panikar & Aiyar's (1939) observations that in the tropics where food is available throughout the year, species with restricted breeding seasons have a much longer breeding season on average than those living in temperate seas.

Since the worms' burrows were only submerged by extreme spring tides during full and new moons, spawning would most likely to occur then when the worms could swim out of their burrows. Swarming has also been observed to be triggerred by the lunar and tidal cycle in other species of *Nereis* (Bradfield & Chapman, 1967; Korringa, 1957). The fertilised eggs must have been carried out with the receding tide and the larvae settled and matured in the subtidal seabed, migrating up the shore mainly for reproduction.

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