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A STUDY OF THE FEEDING BIOLOGY OF DEEP-SEA ECHINOIDS FROM THE NORTH ATLANTIC

by

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ABSTRACT

FACULTY OF SCIENCE

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Doctor of Philosophy

A STUDY OF THE FEEDING BIOLOGY OF DEEP-SEA ECHINOIDS FROM THE NORTH ATLANTIC

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The seasonal downward flux of phytodetrital material to the deep-sea floor is now becoming recognized as a significant pathway of nutrient energy to the bottom-dwelling biota. This material is known to be colonized rapidly by bacteria, flagellates and benthic foraminiferans. The diet of two deep-sea echinoids was investigated in order to evaluate the effect of this food input on their life history: *Phormosoma placenta* Wyville Thomson, a non-seasonal breeder; and *Echinus affinis* Mortensen, a seasonal breeder. Both species were collected during two major deep-sea investigations including both temporal and depth related sampling programmes in the NE Atlantic.

Nonlinear regression and 'lack of fit' analysis have resulted in the identification of a number of seasonal fluctuations in the organ and gut content indices of both *P. placenta* and *E. affinis*. The seasonality in the gut wall and gonad indices were highly correlated for both species. The amount of gut content of *P. placenta* showed an annual cycle with higher values in the spring and summer months. The results of gut content analyses indicated a bi-annual cycle with a marked increase in the proportion of organic matter during the summer and the end of autumn in *P. placenta*. The amount of gut content of *E. affinis* showed an annual seasonal variation with low values in the winter and high values during spring and summer months. The proportion of organic matter in the gut content of *E. affinis*, however, did not appear to change seasonally.

During a cruise to the Bahamas from May 25 to June 7, 1989 fresh samples of regular echinoids were also examined: *Cidaris blakei*, *Stylocidaris lineata*, *Aspidodiadema jacobyi* and *P. placenta*.

Morphological observations, transmission electron microscopy and histological analysis of the gut wall, particle-size distribution and chemical analyses of the gut content were undertaken in the laboratory. The gut content of specimens of each species was identified in detail using a scanning electron microscope. A variety of planktonic and benthic material was identified from the gut content of the different species of echinoids. The gut contents were composed of aggregates of diatoms, flagellates, coccoliths, radiolarians, foraminiferans, sponge spicules, and, especially in the Bahamian samples, fibres of plant material such as *Thalassia* sp and *Sargassum* sp.

The analyses of gut content of these deep-sea urchins support the hypothesis that surface-derived plant material and phytodetritus provide a food source which is relevant to life-history processes such as seasonal growth and reproduction.

Pra mãe,
pro tio Johnny,
pro Mark,
pras crianças,
e pros anjos que me ajudam a voar.

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Chapter 1: General Introduction

For the last twenty-five years great attention has been directed to the deep sea regarding various problems related to physical, geochemical, and biological aspects. Two significant advances achieved in our understanding of deep sea ecology have been the discovery of the hydrothermal vents, and of a predictable seasonality below the thermocline (Tyler 1988).

Physical and chemical properties are considered to behave conservatively in the deep sea in the short and medium time scale (Tyler 1988). The benthic environment and the water column below the thermocline was regarded as stable with little variation (Menzies 1965). Only during the eighties has evidence for a variability in the deep sea been presented as a result of analyses of the physiological variation of its organisms, long term current measurements, and sediment flux studies. According to Tyler (1988) the physical variability can be:

1-) predictable, *e.g.* , seasonal variation in the ocean currents (Dickson *et.al.* 1982, 1986), by diurnal (Gould & McKee 1973) and semi diurnal (Lonsdale & Hollister 1979) tidal variation which would gently disturb the physical environment of the deep sea; or

2-) unpredictable such as turbidity flow, or benthic storms.

Also, the downward flux of detritus can be detected:

1-) seasonally, *e.g.*, by particulate organic aggregates (there is a downward flux throughout the year which increases in intensity with season); or

2-) non-seasonally by large food falls, moults, and turbidity currents (Tyler 1988 and authors therein).

The rain of particles and aggregates derived from surface production is considered to be of greater significance to the flux of organic matter into the deep sea and can be predicted in certain areas of the world oceans such as in the Panama Basin, Sub-Arctic Pacific, central Pacific and off California, Sargasso Sea, in the Porcupine Seabight-Abyssal Plain in the Atlantic Ocean, in the mid North Atlantic, and in the western Atlantic (Honjo 1980, Honjo *et. al.* 1980, Deuser & Ross 1980, Deuser *et.*

al. 1981, Billett *et. al.* 1983, Gardner *et. al.* 1984, Altabet & Deuser 1985, Lampitt 1985, Rice *et. al.* 1986, Deuser 1986, Grassle & Morse-Porteous 1987, Thiel *et. al.* 1990, reviewed by Tyler 1988 and Rice *et. al.* 1991).

Deep sea biologists have made significant contributions to our appreciation of an unexpected seasonality below the thermocline by the examination and determination of: the rate of rapidly sinking detritus to great depths from seasonal surface production; the apparently seasonal reproductive and somatic growth response; and the effects these parameters have on the population structure of particular species in the NE Atlantic. British deep sea ecologists have carried out two major programmes in the NE Atlantic:

1-) a unique seasonal time series over approximately a 10 year period in the northern Rockall Trough (by the Scottish Marine Biological Association - SMBA); and

2-) a depth related sampling programme in the Porcupine Seabight (by the Institute of Oceanographic Sciences - IOS).

The material collected by those institutions has formed the basis of a study of reproduction of deep sea invertebrates, especially echinoderms, gastropod molluscs and other benthic mega-invertebrates. As a result of these studies there is a considerable body of data on the reproductive biology of some 50 species which include both non-seasonal and seasonal breeders. Tyler *et.al.* (1982) have related the seasonal reproduction in the deep sea echinoderms to the rapid sinking of organic matter into the NE Atlantic (Billett *et.al.* 1983, Lampitt 1985). In addition to the reproductive response, Smith & Baldwin (1984) and Smith (1987) have shown that the deep sea sediment community oxygen consumption (SCOC) may also respond to this seasonal influx. However these responses have yet to be linked through the process of feeding at the deep sea bed, as this would "*appear to be the pathway for the energy contained in the surface derived organic matter which is taken in and partioned to somatic and reproductive growth*" (Tyler 1988). It is very difficult to demonstrate such a direct relationship since "*there are in the deep sea many processes which will tend to smooth out the degree to which the* *bottom inputs are pulsed"* (Tyler 1988).

The echinoderms would appear to be one of the most conspicuous benthic groups playing an important role in the complex food web at the deep-sea floor. Jangoux & Lawrence (1982) edited a volume in which the nutrition of echinoderms was reviewed. Knowledge of feeding, absorption and gut structure in shallow water adult echinoderm species is relatively well known but our knowledge of these processes in deep sea species is poorly understood. The examination of nutrition in deep sea echinoderms has rarely been attempted owing to the difficulty in obtaining suitable samples. Most deep sea invertebrate material is collected by means of trawls and sledges thus inducing stress in these animals that may lead to evisceration or the loss of the gut contents.

Almost nothing is known about the feeding biology in the deep sea crinoids. We may assume "passive suspension feeding" by analogy with shallow water species (La Touche & West 1980, Meyer 1982), and by submersible observations (Billett, Tyler, personal communication).

Of the remaining echinoderm classes which often dominate the biomass at bathyal and abyssal depths, feeding in holothuroids and ophiuroids has received some attention. There have been a limited number of studies of feeding in deep sea holothurians (Khripounoff 1979, Sibuet 1980, Khripounoff & Sibuet 1980, Sibuet & Lawrence 1981, Deming *et.al.* 1981, Sibuet *et.al.* 1982, Massin 1984, Suchanek *et.al.* 1985, Briggs 1985, Billett *et. al.* 1988, reviewed by Billett 1991) and a number of these studies have used the French DSRV 'Cyana' (Sibuet, Tyler, personal communication). In general for deep sea holothurians we have an outline of the gut content, biochemical and calorific values and a speculative idea about how this varies on a seasonal basis when related to the input of organic matter to the deep sea. Billett *et. al.* (1988) quantified chloropigments present in the gut content of some holothurians from the Porcupine Seabight and showed that the selection coefficient for *Benthogone rosea*, and probably other holothurians, changes on a seasonal basis concomitantly with the annual cycle in the deposition of detritus.

Data of feeding in deep sea ophiuroids have been reviewed by

Tyler (1980). These data are often the result of "side observations" of major taxonomic studies (e.g. Mortensen 1933, Madsen 1973) although there have been the occasional specialist studies (e.g. Schoener & Rowe 1970, Litvinova & Sokolova 1971, Pearson & Gage 1984). In none of these studies has any analysis been made of the biochemical or calorific content of the gut contents or gut structures.

Of the main echinoderm classes the asteroids appear to show the widest variety of feeding types. Jangoux (1982) has summarized the food of a number of asteroid species including those found in the deep sea. Only two studies have examined the gut content in detail: Carey (1972) and Khripounoff (1979). Brief examination of the gut content of deep sea starfish were made by Pain *et. al.* (1982) and Tyler & Muirhead (1986).

Feeding in the deep sea echinoids has also received very little attention and been restricted to :

1-) submersible observations of the feeding behaviour (Grassle *et.al.* 1975, Pawson 1976, 1982);

2-) the use of deep sea time lapse photography (Lampitt 1985);

3-) limited observation of the gut contents. (Mortensen 1928, 1935, 1943, Lewis 1963, Pawson 1976, 1982, McClintock *et. al.* 1990).

Lewis (1963) briefly described the gut contents of a series of bathyal echinoids species collected off Barbados. Only Suchanek *et.al.* (1985) have studied in detail the diet of the Caribbean deep sea species *Hygrosoma petersi* and *Salencidaris profundi*. Other nutritional studies on echinoids refer to species found in shallow water (Carpenter 1981, Lane & Lawrence 1982, McClintock *et. al.* 1982; Lawrence & Glynn 1984, Coyer *et.al.* 1987, Briscoe *et.al.* 1988).

Mortensen (1935) and Pawson (1982) observed "mud pellets and organic substances in mud" in the gut contents of *P. placenta* which has been observed to feed on petroleum hydrocarbons and plant material (Pequegnat & Jeffrey 1979, De Ridder & Lawrence 1982). *E. affinis* appears to feed on surface derived organic matter showing some preference for patches of the sea bed covered with detritus (Lampitt 1985). Other than these very limited data nothing had been recorded on feeding, gut content, biochemical, or calorific content on individual samples from the deep sea let alone on a seasonal basis.

The aim of this research work was to address two prior fundamental questions:

1-) Do the continually breeding deep sea echinoids benefit from the seasonal input of surface derived material and store it in, for example, the gut wall, or does the seasonally derived material have little or no effect on the feeding processes of these particular animals?

2-) In seasonally breeding deep sea echinoids, is there a direct link between this seasonally derived surface material and the seasonal growth of the gametogenic tissue through the digestive pathway?

In order to answer these questions samples of two species of regular echinoids found in the deep NE Atlantic were examined:

1-) *Phormosoma placenta* (Wyv Thomson) which does not appear to have any seasonal cycle in oogenesis, probably breeding year-round (Tyler & Gage 1984a, Gage *et.al.* 1985) (Figure 1.1a, b); and

2-) *Echinus affinis* (Mortensen) which is seasonally reproductive (Tyler & Gage 1984b) (Figure 1.1c, d).

Samples of both species have been collected by the IOS and the SMBA during their cruises to the Porcupine Seabight and Rockall Trough respectively. Observations on these species include macro- and micro-morphology of the gut wall (Chapter 3), appearance and total organic matter of the gut content, and gonad development (Chapter 4).

A diving cruise to the Bahamas, using the Harbor Branch Oceanographic Institute R/V 'Seward Johnson' and DSRV 'Johnson Sea-Link-II', provided fresh samples of additional species of deep-sea echinoids. This cruise allowed personal observations on the behaviour of several species of echinoids and analyses of the gut contents were undertaken mainly on four species of echinoids including *P. placenta* (Chapter 5).

A general description of the environments where the deep-sea echinoids used in this research work were collected is given in

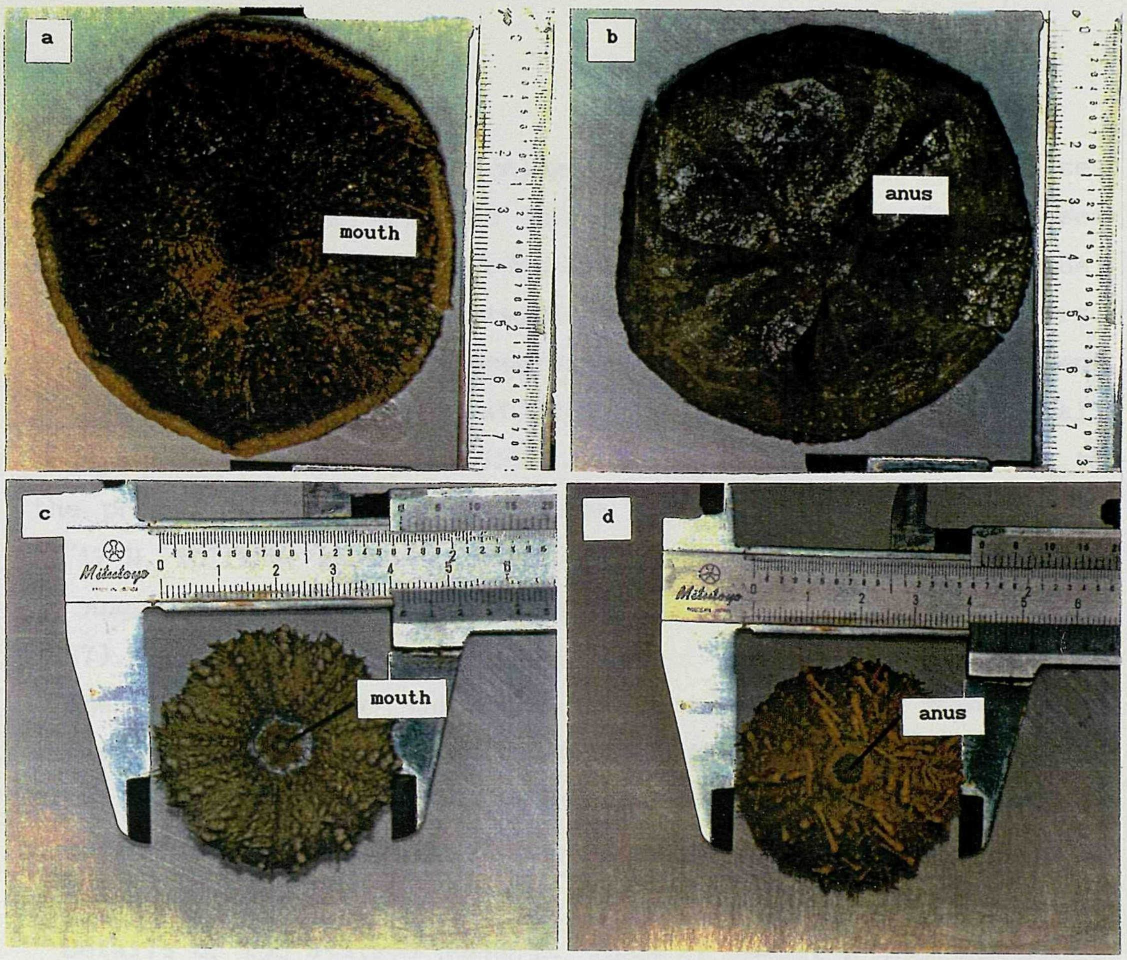


Figure 1.1. Whole urchins as they come on board ship after collection with a semi-balloon otter trawl. *Phormosoma placenta*: **a.** oral view, **b.** dorsal view; and *Echinus affinis*: **c.** oral view, **d.** dorsal view.

Chapter 2: Study sites

2.1. Introduction

An understanding of the ecological processes in the deep-sea benthos of the NE Atlantic has been the target of deep sea biologists from the Scottish Marine Biological Association (SMBA) and the Institute of Oceanographic Sciences (IOS) in recent years. The SMBA has carried out a programme of sampling at the "Permanent Station", 54°14'N, 12°16'W, at 2900m depth in the Southern Rockall Trough at the base of the Irish Continental slope, from 1975 to the present. This station has been supplemented by station "M", 57°17'N, 10°11'W, at the base of the Barra Hebridean Fan from 1978 to August 1983 (Figure 2.1) (Gage *et.al.* 1980, Pain 1983, Colman 1987).

The IOS programme has sampled at approximately 500m depth intervals between 200m and 4800m from the top of the Porcupine Bank down its eastern flank, through the mouth of the Porcupine Seabight and out onto the Porcupine Abyssal Plain (Billett 1988, Rice *et.al.* 1991) (Figure 2.1).

Details of the station numbers, types of sampling gear and areas where *P. placenta* and *E. affinis* have been sampled are listed in the Tables 2.1 and 2.2 respectively.

Echinoids samples collected at depths between 238-893m and through manned submersible dives off the Bahamas were also used in this study as part of a sampling programme of the Harbor Branch Oceanographic Institution. Diving sites are represented in Figure 2.2 and details of the dives are listed in Table 2.3. Most dives were done in the Northwest Channel and South West Reef in the Tongue of the Ocean.

The study sites, sediments, water masses circulation, and fluxes of surface derived material are described and discussed in this chapter.

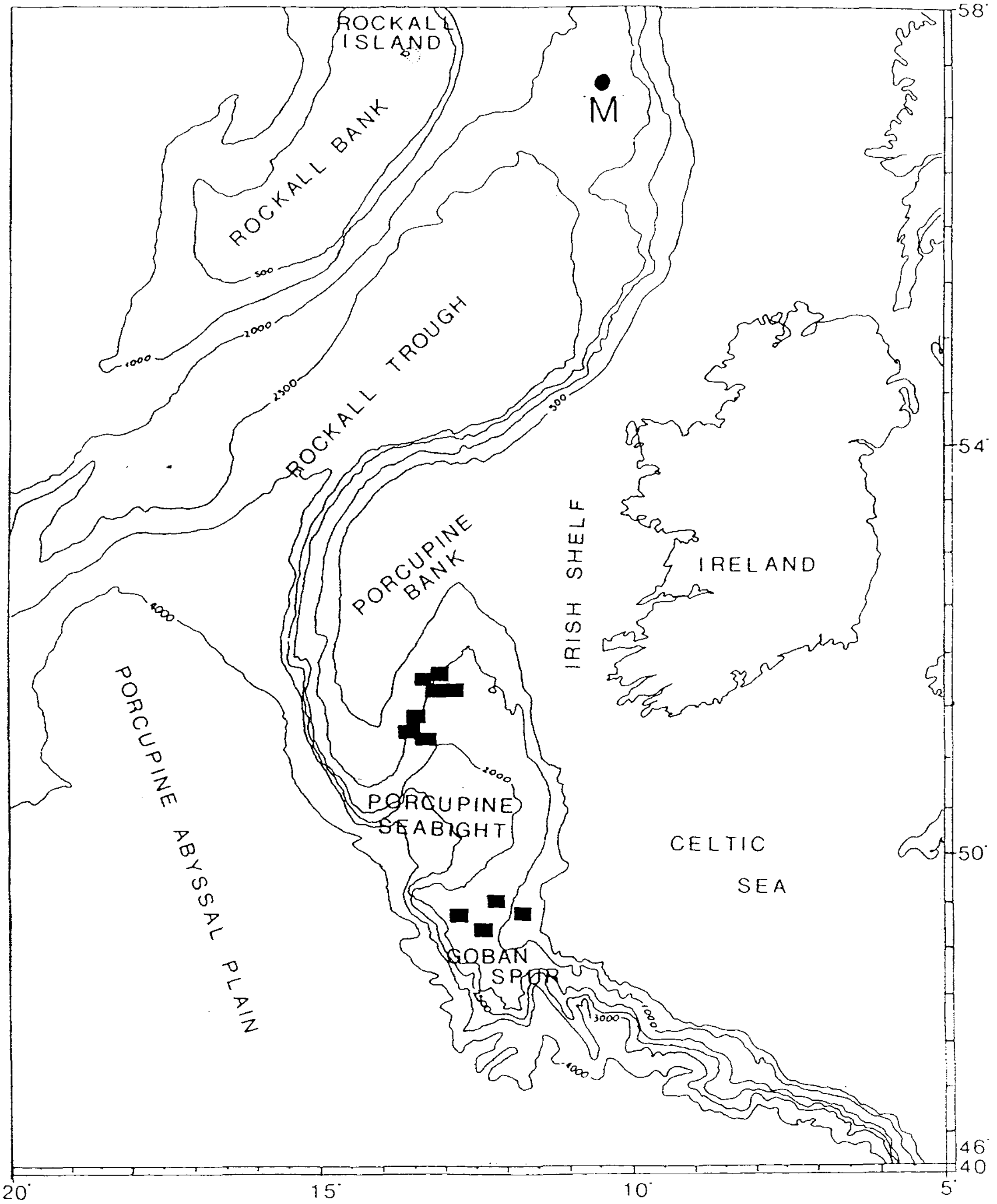


Figure 2.1. ● S.M.B.A. sampling station 'M' in the Rockall Trough where *Echinus affinis* were collected and, ■ I.O.S. sampling stations in the Porcupine Seabight and Goban Spur where *Phormosoma placenta* were collected. Depths in metres.

Table 2.1. *Phormosoma placenta*: details of the gear, station, date, mean depth, and position of the stations where specimens were collected by IOS. OT - semi-balloon otter trawl; GT - Granton trawl; BNC - coarse mesh epibenthic sledge; BNF- fine mesh epibenthic sledge.

GEAR	STATION	DATE	DEPTH (m)	LATITUDE	LONGITUDE
OT	51305-1	18/Feb/82	985	51° 50'N	13° 05'W
OT	51306	19/Feb/82	1005	51° 44'N	12° 53'W
OT	51404-1	26/Mar/82	733	51° 54'N	13° 18'W
BNF	9779-1	24/Apr/78	1401	49° 22'N	12° 49'W
OT	51007-1	02/May/81	1025	51° 46'N	13° 06'W
OT	50509-1	03/Jun/79	1507	51° 14'N	13° 17'W
OT	50522-1	08/Jun/79	983	49° 25'N	11° 45'W
BNC	50610-1	08/Jul/79	980	51° 26'N	13° 24'W
GT	50809-1	01/Aug/80	1250	49° 32'N	12° 10'W
BNF	10109-1	07/Sep/79	1125	49° 12'N	12° 19'W
OT	50904-1	08/Nov/80	1028	51° 20'N	13° 28'W

Table 2.2. *Echinus affinis*: details of where specimens used in this study were collected. Methods of collection were: AT - Agassiz trawl; SWT - small single warp otter trawl; ES - epibenthic sled; OTSB - semi - balloon otter 14 trawl.

GEAR	STATION	DATE	DEPTH (m)	LATITUDE	LONGITUDE
AT	153	15/Jan/79	2200	57° 20'N	10° 27'W
AT	171	03/Mar/80	2225	57° 10'N	10° 17'W
AT	186	12/Apr/81	2170	57° 22'N	10° 19'W
AT	144	19/Apr/78	2240	57° 13'N	10° 20'W
AT	154	21/May/79	2264	57° 08'N	10° 22'W
AT	177	29/May/80	2200	57° 18'N	10° 16'W
AT	151	06/Jun/78	2175	57° 21'N	10° 22'W
AT	245	25/Jul/83	2175	57° 25'N	10° 21'W
AT	247	27/Jul/83	2084	59° 02'N	10° 55'W
AT	271	04/Aug/83	2220	56° 42'N	10° 25'W
AT	273	05/Aug/83	2200	56° 10'N	10° 29'W
AT	228	10/Aug/82	2026	57° 01'N	09° 51'N
AT	195	18/Aug/81	2190	57° 23'N	10° 27'W
AT	181	16/Sep/80	2220	57° 19'N	10° 28'W
ES	184	17/Sep/80	2260	57° 14'N	10° 24'W
SWT	18	22/Oct/77	1809	56° 46'N	09° 42'W
AT	355	24/Oct/87	2190	57° 18'N	10° 24'W

Table 2.3: Details of species and number of specimens collected (N°), dives numbers, positions and depths of collection.

SPECIES	N°	DIVE N°	DATE	POSITION		MEAN DEPTH (m)
				Lat.	Long.	
Stylocidaris lineata	5	1787	25/05	24°53'N	77°33'W	665
Phormosoma placenta	3	1787	25/05	24°53'N	77°33'W	665
Cidaris blakei	18	1788	25/05	no data		550
S. lineata	2	1788	25/05	no data		550
Aspidodiadema jacobyi	20	1789	26/05	25°26'N	78°02'W	426
Conolampus sigsbei	1	1789	26/05	25°26'N	78°02'W	426
A. jacobyi	6	1790	26/05	25°25'N	77°59'W	538
Araeosoma sp.	1	1791	27/05	25°22'N	78°05'W	808
P. placenta	10	1791	27/05	25°22'N	78°05'W	808
Archaeopneustes hystrix	10	1792	27/05	24°51'N	77°51'W	447
S. lineata	12	1793	28/05	24°54'N	77°33'W	463
Coelopleurus floridans	7	1793	28/05	24°54'N	77°33'W	463
P. placenta	1	1795	29/05	no data		506
S. lineata	10	1796	29/05	24°53'N	77°32'W	500
A. jacobyi	5	1796	29/05	24°53'N	77°32'W	500
Calocidaris sp.	3	1799	30/05	25°06'N	77°23'W	238
P. placenta	4	1804	02/06	24°54'N	77°34'W	736
Stereocidaris sp.	5	1804	02/06	24°54'N	77°34'W	736
A. jacobyi	23	1807	04/06	25°23'N	77°55'W	536
Araeosoma sp.	1	1811	05/06	25°26'N	78°02'W	449
P. placenta	15	1812	06/06	no data		893

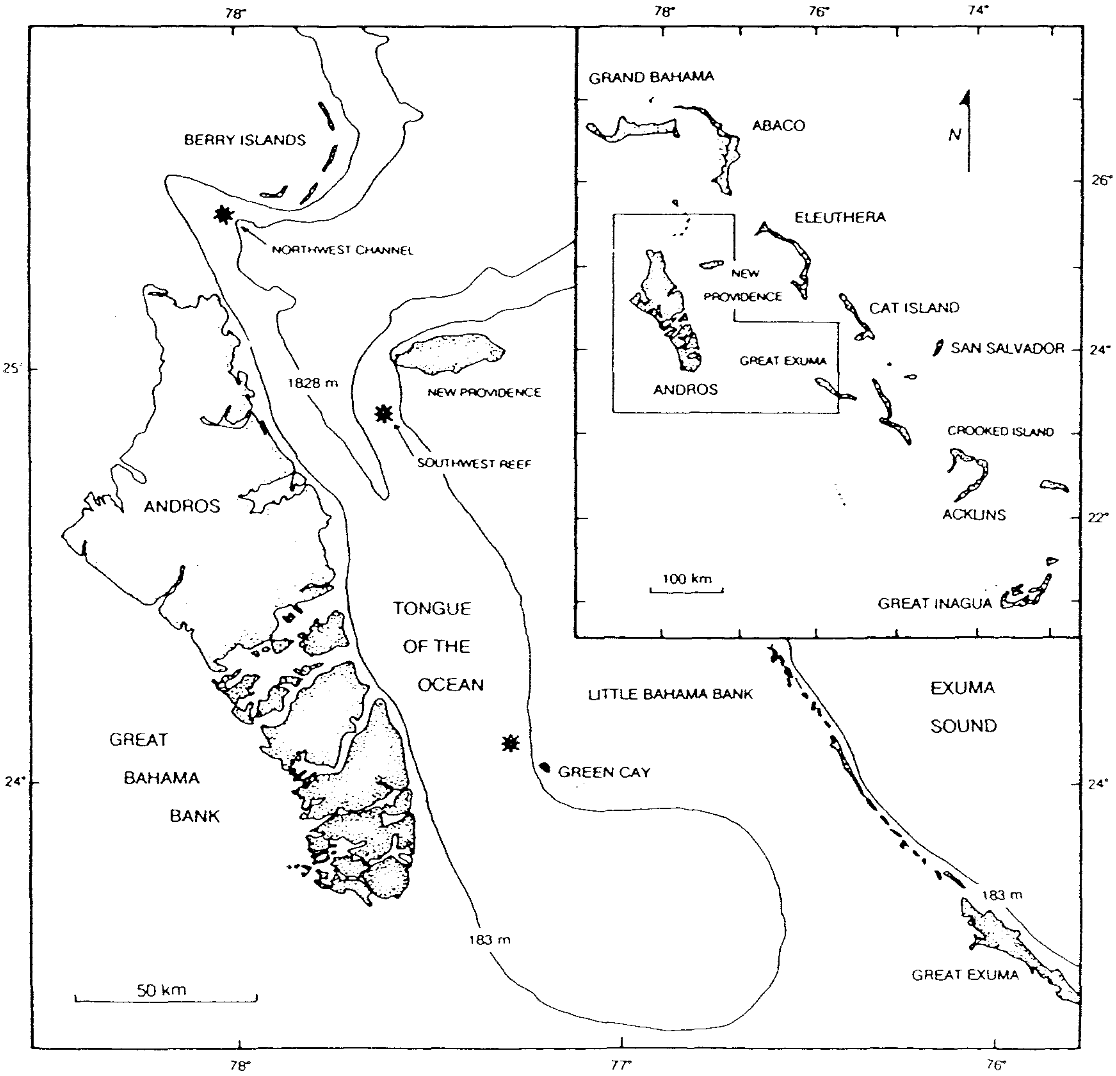


Figure 2.2. ✱ Sampling areas where dives with the D.S.R.V. 'Johnson Sea-Link-II' took place in the Bahamas in 1989.

2.2. Sampling sites

2.2.1. The Rockall Trough

The Rockall Trough is a tongue of deep water, 250 km wide, extending northeastwards into the U.K. continental margin from the North Atlantic Ocean basin to the south (Scrutton 1986) (Figure 2.1). Its depth ranges from 1200m in the north to 3000m in the south (Lonsdale & Hollister 1979, Scrutton 1986). As the Porcupine area, it has developed since the Early Cretaceous by rifting and sea floor spreading between Europe and Greenland, subsequently being partially infilled by sediment (Naylor & Monteney 1975, Roberts *et.al.* 1979, Naylor & Shannon 1982, Pain 1983, Colman 1987, Billett 1988).

The Rockall Trough is delimited by the 1250 m isobath to the north and northwest. This depth is common to the channels separating Rockall Bank, Lousy Bank and Bill Bailey's Bank, all of which rise to within 500 m of the surface (Ellett & Martin 1973). The Faroe Bank Channel, on the northern side of the Wyville-Thomson Ridge, connects the deep water of the Norwegian Sea with the Atlantic Ocean immediately north of the Banks bordering the Rockall Trough (Pain 1983). The Feni Ridge, a broad current deposited sediment drift whose sinuous form follows the contours of the Rockall Plateau, bounds the western margin of the Trough. The Malin and Hebridean shelves, and the Porcupine Bank form the eastern boundary of the Trough (Roberts 1975).

The surface sediments in the Rockall Trough consist mostly of calcareous fine silts with a present rate of deposition of approximately 2-7cm.10^{-3} years (Kuenen 1950, Roberts 1975). In addition, hemipelagic marls, calcareous oozes, and silt and sand are found at deeper levels in the Trough (Roberts & Kidd 1979). Scanning electron microscope photographs have shown that the sediment consists of micro-organisms especially foraminiferans, silt and clay minerals and some opaque materials (Rees *et.al.*,1982). The dominant grain size is fine silt (5-10 μm diameter) with some clay size grains (Gage & Tyler 1982). Occasional irregularly shaped fragments of calcareous material up to 30 μm have been found (Gage & Tyler 1982).

The sediments sampled at the "Permanent Station" and station "M" consist of a light- brown superficial layer of *Globigerina* ooze, a soft clay formed from the breakdown of foraminiferans and coccolithophores (Gage 1978). Below this layer of ooze there is a deep layer of stiff greenish-coloured clay. Gage & Tyler (1982) have shown that the sediment sampled from a spade box core at station "M" in 1979 indicated a carbonate ooze with an organic content of 0.39% determined by the wet oxidation method of Gaudette *et.al.* (1974). A similar value (0.47%) was obtained at the "Permanent Station". The calcium carbonate content, measured by loss on combustion, was 48% at station "M" and considerably higher (67%) at the "Permanent Station" (Gage *et.al.* 1980). Granulometric analysis of the sediment samples from both stations have shown that station "M" which lies at the base of the slope has much sandier sediment (33.2% sand and 43.1% silt/clay) against the soft ooze of the "Permanent Station" (27.4% sand and 57.9% silt/clay) (Gage & Tyler 1982, Pain 1983).

2.2.2. The Porcupine Seabight

The Porcupine Seabight is a large indentation into the northwestern European Continental shelf which slopes gently to a depth of 3000 m but then inclines more steeply at its mouth before opening out to the Porcupine Abyssal Plain at about 4500m (Billett 1988, Rice *et. al.* 1991) (Figure 2.1). The Porcupine Bank bounds the northwest margin of the Seabight which narrows towards its northernmost end. The Celtic Shelf forms the eastern border from which emerge an apparently active system of channels known as the Gollum Channel System (GCS) (Billett 1988, Rice *et. al.* 1991).

The sediments at the IOS stations from the Porcupine Bank down to the Porcupine Abyssal Plain have presented a coccolith-foraminiferan marl with a carbonate content of 45% to 67% sediment dry weight (Lampitt *et. al.* 1986, Rice *et. al.* 1991). The sediments become finer with increasing depth. The distribution of hard shelled multilocular foraminiferans can be used as an indication of sediment transport. There is a downslope sediment transport probably via the channel system or from the steep slopes of the Goban Spur as indicated by samples taken at the mouth of the Seabight (3600 m) and on the Porcupine Abyssal Plain (4100 m) which presented species typical of the upper and the middle slope depths (Weston 1985, Lampitt *et. al.* 1986). The sedimentation rates in the Porcupine Seabight approach 10cm.10^{-3} years (Kidd & Huggett 1981).

It is very difficult to assess the organic content of many marine sediments because of their high carbonate content (Wekley *et.al.* 1983, Hedges & Stearn 1984, Rice *et.al.* 1986, Rice *et. al.* 1991). CHN analysis of the Porcupine Seabight sediments after acid digestion has demonstrated that the organic carbon differs little from 0.5% of the sediment dry weight, and there is no evident relationship between % organic carbon and bathymetric depth (Lampitt *et. al.* 1986). Kidd & Huggett (1981) demonstrated that rock debris from the trawl catches especially at the mouth of the Seabight consisted mainly of clinker rarely exceeding 6cm in maximum dimension although large boulders have been taken occasionally.

2.2.3. The Bahamas

The Bahamas cover 104000km^2 southeast of the Florida Peninsula and are composed of shallow water steep sided carbonate banks forming a limestone platform more than 5km thick, separated by deep troughs (Palmer 1986, Schawb *et. al.* 1989). The Bahamas would have fitted between North and South America and Africa prior to the Atlantic rifting (Palmer 1986). Freeman-Lynde & Ryan (1987) suggest the crust under the Bahamas is thinned continental crust. Production and dispersal of sediment on the platforms is considered to be largely a function of organic growth, pre-Holocene topography and water circulation by winds and tides (Schlager & Ginsburg 1981). The Bahamas are "*at a highly advanced stage of platform growth, deeply dissected by submarine erosion, with flanks unusually high and steep, and the size of individual* *platforms has shrunk considerably through time*" (Schlager & Ginsburg 1981).

Only those deeper areas towards the northern part of the Bahamas were considered for this study: mainly the Northwest Channel and the Tongue of the Ocean. Dives using the D.S.R.V. 'Johnson Sea-Link-II' were accomplished in the proximity of Barry Islands and Andros (Northwest Channel), New Providence Island (Southwest Reef), and Green Cay (Figure 2.2).

The largest group of the Bahama Islands and banks is that known as the Great Bahama Bank, including Andros Island and several smaller outlying cays. Three sides are bounded by the deep ocean channels and the fourth by Andros Island and shallow flats. The Northwest Providence Channel, a 200-2500m deep interplatform, is to the north of Andros Is. Towards the southern end of Andros Island the Great Bahama Bank is continuous with the Exuma Bank lying on the eastern side of the deep ocean pocket known as the Tongue of the Ocean (1460m). Andros Island is divided by three bights into two main parts which lead to the Tongue of the Ocean on the East, and to the shallow bank on the west side. At the northern end of the Andros Island an extensive mud flat, dry in many places at low water, runs some 12 miles northward. To the north of this flat the contour of the bank forms a deep V-shaped indentation of deep water, at the head of which is a narrow 3.5km channel leading onto the bank: the Northwest Channel (Smith 1940). The tidal streams running on and off the bank tend to be complex because of the peculiar relation of the Great Bahama Bank to neighbouring land masses and deep ocean channels (Smith 1940).

The Tongue of the Ocean is an extension of the Atlantic basin into the Bahama Bank (Schawb *et.al.*, 1989). While the periplatform ooze is spread evenly throughout the basin, gravity-flow deposits vary in response to relief and distance from the source in the Tongue of the Ocean (Schlager & Ginsburg 1981). The flows are triggered on the uppermost slope by slumping or collapse of the reef wall, they by-pass the lower slope where they erode a system of shallow gullies, and deposit graded sand and mud on the basin floor. Relatively thick layers of coarse to medium sand are restricted to a belt along the basin margin, only the very distal turbidites of fine sand and mud reach the basin interior. The platform acts as a *line source* of material creating a continuous belt of overlapping small turbidites at the toe-of-slope (Schlager & Ginsburg 1981).

2.2.4. Water masses and circulation in the NE Atlantic

The sources and circulation of the deep NE Atlantic waters have been described in detail by Ellett & Martin (1973) and Ellett *et. al.* (1986). By using the parameters of temperature and salinity plotted against depth at least five distinct water masses have been distinguished:

1-) upper waters to a depth of 600 m are formed in the Bay of Biscay from the mixing of the eastern North Atlantic water and Sub-polar Mode water flowing northeastwards from the south of the Rockall Trough, adjacent to Porcupine Bank (McCartney & Talley 1982);

2-) from approximately 800 m to 1200 m along the Porcupine Shelf the water mass flows northwards into the Trough presenting a high salinity and low oxygen content suggesting a Mediterranean origin (Gibraltar Water) (Cooper 1952, Pain 1983, Colman 1987);

3-) the Labrador Sea Water ranging from approximately 1200 m to 2000 m underlies the Mediterranean water and is marked by a high oxygen content and low salinity (Lee & Ellett 1965, Harvey 1982);

4-) the NE Atlantic Deep Water which presents a northern source and is formed by mixing of Norwegian Sea overflow and eastern basin water (Ellett & Roberts 1973, Roberts 1975). This water mass occurs between 2000 and 2500 m;

5-) Below 2500 m a high silica and low oxygen content water mass indicates a southern source possibly Antarctic bottom water (Lonsdale & Hollister 1979, Vangriesham 1985).

There are no direct current measurements at the "Permanent Station" but an oscillatory tidal flow of 10 $cm.s^{-1}$ or more can be inferred from measurements made elsewhere in the Rockall Trough (Lonsdale & Hollister 1979, Gage *et.al.* 1980, Pain 1983, Colman 1987). Photographs of the sediment bed forms in the sampling area suggest diurnal tidal bottom currents which are of at least 12-15 $cm.s^{-1}$. These photographs also show evidence of current smoothing and sometimes ripples (Lonsdale & Hollister 1979). Current meters deployed at approximately 400 m above the sea bed at station "M" by the Marine Physics Group of the SMBA have indicated oscillatory tidal currents of varying period from semidiurnal to diurnal (Booth 1982).

Near-bed currents in the Porcupine Seabight are tidal and rarely exceed 15 $cm.s^{-1}$ at a height of 1 m above the sea bed (Lampitt 1985; Lampitt *et. al.* 1986, Billett 1988, Rice *et. al.* 1991). Mean residual currents of 6, 2.2 and 9 $cm.s^{-1}$ have been recorded on the slope in the Bay of Biscay, Goban Spur, and to the west of the Porcupine Bank respectively (Dickson *et.al.* 1985, Huthnance 1986, Billett, 1988). The currents approaching the Porcupine Seabight from the south over the Goban Spur have been a major factor in determining the deep circulation of the Seabight. The upper slope has been affected by water descending off the Celtic shelf and Porcupine Bank during the winter in some years (Cooper & Vaux 1949) and by internal waves and tides inducing near bottom motion (Huthnance, 1986) which increases sediment removal. Other slope processes include turbidity currents , especially on the lower slopes and within the channels (Billett 1988). On the Porcupine Abyssal Plain the currents are generally slow, flowing northwards at 1-2 $cm.s^{-1}$ up to the central part of the abyssal plain, turning westwards and southwestwards as the abyssal plain shallows to the north and therefore southwards and westwards along the eastern side of the Mid-Atlantic Ridge (Dickson *et.al.* 1986, Billett 1988).

2.2.5. Water masses circulation in the Bahamas

Little work has been conducted on the circulation in most parts of the Bahamas. This is particularly true for the region of Exuma Sound around Little San Salvador. The only oceanographic study done in that area was the study by Pat Colin of the National Oceanic and Atmospheric Administration (NOAA) showing patterns not at all clear and which remains unpublished (Ned Smith and Craig M. Young, personnal communication).

Four characteristic features serve in the identification of water masses in the upper 1000m east of the Bahamas and north of the Antilles: Subtropical Underwater, Subtropical Mode Water, an oxygen minimum and Antarctic Intermediate Water (Gunn & Watts 1981). Below the surface waters the salinity increases to a maximum in the water column usually between 100 to 200m depth, identifying the core of the Subtropical Underwater. Below the Subtropical Underwater the vertical gradients of temperature and salinity decrease, characteristic of the Subtropical Mode Water. The temperature-salinity characteristics of this water type in the North Atlantic is well documented (17.9±0.3°C and 36.5±0.10%). Oxygen values below the Subtropical Mode Water usually decrease to a minimum above the oxygen rich deep-water. Below the oxygen minimum there is a salinity minimum that identifies the Antarctic Intermediate Water (Gunn & Watts 1981).

South of Grand Bahama Island, in the North West Providence Channel, temperature and salinity transects showed the absence of a coastal water mass because of the narrowness of the shelf and the lack of any substantial land runoff (Lee 1977). The coastal waters were found to be homogeneous in both the horizontal and vertical directions on the shelf. Mixed-layer depths were about 40m in the summer and greater than 80m in the winter. There is a highly variable and unpredictable flow regime in this area. Currents were generally aligned with bottom isobaths with a large number of current reversals. Maximum speeds were about 40cm.s^{-1} towards the west and east. Current means were weak (approximatly 3cm.s^{-1}) and towards the west. Current reversals occurred with semidiurnal, and several days periodicities. Semidiurnal and diurnal tidal currents accounted for 10 to 33% of the observed variability. The greatest part of the variability was produced by currents fluctuations with several-day periods. Fluctuations occurred with periods ranging from 3 to 18 days and had amplitudes of about ± 10cm.s^{-1}. The origin of the low-frequency motions is uncertain (Lee 1977).

The Deep Western Boundary Current in the North Atlantic Ocean is a continuous feature of the mean circulation between Abaco (26.5°N) and Barbados (13°N). Observations of the Deep Western Boundary Current show an intermittent flow of dense waters along the North American Slope and Rise north of the Gulf Stream. Some of this fluid is observed to cross under the Stream just off Cape Hatteras although there are examples of fluid entering the subtropical gyre farther east. South of Cape Hatteras there is a strong deep current to the south along the edge of the Blake Plateau and Blake-Bahama Outer Ridge (Olson *et.al.*1986, and authors therein). The deep circulation east of the Bahamas (between 23° and 27°) show minimum currents near the 1000m level along the southern Bahamas and the interior of the gyre. A mooring along the northern section shows a deeper level of motionless. Flow in the upper 800m is dominated by a baroclinic anticyclonic gyre that appears to be involved in the recirculation of the Gulf Stream water (Lee *et.al.* 1990). The geostrophic flow pattern of currents and water masses east of the Bahamas is quite complicated. The circulation east of the Bahamas seems to represent an extension of the Gulf Stream recirculation rather than a branch of the North Equatorial Current (Olson *et.al.* 1984).

The mean current flow in the North West Providence Channel at 450 and 550m was generally westward along the channel axis at 13 and 8cm.s^{-1}. The mean temperatures were 15.4°C at 450m and 13.5°C at 550m (Pilskan *et.al.* 1989).

The deeper Tongue of the Ocean current is typically oceanic and varies only slightly over the year, whilst the Eastern Great Bahama Bank displays a sharp local and short-term fluctuations that are typical of shoal regions. The currents in the Tongue of the Ocean are weak and variable, and their speed and direction may be attributed to several factors including: the northwest-setting Antilles Current, the prevailing easterly winds, and the funnellike effects of the narrow and shallow passages between the islands and cays. Current speeds are usually less than 1.0cm.s^{-1} at the surface and decreasing with depth. Along its edge tidal currents predominate and reach speeds of 1.5cm.s^{-1}. Below 250m current speeds are usually less than 0.1cm.s^{-1} (Waters, Jr. 1967).

2.3. Fluxes of surface-derived organic matter to the deep-sea

Although the deep sea has been regarded, in the past, as an environment free from seasonal perturbations (Rice *et.al.* 1986) various time series studies have indicated a marked seasonality in the flux of surface derived organic matter into the deep sea in certain areas of the world's oceans such as in the Panama Basin (Honjo 1982, Ittekkot *et.al.* 1984a, Cole *et.al.* 1985); the Sargasso Sea (Deuser & Ross 1980, Deuser *et.al.* 1981a, b, Ittekkot *et.al.* 1984b, Deuser 1986, 1987a, 1987b); and the NE Atlantic (Billett *et.al.* 1983, Lampitt 1985, Rice *et.al.* 1986, Rice *et. al.* 1991). Phytodetritus was found for the first time in a midoceanic region of the NE Atlantic during July and August 1986 at depths of 3800-4590m (Thiel *et.al.* 1990).

After the bloom production on the surface waters, the phytoplankton cells, mainly diatoms, through their mucous secretion, contribute to the formation of aggregates which sink and, doing so, possibly "scavenge" other particles increasing their sinking rates at an average of 100 $m.day^{-1}$ (Smetacek 1985). Organic macroaggregates, zoo and phytoplankton remains are therefore deposited rapidly over a wide bathymetric range following the spring bloom (Billett *et.al.* 1983, Lampitt 1985, Rice *et.al.* 1986, Riemann 1989, Thiel *et.al.* 1990, Gooday & Turley 1990, Rice *et. al.* 1991). Large amounts of this material have been seen to accumulate on the sea bed at various depths during the months May to July in the Porcupine Seabight (Billett 1988, reviewed by Rice *et. al.* 1991).

The quantitative and qualitative aspects as well as the timing of a downward flux of detritus might be an important factor determining abundance, structure and activity of shallow water benthos (Christensen & Kanneworff 1986) and also the deep sea benthos (Billett *et.al.* 1983). The rapid sedimentation of detrital material (Lampitt 1985) may represent a major nutrient input to the deep sea community, being promptly available for consumption by the deep sea biota (Cole *et.al.* 1987, Billett 1988, Lochte & Turley 1988, Gooday 1988, Graf 1989, Gooday & Lambshead 1989, Gooday & Turley 1990, Billett 1991). Although relevant, the contribution of this material to the nutrition of the deep sea living communities such as macrofaunal surface deposit feeders is yet to be established (Rice *et.al.* 1986, Jumars *et.al.* 1990). Gooday (1988) reported the first unequivocal eukaryotic response to fluff where large populations of benthic foraminiferans were found in phytodetrital aggregates from an abyssal site in the NE Atlantic. After deposition, the detrital material is repeatedly resuspended and deposited as seen in the dramatic changes on the sea floor in photographs from the Porcupine Seabight (Lampitt 1985, Rice *et.al.* 1986). The detritus may cover the sea bed completely or occur in localized patches around mounds and within hollows (Billett & Hansen 1982).

According to Fowler & Knauer (1986) the "large" (> 100 μm) particles responsible for the vertical flux in the oceanic waters are primarily biogenic ranging from "*small, discrete faecal pellets and plankton hard parts to large aggregates or 'snow' which contain both organic and inorganic constituents*".

Hecker (1990), from a photographic study at depths from 450-2400m in the continental slope southeast of Georges Bank, suggest that continental margin processes may play an important role in the transfer of organic material to the deep-sea. Several authors (Wiebe *et.al.* 1976, Stockton & De Laca 1982, Smith 1985, 1986, Suchanek *et.al.* 1985, Kojima & Ohta 1989) have pointed out the importance of horizontal transport of terrestrial and coastal materials to the deep-sea benthic communities. This might be particularly important in areas such as the Bahamas where deep-sea basins are physically close to shallow-water bank processes. The effects of hurricane on local sedimentation (transport and redistribution of sandy sediment) in tropical areas such as the Bahamas can be substantial (Pilskan *et. al.* 1989 and authors therein). Pilskan *et.al.* (1989) suggested that a combination of seasonal storms and hurricane events periodically sweep large amounts of ressuspended platform muds into the Northwest Providence Channel where they are incorporated into the periplatform ooze sediment.

Sampling of surface sediment detritus of the NE Atlantic, using the SMBA's multiple corer (McIntyre & Warwick 1984, Barnett *et.al.*

1984) has indicated the presence of mainly diatoms, and discrete gelatinous aggregations, up to 10mm diameter, of coccolithophorids, dinoflagellates, crustacean eggs, small fecal pellets, and amorphous organic material (Billett *et.al.* 1983). It appears that amongst the phytodedritus there is an abundance of the diatoms *Thalassionema nitzschioides*, *Nitzschia delicatissima* and *Chaetoceros (Hyalochaete) spp.* (Rice *et.al.* 1986). Phytodetritus, collected from the mid-oceanic region of the NE Atlantic, were found to be colonized by heterotrophic, nonpigmented bacteria and by photosynthetic, pigmented cyanobacteria (Thiel *et.al.* 1990). Also, the phytodetritus from the mid-oceanic region of the NE Atlantic were found to contain, coupled with surface production, tintinnids, coccolithophorids, silicoflagellates, dinoflagellates, planktonic foraminiferans, crustacean exuviae, and the diatoms *Bacteriastrum delicatulum*, *Rhizosolenia bergonii* and *Thalassionema nitzschioides* (Thiel *et. al.* 1990).

The arrival of the detritus was observed in the Rockall Trough, in 1981-1982 at the S.M.B.A. 'Permanent Station' at 2900m (S.M.B.A. Annual Report 1982, Barnett *et.al.* 1984). It is possible that the Rockall Trough has similar sedimentary environment to that of the Porcupine Seabight, as the surface production over these areas is very similar (Colebrook 1986).

Marine snow is probably an important site of heterotrophic activity in the deep sea presenting populations of bacteria and protozoans (Alldredge & Youngbluth 1985). Although the contribution of bacteria inhabiting the aggregates to the total bacterial production in the oceans is very low (Alldredge & Youngbluth 1985, Alldredge *et.al.* 1986), Caron *et.al.* (1986) have found that enriched marine snow with bacteria in the open water in the NW Atlantic is higher than near shore. The bacterial transformation of organic matter in the deep sea has been considered to be a very slow process (Jannasch *et.al.* 1971, Jannasch & Wirsen 1973, 1977, Austin 1988). Nevertheless, some rapid microbial activity at abyssal depths has been suggested by Deming (1985), Cole *et. al.* (1987) and Lochte & Turley (1988). Lochte & Turley (1988) have reported aggregates containing a rich community of active bacteria and cyanobacteria, and have proposed that the phytodetritus is used rapidly by deep sea adapted bacterial populations indicating that the biological degradation and transformation of the deposited detrital organic material in the deep sea is faster than previously assumed.

Recent studies have proved the existence of a link between the seasonal surface production and the flux of organic matter to the deep sea sediments in the Porcupine Seabight (Billett *et.al.* 1983, Lampitt 1985). It is still to be determined how much of this material is decomposed during sinking and how much bacterial action contributes to this degradation even though some biochemical results in the Porcupine Seabight (Rice *et.al.* 1986) have indicated that the phytodetritus undergoes little degradation during its passage through the water column.

Tyler (1988) has proposed that *"the link between the sedimentation of the seasonally varying surface-derived organic matter and seasonal physiological processes in deep sea animals must be via feeding"*. Observations from underwater photographs in the Porcupine Seabight have shown that some macrobenthic species move towards phytodetritus and recently formed mounds, e.g. faecal pellets on which they apparently feed (Billett *et.al.* 1983, Lampitt 1985). The benthic biota, which respond to this flux, probably obtain their energy: 1-) directly by the effect of digestive enzymes on the ingested freshly arrived phytodetritus; and/or 2-) indirectly through the bacterial activity (Levinton & Stewart 1988). If the energy is mostly obtained through bacterial activity, then other circumstances in which bacterial growth is stimulated would also provoke some sort of benthic reaction such as the feeding on recently formed faecal pellets. Although some deep sea invertebrate species have shown synchronous annual reproductive cycles with spawning in the early spring (Pain *et. al.* 1982, Tyler *et.al.* 1982, Pain 1983, Tyler *et.al.* 1984, Tyler & Gage 1984b, Tyler, 1986) whether this or other physiological processes are responses to the arrival of the phytodetritus arrival is not clear and must be examined in more detail (Rice *et.al.* 1986).

Chapter 3: Revision on the macro and micro-morphology of the alimentary canal of regular echinoids with emphasis on selected deep-sea species and the shallow-water *Psammechinus miliaris* (Gmelin)

3.1. Introduction

The alimentary canal of echinoids is well developed, located in the general body cavity, attached to the body wall by mesenteries, and surrounded by coelomic fluid (Jangoux 1982). It is considered complex and highly specialized (Hamann 1887, Stott 1955, Buchanan 1969, Holland & Ghiselin 1970, Tokin & Filimonova 1977, De Ridder & Lawrence 1982).

Although regular echinoids have a remarkably consistent digestive organization (De Ridder & Jangoux 1982) the gross anatomy of the gut may vary in different orders, or even within the same order as in the cidaroids (personal observation). Generally, regular echinoids have a pharynx, an oesophagus, two circuits of intestine which are currently called stomach and intestine, and the rectum which opens through the anus on the aboral surface (Holland & Ghiselin 1970, De Ridder & Jangoux 1982).

There are some discrepancies in the descriptions of the alimentary canal of regular echinoids in the literature (Hamann 1887, Hyman 1955, De Ridder & Jangoux 1982). This chapter revises the general description of the alimentary canal based on observations of deep-sea species of regular urchins collected in the Rockall Trough, Porcupine Seabight and the Bahamas. A shallow-water echinoid, *Psammechinus miliaris* (P.L. Müller, Gmelin), was used to study the ultrastructure of the gut wall as the deep-sea material dissected on board ship in recent cruises did not preserve well.

Macromorphology of the food canal

The food canal of sea urchins is simple in shape, consisting of a tubular duct looping in the general body cavity (Jangoux 1982). The mouth opens in the centre of the oral surface encircled by a lip-like eminence leading into a small **buccal cavity** which is encircled by the main nerve ring and pierced by the teeth of the **Aristotle's lantern** in all regular echinoids (Hyman 1955, Holland & Ghiselin 1970, De Ridder & Jangoux 1982). The morphology of the Aristotle's lantern in the different orders has diverged during the evolution of the regular echinoids (Lawrence 1987). It is a complex of forty calcareous plates (five teeth, ten demipyramids, ten epiphyses, five rotules and five double elements forming a compass) supported in the peristome and test by muscles which protrude and retract to move the teeth to grasp and chew up food (Hyman 1955, De Ridder & Lawrence 1982, Lawrence 1987).

The broad anatomy of the Aristotle's lantern can vary in different groups of echinoids. There are primarily four types of lantern (De Ridder & Lawrence 1982 and authors therein, Lawrence 1987):

1-) the cidaroid: the most primitive and can move only vertically;

2-) the aulodont (Echinothurioida, Diadematoida, Pedinoida);
3-) the stirodont (Salenioida, Hemicidaroida, Phymosomatoida, Arbacioida);
4-) the camarodont (Temnopleuroida, Echinoida): the most evolved type of lantern, with increased pulling and tearing power.

The **pharynx** is placed in the centre of the Aristotle's lantern and passes at once into the oesophagus as it emerges from the distal part of the lantern (Hyman 1955). The **oesophagus**, generally an incurved segment, descends orally making a loop (Hyman 1955, De Ridder & Jangoux 1982), and is connected to the **stomach** which curves around inside the test in a clockwise direction as viewed from the oral surface. Towards the end of the first loop, the stomach becomes the **intestine** (Figures 3.1). The latter curves anticlockwise below the stomach making an almost complete circuit followed by the **rectum** (Figure 3.1) which ascends to the underside of the periproct and opens at the **anus** (Hyman 1955). This arrangement of the stomach and intestine is related to the body pentamery (Hyman 1955).

Most echinoids have a bypass in the inner border of the stomach which extends from its beginning to where the intestine starts: the **siphon**. The siphon, a slender tube, is believed to conduct water from the proximal to the distal end (Cuénot 1900, Stott 1955). This may be a way of removing water from the food that is ingested to be pumped off to the intestine, concentrating the food for digestion in the oral intestine (Hyman 1955). The function of the siphon in any order, however, is still speculative (De Ridder & Jangoux 1982).

Prouho (1885), Holland & Ghiselin (1970), De Ridder & Jangoux (1982) reported that the cidaroids, diadematoids, and echinothurioids possess a siphonal groove, which runs the entire length of the gut, instead of a siphon.

Micromorphology of the food canal

The histology of the gut wall in echinoderms is similar throughout the phylum with four layers of tissue: a digestive epithelium often associated with an epineural nerve plexus, a connective tissue layer, a muscle layer associated with a hyponeural nerve plexus, and a peritoneum (Hyman 1955, Stott 1955, Rosati 1968, Tokin & Filimonova 1977, Buchanan *et. al.* 1980, Jangoux 1982, De Ridder & Jangoux 1982, La Haye & Holland 1984, Lawrence *et. al.* 1989). Stott (1955) described a fifth layer of very fine neurofibrilar meshwork in *Echinus esculentus*. The thickness of the gut wall may vary probably because of different functions in different parts of the digestive tract (Stott 1955).

The cells which form the digestive lining epithelium are called **enterocytes**. The enterocytes may play different functions in different portions of the digestive tract (Rosati 1968, De Ridder & Jangoux 1982). They are believed to produce currents in the oesophagus and absorb and store nutrients (glycogen and lipid) in the stomach and intestine (Fuji 1961, Tokin & Filimonova 1977, De Ridder & Jangoux 1982). The enterocytes bear microvilli throughout the whole digestive tract.

The lining epithelium of the oesophagus has enterocytes, the **mucous cells**, which produce and accumulate mucus intracellularly (Hyman 1955, Holland & Nimitz 1964, Holland & Ghiselin 1970, Tokin & Filimonova 1977).

Mucous cells specialize in the secretion of different types of mucus which will help in the digestion of food particles (Tokin & Filimonova 1977). Food particles are formed into pellets and covered with mucus in the pharynx and oesophagus (Tokin & Filimonova 1977, De Ridder & Jangoux 1982 and authors therein, Lawrence 1982). This mucoid coat remains throughout the passage of the pellets in the gut as observed by previous authors (De Ridder & Jangoux, 1982 and authors therein), although the pellets themselves may become smaller (Stott 1955).

Cidaroids have mucous cells widely distributed through all regions of the gut (Holland & Ghiselin 1970), whereas aulodonts, stirodonts and camarodonts have mucous cells located especially in the oesophagus and stomach (Holland & Ghiselin 1970).

Reticular fibres form dense networks underneath epithelial membranes forming a **basal lamina** (Green 1984). The basal laminae in echinoderms are very distinct and possibly function as barriers to particles and substances passing through the parenchymal cells and the connective tissue space, holding back molecules on the basis of their size, shape and electrostatic change (Green 1984).

The lining epithelium, connective tissue and peritoneum often carry 'blood cells' or **coelomocytes**. Stott (1955) described reddish-purple spherules, which were seen throughout the whole gut wall of *Echinus esculentus*, as granulocytes. These granulocytes are, in fact, coelomocytes packed with granules (Hyman 1955 and authors therein, Rosati 1968).

Four types of coelomocytes have been described previously (Höbaus 1978 and authors therein, Messer & Wardlaw 1979):

1-) vibratile cells which possess a long flagellum and swim rapidly through the coelomic fluid;

2-) bladder amoebocytes, also known as phagocytic leucocytes;
3-) colourless spherule cells; and
4-) red spherule cells (granulocytes) which possessed the pigment echinochrome giving the coelomic fluid of the sea urchin its pink colour.

Coelomocytes are part of the haemal system, and their function is very controversial (Lawrence 1982, Bamford 1982). They enter the gut in great numbers taking up various types of particulate matter, which are mostly non-nutritive in nature (Ferguson 1982).

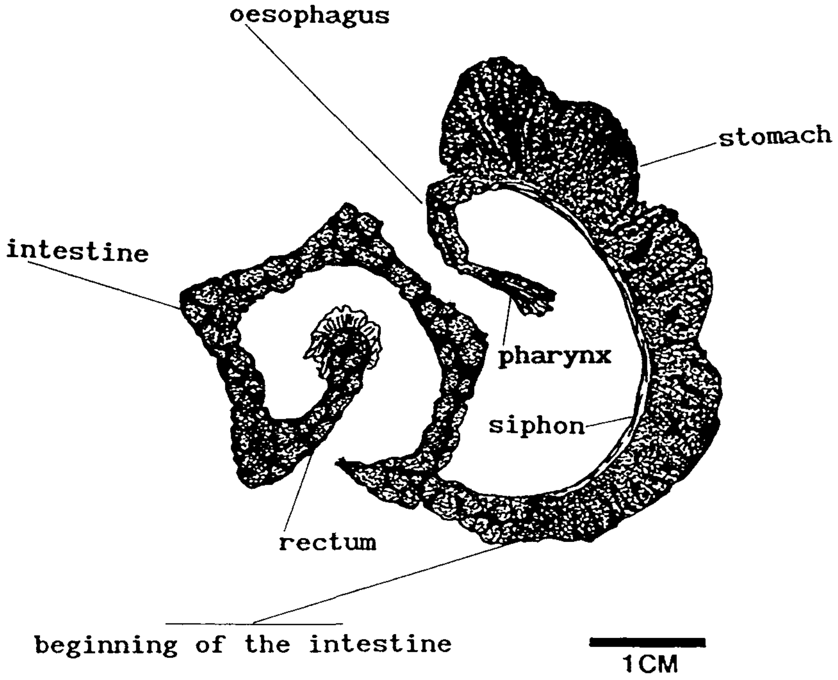


Figure 3.1. *Echinus affinis*. Drawing of the gut wall as dissected out of the test viewed orally.

3.2. Materials and methods

Samples of *Phormosoma placenta* and *Echinus affinis* were fixed in 7% seawater formalin and subsequently stored in 70% alcohol. Ten individuals each of *P. placenta* and *E. affinis* were dissected.

Samples of *Stylocidaris lineata*, *Cidaris blakei*, *Aspidodiadema jacobyi*, and *P. placenta* collected in the Bahamas were dissected on board ship immediately after collection and fixed in 5% formalin-buffered for 24 hours and subsequently transfered to 70% alcohol for storage prior to other laboratory preparations for histology.

The food canals of the dissected urchins were separated into oesophagus, stomach and intestine to be processed through to paraffin wax, sectioned at 7μm and stained with Mallory (Grimstone & Skaer, 1972). Dissected parts to be sectioned were taken from the central part of each organ.

The macromorphlogy of the study species was observed as dissections took place.

During two recent cruises to the Rockall Trough (Challenger 67/90 and Challenger 71/90) samples of *E. affinis* and *P. placenta* were fixed for Transmission Electron Microscopy. Animals were dissected immediately after collection and fixed in 2.5% glutaraldehyde - caccodylate buffer. They were processed through in 1% Osmium, dehydrated and embedded in epoxy resin. Because of the time lapse between the two fixations (glutaraldehyde to osmium), these samples were poorly preserved, and most of the ultrastructure lost.

A shallow-water sea urchin, *Psammechinus miliaris*, was used in order to improve the fixation for transmission electron microscopy, and so compare the ultrastructure of the gut wall with the results obtained from *E. affinis* and *P. placenta*. One individual was injected with 2.5 % glutaraldehyde-caccodylate buffer prior to dissection. The sea urchin had been fed on a high protein formulated food with agar (Lawrence *et. al.* 1989, Crooks 1991) for more than six months which caused it to have gut clear of sediment or hard particles. Parts of its gut extracted from the central area of each organ were processed for transmission electron microscopy in the same week of dissection and fixation.

The following species were observed in this study regarding the presence of a siphon or a siphonal groove with the alimentary canal:

1-) cidaroids: *Stylocidaris lineata* Mortensen, *Cidaris blakei* (Pruho), *Notocidaris gaussensis* Mortensen, *Prionocidaris baculosa* (Lamarck);

2-) diadematoids: *Diadema setosum*, *Plesiodiadema antillarum* (Agassiz), *Aspidodiadema jacobyi* (Agassiz) (Figure 3.3);

3-) echinothurioids: *Calveriosoma hystrix* Wyv. Thomson, *Phormosoma placenta* Wyv. Thomson, *Araeosoma belli* Wyv. Thomson.

3.2. Results

Cidaroids

The cidaroids, *Stylocidaris lineata* Mortensen and *Cidaris blakei* (Pruho), have a short intestine followed by the rectum which is approximately one third the length of the stomach (Figure 3.2). The intestine does not make a complete loop anticlockwise in these species as in the other regular echinoids.

The macromorphology of the digestive tract of cidaroids varies slightly amongst species. *Notocidaris gaussensis* Mortensen, an antarctic species, possesses a marked contriction at the point where the intestine curves itself anticlockwise when viewed orally. This is not clear in *S. lineata* or *C. blakei*. *Prionocidaris baculosa* (Lamark) has a much more convoluted intestine than the other cidaroids observed.

Histologically, the preservation of the food canals of *S. lineata* and *C. blakei* was very poor because the specimens were fixed whole. The injection of fixative through the test was not sufficient to fix the gut wall adequately. In retrospect, the test should have been broken carefully to allow draining of the coelomic fluid and proper penetration of the fixative.

The basic structure of the gut tissues was the same through its length in each species. The lining epithelium was not clear in either species, possibly because of the poor preservation of the gut wall.

C. blakei had granulocytes close to the basal laminae and few distributed amongst the enterocytes. It had a thin layer of connective tissue and distinct peritoneum. 'Vessels' of the haemal system were observed between the peritoneum and the connective tissue where coelomocytes were more abundant.

S. lineata had a very distinct basal laminae, and more conspicuous connective tissue than *C. blakei*. Granulocytes were found mainly in the connective tissue, but also close to the peritoneum, and at the edge of the lining epithelium especially in the intestine.

Aspidodiadema jacobyi (Agassiz)

Aspidodiadema jacobyi, a diadematoid, aulodont, has a long oesophagus and a distinct siphon which runs parallel to the stomach (Figure 3.3). The stomach and intestine are surrounded by well pigmented channels which form part of the haemal system.

A transversal section of the gut wall showed the oesophagus had a conspicuous connective tissue layer. This was not so evident in the stomach and intestine walls. The stomach and intestine walls had a thin layer of connective tissue and more distinct lining epithelium than the oesophagus.

Coelomocytes were found through all layers of tissue in each portion of the gut examined, but no granulocytes were observed.

The siphon resembled the stomach histologically, and was surrounded by vessels of the haemal system containing coelomocytes.

Phormosoma placenta Wyville Thomson

Phormosoma placenta, an echinothurioid, aulodont, possesses a long oesophagus running anticlockwise as viewed from the oral surface. The festoons of the stomach are enlarged and form 10 pouches arranged 'in pairs' over the 5 radial regions of the inside test. It appears that the intestine starts between the 9th and 10th pouches where there is a remarkable constriction, just before the turning loop where the digestive tract runs anticlockwise again. Although the intestine is enlarged interradially, it does not form double pouches as does the stomach. The rectum ends widely on the underside of the periproct, where it is opened by the anus (Figure 3.4). The gut wall has a dark brown colour which is usually darker in the stomach of fresh specimens (Figure 3.5).

Phormosoma placenta has the basic four layers of tissue (Figure 3.6a, b, c). The enterocytes of the lining epithelium in the oesophagus was not well preserved. But the presence of mucous cells was evident during observations of this organ at the electron microscope. In all portions of the gut a marked basal lamina was followed by the connective tissue, a well distinguished muscular layer, and the peritoneum. Granulocytes were not found throughout the gut wall, as in *A. jacobyi*.

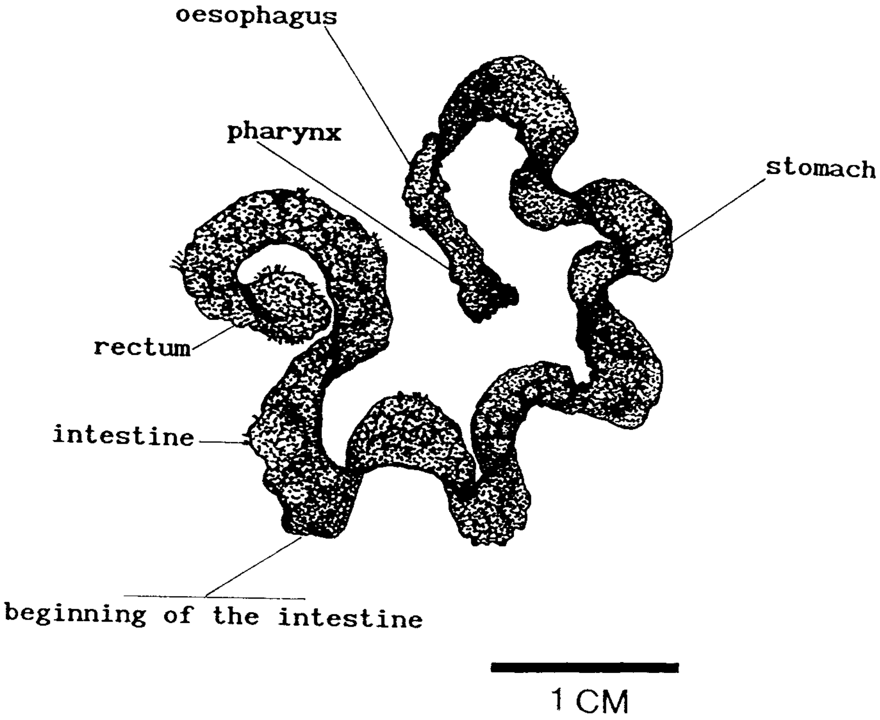


Figure 3.2. *Stylocidaris lineata.* Drawing of the gut wall as dissected out of the test viewed orally.

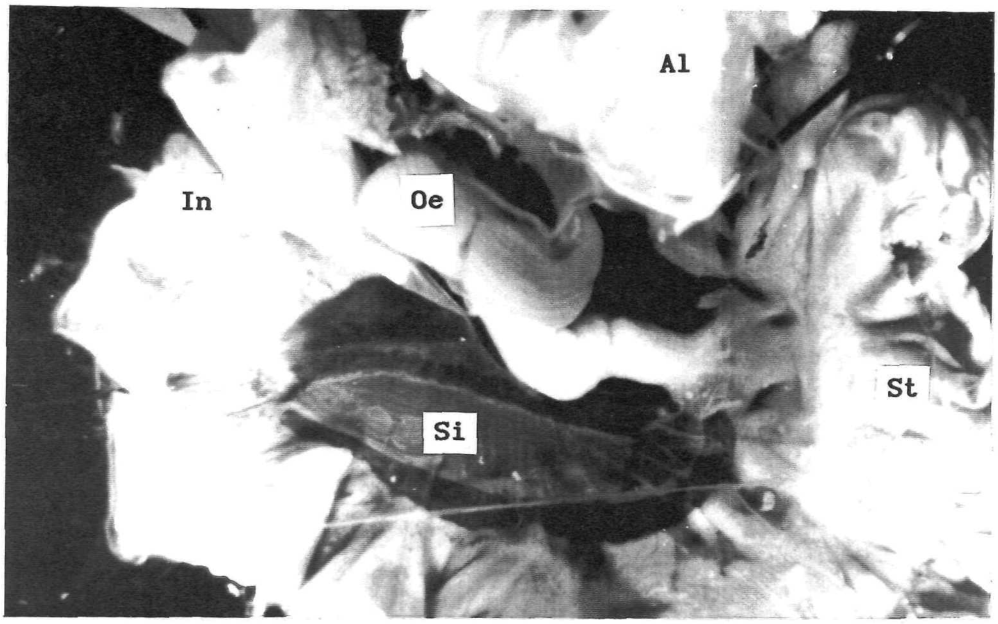


Figure 3.3. *Aspidodiadema jacobyi*. Photograph of the gut wall in the test viewed orally. **Al**=Aristotle's lantern, **Oe**=oesophagus, **St**=stomach, **In**=intestine, **Si**=siphon.

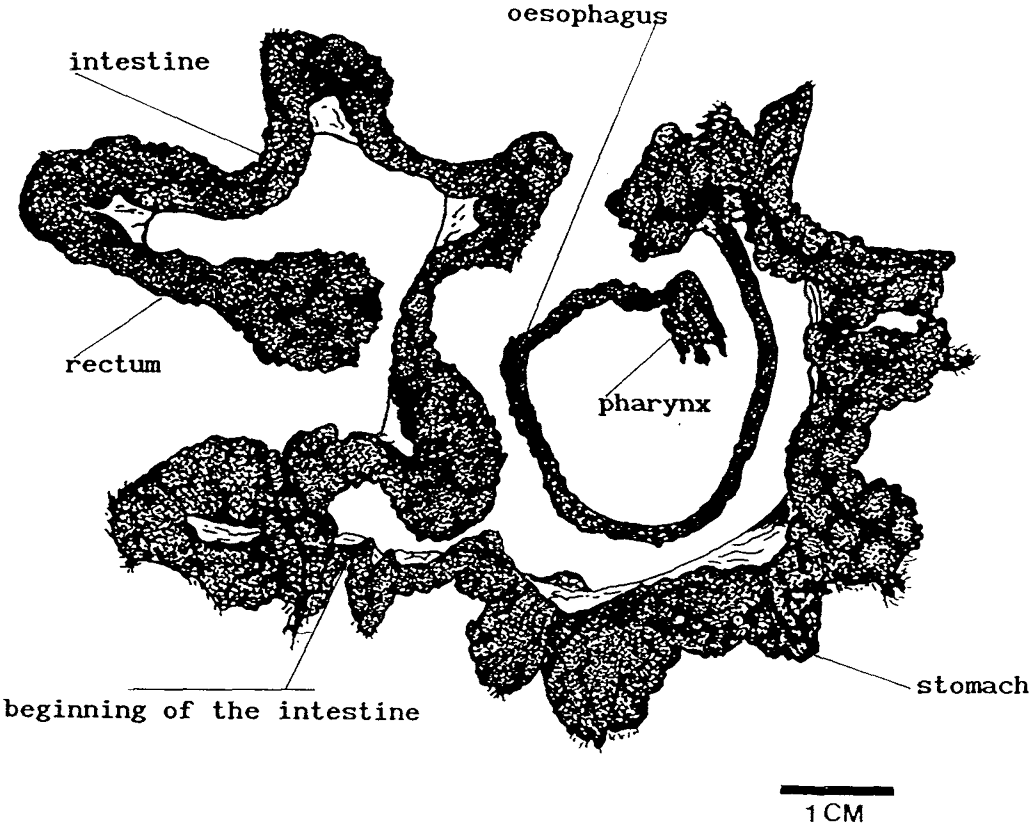


Figure 3.4. *Phormosoma placenta.* Gut wall as dissected out of the test viewed from the oral surface.

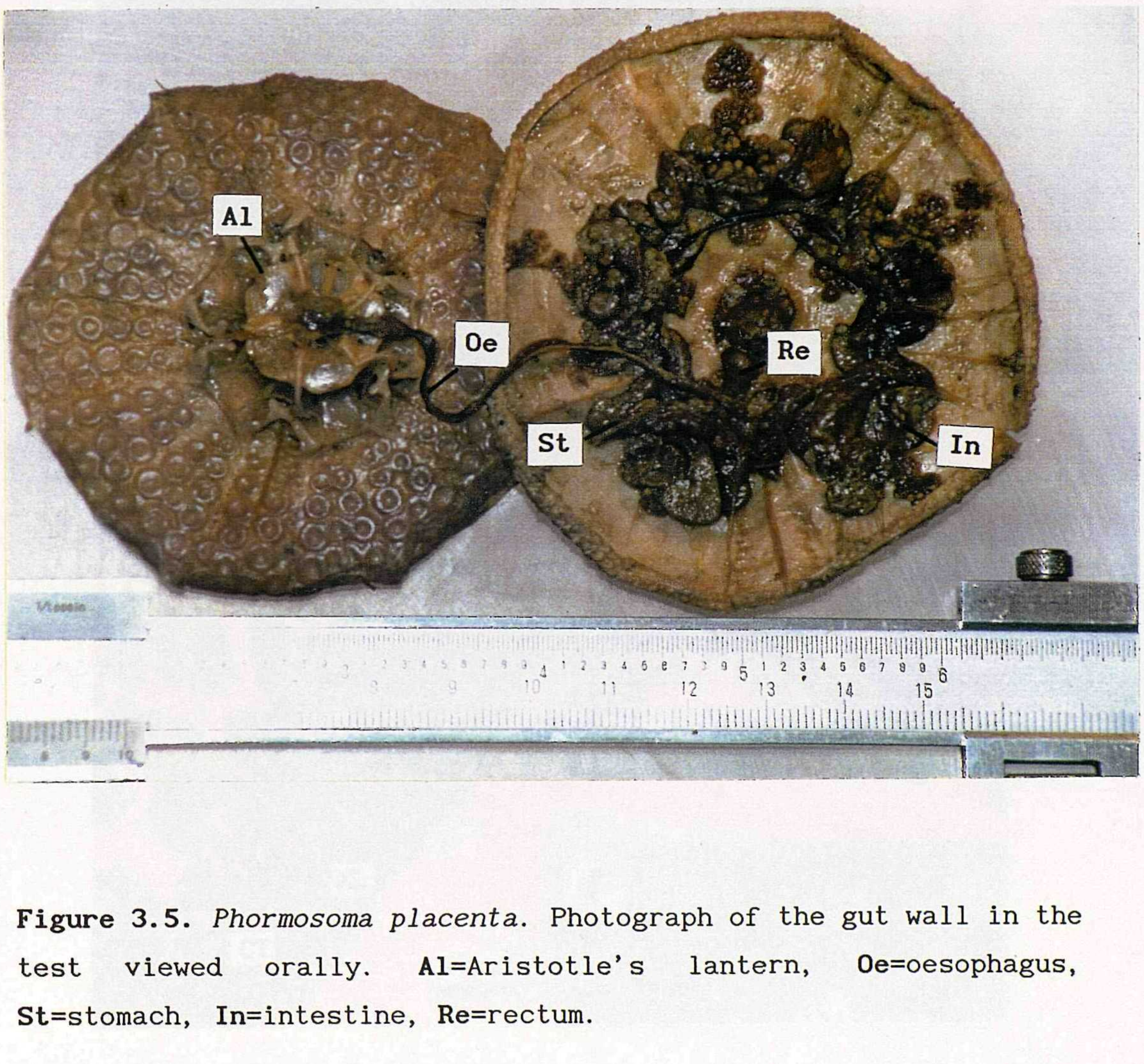


Figure 3.5. *Phormosoma placenta*. Photograph of the gut wall in the test viewed orally. Al=Aristotle's lantern, Oe=oesophagus, St=stomach, In=intestine, Re=rectum.

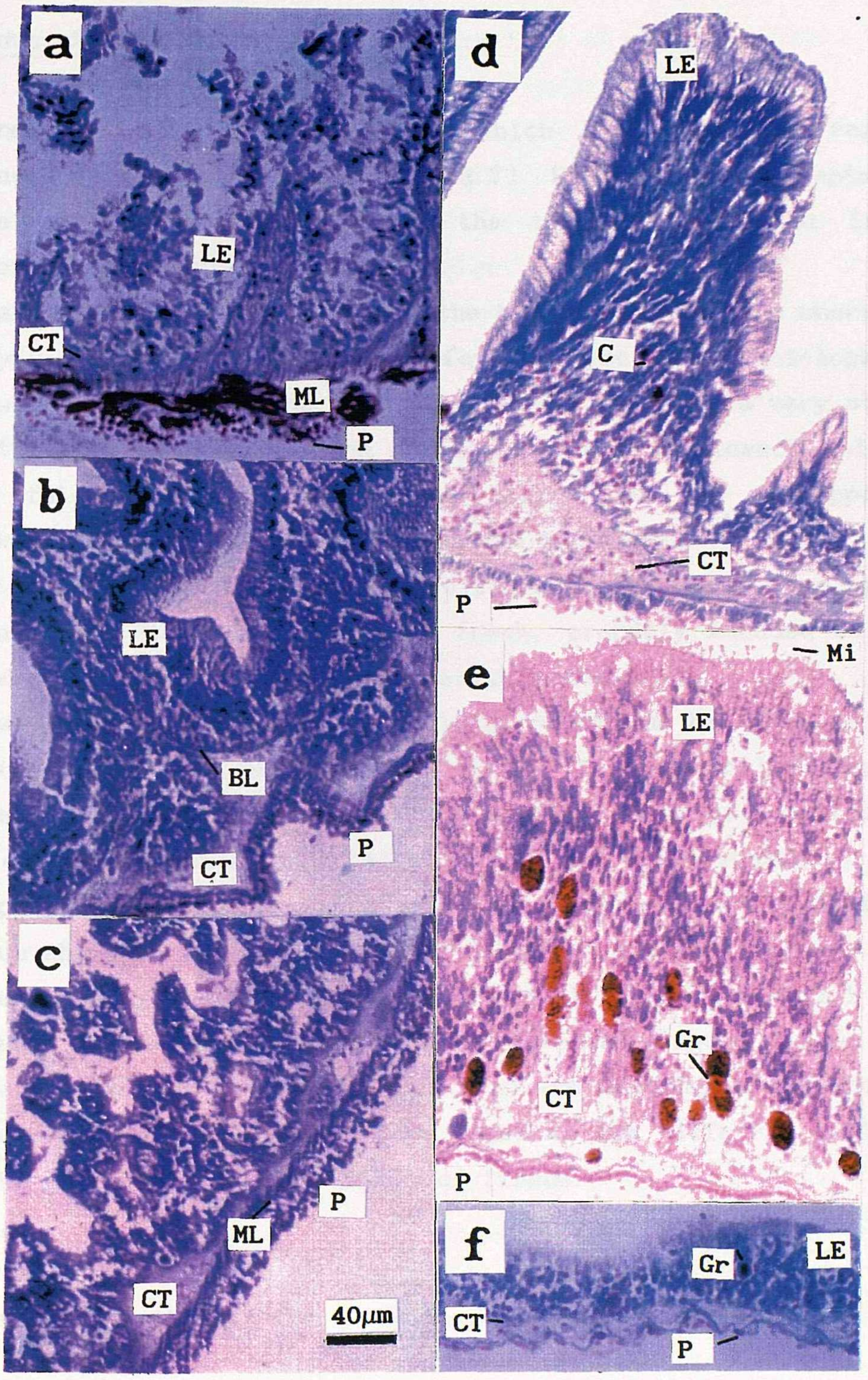


Figure 3.6. Light microscopy using Mallory stain. Scale is the same for all photographs. *Phormosoma placenta*: a-) oesophagus wall; b-) stomach wall; c-) intestine wall. *Echinus affinis*: d-) oesophagus wall; e-) stomach wall; f-) intestine wall. **LE**=lining epithelium, **BL**=basal laminae, **CT**=connective tissue, **ML**=muscle layer, **P**=peritoneum, **Gr**=granulocyte, **C**=coelomocyte, **Mi**=microvilli.

Echinus affinis Mortensen

Echinus affinis is a camarodont which has a typical regular echinoid food canal (Figures 3.1, 3.7). Figure 3.1 represents the whole gut as dissected out of the test and laid out in an approximately natural arrangement.

A short oesophagus starts from the base of the lantern where the pharynx ends. The stomach ends before the gut loops anticlockwise (viewed orally) where the intestine starts. There is a very slight constriction between stomach and intestine. The stomach wall is also thicker than the intestine wall. There is no apparent thickness distinction between intestine and rectum.

E. affinis gut wall has a yellowish-brown colour, slightly pigmented in red when dissected fresh, probably because of the presence of coelomocytes in the haemal system.

Histologically, the lumen or lining epithelium had tall slender enterocytes, and an obvious basal lamina. This was followed by the connective tissue, a well developed muscular layer of longitudinal and circular fibres (especially evident in the oesophagus), and the peritoneum (Figures 3.6d, e, f). An electron micrograph of the rectum shows more clearly the four layers of tissue (Figure 3.8).

The oesophagus had large enterocytes with mucus. Microvilli were evident in the enterocytes of the stomach (Figure 3.6e). The intestine had a much thinner wall than the oesophagus and stomach (Figure 3.6f). Granulocytes were found in the different portions of the gut throughout all layers of tissue.

Psammechinus miliaris (P. L. Müller, Gmelin)

Psammechinus miliaris, camarodont, has a similar organization of the digestive tract to *Echinus affinis*. A fresh dissected digestive tract of *P. miliaris*, however, had a darker yellow-brown colouration than *E. affinis*.

The lining epithelium of the oesophagus has mucous cells which appear to be attached to each other mainly at the outer edge by some type of junction, possibly macula adherens (Figure 3.10). The enterocytes of the stomach are composed of Zymogenic-like cells with granules filling the cytoplasm (Figure 3.10).

Microvilli are obvious especially in the enterocytes of the stomach, intestine and rectum walls (Figures 3.10, 3.11, 3.12). Notably in the intestine, the enterocytes may form a projection which could be related to excretion of waste material (Figure 3.13). The cells would loose parts of themselves which are old or containing excretory substances (M. Jensen, personal communication). Cilia are often found through the whole gut lumen (Figures 3.10, 3.12, 3.14). These could have sensory function (Ridder & Jangoux 1982 and authors therein).

Electron micrographs of the lining epithelium from different portions of the gut wall of *P. miliaris* and *E. affinis* show that the enterocytes have conspicuous intercellular spaces (Figures 3.8 to 3.14) where various kinds of coelomocytes (Figure 3.15), unidentified material and bacteria can be found especially in the intestine (Figure 3.14).

Granulocytes were found dispersed throughout the gut wall in the different layers of tissue (Figure 3.16) not only in *P. miliaris* but also in *E. affinis*. These granulocytes contained amorphous granules of various sizes (Figure 3.15c). The granulocytes were X-rayed and no metal elements, such as ferric iron, were found. Possibly this result occurred because the specimen of *P. miliaris* used for the electron microscopy had been fed for six months on a special sediment-free diet.

Siphon or siphonal groove?

A siphonal groove, which runs the entire length of the gut of cidaroids, diadematoids, and echinothurioids, is reported in the literature (Prouho 1885, Holland & Ghiselin 1970, De Ridder & Jangoux 1982). However, the macroscopic visual observation of the morphology of the alimentary canal of the cidaroids (*Stylocidaris lineata*, *Cidaris blakei*, *Notocidaris gaussensis*, *Prionocidaris baculosa*), diadematoids (*Diadema setosum*, *Plesiodiadema antillarum*, *Aspidodiadema jacobyi*) and echinothurioids

(*Calveriosoma hystrix*, *Phormosoma placenta*, *Araeosoma belli*) indicated that, except for the cidaroids, most species examined have a siphon. A siphon could be seen clearly in all diadematoids studied as shown in *A. jacobyi* (Figure 3.3). Further histological investigation on the echinothurioids is yet necessary in order to substantiate these observations.

It is possible that the siphonal groove, as observed in the cidaroids, is a 'tubular' part of the haemal system. This structure usually appeared well pigmented in all cidaroids.

More thorough analysis of the siphon and siphonal groove need to be pursued in different species to evaluate their real micro-, macro-morphology and function.

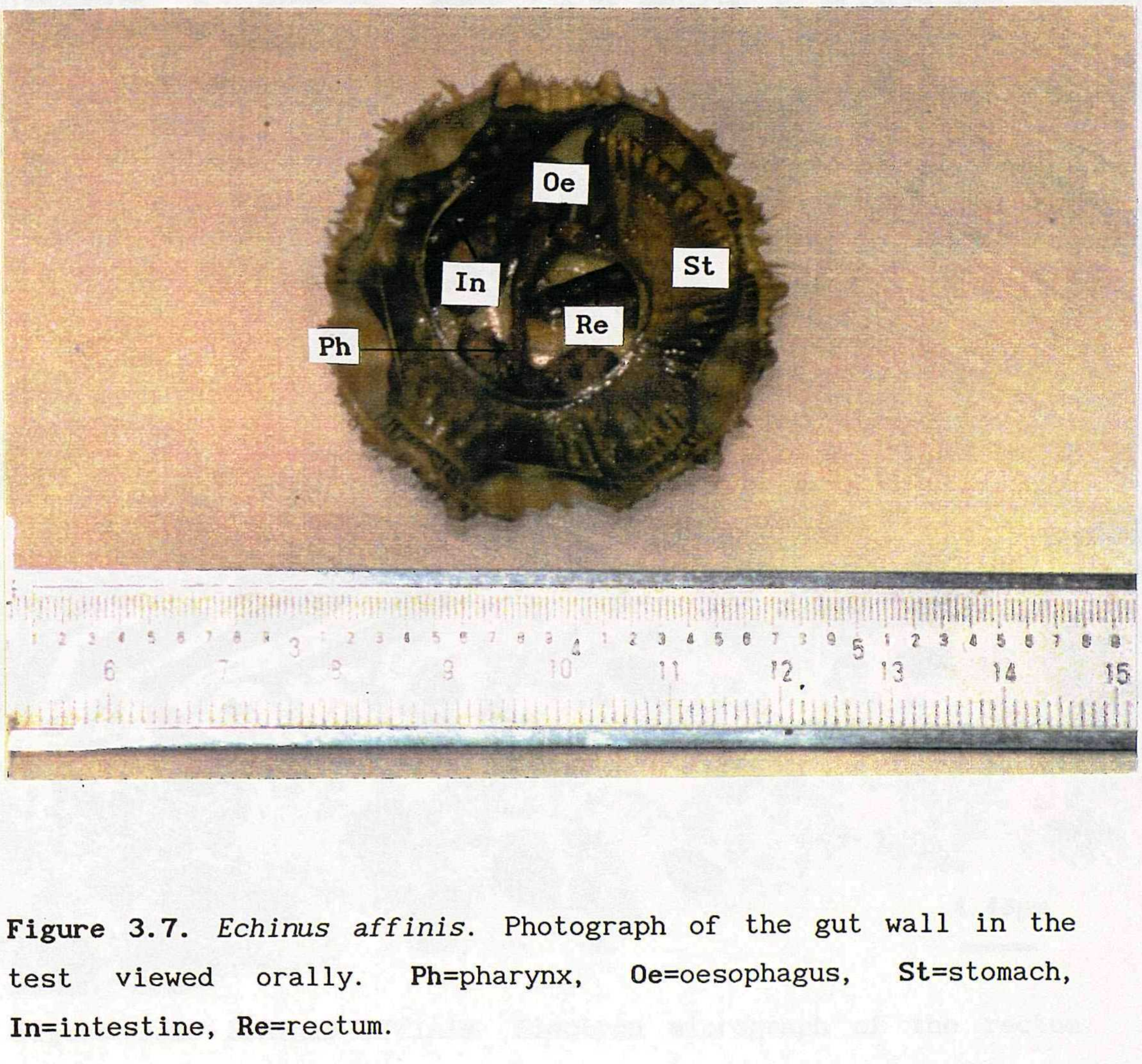


Figure 3.7. *Echinus affinis*. Photograph of the gut wall in the test viewed orally. **Ph**=pharynx, **Oe**=oesophagus, **St**=stomach, **In**=intestine, **Re**=rectum.

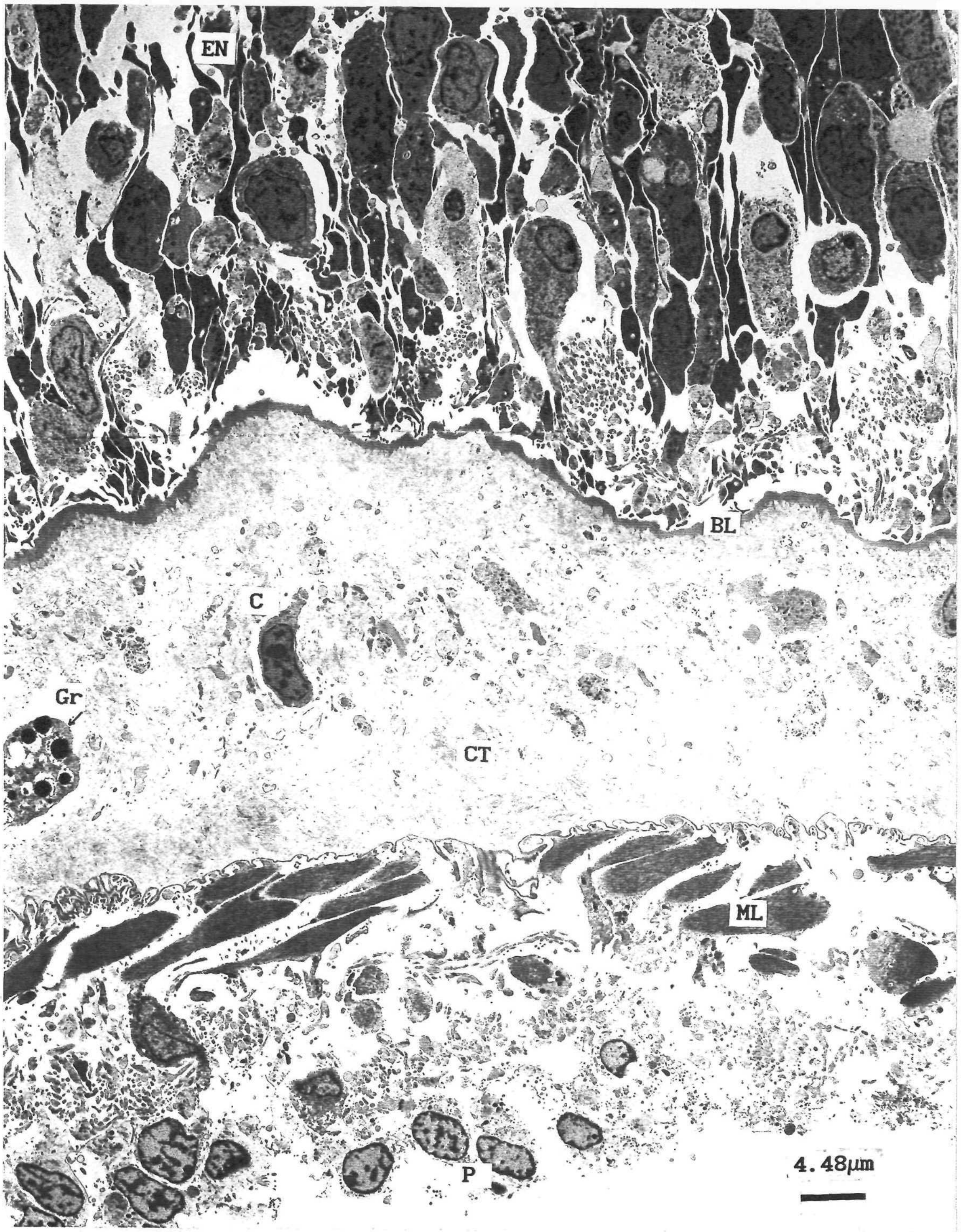


Figure 3.8. *Echinus affinis*. Electron micrograph of the rectum wall. **EN**=enterocytes, **BL**=basal lamina, **CT**=connective tissue, **ML**=muscle layer, **P**=peritoneum, **C**=coelomocyte (phagocyte), **Gr**=granulocyte. Mag. x2230.

Figure 3.9. *Psammechinus miliaris.* Electron micrograph of the oesophagus mucous cells in the lining epithelium. **Mu**=goblets of mucus, **Nu**=nucleus, **Cy**=cytoplasm, **Mi**=microvilli. Mag. x2230.

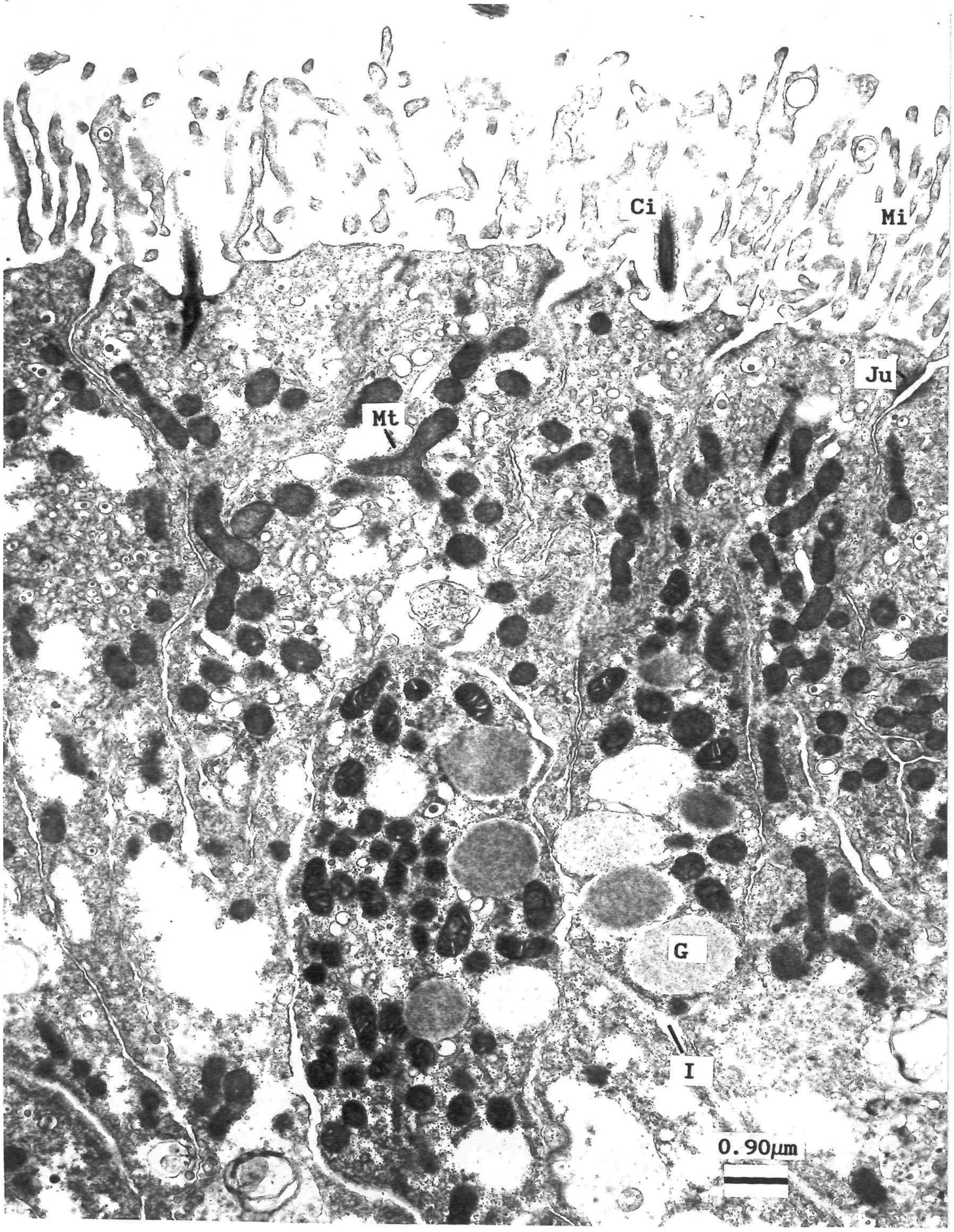


Figure 3.10. *Psammechinus miliaris*. Electron micrograph of the lumen portion of stomach enterocytes in the lining epithelium. **Mi**=microvilli, **Mt**=mitocondria, **Ju**=junction (possibly macula adherens), **Ci**=cilia, **G**=granules, **I**=intercellular space. Mag. x11150.

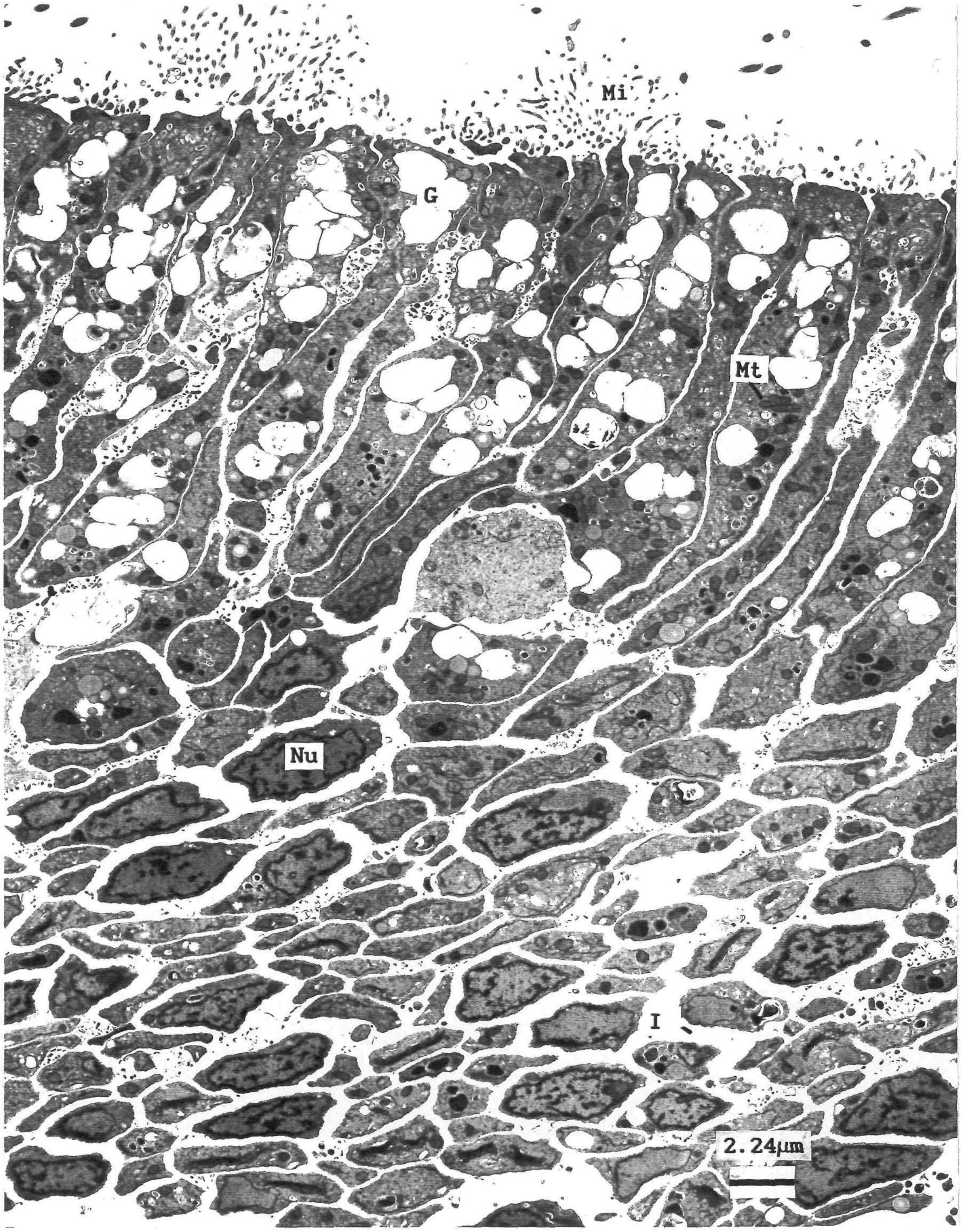


Figure 3.11. *Psammechinus miliaris*. Electron micrograph of the lumen portion of intestine enterocytes in the lining epithelium. **Mi**=microvilli, **Mt**=mitocondria, **Nu**=nucleus, **G**=granules, **I**= intercellular space (with unidentified particles). Mag. x4460.

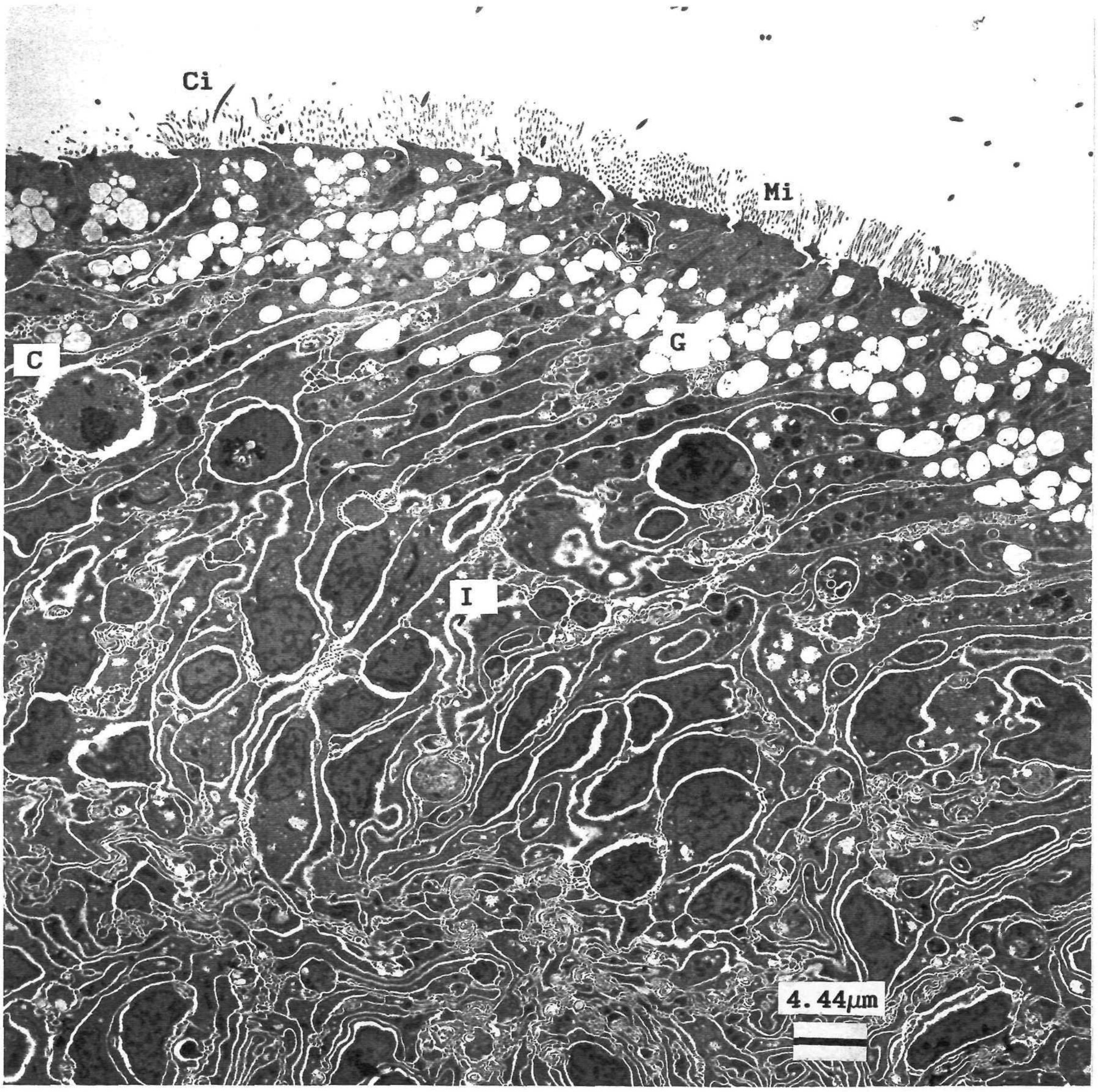


Figure 3.12. *Psammechinus miliaris*. Electron micrograph of the lumen portion of rectum enterocytes in the lining epithelium. **Mi**=microvilli, **Ci**=cilia, **G**=granules, **I**=intercellular space, **C**=coelomocyte. Mag. x2250.

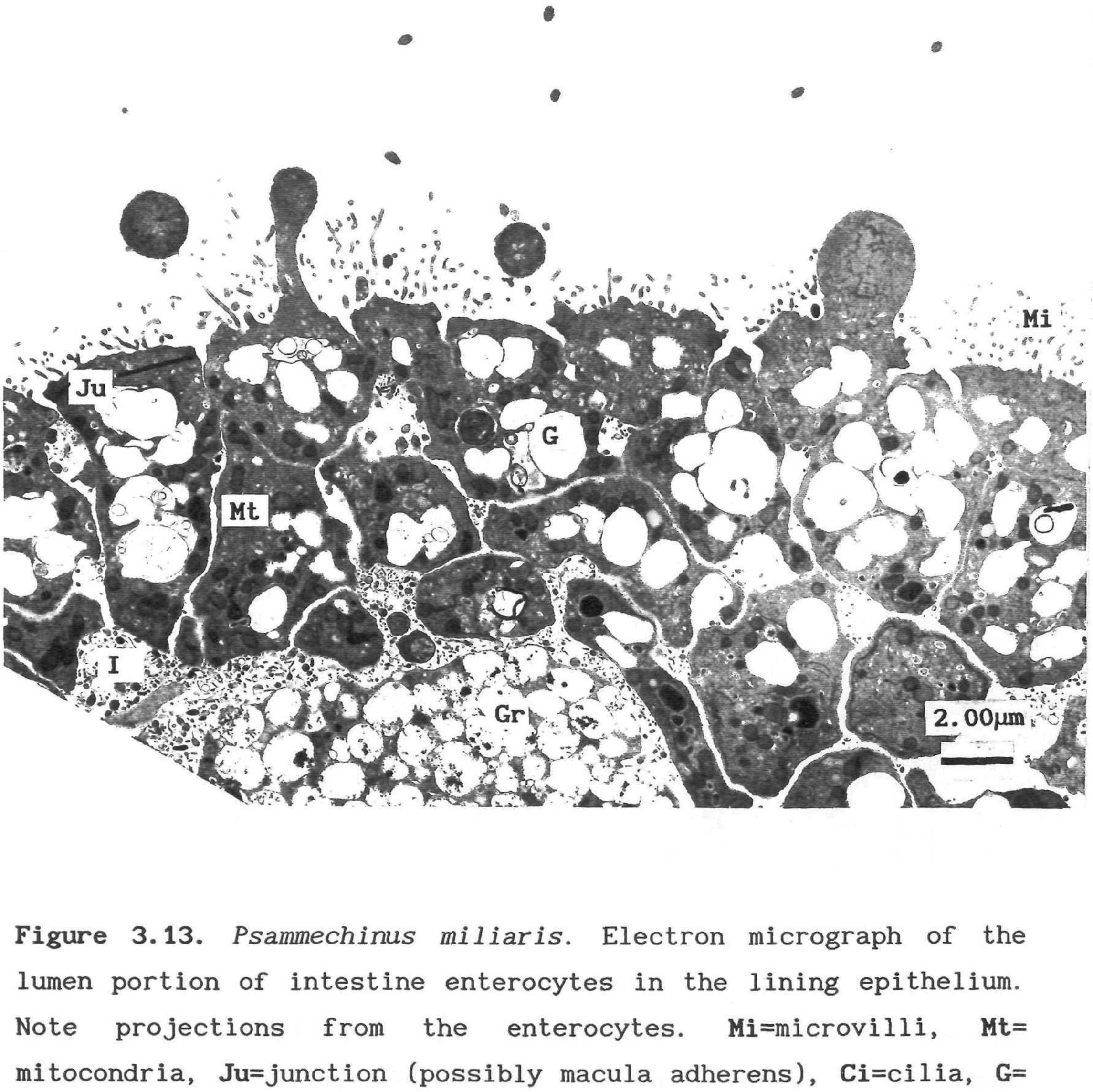


Figure 3.13. *Psammechinus miliaris.* Electron micrograph of the lumen portion of intestine enterocytes in the lining epithelium. Note projections from the enterocytes. **Mi**=microvilli, **Mt**= mitocondria, **Ju**=junction (possibly macula adherens), **Ci**=cilia, **G**= granules, **Gr**=granulocyte, **I**=intercellular space. Mag. x5010.

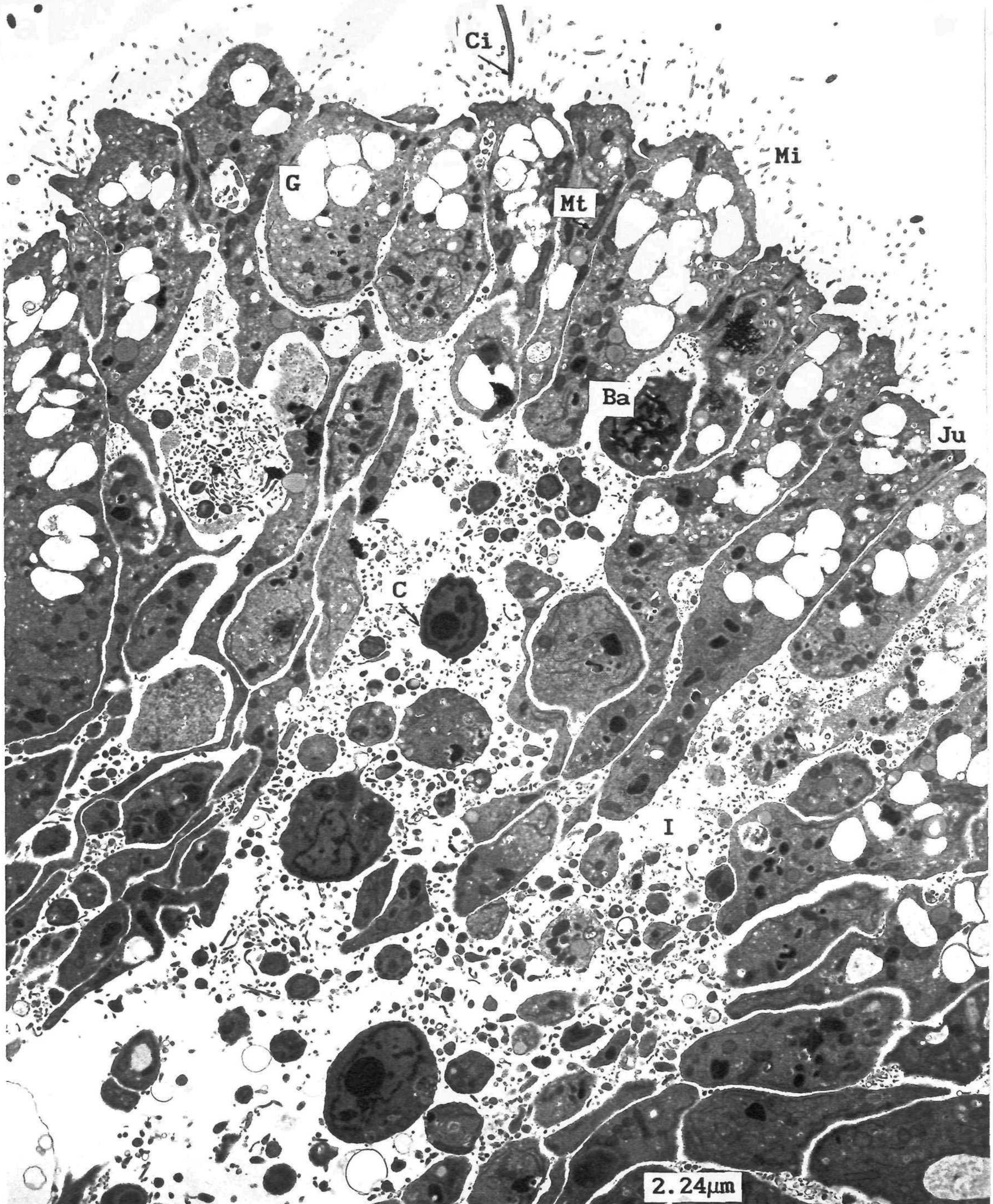


Figure 3.14. *Psammechinus miliaris*. Electron micrograph of the lumen portion of intestine enterocytes in the lining epithelium. **Mi**=microvilli, **Mt**=mitocondria, **Ju**=junction (possibly macula adherens), **Ci**=cilia, **G**=granules, **C**=coelomocyte, **Ba**=bacteria, **I**=intercellular space. Mag. x4460.

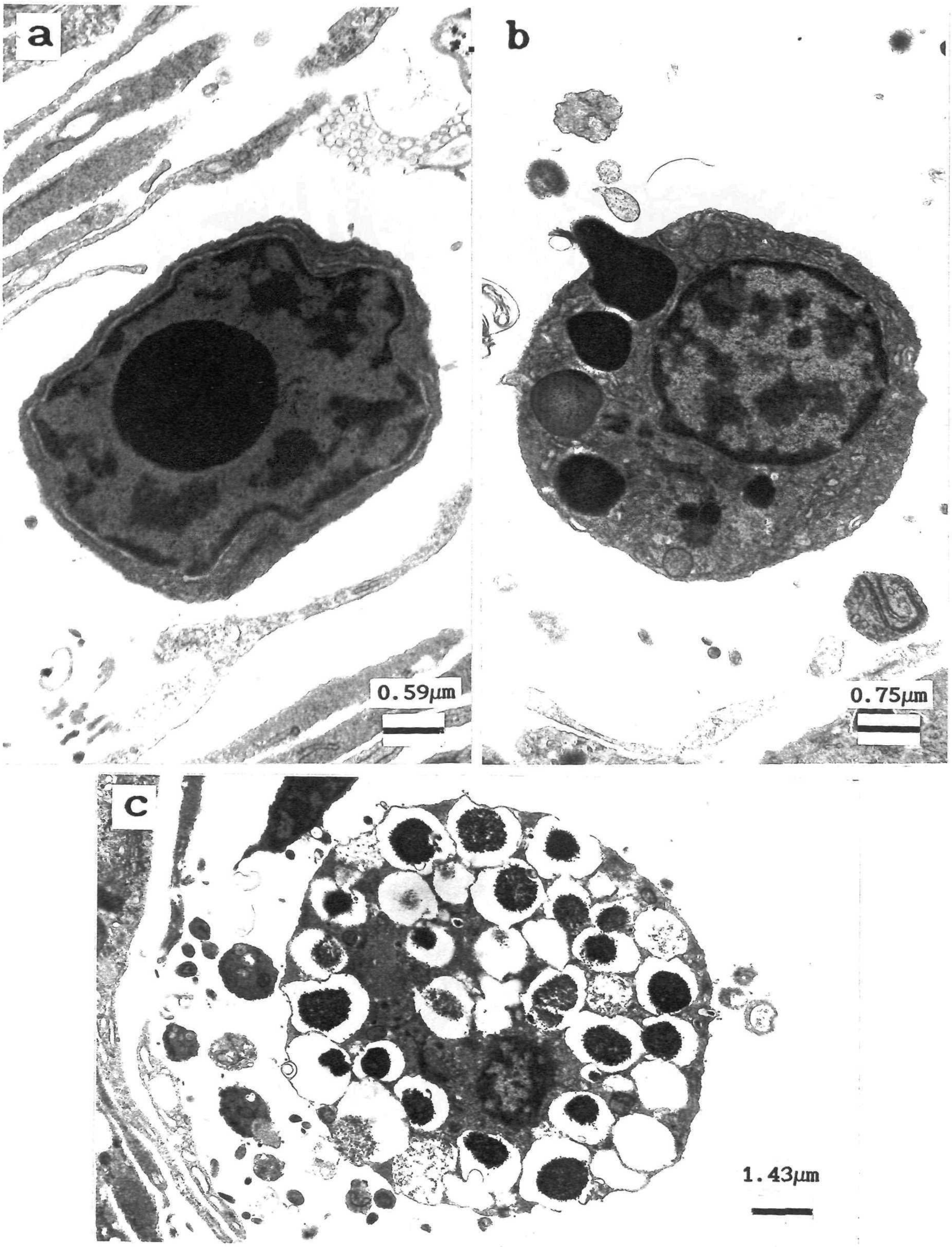


Figure 3.15. *Psammechinus miliaris.* Electron micrograph of coelomocytes found in the intercelullar spaces of the lining epithelium: a-) leucocyte (oesophagus), Mag. x16800; b-) phagocyte (intestine), Mag. x13360; c-) granulocyte (oesophagus), Mag. x7000.

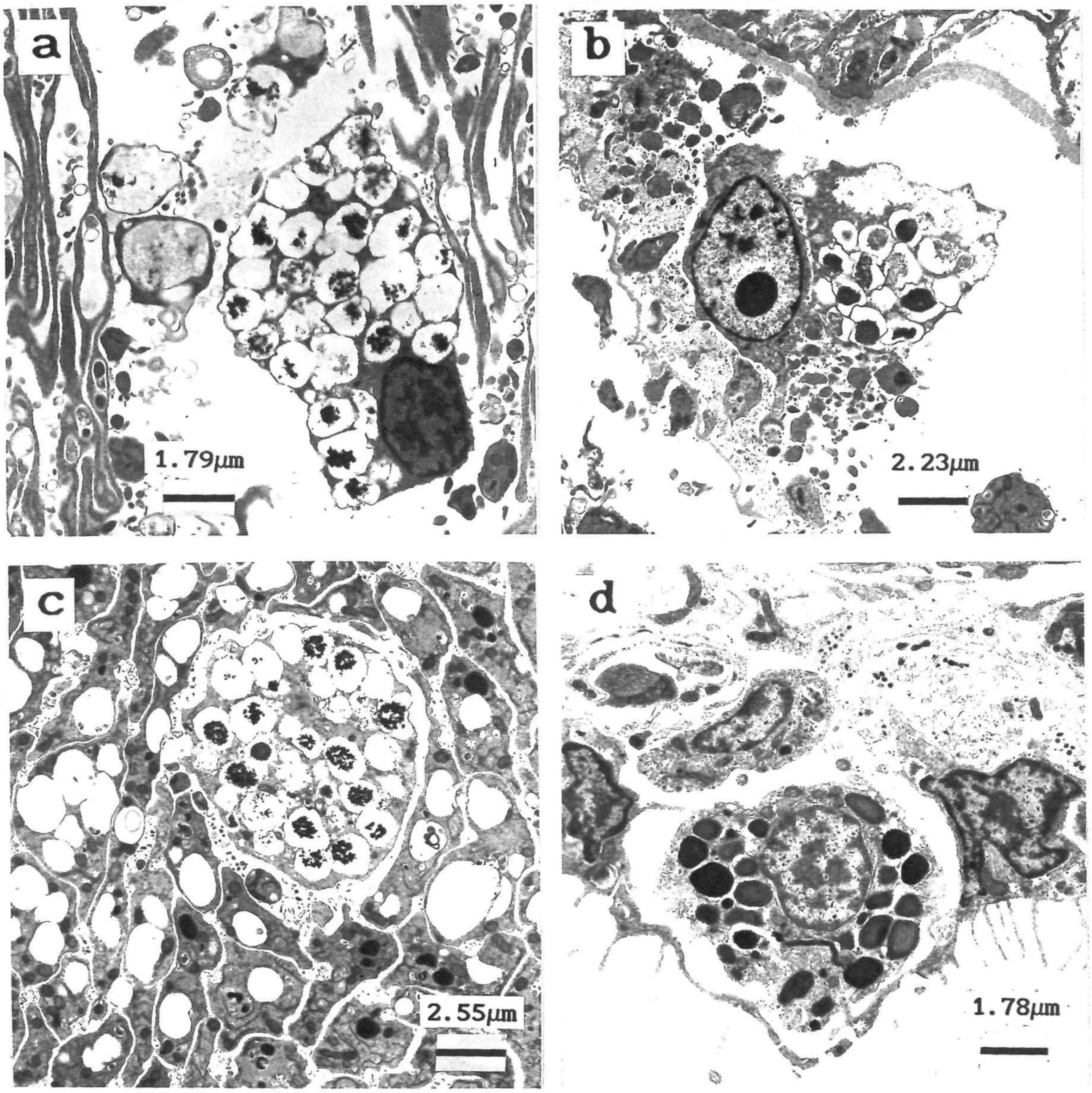


Figure 3.16. Electron micrograph of coelomocytes found in different layers of the gut wall of *Psammechinus miliaris*: a-) granulocyte between enterocytes of the oesophagus wall, mag. x5600; b-) granulocyte in the connective tissue of the stomach wall, mag. x4480; c-) granulocyte between enterocytes of the intestine wall, mag. x3920; d-) granulocyte between the peritoneum cells of the rectum wall, mag. x5600.

3.4. Discussion

The literature concerning the general morphology, histology and histochemistry of the alimentary canal of regular echinoids is very limited (reviewed by De Ridder & Jangoux 1982).

Current terms such as stomach and intestine were used for the two circuits of the digestive tract of regular echinoids in order to avoid confusion in the presentation of this work. This terminology, however, is possibly not appropriate considering the function of the different parts of the alimentary canal. It would be more suitable to follow Hamann (1887) nomenclature if functional morphology of the cells and structure of the tissues were taken into account. Hamann (1887) was the first author to argue the naming of different portions of the digestive tract of echinoids based on their function. He called what is currently named as pharynx the oesophagus, and oesophagus the stomach based on his histological work.

Hyman (1955) preferred the terms small and large intestine instead of stomach and intestine for the two circuits of the alimentary canal. It would be more appropriate to call these two circuits: oral circuit and apical circuit of the intestine respectively, based on their position inside the test rather than the size. The oral circuit is often larger than the apical circuit of the intestine. Most authors in recent years, however, call the oral and apical circuits stomach and intestine respectively (De Ridder & Jangoux 1982 and authors therein) (Table 3.1).

Hamann's ideas were rejected at his time, and remained so. However, detailed histology, histochemistry, electron microscopy would be necessary to identify the real function of each portion of the gut wall.

A meticulous study of the digestive tract of cidaroids is needed. This order is considered the most primitive of the regular echinoids, although this is based only on the study of the hard parts of the animals (Kier 1974, Lawrence 1987). Observations of the macromorphology of soft parts of cidaroids conducted during this work showed that the alimentary canal varies between species. Unfortunately the preservation of the alimentary canals of *Cidaris* *blakei* and *Stylocidaris lineata* was very poor. Study of the macro- and micro-morphology of the soft parts of cidaroids could reveal essential clues about the evolution of the order, not yet discussed in the literature (M. Jensen personal communication). Within the diadematoids Holland & Ghiselin (1970) reported a siphonal groove in several species including *Diadema setosum.* This species along with other diadematoids have been dissected and carefully examined. As a result a siphon rather than a siphonal groove was recognized.

Several authors have shown that the alimentary canal of sea urchins may play an important role in accumulating nutritive substances (Fuji 1961, Farmanfarmaian & Phillips 1962, Lawrence *et. al.* 1966, Tokin & Filimonova 1977, Bamford 1982, Nichols *et.al.* 1985). It is possible that the gut wall nutritive reserves can be used for the maturation of the gonads (Lawrence *et. al.* 1966, Lawrence 1970, 1971, 1976, Fenaux *et. al.* 1975, Giese 1976, Tokin & Filimonova 1977, De Ridder & Lawrence 1982, Nichols *et. al.* 1985). Comely & Ansell (1989), however, suggest that the coincident development of the condition of the gonads and gut wall give little support to this hypothesis in their study of *Echinus esculentus* from Scottish west coast.

Food particles are covered with mucus in the first portion of the gut: the pharynx and oesophagus (Holland & Nimitz 1964, Buchanan 1969, Tokin & Filimonova 1977). The echinoids can have intra- and extracellular digestion, absorption and elimination of food products in the oesophagus, stomach and intestine (Holland & Nimitz 1964, Holland & Ghiselin 1970, Tokin & Filimonova 1977, De Ridder & Jangoux 1982, Lawrence 1982). The functional heterogeneity of cells in different regions of the digestive tract agrees with the relatively slow movement of food through the gut (Lasker & Giese 1954, Tokin & Filimonova 1977).

What would be the implications of having a lengthy gut such as in *Aspidodiadema jacobyi* and especially in *Phormosoma placenta*? Also, what would it happen in the pouches of the stomach of *P. placenta*? These species may be non-selective and even if they process the sediment rapidly, they would make the most of what they consume by having a more extensive absorbtive area. *P.*

placenta possibly retains portions of the sediment ingested for more effective digestion of the nutrients present in it. However, these are hypotheses which have yet to be tested.

It is important to understand how substances are processed and taken up by cells that form the gut wall throughout its length in order to follow the destination of the nutrients. The functional significance of the basic structures of the gut wall of both regular and irregular echinoids regarding the food which is eaten and its fate needs to be studied in more detail (De Ridder & Jangoux 1982).

The distribution mechanisms of nutrients at cellular level are still unknown (Rosati 1970). The molecules taken up by the lining epithelium cells are usually originated from larger particles which are first physically and chemically broken down (Huvard & Holland 1986). Huvard & Holland (1986) suggested that macromolecules are taken up by pinocytosis, and small molecules by absorption via carrier-mediated transport systems. '*The extensiveness of endocytosis by gut epithelial cells as shown by ultrastructural studies indicates that the potential significance of intracellular digestion may be greater than previously anticipated*' (Lawrence 1982).

According to Rosati (1968), cells of the lining epithelium of holothurians adhere closely only at their flattened apical portion. Although they follow the shape of the neighbouring cells, they never adhere closely to them. This facilitates the transport of particles and movement of coelomocytes through this tissue (Rosati 1968). The results shown in this work on *Echinus affinis* and *Psammechinus miliaris* agree with Rosati's results, but there is a possibility that the intercellular spaces observed are an artefact caused by shrinkage of cells in the process of fixation for electron microscopy.

Stott (1955) suggested that the haemal system acts as a pathway for migrations of the nutritive granulocytes as well as providing a system for the possible diffusion outwards from the gut of dissolved substances. Granulocytes would migrate from the haemal system to the lumen of the digestive tract as a form of excretion (Rosati 1968 and authors therein). This proposition would be correlated with a poorly developed haemal system which lacks a pumping mechanism, with omnivorous feeding and a slow passage of food through the gut (Stott 1955).

Some authors (Cuénot 1891,a, b, Chapeaux 1893, Barrington 1962) have exaggerated the nutritional function of the coelomocytes (Ferguson 1982, Bamford 1982). The proposition that these cells are significant in translocating nutrients from absorptive and storage sites in the gut to other areas of utilization is based on very little experimental evidence (Farmanfarmaian & Phillips 1962), and their relationship with the haemal fluid implies only a local nutritive role (Ferguson 1982). It is possible that the release of the coelomocytes is a defensive reaction protecting the mucosal lining (Messer & Wardlaw 1979) and "cleaning up" after normal cell replacement and minor injuries (Ferguson 1982).

Here coelomocytes were viewed amongst cells of the four layers of tissue in all regions of the gut walls of the different species observed. However, no conclusion can be made from this study of whether these 'blood cells' migrate from or to the gut lumen, or the kind of material they carry. More careful controlled analyses of the composition of coelomocytes is needed in order to understand their nature and origin.

In conclusion, differences in functional morphology of cells present in different parts of the digestive tract in regular echinoids indicate that the nomination of each section should follow that of Hamann (1887). However, more analyses of the ultrastructure of cells present in different layers of tissue of the alimentary canal are necessary for a better understanding of the function of pharynx, oesophagus, stomach, intestine, rectum, and siphon.

The cidaroids need more attention because of differences in the macromorphology of the digestive tract in different species of the group.

Well elaborated experiments are needed to investigate the real function of the siphon and also to understand the role of coelomocytes within the alimentary canal.

Chapter 4: Analyses of the gut contents of two species of deep-sea regular echinoids collected in the NE Atlantic: *Phormosoma placenta* Wyville Thomson and *Echinus affinis* Mortensen

4.1. Introduction

During the last ten years one of the significant developments in our understanding of the functional ecology of the deep-sea has been the recognition of seasonal processes in this apparently constant environment (reviewed by Tyler 1988). Support for the seasonal concept comes from studies in the NE Atlantic of the rapid downward flux of surface-derived phytodetritus to the deep-sea bed (Billett *et. al.* 1983, Lampitt 1985, Rice *et. al.* 1986), the colonization of this material by flagellates and foraminiferans (Lochte & Turley 1988, Gooday 1988, Gooday & Turley 1990), and evidence that certain deep-sea invertebrates exhibit seasonal growth and reproductive cycles, whose timing can be related to this vertical flux (Gage & Tyler 1981, Tyler *et. al.* 1982, Van Praët *et. al.* 1984, Gage & Tyler 1985, Gage 1987, Harrison 1988, Van Praët *et. al.* 1990). Seasonal variation in vertical flux (Deuser 1986 and references therein) and seasonal reproduction (Schoener 1968, Rokop 1974) in deep-sea invertebrates have also been established elsewhere in the world oceans (Tyler 1988).

There is, however, no direct evidence from a long time series to link the seasonal availability of phytodetritus at the deep-sea bed with growth and reproduction in deep-sea metazoans. Phytodetritus has been suggested to fuel rapid growth in the deep-sea barnacle *Poecilasma kaempferi* (Lampitt 1990). Data on chlorophyll-derived pigments in the gut contents of deep-sea holothurians have shown that they feed exclusively on phytodetritus when first deposited as a homogeneous layer overlying the sediment (Billett *et. al.* 1988), and detrital particles rich in pigments were selected when the detritus was distributed in patches.

Furthermore, photographic evidence suggested *Echinus affinis* feeds on this detritus as well as on faecal mounds of holothurians (Lampitt & Burnham 1983, Lampitt 1985, Lampitt & Billett 1988). However, data from the Norwegian Sea showed that faecal pellets deposited at the sea bed in spring and summer were rapidly buried (Graf 1989). This would diminish the chance of invertebrates like *E. affinis* to feed on faecal material because of their morphological non-capability of gathering buried substances.

This chapter focuses on two deep-sea regular echinoids found in the NE Atlantic. *Phormosoma placenta* **Wyville Thomson** which does not appear to have any seasonal cycle in oogenesis, probably breeding year round (Tyler & Gage 1984a, Gage *et. al.* 1985), and *Echinus affinis* **Mortensen**, which is seasonally reproductive (Tyler & Gage 1984b). The gut contents of preserved samples of *P. placenta* and *E. affinis* were analysed. Samples of *P. placenta* collected from 733-1507m in the Porcupine Seabight and Goban Spur over a five-year period by the Institute of Oceanographic Sciences (I.O.S.); and samples of *E. affinis* collected from approximately 2200m (Station 'M') in the Rockall Trough since 1978 at different times of the year by the Scottish Marine Biological Association (S.M.B.A.) were used for this study.

The material used has limitations as seasonal freshly collected samples are necessary for more detailed biochemical analyses of the gut contents. It was possible, however, to begin the study on the determination of whether the diet of these species varies in both quantity and quality with season using these preserved samples.

4.2. Materials and methods

Samples of *Phormosoma placenta* were collected using a semi-balloon otter trawl, Granton trawl or an IOS epibenthic sledge with a coarse 4mm mesh net or a fine 1mm mesh net (Rice *et. al.* 1982). *Echinus affinis* were collected with a 3m-wide Agassiz trawl, a single warp trawl, semi-balloon otter trawl or a Woods Hole epibenthic sledge (Hessler & Sanders 1967). The reproductive biology of these species was examined previously (Tyler & Gage

1984a, b). The samples used are listed in Tables 2.1 and 2.2; the collection sites are shown in Figure 2.1 (Chapter 2).

Samples were fixed in 7% seawater formalin and subsequently stored in 70% alcohol. *P. placenta* (110 individuals) and *E. affinis* (170 individuals) were dissected after being damp-dried and their test diameter noted. Because the material was preserved a set protocol was observed for the analyses of each specimen. Three portions of the gut (oesophagus, stomach, and intestine) were isolated, as well as the gut contents from each part. All parts were dried at 70°C to constant weight and ashed for four hours at 500°C to determine the ash-free dry weight (AFDW) in grams representing the amount of organic matter, and AFDW percentage representing the proportion of total organic matter-TOM (Paine 1971, Sibuet & Lawrence 1981). The dry weight was used to calculate the organ and gut content indices as follows:

1-) the organ index (OI):

$$\mathrm{OI} = \left(\frac{\text{organ total dry weight (g)}}{\text{test total dry weight (g)}} \right) \times 100$$

2-) the gut total content index (GTCI):

$$\mathrm{GTCI} = \left(\frac{\text{gut total content dry weight (g)}}{\text{test dry weight (g)}} \right) \times 100$$

3-) the gut organic content index (GOCI):

$$\mathrm{GOCI} = \left(\frac{\text{gut content ash-free dry weight (g)}}{\text{test dry weight (g)}} \right) \times 100$$

Indices were used to standardize the weight of the organs, gut total content and gut organic content to the weight of the animals in order to determine seasonal changes in these organs or gut content (Nichols *et. al.* 1985).

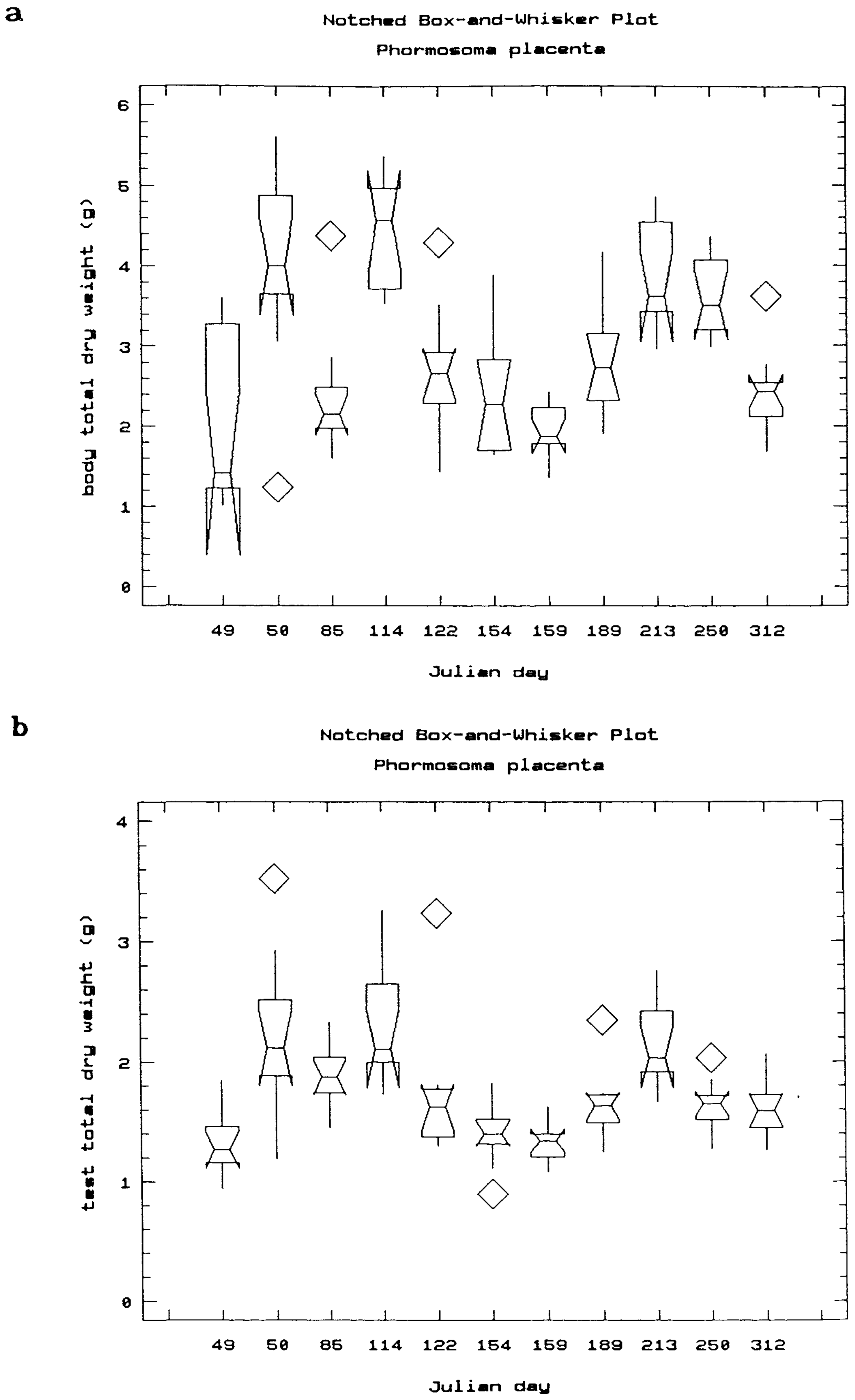


Figure 4.1. *Phormosoma placenta.* Notched Box-and-Whisker plot of: **a.** body dry weight against Julian day; **b.** test dry weight against Julian day.

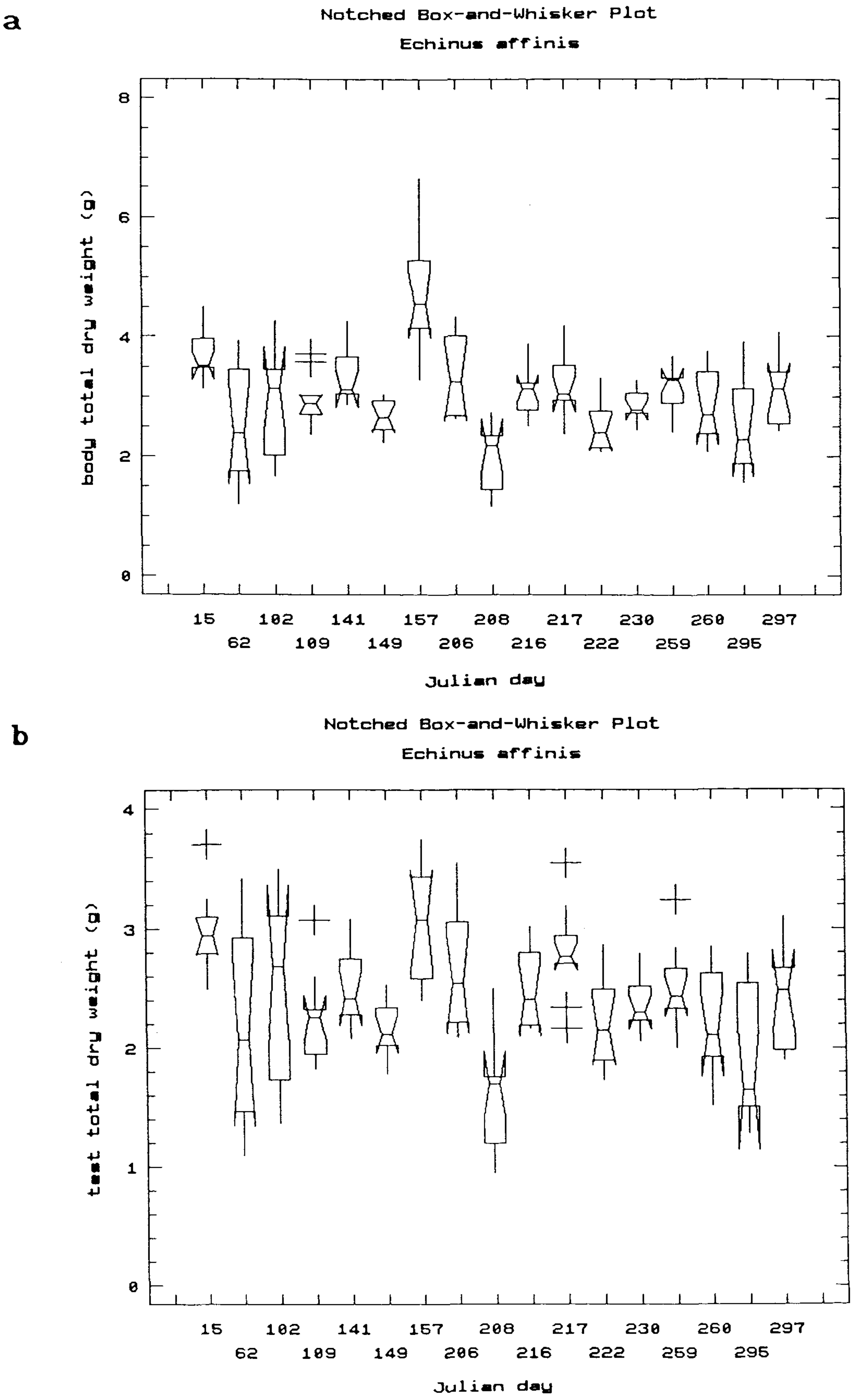


Figure 4.2. *Echinus affinis*. Notched Box-and-Whisker plot of: **a.** body dry weight against Julian day; **b.** test dry weight against Julian day.

Results of both body total dry weight and test total dry weight are shown in Figures 4.1a, b and 4.2a, b. The notched Box-and-Whisker plot was chosen because the data was not normally distributed. The horizontal bars represent the medians, the box represents the interquartile range, the vertical bars extending from each box represent the replicates outside of the interquartile range, and the diamonds or crosses are outliers. The notches surrounding the medians are a measure of the significance of differences between the values. If the notches about two medians do not overlap, the medians are significantly different at 95% confidence level. A box with a protruding notch lying outside either hinge indicates low confidence for the quartile, and a skewed distribution (McGill *et. al.* 1978). *Phormosoma placenta* data will be always represented with diamonds and *Echinus affinis* data with crosses in all graphs shown in this and the results sections.

Calculation of organ indices using total body weight will produce biased indices resulting from changes in the weight of different organs or gut content through the year. A non-parametric Spearman correlation test was applied to compare total body weight and test weight and verify if the test weight was a good substitute for the calculation of the organ indices instead of total body weight. Both are significantly correlated in *P. placenta* and in *E. affinis* as shown in Table 4.1. The weight of the test was therefore used in the calculation of the organ indices rather than total body weight.

Table 4.1. Spearman rank correlation between test dry weight and body dry weight.

SPECIES	CORRELATION COEFFICIENT	n	p ≤
Phormosoma placenta	0.74	110	0.0001
Echinus affinis	0.94	170	0.0001

Data from different years were arranged on a single annual axis in a 'composite' year because of the problems of resolution of temporal sampling in the deep-sea. A single data set for any particular month must be interpreted with reservation because of the interannual variation between samples .

A sample from the gut content of one specimen from each month was examined under the scanning electron microscope (SEM). The gut contents were treated with $2.5g.l^{-1}$ sodium hexametaphosphate to disaggregate the particles from the faecal pellets (Briggs 1985). A particle sample size of $0.5cm^3$ was diluted in 10ml of distilled water. One drop of the mixture was air-dried on to coverslips and mounted on EM stubs using silver paint. After air-drying, the stubs were gold-coated and examined under a Joel-35C scanning electron microscope. Other visual observations were made under a Wild-M8 stereomicroscope.

Particle-size distribution of the gut content was measured after disaggregation of total faecal pellets from each stomach and intestine in $2.5g.l^{-1}$ sodium hexametaphosphate. The gut content particles were then separated with the use of sieves of mesh size 500, 250, and 63 μm. Each fraction was washed into centrifuge tubes and gently centrifuged at 60 revolutions per minute for one hour. The total amount of gut content of each particle size fraction was calculated from total packed volume of the content.

Statistical analysis

For statistical analysis one non-seasonal and two seasonal regression models were defined. The seasonal models were sinusoidal functions defined as follows:

$$\text{Model I:} \quad Y = \theta_1 + \theta_2 \cos \left[\frac{2\pi\ (X-\theta_3)}{365} \right]$$

$$\text{Model II:} \quad Y = \theta_1 + \theta_2 \cos \left[\frac{2\pi\ (X-\theta_3)}{182.5} \right]$$

where,

Y = dependent variable,

X = independent variable (Julian day), and

θ_n = adjustable parameters.

Model I represents annual seasonality, and model II represents bi-annual sesonality (with peaks twice a year). These models were fitted to the data by reducing the mean square of the residuals using nonlinear regression.

The non-seasonal model was defined as $Y = \theta$. This model assumes that the dependent variable remains constant throughout the year.

The adequacy of the models was investigated by the examination of the residuals:

1-) evaluating the coefficients of skewness and kurtosis of the residuals (Sokal & Rohlf 1981) as represented graphically in a normal probability plot, and

2-) plotting the residuals against Julian day to examine the randomness of the residuals (Draper & Smith 1980).

A 'lack of fit' analysis of the models (Bates & Watts 1988) was applied to the data of organ indices and gut content analyses in order to reject those models which did not fit the data.

The assumption of normally distributed errors is a prerequisite for applying the lack of fit analysis (Bates & Watts 1988). Student's t-test was used to check that the residuals did not deviate significantly from a normal distribution (Sokal & Rohlf 1981). Any distribution of residuals with a coefficient of skew (g_1) greater than 0.77 and a coefficient of kurtosis (g_2) greater than 1.50 for *Phormosoma placenta* data deviates significantly from a normal distribution at the 0.1% level. Likewise $g_1>0.62$ and $g_2>1.24$ for *Echinus affinis* data deviate significantly from a normal distribution at 0.1% level (Sokal & Rohlf 1981, p. 174).

The model which had the best fit to the data was chosen based on the following criteria:

1-) a more linear normal probability plot of the residuals (because of lower skew and kurtosis),

2-) a lower mean square of the residuals,

3-) a more random plot of residuals against Julian day, and

4-) a non-significant lack of fit of the model.

The normal probability plot was a useful aid to identify outliers which could generate high levels of skew or kurtosis (Bates & Watts 1988).

The acceptance of the seasonal model I or seasonal model II was based on that which had a higher r^2. The r^2 indicated how much variation of the dependent variable was explained by the model.

In order to display the amplitude of seasonal variation (θ_2) and the time of year at which the indices reach their peaks (θ_3, Julian day), the graphs presented in the results section below show the seasonal model fitted to the data, regardless of whether the seasonality was found to be significant.

4.3. Results

4.3.1. *Phormosoma placenta* Wyville Thomson

4.3.1.1. Organ indices

The statistical analysis of the data obtained from specimens of *Phormosoma placenta* is summarised in Table 4.2. All statistics quoted in this section (excluding correlation coefficients) refer to this table.

Gonad Index: A comparison between the coefficients of skew (g_1) and kurtosis (g_2) of the seasonal model I and the non-seasonal model in *Phormosoma placenta* gonad index data suggest that the seasonal model has a better fit to the data. Although the lack of fit analysis was applied to both models, the F-test was stronger on the seasonal model because in this case the residuals were more normally distributed (g_1=0.953, g_2=1.378). The seasonal model I had a non-significant lack of fit to the gonad index data (F-ratio=0.210, n=110, $p \leq 0.001$), therefore being accepted in preference to the non-seasonal model (Figure 4.3). The indication of a seasonal fluctuation in the gonad index does not necessarily mean the species is a seasonal breeder, as the gonad may be used as a storage organ.

Oesophagus Wall Index: There was insufficient evidence to suggest that the oesophagus wall index had a seasonal fluctuation. Although the F-test showed no significant lack of fit for both seasonal model I and the non-seasonal model, the analysis is invalidated by the high values of g_1 and g_2. The plot of the seasonal model I (figure 4.4) shows that any seasonal fluctuation is very small and therefore difficult to detect.

Stomach Wall Index: The stomach wall index had a significant correlation with the gonad index (Spearman $r=0.45$, $p\leq 0.0001$), suggesting that the stomach wall index fluctuates seasonally . The seasonal model II, however, showed a better fit to the stomach wall index than the seasonal model I, indicating a fluctuation which increased from the spring to higher values in July and decreased to low values in the end of the summer (Figure 4.5). Despite the fact that the lack of fit analysis indicated that both the seasonal and non-seasonal models were inadequate (F-ratio=4.190, F-ratio=16.383 respectively, $n=110$, $p\leq 0.001$), the choice of the seasonal model II rather than the non-seasonal model was based on its lower values of g_1, g_2, and F-ratio. More data are necessary in order to evaluate the seasonal fluctuation.

Intestine Wall Index: Stomach and intestine wall indices were also significantly correlated (Spearman correlation $r=0.44$, $n=110$, $p\leq 0.0001$). As with the stomach, the intestine wall index had a fluctuation with high values in the summer. The non-seasonal model showed a significant lack of fit to the data at 1.0% level (F-ratio=2.971, $n=110$). This implies that the intestine wall index data must have a seasonal fluctuation. Although the F-test for the seasonal model was weakened because the residuals were more skewed ($g_1>0.77$), the F-ratio of 1.97 ($n=110$) indicated a non-significant lack of fit of this model at the 2.5% level ($p\leq 0.025$). Therefore seasonal model II was accepted as showing a better fit to the data (Figure 4.6).

SUBJECT	MODEL	ANALYSIS OF VARIANCE		RESIDUAL ANALYSIS			'LACK OF FIT' ANALYSIS				
		ERROR	R-SQUARED	RESIDUAL STANDARD DEVIATION	COEFF. OF SKEWNESS	COEFF. OF KURTOSIS	SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F-RATIO
GONAD INDEX	■ Seasonal I	337.2	0.124	1.759	0.953	1.378	Lack of fit	7.0	10	0.701	0.210
							Replication	330.2	99	3.335	
							Residuals Error	337.2	109	3.094	
	Non-Seasonal	385.0	0	1.879	1.360	1.707	Lack of fit	54.8	10	5.482	1.644
							Replication	330.2	99	3.335	
							Residuals Error	385.0	109	3.532	
OESOPHAGUS WALL INDEX	Seasonal I	4.621	0.050	0.206	2.052	6.529	Lack of fit	0.392	10	0.039	0.912
							Replication	4.229	99	0.043	
							Residuals Error	4.621	109	0.042	
	☐ Non-Seasonal	4.864	0	0.211	1.875	5.698	Lack of fit	0.635	10	0.064	1.477
							Replication	4.229	99	0.043	
							Residuals Error	4.864	109	0.045	
STOMACH WALL INDEX	☐ Seasonal II	76.83	0.145	0.840	0.357	0.175	Lack of fit	22.84	10	2.284	4.190
							Replication	53.99	99	0.545	
							Residuals Error	76.83	109	0.705	
	Non-Seasonal	89.83	0	0.908	0.569	0.440	Lack of fit	89.29	10	8.928	16.383
							Replication	53.99	99	0.545	
							Residuals Error	89.83	109	0.824	
INTESTINE WALL INDEX	■ Seasonal II	28.72	0.100	0.513	0.828	0.680	Lack of fit	6.28	10	0.628	1.970
							Replication	22.43	99	0.319	
							Residuals Error	28.72	109	0.263	
	Non-Seasonal	31.91	0	0.541	0.622	0.096	Lack of fit	9.48	10	0.948	2.971
							Replication	22.43	99	0.319	
							Residuals Error	31.91	109	0.293	
STOMACH TOTAL CONTENT INDEX	☐ Seasonal I	43492	0.073	19.975	0.546	-0.425	Lack of fit	12184	10	1218	3.853
							Replication	31307	99	316	
							Residuals Error	43492	109	399	
	Non-Seasonal	46905	0	20.744	0.371	-0.673	Lack of fit	15598	10	1560	4.932
							Replication	31307	99	316	
							Residuals Error	46905	109	430	

Statistics of 'best-fit' model:

■ Significantly good fit to the data.

☐ Best fit, but refinement of model / more data needed.

Table 4.2: Statistical analysis of data obtained from *Phormosoma placenta* (continued on following page)

(Table 4.2 continued)

SUBJECT	MODEL	ANALYSIS OF VARIANCE		RESIDUAL ANALYSIS			'LACK OF FIT' ANALYSIS				
		ERROR	R-SQUARED	RESIDUAL STANDARD DEVIATION	COEFF. OF SKEWNESS	COEFF. OF KURTOSIS	SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F-RATIO
INTESTINE TOTAL CONTENT INDEX	□ Seasonal I	43431	0.151	19.961	0.973	0.597	Lack of fit	15275	10	1527	5.371
							Replication	28157	99	284	
							Residuals Error	43431	109	398	
	Non-Seasonal	51182	0	21.669	0.628	-0.285	Lack of fit	23025	10	2303	8.096
							Replication	28157	99	284	
							Residuals Error	51182	109	470	
STOMACH ORGANIC CONTENT INDEX	Seasonal II	346.4	0.030	1.783	1.079	2.224	Lack of fit	88.9	10	8.894	3.421
							Replication	257.4	99	2.600	
							Residuals Error	346.4	109	3.178	
	□ Non-Seasonal	357.0	0	1.810	1.104	2.370	Lack of fit	99.5	10	9.954	3.828
							Replication	257.4	99	2.600	
							Residuals Error	357.0	109	3.275	
INTESTINE ORGANIC CONTENT INDEX	□ Seasonal I	187.0	0.167	1.310	1.147	2.418	Lack of fit	47.2	10	4.723	3.345
							Replication	139.8	99	1.412	
							Residuals Error	187.0	109	1.716	
	Non-Seasonal	224.5	0	1.435	0.734	1.046	Lack of fit	84.7	10	8.471	5.999
							Replication	139.8	99	1.412	
							Residuals Error	224.5	109	2.060	
STOMACH CONTENT TOTAL ORGANIC MATTER	□ Seasonal II	1975	0.221	4.257	1.071	2.510	Lack of fit	957	10	95.69	9.304
							Replication	1018	99	10.28	
							Residuals Error	1975	109	18.12	
	Non-Seasonal	2534	0	4.822	0.295	0.192	Lack of fit	1516	10	151.64	14.745
							Replication	1018	99	10.28	
							Residuals Error	2534	109	23.25	
INTESTINE CONTENT TOTAL ORGANIC MATTER	■ Seasonal II	1349	0.296	3.518	-0.053	0.845	Lack of fit	294	10	29.41	2.760
							Replication	1055	99	10.65	
							Residuals Error	1349	109	12.37	
	Non-Seasonal	1916	0	4.193	0.178	0.154	Lack of fit	862	10	86.18	8.090
							Replication	1055	99	10.65	
							Residuals Error	1916	109	17.58	

Statistics of 'best-fit' model:

■ Significantly good fit to the data.

□ Best fit, but refinement of model / more data needed.

Table 4.2 (continuation)**: Statistical analysis of data obtained from *Phormosoma placenta***

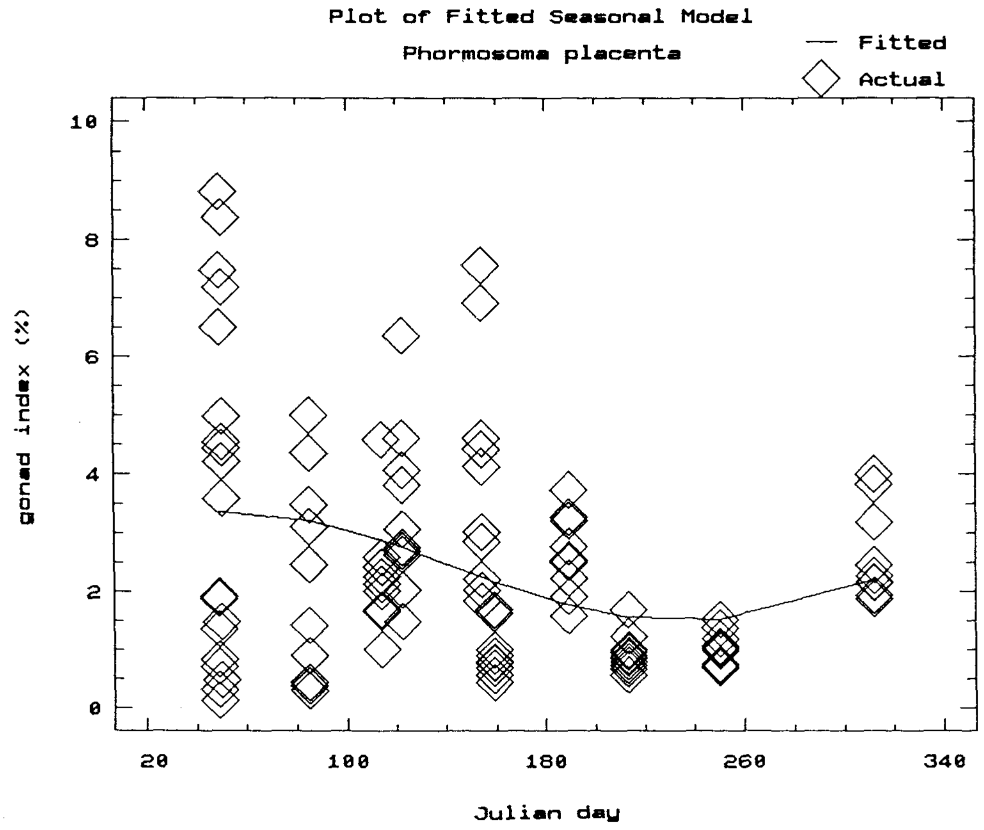


Figure 4.3. *Phormosoma placenta.* Plot of seasonal model I fitted to gonad index data.

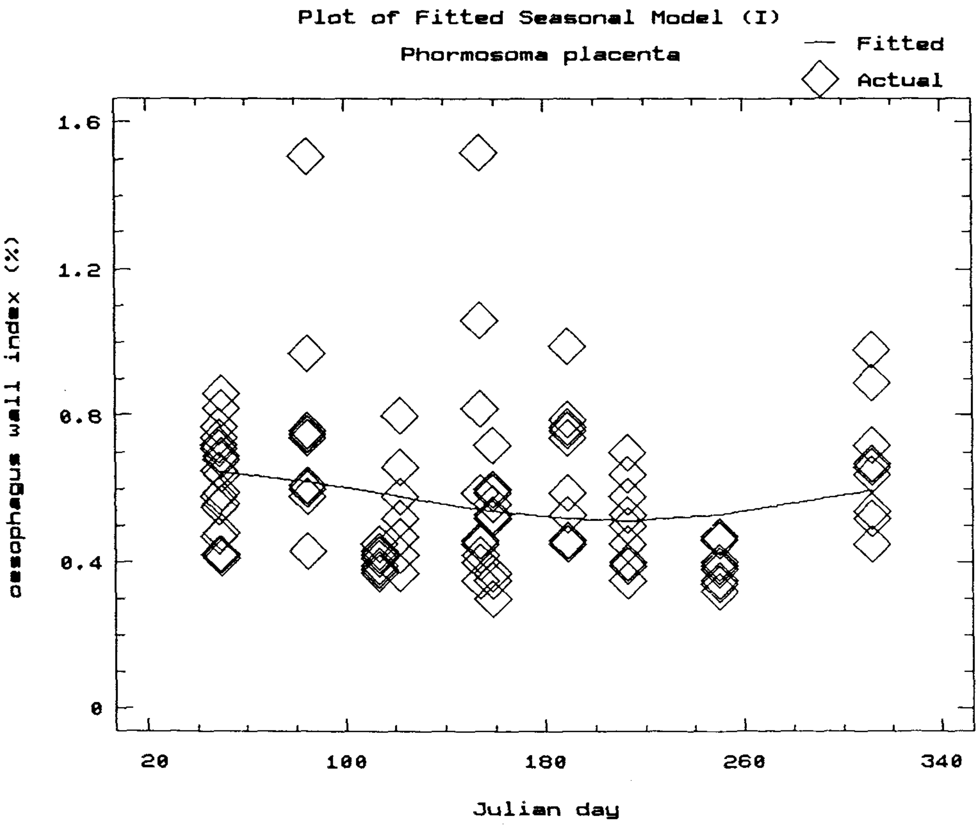


Figure 4.4. *Phormosoma placenta.* Plot of seasonal model I fitted to oesophagus wall index data.

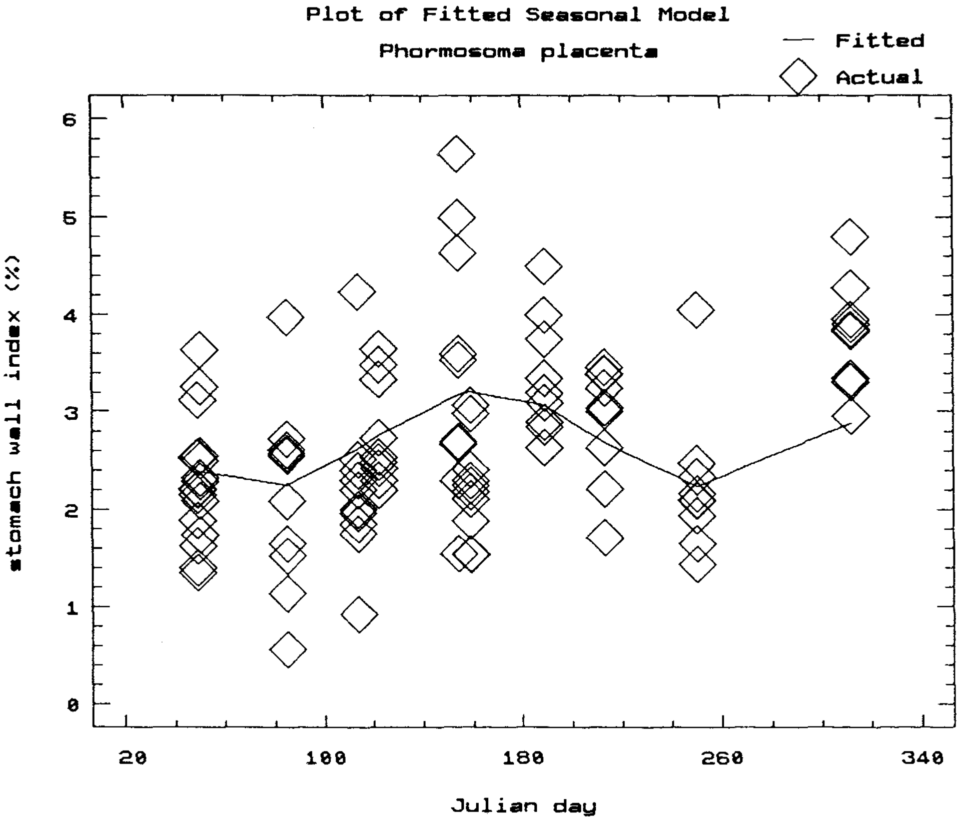


Figure 4.5. *Phormosoma placenta*. Plot of seasonal model II fitted to stomach wall index data.

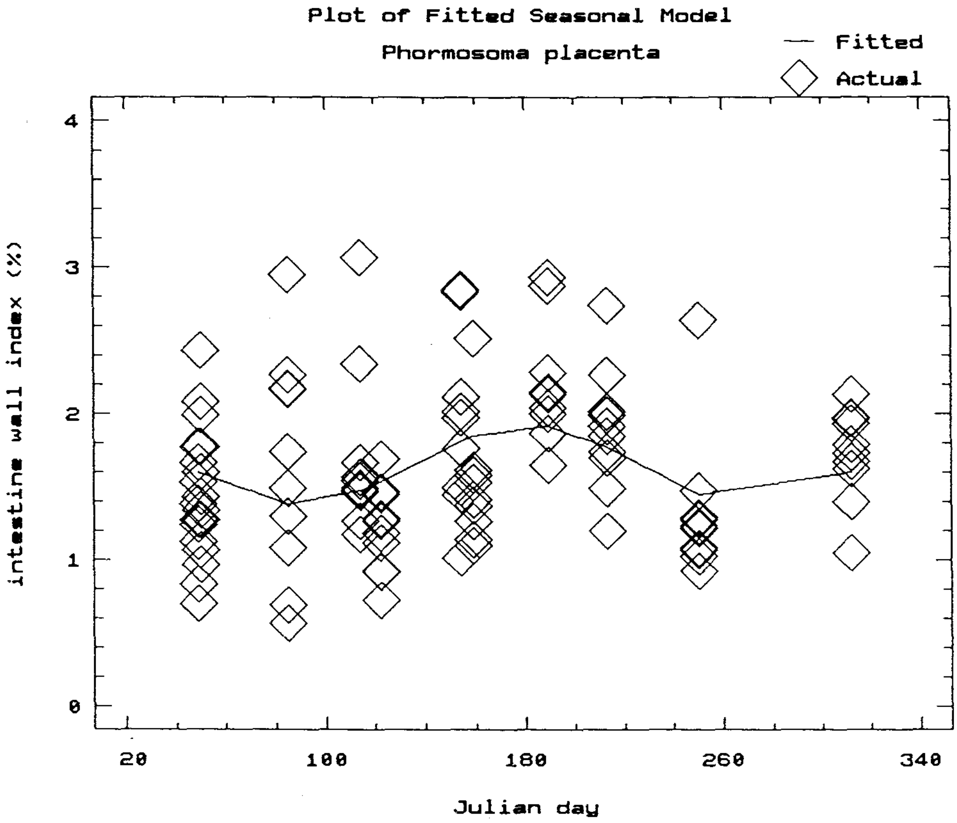


Figure 4.6. *Phormosoma placenta.* Plot of seasonal model II fitted to intestine wall index data.

4.3.1.2. Gut Content Indices

Statistical analysis of *Phormosoma placenta* gut contents data is summarised in Table 4.2.

Stomach and Intestine Total Content Indices: The non-seasonal model had a significant lack of fit when applied to both stomach and intestine total content indices (F-ratio=4.932, and F-ratio=8.096 respectively, $n=110$, $p \leq 0.001$), therefore implying some kind of seasonal variation. More data are required to explain the seasonality because a lack of fit was also found for the sinusoidal model I, suggesting this model was not sufficient to explain the residuals (Figure 4.7a, b).

Stomach and Intestine Organic Content Indices: The stomach organic content index data had high skewness and kurtosis indicating the errors were not normally distributed and therefore invalidate the F-test for the lack of fit analysis (Table 4.2). Because the data were too unevenly distributed to test one model against the other, in the absence of evidence for seasonality a non-seasonality was accepted (Figure 4.8a).

The non-seasonal model skewness and kurtosis indicated normally distributed errors for the intestine organic content index data ($g_1=0.734$, $g_2=1.046$) validating the F-test of the lack of fit analysis for this model. A seasonal variation was suggested as the non-seasonal model showed a significant lack of fit to the data (F-ratio=5.999, $n=110$, $p \leq 0.001$), although seasonal model I, used in this case (Figure 4.8b), was not sufficient to explain the residuals.

Stomach and Intestine Content Total Organic Matter (%): The non-seasonal model showed a significant lack of fit to the stomach content total organic matter data (F-ratio=14.745, $n=110$, $p \leq 0.001$), and therefore implied a seasonal model was required. The seasonal model II assigned in this case explains a relatively large proportion of the residuals ($r^2=0.221$), but still leaves much of the residual error unexplained (Figure 4.9a).

The seasonal model II had a significantly better fit to the intestine content total organic matter data. Skewness was reduced for this model in comparison with the non-seasonal model (Seasonal II: g_1=-0.053; Non-seasonal: g_1=0.178). Lack of fit analysis showed that the non-seasonal model did not fit the data (F-ratio=8.090, n=110, $p \leq 0.001$), whereas the seasonal model II had a non-significant lack of fit to the data (F-ratio=2.760, n=110, $p \leq 0.001$). A marked increase in the total organic matter of the gut content occurred from April until June coincident with the first arrival of the phytodetritus to the sea bed. The total organic matter of the intestine content decreased from July/August to lower values in September (Figure 4.9b). The high values shown in November could be an effect of a second arrival of phytodetritus to the sea-bed in the autumn.

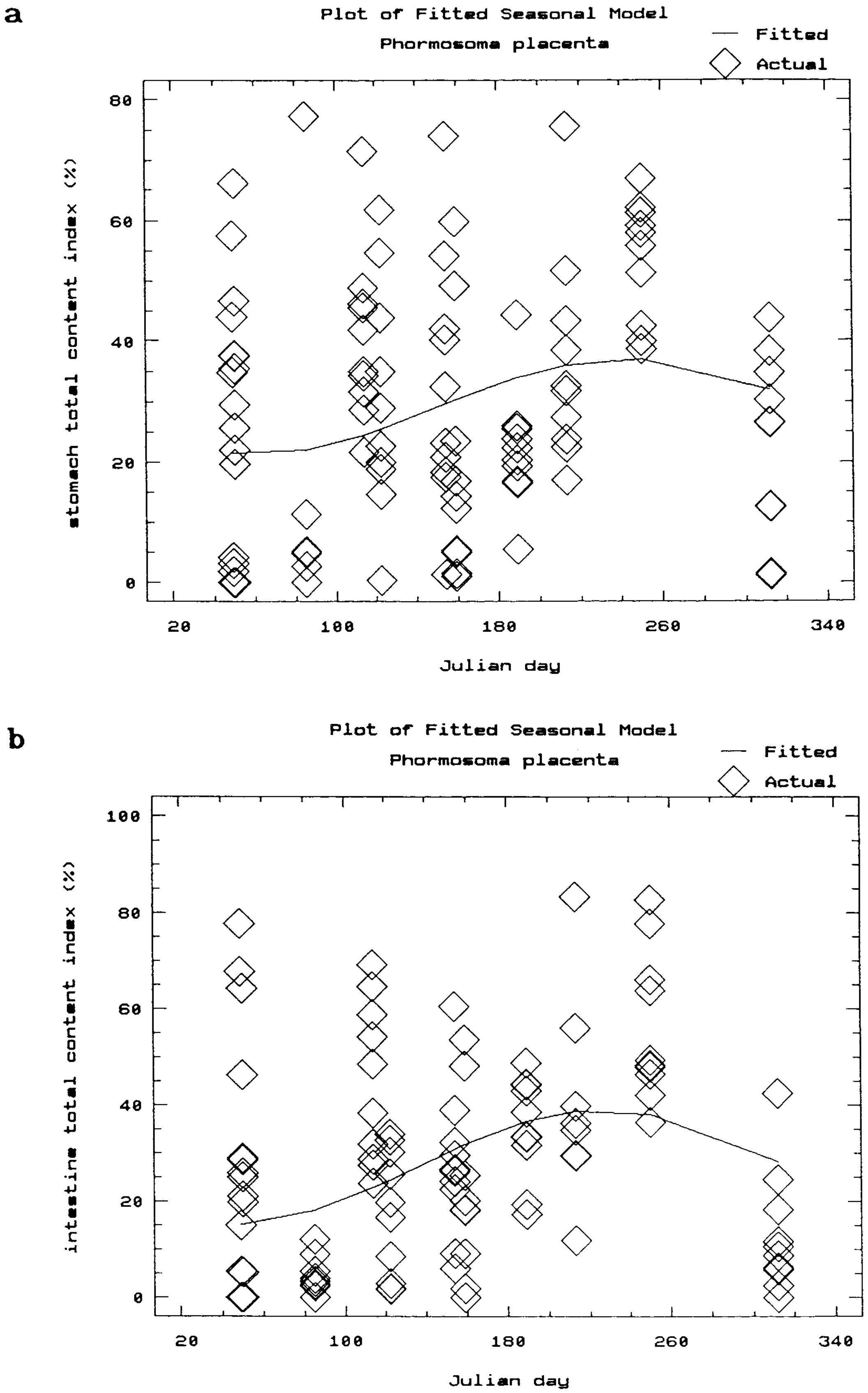


Figure 4.7. *Phormosoma placenta.* Plot of seasonal model I fitted to: **a.** stomach total content index data; **b.** intestine total content index.

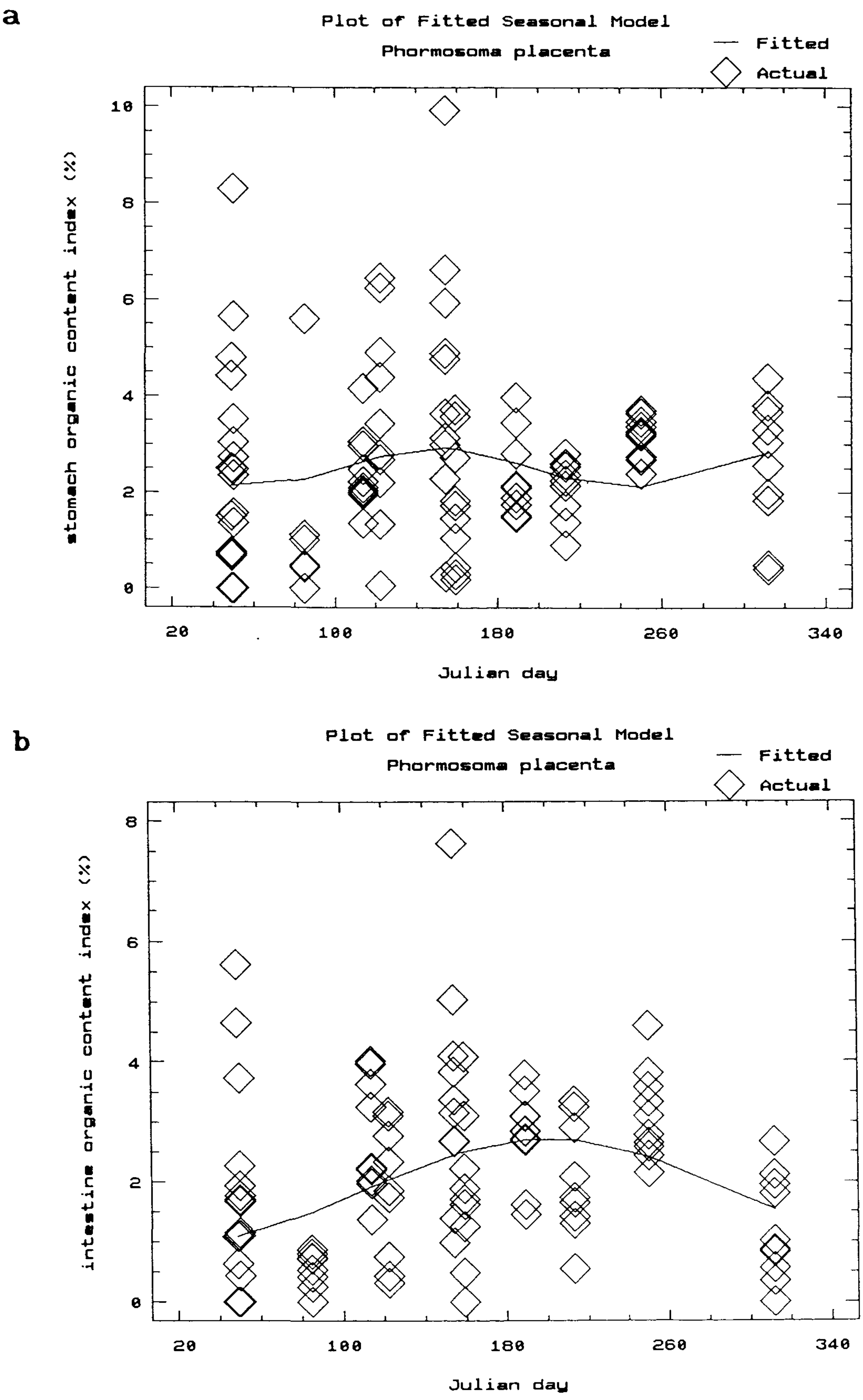


Figure 4.8. *Phormosoma placenta.* **a.** Plot of seasonal model II fitted to stomach organic content index data; **b.** plot of seasonal model I fitted to intestine organic content index.

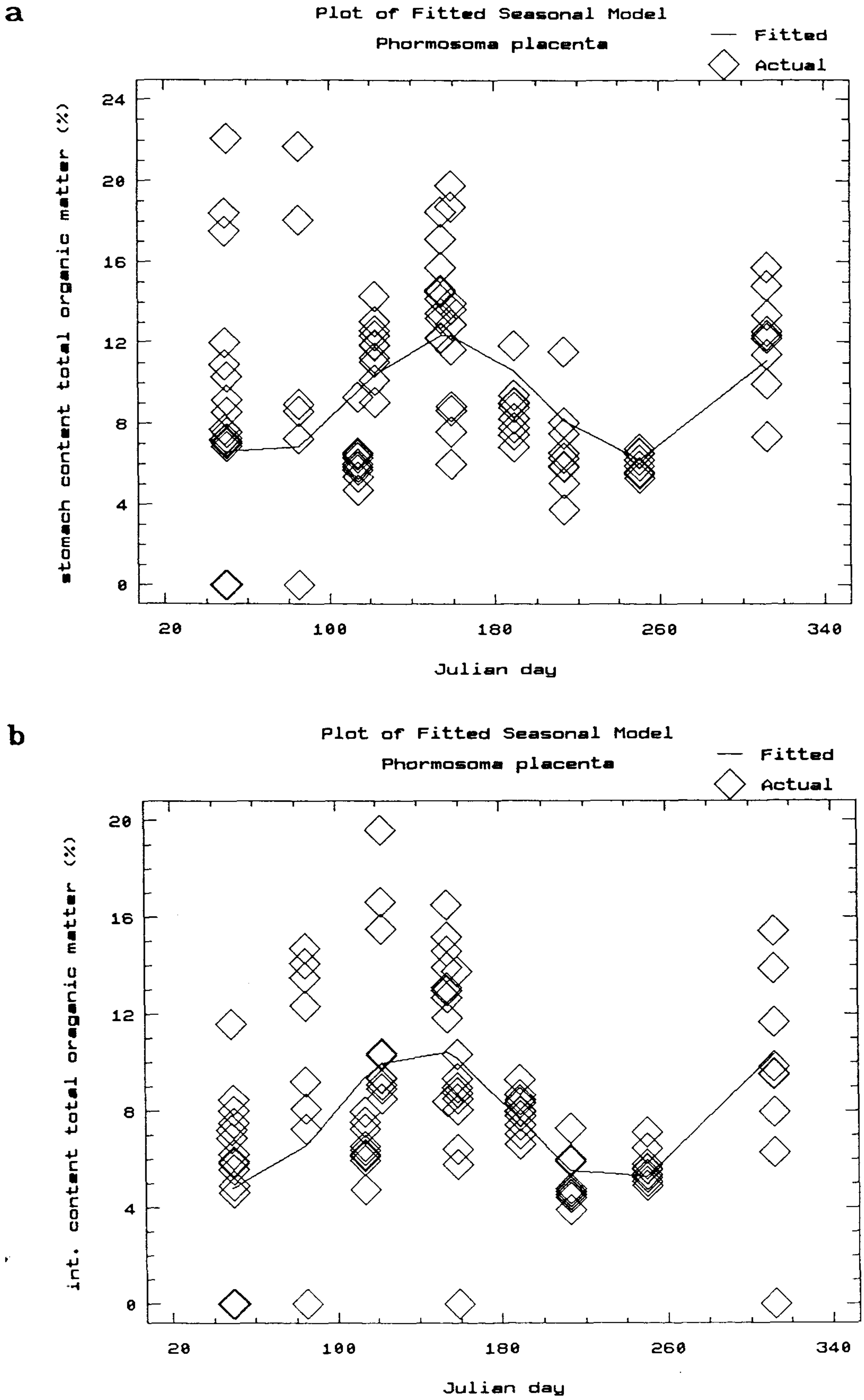


Figure 4.9. *Phormosoma placenta.* Plot of seasonal model II fitted to: **a.** stomach content total organic matter data; **b.** intestine content total organic matter data.

4.3.1.3. Visual observation of the gut content

As in other regular echinoids *Phormosoma placenta*, as well as *Echinus affinis*, form food pellets in the buccal cavity and the pharynx (De Ridder & Lawrence 1982, De Ridder & Jangoux 1982). The pellets are covered by a mucoid coat which remains throughout the passage of the pellets through the gut (De Ridder & Jangoux 1982 and references therein, see also chapter 2).

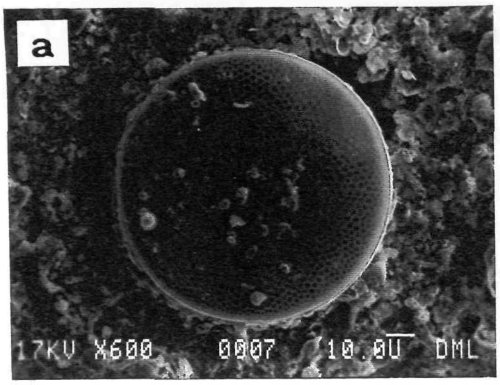
SEM and light microscope examination of the pellets from *P. placenta* showed, qualitatively, a wide variety of planktonic and benthic material. The pellets consisted of various phytoplankton cells, foraminiferans, radiolarians, sponge spicules, and small unidentified particles (Table 4.3; Figure 4.10). Amongst the diatoms *Thalassiosira* species appeared more commonly in the summer samples. *Chaetoceros* spp as well as flagellates cysts were found mainly in the spring samples. Several species of coccoliths were observed in all samples of gut content of *P. placenta.* Radiolarians were also common through spring and summer samples. Pelagic foraminiferans appeared mainly in the summer samples possibly as a result of their presence in the phytodetritus flux characteristic of that time of the year. Although the tintinids were only identified in the sample from November, they appeared to be present in abundance.

No attempt was made to quantify the various structures found in the gut content because depending on the magnification one or other organism appeared more abundantly. For example, at mag. 3000x many coccoliths and diatoms species were seen whilst at mag. 100x more foraminiferans and unrecognizable material were observed. A good quantitative survey on the gut contents of these echinoids would require the examination of at least 6-10 specimens from each month, each specimen observed at various defined magnifications. This way any seasonality in the appearance of different structures in the gut content could be detected.

Table 4.3. *Phormosoma placenta* list of the structures found in the gut in different months identified from SEM photographs.

STRUCTURES IDENTIFIED	win			spr			sum			aut			
	D	J	F	M	A	M	J	J	A	S	O	N	D
DIATOMS													
Thalassiosira sp.	.	.	.	.	.	.	+	+	+	.	.	+	.
Chaetoceros sp.	.	.	+	+	+	+	.	.	+	.	.	.	.
Chaetoceros resting spore	.	.	.	+	.	+	+	.	.	.	.	.	.
Coscinodiscus sp.	.	.	.	+	.	.	.	.	.	.	.	+	.
Nitzschia sp.	.	.	.	+	+	.	.	+	+	.	.	.	.
Thalassionema sp.	.	.	.	.	.	+	.	.	.	.	.	.	.
FLAGELLATES													
dinoflagellate cysts	.	.	+	.	+	+	+	.	.	.	.	.	.
silicoflagellates	.	.	.	.	.	+	+	.	.	.	.	.	.
COCCOLITHS	.	.	+	+	+	+	+	+	+	+	.	+	.
RADIOLARIANS	.	.	+	+	+	+	+	+	.	.	.	.	.
BENTHIC FORAMS	.	.	.	.	+	.	.	.	.	.	.	.	.
PELAGIC FORAMS	.	.	.	.	+	.	+	+	+	.	.	+	.
TINTINNIDS	.	.	.	.	.	.	.	.	.	.	.	+	.
sponge spicules	.	.	.	.	+	+	.	+	.	.	.	.	.
radula	.	.	.	.	.	.	.	.	+	.	.	.	.

Figure 4.10. *Phormosoma placenta*. Scanning electron micrographs of the gut contents: **a.** *Coscinodiscus* sp; **b.** *Chaetoceros* sp; **c.** *Thalassiosira* sp, coccolith, unrecognisable debris; **d.** parts of the coccolithophore (?) *Emiliania huxleyi*; **e.** silicoflagellate; **f.** *Actiniscus pentasterias*; **g.** planktonic foraminiferan; **h.** juvenile Nodosariacea (?) *Marginulinopsis* sp.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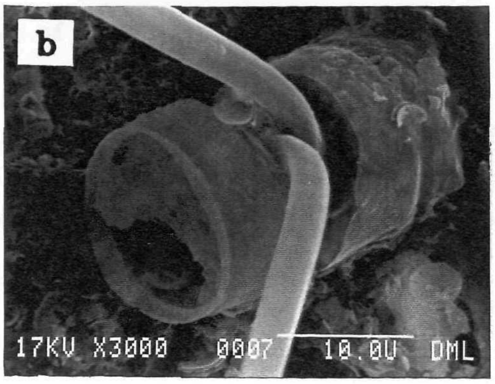
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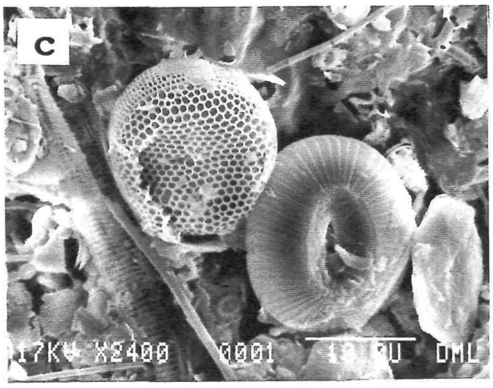
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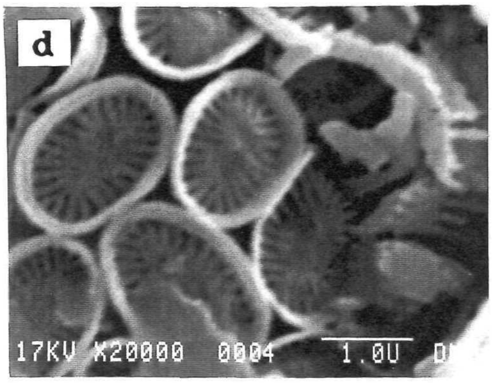
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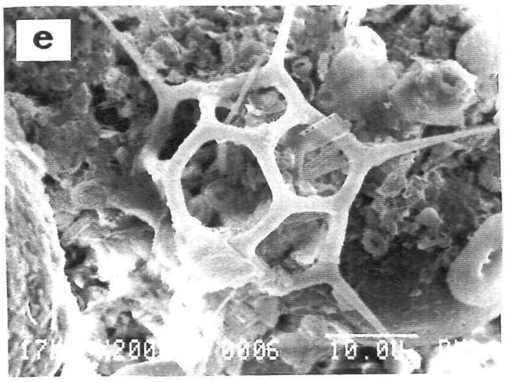
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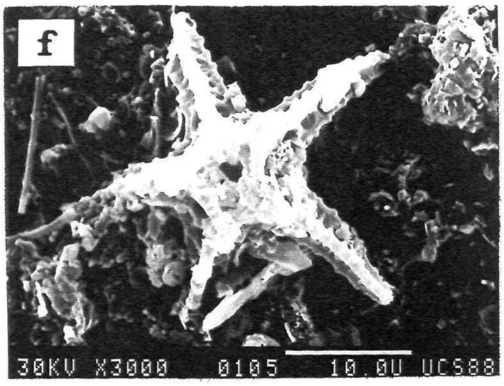
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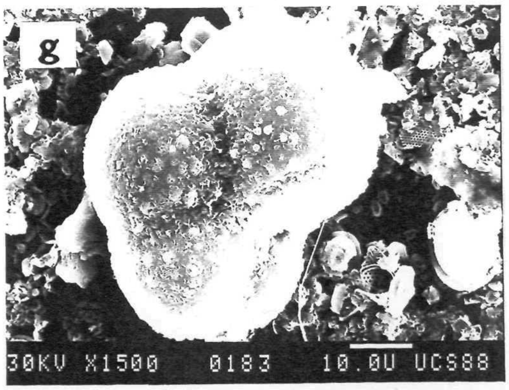
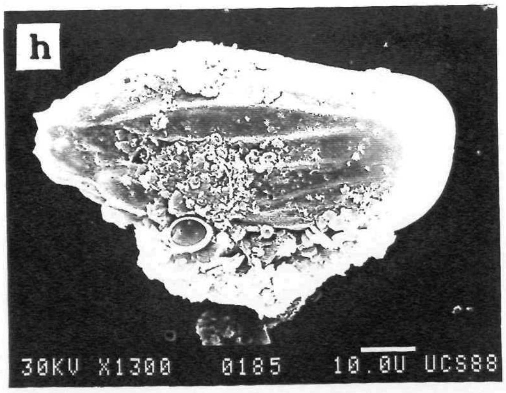

g
30KV X1500 0183 10.0U UCS88


h
30KV X1300 0185 10.0U UCS88

4.3.1.4. Particle-size distribution of the gut content

Stomach and intestine contents had most particles < 250μm, especially those < 63μm (Table 4.4; Figure 4.11). There was no significant difference in particle-size distribution between stomach and intestine content particles.

Table 4.4. *Phormosoma placenta.* Particle-size distribution of the stomach and intestine content found from packed volume (cm^3) from different size fractions.

SIZE (μm)	n	STOMACH CONTENT (cm^3) $\bar{X}$ ± 1SD	INTESTINE CONTENT (cm^3) $\bar{X}$ ± 1SD
P > 500	6	0.13 ± 0.07	0.09 ± 0.03
250 < P < 500	6	0.09 ± 0.03	0.06 ± 0.03
63 < P < 250	6	0.24 ± 0.13	0.22 ± 0.20
P < 63	6	0.66 ± 0.40	0.81 ± 0.51
total volume	6	1.11 ± 0.37	1.39 ± 0.26

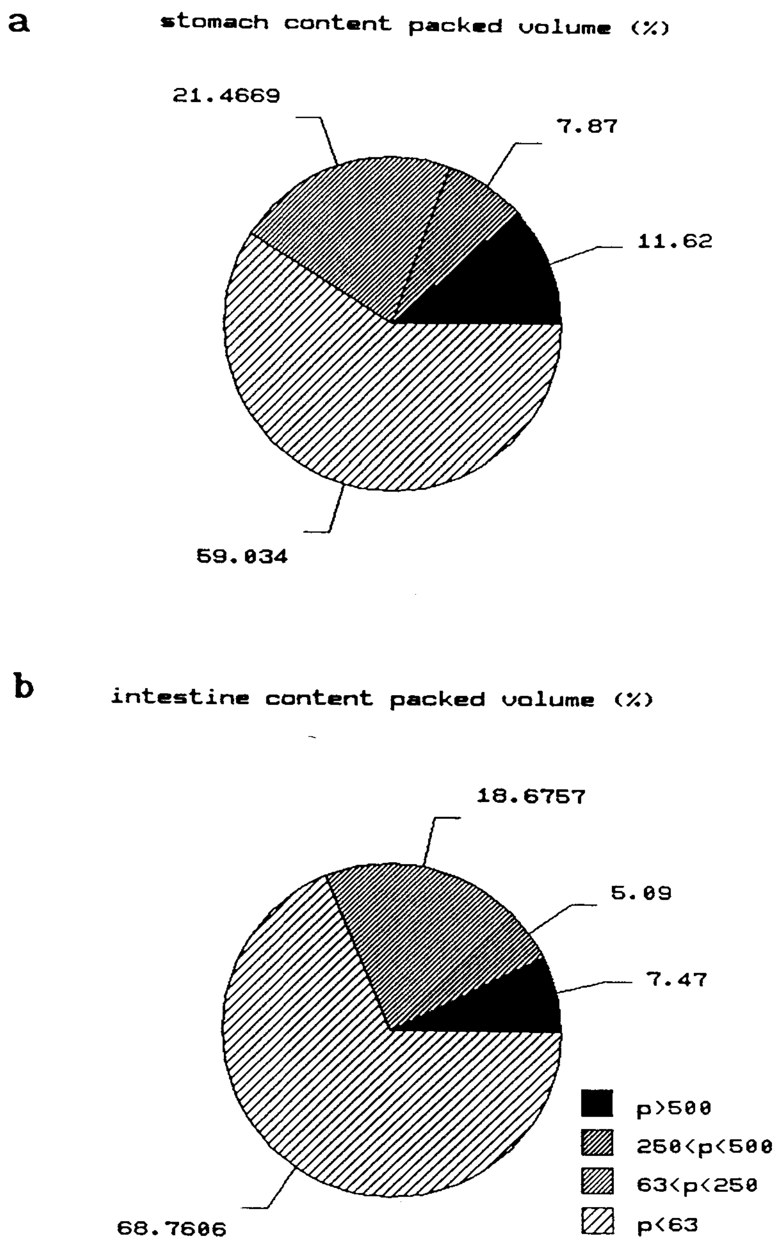


Figure 4.11. *Phormosoma placenta.* Particle-size distribution of the gut content found from the packed volume (cm^3) from different size fractions: **a.** stomach content; **b.** intestine content. Pie chart represent the percentage of each size fraction.

4.3.2. *Echinus affinis* Mortensen

4.3.2.1. Organ indices

The statistical analysis of the data obtained from specimens of *Echinus affinis* is summarised in table 4.5. All statistics quoted in this section refer to this table.

Gonad index: A comparison of gonad AFDW as a function of Julian day and test diameter for *Echinus affinis* is shown in Figure 4.12. Full reproductive maturity is reached at a test diameter >30mm although the gonads start developing at much smaller size (Gage & Tyler, 1985). Although the skew (g_1) was higher than 0.62 when the sinusoidal model I was applied to the gonad index, the kurtosis (g_2) indicated a normal distribution of residuals validating the lack of fit analysis (Table 4.5). The unexplained seasonal fluctuation would require a more complex seasonal model. Some variation throughout the year was suggested because the non-seasonal model also failed to fit the data (g_1=1.337, g_2=1.436, F-ratio=21.68; $p \leq 0.001$). The residuals of both seasonal and non-seasonal models were tested with a random plot of the residuals and a normal probability plot which confirmed that the seasonal pattern fitted the data better than the non-seasonal pattern. Higher values of gonad index were found during the winter months decreasing from spring towards the summer and increasing again from the autumn months towards the winter (Figure 4.13).

Oesophagus Wall Index: The oesophagus wall index was not significantly fitted by the non-seasonal model (F-ratio= 8.575, n=170, $p \leq 0.001$). Although not adequate, the seasonal model II was applied showing a weak seasonal fluctuation with increasing values from the winter months towards spring, decreasing slightly from April to the summer months and increasing again in September (Figure 4.14).

SUBJECT	MODEL	ANALYSIS OF VARIANCE		RESIDUAL ANALYSIS			'LACK OF FIT' ANALYSIS				
		ERROR	R-SQUARED	RESIDUAL STANDARD DEVIATION	COEFF. OF SKEWNESS	COEFF. OF KURTOSIS	SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F-RATIO
GONAD INDEX	☐ Seasonal I	2386	0.169	3.757	0.894	1.241	Lack of fit	1508	16	94.23	16.416
							Replication	878	153	5.74	
							Residuals Error	2386	169	14.12	
	Non-Seasonal	2870	0	4.121	1.337	1.436	Lack of fit	1992	16	124.47	21.684
							Replication	878	153	5.74	
							Residuals Error	2870	169	16.98	
OESOPHAGUS WALL INDEX	☐ Seasonal II	1.340	0.070	0.089	0.920	1.039	Lack of fit	0.586	16	0.037	7.325
							Replication	0.754	153	0.005	
							Residuals Error	1.340	169	0.008	
	Non-Seasonal	1.441	0	0.092	0.849	0.661	Lack of fit	0.686	16	0.043	8.575
							Replication	0.754	153	0.005	
							Residuals Error	1.440	169	0.009	
STOMACH WALL INDEX	☐ Seasonal I	50.29	0.164	0.545	0.643	-0.116	Lack of fit	31.06	16	1.941	15.408
							Replication	19.22	153	0.126	
							Residuals Error	50.287	169	0.298	
	Non-Seasonal	60.13	0	0.597	0.681	0.200	Lack of fit	40.91	16	2.557	20.292
							Replication	19.22	153	0.126	
							Residuals Error	60.13	169	0.356	
INTESTINE WALL INDEX	■ Seasonal I	4.350	0.080	0.160	0.505	0.035	Lack of fit	0.737	16	0.046	1.919
							Replication	3.613	153	0.024	
							Residuals Error	4.350	169	0.026	
	Non-Seasonal	4.728	0	0.167	0.519	-0.045	Lack of fit	1.115	16	0.070	2.904
							Replication	3.613	153	0.024	
							Residuals Error	4.728	169	0.025	
STOMACH TOTAL CONTENT INDEX	■ Seasonal I	2332	0.124	3.715	0.712	1.007	Lack of fit	423	16	26.42	2.117
							Replication	1909	153	12.48	
							Residuals Error	2332	169	13.80	
	Non-Seasonal	2663	0	3.970	0.911	1.228	Lack of fit	754	16	47.12	3.776
							Replication	1909	153	12.48	
							Residuals Error	2663	169	15.76	

Statistics of 'best-fit' model:

■ Significantly good fit to the data.

☐ Best fit, but refinement of model / more data needed.

Table 4.5: Statistical analysis of data obtained from *Echinus affinis* (continued on following page)

(Table 4.5 continued)

SUBJECT	MODEL	ANALYSIS OF VARIANCE		RESIDUAL ANALYSIS			'LACK OF FIT' ANALYSIS				
		ERROR	R-SQUARED	RESIDUAL STANDARD DEVIATION	COEFF. OF SKEWNESS	COEFF. OF KURTOSIS	SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F-RATIO
INTESTINE TOTAL CONTENT INDEX	□ Seasonal I	4373	0.034	5.087	0.649	0.219	Lack of fit	1491	16	93.17	4.947
							Replication	2882	153	18.84	
							Residuals Error	4373	169	25.87	
	Non-Seasonal	4528	0	5.176	0.603	-0.049	Lack of fit	1646	16	102.90	5.463
							Replication	2882	153	18.84	
							Residuals Error	4528	169	26.79	
STOMACH ORGANIC CONTENT INDEX	Seasonal I	85.08	0.020	0.710	1.291	1.735	Lack of fit	10.44	16	0.652	1.337
							Replication	74.64	153	0.488	
							Residuals Error	85.08	169	0.503	
	□ Non-Seasonal	86.86	0	0.717	1.267	1.940	Lack of fit	12.22	16	0.763	1.564
							Replication	74.64	153	0.488	
							Residuals Error	86.86	169	0.514	
INTESTINE ORGANIC CONTENT INDEX	■ Seasonal I	62.40	0.053	0.608	0.832	0.939	Lack of fit	0	16	0	0.000
							Replication	62.397	153	0.408	
							Residuals Error	62.397	169	0.370	
	Non-Seasonal	65.89	0	0.624	0.878	0.981	Lack of fit	3.497	16	0.219	0.536
							Replication	62.397	153	0.408	
							Residuals Error	65.894	169	0.390	
STOMACH CONTENT TOTAL ORGANIC MATTER	Seasonal I	16793	0.026	9.968	1.017	1.480	Lack of fit	5235	16	327.16	4.331
							Replication	11559	153	75.55	
							Residuals Error	16793	169	99.37	
	□ Non-Seasonal	17240	0	10.100	1.172	1.431	Lack of fit	5681	16	355.07	4.700
							Replication	11559	153	75.55	
							Residuals Error	17240	169	102.01	
INTESTINE CONTENT TOTAL ORGANIC MATTER	Seasonal I	4796	0.009	5.327	1.070	1.423	Lack of fit	1914	16	119.65	6.352
							Replication	2882	153	18.84	
							Residuals Error	4796	169	28.38	
	□ Non-Seasonal	4840	0	5.352	1.017	1.439	Lack of fit	1959	16	122.42	6.500
							Replication	2882	153	18.84	
							Residuals Error	4840	169	28.64	

Statistics of 'best-fit' model:

■ Significantly good fit to the data.

□ Best fit, but refinement of model / more data needed.

Table 4.5 (continuation): **Statistical analysis of data obtained from *Echinus affinis***

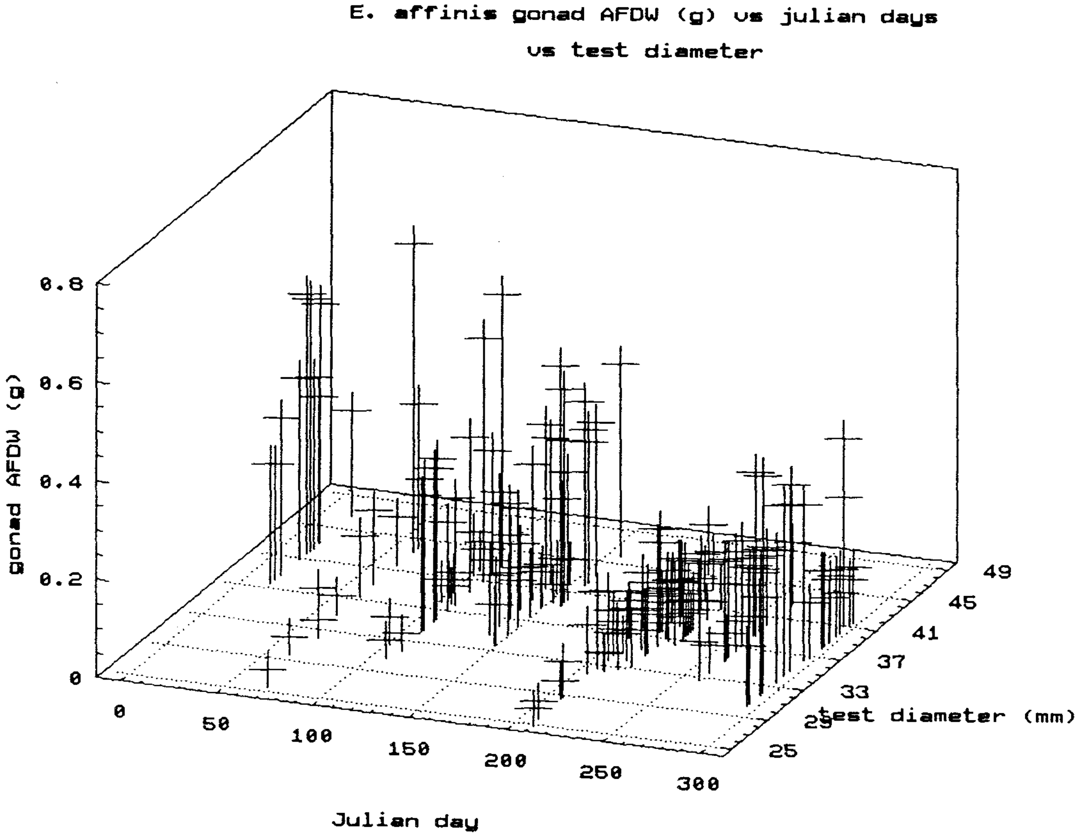


Figure 4.12. *Echinus affinis*. Scatterplot of gonad AFDW (g) as a function of Julian day and test diameter.

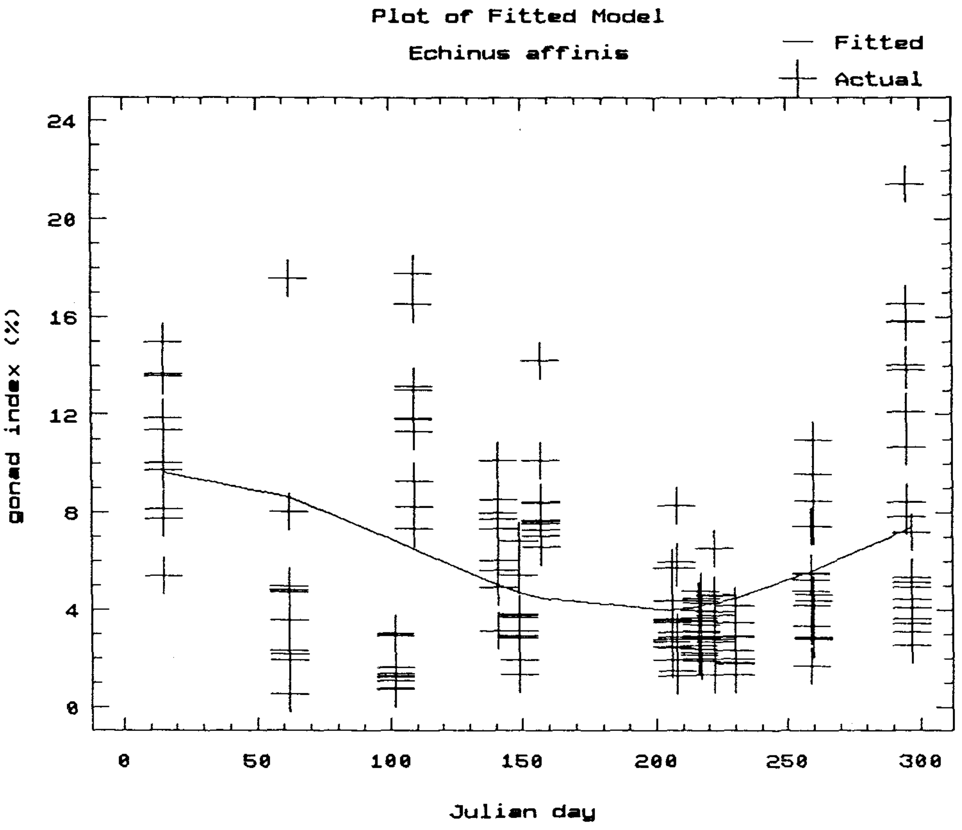


Figure 4.13. *Echinus affinis*. Plot of seasonal model I fitted to gonad index data.

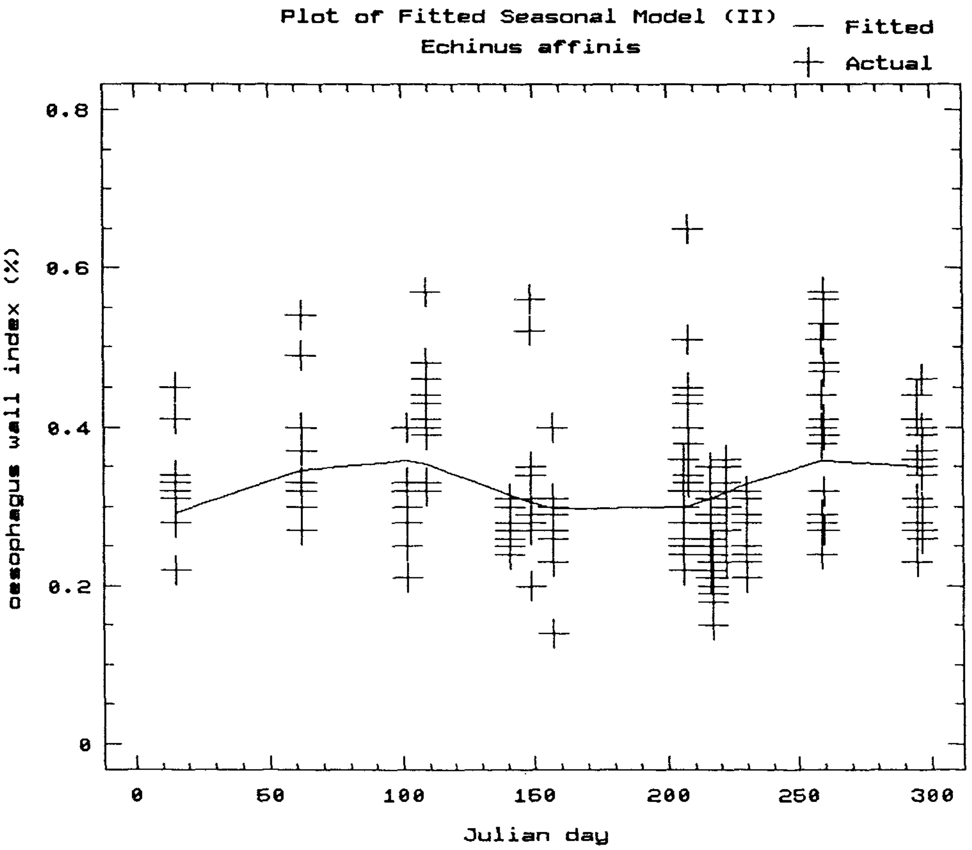


Figure 4.14. *Echinus affinis.* Plot of seasonal model II fitted to oesophagus wall index data.

Stomach wall index: There appeared to be a seasonal fluctuation throughout the year in the stomach wall ash-free dry weight (g) (AFDW) when these data are plotted as a function of Julian day. During the spring and summer there was a considerable variation between samples, but the summer and autumn samples show a steady increase in the AFDW of the stomach wall (Figure 4.15). There was a significant correlation between the stomach wall AFDW and stomach wall index (Spearman r=0.69, n=170, $p \leq 0.001$). The stomach wall AFDW, plotted as a function of Julian day and test diameter, was used to obviate the effect of overall size of the echinoids (Figure 4.16).

The fluctuation pattern of the stomach wall index was not so clear (Figure 4.17). The significant lack of fit of the non-seasonal model (F-ratio=20.35, $p \leq 0.001$) suggested a better fit to the stomach wall index would be achieved by a seasonal model. Values of g_1 and g_2 were reduced in comparison with the non-seasonal model when seasonal model I was applied to these data (Figure 4.18). However, this model also showed a significant lack of fit to the data (F-ratio=15.408, $p \leq 0.001$).

A significant correlation between stomach wall index and gonad index was found (Spearman r=0.52, n=170, $p \leq 0.001$), both showed higher values in the winter decreasing in the spring towards the summer and increasing again towards the autumn/winter. The stomach wall index, however, increased steadily through the summer, autumn and winter from a lower value in May, whilst the gonad index increased from July/August (Figures 4.13 and 4.18).

Intestine wall Index: The relationship between intestine wall AFDW and Julian day is shown in Figure 4.19. No obvious seasonal trends were apparent. Intestine wall AFDW was also correlated to the intestine wall index (Spearman r=0.67, n=170, $p \leq 0.001$). There was a significant lack of fit of the non-seasonal model when applied to the intestine wall index data (F-ratio=2.904; n=170, $p \leq 0.001$), but a non-significant lack of fit of the seasonal model I (F-ratio=1.919, n=170, $p \leq 0.001$) (Figure 4.20).

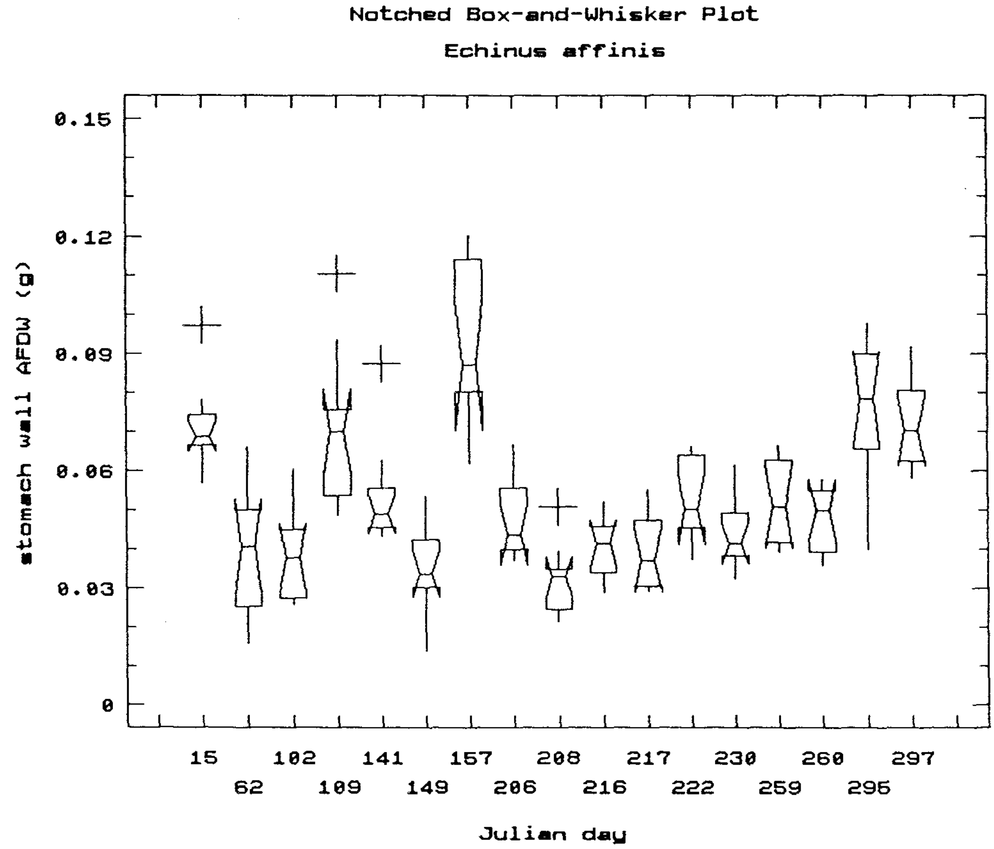


Figure 4.15. *Echinus affinis.* Notched Box-and-Whisker plot of stomach wall ash-free dry weight (g) as a function of Julian day.

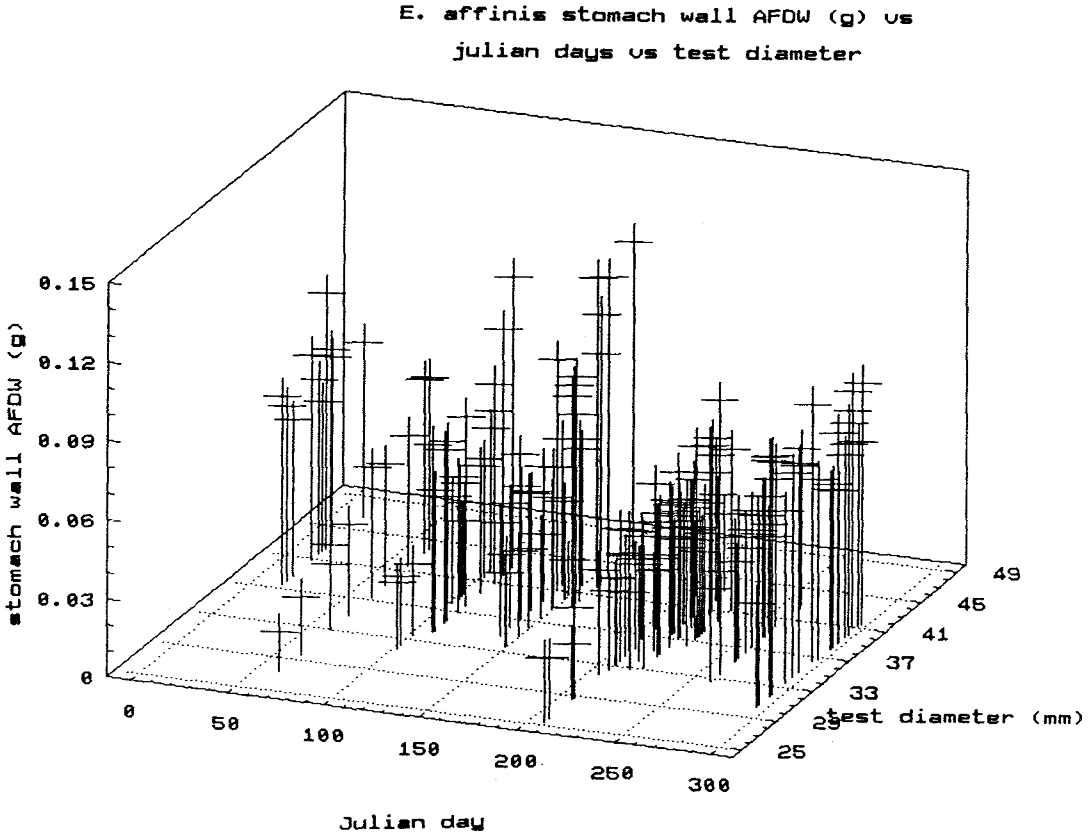


Figure 4.16. *Echinus affinis.* Scatterplot of stomach wall ash-free dry weight (g) as a function of Julian day and test diameter (mm).

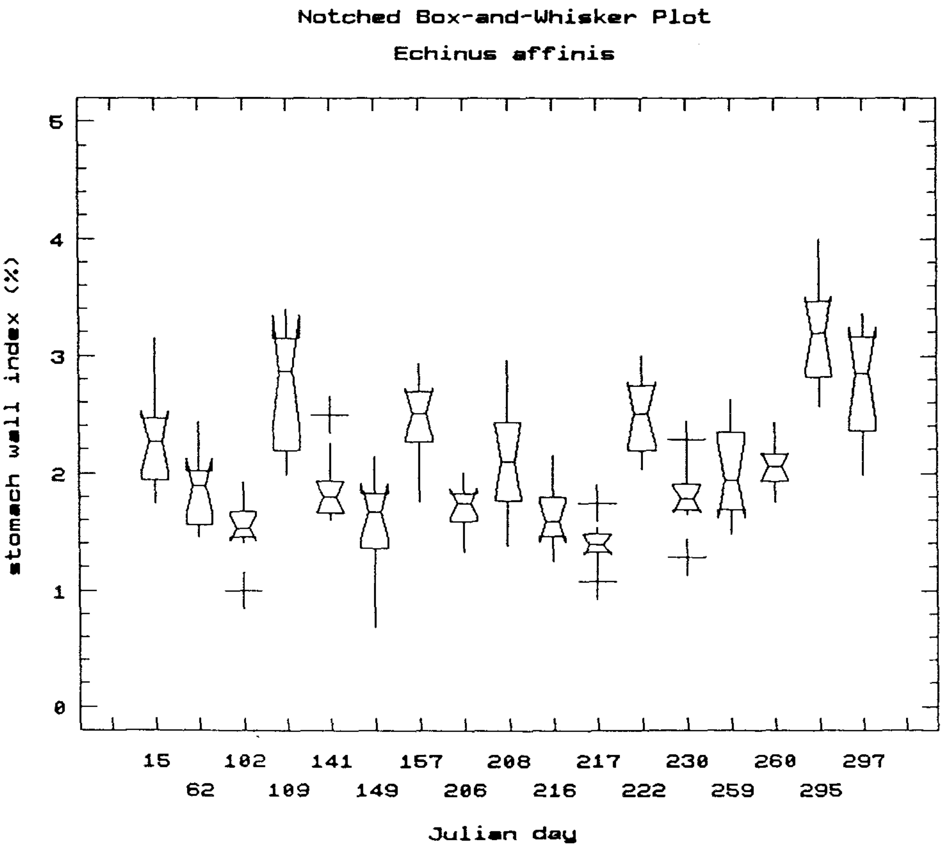


Figure 4.17. *Echinus affinis*. Notched Box-and-Whisker plot of stomach wall index as a function of Julian day.

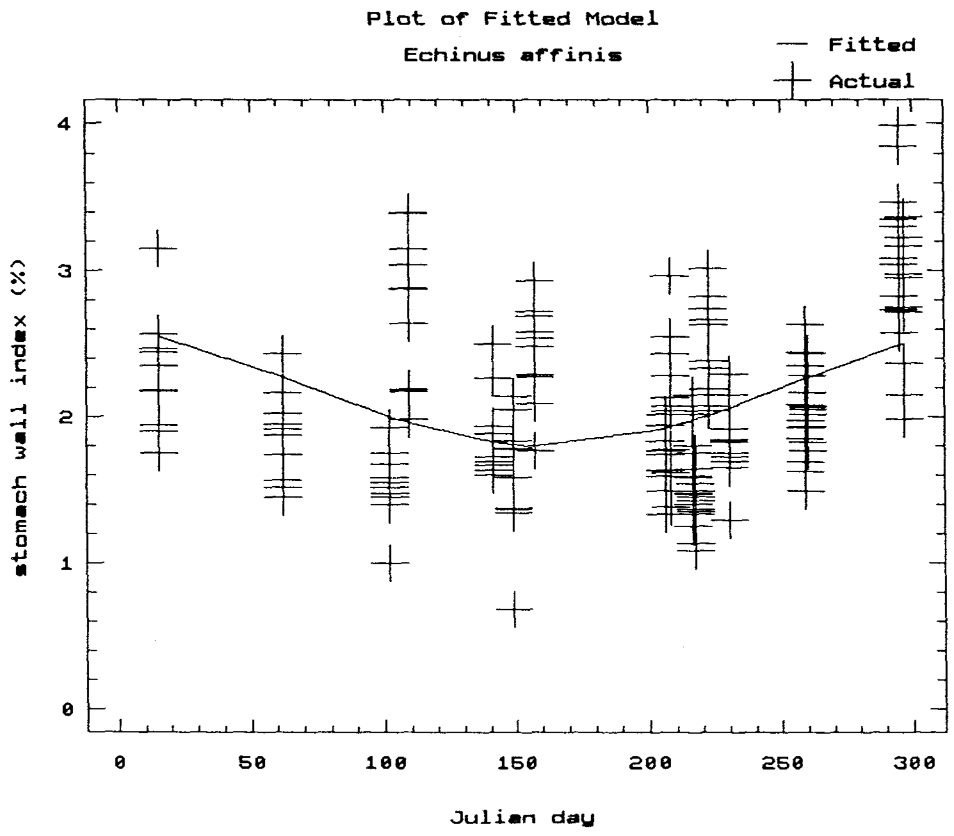


Figure 4.18. *Echinus affinis.* Plot of seasonal model I fitted to stomach wall index data.

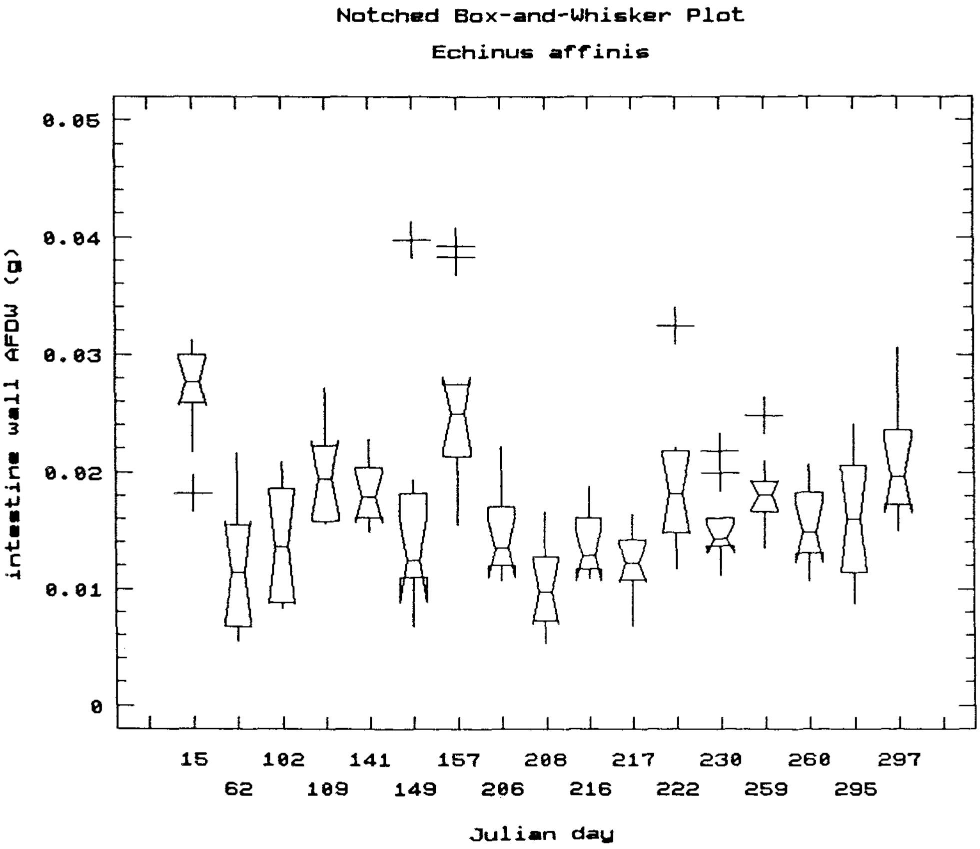


Figure 4.19. *Echinus affinis*. Notched Box-and-Whisker plot of intestine wall ash-free dry weight (g) as a function of Julian day.

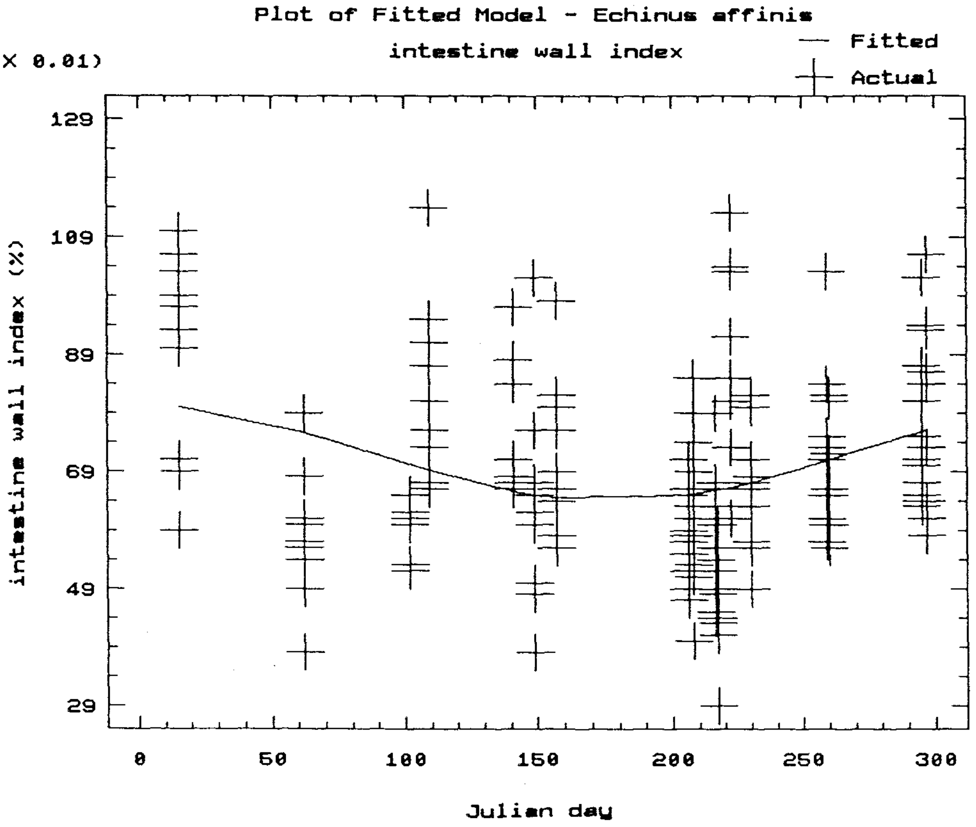


Figure 4.20. *Echinus affinis.* Plot of seasonal model I fitted to intestine wall index data.

4.3.2.2 Gut Content Indices.

Statistical analysis of the gut content data for *Echinus affinis* is summarised in Table 4.5.

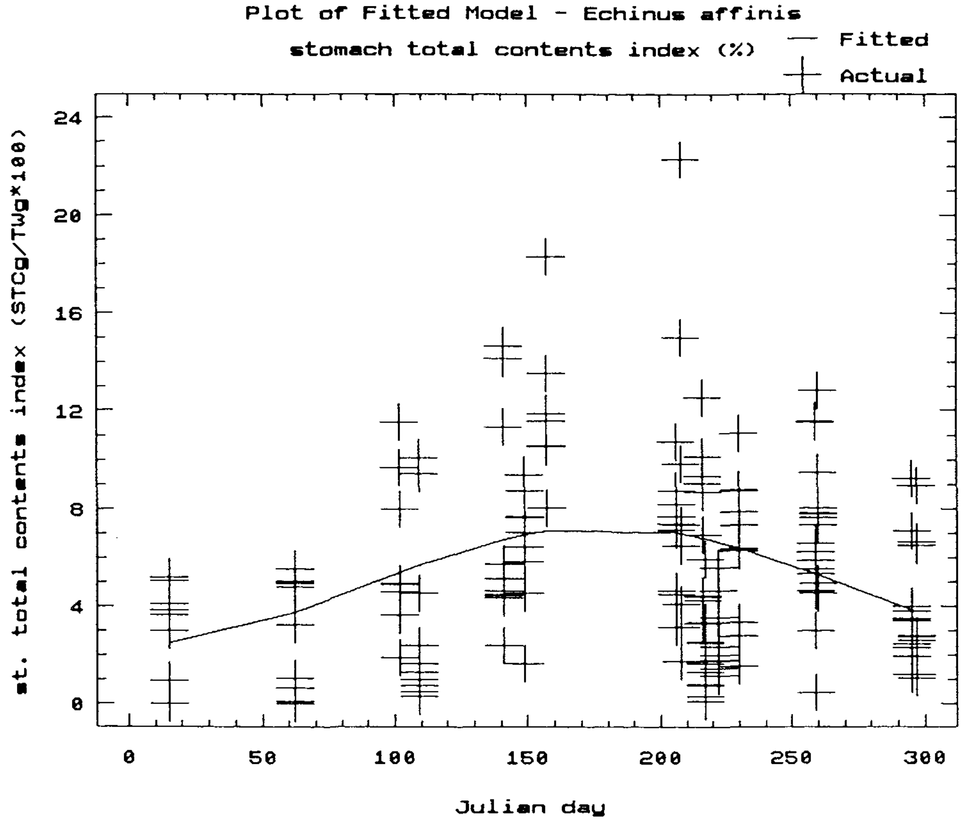
Stomach and Intestine Total Content Indices: The non-seasonal model showed a significant lack of fit when applied to the stomach total content index (F-ratio=3.776, n=170, $p \le 0.001$), which suggested that the amount of food in the stomach varied throughout the year. When the seasonal model I was applied to these data a non-significant lack of fit was observed (F-ratio=2.117, n=170, $p \le 0.001$). The data suggest that the variation is represented by an increase in the month of June coincident with the deposition of phytodetritus at the deep-sea bed (Figure 4.21a).

The non-seasonal model showed a significant lack of fit to the intestine total content index data (F-ratio=5.463, n=170, $p \le 0.001$), and so it was concluded that there was some variation through the year, partially explained by seasonal model I (Figure 4.21b).

Stomach and Intestine Organic Content Indices: The lack of fit analysis cannot be applied to the data of stomach organic content index because g_1 and g_2 for both seasonal and non-seasonal models were high indicating a non-normal distribution of residuals. However, because the skew was reduced in the non-seasonal model this was accepted as a better model (Figure 4.22a, Table 4.5).

In contrast, both the seasonal model I and the non-seasonal model showed no significant lack of fit ($g_1 < 0.62$, $g_2 < 1.24$, seasonal model F-ratio=0, non-seasonal model F-ratio=0.536, n=170, $p \le 0.001$) when applied to the intestine organic content index data (Table 4.5). The residual error left after fitting the seasonal model was solely because of the replication in each sample. For this reason the seasonal model was accepted, indicating an increase in the intestine organic content index in the spring and summer (Figure 4.22b).

a



b

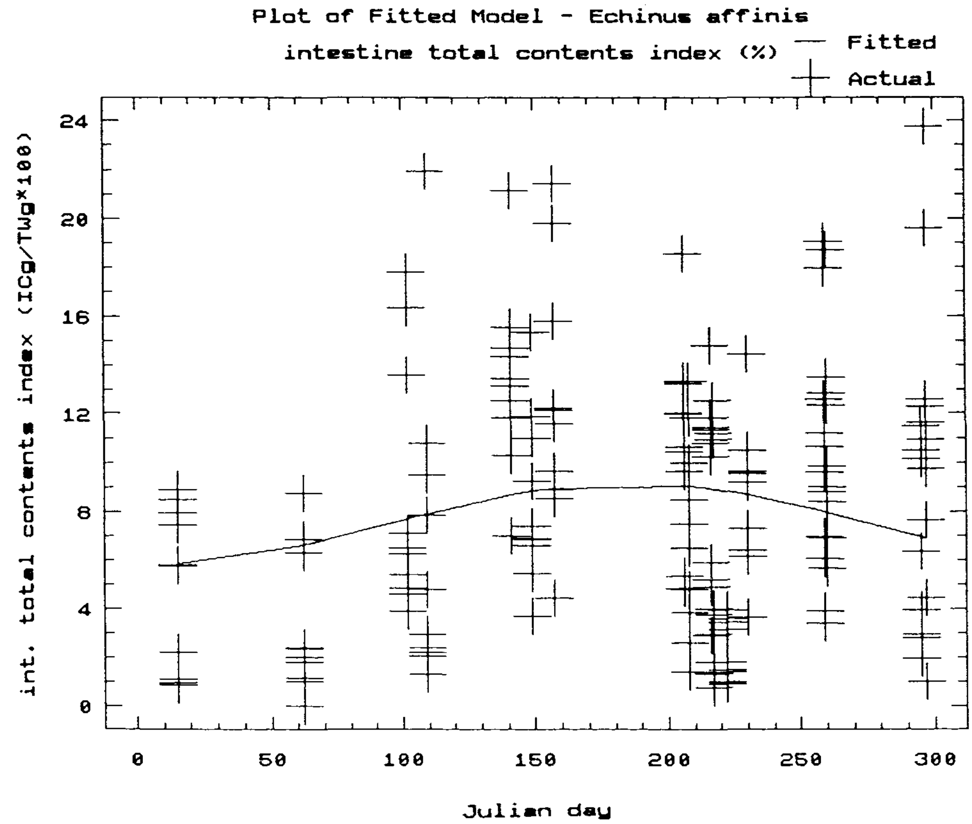


Figure 4.21. *Echinus affinis*. Plot of seasonal model I fitted to: **a.** stomach total content index data; **b.** intestine total content index data.

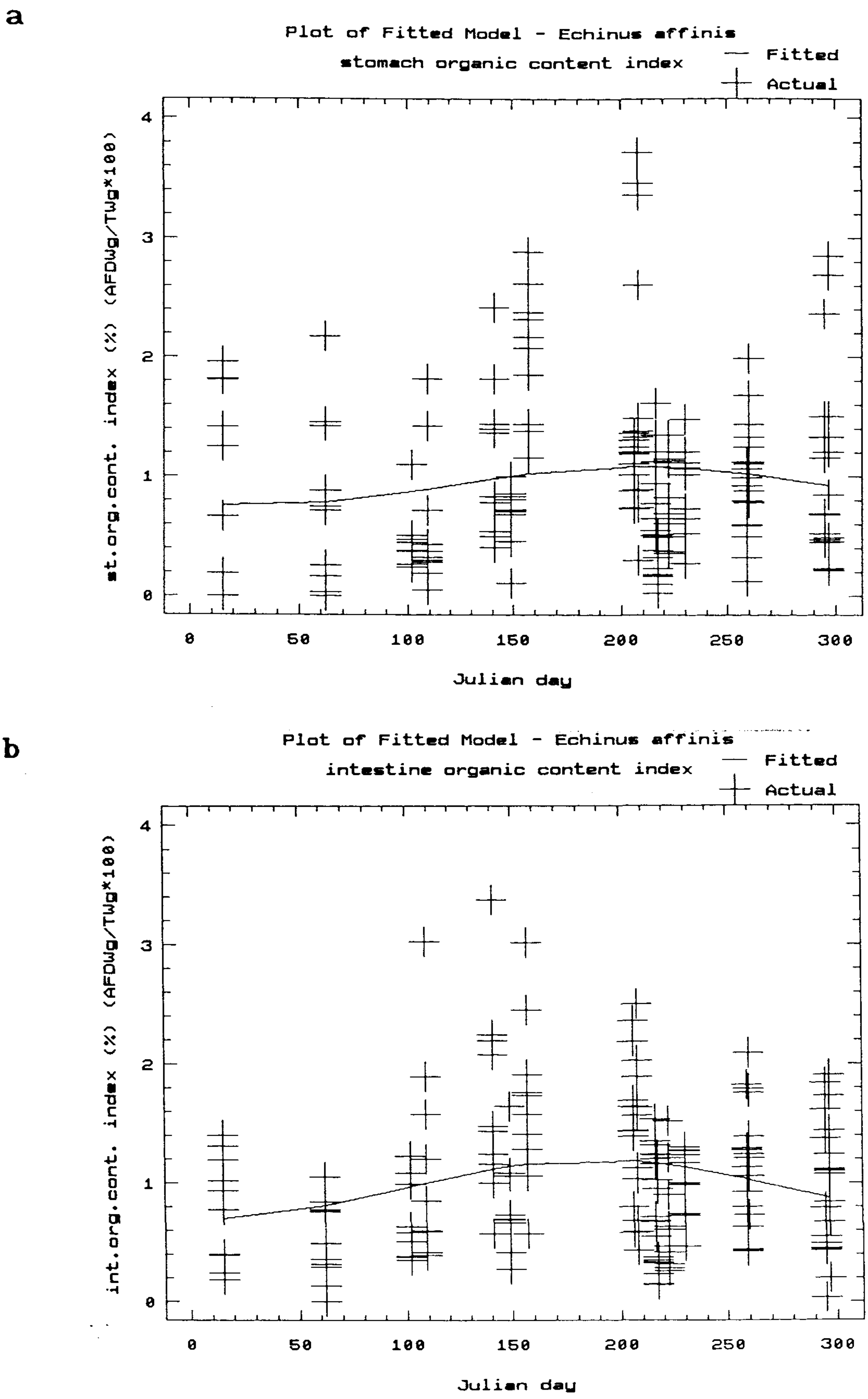


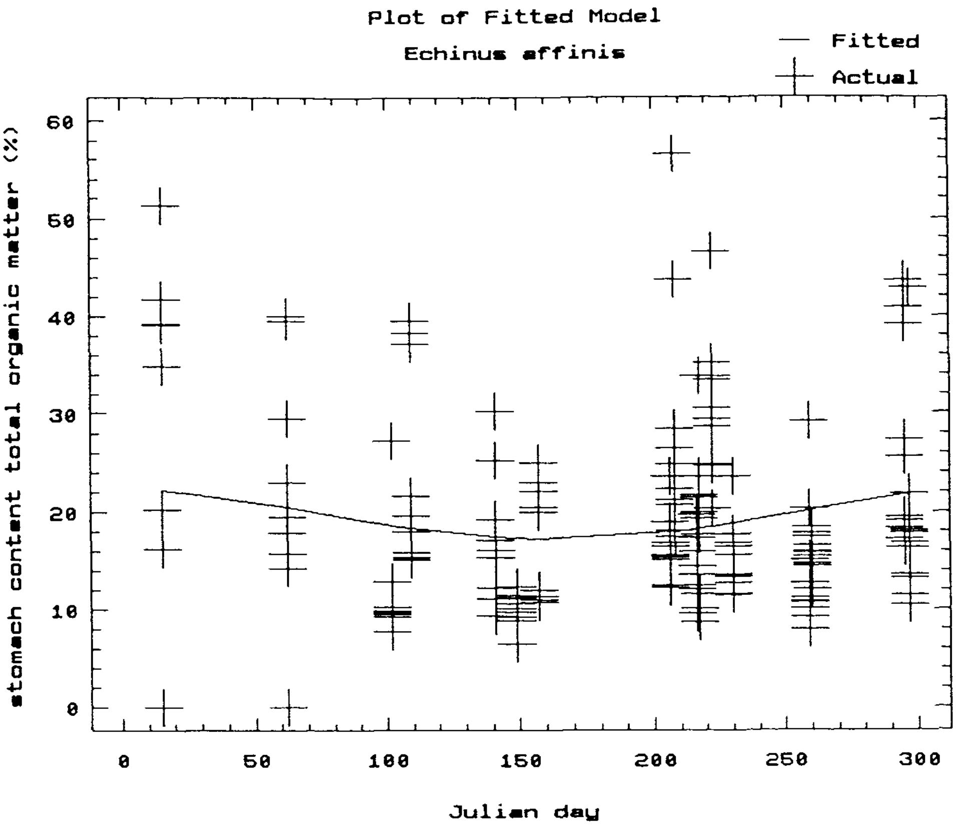
Figure 4.22. *Echinus affinis*. Plot of seasonal model I fitted to: **a.** stomach organic content index data; **b.** intestine organic content index data.

Stomach and Intestine Total Organic Matter (%): The residuals were abnormally skewed (g_1>0.62) when seasonal and non-seasonal models were fitted to the stomach total organic matter data, although g_1 was slightly lower for the seasonal model (Table 4.5). In spite of the weakness of the lack of fit analysis, it was possible to infer a slight seasonality because the non-seasonal model had a significant lack of fit to the data. The seasonal model I did not fully explain the fluctuations (Figure 4.23a).

A similar interpretation was given to the intestine total organic matter where the F-test of the lack of fit analysis was weak, but as g_1 for the non-seasonal model was reduced, this was accepted as a better model to explain the data (Table 4.5, Figure 23b). The proportion of organic matter present in both the stomach and intestine content do not have a distinct seasonal pattern through the year.

The observations on gut content suggested that *Echinus affinis* was feeding more actively during the phytodetritus pulse. These urchins, however, were not ingesting an increased proportion or quantity of organic matter, but rather an increased overall quantity of detritus including inorganic material.

a



b

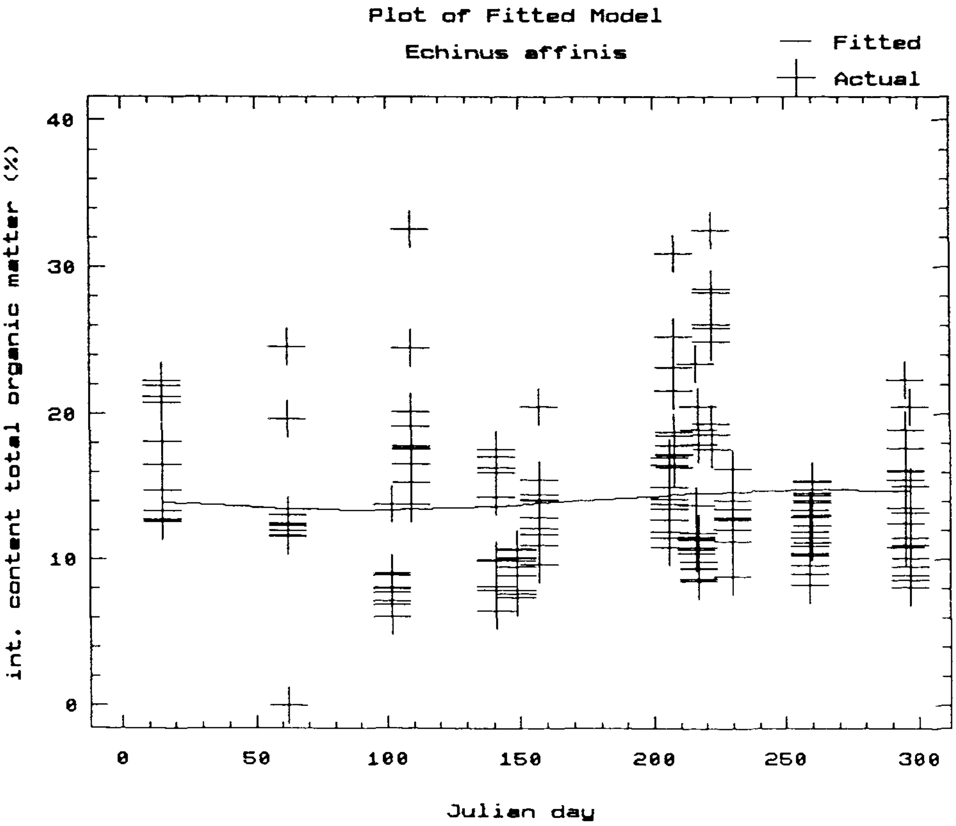


Figure 4.23. *Echinus affinis*. Plot of seasonal model I fitted to: **a.** stomach total organic matter data; **b.** intestine total organic matter data.

4.3.2.3. Visual observation of the gut content

SEM and light microscope examination of the gut contents of spring and summer samples showed, qualitatively, a wide variety of phytoplanktonic and protozoan material (Table 4.6; Figure 4.24).

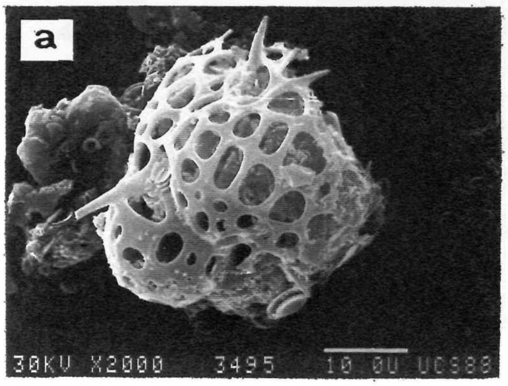
The dominant diatom genera found in the stomach contents in late spring/summer samples of *E. affinis* were *Thalassiosira* (spring and summer), *Chaetoceros*, especially resting spores (summer), *Thalassionema*, *Rhizosolenia*, *Nitzschia* and *Coscinodiscus* (mainly summer). The occurrence of these species in the gut content of *E. affinis* conform to those found in the Porcupine Seabight (Rice *et. al.* 1986). Dinoflagellates were found in the late summer samples. The remains of coccolithophorids, radiolarians and both benthic and planktonic foraminiferans were found throughout the year.

Despite the fact that no quantitative assumption can be made from the SEM photographs of the gut contents it appears that the diversification of the organisms and other structures found in the gut during spring and summer is higher than in the winter and autumn months (Table 4.6). There is a considerable increase in the different types of diatoms observed in the gut of *E. affinis* during spring-summer, conforming with the information from the Continuous Plankton Recorders (CPR_s) (Reid *et. al.* 1987) in which surface blooms of diatoms are reported from May to October in the NE Atlantic.

Table 4.6. *Echinus affinis* list of the organisms and structures found in the gut in different seasons identified from the SEM photographs.

STRUCTURES IDENTIFIED	win			spr			sum			aut			
	D	J	F	M	A	M	J	J	A	S	O	N	D
DIATOMS													
Thalassiosira leptopa	.	.	.	+	+	.	.	.	.	.	.	.	.
T. eccentrica	.	.	.	.	+	+	.	.	+	+	.	.	.
T. rotula	.	.	.	.	.	+	.	.	+	.	.	.	.
T. angulata	.	.	.	.	.	.	.	.	+	.	.	.	.
Thalassiosira sp.	.	+	.	.	+	.	+	.	+	+	.	.	.
Chaetoceros affinis	.	.	.	.	+	+	.	.	+	.	.	.	.
C. subsecundus (resting spore)	.	.	.	.	.	+	.	.	+	.	.	.	.
C. laciniosus (resting spore)	.	.	.	.	.	.	+	.	+	.	.	.	.
C. debilis (resting spore)	.	.	.	.	.	.	+	.	.	+	.	.	.
C. compressum (resting spore)	.	.	.	.	.	.	.	+	.	.	.	.	.
C. diadema (resting spore)	.	.	.	.	.	.	.	+	.	.	.	.	.
C. radians	.	.	.	.	.	.	.	.	+	.	.	.	.
C. cinctus (resting spore)	.	.	.	.	.	.	.	.	.	+	.	.	.
Chaetoceros sp.	.	.	.	.	.	.	+	.	+	+	+	.	.
Thalassionema nitzschioides	.	.	.	+	+	.	.	.	+	.	.	.	.
T. bacilaris	.	.	.	.	.	.	.	.	.	+	.	.	.
Thalassionema sp.	.	.	.	.	.	.	.	.	+	+	+	.	.
Coscinodiscus cf.*curvatulus*	.	.	.	.	.	+	.	.	+	.	.	.	.
C. perforatus	.	.	.	.	.	.	.	.	+	.	.	.	.
C. wailesii	.	.	.	.	.	.	.	.	.	+	.	.	.
Coscinodiscus sp.	.	.	.	.	.	.	.	.	.	+	.	.	.
Rhizosolenia hebetata fo. *semispina*	.	.	.	.	+	.	.	+	+	.	.	.	.
R. shrubsolei	.	.	.	.	.	.	.	.	.	+	.	.	.
Rhizosolenia sp.	.	.	.	+	.	.	.	+	.	+	.	.	.
Nitzschia seriata	.	.	.	.	.	.	+	.	.	.	.	.	.
Nitzschia sp.	.	.	.	.	+	+	.	+	+	.	.	.	.
Paralia sulcata	.	.	.	.	.	.	.	.	+	.	.	.	.
Roperia tesselata	.	.	.	.	.	.	.	.	+	.	+	.	.
Actinocyclus sp.	.	.	.	.	.	.	.	.	+	.	.	.	.
FLAGELLATES													
Actiniscus pentasterias	.	.	.	.	.	.	+	.	.	.	.	.	.
dinoflagellate cysts	.	.	.	.	.	.	.	.	.	.	+	.	.
silicoflagellates	.	+	.	.	.	.	.	.	+	.	.	.	.
COCCOLITHS	.	+	.	+	+	+	+	+	+	+	.	.	.
RADIOLARIANS	.	.	.	.	.	+	+	.	.	.	.	.	.
FORAMS	.	+	.	+	+	+	+	+	+	+	+	.	.
sponge spicules	.	.	.	+	+	+	.	.	+	+	+	.	.

Figure 4.24. *Echinus affinis*. Scanning electron micrographs of the gut contents: **a.** Radiolarian; **b.** *Rhizosolenia* sp (opened), coccoliths; **c.** diatoms (>) *Thalassiosira leptotus* and (<) *T. eccentrica*, coccoliths, debris; **d.** *Nitzschia* sp; **e.** *Chaetoceros* sp; **f.** unidentified form (possibly a foraminiferan); **g.** *Chaetoceros subsecundus* resting spore (centre), coccoliths; **h.** *Thalassionema* sp.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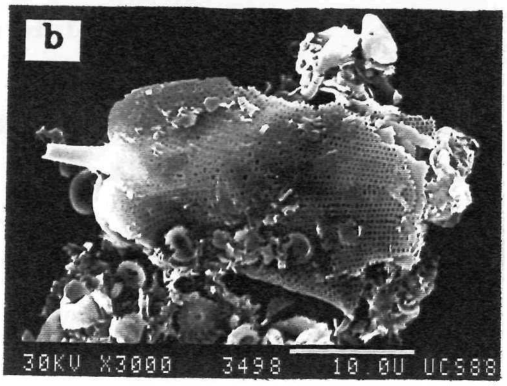
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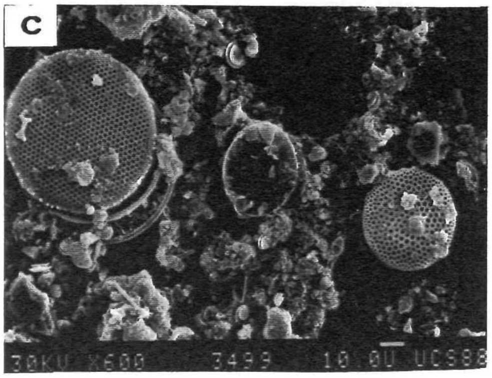
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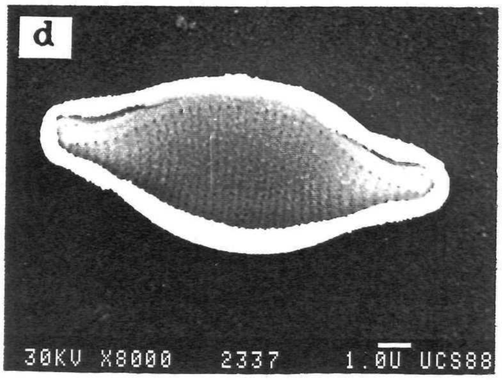
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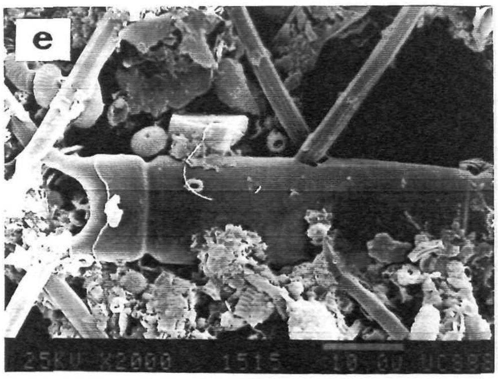
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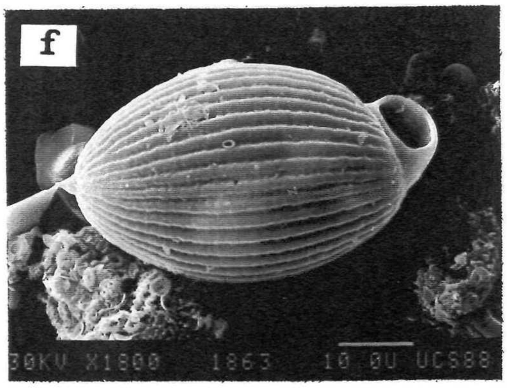
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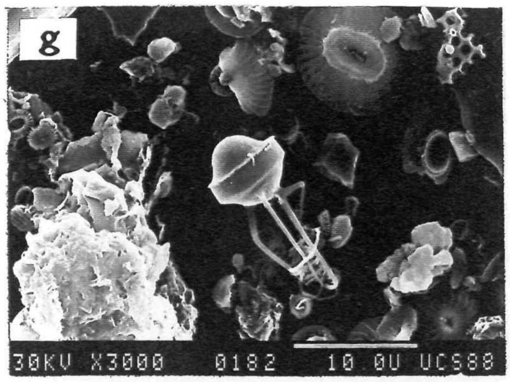
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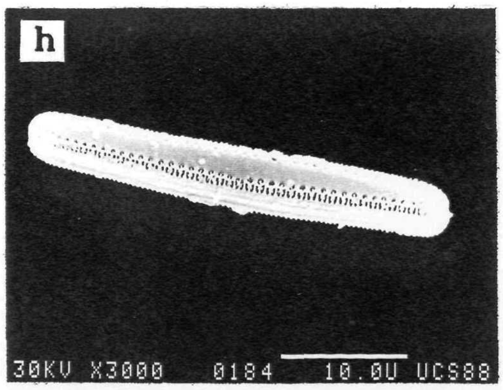
h
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4.3.2.4. Particle-size distribution of the gut content

Echinus affinis particle-size distribution of stomach and intestine content showed that the most abundant fraction sizes were particles < 250μm (Figure 4.25a, b). The percentage of particles > 500μm was higher than those 250μm < p < 500μm (p>500μm ≅ 19-20% for the stomach and intestine content respectively). There was no significant difference between the size of particles present in the stomach or the intestine.

Table 4.7 summarizes the particle-size distribution of *E. affinis* gut contents based on the packed volume (cm^3) of each fraction.

Table 4.7. *Echinus affinis.* Particle-size distribution of the stomach and intestine content found from packed volume (cm^3) from different size fractions.

SIZE (μm)	n	STOMACH CONTENT (cm^3) $\bar{X} \pm 1SD$	INTESTINE CONTENT (cm^3) $\bar{X} \pm 1SD$
P > 500	6	0.09 ± 0.03	0.11 ± 0.03
250 < P < 500	6	0.06 ± 0.03	0.05 ± 0.02
63 < P < 250	6	0.18 ± 0.09	0.20 ± 0.14
P < 63	6	0.15 ± 0.06	0.20 ± 0.12
total volume	6	0.47 ± 0.15	0.56 ± 0.16

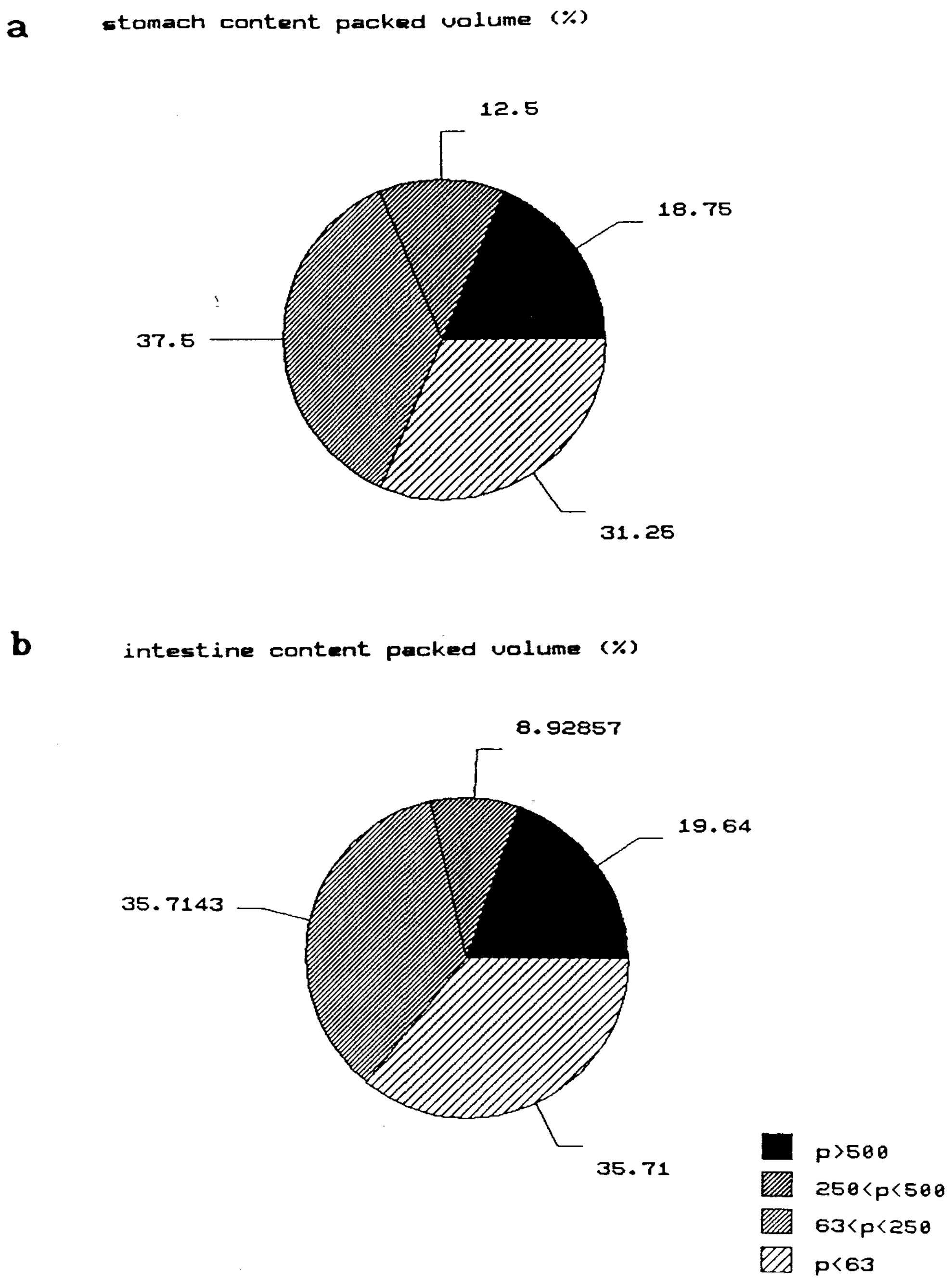


Figure 4.25. *Echinus affinis.* Particle-size distribution of the gut content found from the packed volume (cm^3) from different size fractions: **a.** stomach content; **b.** intestine content. Pie chart represent the percentage of each size fraction.

4.3.2.5. ^{210}Po in *Echinus affinis*

From a very limited data set (Table 4.8; J. Pentreath personal communication) it appeared that ^{210}Po level in the gut, gonad and test of *E. affinis* increased after the deposition of phytodetritus. This seasonally occuring short-lived radionuclide showed a seasonally varying downward flux associated with the flux of organic matter (Bacon *et. al.* 1985) and was used as a natural tracer in the dietary studies (Heyraud *et. al.* 1988).

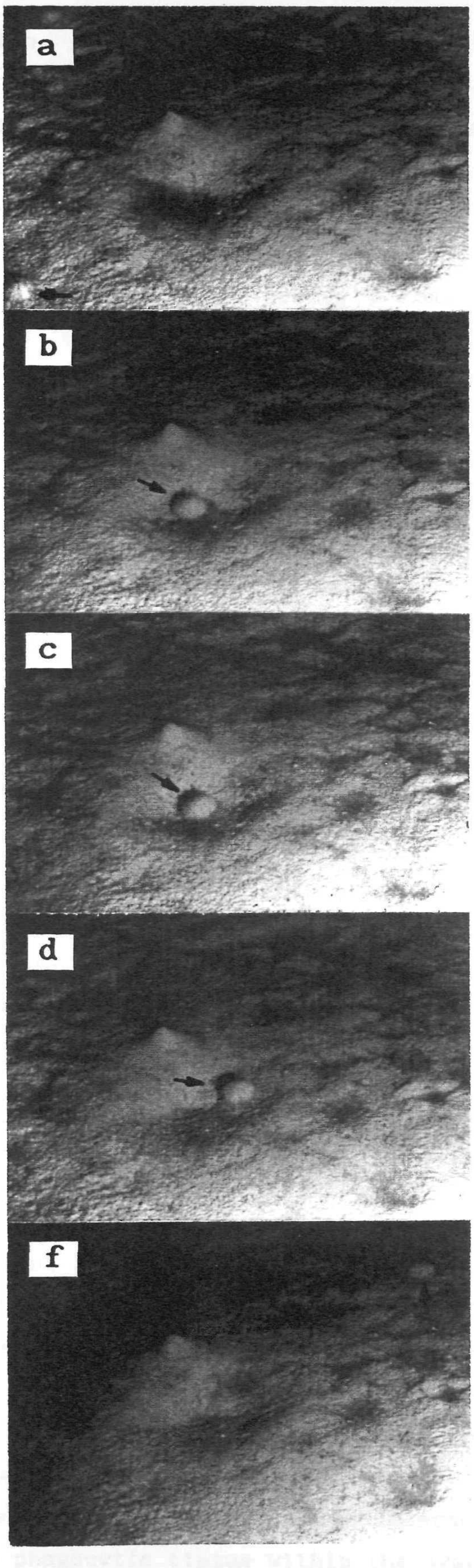
Table 4.8. *Echinus affinis.* ^{210}Po content (Bq Kg-1) of the intestine, gonads and test.

DATE	INTESTINE	GONAD	TEST
03/Mar/80	154+/-6	13+/-0.6	12.5+/-0.5
28/May/80	173+/-12	12+/-0.7	13+/-0.9
19/Aug/80	234+/-26	42+/-1.8	18+/-0.7

4.3.2.6. Observations of feeding behaviour in *Echinus affinis*

Data from Bathysnap (I.O.S. station 51418#1, Lat. 51° 04.7' - Lon. 12° 54.5', at 2000m depth from April to May/82) showed that on several occasions specimens of *E. affinis* came close to hollows where phytodetritus had accumulated. In one of the frame sequences one *E. affinis* is seen approaching a hollow containing phytodetrital material. Over a period of 4 hours the individual appeared to consume the phytodetrital material in the hollow before moving out of the area of the photograph (Figure 4.26a-e). In the same film *E. affinis* appeared to be feeding on faecal mounds of holothurians as previously reported (Lampitt & Burnham 1983, Lampitt 1985 and Lampitt & Billett 1988).

Figure 4.26. A sequence of 'bathysnap' photographs showing *Echinus affinis* feeding in a hollow at 2000m depth, in the Porcupine Seabight. Frame interval is 64 minutes, area of camera view $2m^2$. Photographs courtesy of Dr. R.S. Lampitt.


a
b
c
d
f

4.4. Discussion

The rapid sink of phytodetritus to the deep-sea, as discussed in chapter 2, may represent a significant nutrient input promptly available for rapid colonization and consumption by the deep-sea biota (Billett *et. al.* 1983, Cole *et. al.* 1987, Lochte & Turley 1988, Gooday 1988, Graf 1989, Gooday & Lambshead 1989, Gooday & Turley 1990, Billett 1991).

What effect does the seasonal input of phytodetritus have on the biology of deep-sea echinoids?

We have no evidence that *Phormosoma placenta* or *Echinus affinis* show any seasonal variation in the rate of metabolism such as respiration. There is some evidence of a seasonal variation in deep-sea sediment community oxygen consumption (Smith 1987) in the NE Pacific, but data for respiration in deep-sea echinoderms (Smith 1983) were not taken on a seasonal basis. Very little, other than maximum size, is known about growth of echinothuriids (Smith 1980). It is possible, however, to relate variation in growth and reproduction in *E. affinis* to seasonal vertical flux. Growth rate in sea urchins is closely related to feeding (Ebert 1968, Jensen 1969). In *E. affinis* annual growth zone banding in skeletal plates (Gage & Tyler 1985) may be a result of skeletal growth coincident with the presence of phytodetritus on the sea bed. In addition, Tyler & Gage (1984b) have shown that maximal vitellogenic activity occurs during the latter part of, and subsequent to, phytodetrital deposition. A similar relationship has been observed in other species with seasonal reproduction (Tyler *et. al.* 1982, Harrison 1988, Van Praët *et. al.* 1990). Furthermore, the depletion of food in a previous year may retard gonad development and the spawning period of the subsequent year. Therefore, there will be interannual variation in the gonad development depending on the availability of food each year.

Although *P. placenta* is considered a non-seasonal breeder (Tyler & Gage 1984a, Gage *et. al.* 1985), the gonad index data fluctuated throughout the year with higher values in the winter (Table 4.9). This could be a result of the complex interaction of gametogenic and nutritive phagocytic tissue within the ovary (Holland 1969).

The quantity of food in the gut of *P. placenta* appeared to vary annually with lower values in February increasing towards the summer months and decreasing in November. There was, however, a high variation in the quantity of food throughout the year and within samples (*e. g.*, 67% of the residuals in the stomach total content index data are attributable to replication error with a further 7% explained by the seasonal model applied). In spite of this variation, the proportion of organic matter present in the gut content of *P. placenta* showed a bi-annual variation with low values in February, a marked increase in the spring months, low values towards the end of the summer and an autumn increase (Table 4.9). The high values in the autumn coincide with a second bloom of surface production in the NE Atlantic during this season (Reid *et. al.* 1987).

Compared with the surrounding environment, the mean values of the percentage organic matter in the gut content of both *P. placenta* and *E. affinis* was very high (4-10% and 15-20% respectively). This could be an indication of selection in the acquisition of food particles. *P. placenta*, however, appears to feed on sedimentary material of lower total organic matter than *E. affinis*. This might be a result of the ambient sedimentary environment of *P. placenta* being poorer in organic matter than that of Station 'M' where *E. affinis* were collected. Unfortunately we do not have sediment organic matter values for each sampling time, although Gage and Tyler (1982) reported an organic carbon value of 0.39% at Station 'M' and Rice *et. al.* (1986) have shown that levels of organic carbon vary from 0.56% to 1.28% in the Porcupine Seabight. These latter data are lower than those previously reported (6% organic carbon) for the Porcupine Seabight (Billett *et. al.* 1983). It is likely that the Rockall Trough has a similar temporal deposition of organic matter as the Porcupine Seabight, because the surface production in those areas is apparently similar (Colebrook 1986).

Assuming that station 'M' and the stations in the Porcupine Seabight and Goban Spur have similar seasonal events and are alike in their other physical characteristics such as the sediment composition, it appears that *P. placenta* is a less selective feeder than *E. affinis*. *E. affinis* did not show any clear seasonal variation throughout the year in the proportion of organic matter present in the gut, although photographic evidence from the Porcupine Seabight has shown that *E. affinis* was attracted to hollows containing lumps of phytodetritus (Lampitt 1985) or holothurian faecal pellets (Lampitt & Billett 1988).

Despite the fact that *E. affinis* feeds on nutritious food independent on the season, the total quantity changes considerably in spring and summer (see the summary of results in Table 4.10). Small amounts of food are recorded in winter months. Prior to spawning in the early months of the year the gonads will occupy much of the interior of the test, but this does not explain the reduced absolute amount of material that can be taken into the gut as reported for *Strongylocentrotus intermedius* (Fuji 1967). Some of the *Echinus affinis* winter specimens had ripe gonads as well as guts full of detritus material.

A study on the gut wall and gonad variations in *Echinus esculentus* from the Scottish coast showed that the "*gut weight seems to reflect seasonal differences in the flux of nutrients taken up through this route, increasing when food availability is high and the animals are actively feeding, and decreasing when food availability is low or when other factors cause nutritive stress*" (Comely & Ansell 1989). In accord with the results shown in this chapter, Comely and Ansell (1989) also found coincident trends between gonad and gut wall indices. However, they gave little support to the hypothesis of the gut wall being used as a storage organ to support gonadal development as previously suggested by several authors (Lawrence *et. al.* 1966, Lawrence 1970, 1971, 1976, Fenaux *et. al.* 1975, Giese 1976, Tokin & Filimonova 1977, De Ridder & Lawrence 1982, Nichols *et. al.* 1985). Yet, it is possible to suggest that the stomach wall of *E. affinis* is used as a storage organ in addition to the gonads. The marked increase in the ^{210}Po in the gut of *E. affinis* is possibly associated with the increased feeding on phytodetritus as ^{210}Po is related to the intensity of vertical particle flux in the water column (Bacon *et. al.* 1985). The elevated levels of ^{210}Po in the gonads in late summer suggests an energy transfer from the gut to the gonad. By winter when the growth rate has slowed and gonad development is complete, the gut wall would supply the energy for the maintenance metabolism. This hypothesis needs testing through more detailed chemical, histological and transmission electron microscopy analyses of the gut structure.

The visual gut content analyses, by differentiating the types of food present in the gut, provide more evidence on feeding behaviour (Briggs 1985) rather than on the nutritional quality of the food sources. It is apparent that the results represented in tables 4.5 and 4.8 resemble those for the surrounding sediment and accord with information of surface production from the Continuous Plankton Recorder Survey (Reid *et. al.* 1987). Sampling of surface sediment detritus using the SMBA multiple corer (Barnett *et. al.* 1984, McIntyre & Warwick 1984) has indicated the presence mainly of diatoms, and discrete gelatinous aggregations, up to 10mm diameter, of coccolithophorids, dinoflagellates, crustacean eggs, small faecal pellets, and amorphous organic material (Billett *et. al.* 1983). It appears that amongst the phytodetritus from the Porcupine Seabight there is an abundance of *Thalassionema nitzschioides*, *Nitzschia delicatissima* and *Chaetoceros (Hyalochaete)* spp. (Rice *et. al.* 1986).

Table 4.9. *Phormosoma placenta*. Summary of the results for organ and gut content indices, and total organic matter of the gut content.

SUBJECT	SEASONALITY	PEAK PERIOD(S)
gonad index	annual	higher in February/lower in August/increasing in November
oesophagus wall index	none	-
stomach wall index	bi-annual	lower in March / higher in June/ lower in September/ higher in November
intestine wall index	bi-annual	lower in March / higher in July/ lower in September/ higher in November
stomach total content index	annual	lower in February/ higher in September/lower in November
intestine total content index	annual	lower in February/ higher in August/lower in November
stomach organic content index	none	-
intestine organic content index	annual	lower in February/ higher in June-July/lower in November
stomach total organic matter	bi-annual	lower in February/ higher in May/lower in September/higher in November
intestine total organic matter	bi-annual	lower in February/ higher in April-May / lower in August-September/ higher in November

Table 4.10. *Echinus affinis*. Summary of the results for organ and gut content indices, and total organic matter of the gut content.

SUBJECT	SEASONALITY	PEAK PERIOD(S)
gonad index	annual	higher in January / lower in July-August/ higher in October
oesophagus wall index	bi-annual	lower in January / higher in April/ lower in June - August/ higher in September/decreasing in October
stomach wall index	annual	higher in January / lower in May/ higher in October
intestine wall index	annual	higher in January / lower in May/ higher in October
stomach total content index	annual	lower in January / higher in May-August/ lower in October
intestine total content index	annual	lower in January / higher in May-August/ lower in October
stomach organic content index	none	-
intestine organic content index	annual	lower in January / higher in May-August/ lower in October
stomach total organic matter	not distinct	-
intestine total organic matter	not distinct	-

In conclusion, *P. placenta* showed a seasonal variation in the quality of diet whereas in *E. affinis* there was seasonal variation in the quantity of food consumed. There is some evidence for coupling between the seasonal deposition of phytodetritus and the diet, growth and reproduction of *E. affinis*, although more detailed organic analyses of the gut content are essential to test this hypothesis. *P. placenta* was also affected by this flux, but, responding in a different way, perhaps because of morphological and physiological differences of the gut, and differences in the utilization of the deposit sediments. Similar couplings would be expected in other species with seasonal reproduction that might be expected to assimilate deposited or resuspended phytodetritus (Tyler *et. al.* 1982, Harrison 1988, Van Praët *et. al.* 1990).

Further analyses on fresh seasonal samples of deep-sea species and sediments are necessary to better establish the link between surface production, fluxes of organic matter, megafauna usage of this material and their life-histories.

Chapter 5: Examination of nutrition and gut content of deep-sea echinoids from the Bahamas

5.1. Introduction

A cruise of the R/V Seward Johnson and the DSRV Johnson Sea-Link-II, Harbor Branch Oceanographic Institution, to the Bahamas, from May 25 to June 7, 1989, provided the material for this study. The main objectives of the cruise were related to studies of larval development of deep-sea echinoderms, but individual adult urchins were also used for the study of gut contents and feeding biology. Furthermore, this was an opportunity to examine *Phormosoma placenta* from a different area than the NE Atlantic. Some of the observations made during submersible dives in the cruise are reported here.

The aim of this investigation was to study in greater detail than previously reported (Mortensen 1935, Lewis 1963, Pawson 1976,. 1982) the types of food sea urchins were utilizing at bathyal depths off the Bahamas. The question of whether these urchins also use surface derived material as essential energy source for their metabolism was to be addressed through visual observation and evaluating particle-size distribution of the gut contents. Organic analyses of the gut contents were observed where enough material was available.

The analyses of gut contents of four species of echinoids are reported here.

5.2. Materials and methods

Echinoids were collected from bathyal depths (463m to 893m)in the Bahamas, using the manned submersible 'Johnson Sea-Link-II' (Table 2.3). The D.S.R.V. 'Johnson Sea-Link-II' (Figure 5.1) is provided with 12 buckets to store samples during the dive, a basket, mechanical arm, a system to collect delicate specimens, a video camera, and a camera which takes sharp photographs with a laser system for focusing. The samples are generally collected in very good condition because they are put in separate containers. Also, as soon as the material collected with the submersible is brought on board ship, it is taken to the cold room (10°C) to limit stress to live animals.

Although 162 echinoids of several species were dissected on board the ship, only four species were examined in more detail because of the limited number of individuals of each species collected. These were specimens of *Cidaris blakei*, *Stylocidaris lineata*, *Phormosoma placenta*, and *Aspidodiadema jacobyi*. Table 2.3 (Chapter 2) presented details of the species, dive numbers, dates, positions and depths of collection. The area of collection is represented in Figure 2.2. Information about their gut morphology was described in Chapter 3.

After test diameter and test height were noted, specimens were fixed in 5% buffered formalin or frozen for further examination in the laboratory. When possible the oesophagus, stomach and intestine of specimens in good condition were separated as well as their gut content.

For particle-size distribution the gut content of 6 individuals each of *Cidaris blakei*, *Stylocidaris lineata*, *Phormosoma placenta*, and *Aspidodiadema jacobyi* were disaggregated in $2.5g.l^{-1}$ sodium hexametaphosphate and sieved at 500μm and 63μm mesh. A model PCA I Coulter Electronics coulter-counter was used to count the number of particles in a subsample ($0.1cm^3$) of particles smaller than 63μm. A Kontron Image Processing System (IPS) was used to measure subsamples ($0.1cm^3$ of each size fraction: $63<p<500\mu m$ and $p>500\mu m$) of particles bigger than 63μm.

For scanning electron microscopy a subsample from the gut content of $0.5cm^3$ of particles smaller than 63μm was diluted in 10ml of distilled water. One drop of the mixture was dried in a SAMDRI-790 critical point drier or directly dried and mounted onto coverslips with carbon and silver paint on EM stubs. Stubs were gold-coated and examined under a NOVASCAN scanning electron microscope. Particles were also observed under a Wild Heerbrugg stereomicroscope some of which were mounted onto a palaeontological slide for further identification.

The ash-free dry weight (AFDW), the proportion of organic matter in the gut content, and gut wall were determined gravimetrically. Samples of gut content were dried at 60°C for 24 hours to a constant weight in a model 19 Thelco vacuum oven and subsequently ashed at 500°C for 4 hours in a muffle-furnace.

Freezed-dried gut content were analysed for soluble protein (Lowry *et.al.* 1951), carbohydrate (Dubois *et.al.* 1956), and organic carbon (Gaudette *et. al.* 1974.

Owing to submersible constraints it was not possible to collect a sediment core next to the animals as a control for the gut content analyses.

Statistical analysis

Student's t-test was used for pairwise comparison between species to identify significant differences in the mean values of the following:

a-) particle-size distribution;

b-) total organic matter (AFDW%) of the gut wall;

c-) total organic matter (AFDW%) of the gut content.

A two-tailed t-test was applied to test the hypothesis $H_o : \mu_1 = \mu_2$ against $H_1 : \mu_1 \neq \mu_2$ (where μ_i is the population mean of species i) :

$$Z_o = \frac{\bar{x}_1 - \bar{x}_2}{\sigma \sqrt{\left(\frac{1}{n_1} + \frac{1}{n_2} \right)}}$$

where $\bar{x}_i$ is the sample mean for species i; n_i is the number of specimens within the sample; and σ is the standard deviation of the populations of species 1 and 2. Because σ is unknown, an estimate of the population standard deviation is given by the standard deviation of the two samples, s, where

$$s = \sqrt{\frac{s_1^2\,(n_1 - 1) + s_2^2\,(n_2 - 1)}{(n_1 + n_2 - 2)}}$$

where s_i is the sample standard deviation of species i.

The significance test depends on the assumptions that the sets of observations are normally distributed and that the populations have the same variance σ^2 (Chatfield 1983).

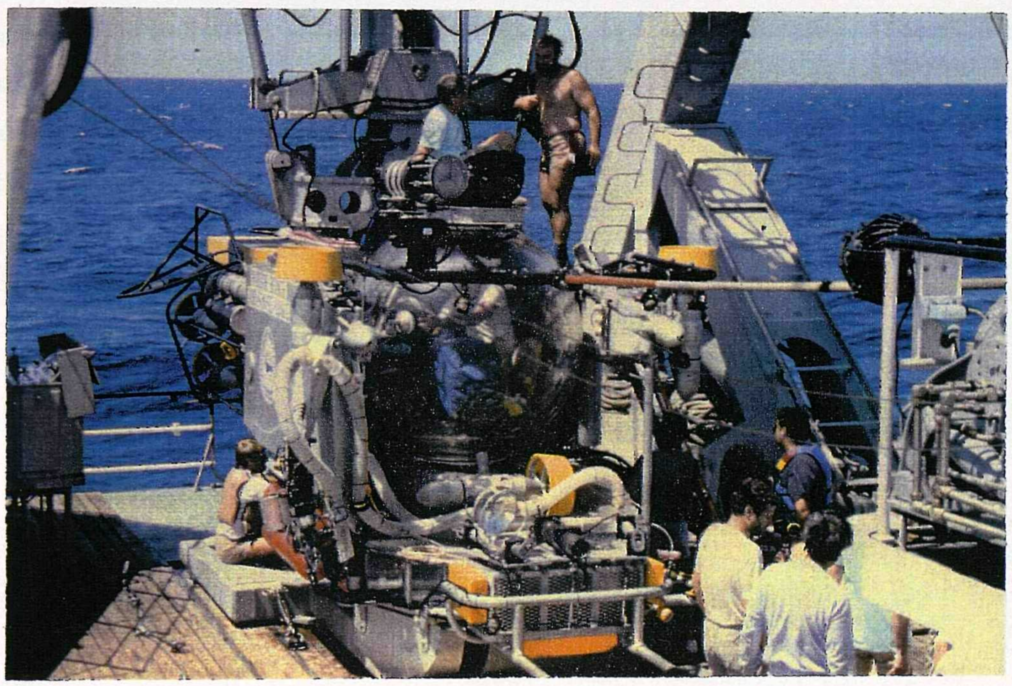


Figure 5.1. Photograph of the D.S.R.V. 'Johnson Sea-Link-II' on board the R/V 'Seward Johnson' (Harbor Branch Oceanographic Institution) after a dive in the Bahamas.

5.3. Results

5.3.1. General observations

Specimens of *Phormosoma placenta* were generally found in deeper waters (Table 2.3), not in herds as observed by Grassle *et.al.* (1975) but as a large number of individuals spaced aproximately 1 - 3m apart from each other. Specimens of *C. blakei* and *S. lineata* were observed sitting on rocks, or generally where the sediment was apparently coarser on the slope. Also, *S. lineata* were collected in the same localities where *A. jacobyi* and *P. placenta* were found (Table 2.3). Photographs of these four species of regular echinoids are shown in Figures 5.2a, b, and 5.3a, b.

Many broken pieces of plant material mainly of the seagrass *Thalassia* sp and algae *Sargassum* sp were observed on the seabed. Echinoid species such as the echinothuriids *Araeosoma belli* and *A. fenestratum* appear to depend entirely on macrodetrital falls: these urchins contained only fragments of *Sargassum*, *Thalassia* and other macrophytes as part of their dietary material. All the other species dissected contained products of algal falls to some extent as part of their gut content together with other sedimentary elements.

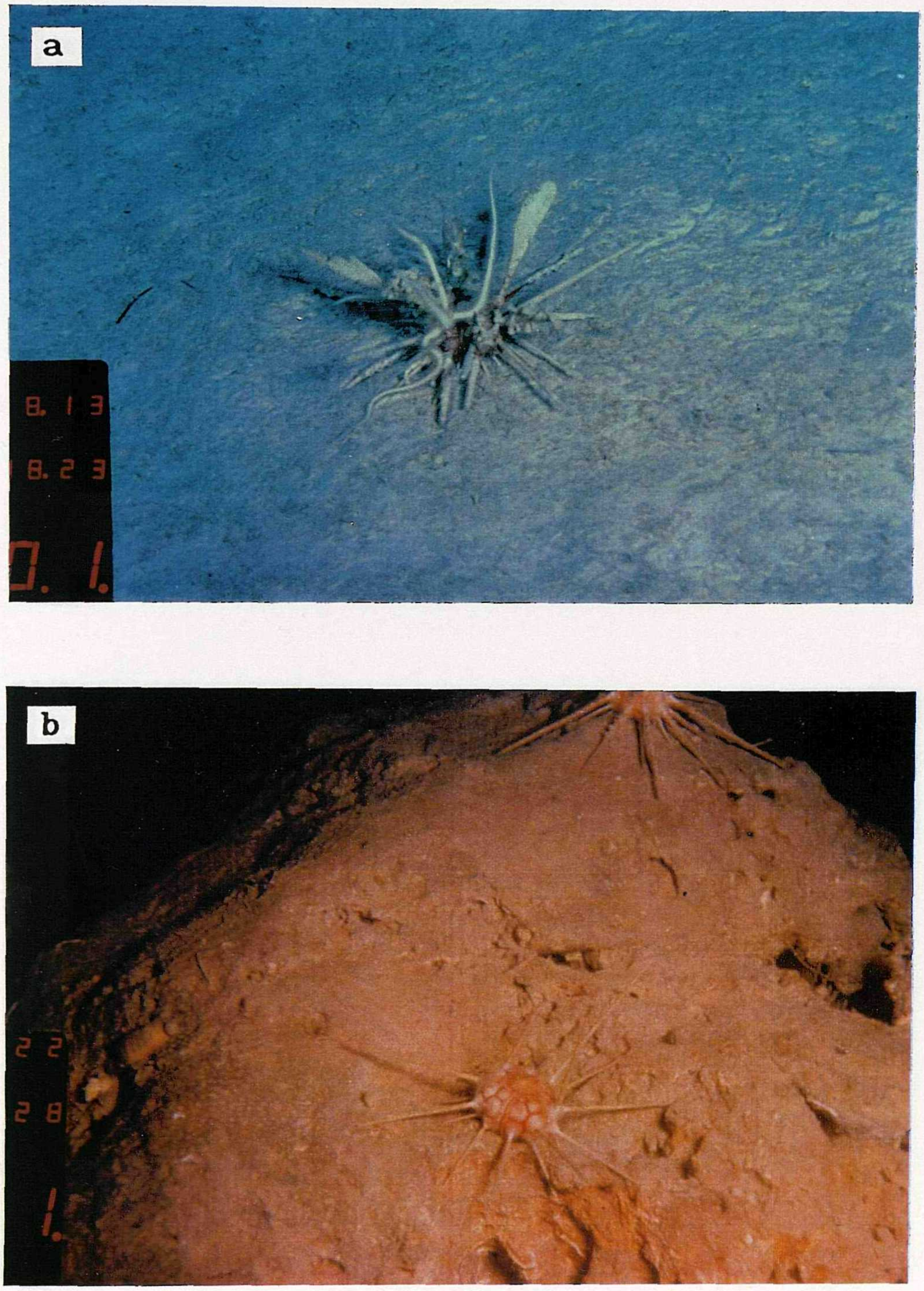


Figure 5.2. Photographs taken in the Tongue of the Ocean using the D.S.R.V. 'Johnson Sea-Link-II' in 1989. **a-)** *Cidaris blakei*; **b-)** *Stylocidaris lineata*. Courtesy of Dr. C.M. Young (Harbor Branch Oceanographic Institution).

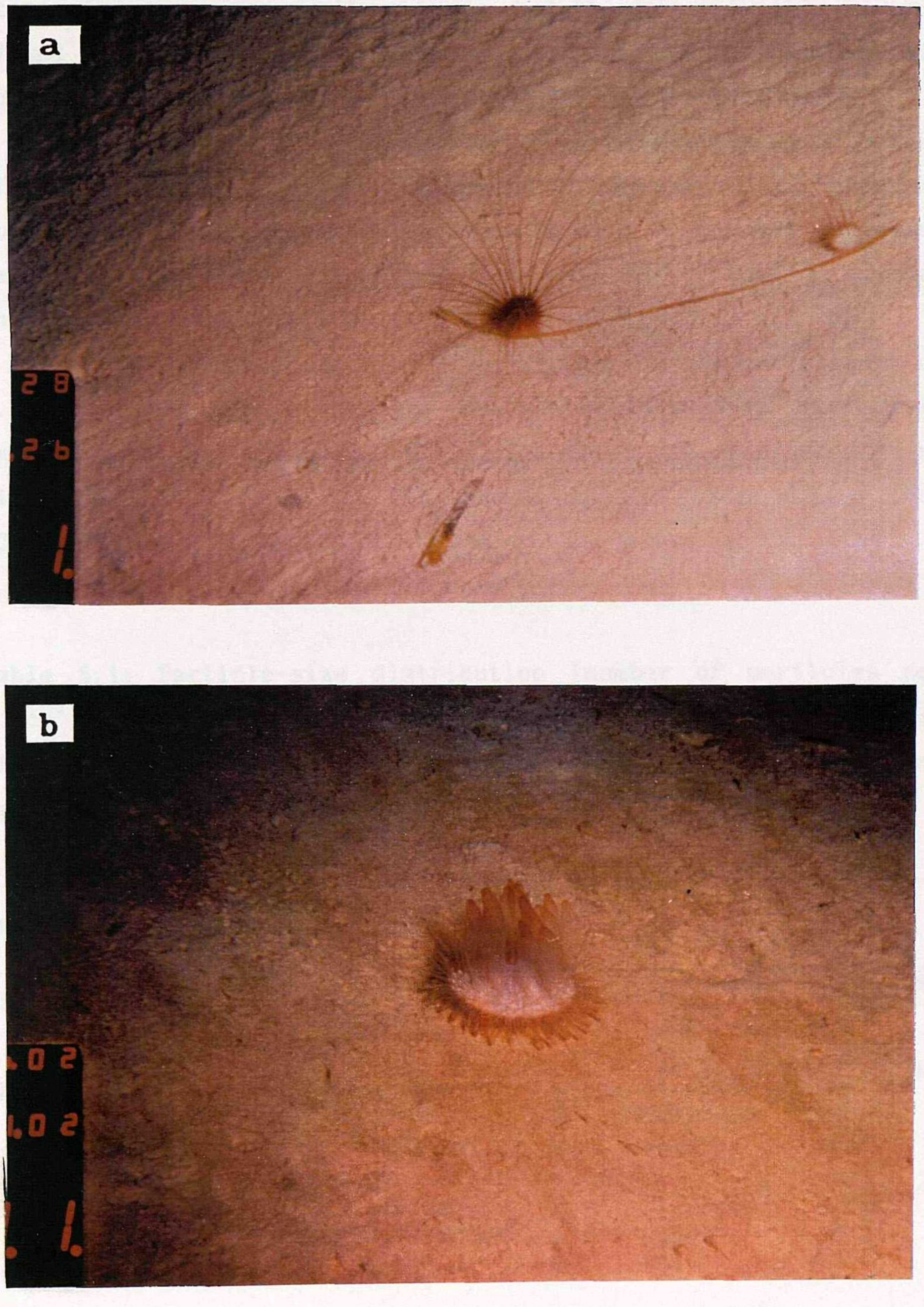


Figure 5.3. Photographs taken in the Tongue of the Ocean using the D.S.R.V. 'Johnson Sea-Link-II' in 1989. **a-)** *Aspidodiadema jacobyi*; **b-)** *Phormosoma placenta*. Courtesy of Dr. C.M. Young (Harbor Branch Oceanographic Institution).

5.3.2. Particle-size distribution of the gut contents

Stereomicroscope observations suggested the two cidaroids *Cidaris blakei* and *Stylocidaris lineata* had much coarser particulate gut content than *Aspidodiadema jacobyi* and *Phormosoma placenta*.

C. blakei and *S. lineata* contained a significantly lower number of particles <63μm than *A. jacobyi* and *P. placenta*. *P. placenta* had the highest number of particles <63μm whereas *A. jacobyi* contained less than half the number of particles for $0.1cm^3$ of p<63μm counted in *P. placenta* (Tables 5.1, 5.2; Figure 5.4).

The number of particles bigger than 500μm for the gut content of *S. lineata* was higher than for the gut content of *C. blakei*, *A. jacobyi* and *P. placenta*. The last three species showed no significant difference in the number of particles bigger than 500μm (Figure 5.5; Tables 5.1, 5.3).

Table 5.1: Particle-size distribution (number of particles per $0.1cm^3$ of each fraction size) of gut content of *Cidaris blakei*, *Stylocidaris lineata*, *Phormosoma placenta*, and *Aspidodiadema jacobyi*.

SPECIES	SIZE FRACTION	n	$\overline{X}$	SD
Cidaris blakei	p<63μm	6	$2.29x10^6$	$5.98x10^5$
	p>500μm	6	204	32.0
Stylocidaris lineata	p<63μm	6	$4.79x10^6$	$1.37x10^6$
	63μm<p<500μm	6	1310	106
	p>500μm	6	614	161
Phormosoma placenta	p<63μm	6	$1.92x10^7$	$1.82x10^6$
	63μm<p<500μm	6	453	14.2
	p>500μm	6	219	16.0
Aspidodiadema jacobyi.	p<63μm	6	$8.54x10^6$	$1.52x10^6$
	p>500μm	6	213	55.4

Table 5.2: T-test statistics (Z_o values) for pairwise comparison of particle-size distribution mean values (p<63µm) of *Cidaris blakei*, *Stylocidaris lineata*, *Phormosoma placenta*, and *Aspidodiadema jacobyi*. Z_o values relate to data in Table 5.2; t(0.05, 8)=2.31.

	S. lineata	*P. placenta*	*A. jacobyi*
C. blakei	4.10	15.27	9.41
S. lineata		15.47	4.50
P. placenta			-11.01

Table 5.3: T-test statistics (Z_o values) for pairwise comparison of particle-size distribution mean values (p>500µm) of *Cidaris blakei*, *Stylocidaris lineata*, *Phormosoma placenta*, and *Aspidodiadema jacobyi*. Z_o values relate to data in Table 5.2; t(0.05, 8)=2.31.

	S. lineata	*P. placenta*	*A. jacobyi*
C. blakei	6.13	1.02	0.36
S. lineata		-6.00	-5.78
P. placenta			-0.23

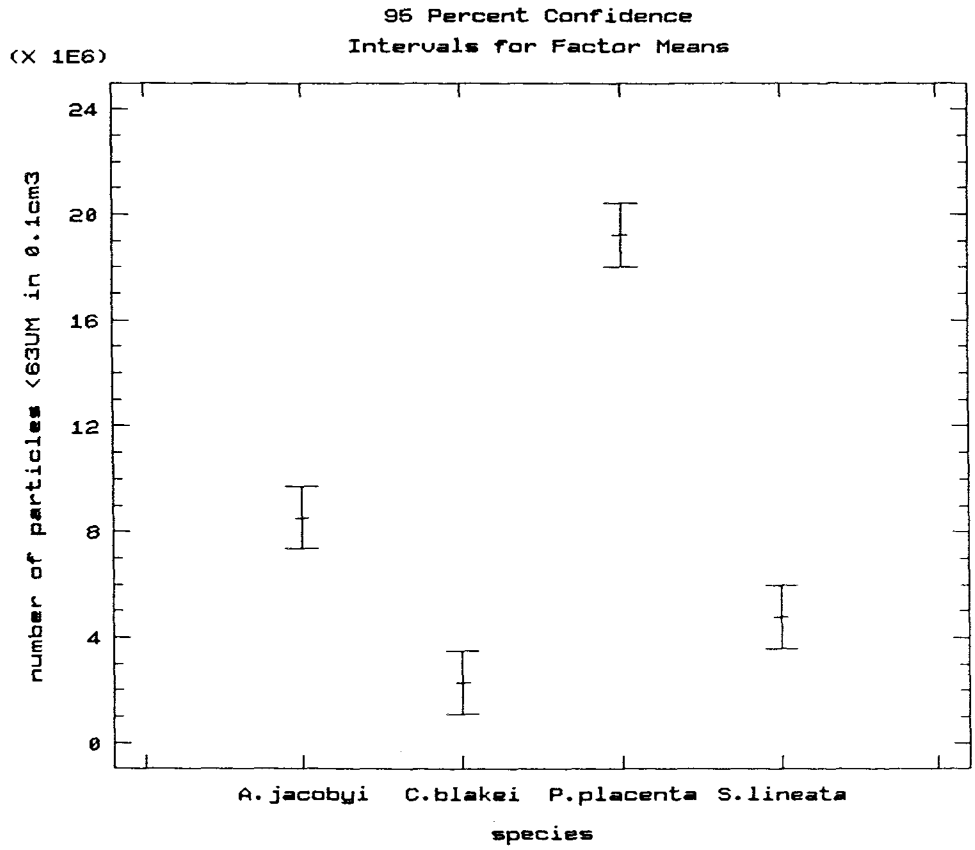


Figure 5.4. *Aspidodiadema jacobyi*, *Phormosoma placenta*, *Stylocidaris lineata*, and *Cidaris blakei*. Plot of the particle-size distribution of the gut content. Number of particles in 0.1cm^3 of p<63μm.

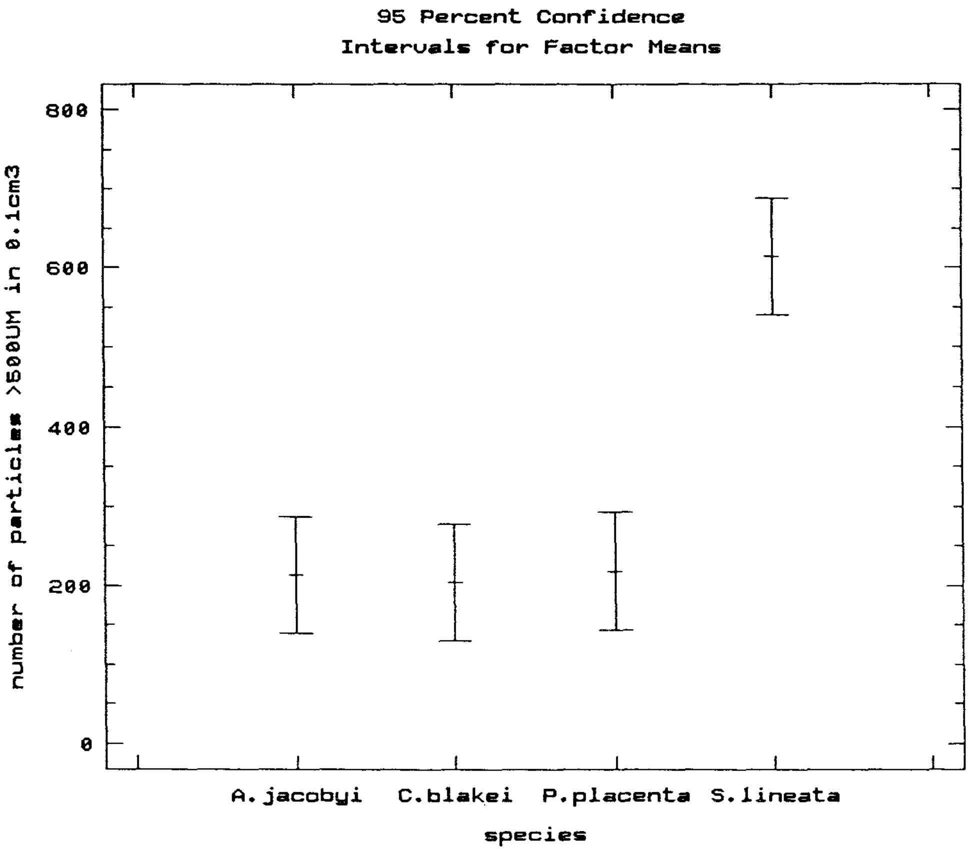


Figure 5.5. *Aspidodiadema jacobyi*, *Phormosoma placenta*, *Stylocidaris lineata*, and *Cidaris blakei*. Plot of the particle-size distribution of the gut content. Number of particles in 0.1cm^3 of p>500μm.

These results confirm visual observations that *S. lineata* had higher number of particles >500µm in the gut content than the other species. However, the fact that *A. jacobyi*, *P. placenta*, and *C. blakei* had less particles >500µm only indicates their particles, within this size fraction, were possibly bigger than those from *S. lineata*.

The intermediate fraction size (63µm<p<500µm) was measured only for *S. lineata* and *P. placenta*. The gut content of *P. placenta* had twice as many particles of the fraction size 63µm<p<500µm per 0.1cm^3 than *S. lineata* (Table 5.1; Student's t-test $Z_o=19.59$, t(0.05, 8)=2.31). This indicates that within this fraction *P. placenta* had particles of smaller size than *S. lineata*.

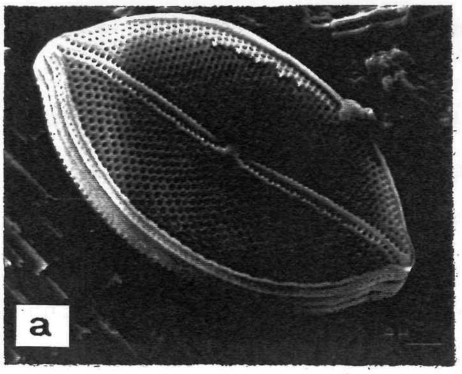
5.3.3. Scanning electron microscopy observations

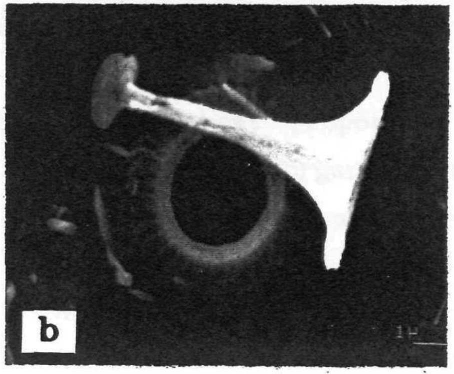
The types of particles found in the gut content of the four species, as observed under the scanning microscope are listed in Table 5.4, some of which are shown in Figure 5.6. *C. blakei* and *S. lineata* had pellets consisting of plant material and fibres, broken shells, calcareous aggregates, foraminiferans, diatoms, radiolarians, sponge spicules, and other elements. *A. jacobyi* and *P. placenta* had pellets consisting mainly of small mud balls as observed by Mortensen (1935) and Pawson (1982). These 'mud balls' consisted of fine sediment composed of aggregates of diatoms, frustules, foraminiferans (benthic and displaced from shallow waters), and also included fibres of plant material, protozoans (Table 5.4). Amongst the diatoms present in the gut contents of the different species of echinoids the *Amphora* spp and *Mastogloia* spp were found. These diatoms are characteristic epiphytes on larger algae such as *Sargassum*.

Table 5.4. List of items found in the gut content of *Aspidodiadema jacobyi*, *Phormosoma placenta*, *Stylocidaris lineata*, and *Cidaris blakei*.

FOOD TYPES	*A. jacobyi*	*P. placenta*	*S. lineata*	*C. blakei*
DIATOMS				
Mastogloia sp	+	+	+	+
Synedra sp	+			
Amphora sp		+	+	
Paralia sulcata		+		
Hantzschia sp		+		
Asteromphalus sp	+			
Rhizosolenia sp		+		
Thalassiosira sp	+	+	+	+
Chaetoceros sp	+		+	+
Coscinodiscus sp	+	+		+
COCCOLITHS				
Gephyrocapsa sp	+			+
Cyclococcolithus sp	+			+
Umbilicosphaera sebagae		+	+	
Emiliana huxleyi		+		
Acanthoica sp			+	
Amphisolenia cf *brasiliense*		+	+	
Synacosphaera sp		+		
Discosphaera tubifera		+	+	+
Scyphosphaera cepsteinii	+	+		
SILICOFLAGELLATES	+	+		+
RADIOLARIANS	+		+	+
CYSTS	+	+		
SPORES	+			
POLLEN GRAIN		+		
SPONGE SPICULES				+
FORAMINIFERANS				
Fissurina sp				+
Quinwquiloculina sp			+	+
Hoeglundina sp	+			+
Globorotalia sp				+
Psammosphaera sp				+
Trochammina sp				+
Nonionella sp			+	+
Globigerina sp	+	+	+	+
Reophax sp	+			
Uvigerina sp	+		+	
Chilostominella sp	+			
Rhizammina sp	+			
OSTRACOD			+	
BIVALVE			+	
BRIOZOANS				+
CRUSTACEANS	+		+	+
NEMATODES	+			
FIBRES OF PLANT MATERIAL	+	+	+	+

Figure 5.6. Scanning electron micrographs of the gut content of Bahamian regular echinoids. *Cidaris blakei*: **a.** *Mastogloia* sp, **b.** *Dicosphaera tubifera* over *Umbilicosphaera* sp; *Stylocidaris lineata*: **c.** whole frustule of *Amphora* sp, **d.** benthic foraminiferan; *Aspidodiadema jacobyi*: **e.** benthic diatom *Synedra* (?), **f.** cyst; *Phormosoma placenta*: **g.** *Scyphosphaera cepsteinii*, **h.** *Amphora* sp.


a
4μ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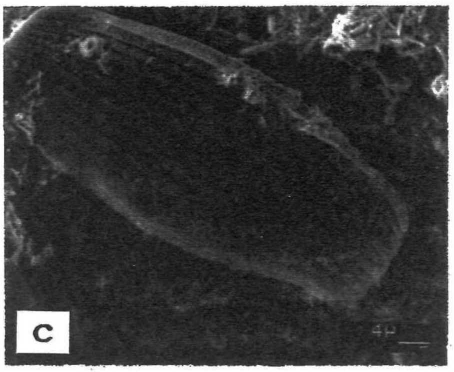
c
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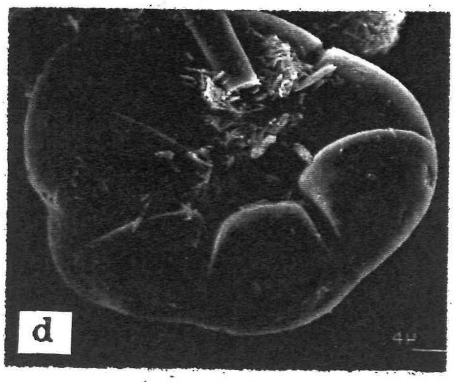
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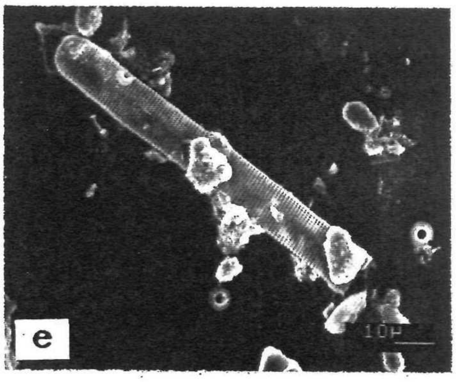
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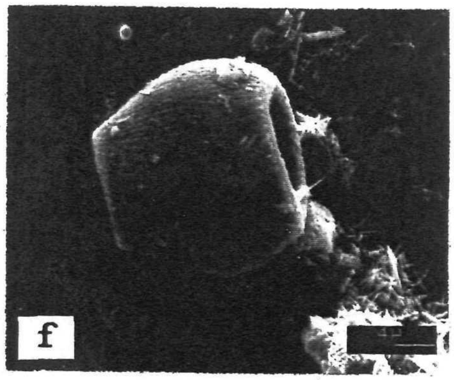
f
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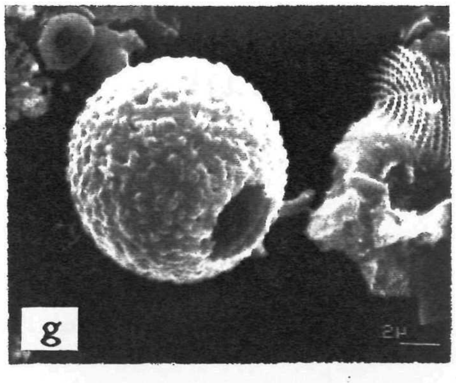
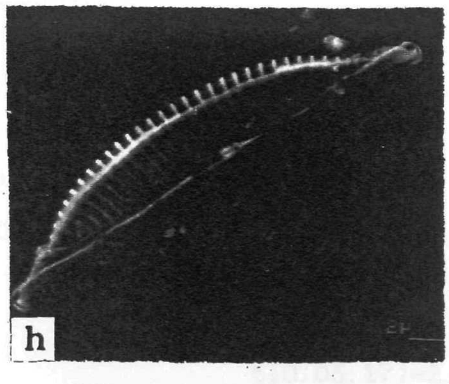

g
5μ


h
5μ

5.3.4. Organic matter of the gut wall and gut content

Gut wall: *Stylocidaris lineata* had the highest proportion of organic matter in the gut wall. The proportion of organic matter in the gut wall varied significantly between *S. lineata* and *Cidaris blakei*, and also between *S. lineata* and *Phormosoma placenta*. Pairwise comparisons showed no other significant differences between species (Tables 5.5, 5.6; Figure 5.7).

Table 5.5: Results of total organic matter (AFDW%) of the gut wall of *Cidaris blakei*, *Stylocidaris lineata*, *Phormosoma placenta*, and *Aspidodiadema jacobyi*.

SPECIES	**n**	**$\bar{x}$**	**SD**
Cidaris blakei	5	57.60	9.24
Stylocidaris lineata	4	71.58	6.29
Phormosoma placenta	7	47.60	19.79
Aspidodiadema jacobyi	12	55.63	9.97

Table 5.6: T-test statistics (Z_o values) for pairwise comparison of gut wall total organic matter (AFDW%) mean values of *Cidaris blakei*, *Stylocidaris lineata*, *Phormosoma placenta*, and *Aspidodiadema jacobyi*. Z_o values relate to data in Table 5.5.

	S. lineata	*P. placenta*	*A. jacobyi*
C. blakei	Z_o=2.57 t(0.05,7)=2.37	Z_o=-1.04 t(0.05,10)=2.23	Z_o=-0.38 t(0.05,15)=2.13
S. lineata		Z_o=-2.31 t(0.05,9)=2.26	Z_o=-1.19 t(0.05,14)=2.15
P. placenta			Z_o=1.19 t(0.05,17)=2.11

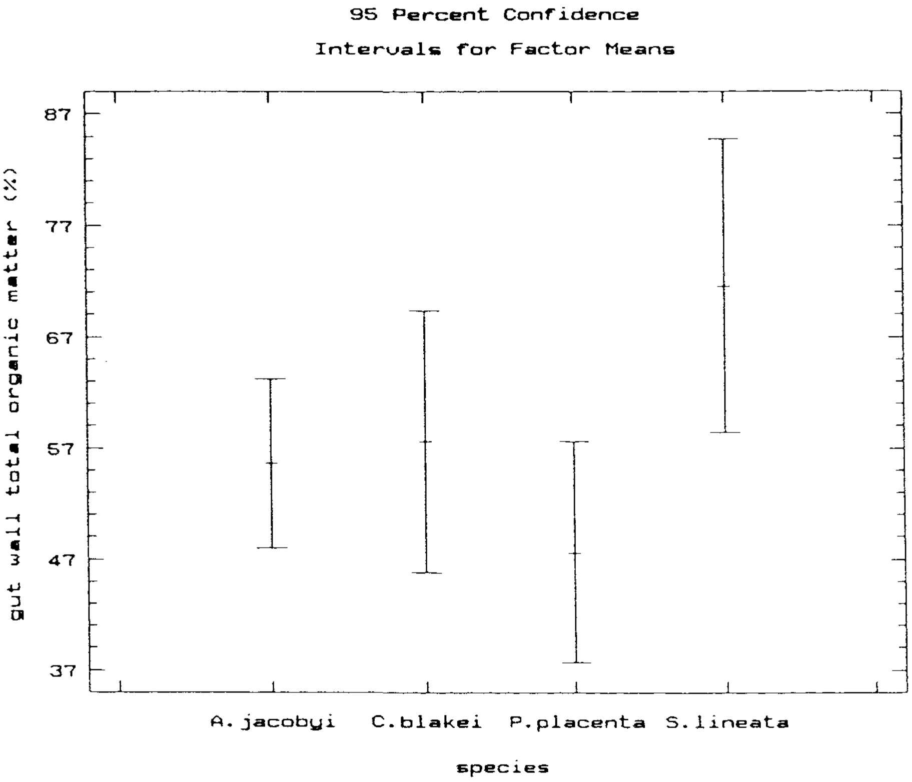


Figure 5.7. *Aspidodiadema jacobyi*, *Phormosoma placenta*, *Stylocidaris lineata*, and *Cidaris blakei*. Plot of the total organic matter (AFDW %) of the gut wall.

Gut content: The total organic matter of the gut content of *S. lineata* did not vary significantly from those of *C. blakei* and *A. jacobyi*. The mean values were high (29.55%, 54.95%, and 33.93% respectively), but the variation within samples was also high (Table 5.7). Owing to the lack of sediment control it is difficult to evaluate the extent to which these species were feeding on highly organic material. The variation within samples could be a result of patchiness in the food resources in the deep-sea environment. Young *et. al.* (in press) found that *S. lineata* feeds on the flesh of broken echinoids and on dead asteroids both in aquaria and *in situ*, suggesting that this species consume organic matter of diverse origin. This would partially explain the high values of organic matter found in the gut content of *S. lineata* collected for this project.

P. placenta had the lowest mean value of total organic matter in the gut content ($\bar{X}$±1SD = 8.27±1.35) and significantly different from the other three species (Tables 5.7, 5.8, Figure 5.8). Although specimens of *P. placenta* used for this analysis were fresh, the result was similar to that found for specimens of *P. placenta* collected in the NE Atlantic ($\bar{X}$≅4%-15%, see chapter 4).

Table 5.7: Results of total organic matter (AFDW%) of the gut content of *Cidaris blakei*, *Stylocidaris lineata*, *Phormosoma placenta*, and *Aspidodiadema jacobyi*.

SPECIES	n	$\bar{X}$	SD
Cidaris blakei	6	54.95	18.82
Stylocidaris lineata	3	29.55	15.52
Phormosoma placenta	7	8.27	1.35
Aspidodiadema jacobyi	12	33.93	14.35

Table 5.8: T-test statistics (Z_o values) for pairwise comparison of gut content total organic matter (AFDW%) mean values of *Cidaris blakei*, *Stylocidaris lineata*, *Phormosoma placenta*, and *Aspidodiadema jacobyi*. Z_o values relate to data in Table 5.7.

	S. lineata	*P. placenta*	*A. jacobyi*
C. blakei	$Z_o=2.00$ t(0.05,7)=2.37	$Z_o=6.59$ t(0.05,11)=2.20	$Z_o=2.65$ t(0.05,16)=2.12
S. lineata		$Z_o=3.93$ t(0.05,8)=2.31	$Z_o=0.47$ t(0.05,13)=2.16
P. placenta			$Z_o=4.66$ t(0.05,17)=2.11

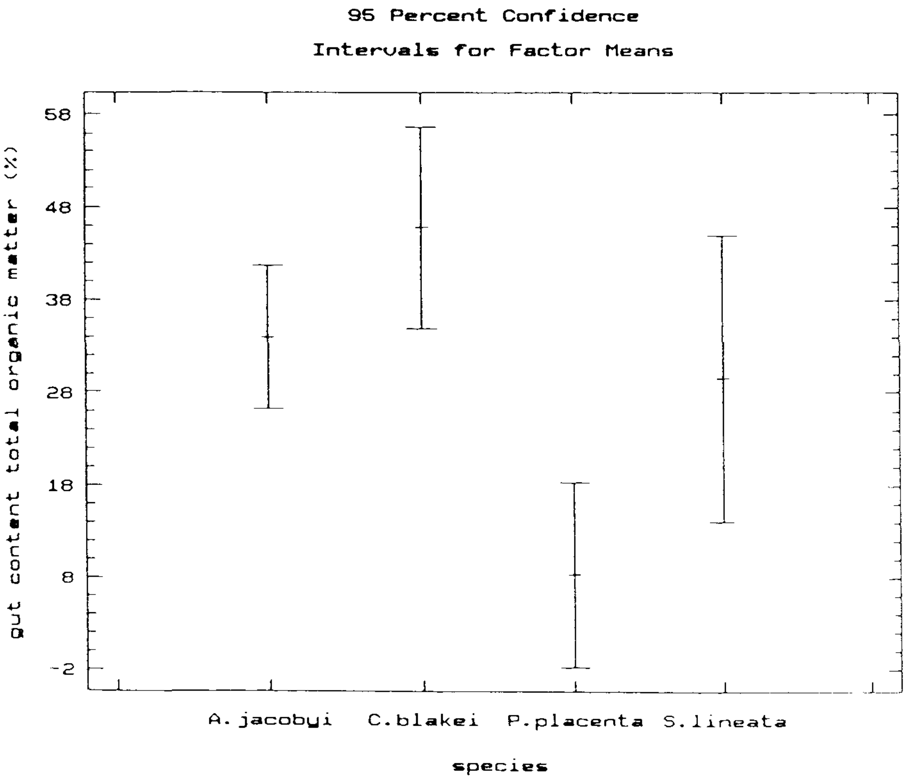


Figure 5.8. *Aspidodiadema jacobyi*, *Phormosoma placenta*, *Stylocidaris lineata*, and *Cidaris blakei*. Plot of the total organic matter (AFDW %) of the gut contents.

Chemical analyses of the gut wall and gut content

The gut content and gut wall of only a few species were observed through biochemical analyses. These were the regular urchins *Coelopleurus floridanus*, *Cidaris blakei*, *Stylocidaris lineata*, *Aspidodiadema jacobyi*, *Phormosoma placenta*, and the irregular urchins *Archaeopneustes hystrix* and *Conolampas sigsbei*. The reason for missing values in these analyses is because each specimen was treated individually in each sample. Sometimes the amount of freeze-dried material per specimen was not enough to perform all the analyses. It would have been necessary to put together the gut contents or gut wall of more than one specimen from a sample in order to carry out all the proposed analyses (organic carbon, soluble protein, lipids and carbohydrates). This would make the results less accurate, although it has been done in the past by different authors (Féral 1985, 1989, McClintock *et. al.* 1989).

The percentage of organic carbon in the gut content of *Coelopleurus floridanus* and *Phormosoma placenta* was higher than that found for *Archaeopneustes hystrix* and *Conolampas sigsbei* (Table 5.9). These latter species, however, had highest values for soluble protein and lipids. *Coelopleurus floridanus* showed a very high value for carbohydrate (6.3%) compared to *Archaeopneustes hystrix* and *Conolampas sigsbei* (2.5% and 3.5% respectively). The variation in the results of carbohydrates for *A. hystrix* and *C. sigsbei* between specimens of each species was very high (Table 5.9). The same can be said from the lipid analysis. The high variation in the lipids analysis for all species studied is possibly a consequence of the use of the gravimetric method. This method is not sufficiently sensitive when small amounts of material are used. Outliers were produced and not disregarded because the sample size was also small.

Table 5.10 shows the results of soluble protein lipid and carbohydrate for the gut wall and gut content together of *Cidaris blakei*, *Stylocidaris lineata* and *Aspidodiadema jacobyi*. In these cases it was not possible to freshly separate gut wall from gut content because of the small sizes of the urchins, and also the softness of the fresh gut wall which did not facilitate manipulation of the material. The high values observed are probably to the greatest extent because of the chemical extraction from the gut wall (Table 5.10).

Table 5.9: Results of the organic analyses of gut content of some of the echinoid species collected in the Bahamas. Values are given in percentage dry weight (means ± 1SD).

SPECIES	n	ORGANIC CARBON	PROTEIN	LIPID	CARBOHYDRATE
Coelopleurus floridanus	5	3.0±1.3	4.3±2.0	1.1±0.9	6.3±1.9
Phormosoma placenta	10	4.0±1.4	5.2±1.8	-	-
Archaeopneustes hystrix	12	0.7±0.1	7.8±0.4	1.5±1.0	2.5±1.4
Conolampas sigsbei	8	1.8±0.4	15.3±0.9	4.6±3.9	3.5±1.4

Table 5.10: Results of the organic analyses of gut content and gut wall together of some of the echinoid species collected in the Bahamas. Values are given in percentage dry weight (means ± 1SD).

SPECIES	n	ORGANIC CARBON	PROTEIN	LIPID	CARBOHYDRATE
Cidaris blakei	11	-	19.9±2.2	6.4±2.6	15.1±2.6
Stylocidaris lineata	6	-	13.6±6.3	-	10.6±4.1
Aspidodiadema jacobyi	12	-	12.4±1.2	-	9.8±1.3

Although few conclusions can be made from the results presented in Tables 5.9 and 5.10, the biochemical analyses have shown that there is a high variation within samples. It would be necessary to observe many specimens per sample individually to have a realistic insight of what is occuring biochemically with the gut wall and gut content of each population.

5.4. Discussion

The shallow waters and banks, together with currents and tidal movements, and more drastic phenomena such as hurricanes will influence the types of material deposited in different deep-sea regions of the northern parts of the Bahamas (Pilskan *et. al.* 1989). The sediment apparently changes considerably through the slopes off the northern Bahamian islands from coarser to finer grains with depth (Droxler 1985). The sediments deposited between graded beds of lime sand and mud from sediment gravity flows are either a periplatform carbonate ooze (60-95% carbonate) in the Bahamian basins or a marl to hemipelagic mud (2-72% carbonate) in the adjacent Atlantic Ocean. The sources of this deposited sediment are :

1-) calcite (tests of planktonic foraminifera and plates of coccoliths), and aragonite (tests of pteropods), both of pelagic origin;

2-) fine aragonite (organically or inorganically precipitated) and some of the magnesian calcite are derived from either the margin or the top of the shallow carbonate banks which are neritic in origin;

3-) a non-carbonate fraction (clays, quartz, and feldspar) transported into the region by oceanic currents mainly through the action of the Western Boundary Undercurrent (Droxler 1985).

Seagrasses such as *Thalassia testudinum* can form dense, highly productive beds over extensive areas of shallow water in the Bahamas (Young personal communication, personal observation). *Thalassia* and the macroalgae *Sargassum* have often been observed on the sea floor in the Bahamas (Pawson 1982, personal observation, Young personal communication). Once negatively buoyant, the *Sargassum* can sink to approximately 5000m in about 40 hours (Johnson & Richardson 1977). Food falls like this may represent an important local energy enrichment to the deep-sea environment affecting the faunal diversity, composition, biomass levels and ecology of deep-sea benthos (Hessler & Sanders 1967, Sanders & Hessler 1969, Harrison & Mann 1975, Stockton & De Laca 1982, Jumars & Eckman 1983, Suchanek *et. al.* 1985, Grassle & Morse-Porteous 1987). The role of large food falls, such as seagrass, macroalgae and associated microorganisms and other large organisms, in the ecology of deep-sea benthos have received very little direct study (Smith 1985, 1986).

De Ridder & Lawrence (1982) suggested the composition of the gut contents of regular echinoids strongly indicate that they are opportunistic feeders. This certainly appears to be true for the deep-sea echinoids found off the northern Bahamian islands. Young *et. al.* (in press) have shown that *Stylocidaris lineata* is attracted to food of diverse origin. All species of regular echinoids dissected for this study contained, as part of their dietary content, varied elements from the sediment and surface derived material, particularly pieces of seagrass, apart from other smaller organisms found in the sediment. It is still unknown how the input of seagrass affects the general metabolism of the sea urchins in this tropical area. Are the sea urchins capable of digesting such material? Or are the urchins taking advantage of the subproducts of microorganisms degradation? How is the gametogenic growth affected by this input of seaweed? Is the general growth of the echinoids influenced by these surface originated plants? And how? Do the echinoids living in the Tongue of the Ocean respond similarly to surface production as has been demonstrated for species from the NE Atlantic? These questions are still to be resolved. It is known that the seaweed detritus can be formed through mechanical breakdown by physical or biological grinding, autolysis, leaching (removal of water soluble components), and digestion of debris by bacterial and fungal extracellular enzymes (Harrison & Mann 1975). *Sargassum* spp arriving at the deep-sea can be at different levels of degradation as their descent can take hours or days. Once negatively buoyant, either because of natural decay or by wave action passing through the deep mixed layer the seagrasses will sink fairly rapidly (Johnson & Richardson 1977).

Apart from large food falls rapid sinking of small particulate matter could supply approximately 14% of the benthic requirements in areas such as the Tongue of the Ocean (Wiebe *et. al.* 1976). Wiebe *et. al.* (1976) suggested 7% to 25% of the total carbon reaching the sea floor in that area is organic in origin.

The four species chosen for more detailed analyses of the gut contents showed marked differences in the proportion of organic matter present in the gut contents. *P. placenta*, a species usually collected from deeper dives, feeds mainly on the sediment as previously reported by Mortensen (1935) and Pawson (1986),but also contained pieces of *Thalassia* sp in the gut. The proportion of organic matter found in the gut content of *P. placenta* from the Bahamas was similar to that found for the same species in the NE Atlantic (4%-15% AFDW). *Aspidodiadema jacobyi*, *Cidaris blakei* and *Sylocidaris lineata* showed higher values of organic content in the gut contents. *S. lineata* is known to feed on material of high organic contentif that is available in the surrounding environment (Young *et. al.* in press).

Differences in the proportion of organic matter as well as in particle-size distribution of the gut contents could be a consequence of selection of food particles or even patchiness of enriched sediment where the collection took place. It was not possible to confirm these ideas without having a control sample of the sediment where the animals were collected, and these important data are missing. Even in the possession of local sediment cores, the great spatial variability in the sediment would not be represented in the cores (Wolff 1976, 1979).

Akhmet'ieva (1987) stressed that the maximum organic carbon present in the gut contents of holothurians, collected in different regions of the Pacific and Indian Oceans, was 1.7 to 12 times greater than the surrounding sediment. Similar results had been previously found by Khripounoff & Sibuet (1980) and Sibuet *et. al.* (1982) for holothurians from the NE Atlantic. These authors found a direct relationship between the organic carbon concentrations present in the gut of holothurians and the surrounding environment. It is possible that, similarly, echinoids have higher concentrations of organic carbon in their guts when the surrounding environment is enriched.

A previous study on the organic analyses of Bahamian echinoderms (McClintock *et.al.* 1990) claimed its data can be used to estimate energetic densities of bathyal echinoderm populations. Although valuable, it is a very ambitious utilization for data which is based on the analyses of one or very few individuals (1-3) of each species studied. The paper produced by McClintock *et.al.* (1990) is useful to have an idea of the order of magnitude of the results such as organic carbon and proteins. However, the results shown here, based on at least 5 individuals within a sample, indicated that variation between individuals of the same species in the same sample can be large. Therefore a large number of individuals of each species from a sample is required in order to estimate energetic densities of bathyal echinoderm populations with any accuracy.

This study characterized food types, and showed that there are variations within sampled species which must be considered without prejudice. These differences show the importance of the relationship between the echinoids and their surrounding environment.The patchiness of the substrate is more likely to be the cause for these differences than morphological or physiological requirements.

More detailed study of the feeding requirements of different species of deep-sea urchins from the Bahamas and elsewhere is necessary in order to understand their general biology, distribution and ecology. Apart from careful collection of these deep-sea animals, with more specimens per sample for each analysis, the substrate sediment must be sampled for comparison with the material present in the gut content. Also, the gut morphologies of the different species must be taken into account as well as their working mechanisms. Cidaroids have a relatively short gut, with a stout Aristotle's lantern attached to the test by strong muscular ligaments; the physiology of the alimentary canal is not well known (Hyman 1955, De Ridder & Jangoux 1982, see chapter 3). *A. jacobyi* and *P. placenta* have the same type of lantern (aulodont) with teeth lacking a keel (Hyman 1955), long oesophagus and enlarged pouches in the stomach (see chapter 3). Information about their physiology is also limited. There is very little known about how these urchins process the material ingested. Most of the behavioural and physiological work has been done on shallow water echinoids (reviewed in Jangoux & Lawrence 1982, Féral & Magniez 1988, Féral *et. al.* 1990). Only recently a more controlled experiment was designed for the observation of feeding behaviour on deep-sea echinoids using a manned submersible (Young *et. al.* in press).

Because of the difficult access to the deep-sea environment, a network of information is required to solve the puzzle of the life styles of deep-sea echinoids. Any information is crucial in understanding their adaptations and evolution.

Chapter 6: General discussion and summary

The purpose of this study was to investigate whether the seasonality of reproduction in deep-sea echinoids is directly related to the seasonal flux of surface derived material. If seasonality in reproduction is related to surface production and the flux of organic matter to the deep-sea, how do deep-sea species which are not seasonally reproductive partition energy for continuous breeding?

Two species of echinoids were chosen for this investigation in an attempt to answer these questions:

1-) the non-seasonal breeder *Phormosoma placenta*; and

2-) the seasonally reproductive *Echinus affinis*.

The breeding cycles of *P. placenta* and *E. affinis* were previously reported by Tyler & Gage (1984a, b respectively).

The main approach of this work was to study the origin of energy in the form of food and the pathways by which energy enters the urchins, *viz* the gut contents and the digestive system. Deep-sea echinoids were chosen because very little is known about the feeding biology of these animals apart from general description of the digestive tract, general observations of food types, behaviour and biochemistry (Mortensen 1928, 1935, 1943, Lewis 1963, Grassle *et. al.* 1975, Pawson 1976, 1982, Suchaneck *et.al.* 1985, McClintock *et. al.* 1990).

The first step was to try to understand how the digestive system of regular echinoids work, how the sediment taken in by the urchins is processed and, to a certain extent, how the condition of the gut content was in relation to the gonad development, alimentary canal, and the surrounding environment.

For the analyses of the samples, organ and gut content indices, and total organic matter (ash-free dry weight) of the gut content were used rather than more sophisticated chemical analyses. This was because most of the samples available for the project had been fixed and preserved for several years from previous cruises to the Rockall Trough and Porcupine Seabight. The results obtained were interpreted with appropriate reservations respective to the quality of the samples.

The first opportunity to work on fresh samples of deep-sea regular echinoids arose from a diving cruise made in the Bahamas with Dr. C.M. Young by the Harbor Branch Oceanographic Institution using the R/V 'Seward Johnson' and DSRV 'Johnson Sea-Link-II'. Several species of echinoids were collected with the purpose of larval development studies, but also for any other observation of general biology. The usage of that material was an opportunity to try some techniques for the chemical analyses of the gut content and gut wall, and electron microscopy of the gut wall of regular echinoids. Also, there was the opportunity to use similar techniques to those applied on preserved samples from the NE Atlantic. Although more experiments are needed on increased sample size, the use of dry and ash-free dry weights did not appear to differ significantly between fresh and preserved samples. At least one species was found both in the Bahamas and in the NE Atlantic: *Phormosoma placenta*. Three other species from the Bahamas were studied in more detail: *Aspidodiadema jacobyi*, *Cidaris blakei* and *Stylocidaris lineata*.

Particle-size distribution of the gut contents was determined in order to evaluate differences in urchins found in the same locality (samples from the NE Atlantic, and the Bahamas). Particle-size distribution was also used to find out if in individual animals there was a difference between stomach content and intestine content because of breakage of food particles or absorption (samples from the NE Atlantic only). Two attempts were made to collect sediment cores on the site of urchins collection, one using a core held by the mechanical arm of the submersible in the Bahamas, other using a multiple-corer in the NE Atlantic. Both failed because of faults in the respective equipment.

The main objective of using histology and electron microscopy in this project was the familiarization with the gut microstructure of regular echinoids which had been generally described for other species of shallow-water urchins by different authors (De Ridder & Jangoux 1982 and authors therein, Lawrence *et. al.* 1989). The gut wall of echinoids desintegrates very rapidly once dissection is started, even if dissection takes place in a 4°C cold room. It was evident from the material which had been fixed in the usual way ('throwing' urchins in a bucket filled with 5-10% formalin) that preservation of the soft parts was poor. In order to overcome this problem it is important to guarantee that the test is broken carefuly to allow the fixative to penetrate the soft tissues. It was more efficient to inject glutaraldehyde in the gut and through the test prior to dissection than the common procedure of fixing the tissue after dissection, as in the processing of the gut wall of *Psammechinus miliaris*. The electron microscopy results showed much clearer images of the microstructures of different parts of the gut wall of *P. miliaris* than *E. affinis* or *P. placenta*.

The dissection of several diadematoids revealed the presence of a siphon instead of a siphonal groove which had been previously described by Holland & Ghiselin (1970).

Observations on the gut macro-morphology of echinothuroids prompted questions on the importance of having such a long gut: How do these urchins process their food?; What is the function of the long oesophagus?; Are the cells present in the pouches of the stomach exerting the same function as those in the narrower parts of the stomach wall?; How does the intestine wall functionally differ from the stomach wall?; etc.. These are areas that need more careful investigation.

Differences in the macromorphology of the gut wall of different species of cidaroids might reveal new insights about their evolution and their relationship with other regular echinoids. So far, much importance has been given to the hard parts of the animals such as the test and Aristotle's lantern (Lawrence 1987 and authors therein). Cidaroids are difficult to fix properly. Their test is thick and hard to break without damaging the soft parts, mainly the gut wall. Quick drainage of the coelomic fluid prior to injection of fixative is possibly a better way of dealing with the animal during dissection because the fixative would not be diluted.

Deep-sea echinoids are mainly opportunistic in the way of feeding and can be considered as stress-tolerating echinoderms mainly because of the limitations in food supply (Lawrence 1990). Exceptions are likely to be amongst those species of echinothurioids which highly select their food in the deep-sea (Mortensen 1935, Lewis 1963, Pawson 1982, Suchaneck *et. al.* 1985), such as *Araeosoma belli* and *A. fenestratum.* These species feed almost exclusively on plant material especially seagrasses derived from the surface and shallow coastal areas. These seagrasses can be rapidly sink down to the sea floor forming a food source very low in nitrogen which decomposes very slowly (Gage & Tyler 1991 and authors therein). This occurrence is typical in the Bahamas where the slope is fairly close to islands, sand banks and narrow platforms (Wiebe *et. al.* 1976). A recent study in the Bahamas by Young *et.al.* (in press) has shown that the cidaroid *Stylocidaris lineata*, apart from consuming organic matter of diverse origin such as dead asteroids, is also "*attracted to packages of macroalgal debris which it is able to discriminate from inert material*".

Phormosoma placenta from the Bahamian environment had gut content composed of fine sediment made into small 'mud balls' as described by Mortensen (1935) and Pawson (1982), but also contained pieces of seagrass. The same species from the NE Atlantic had gut content of similar appearance however, with no pieces of seagrass because these were not readily available in their environment.

The observed differences in the particle-size distribution of the gut content may be related to the type of apparatus to collect food. Not only the Aristotle's lantern, but spines and different types of tube feet and maybe other characteristics of the micro- and macro-morphology of different species of sea urchins would contribute to the gathering of different food particles (Lawrence 1987). This is a hypothesis which needs testing through experimental work preferably on live animals. The real importance of gut content particle-size, where nutrients may be limited in the environment, is probably related to the capacity of the urchins to process the material that they take in, according to the morphological structures they possess. Basically, how fast they process that material, how efficiently they select nutritive particles, how efficiently the particles are digested. Morphological characteristics influence the efficiency of skimming, general feeding behaviour, other physiological responses and even the patterns of distribution of the species (De Ridder and Lawrence 1982, Lawrence 1987). Morphophysiology and food availability determine the feeding habits of organisms (Lopez and Levinton 1987). Food availability may influence the distribution of deep-sea species, and the composition and biomass of the communities (Sokolova 1959, Grassle *et. al.* 1975, Thiel 1979, Sloan and Campbell 1982, De Ridder and Lawrence 1982, Jumars and Eckman 1983, Lampitt *et. al.* 1986). The energy availability for the metabolic processes may be a result of quantity and quality of the food supply (Jumars *et. al.* 1990). A realistic field study of deposit feeders needs to incorporate both seasonal sampling and some sort of bioassay which can estimate the changing usage of the sedimentary particles by the benthos (Levinton and Stewart 1988). In this work a seasonal component is present for the NE Atlantic samples but information regarding the conditions in the sediment and other bio-physical parameters is lacking not only for the NE Atlantic but also for the Bahamian samples.

Particle-size distribution of the gut content of *Echinus affinis* and *Phormosoma placenta* had no significant difference. However, the first species appeared to be more selective in the nutrient intake. *E. affinis* fed less during winter and autumn months, but in these months had as high values of total organic matter of the gut content as during the spring and summer months. On the other hand, *P. placenta* had a large volume of gut contents in every month of the year. *P. placenta* have an increased digestive surface in contact with the sediment taken in because of their long gut. One could speculate whether this factor would allow them to extract as much nutrients as possible from the gut content ingested from a 'poor' environment. In terms of total organic matter however, the gut contents of *P. placenta* showed high values in the spring and autumn in a bi-annual cycle which reflected the condition of the surrounding environment supposedly richer from surface derived material (Reid *et. al.* 1987, Rice *et. al.* 1991).

In spite of the environmental differences the average total organic matter of the gut content of *P. placenta* from the NE Atlantic was not significantly different from that of *P. placenta* from the Bahamas (4-15%).

How did the seasonality of 'food' supply and the fluctuations of gut content (either qualitatively or quantitatively) influence the gonads and gut wall of both *P. placenta* and *E. affinis*?

Gonad and gut wall indices calculated for *P. placenta* and *E. affinis* in chapter 4 indicated that the condition of these organs were highly correlated in both species. These results suggest that the nutrients obtained through the gut wall are transferred to the gonads, but the gut wall can also be a site for energy reserve. This had been suggested before by several authors (Lawrence *et. al* 1966, Lawrence 1970, 1971, 1976, Fenaux *et. al.* 1975, Nichols *et. al.* 1985). Yet, this is still highly speculative. More experimental work is needed in order to identify storage sites other than the gonads in several species of echinoids from different locations, and the reasons for this storage if these are not only directed to reproduction (Comely & Ansell 1989).

Although the non-seasonal reproductive cycle of *P. placenta* had been previously reported (Tyler & Gage 1984a), the gonad index indicated a seasonal fluctuation. This result might be only a consequence of vitellogenesis.

From the analysis of few echinoid species from the Bahamas, it was clear that more specimens per sample are necessary in order to study the gut wall and gut content biochemically. This is because variation within samples is very high. The variation between individuals collected in the same locality at the same time is likely to be a result of patchiness of nutrients on the sea floor.

Conclusions and directions for further research

1-) Non-linear regression was found to be essential in testing for seasonality. 'Lack of fit' analysis was needed to test for significant differences between seasonal and non-seasonal models. The statistical analyses have resulted in the identification of a number of seasonal fluctuations in the organ and gut content indices of both *Phormosoma placenta* and *Echinus affinis*.

2-) *Phormosoma placenta* had high quantities of gut content independent of the season. However, the results of total organic matter of the gut content showed a biannual cycle with higher values in the spring and autumn coincident with the main periods of arrival of surface derived material on the sea floor. The average total organic matter of the gut content of *P. placenta* collected in the NE Atlantic did not differ significantly from the average of total organic matter of *P. placenta* collected in the Bahamas. It is still not clear how *P. placenta* stores energy for its reproduction.

3-) *Echinus affinis* had less food in the gut when gonads were fully developed. Although the quantity of gut content increased from spring and during the summer months, the percentage organic matter in these seasons did not vary significantly from the autumn or winter. Gonad and gut wall indices were highly correlated indicating that the stomach is possibly used as a site for storage of nutrients which can be used for the maintenance metabolism when energy source in the gonads is directed to complete gametogenesis.

4-) More samples from the autumn and winter are needed within the two seasonal programmes in the NE Atlantic. But this limitation is consequence of unavoidable factors such as weather conditions, ship time not always available.

5-) A siphon was observed in several species of diadematoids. Recent descriptions of gut morphology repeat old descriptions without much detail to functional morphology. It would be useful to use histochemistry and morphometry on seasonal samples as tools for observing, for example, if there are changes in the gut wall throughout the year that would explain seasonal changes observed in the stomach and intestine wall index of *Echinus affinis*. Also, these analyses would be useful to compare each region of the gut, for example, how the pouches of the stomach of *P. placenta* differ from narrower areas of the stomach. The injection of the fixative in the gut of the echinoids prior to dissection proved to be a better way of preserving the ultrastructure of the gut wall. More careful electron microscopic work as well as chemical analyses are needed in different species of echinoids to evaluate the real function of each portion of the gut in the different species.

6-) Scanning electron microscopy observations provided the identification of a wide range of food types which are largely represented by surface derived material.

7-) The chemical analyses of the Bahamian species of regular echinoids showed that variation within samples can be high. More individuals of the same species within a sample should be submitted to biochemical tests in order to have a good representation of the population. Particle-size distribution of the gut content indicated that different species living in the same locality have different particle size in the gut. This could be because of particle selection and/or the capability of ingesting specific size fractions of the sediment. This hypothesis needs testing together with proper collection of sediment cores where the urchins are sampled.

The work presented in this thesis provides a significant contribution to the natural history information about feeding in deep-sea regular echinoids. It has shown that more samples are necessary to assess fully the seasonal fluctuation on the feeding of these echinoids. An understanding of how the gut content is processed through the digestive system of the echinoids is necessary to link food and gonad development. It is essential to have a sediment control of the surrounding environment where the echinoids are collected in order to evaluate nutritional changes in their gut content, and ultimately relate these changes with other aspects of their metabolism.

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