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The Reproductive Ecology of Elasipodid Holothurians from the N. E. Atlantic

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Abstract *The reproductive and population biology of the deep-sea elasipodid holothurian families Psychropotidae and Deimatidae from the North Atlantic is described. In the psychropotid species the ovary is a large thick-walled nodular structure containing oocytes with an interspecific maximum size of 1.2 mm to 3 mm. In the deimatid species the ovaries consist of thin-walled elongate tubules containing oocytes up to a maximum diameter of 900 μm. In the psychropotids all the oocytes undergo vitellogenesis whereas in the deimatids some could act as nurse cells for those oocytes continuing to grow. Fecundity is low in both families. The soluble lipid content of the ovary in all species is high when compared to other tissues. The calorific content of the whole ovary varies from 25.46 to 28.08 Jmg^{-1}AFDW. There is no evidence for any reproductive periodicity in any of the species examined.*

In the psychropotids the testes are well-developed structures packed with spermatozoa except in those specimens that are infested with protozoan parasites. In the deimatids functional males were never observed. About 50% of the specimens from any population are female but the other half of the population are sexually inactive and are assumed to be predominantly males.

The population structure of the species examined is very variable with single modes skewed towards small or large sizes and in some cases a bimodal distribution. Very small specimens were taken rarely in benthic samples although juveniles of Benthodytes sordida *and* Psychropotes longicauda *have been taken in rectangular midwater trawls at distances of 17 to 1000m above the seabed. This suggests the possibility of a pelagic juvenile capable of dispersal by deep-ocean currents and may account for the cosmopolitan distribution of many of these species.*

Introduction

The elasipodid holothurians dominate the deep-sea invertebrate megafaunal biomass in many areas of the world's oceans. This group has been studied extensively

by Hansen (1975) who described their taxonomy, zoogeography, bathymetry, and biology from material taken by the great oceanographic expeditions. In the N. E. Atlantic, Gage et al. (1985) have described the distribution and known biology of the elasipodids of the Rockall Trough and adjacent areas whilst Lampitt et al. (1986) have reported the variation in biomass of the echinoderms, including the holothurians, with depth. Most elasipodids are epibenthic holothurians feeding on superficial sediment as they roam across the seabed. Khripounoff and Sibuet (1980) and Sibuet et al. (1982) have suggested that epibenthic holothurians are selective deposit feeders taking the detrital particles richest in bioavailable compounds and showing a negative selection for living organisms. Sibuet and Lawrence (1981) studied the biochemistry and calorific content of two elasipodid species and these data have been extended to additional species by Walker, Tyler, and Billett (1987).

Details of reproduction in the elasipodids are limited owing to the general paucity of material. Théel (1882) described the morphology of the gonads in several species but little else was known until Hansen (1975) noted the egg size found in many elasipodids, including the massive egg (ca. 4 *mm* diameter) produced by psychropotids, and described the intraovarian brooding of *Oneirophanta mutabilis affinis*, the only known case of brooding in deep-sea holothurians (see also Hansen, 1968). More detailed analyses of the reproductive biology of elasipodid holothurians has been aided by the deep-sea sampling programs of the Institute of Oceanographic Sciences in the Porcupine Seabight and Abyssal Plain and Madeira Abyssal Plain, and the Scottish Marine Biological Association's campaign in the Rockall Trough. Material from these areas has been examined in detail in two species of Laetmogonidae (Tyler, Muirhead, Billett, and Gage, 1985b), and two species of Elpidiidae, one benthic and one benthopelagic (Tyler, Gage, and Billett, 1985a), whilst preliminary observations of *Deima validum* and *Oneirophanta mutabilis*, are given in Tyler, Muirhead, Gage, and Billett (1985c). All these data suggest direct development with no evidence of a reproductive seasonality although reproduction may be periodic in another elpidiid *Kolga hyalina* (Billett and Hansen, 1982). This paper extends the observations of reproduction to *Psychropotes longicauda, P. depressa, P. semperiana*, and *Benthodytes sordida* (family Psychropotidae) and to *Deima validum* and *Oneirophanta mutabilis* (family Deimatidae), and interprets their reproductive biology in relation to their ecology.

All the species considered in this paper have particularly wide geographic distributions. Two species, *P. longicauda* and *O. mutabilis*, have been found in almost every area of the deep sea at abyssal depths (Hansen, 1975). Other species, such as *P. depressa*, may also have cosmopolitan distributions since their apparent absence from some regions may be an artifact produced by insufficient sampling (Hansen, 1975). The dispersal of psychropotid holothurians is aided by the pelagic development of juveniles which have been found hundreds, and even thousands of meters above the seafloor (Billett et al., 1985).

All but one of the species examined in this study live at abyssal depths (>3000 m) and have a wide bathymetric range. *P. depressa* is an exception, and is usually found within a restricted range at midslope depths (Hansen, 1975). In the Porcupine Seabight this species is abundant only in a narrow depth range between 2300 m and 2440 m.

The species of *Benthodytes* in the northeast Atlantic are imperfectly known partly because species of this genus are difficult to distinguish taxonomically

(Hansen, 1975). The specimens from the Porcupine Abyssal Plain agree with the descriptions of *B. sordida* (Théel, 1882) and *B. janthina* (von Marenzeller, 1893) which Perrier (1902) considered to be identical, but also show similarities to *B. lingua* (Perrier, 1896). Body wall calcareous ossicles are rare in the Porcupine Abyssal Plain specimens but when present are the same as those in *B. lingua*. However, the specimens do not have filiform papillae as in the latter species. The present material therefore is referred to as *B. sordida*, which may be synonymised with *B. lingua* in the future. *B. sordida* occurs in the Antarctic Ocean south of Australia and the Atlantic Ocean. Like the other psychropotids considered, it too has a wide geographic distribution but its precise bathymetric range is uncertain until the various synonymies can be ascertained. On the Porcupine Abyssal Plain it occurs at depths between 3310 m and 4800 m (the greatest depth sampled).

Materials and Methods

The samples that form the basis of this study were collected by the Institute of Oceanographic Sciences in the deeper parts of the Porcupine Seabight, the Porcupine Abyssal Plain, and the Madeira Abyssal Plain (Tables 1 and 2). Additional samples of *Oneirophanta mutabilis* were collected by the Scottish Marine Biological Association in the Rockall Trough. All samples were collected with either an epibenthic sled (Hessler and Sanders, 1967; Aldred et al., 1976) or semiballoon otter trawl (Merrett and Marshall, 1981). On capture all specimens for preservation were fixed in 8% seawater formalin and subsequently transferred to 70% alcohol for storage. Material to be used for biochemical and calorific content analysis was deep frozen.

For the determination of the reproductive biology on *preserved material* the adult was measured prior to dissection of the gonads. To remove the gonad an incision was made on one side of the mid-dorsal axis near the anterior region. In most specimens the gonopore was evident and the incision extended to this point. In all five species examined the gonad is a paired structure with the gonoduct from each half uniting close to the gonopore. One half of the gonad was removed by cutting a gonoduct just distal of this junction and its length noted. Owing to the very large size of the gonads a portion was selected for processing to paraffin wax. Sections were cut at 7–10 μm (the thicker sections being necessary to produce intact sections of large eggs). A section of every specimen examined was stained with Mayers haemalum and eosin to determine the general histology. Sections of particular interest were stained with 0.5% aqueous toluidine blue, methyl-green pyronin, and PAS whilst a selection of female sections were stained with alcoholic Sudan Black B and a selection of male sections stained with the Feulgen reaction. Actual fecundity (the total number of large oocytes produced at one time) was determined by direct counting of oocytes in a whole ovary, whilst potential fecundity (the total number of oocytes in the ovary irrespective of size) was determined by counting the number of oocytes in an ovary nodule and multiplying up by the total number of nodules.

For biochemical and calorific determination the gonads were dissected out of thawed material, refrozen and freeze dried.

For the determination of protein, lipid and carbohydrate the freeze dried tissue

Table 1
Station Data for Samples Used in this Study

Station No.	Date D M Y	Latitude N	Longitude W	Depth (m)
ES28	03/11/73	54° 33′	12° 21′	c.2880
AT119	28/01/77	54° 40′	12° 14′	2908
AT121	29/01/77	54° 37′	12° 09′	2910
ES129	07/04/77	54° 39′	12° 17′	c.2900
AT130	07/04/77	54° 46′	12° 19′	c.2900
SWT15	11/08/77	49° 30′	16° 12′	4810
9638#2	09/11/77	49° 50′	14° 09′	4043–4104
9640#1	13/11/77	50° 05′	13° 51′	3749–3757
ES136	21/02/78	54° 29′	12° 18′	2900
9756#3	11/04/78	49° 48′	14° 16′	4080–4156
9756#5	12/04/78	49° 50′	14° 08′	4012–4020
9756#9	13/04/78	49° 48′	14° 02′	4039–4069
9756#14	15/04/78	50° 04′	13° 54′	3680–3697
SWT27	04/05/78	54° 26′	12° 52′	2965
50511#1	04/06/79	50° 32′	13° 01′	2405–2435
50514#1	05/06/79	49° 40′	14° 01′	4017–4095
50515#1	06/06/79	49° 44′	15° 06′	4505–4515
50603#1	02/07/79	49° 45′	14° 01′	4000
50613#1	09/07/79	50° 29′	13° 03′	2440
ES164	11/08/79	54° 37′	12° 22′	2925
10106#1	04/09/79	50° 41′	12° 50′	2300–2315
10114#1	10/09/79	49° 45′	14° 08′	4050
50711#1	18/10/79	49° 53′	15° 36′	4780–4795
50811#1	02/08/80	49° 39′	14° 34′	4375
50812#1	03/08/80	49° 45′	14° 10′	4090
50812#2	03/08/80	49° 53′	14° 17′	4080
50910#1	10/11/80	49° 50′	14° 45′	4312
51414#1	30/03/82	49° 42′	14° 14′	4245
51608#1	19/07/82	49° 36′	14° 27′	4320
11116#1	24/05/84	47° 46′	15° 23′	4800
52215#1	22/06/85	49° 30′	14° 49′	4561–4565
11261#44	01/07/85	31° 07′	25° 05′	5440
11261#50	02/07/85	31° 13′	25° 18′	5440
11261#52	03/07/85	31° 13′	25° 13′	5440
11261#58	04/07/85	31° 06′	25° 04′	5400–5440
11262#19	18/07/85	31° 20′	25° 29′	5432
52403#13	05/12/86	48° 56′	16° 00′	4805–4810

was powdered, fractionated, and measured as described by Walker, Tyler, and Billett (1987). The ash content was determined by incineration of freeze-dried tissue at 450 ± 50°C for 24 hrs. Calorific content of the tissue can be determined by using the conversion factors of 17.15J/mg for carbohydrate, 39.55J/mg for lipid, and 23.64J/mg for protein (Brody, 1945). The population structure was determined

Table 2
Samples Used in this Study to Examine Reproductive Biology, Showing the Number of Females and Males

	Benthodytes sordida		*Psychropotes longicauda*		*Psychropotes depressa*		*Psychropotes semperiana*		*Oneirophanta mutabilis*			*Deima validum*	
Species:	F	M	F	M	F	M	F	M	F	M	No Gonad	F	M
Stn. No.													
ES28									1	0			
AT119									1	0			
AT121									5	6	1		
ES129									2	1			
AT130									0	1			
SWT15									5	6	3		
9638#2	1	0	4	6									
9640#1	3	1											
ES136											1		
9756#3	0	1	3	7					1	2		1	0
9756#5	0	1							3	0			
9756#9									2	0			
9756#14	2	0											
SWT27									0	1			
50511#1					4	6							
50514#1	2	2											
50515#1	1	0	5	5					7	11	2	2	8
50603#1	1	0	1	1					7	11			
50613#1					4	6							
ES164									0	1			
10106#1					6	4							
10114#1												1	0

(continued)

Table 2 (*continued*)

	Benthodytes sordida		*Psychropotes longicauda*		*Psychropotes depressa*		*Psychropotes semperiana*		*Oneirophanta mutabilis*			*Deima validum*	
Species:	F	M	F	M	F	M	F	M	F	M	No Gonad	F	M
50711#1	1	0	5	3					9	11		0	1
50811#1									12	8			
50812#1												2	0
50812#2												2	1
50910#1									9	11			
51414#1									2	3		1	1
51608#1									4	5		0	2
11261#44							1	1					
11261#50							0	2					
11261#52							2	1					
11261#58							0	1					
11262#19							2	0					
52403#1			3	7									

by measurement of individuals from the head to the posterior end of the body, excluding the unpaired dorsal appendage ("tail") in the *Psychropotes* species.

Observations and Results

Gross Morphology of the Ovary and Testis

In all species examined the gonad is a paired structure lying in the anterior coelom. Each description, however, refers to only one branch of the gonad. The sex ratios are given in Table 2.

In *Benthodytes sordida* the ovary extends up to 20 mm long (Fig. 1a). A narrow (ca. 2 mm diameter) central tube or gonoduct runs the entire length of the ovary. The gonoduct branches at regular intervals and each branch gives rise to an irregular nodular structure in which the oocytes develop. Up to 12 of these branches have been found to arise from the central gonoduct. Oocytes in excess of about 500 μm diameter can be seen as light brown spheres against the pale lilac of the ovary wall. In well-developed specimens oocytes larger than 1 mm are clearly visible through the ovary wall (Fig. 1a). Analyses of the fecundity of *Benthodytes sordida* suggest that there is an actual fecundity of about 260 eggs per female and a potential fecundity of about 4000 per female.

In *Psychropotes longicauda* the gonoduct and ovary extend up to 70 mm long (Fig. 1b). Some 50% of this length is taken up by a very wide gonoduct (c. 8 mm diameter) in which the longitudinal muscles can be seen clearly. Beyond this midpoint alternating globose structures (sacs) arise on either side of the gonoduct. These structures appear to be larger distally. In some well-developed specimens it appears that the "newer" sacs develop proximally along the gonoduct. Owing to the opaque nature and thickness of the ovary wall only the largest oocytes (ca. 2 mm diameter) are visible within the ovary. Occasional oocytes in excess of 3 mm diameter were observed. Despite the apparent large size of the ovary it was rare for one of these sacs to contain a large oocyte. In one specimen in which half the ovary supported 18 sacs only four oocytes larger than 1 mm were found and of these, two were found together. This suggests an actual fecundity as low as eight per individual and a potential fecundity of <250. Much of the interior of the ovary would appear to be packed with pale yellow connective tissue.

The ovary of *Psychropotes depressa* is not as magnificent as its congener *P. longicauda*, growing only to a length of 35 mm (Fig. 1c). The gonoduct forms a central core from which arise numerous sacs which give the ovarian branch the impression of a "corn on the cob" (Hansen, 1975). In excess of 100 of these sacs may be found on each ovarian branch. In most of these sacs one or two oocytes greater than 750 μm are visible suggesting an actual fecundity of about 250 per individual and a potential fecundity of about 5000 per individual. A similar gross ovarian morphology is seen in *P. semperiana*. Eggs up to 3 mm in diameter have been found in *P. semperiana*.

In newly developing females of both *Deima validum* and *Oneirophanta mutabilis* the gonad consists of a few translucent strands lying in the interradius CD (*sensu* Hyman, 1955). The proximal part of the ovary consists of an opaque base from which the gonad tubules develop distally. As the gonad tubules elongate the gonad wall remains thin and vitellogenic oocytes can be recognized through the

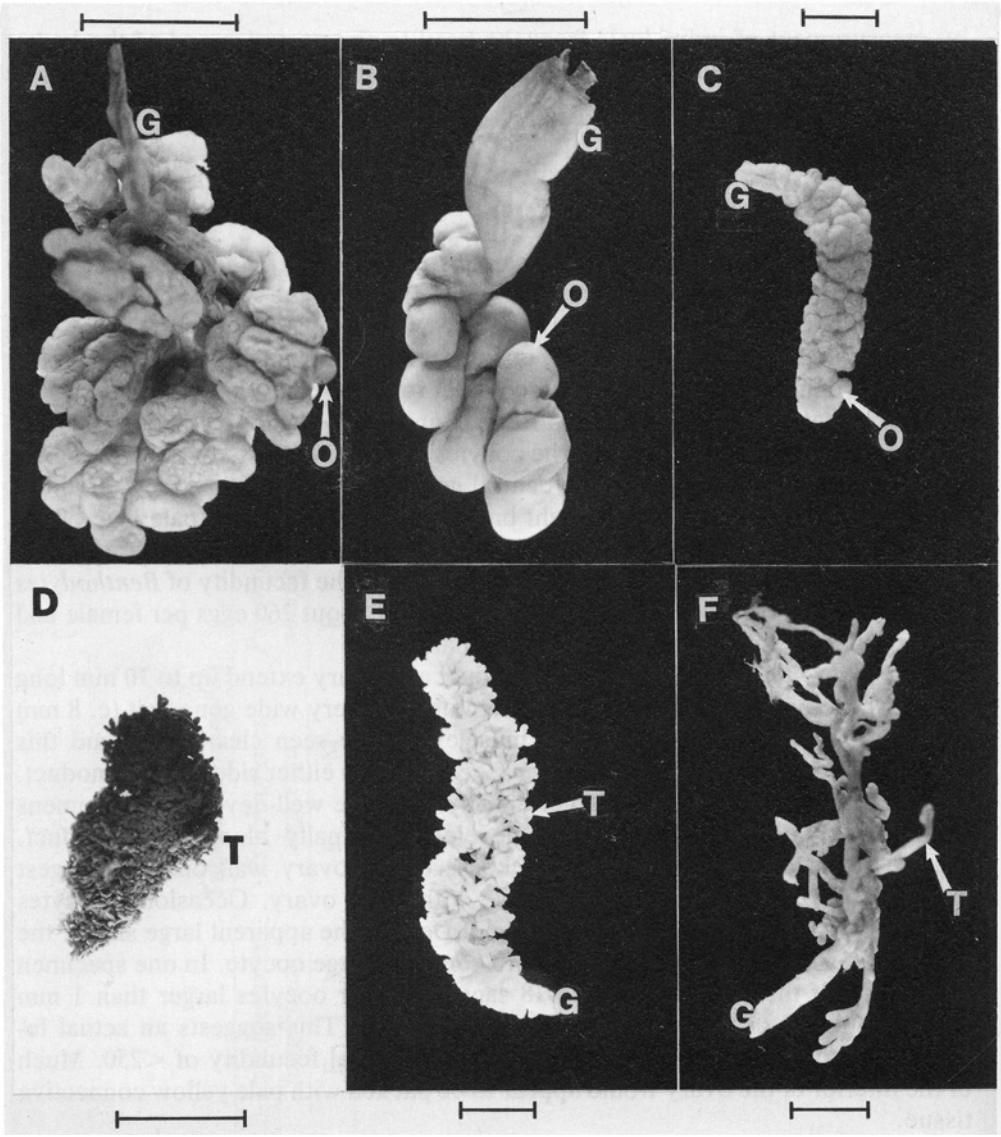

Figure 1. Gross morphology of the gonads: (a) Female *Benthodytes sordida*. (b) Female *Psychropotes longicauda*. (c) Female *P. depressa*. (d) Male *B. sordida*. (e) Male *P. longicauda*. (f) Male *P. depressa*. G = Gonoduct; O = Oocyte; T = testis tubule. Bar = 10 mm.

wall as brown spheres up to 1 mm diameter. In well-developed specimens the gonad is loosely packed with large primary oocytes. Of all the specimens of each species examined, about 50% were obviously female (Table 2) determined by macroscopic or microscopic means. However, the remaining 50% of specimens were *not* obviously male even when examined microscopically (see below). In these specimens (which we presume to be male), the gonad, even in the largest specimens, appears as a collection of translucent tubules with no evidence of spermatozoa as seen in other holothurians examined previously.

In the psychropotidae the testes are well-developed structures. The testis of *Benthodytes sordida* extends up to 30 mm long and is pear shaped with the pointed end directed posteriorly (Fig. 1d). The gonoduct gives rise to a series of short side tubules which bifurcate repeatedly to terminate in a cluster of tubules. Thus the whole testis branch appeared as a compact violet structure which consisted of the central gonoduct surrrounded by a mass of germinal tubules.

The testis of *Psychropotes longicauda* (Fig. 1e) is a much more etiolated structure than that of *Benthodytes sordida*. It consists of a central gonoduct that runs the entire length of the testis. From this central gonoduct arise occasional side tubules which rarely bifurcate except at their distal ends. In most testes examined the pale fawn color is flecked with numerous white spots which may be the protozoan parasite *Ixoreis psychropotae* (Massin, Jangoux, & Sibuet, 1978).

The testis of *Psychropotes depressa* also has a central gonoduct which runs its entire length (Fig. 1f). However, numerous side branches arise from this central rachis, each side branch giving rise to a cluster of stout tubules.

Microscopic Observations of Oogenesis

In *Benthodytes sordida* the ovary wall is covered with a thin coelomic epithelium along which the nucleii of the cells are irregularly lined. Immediately below this epithelium is a β-metachromatic, PAS-positive layer less than 10 μm thick and beneath this layer is a wide highly fibrous connective tissue layer up to 800 μm deep, which forms the main part of the ovary wall (Fig. 2a).

Oocyte development appears to take place in this wall, although definitive oogonia are not recognized. Young primary oocytes (<20 μm diameter) are found along the internal epithelium. These oocytes have the large nucleus and basophilic cytoplasm typical of their developmental stage. By the time these young primary oocytes reach 50 μm diameter they are embedded in a shallow cavity and are covered by a cellular layer continuous with the inner lining of the wall. The primary oocyte continues to grow in its cavity and in some specimens the wall is a continuous series of cavities which contain developing oocytes. These cavities are separated from the lumen of the ovary by a cell layer. These previtellogenic oocytes continue to grow to about 500 μm before there is any evidence of vitellogenesis. The next size of ooyctes readily recognized are those of about 1200 μm diameter in which the yolk is fully formed. This suggests that the actual process of vitellogenesis is very rapid. Vitellogenic oocytes contain amorphous vividly PAS-positive material. However, the periphery of some vitellogenic oocytes has a globular yolk whereas the centre is amorphous. This may be interpreted as a fixation artefact since the dense yolk of the vitellogenic oocyte will prevent rapid fixative penetration.

As development proceeds the wall appears to get thinner but even the vitellogenic oocytes remain covered by a sheet of accessory cells. It is suggested that most of these vitellogenic oocytes are spawned as phagocytosis is observed only occasionally. In many specimens there is evidence of protozoan parasitic infestation.

In *Psychropotes longicauda* the pattern of oocyte development and location appear to be similar to that in *Benthodytes sordida*. Primary oocytes < 1200 μm diameter rest on the inner surface of the wall (Fig. 2b), but at sizes greater than

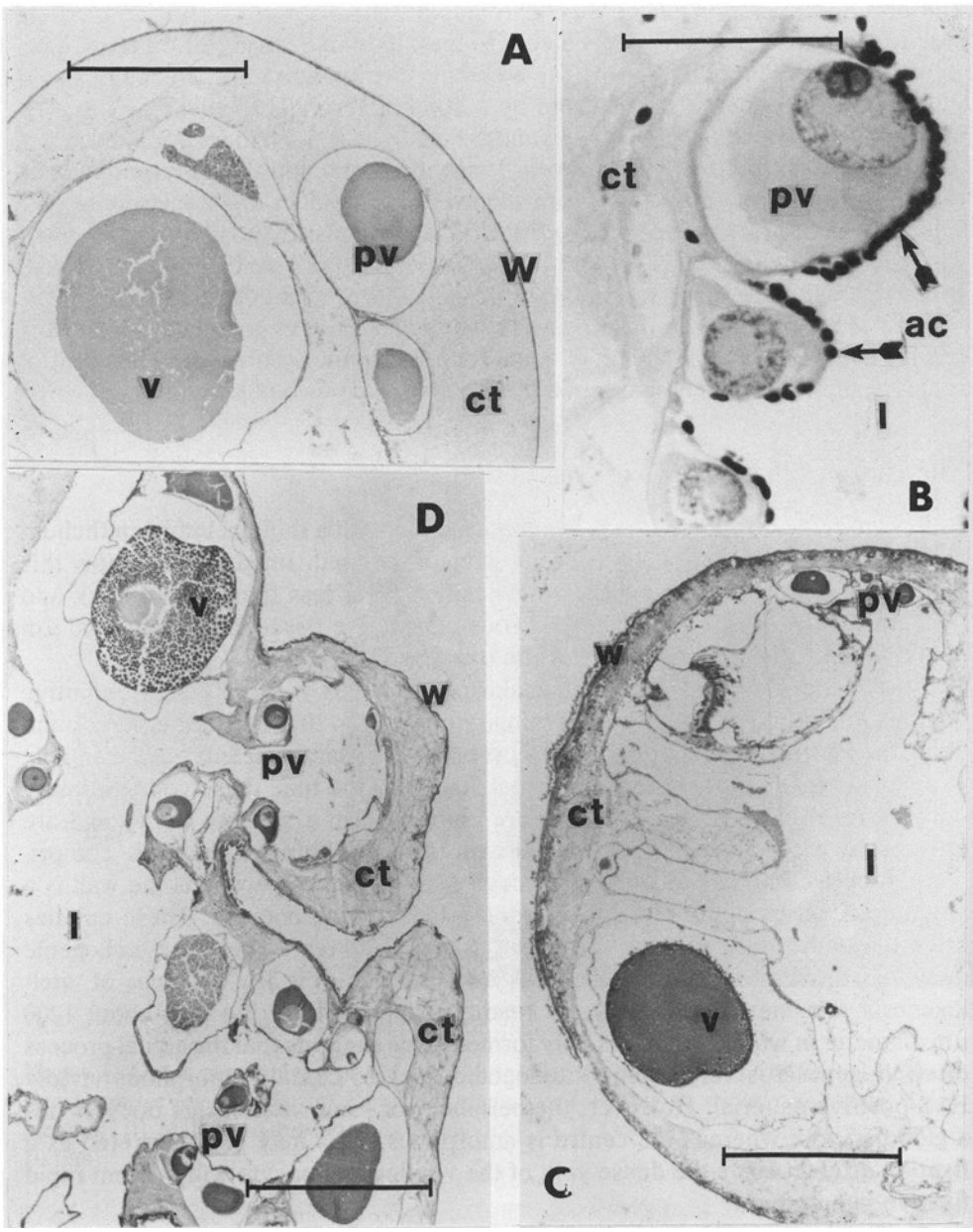

Figure 2. Oogenesis: (a) *Benthodytes sordida* (Methy green pyronin) Bar = 1 mm. (b) *Psychropotes longicauda* (H & E) Bar = 100 μm. (c) *P. longicauda* (Sudan black) Bar = 1 mm. (d) *P. depressa* (Sudan black) Bar = 1 mm. w = gonad wall; v = vitellogenic oocyte; pv = previtellogenic oocyte; ct = connective tissue; ac = follicle cell; l = gonad lumen.

this they appear to develop in cavities in the connective tissue of the wall (Fig. 2c). From this period the oogenic process is very similar to that of *B. sordida*. However, the layer surrounding the developing oocyte is distinctly PAS-positive and the maximum oocyte size produced is in excess of 3 mm. As in *B. sordida* poor fixation penetration is believed to have led to the amorphous appearance of the yolk in these large oocytes. Histochemical staining suggested that the yolk is a bound lipid-carbohydrate complex, although the reaction to Sudan Black B is always less than the reaction to PAS. In some specimens there is oocyte degeneration and this material is very PAS and Sudan Black B-positive.

This pattern of oocyte development is observed also in *Psychropotes depressa* and *P. semperiana*. The main difference between the gametogenic biology of these species and that of *P. longicauda* is that there are more previtellogenic, and that the vitellogenic oocytes, which grow up to 1800 μm in diameter, contain numerous PAS-positive and Sudan Black B-positive globules (Fig. 2d).

The gametogenic process in the Psychropotidae differs from that of the Deimatidae, although the pattern examined in the two species of this latter family is very similar.

From the histology, the ovary wall in the Deimatidae is very thin. The outer wall is lined with coelomic epithelium over a thin layer of muscle and connective tissue. Lining the inner wall is a germinal layer consisting of numerous cells. Of the cells some are obviously oogonia, but we believe the majority are non-reproductive cells.

In the developing ovary individual primary oocytes are found scattered in the germinal epithelium (Fig. 3a,b). These primary oocytes are basophilic and are anchored to the wall by a β-metachromatic, PAS-positive follicle cell layer that is continuous with the germinal epithelium of the inner ovary wall (Fig. 3c—arrows). The lumen of the gonad contains a reticulate material, which is β-metachromatic. These oocytes continue to develop and at a diameter of about 120 μm they are still previtellogenic and are surrounded by up to 20 overlapping follicle cells in section. Vitellogenesis begins at about 170 μm diameter in *Deima validum* and 250 μm diameter in *Oneirophanta mutabilis* whilst the oocyte is still attached to the ovary wall. At this point the fate of these oocytes may follow one of two paths.

Some continue to undergo vitellogenesis. In *Deima validum* the oocyte fills with finely granular PAS-positive material whilst in *Oneirophanta mutabilis* the PAS-positive material is granular but the cytoplasm also contains clear vacuoles suggesting the presence of neutral fats. These vitellogenic oocytes grow to their maximum size of about 700 μm in *Deima* and about 950 μm in *Oneirophanta* but are not as tightly packed in the lumen as seen in other echinoderm species. At this stage the wall of the ovary has become exceptionally thin.

Alternatively, previtellogenic oocytes may undergo degeneration before they reach 250 μm diameter. In these oocytes, often still closely associated with the germinal epithelium, the cytoplasm is invaded by numerous phagocytes. All the material becomes β-metachromatic and some of these degenerating oocytes contain occasional PAS-positive clumps (Fig. 3d). However, the almost complete absence of PAS-positive material suggests that this degenerative process is almost exclusively confined to previtellogenic oocytes. It is possible that this degenerated material is used as nutriment for those oocytes that successfully develop through to maximum size.

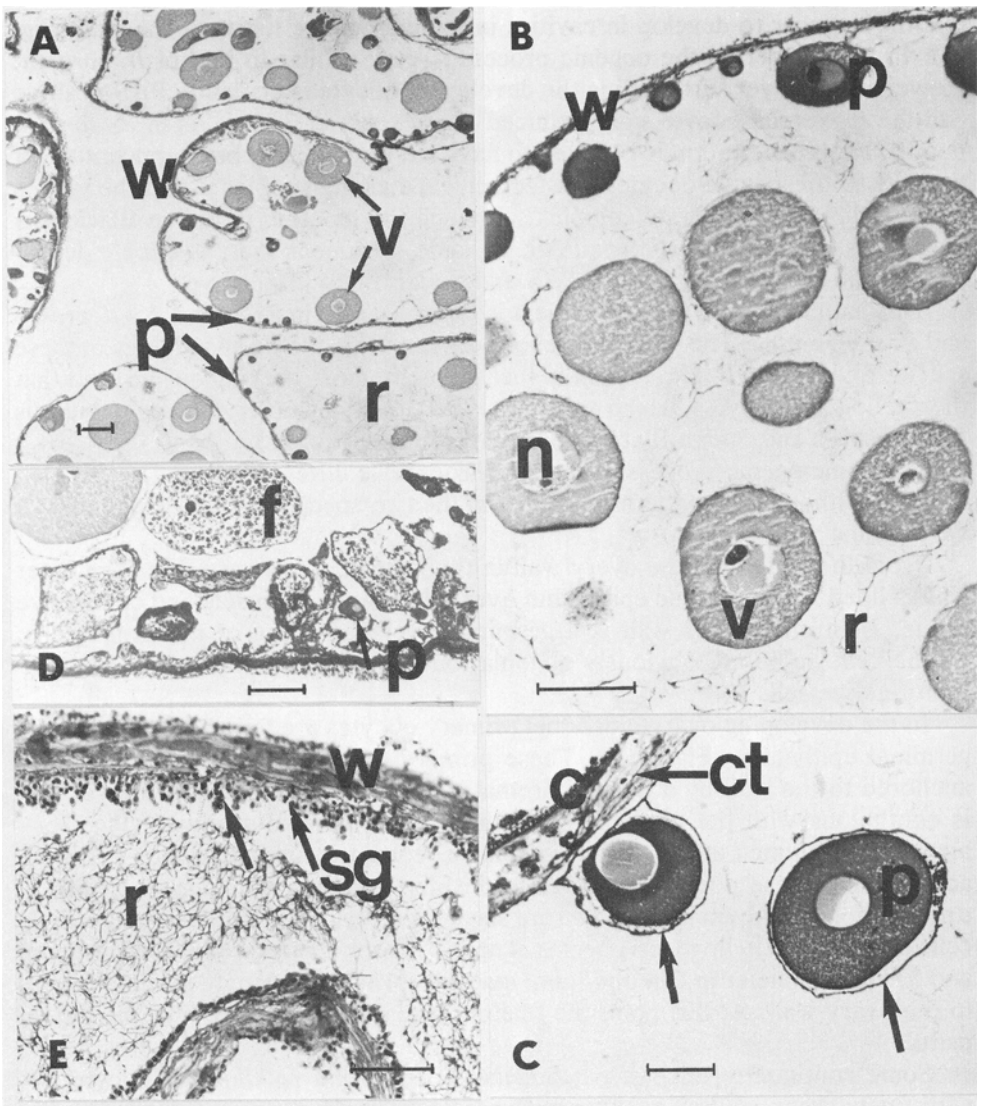

Figure 3. Gametogenesis: (a) *O. mutabilis* general view (H & E). (b) *O. mutabilis* high magnification of (a) to show relationship of gonad wall, previtellogenic and vitellogenic oocytes (H & E). (c) *D. validum* young previtellogenic oocytes with surrounding follicle cells (H & E). (d) *Oneirophanta mutabilis* degenerating oocytes (PAS). (e) *D. validum* testis (PAS). w = gonad wall; v = vitellogenic oocyte; p = previtellogenic oocyte; r = reticulate material; n = nucleus; ct = connective tissue; f = phagocytosed oocyte; sg = spermatogonial layer; arrows = β-metachromatic follicle cell layer. Bar = 100 μm.

In well-developed ovaries the ovary tubules join into a basal mass. This basal mass consists of a considerable amount of β-metachromatic connective tissue, through which runs the proximal part of the ovarian tubules. In some cases these channels are lined with eosinophilic cells of unknown function.

None of the species examined showed any evidence of reproductive season-

ality. There is also no evidence of brooding which suggests that eggs and sperms are released into the water column before fertilization takes place.

Microscopic Observations of Spermatogenesis

In developing specimens of *Benthodytes sordida* the testis tubule is a thin-walled quadripartite structure. The outer layer is the coelomic epithelium. Beneath this there is a β-metachromatic muscle layer followed by a connective tissue layer and the β-metachromatic genital haemal sinus. Although these structures are distinguishable by aqueous toluidine blue, the whole wall appears to be PAS-positive. The β-metachromatic genital haemal sinus makes great infoldings into the testis lumen normal to the wall and these infoldings appear to contain a core of the connective tissue which swells slightly at the distal end (Fig. 4a). These infoldings are lined with spermatogonia and spermatocytes and the whole structure is reminiscent of the "colonettes" found in asteroids (Tyler, Pain, and Gage, 1982). These spermatocytes give rise to spermatids which differentiate into round-headed spermatozoa and cluster in the lumen of the testis. As development proceeds the lumen becomes packed with spermatozoa and the colonettes retract. A high proportion of the males (80%) had a low level of sporozoan infestation.

In the early stages of spermatogenesis in *Psychropotes longicauda* the wall is very thick. It does, however, consist of the quadripartite structure seen in *B. sordida*. The outer layer is coelomic epithelium overlying a β-metachromatic muscle layer. As in the ovary of *P. longicauda* the connective tissue layer is very thick (up to 300 μm). The inner surface of the connective tissue is lined with a β-metachromatic structure, the genital haemal sinus. The whole structure is PAS-positive. The spermatogonia develop in pits in the wall and these pits can be almost as deep as the wall itself (Fig. 4d). No spermatogonial development takes place on the wall surface that immediately lines the lumen. As spermatogenesis proceeds the hollows fill up and the wall becomes much thinner and adopts the colonette structure seen in *B. sordida*. The lumen becomes packed with spermatozoa as spermatogenesis continues. Parasitic infestation by a sporozoan (Fig. 4c) is very common (24 out of 27 males examined) and to an extent that at least *two* testes are completely parasitized and show no evidence of spermatogenic function. In at least one parasitized specimen the lumen is packed with eosinophilic Feulgen-positive cells, of a similar size to the spermatozoa, but of unknown function.

In the early stages of spermatogenesis in *P. depressa* and *P. semperiana* the internal epithelium is highly convoluted and lined with spermatogonia and spermatocytes (Fig. 4b). As spermatogenesis proceeds the colonette structure is observed. The process of spermatogenesis appears to continue in a very similar way to the preceding two species. No sporozoan parasites were observed in *P. depressa* or *P. semperiana*.

Development in the testis in the species of Deimatidae (Fig. 3e) presents much more of an enigma. In all the specimens examined, of both species, the testis consists of a few thin translucent strands. Microscopically the tubule wall is similar to that of the ovary but at most, the germinal epithelium consists of numerous cells forming a layer up to four cells deep along the epithelium. There are no recognizable spermatocytes or spermatids.

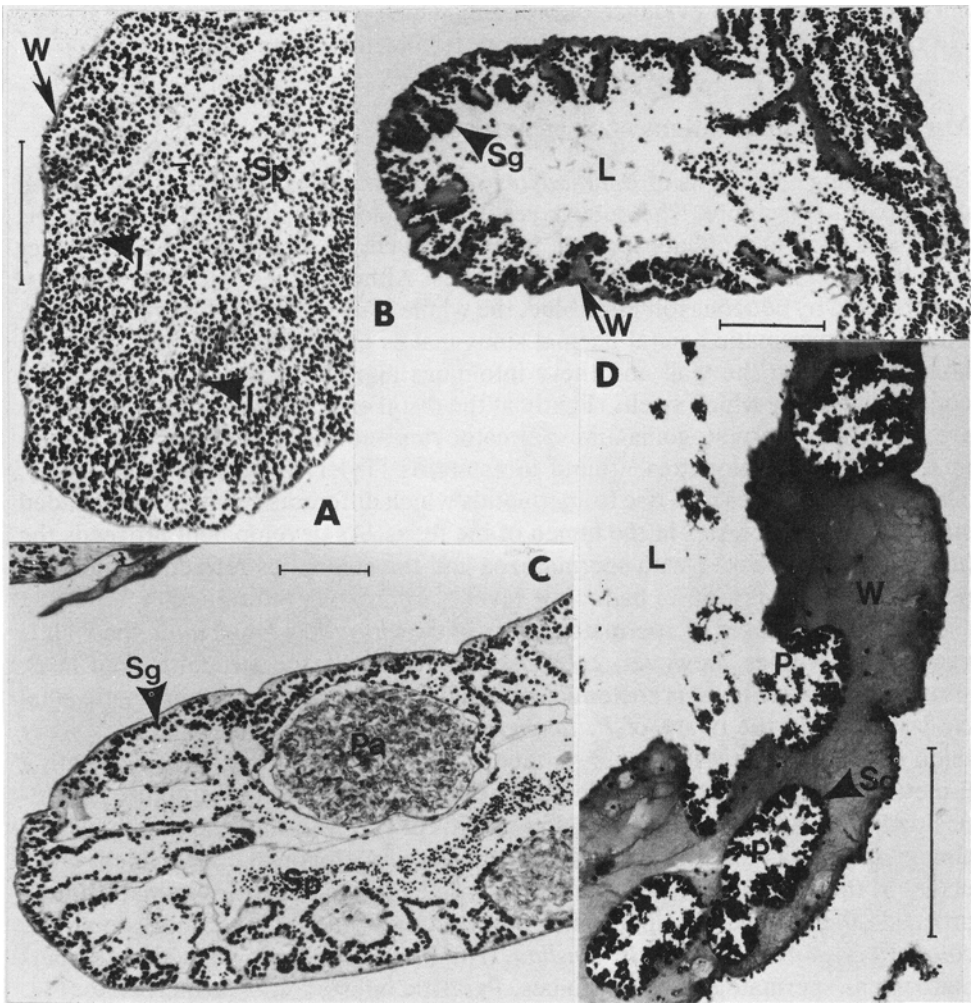

Figure 4. Spermatogenesis: (a) *Benthodytes sordida* (PAS). (b) *Psychropotes depressa* (PAS). (c) *P. longicauda* (PAS). (d) *P. longicauda* (PAS). Sg = spermatogonia; Sp = spermatozoa; w = wall; l = infolding; L = lumen; P = pit in testis wall; Pa = parasitic inclusion. Bar = 100 μm.

Biochemical and Calorific Analysis

The biochemical analysis of the gonad tissue is given in Table 3. In all species examined, in both the ovary and testis, the dominant fraction is insoluble protein. Soluble lipid is high in the ovary whereas soluble protein is high in the testis. Soluble protein would appear to be generally higher in ovaries of the Psychropotidae than the Deimatidae possibly as a function of the thick ovary wall found in the former. No data are available for the males of the Deimatidae (see above). The calorific content of whole ovary varies from 25.46 J mg^{-1}AFDW in *Benthodytes sordida* to 28.68 J mg^{-1}AFDW in *Deima validum*, the difference being most influenced by the calorific conversion value of the soluble lipid fraction in

Table 3
Biochemical and Calorific Content of the Gonads of Elasipodid Holothurians in the NE Atlantic

			Soluble Protein	Soluble Lipid	Soluble C.hydrate	Average Remainder	% Ash	Calorific Content J mg.$^{-1}$ ash-free dry weight
		n	μg. mg.$^{-1}$ dry weight					
Oneirophanta mutabilis	Ovary	17	38.9 ± 18.0	162.2 ± 47.1	11.7 ± 5.5	646.3 ± 76.1	14.0	26.55
Deima validum	Ovary	1	41.4	213.6	16.8	381.3	34.7	28.68
Benthodytes sordida	Ovary	2	60.5 ± 7.6	105.6 ± 22.3	5.6 ± 1.3	726.8 ± 34.3	10.1	25.46
	Testis	2	149.8 ± 21.7	127.6 ± 9.7	17.1 ± 3.8	590.7 ± 51.8	11.4	25.78
Psychropotes longicauda	Ovary	6	76.0 ± 16.7	123.9 ± 57.3	11.4 ± 6.5	547.7 ± 22.3	25.0	26.11
	Testis	4	138.2 ± 31.5	61.7 ± 23.3	8.2 ± 5.0	619.1 ± 32.3	17.2	24.74

the respective ovaries. The calorific value of the testis of the psychropotid species is very similar owing to the very high protein levels.

Adult Size Distributions

Populations of *P. longicauda* on the Porcupine Abyssal Plain are dominated by large specimens generally longer than 130 mm, some reaching a length of 280 mm (Fig. 5). The number of modes in each population is unclear in most samples because of their small sample size (6 samples with <21 specimens each), but in the larger populations sampled, a biomodal distribution is evident. This may reflect spatial and/or temporal variability in recruitment of juveniles to the benthic population since no periodicity is evident in gametogenesis. The smallest specimens of *P. longicauda*, about 20 mm long, correspond to the largest juveniles taken in pelagic nets in the northeast Atlantic (Billett et al., 1985).

Two of the three size distributions of *P. depressa* show a single well-defined mode but the third has a wider size range of specimens and could be bimodal (Fig. 6).

D. validum occurred in abundance in two samples only (n = 24 and 28) from the Porcupine Abyssal Plain. The size distributions are completely different. One sample is dominated by large specimens longer than 80 mm and the other by small specimens generally less than 70 mm in length (Fig. 7). Despite intensive sampling in the Porcupine Seabight, *D. validum* has been collected in two samples only at depths shallower than 3950 m. Although 11 *D. validum* were collected in one of these samples (3000 m) all the specimens were small, only 20 mm to 50 mm long, while the other sample (2750 m) contained just one small juvenile only 6.2 mm long. The latter specimen has plate ossicles in the body wall typical of deimatid holothurians and has retracted tentacles. These features suggest that the juvenile is *D. validum* although further specimens are needed to link this small specimen with larger deimatids for the identification to be substantiated. A similar juvenile, only 4.7 mm long, has been found at 4800 m on the Porcupine Abyssal Plain.

The population size distributions of *O. mutabilis* show great variability (Fig. 8). There is an indication that large specimens, up to 165 mm long, are more abundant close to the base of the continental slope while smaller specimens occur further out on the abyssal plain (compare Stas 50514 and 50515 taken on consecutive days). However, it is clear from the size distributions of Stas. 11116#1 and 52403#13 that there is no general trend in *O. mutabilis* size with depth or with distance from the base of the continental slope. Some populations are dominated by small specimens which may indicate that recruitment to the adult population varies spatially and/or temporally.

Discussion

In all the species examined the gonad is a large structure (up to 20% of ash free dry weight) lying in the interradius CD (*sensu* Hyman, 1955). In *Psychropotes longicauda* as few as eight vitellogenic oocytes are found in the ovary at any time. Developing females, in which the ovary contained numerous small oocytes, were found in the Deimatidae whilst in the Psychropotidae all the ovaries contained well-developed eggs. This may be a function of bias in sampling or may suggest

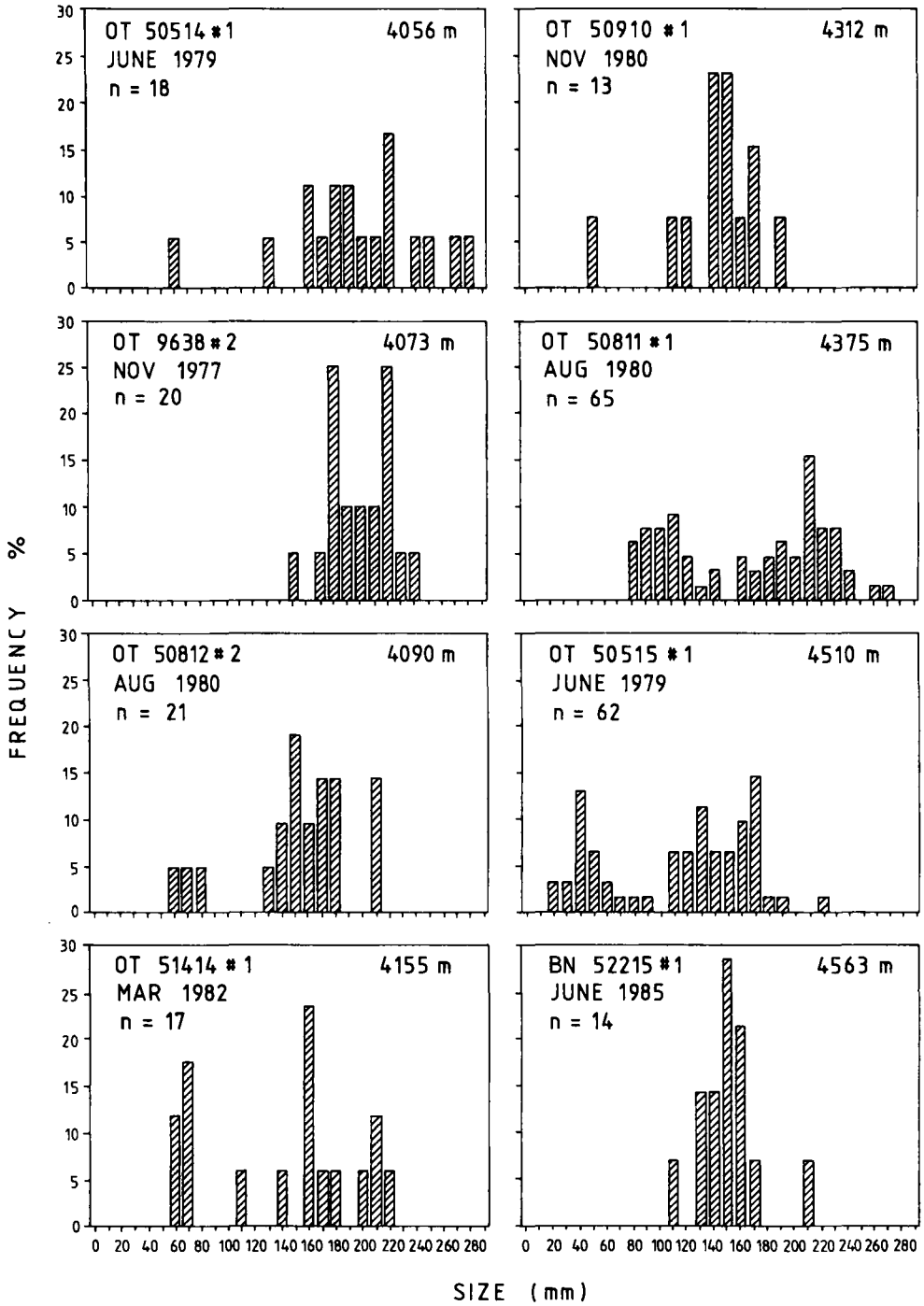

Figure 5. Population size distributions for *Psychropotes longicauda* arranged according to increasing depth. Station number, date and number of specimens measured (n) also given.

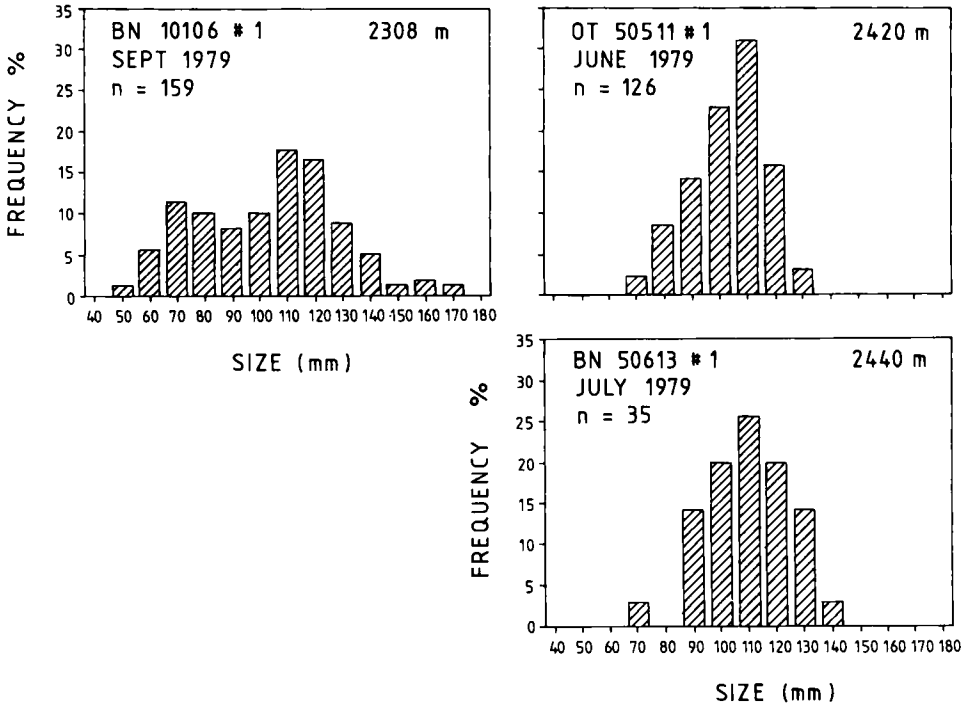

Figure 6. Population size distributions for *Psychropotes depressa*.

that each specimen of *Deima* and *Oneirophanta* spawns all its large oocytes in one go whereas psychropotids spawn only a few eggs at any one time. The possibility of reproductive periodicity in deimatids was noted by Hansen (1975).

Many of the structures seen in the ovaries of species of the two families show subtle but significant differences. The wall of both ovary and testis in the psychropotid species is very thick containing much connective tissue whilst in both deimatid species the wall is very thin.

Gametogenesis and final oocyte size varies between each species. In the Deimatidae the eggs have a maximum size of 950 μm whereas in the psychropotidae the eggs vary between 1.2 mm and 3 mm. Hansen (1975) noted a maximum egg size of 4.4 mm for *P. longicauda*, the largest egg known in holothurians. Hansen (1975) also reported a maximum egg size of 0.5 mm for *P. semperiana*. However, it is evident from our work that this species produces eggs up to at least 3 mm in diameter in common with the other psychropotids. In the well-developed oocytes of the psychropotid species there is dense yolk. Much of this is PAS-positive although the biochemical analysis suggests low carbohydrate content. Histochemical analysis suggests a lipid-carbohydrate complex and lipids, as demonstrated by Sudan Black B, are common in degenerated oocytes suggesting active recycling of material. Although there is considerable soluble lipid in the developing oocytes the proteins in the thick gonad wall account for more of the calorific content than in the ovaries of the Deimatidae. In the cytoplasm of the developing oocytes in *Deima* and *Oneirophanta* are found numerous vacuoles. From these observations and biochemical data we believe this may represent a neutral lipid

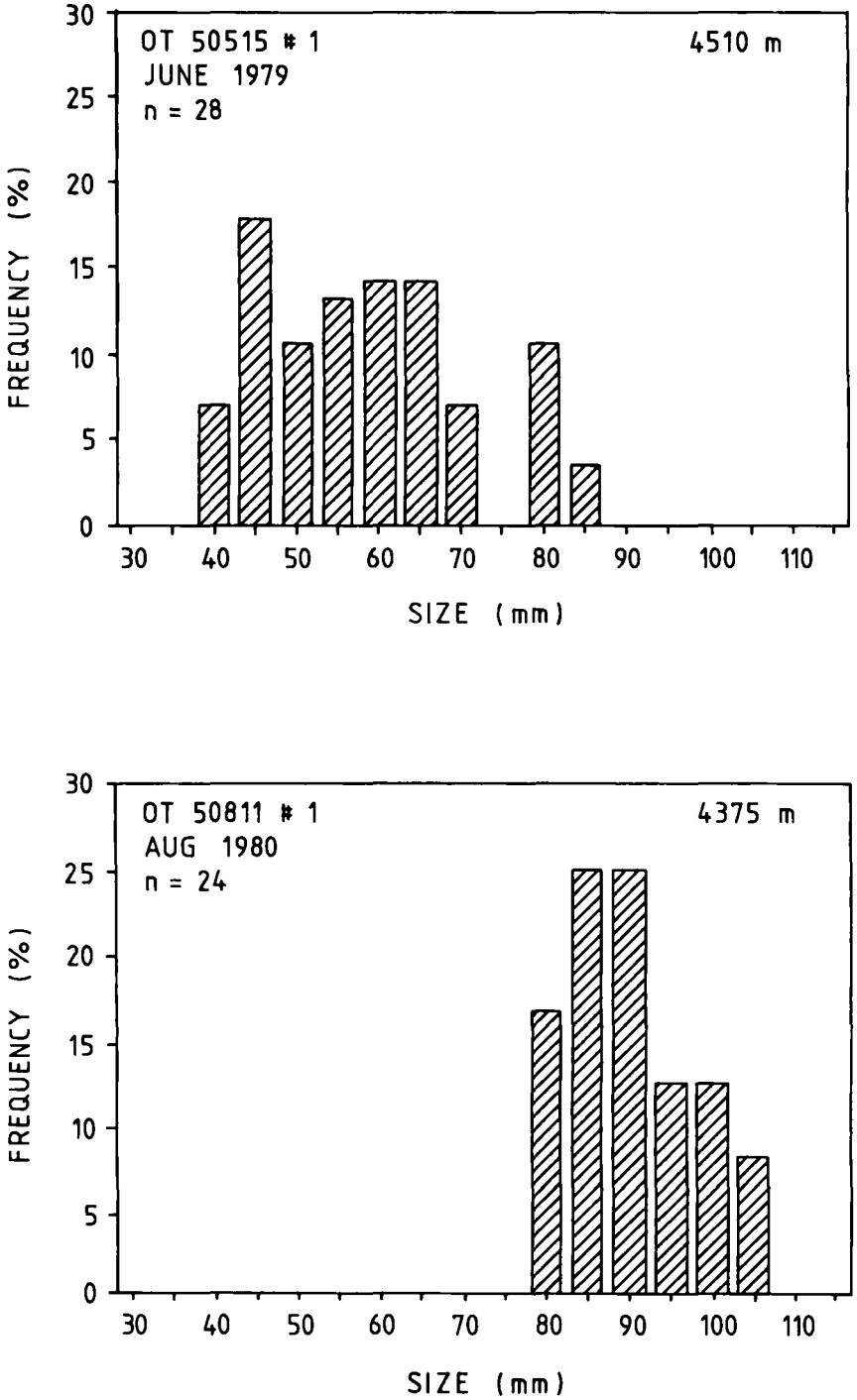

Figure 7. Population size distributions for *Deima validum*.

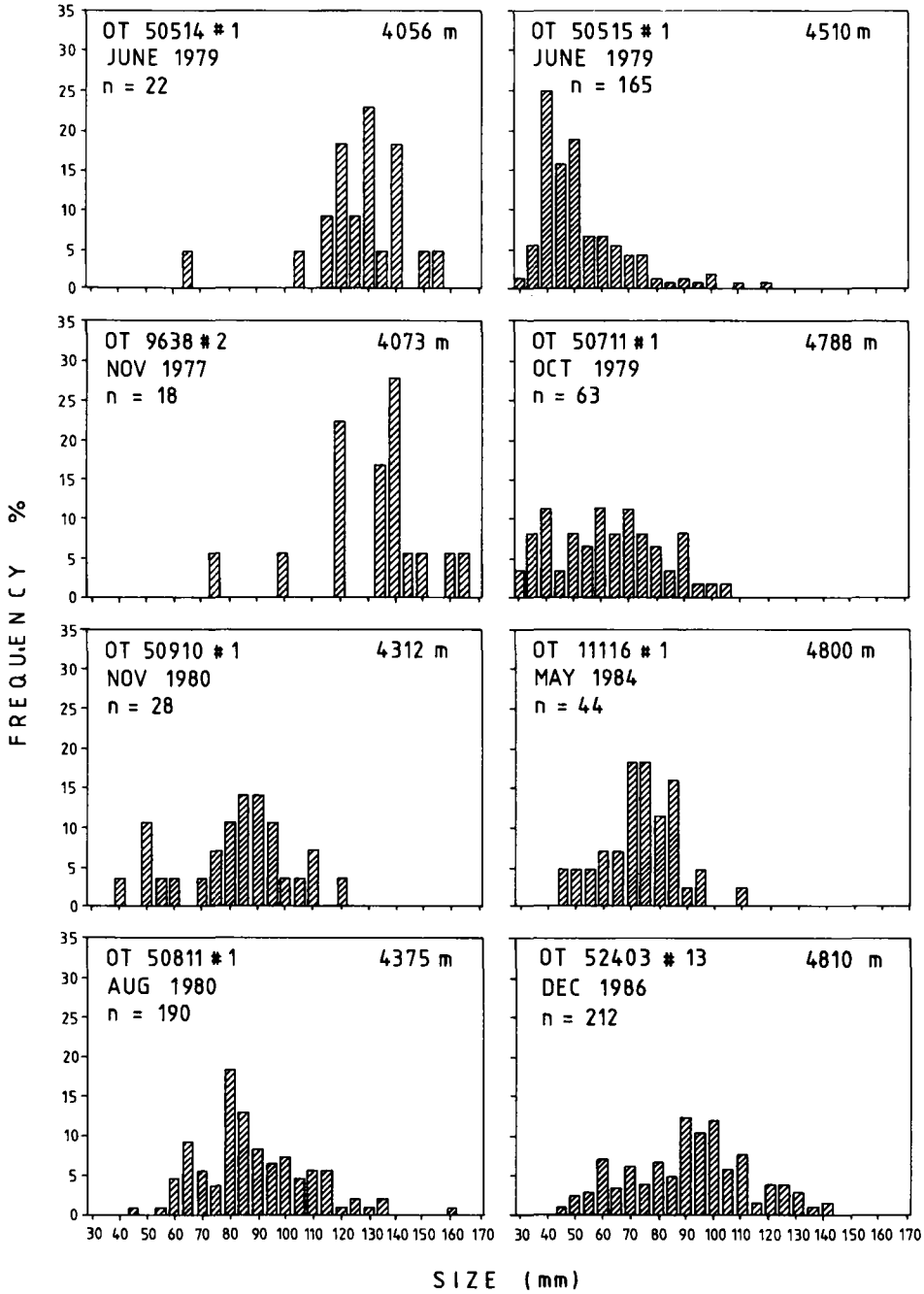

Figure 8. Population size distributions for *Oneirophanta mutabilis* arranged according to increasing depth. Station number, date and number of specimens measured (n) also given.

that is dissolved out during processing. Within the ovaries of the deimatid species there is considerable phagocytic breakdown of oocytes <300 μm diameter suggesting a "nurse cell" function for these oocytes.

The very large size of psychropotid eggs suggests that these holothurians may undergo direct development omitting a larval stage and the egg develops directly to the juvenile. The lipid-rich yolk forms an important energy store for development and acts as buoyancy for the egg. Juveniles of *Benthodytes lingua* (*? B. sordida*) and *Psychropotes longicauda* have been taken in rectangular midwater trawl (RMT) tows at 3485–3515 m (ca. 600 m above the seabed) and at 3470–4008 m (17–1000 m above the seabed) respectively in the northeast Atlantic (Billett et al., 1985). A single juvenile of *P. depressa* has been taken at 1000–1500 m (2500–3000 m above the seabed) in the Porcupine Seabight area (Billett et al., 1985), whilst other psychropotids have been found in pelagic samples elsewhere (Grieg, 1921; Belyaev and Vinogradov, 1969). The large egg produced by psychropotids, therefore, appears to lead to the direct development of the holothurian in the pelagic realm. This allows the wide dispersal of the holothurian and confers the added advantage of allowing development to occur in an environment where biomass (Angel and Baker, 1982) and hence predation is lower than at the sediment surface. Juveniles up to 35 mm long have been taken in pelagic nets (Belyaev and Vinogradov, 1969) indicating that the juvenile develops for some time in the plankton and may be carried a great distance before starting a benthic life. Psychropotids with this type of development are amongst the few deep-sea holothurian species that have wide, even cosmopolitan, geographic distributions. With the possible exception of *P. depressa* it is unlikely that the juvenile would be carried out of an area where the bathymetric range is suitable for adult development.

The smaller egg size of the deimatids in comparison with those of the psychropotids suggests that development in these two families will be different although direct development is still indicated. Juveniles of *Deima* and *Oneirophanta* have never been taken in midwater trawls suggesting that development of their eggs may occur on the seabed or immediately above it. This is substantiated by Hansen (1975) who considered that the clear local variation between populations of *O. mutabilis* in the Kermadec Trench indicated limited dispersal. Hansen (1968) described intraovarian brooding in *O. mutabilis affinis*, a subspecies restricted to the Panama Basin, and believed that the limited dispersal evident in *O. mutabilis mutabilis* indicated that this subspecies brooded its young as well. In addition, he found that *O. mutabilis affinis* brooded juveniles up to a length of 30 mm, a size corresponding to the smallest known specimens of *O. mutabilis mutabilis* (Hansen, 1975). Population size distributions of this species and *D. validum* from the Porcupine Abyssal Plain show a similar lower limit of 30 mm (Figs. 7,8), but no evidence of brooding has been found.

Despite the evidence of limited dispersal (brooding?) in deimatids, a number of features suggest that a pelagic dispersal phase could occur. First, the deimatids are amonst the few deep-sea holothurian species that are found all over the world. Other species with a similar distribution, such as *P. longicauda*, have a pelagic development phase. Second, the ovary of most deimatid specimens is large and contains many eggs, more in keeping with a species that broadcasts its eggs rather than broods them. Third, the presence of small juvenile *D. validum* at shallower depths in the Porcupine Seabight (2750 m to 3000 m) than the depth at which adult

specimens occur (3950 m) suggests that the eggs are transported away from the adult population.

The other enigmatic feature of the deimatids is the absence of reproductively viable males in any sample taken to date. In each population sampled on the Porcupine Abyssal Plain half the specimens are reproductively inactive and these are assumed to be male holothurians (Hansen, 1975; Tyler et al., 1985c). The reason for the absence of spermatogenesis is not known. Spawning on capture may be ruled out as some spermatozoa are always left in the testis. It is possible that the samples have been taken whilst the males are "resting" but evidence from other deep-sea holothurians (Tyler et al., 1985a,b) suggests that individual males remain fully ripe so that when they encounter a ripe female they are ready to spawn. Although sporozoan parasites do occur in *O. mutabilis* (Massin, 1984) and *D. validum* (Billett, pers. obs.) they are not as common as in *P. longicauda* and do not appear to lead to parasitic castration as in the latter species. A seasonal cycle in spermatogenesis is unlikely since no spermatogenic, or oogenic cycle, has been observed, in the present samples which cover eleven months of the calendar year over seven years. However, periodic reproduction on a time scale greater than an annual cycle cannot be ruled out.

Hansen (1968, 1975) noted that where brooding was found in *O. mutabilis affinis* from the Panama Basin, all the brooded juveniles were at a similar stage of development. Sampling occurred in an area of seasonal intense upwelling and at the end of the upwelling period. Development of the young coincided, therefore, with a period of rich surface-water production and Hansen (1975) suggested that seasonal variation in surface productivity may induce reproductive periodicity at abyssal depths. It is now known that the flux of organic carbon to the seabed in the Panama Basin does indeed vary seasonally and that is correlated to surface water primary production (Honjo, 1982). Similar seasonal changes in the quantity of organic matter reaching the seabed have been noted in the northeast Atlantic (Billett et al., 1983; Lampitt, 1985; Rice et al., 1986), but no correlation between deimatid reproduction and the seasonal deposition of organic matter has been noted. The relationship between reproduction and organic matter supply remains tenuous, therefore, although it should be noted that the deimatids occur primarily in regions where the supply of organic matter to the seafloor is known to, or would be expected to, vary seasonally.

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