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12. We thank Dr. F. J. Wolf for his support of these studies and Dr. S. Rooney for suggestions in the preparation of this report. We also thank Dr. L. L. Hsu and Dr. A. J. Mandell of the University of California at San Diego for discussion concerning several aspects of this work. After this manuscript was submitted for publication, studies in their laboratory with rat brain enzymes and other TLC solvent systems for the product identification have confirmed our results.

1 June 1974; revised 12 July 1974 ■

Reproductive Patterns in the Deep-Sea Benthos

Abstract. *The reproductive condition of a variety of benthic invertebrates from the bathyal San Diego Trough was monitored periodically. In this physically seasonless, deep-sea environment most species reproduce throughout the year, and only a few display highly synchronous, annual reproductive cycles. These few are not typical deep-sea species; they are known from shallow water and belong to groups which are relatively unimportant in abyssal depths. Of the 11 species examined, 3 bivalves, 2 ophiuroids, 2 isopods, 1 amphipod, and 1 polychaete breed year-round, and 1 brachiopod and 1 scaphopod spawn seasonally.*

The deep sea has long been considered the most constant of environments, the physical parameters changing only very slowly over thousands of years and displaying little or no seasonal variation (*1*). In shallow-water environments, most marine animals reproduce in a cyclic manner, seemingly in response to the demands of fluctuating external conditions (*2, 3*). Hence, it might be expected, as Orton (*4*) first suggested, that organisms inhabiting such a constant physical environment as the deep sea would reproduce throughout the year.

However, of the past attempts to investigate this question, indications of reproductive periodicity were found in a surprisingly large percentage of cases (*5*). Unfortunately, in all these instances the data are equivocal, having been derived from deep-sea bottom samples not specifically intended for reproduction studies. The samples were in most cases obtained from a variety of locations, depths, and years, often with samples either poor or lacking for several critical periods.

In order to avoid these difficulties in the investigation reported here, deep-sea populations were systematically monitored at regular intervals. Epibenthic sled (*6*) and otter trawl samples were taken every 13 weeks from October 1970 through October 1971, from the same station in the San Diego Trough (32°26.5′N, 117°28.5′W) at a depth of 1240 m.

The San Diego Trough is a relatively flat, sediment-filled basin 24 km off the coast of southern California. The sediments are a clayey-silt with a mean grain diameter of 8 μm (*7*) and an organic carbon content of 1 to 3 percent by weight (*8*). The three important physical factors—photoperiod, temperature, and salinity—changes in which are deemed to control the timing of reproduction in marine invertebrates (*2*), are nonexistent or do not vary throughout the year. Sunlight does not penetrate the depths of the trough (*9*) and the near-bottom temperature and salinity are nearly constant, ranging only 0.3°C and 0.02 per mil during the year (*10*).

Most of the species examined from this physically seasonless environment reproduce throughout the year. This is achieved in two different ways: (i) asynchronously, with the individuals of a population having distinct gametogenic cycles but being out of phase with each other, so that a relatively constant proportion of individuals breeds at any one time; and (ii) continuously, with all adult individuals in the population being reproductively active throughout the year. Only a few gametes are released at any one spawning so that complete spawn-out and recovery does not occur as it does in the asynchronous type.

Asynchronous year-round reproduction was seen among the echinoderms (*Ophiomusium lymani* and *Ophiacantha normani*) and the crustaceans (*Eurycope californiensis, Harpiniopsis excavata,* and *Ilyarachna profunda*). For example,

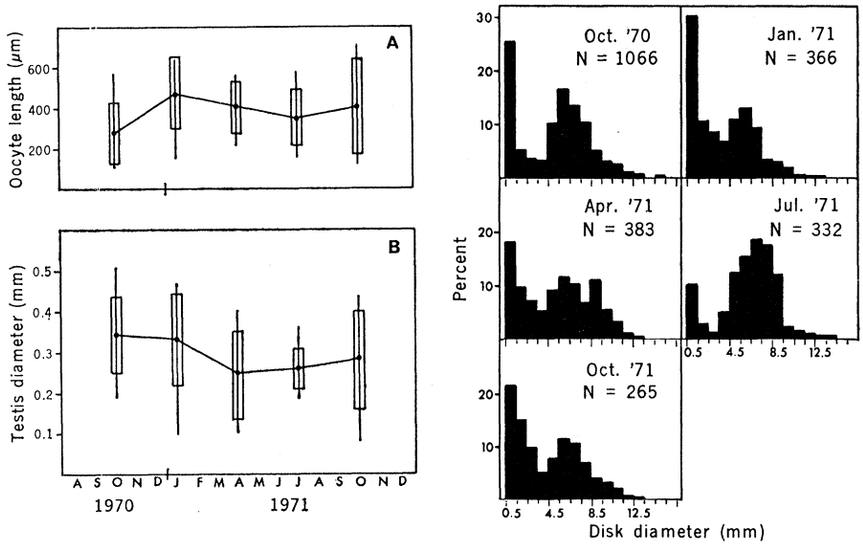

Fig. 1 (left). Year-round reproduction in the brittle star *Ophiacantha normani*. (A) Oocyte size of females during the year. (B) Testis size of males during the year. Each data point represents the mean for ten individuals. Oocyte length for an individual female was obtained by measuring the largest oocyte present in the gonads of one randomly selected genital bursa. Testis diameter for an individual male represents the mean of ten testes from one randomly selected genital bursa. A vertical line represents the 95 percent confidence limits of the mean. Fig. 2 (right). Size-frequency distributions of the sample population of *Ophiacantha normani*. Histograms for each season were prepared from epibenthic sled (*6*) samples.

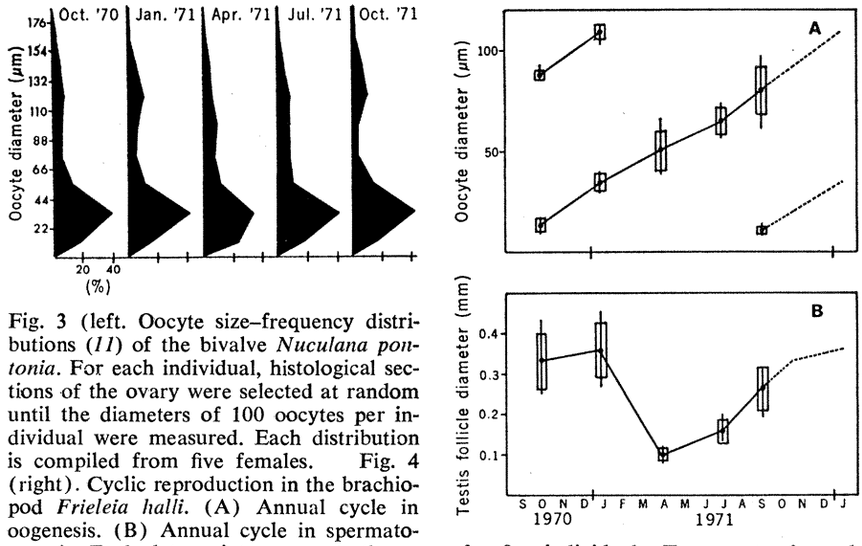

Fig. 3 (left. Oocyte size–frequency distributions (*11*) of the bivalve *Nuculana pontonia*. For each individual, histological sections of the ovary were selected at random until the diameters of 100 oocytes per individual were measured. Each distribution is compiled from five females. Fig. 4 (right). Cyclic reproduction in the brachiopod *Frieleia halli*. (A) Annual cycle in oogenesis. (B) Annual cycle in spermatogenesis. Each data point represents the mean for five individuals. Ten oocytes in each size class were measured in each female and ten testis follicles were measured in each male. A vertical line represents the range of the individual means; a vertical bar represents the 95 percent confidence limits of the overall mean.

in the brittle star *O. normani* at any one time some individual females have only small oocytes, some have oocytes of intermediate size, and others have large oocytes. The overall population mean for oocyte size, however, does not change significantly throughout the year (Fig. 1A). Likewise, the males show no significant seasonal cycle at the population level (Fig. 1B). In all seasons, individual males of the population have either small, intermediate, or large testes. Thus, only a relatively few individuals are capable of spawning at any one time, but at the population level breeding occurs throughout the year.

Year-round recruitment in *O. normani* is clearly seen from the size structure of the population. In all seasons the smallest size class, 0.50 to 1.49 mm in disk diameter, is represented by a stationary peak of between 10 and 30 percent of the population (Fig. 2). Furthermore, the size-frequency histograms are very similar from season to season, without evidence of year classes.

Continuous reproduction was seen among the bivalves (*Nucula darella*, *Nuculana pontonia*, and *Bathyarca* sp.) and a polychaete (*Fauveliopsis glabra*). Typically, as in the bivalve *N. pontonia*, gametogenesis is noncyclic, with oocyte size–frequency distributions (*11*) of the population being identical from season to season (Fig. 3). Furthermore, individuals show similar oocyte size distributions, with both large and small oocytes present in relatively constant proportions. All adult males show all stages of spermatogenesis. These data suggest that for each individual gametogenesis is continuous, with only partial spawning occurring at any one time. This hypothesis is verified by constant recruitment of young into the population. Small individuals, less than 4 mm in shell length, constitute 15 to 24 percent of the population in all seasons.

While year-round reproduction dominates, cyclic reproduction does occur. The brachiopod *Frieleia halli* has a distinct gametogenic cycle with very close synchrony among individuals. Oogenesis is initiated in early fall, more than a year in advance of spawning. Thus, two sizes of oocytes are present in the gonads during part of the year (Fig. 4A). The larger, fully developed eggs are spawned between winter and early spring. A complementary pattern is displayed by the males, but with spermatogenesis occurring shortly after spawning instead of before (Fig. 4B). Mature spermatozoa are present in all individuals only during fall and winter.

Although the brachiopod spawns between January and April this should not be construed as the "breeding season" for bathyal species. A scaphopod, *Cadulus californicus*, from the San Diego Trough also has a synchronous reproductive cycle, but spawns in late summer, between July and October (*12*).

If there is a seasonal influx of food to the bottom, the reproductive patterns of most of the bathyal inhabitants are uninfluenced by it. For these year-round reproducers there must be little or no environmental selection pressure favoring reproduction at any single time of the year. A seasonal food supply may influence the seasonal reproducers, but not in the same way as evidenced by their different spawning periods.

A highly synchronous reproductive cycle may be necessary to ensure maximum fertilization in spawning. The seasonally reproducing species are much less abundant than the other species examined and are sessile or possess rather limited mobility. The attached brachiopod, *Frieleia halli*, is approximately one-hundredth and one-tenth as abundant as *Ophiacantha normani* and *Nuculana pontonia*, respectively. The higher densities and vagility of the other species make epidemic spawning unnecessary—ripe individuals can easily locate mating partners.

The question of the factors influencing the control and synchronization of gametogenesis and the maintenance of a year-long cycle remains unanswered. Periodicity in food supply seems an unreliable and doubtful proximal cause. Annual fluctuations in surface production are not very large and the timing is subject to variation from year to year (*13*). Data for the brachiopod show that it has maintained a predictable annual gametogenic cycle for more than 3 years (*12*).

Circannian endogenous reproductive rhythms have been demonstrated (*14*), but these cycles require external physical cues for synchronization. In the deep sea the physical cue could be tidal. Marine organisms are known to be influenced by tidal cycles, several species having tidal breeding rhythms (*15*). Internal tides at a depth of 1000 m have been shown to cause the cyclic displacement of isotherms as much as 0.5°C (*16*). Near-bottom currents with a clear relation to tidal cycles have been measured in the San Diego Trough (*17*).

However, cyclic reproduction probably is very rare in abyssal depths. The two seasonally reproducing species examined are not really typical deep-sea species. Both have been recorded from continental shelf depths (*18*). Furthermore, brachiopods and scaphopods are of very minor importance in the deep sea, only 12 scaphopod and 15 brachiopod species having been recorded from depths greater than 2000 m in the Pacific Ocean (*19*). Off San Diego the scaphopod *Cadulus californicus* has been collected as shallow as 110 m (*20*), and locally its reproductive cycle may be influenced and synchronized by populations residing in shallower water.

In contrast, the year-round reproducing species belong to groups which are

very common in the deep sea (*19, 21*). The continuous reproducers, in particular, can be considered typical deep-sea species. The bivalves examined have not been recorded from depths shallower than the San Diego Trough and the polychaete belongs to a family which is almost exclusively deep-sea, *Fauveliopsis glabra* being the shallowest recorded species (*22*). Thus, the available data suggest that year-round reproduction is the common pattern in the deep-sea benthos.

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23. I thank R. R. Hessler, W. A. Newman, P. A. Jumars, and D. Thistle for critically reading the manuscript. Supported by NSF grant GA 32429.

24 June 1974; revised 9 August 1974

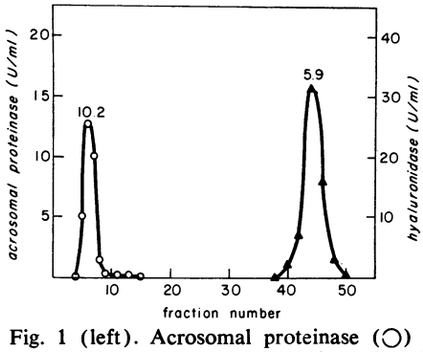
Amino Acid Content of Rabbit Acrosomal Proteinase and Its Similarity to Human Trypsin

Abstract. *Rabbit acrosomal proteinase from epididymal spermatozoa of 44 male rabbits was purified by subcellular fractionation, sucrose density gradient centrifugation, and electrofocusing; the specific activity of the purified product was 20,047 units per milligram, a value similar to that observed for pancreatic trypsins from various sources. The molecular weight determined from the amino acid analyses and ultracentrifugation was about 22,000. This rabbit acrosomal proteinase showed great similarity to pancreatic trypsin, especially to human pancreatic trypsin, both in the number of individual amino acids and in the total number of residues. This similarity was confirmed by an antigenic cross reaction between rabbit antiserum to bovine pancreatic trypsin with human, rabbit, rhesus monkey, and bull acrosomal proteinase.*

Acrosomal proteinase is present in the acrosomes of spermatozoa from numerous species, and its proteolytic activity is essential to fertilization (*1, 2*). Its enzymic properties are similar to those of pancreatic trypsin (*1–7*), and evidence for a trypsinogen-like precursor has also been reported (*8*). However, molecular weight estimations for this enzyme have ranged from 27,300 to 59,000, values considerably higher than those reported for pancreatic trypsin (*2, 4, 5, 7*). It was apparent that an amino acid analysis might help to explain some of the similarities and differences between pancreatic trypsin and acrosomal proteinase, and this was the object of our study.

Rabbit (New Zealand White) epididymal spermatozoa were collected and fractionated subcellularly by means of sonication and sucrose density gradient centrifugation (*2*). The isolated sperm heads were extracted at 37°C with 0.2 percent Hyamine 2389 for 90 minutes, and the extract was centrifuged. This supernatant was fractionated on sucrose gradients at 216,000*g* for 17 hours at 5°C (*2*), and 87 percent of the enzyme was recovered in this step. The fractions containing both hyaluronidase and acrosomal proteinase in the region of 4.3*S* proteins were collected, dialyzed, and subjected to electrofocusing in a sucrose gradient (*p*H 7–10; 2 percent Ampholine, carrier ampholytes) for 70 hours at 3°C (LKB 8100 apparatus), a procedure providing good resolution of acrosomal proteinase (isoelectric *p*H, 10.2) and hyaluronidase (isoelectric *p*H, 5.9) (Fig. 1). The peak fractions of acrosomal proteinase collected from this column had a specific activity of 20,047 unit/mg, and this value represents a 118-fold purification of the enzyme extracted from whole, washed epididymal sperm prior to subcellular fractionation. No further increase in specific activity was obtained after a second electrofocusing.

This specific activity is similar to that observed for pancreatic trypsins from various sources, and similar specific activities were observed in three separate runs by this purification procedure. When the molecular weight of this hyaluronidase-free proteinase was determined by ultracentrifugation in sucrose gradients containing 2 percent acetic acid to prevent aggregation of the enzyme, the proteinase sedimented precisely with pancreatic trypsin in the region of 2.7*S* proteins. The peak fractions from electrofocusing were used



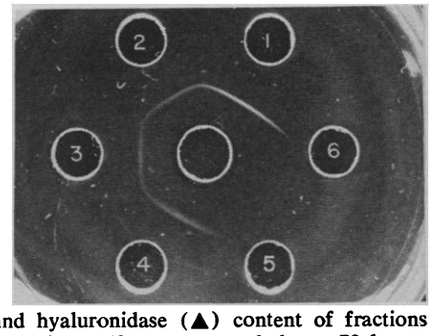


Fig. 1 (left). Acrosomal proteinase (○) and hyaluronidase (▲) content of fractions collected from the LKB 8100 electrofocusing column (2 percent ampholytes, 70 hours, 1000 volts, 3°C). Fig. 2 (right). Immunodiffusion plate with rabbit antiserum to bovine trypsin in the center well. The other wells contained (1) bovine trypsin, (2) human acrosomal proteinase, (3) bull acrosomal proteinase, (4) rabbit acrosomal proteinase, (5) human thrombin, and (6) bovine chymotrypsin.