UNIVERSITY OF SOUTHAMPTON

The '*Amperima* Event': analysis of community change in the abyssal Northeast Atlantic Ocean

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ABSTRACT

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THE '*AMPERIMA* EVENT': ANALYSIS OF COMMUNITY CHANGE IN THE DEEP NORTHEAST ATLANTIC OCEAN

by Benjamin Doull Wigham

The deep sea was long considered to be a relatively stable, food-limited environment. We now know that the deep-sea benthic environment is a very dynamic ecosystem, both physically and biologically. However, in the deep sea there is still little information available of how the environment changes naturally over time.

More than 10 years of sampling on the Porcupine Abyssal Plain (PAP) has highlighted a radical change in the abundance of invertebrate megafauna over the period from 1996 to at least 2000. Actinarians, annelids, pycnogonids, tunicates, ophiuroids and holothurians increased significantly in abundance. Two holothurian species, *Amperima rosea* and *Ellipinion molle*, increased in abundance by more than two orders of magnitude and dominated the community from 1996 onwards. Samples from the PAP dating back to 1977 show that prior to 1996 these holothurians were always a minor component of the megafauna. From 1996 to 2000 *A. rosea* was abundant over a wide area of the PAP indicating that the phenomenon was not a localised event.

The PAP benthos is subjected to an episodic pulse of organic input in the form of phytodetritus. This signal provides a nutritional cue for the seasonal reproduction and recruitment of several deep-sea invertebrate species, including some echinoderms. The reproductive biology of *Amperima rosea* was examined. *A. rosea* produces small eggs ($\leq$ 200μm) and has a greater fecundity (~ 12,000) compared to other abyssal species. It appears to grow quickly and reaches sexual maturity at a very small size. These features indicate that the species has the potential to increase rapidly in population size and to colonise large areas under optimal conditions.

Time-lapse photography has shown that the true abundance of *A. rosea* (~7,000 ind. ha^{-1}), and other smaller components of the megafauna, is greatly underestimated by trawl catches. During the period 1997 to 2000 the feeding activity of *A. rosea* removed phytodetritus from the seafloor, covering 100% of the seafloor in ~ 2 to 4 months. The abundance of *A. rosea* was shown to be temporally patchy, possibly indicating the movement of loose aggregations of this holothurian.

Chlorophyll and carotenoid pigments found in the gut contents of six species of abyssal holothurian indicate a high degree of selectivity for different fractions of the phytodetritus. Exceptionally high concentrations of chlorophyll *a*, zeaxanthin and β-carotene indicate that *A. rosea* is highly selective for fresh phytodetritus with a possible preference for particles rich in cyanobacteria, while at the same time not selecting particles of dinoflagellate origin.

The analysis of genetic variation in *A. rosea* populations on the PAP have produced a species-specific set of primers and amplified and sequenced a 310bp sequence for the 16S region.

The results of this study show that natural change in deep-sea populations can be rapid and on a large-scale, both in terms of the number of taxa and area affected. The biology of *A. rosea* appears suited to an opportunistic life history with the capacity to select and rapidly utilise certain fractions of fresh phytodetritus. It is hypothesised that climate change can have an affect on the abyssal fauna living at more than 4000m water depth.

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Welcome to the:
"The Amperima event"
FRESH
PHYTODETRITUS

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Chapter One – General Introduction

1.1. From Sail to Subs: a brief history of deep-sea exploration.

The deep sea represents the largest ecosystem on earth, with over 50% of our planet lying beneath water deeper than 3000m. However, it was not initially a financially well-supported field of science, and research into the deep-sea environment did not begin in earnest until the mid-nineteenth century. Then, as is still the case today, funding for expeditions was hard to come by and those ships that managed to leave port were often poorly equipped for the tasks that lay ahead. In spite of the many obstacles placed in their path, the scientists and crew of these early voyages of discovery set in motion the development of a branch of science that fascinated and inspired those who followed in their wake.

During 1841 and 1842 the British vessel H.M.S. *Beacon* undertook what can be considered as the first deep-sea biological cruise. Working in the Aegean Sea, her crew made over 100 dredge hauls down to a depth of 230 fathoms (420m) (Menzies *et al.*, 1973; Rice, 1986). On board the *Beacon* was Edward Forbes, a young professor from Edinburgh University. He noted that deeper dredges contained fewer and fewer species, which led him to conclude that the oceans did not support life below 300 fathoms (600m), and so the concept of the 'Azoic Zone' was born. Although it has since been proved to be incorrect, this theory generated much debate and enthusiasm for the exploration of a hitherto ignored environment.

In 1868 the heroic age of deep-sea research began when Charles Wyville Thomson, also a professor at Edinburgh University, and W.B. Carpenter persuaded the Royal Society of London and the British Royal Navy to help them organise a cruise with the express aim of exploring deep water. Stimulated by the collections of Norwegian naturalist Michael Sars, gathered in the deep Lofoten Fjord (>660m), Wyville Thomson proceeded to undertake five cruises during the years 1868-1870, the first aboard H.M.S. *Lightning* (1868) and the latter aboard H.M.S. *Porcupine* (1869-70). During these cruises the scientists explored the deep water to the north and west of Britain and off the Iberian peninsula, recovering new forms of life from depths as great as 4289m (Gage and Tyler, 1991).

Historically, the most important discovery of these cruises was the recording of negative bottom temperatures 10 to 30' north of 60°N, and positive temperatures south of 50°N. This led Wyville Thomson to speculate on the existence of a submarine ridge separating the North Atlantic from the subzero waters of the deep Norwegian Sea (Thomson, 1874; Murray and Hjort, 1912; Menzies *et al.*, 1973). However, it was not until after the death of Wyville Thomson in 1882, that John Murray aboard H.M.S. *Triton*, actually charted the submarine ridge that to this day bears the name of Wyville Thomson. Murray went on to become an important figure in the fledgling science of oceanography, having begun his scientific career working alongside Wyville Thomson on the famous *Challenger* expedition of 1872-1876.

The success of the *Lightning* and *Porcupine* cruises led Wyville Thomson to put forward proposals for a circumnavigation of the globe, and in the December of 1872 H.M.S. *Challenger* set sail from Sheerness. The *Challenger* and her crew spent three and a half years traversing the world's oceans and sampling their untouched depths. The results of this groundbreaking scientific expedition are considered by many to represent the true birth of modern oceanography (Murray and Hjort, 1912; Menzies *et al.*, 1973; Rice, 1986).

After *Challenger* there followed a great era of pioneering deep-sea research, involving numerous cruises by vessels and scientists from several countries. The American Alexander Agassiz sampled off the East Coast of North America and in the seas of the Caribbean, during three cruises aboard the *Blake* (1877-1880), and later, in 1891, off Central America aboard the U.S. *Albatross*. In Europe, Alphonse Milne-Edwards and others aboard the French research vessels *Travailleur* (1880-82) and *Talisman* (1883) were sampling in the deep waters of the Mediterranean, Azores and Sargasso Sea (Menzies *et al.*, 1973; Rice, 1986). It was during these cruises that the first discovery and description of *Amperima rosea* Pawson (Perrier) was made (Hansen, 1975).

This era effectively came to a close with the voyages of the Swedish *Albatross* (1947-48) in the Atlantic and the Danish round-the-world voyage of the *Galathea* (1950-52) (Gage and Tyler, 1991). However, the work carried out on both these cruises made important technological contributions to deep-sea research. The voyage of the *Albatross* saw the development of the now widely used piston corer and single-wire otter trawl, and it was

during the latter expedition that the long search for life at the greatest depths of the ocean finally bore fruit, when those aboard the *Galathea* successfully recovered animals from the floor of the Philippines Trench (10,190m) (Gage and Tyler, 1991).

The 1960s and 1970s saw an increase in the amount of ecological and experimental research taking place in the deep sea, a field dominated by American researchers, such as Howard L. Sanders and Robert R. Hessler, working out of the growing oceanographic institutions at Woods Hole, Massachusetts and Scripps, University of California, San Diego.

The 1980s and 1990s ushered in an era of great proliferation in the number of deep-sea research programmes being undertaken, with institutions in Europe and the USA sharing expertise and technology in order to collectively overcome the challenges presented by one of our planet's most inaccessable environments. The effort and money invested in these programmes has certainly not been wasted as researchers have now monitored vertical organic flux from surface waters (e.g. JGOFS), measurements and samples have been taken at fixed stations over periods of several years, both in the Atlantic and Pacific oceans (i.e. BENGAL and STATION 'M'), and the discovery of new sites of hydrothermal venting has led to the discovery of new species and a vastly increased understanding of the biology and ecology of those already known (e.g the research programmes BRAVEX, BRIDGE, and AMORES). The discovery of an unexpected seasonal pattern of reproduction (Tyler and Gage, 1980; Tyler and Pain, 1982; Tyler *et al.*, 1984a; Tyler *et al.*, 1990) and respiration (Smith and Baldwin, 1984; Smith, 1987), within the deep-sea benthic invertebrate communities, has challenged the previously held views of constancy at the deep-sea floor (Tyler, 1995). Yet, even when coupled with the recent discoveries of hydrothermal activity at oceanic spreading centres and their chemosynthetically-based biological communities, our knowledge of the nature of life at deep ocean depths is still constantly expanding and is far from complete.

The future for deep-sea research is moving in a very technology-driven direction. The desire for knowledge and more importantly the need for experimental testing of scientific hypotheses can no longer be satisfied by the surface sampling techniques employed since the days of Wyville Thomson and the *Challenger*. The development of diving technology has been ongoing since the 17th century, when diving bells were first used to work down

to depths of 18m. However, it took a further three hundred years for the first deep water research vehicle, Beebe's Bathysphere, to be built and used in 1934 (Beebe, 1939; Busby, 1976; Welham, 1994). From that starting point the past 60 years have seen a rapid advancement in the level of technology and finance available for the development of manned research submersibles. Today scientists can dive to depths of over 6000m in the Indo-Pacific or cruise at 800m in the Bahamas using a wide selection of submersibles, such as the French *Nautile* and *Cyana*, the American *Alvin*, *Sea-Cliff* or *Johnson Sea-Link*, the twin Russian subs *Mir I* and *II*, or the deep-diving Japanese *Shinkai 6500* (Busby, 1976; Gage and Tyler, 1991; Welham, 1994). The introduction of Remotely Operated Vehicles (ROVs) such as the American *ANGUS* and *Jason*, the French *Victor* and the Japanese *Kaiko* (Busby, 1976; Gage and Tyler, 1991; Welham, 1994), has given scientists the opportunity to conduct experiments and collect samples at the deep-sea floor without the associated risks and costs of manned dives.

1.2. The deep-sea environment.

When considering the topography of the ocean floor, it is generally agreed that the deep sea starts at the edge of the continental shelf (Gage and Tyler, 1991). However, in terms of hydrographical parameters the deep sea is usually considered to be the region below the permanent thermocline, where stable temperatures of 4°C are eventually reached and the downward temperature gradients are small. In the majority of the world's oceans this more stable temperature regime is reached at 800-1300m depth, with the exception of the North Atlantic, where the outflow of warmer Mediterranean water depresses the 4°C isotherm to approximately 4000m. The water covering most of the abyssal plains of the world's oceans is ultimately derived from the sinking of surface water in the Norwegian Sea, and is termed North Atlantic Deep Water. In the Northeast Atlantic most of the water enters via either the 800m deep Faeroe Bank Channel or over the 450m crest of the Faeroe-Iceland Ridge. Cold Arctic Bottom Water also spills over the Wyville Thomson Ridge from the Faeroe Bank Channel into the northern Rockall Trough. The Northwest Atlantic basins are fed by water entering via the Denmark Strait, bisecting Iceland and Greenland.

With the exception of hydrostatic pressure and current energy the physical properties of the deep-sea environment exhibit a very narrow range at any site below the permanent

thermocline. Sunlight only has a secondary influence at depths below the photic zone (1000m), where surface derived organic material creates an important link in the deep-sea food chain, coupling surface primary production with the consumer community of the deep-sea benthos. Levels of salinity are also remarkably constant and at depths greater than 2000m it remains close to 34.8, only dropping to 34.65 at the very deepest parts of the ocean (Menzies, 1965).

Values of *in situ* oxygen concentrations are considered to be representative of the time since that body of water was at the surface and the reduction resulting from the respiration of the benthic and pelagic fauna (Tyler, 1995). Consequently the deep waters of the North Atlantic and Antarctica exhibit the highest values of oxygen concentration (6-7ml L^{-1}) whereas those waters with the greatest sub-surface age, i.e. the North Pacific, have values <3.6ml L^{-1} (Mantyla and Reid, 1983). Despite these low values, no organism at the abyssal plain depths will suffer anoxic conditions, except for highly localised effects. Truly anoxic conditions only prevail at the immediate exit of hydrothermal vents, enclosed basins such as the Black Sea and Cariaco Trench, and regions of upwelling such as the Arabian Sea (Tyler, 1995).

The hydrostatic pressure at any one point in the sea is a function of the water density and depth (Menzies, 1965). The increase in pressure with depth in the water column represents one of the longest environmental gradients on earth, increasing by 10^5Pa every 10m. It has been invoked as the cause of the observed decrease in diversity at the higher taxon level as depth increases (Tyler, 1995), although it now widely thought that food availability may be a controlling factor. Representatives of invertebrate taxa, such as the crustaceans, actinarians and echinoids, have not been observed at depths >6000m in the hadal (Gage and Tyler, 1991) or ultra-abyssal (Vinogradova, 1962) zone. Conversely, other taxa, especially holothurians and polychaetes, often appear to show an increased abundance below this depth, when compared to those communities at bathyal and abyssal depths. However, there are always exceptions and it often depends on where samples are taken as to how well they fit this general trend. For example some species of holothurian may be found in exceedingly large numbers at bathyal depths, e.g. *Kolga hyalina* (Billett and Hansen, 1982).

The seafloor at abyssal depths is predominantly a soft-sediment environment. Exposed hard rock tends to be confined to steep continental slopes, seamounts and along the mid-

oceanic ridges. Any hard substratum to be found on the abyssal plains tends to be sunken wood or, in the North Atlantic, large amounts of clinker (pers. obs.) or the occasional sunken vessel. In areas such as the South Bermuda Rise, South West Pacific, and Bellinghausen Basin, manganese nodules provide an abundant source of hard substratum and have their own distinctive fauna (Heezen and Hollister, 1971; Mullineaux, 1987).

The vast areas of seafloor at the flanks of the Mid-Atlantic Ridge and across the abyssal plains are covered with either biogenic oozes or clays, depending on the productivity of the overlying water. Biogenic pelagic sediments (oozes) contain >30% biogenic skeletal material, often derived from diatoms, foraminifera or radiolaria. The type of biogenic ooze found in deep-sea sediments varies with depth and geographic location as the corresponding carbonate compensation depth (CCD) also varies. These biogenic oozes may accumulate at a high rate, sometimes as much as several centimetres per thousand years (Gage and Tyler, 1991). Deep-sea sediments overlying the oceanic crust vary in thickness depending on the age of the underlying crust and on the rate of sedimentation. Sediment thickness tends to increase with increasing distance from the mid-ocean ridge, a pattern that can be observed using seismic profiles (Heezen and Hollister, 1971). Sediments on the abyssal plains may be thousands of metres thick, giving them their monotonous flat topography broken only by undulating abyssal hills, plateaux and seamounts.

In order to understand how the deep-sea benthos responds to its environment it is also important to have a good understanding of the physical properties and processes of the water column immediately adjacent to the seabed. One of the most relevant of these properties, from an ecological standpoint, is the Benthic Boundary Layer (BBL). This layer extends a few tens of metres into the water column and its thickness is limited by the attenuation of turbulent mixing (Richards, 1990). The motion of the BBL affects numerous parameters including recruitment of larvae to the benthos, and fluxes of nutrients to, and waste from, the seafloor. The resuspension of sediment into the BBL can affect the survival of filter feeders, many of which show adaptive body forms designed to overcome this problem (e.g. stalked crinoids). The sediment load in the near-benthic layers of the water column can also drastically increase during the period of a 'benthic storm' (Hollister *et al.*, 1984). These aperiodic physical phenomena occur when the energy created by mesoscale eddies in the surface waters is transmitted to the deep sea,

creating 'storms' which may last for a few days up to several weeks. During these 'storms' deep, cold currents flowing toward the equator are reversed and intermittently strong currents are formed with greater than daily average velocities of >15cm s^{-1} (Hollister *et al.*, 1984).

The discovery of these aperiodic perturbations along with periodic perturbations such as the seasonal deposition of phytodetritus (Billett *et al.*, 1983; Rice *et al.*, 1994) has challenged the previously held perceptions of constancy and continuity of the deep, non-vent, benthic environment. Fluctuations such as these may play an important role in the structuring of deep-sea communities.

1.2.1 The Northeast Atlantic Ocean

The NE Atlantic Ocean extends northwards from the equator and encompasses all the water masses to the east of the mid-Atlantic ridge up to the continental boundaries of Western Europe and Africa. It has a complex topography that divides the ocean into several basins separated by ridges and banks. It is delimited to the northwest by the Reykjanes Ridge, by Iceland to the north and the Iceland-Faroe-Shetland Ridge to the northeast. To the east it is bordered by the continent of Europe and on the continental slope southwest of Ireland, can be found the Porcupine Bank, Porcupine Seabight and Goban Spur. Heading further south the Porcupine Abyssal Plain is present as part of the West European Basin that extends down to the equator and includes both the Madeira and Cape Verde Abyssal Plains. The northeast Atlantic is a well-studied area and there are numerous oceanographic and biological data available for this area. Particularly well-studied localities include the Faroe-Shetland Channel (Turrell *et al.*, 1999; Bett, 2001), the Rockall Trough (Gage *et al.*, 1983; Gage *et al.*, 1985; Holliday and Reid, 2001; New and Smythe-Wright, 2001), the Porcupine Seabight (Tyler and Billett, 1987; Rice *et al.*, 1991) and the Porcupine Abyssal Plain (Rice *et al.*, 1994; Thurston *et al.*, 1998; Billett *et al.*, 2001).

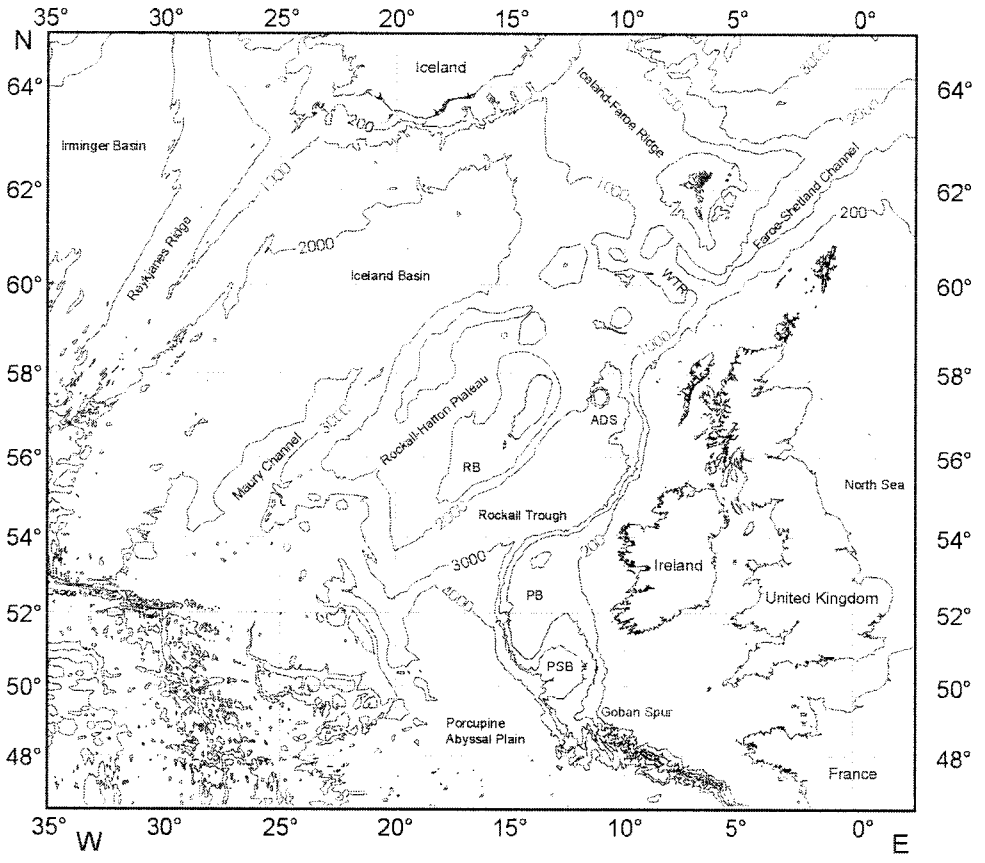

Figure 1.1. Bathymetric map of the NE Atlantic Ocean showing the major topographic features. ADS=Anton Dohrn Seamount; PB=Porcupine Bank; PSB=Porcupine Seabight; RB=Rockall Bank; WTR=Wyville Thomson Ridge.

1.2.1.1 Seabed character: sediments and water masses.

The sediments in the Porcupine Seabight are coccolith-foram marls with a carbonate ($CaCO_3$) content of 45-67% (Lampitt *et al.*, 1986), similar to those marls found in the northern Rockall Trough that have a $CaCO_3$ content of 51-57% (Thomson *et al.*, 2000). The coarsest sediments are found at the top of the slope (median Φ = 4.4, at 510m), but become finer with increasing depth (median Φ = 7.4, at 960m). From depths of 1500-4100m the median Φ is in the range 8.0 to 8.6 (Lampitt *et al.*, 1986). The sediments of the Goban Spur follow a similar trend changing from terrigenous sandy shelf sediments to clayey silts as depth increases. Current-influenced reworking of the sediment has produced an area of slightly coarser-grained sediment deeper down slope. On the steep western edge of the Goban Spur recent sediment cover is thin with approximately 40% rock outcrop and on the western flank of the Porcupine Bank sediment cover is thinner giving approximately 50-80% rock outcrop (Masson *et al.*, 1989). The sediments on the

Porcupine Abyssal Plain consist of a light grey *Globigerina* ooze (Patching *et al.*, 1986). Clinker is present throughout the entire area, particularly in the mouth of the Seabight and can be more abundant on abyssal seafloors than debris material deposited by ice-rafting or other geological processes. Ice rafted debris consists mainly of sedimentary and igneous rock, large boulders are rare and individual rocks rarely exceed 6cm diameter (Kidd and Huggett, 1981). The clinker and rock debris serve as a suitable substratum for sessile organisms (Rice *et al.*, 1991).

The deepest waters found in the ocean are formed in the cold surface layers close to the coast of Antarctica, and are given the generic term Antarctic Bottom Water (AABW)(Mantyla and Reid, 1983). This water sinks to form a circumpolar bottom water with branches penetrating all the main oceans. In the Atlantic this water flows up the west side at depths >5km but is prevented from doing so on the eastern side by the Walvis Ridge (Gage and Tyler, 1991). Some of this water may penetrate the northeast Atlantic by passing through the major fracture zones of the Mid-Atlantic Ridge. Overlying the AABW and covering most of the abyssal plains of the world's ocean is slightly less dense water. This water is ultimately derived from the sinking of surface water in the Norwegian Sea and is termed North Atlantic Deep Water (NADW). The interaction between Norwegian Sea Deep Water (NSDW), as it overflows the Wyville-Thomson Ridge into the Rockall Channel, and Subpolar Mode Water (SPMW)(van Aken and Becker, 1996) creates Iceland-Scotland Overflow Water (ISOW). The ISOW is a saline water mass, with temperatures around 2.5°C. It gradually becomes mixed with less dense waters as it moves south along the west side of the Rockall-Hatton Plateau, forming a modified water mass called North-East Atlantic Deep Water (NEADW). The NEADW is found in a salinity minimum around 2600m depth (van Aken and Becker, 1996). In the south of the Iceland basin, Porcupine Abyssal Plain and Southern Rockall Trough the NEADW is found below the Labrador Sea Water (LSW) and above another water mass, the Lower Deep Water (LDW). The LDW (<2.5°C) occurs at depths below 3000m in the Porcupine Abyssal Plain and at around 2500m in the southern part of the Rockall Channel (van Aken, 2000). LSW enters the PAP area from the northwest, turning largely southward near 20°W but retains a secondary branch that continues eastward and south-eastward into the northern Bay of Biscay (Paillet *et al.*, 1998). Temperatures in the range of 3.2-4°C have been recorded for LSW in this region (New and Smythe-Wright, 2001). NEADW, in some form, may enter the PAP from the northeast and the PSB from the

southwest as the result of deep-water circulation patterns. NEADW has a temperature of around 3°C. LDW enters the Porcupine region from the southeast (New and Smythe-Wright, 2001), has a temperature of less than 2.4°C (Harvey, 1982) and salinity of less than 34.90 (van Aken and Becker, 1996). The seasonal thermocline in this area is around 50m depth during the summer months, where as the permanent thermocline can be found between 600-1400m depth with an associated temperature decrease of 10°C to ~4°C (Rice *et al.*, 1991). The benthic fauna of the PAP is generally only ever in contact with the Lower Deep Water (LDW) that is believed to enter the PAP via a deep gap in the Azores-Biscay Rise. Evidence from direct and modelled measurements suggest that deep currents on the PAP have a cyclonic circulation, flowing northwards at 1-2cm s^{-1}, up the central and eastern part of the abyssal plain. They then turn westward and southward as the basin shoals to the north, then southward or westward along the eastern flank of the Mid-Atlantic Ridge (Dickson *et al.*, 1985; van Aken, 2000; New and Smythe-Wright, 2001). This circulation pattern is described as systematic and vigorous and superimposed on this residual current are semi-diurnal oscillations that produce current speeds of around 5cm s^{-1}, though they may be as large as 13cm s^{-1} (Vangriesheim *et al.*, 2001).

1.3 Benthic communities: the deep-sea perspective.

Over 90% of known marine animal species and nearly all of the larger marine plants live in close association with the sea floor. Collectively these organisms form what is referred to as the benthos. Benthic animals occupy the interface between the sea floor and the overlying water column, and they are adapted for life on or in particular bottom types. The structure of the benthic community is dependent on the properties of the seabed, which can vary from solid rock surfaces to very soft, muddy sediments. The distribution patterns of benthic plants and animals are strongly influenced by the texture and stability of their substratum. These features determine the effectiveness of locomotion or, for sessile species, the persistence of their attachment to the seabed. The interaction between animals also plays an important role in the structuring of benthic communities. Animals mix and sort the sediments through their burrowing and feeding activities, bringing oxygen and water from the sediment surface down into the deeper sediment layers via tubes and burrows. This activity can increase the vertical distribution of many species by increasing the depth of the oxygenated layer.

In shallow-water, benthic animals occupy a range of habitat types from the rocky intertidal to the soft-sediment estuaries. It is probably the soft-sediment communities of intertidal and shallow sub-littoral mudflats that share the greatest similarity to the majority of deep-sea benthic communities. Excluding the anomalies of hydrothermal vents and cold seeps, the faunal composition within deep-sea benthic communities is similar to that observed in shallow-water. Meio-, macro- and megafauna are all present, both in sessile and errant forms. In the deep sea the meiofauna is dominated by nematodes and foraminifera, with each taxa exhibiting a high degree of diversity (Lambshead, 1993). The macrofauna comprise a number of metazoan taxa, and are dominated by polychaetes, peracarid crustaceans and bivalve molluscs (Grassle and Maciolek, 1992). The errant megafauna is dominated by echinoderms, decapod crustaceans and fish, whilst the crinoids, poriferans and anthozoans are amongst the most common sessile megafaunal taxa observed on hard substratum areas (Lampitt *et al.*, 1986; Gage and Tyler, 1991).

In contrast to the shallow-water benthos, the deep-sea benthos is depauperate with respect to suspension feeders. Jumars and Gallagher (1982) stated that no more than 7% of deep-

sea benthic species are suspension feeders. Carnivores also appear to be less abundant among deep-sea invertebrates, although this is uncertain as separating omnivores and scavengers from the true predatory carnivores has proved difficult. The majority of deep-sea animals, some 80%, are deposit feeders ingesting sediment and utilising any organic matter contained therein.

1.3.1 Changing patterns within benthic communities.

The low density of benthic populations, the small size of most invertebrates, and the paucity of samples led to the deep sea being considered a biological desert for many years, with expectations of low diversity and a predominance of habitat-generalist species (Madsen, 1961). However, during the 1960s the known levels of species diversity within benthic communities increased dramatically as sampling efforts intensified and new techniques of sample collection and processing were developed. Sanders (1968) first postulated that the deep sea is a highly diverse community, an idea based on the relative abundance patterns of the deep-sea fauna. The total number of individuals tends to be sparse, with each deep-sea species commonly represented by only a few individuals. Grassle and Maciolek (1992) reported that over 90% of macrofaunal species were represented numerically by less than 1% of individuals sampled. Estimates of species numbers within the deep sea are largely based on the extrapolation of data from box core samples and rarefaction curves at depths where diversity has been shown to be relatively high (Grassle and Maciolek, 1992; Lambshead, 1993). Grassle (1991) suggested that the total number of benthic deep-sea species could number as much as 10 million, whilst Lambshead (1993) proposed that such a figure may account for only the benthic macrofaunal species. Figures such as these are comparable to those proposed by Erwin (1982, 1983) for tropical beetles, an indication that the deep sea maybe as diverse an environment as the terrestrial rainforests.

The possible processes driving such high diversity are still unknown, although Grassle (1991) cited four major potential factors: (a) Large scale environmental stability (e.g. oxygen, salinity and hydrographic disturbances), (b) Patchy food resources, (c) Localised autochthonous disturbances, and (d) Few large-scale barriers to dispersal.

Sanders (1968, 1969) proposed a stability-time hypothesis that stated that diversity should increase with increasing stability of the physical regime governing the habitat. Sanders proceeded to contrast two hypothetical, end-point communities, (1) a physically-controlled community and (2) a biologically-accommodated community. It was proposed that a physically-controlled community tends not to accumulate species because few species are physiologically capable of living there. Alternatively, a biologically-accommodated community would tend to accumulate species because of the lack of physiological barriers to immigration and the tendency of existing community members to evolve into specialised niches. Thus the hypothesis predicts that physically-controlled communities should be less diverse than biologically-accommodated ones. Sanders (1968) used this hypothesis to explain the increased diversity of polychaetes and bivalves with depth in the deep sea and it has also been used in other contexts to explain patterns of diversity in benthic communities (Gage, 1972). However, the hypothesis has also been criticised, on empirical and theoretical grounds (see Gray, 1974; Abele and Walters, 1979a, 1979b), and contradicted (see Thistle, 1983).

In contrast, Dayton and Hessler (1972) put forward another hypothesis in an attempt to explain the observed diversity patterns in the deep-sea benthos. The Disturbance Theory operates on the basic premise that organisms will generally increase in number until they reach the limit of some resource that is in least abundance. At this point competition occurs and species are eliminated. In the deep sea, the least abundant resource is most likely to be food, as low population densities mean competition for space does not occur. The theory suggests that deep-sea animals are all generalists, rather than specialists, in terms of their feeding strategies. It is suggested that high diversity is maintained because the intensity of indiscriminate feeding, by all levels and sizes, prevents any one species from increasing its population to the point where it is competing with another for the same food resource.

Jumars (1975, 1976) extrapolated the stability-time hypothesis to include the partitioning of trophic resources as an environmental parameter. This adaptation, coupled with the observation that species diversity is greater in oligotrophic than eutrophic regions (Hessler, 1974), appeared to confirm the hypothesis, but the role of dietary specialisation remains uncertain (Gage, 1996).

The distribution of food resources in the deep sea is often highly patchy and it has been proposed that these patchy resources may act not only as direct organic inputs, but also as disturbances (Gage, 1996). Examples of patchy food inputs include phytodetrital material, zooplankton remains, macroalgal and terrestrial plant remains, and vertebrate carcasses (Rowe and Staresinic, 1979). Around these nutrient rich 'islands', species diversity may increase (Gooday, 1988; Gooday and Lambshead, 1989) and the local community may become dominated by organisms that are rare in the background community, such as the vesicomyid clams found on and around the skeletons of Grey whales in the Santa Catalina Basin (Smith *et al.*, 1989).

However, it would appear that neither of these two hypotheses, plus others put forward such as the area hypothesis (Rex, 1981), are by themselves adequate to explain all deep-sea diversity patterns. It is likely that the observed patterns are the result of a combination of many factors interacting simultaneously, with certain factors having more influence at certain locations, depths, and times.

1.3.2 Benthos of the NE Atlantic: Porcupine Seabight and Abyssal Plain.

The Porcupine Seabight (PSB) is an amphitheatre-shaped embayment in the continental margin to the south-west of Ireland, measuring roughly 180 nautical miles (333km) from north to south and 100 nautical miles (185km) from east to west (Rice *et al.*, 1991). It is bounded by the Porcupine Bank to the west and north-west, the Irish and Celtic shelves to the north and east, the Goban Spur to the south and east, and opens onto the Porcupine Abyssal Plain (PAP) through a relatively narrow (approx. 40 nautical miles) entrance to the south-west. The Abyssal Plain itself covers an area of seafloor, more than twice the size of the landmasses of Great Britain and Ireland. It is bordered to the north by the Rockall Trough and by the Iberian and Madeiran abyssal plains to the south. To the west the plain extends to the foothills of the Mid-Atlantic Ridge and stretches east to the edge of the continental margin. It is generally accepted that the true abyssal plain is separated from the continental slope by the 4000m isobath, although some 'abyssal' species are found at shallower depths in the gently sloping mouth of the Rockall Trough. The abyssal plain, like many others in the world's oceans, is characterised by its monotonous flat terrain, punctuated occasionally by undulating abyssal hills and plateaux.

Factors affecting the benthic distribution of adult animals in the NE Atlantic are probably related to the hydrography and dynamics of water masses in the area. The depth of the winter mixed layer is around 600m and may influence the presence of the 'mud line' (Gage and Tyler, 1991) where Gage (1986) described the presence of an apparent maximum in the rate of faunal change at around 1000m depth. Distributions of animals may also be related to tolerances for pressure, particularly during the larval development phases of the life cycle. Young and Tyler (1993) and Young *et al.* (1996) report how embryonic pressure requirements may set the upper and lower bathymetric limits of a species adult distribution.

There are several studies reporting on the abundance and biomass of benthic invertebrates at the PSB and PAP sites, some of them comparing community structure with other deep-sea sites in the NE Atlantic (Pfannkuche, 1985; Rice *et al.*, 1991; Thurston *et al.*, 1994). Lampitt *et al.* (1986) completed a depth transect in the PSB, where a significant negative correlation between megafaunal biomass and depth was recorded. Megafauna were divided into three groups, crustaceans, echinoderms and "other phyla" (mainly sessile suspension feeders). The three groups were affected to different degrees by increasing depth, with crustacean biomass and abundance declining more rapidly with depth than those values recorded for echinoderms. "Other phyla" were found to fall between these two extremes. These differences caused a shift in relative importance of the three groups. The crustaceans (mainly decapods) were found to dominate only at depths <1000m, the suspension-feeders were also present at these depths, but between depths of 1100 and 1400m they contributed about 95% of the total biomass. The echinoderms were important members of the megafauna at all depths, but they particularly dominated at the deeper levels of the Seabight (Billett, 1991). Similar depth-related patterns of dominance have also been observed within the meiofaunal communities of the PSB (Pfannkuche, 1985).

Much of our knowledge concerning the benthic community structure of the PAP comes from numerous studies conducted at a central location, the recently named BENGAL site. In addition to the recent BENGAL time-series programme this site has been sampled periodically since the late 1970s. The megafaunal community of the deeper abyssal plain is dominated by echinoderms, most notably the holothurians, which account for >90% of the significant invertebrate taxa. In contrast, the megafauna of the Madeiran Abyssal Plain is dominated by asteroids, whereas ophiuroids dominate at the Hatteras Abyssal

Plain (Thurston *et al.*, 1994). On the PAP there can be found several species of dominant holothurian, the majority being members of the exclusively deep-sea order Elasipodida.

1.4 Holothurian Biology and Ecology.

1.4.1 General Biology.

The holothurians, or sea-cucumbers, constitute one of the five classes of the phylum Echinodermata. They are similar to echinoids in that they lack the arms of their nearest cousins, but in holothurians the oral-aboral axis is greatly lengthened and the animal tends to be differentiated dorso-ventrally, adopting a 'cucumber-like' shape. Most holothurians lie on their side, across interadii B, A and E, which is adapted to form a ventral sole (Hyman, 1955). Unlike other echinoderms, the endoskeleton is often reduced to the form of microscopic ossicles embedded in the body wall or is lost completely, e.g. *Benthothuria funebris* (pers. obs.). Ossicles can be used in taxonomic studies as an effective character for the identification and classification of many holothurian species (Hansen, 1975). At the oral end of the body, a circle of tentacles representing modified podia surrounds the mouth, which may be terminal or displaced ventrally or dorsally. The tentacles of holothurians are highly disparate in their form and structure. In many shallow-water forms the tentacles often surround a terminal mouth and can be outstretched to collect plankton or detrital particles from the surrounding water column or seafloor. They are then retracted and pushed one at a time into the mouth (Roberts and Moore, 1997; Roberts *et al.*, 2000).

The majority of deep-sea forms are deposit feeders, moving across the seafloor, feeding on the uppermost millimetres of the sediment (Billett, 1991). Most deep-sea holothurians have a crown of ventrally orientated tentacles, which they use to sweep up or delicately pick at, particles of organic matter on the sediment surface. One notable exception to this can be seen in the bathyal dendrochirotid *Psolus squamatus*. This animal has a dorsally located mouth and anus, whilst the ventral surface has become adapted to form a highly differentiated, and adhesive, sole. This curious species can be found attached to hard substrata, where they evert a very delicate crown of 'feathery' tentacles to trap organic particles from the water column (pers. obs.). Different modes of feeding can be related to

the highly disparate nature of the tentacle structure (Hansen, 1975; Roberts and Moore, 1997; Roberts *et al.*, 2000).

Most holothurians move by means of locomotory podia, located on their ventral sole. These podia, or tube feet, are hollow tubular projections of the body wall containing a branch of the water vascular system and terminating in a concave expansion acting as a sucker in most species (Hyman, 1955). In lacking a pentamerous arrangement, the reproductive system of holothurians differs from that of all other existing echinoderms. The single gonad is located in the anterior part of the coelom and opens to the exterior via a gonopore located in the mid-dorsal line, and often situated between the tentacles. The majority of holothurians are dioceous, however there are some hermaphroditic species such as *Cucumaria laevigata*, *Mesothuria intestinalis* and *Paroriza pallens*.

1.4.2 Deep-sea holothurians.

The class Holothuroidea comprises about 1100 species, 400 of which are from the deep-sea (>400m depth). Holothurians are the dominant epibenthic invertebrate taxon in many areas of the deep sea, featuring prominently in photographic collections of both bathyal and abyssal biota (Billett, 1991). In many areas they dominate the invertebrate megafauna both numerically (Rice *et al.*, 1982; Sibuet *et al.*, 1984; Sibuet, 1985) and in terms of biomass (Sibuet, 1984; Thurston *et al.*, 1994). Holothurians are one of the few faunal groups that can penetrate the deepest parts of the oceans; indeed they overwhelmingly dominate the fauna at hadal (>6000m) depths (Belyaev, 1972). There are six major orders within the class Holothuroidea, all of which have representative genera in the deep sea. However it is the order Elasipodida that tends to receive the most attention, as they are confined to the deep sea. Elasipodid holothurians dominate the deep-sea invertebrate megafaunal biomass in many areas of the world's oceans and the order contains many of the most widely distributed deep-sea species. The biology and ecology of elasipodid holothurians has been well reviewed in two comprehensive works (Hansen, 1975; Billett, 1991).

Hansen's volume of the *Galathea Report* (Hansen, 1975) provides a detailed classification and key to the five benthic families of the Elasipodida, as well as descriptions of their distribution, biology and evolutionary relationships. The

geographical distribution of many species suggests the presence of a pelagic phase during development or the faculty of leaving the seafloor as adults. There is evidence for juvenile and adult holothurians leaving the seafloor and being caught in pelagic nets, as well as the existence of truly pelagic holothurians (Billett *et al.*, 1985; Gebruk *et al.*, 1997), although as yet there is only one case of a special pelagic larval stage in the life-cycle of benthic holothurians (Gage, unpublished data).

The deep-sea environment, especially at abyssal depths, is so uniform that it is difficult to imagine distributional barriers for many elasipodid species. Sewell (1948) has already shown how deep-sea currents have been used for the dispersal of pelagic deep-sea copepods. In spite of this hardly two species of elasipodid have the same distribution, and only a few approach anything near a cosmopolitan distribution (Hansen, 1975). The absence of certain species from certain localities may be a reflection of the physical environment of the deep-sea acting to alter the conditions for competition. Turbidity currents may play a major role in the supply of phytodetritus to the deep-sea floor and Heezen *et al.* (1955) suggest that they may convey large amounts of nutritive matter from shallower waters to the abyssal fauna. If a region with an abundant food supply is bordered by relatively barren regions, then geographical isolation of certain species may occur, especially those which are thought of as specialist rather than generalist feeders. Billett (1991) reviews the deep-sea representatives of all six orders, but it is his detailed review of abundance, reproductive biology, locomotion and nutrition that provides the greatest insight into the life-histories of these animals.

1.4.2.1. Elasipodida of the Northeast Atlantic.

Since the late 1970s, the benthic megafauna of the Northeast Atlantic has received considerable scientific attention, particularly during long-term surveys at three major locations; a) the Rockall Trough (Gage *et al.*, 1983; Gage *et al.*, 1985; Gage, 1987), b) the Porcupine Seabight (Tyler *et al.*, 1985b; Walker *et al.*, 1987a, 1987b; Rice *et al.*, 1991), and c) the Porcupine Abyssal Plain (Tyler and Billett, 1987: Billett, 1991; Billett and Rice 2001; Billett *et al.*, 2001). Elasipodid holothurians dominate the fauna at abyssal depths where the key species have been extensively sampled and studied. Hansen (1975) refers to the North Atlantic having three key species of elasipodid holothurian, the cosmopolitan *Psychropotes longicauda* and *Oneirophanta mutabilis* and the rarer

Amperima rosea. Although not recorded from other geographical localities *Amperima* may have a much wider distribution within the abyssal zone than indicated by the paucity of earlier (pre-1996) samples. The elasipodids tend to dominate the holothurian megafauna at abyssal depths, although sampling on the PAP has revealed that a certain proportion of the holothurian biomass is attributable to the aspidochirotids, namely two species of the genus *Pseudostichopus*. Only two species of north Atlantic elasipodid holothurian have not been recorded in other oceans, one of them being *Amperima rosea*. At shallower, bathyal depths, as found in the Seabight and Rockall Trough, the holothurians compete with asteroids for dominance within the megafaunal community. In these localities, deep-sea representatives of the other holothurian orders (Tyler *et al.*, 1987; Billett, 1988; Tyler *et al.*, 1992; Tyler *et al.*, 1994) begin to make a contribution toward the megafaunal biomass. However, several abundant species still occur at bathyal depths (Tyler *et al.*, 1985a). Table 1.1 lists some of the key species of the order Elasipodida, including those that may be found in abundance at the PAP sampling site.

FAMILY	KEY SPECIES	REFERENCE
DEIMATIDAE	***Oneirophanta mutabilis***	Hansen (1968)
		Tyler and Billett (1987)
	Deima validum	Tyler and Billett (1987)
		Billett (1991)
LAETMOGONIDAE	*Laetmogone violacea*	Tyler *et al.* (1985b)
	Benthogone rosea	Tyler *et al.* (1985b)
PSYCHROPOTIDAE	***Psychropotes longicauda***	Tyler and Billett (1987)
		Billett (1991)
	Benthodytes typica	Tyler and Billett (1987)
ELPIDIIDAE	*Peniagone azorica*	Tyler *et al.* (1985a)
	Peniagone diaphana	
	Elpidia sp.	Kaufmann and Smith (1997)
	Amperima rosea	This study
	Kolga hyalina	Billett and Hansen (1982)
PELAGOTHURIIDAE	*Pelagothuria* sp.	Billett (1991)
	Enypniastes diaphana	Billett *et al.* (1984)

Table 1.1 Families and some key PAP species (bold) of the order Elasipodida (Holothuroidea: Echinodermata).

1.4.3 Amperima rosea (Family Elpidiidae: Order Elasipodida).

Amperima rosea Pawson 1965 is a small elasipodid holothurian of the family Elpidiidae. One of 8 species in the genus *Amperima* it is a true abyssal species with the upper limit of its bathymetric distribution lying around the 4000m isobath. Prior to the start of the IOS sampling programme at the PAP, the only recorded specimens of *Amperima rosea* were taken by Perrier in the late 1880s and by Hèrouard in the early 1900s. All these samples were collected south of the PAP between the Azores and Portugal (4060-5005m depth). *Amperima rosea* is a small (<80mm), gelatinous organism similar in overall appearance to its relatives *Ellipinion molle* and *Kolga hyalina* (Hansen, 1975; Billett and Hansen, 1982). *A. rosea* has an ovoid body supported by 9-10 pairs of tube feet, which border the entire ventral sole. At its anterior end it has a well-developed velum formed from two pairs of fused dorsal papillae and a ventrally orientated crown of 10 oral tentacles (see Figure 1.2). The easiest way that *Amperima rosea* can be distinguished from its conspecifics and congeners is by examining the calcareous deposits found in its body tissue. The shape of these deposits, or spicules, are subtly different in every species of holothurian. *A. rosea* has distinctive tripartite spicules, which under close inspection are found to possess undivided apophyses on each of their three arms (see Figure 1.3).

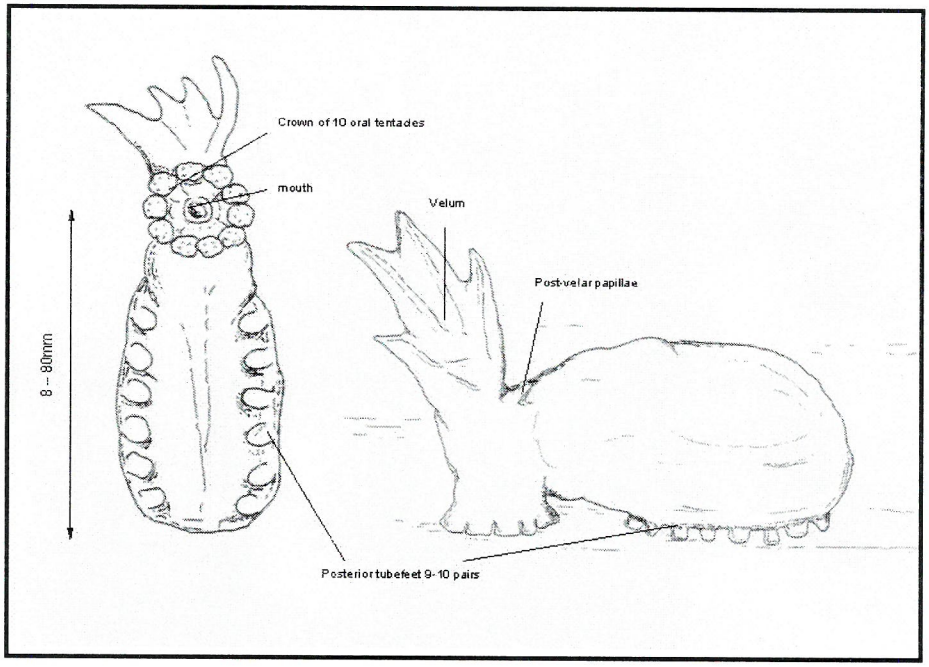

Figure 1.2. Diagram of *Amperima rosea*. Ventral and side views showing major taxonomic characters.

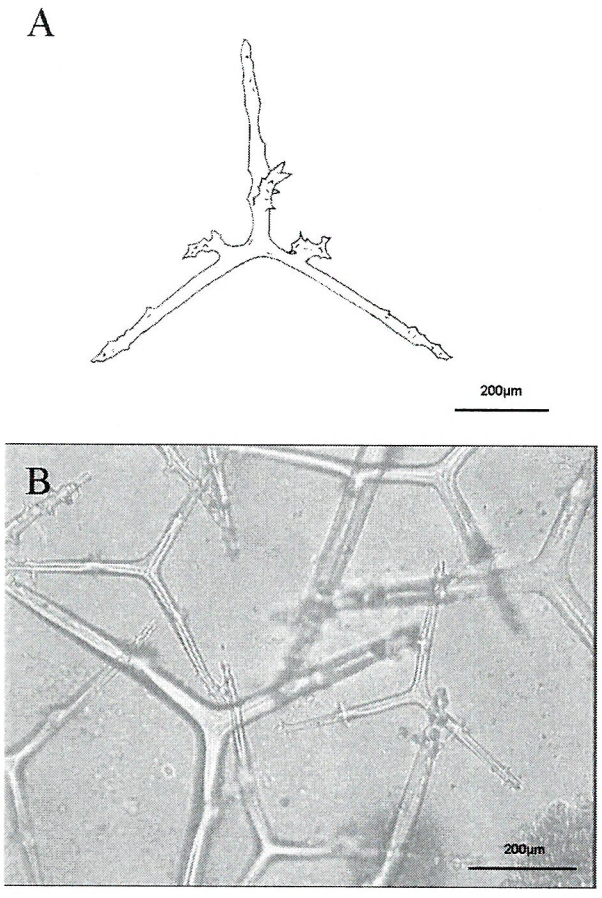

Figure 1.3. Calcareous deposits (spicules) from the body wall tissue of *Amperima rosea*. A, diagram showing tripartite structure; B, light microscopy image of spicules.

1.5 Response of benthic organisms to organic input: phytodetritus and the concept of seasonality in the deep sea.

1.5.1. Organic input to the benthos: phytodetritus.

On an area basis, by far the greater part of the aquatic benthos is found at depths where the light intensity does not permit plants to live by the chemical process of photosynthesis. Under these conditions the benthic community relies, for its food, on that sinking from the euphotic zone above.

The deep sea is considered a food-limited environment (Dayton and Hessler, 1972; Thiel, 1979) in which the abundance and biomass of benthic organisms is related directly to the amount of food reaching the sediment surface (Tseytlin, 1987). This relationship has been described for the majority of benthic groups including the meiofauna (Gooday, 1988; Gooday and Lambshead, 1989; Loubere, 1991), the macrofauna (Carey, 1981; Galéron *et al.*, 2001) and the deposit-feeding megafauna (Lampitt *et al.*, 1986).

Only 1-3% of the primary production generated by photosynthesis reaches the deep-sea floor (Gage and Tyler, 1991), the majority via particles settling through the water column. These 'particles' of organic material can be classified into four main groups; i) large animal remains, i.e. vertebrate carcasses, (Smith, 1985), ii) large plant remains, i.e. macroalgae and terrestrial material (Schoener and Rowe, 1970; Grassle and Morse-Porteus, 1987), iii) larger particles, i.e. faecal pellets, zooplankton moults, phytoplankton remains, (Fowler and Knauer, 1986) and iv) macroaggregates, i.e. 'marine snow', (Alldredge and Silver, 1988; Alldredge and Gotschalk, 1989).

Within the context of this study the most relevant groups for consideration are those of the particulate organic matter (POM) and the associated macroaggregates, or 'marine snow'. The application of sediment traps (Honjo and Doherty, 1988) and deep-sea photography (Lampitt, 1984) has increased our collective understanding of the nature of the downward flux of this material. It has been shown that this flux can be traced into water deeper than 4000m, and in some areas of the world's oceans it arrives as a seasonally predictable pulse. It has also been shown that sinking rates are much greater

than previously thought, with particles sinking at an approximate rate of 100m d^{-1} (Billett *et al.*, 1983).

The flocculent material that constitutes 'marine snow' is probably derived from a variety of sources including gelatinous pelagic fauna, such as salps, and aggregated diatoms (Alldredge and Silver, 1988; Alldredge and Gotschalk, 1989). In the northeast Atlantic Ocean the arrival of a surface-derived phytoplankton pulse was first reported by Billett and co-workers in 1983. By using photo-transects obtained from an epibenthic sledge (Aldred *et al.*, 1976) and time-lapse photography using the '*Bathysnap*' system (Lampitt and Burnham, 1983) they were able to observe a patchy layer of detrital material settling on the seafloor in the Porcupine Seabight (PSB), at depths ranging from 1370-4100m. The settling of phytodetrital material on the seafloor has also been observed at depths approaching 5000m on the Porcupine Abyssal Plain (PAP) (Thiel *et al.*, 1989). The timing of the phytodetrital 'drop' has been shown to be highly variable, with the initial arrival of phytodetritus on the seafloor reported as early as mid-April, at bathyal depths in the PSB (Billett *et al.*, 1983), and at the mid-oceanic BIOTRANS site (Pfannkuche, 1993), late May on the PAP (Rice *et al.*, 1994) and as late as mid-June at the mouth of the PSB (Lampitt, 1985).

1.5.2. Seasonality: the influence of a pulsed arrival of phytodetritus.

As early as 1880, Moseley identified the possibility of a periodic variation in the food supply to the deep sea. He noted that it "may give rise to a little annual excitement amongst the inhabitants" of the deep-sea benthos (Tyler, 1988). In shallow water ecosystems, both marine and freshwater, benthic communities have been shown to respond rapidly to the deposition of phytoplankton blooms (Graf *et al.*, 1983; Goedkoop and Johnson, 1996). Graf (1989) has also shown how a benthic community, at bathyal depths in the Norwegian Sea, can react within days to a pulse of phytodetritus, while Drazen *et al.* (1998) have suggested that protozoans, at abyssal depths in the northeast Pacific, may respond to phytodetrital pulses on time scales as short as weeks.

Analysis of deep-sea meiofaunal communities has revealed how phytodetritus is rapidly colonised by some benthic foraminiferal species, with species exhibiting high densities in the sediments beneath areas with a phytodetrital input compared to those without

(Gooday and Lambshead, 1989; Pfannkuche, 1993). Phytodetritus has also been shown to be an important site for microbial activity, with patches being rapidly colonised by a range of prokaryote micro-organisms (Lochte and Turley, 1988; Turley and Lochte, 1990). In addition, Thiel *et al.* (1989) found higher bacterial abundance and biomass in sediments sampled during periods of phytodetritus coverage. These microbial organisms in turn provide a food source for the foraminiferan and nematode communities, both of which rapidly proliferate in the phytodetritus (Gooday, 1988; Gooday and Lambshead, 1989; Gooday and Turley, 1990). The foraminifera, at least, will in turn be consumed by the larger megafaunal deposit feeders, such as the holothurians, which appear to selectively graze on the phytodetritus-rich surface sediment (Billett *et al.*, 1988).

The influence of phytodetritus as a food source for invertebrate megafauna has been highlighted in numerous studies, some of which have shown that certain species of holothurian and ophiuroid feed almost exclusively on phytodetritus when it is deposited on the seafloor (Billett *et al.*, 1988; Iken *et al.*, 2001). The vertical flux of organic material has been shown to have a distinct seasonal variation at certain locations in the world's oceans (Billett *et al.*, 1983; Lampitt, 1985; Deuser, 1986) so it can be expected that the benthic organisms that rely on this sinking material for a food source may exhibit some form of seasonal pattern in their critical physical attributes of respiration, growth and reproduction (e.g. Tyler *et al.*, 1982a).

Drazen *et al.* (1998) reported how the sediment community oxygen consumption (SCOC), at an abyssal site in the northeast Pacific, exhibits a significant seasonal pattern and shows a strong temporal correlation with particle flux. SCOC has also been shown to increase dramatically in response to increased particle flux at other sites in the eastern North Pacific (Smith, 1987), the North Atlantic (Pfannkuche, 1993), and the Norwegian Sea (Graf, 1989). The long-term time series measurements, reported by Drazen *et al.* (1998), also suggest that seasonal variation in SCOC is a regular seasonal occurrence in the eastern North Pacific.

Respiration at both the community level and individual level has been measured using *in situ* techniques, such as the Free Vehicle Grab Respirometer (FVGR) (Smith *et al.*, 1997). Although there is considerable evidence for seasonal variation in oxygen

consumption at the community level, there is so far no evidence for any seasonality in the respiration rates of individual species (Smith, 1983).

Seasonal variation in the growth of individuals is well documented for shallow water animals. In those groups that possess some form of calcareous skeletal structure, such as the molluscs and echinoderms, seasonal growth may be represented by a banding or ring-like pattern in the plate or shell. These patterns are considered to be indicative of an annual periodicity in the rate of somatic growth, but in the deep sea the presence of such banding patterns is unexpected given the assumed constancy of the conditions (Tyler, 1988). In spite of this expectation a ring-like pattern reflecting growth checks, rather than shell sculpture, has been observed on the shells of *Ledella pustulosa*, a protobranch bivalve, found at 2900m in the Rockall Trough (Gage, 1985 cited in Gage and Tyler, 1991). Data from the Rockall Trough time-series (Tyler and Gage, 1980; Gage and Tyler, 1982b; Gage and Tyler, 1985; Gage, 1987; Gage, 1990) have also provided evidence of seasonal growth banding in echinoids, that is most likely to be associated with the observed seasonal variation in phytodetrital flux, as two of the species under consideration, *Echinosigra phiale* and *Hemiaster expergitus*, show no evidence of seasonal reproduction.

The possibility of seasonal reproduction occurring in deep-sea species was first suggested in the late 1960s (George and Menzies, 1967, 1968). Measurements of body size in samples of the ophiuroids *Ophiura ljungmani* and *Ophiomusium lymani*, showed a greatly increased number of juvenile sizes during the summer compared with winter, indicating a seasonal pattern of recruitment and therefore a possible seasonal pattern of reproduction (Schoener, 1968).

Investigations into the prevalence of seasonal reproduction as a viable strategy for deep-sea invertebrates really gathered momentum in the late 1970s and early 1980s, culminating in the discoveries made during the SMBA-led Rockall Trough time series programme. The analysis of size-frequencies of various echinoderm species collected at depths of 2200m and 2900m also suggested a seasonal pattern of reproduction, similar to that proposed by Schoener back in 1968 (Gage and Tyler, 1981a, 1981b; Gage and Tyler, 1985). An examination of the reproductive biology of deep-sea species provides one of the most effective ways of determining patterns of seasonality, and is often achieved by

tracing variation in gametogenic biology using gonad indices or by oocyte sizing (Tyler, 1988). These methods have been employed successfully to show patterns of seasonal reproduction in several taxa. However, it is from the San Diego and Rockall Troughs that the best evidence for reproductive seasonality has been obtained. Harrison (1988) provided evidence for seasonality of breeding intensity in deep-sea isopods from the Rockall Trough. A significantly greater percentage (25%) of females were found to be brooding during the winter, than in the summer (7%). It was also noted that the onset of vitellogenesis coincided with the summer deposition of phytodetritus. The Rockall Trough fauna has also provided several other species, from numerous taxa, which exhibit patterns of seasonal reproduction. Bishop and Shalla, (1994) investigated the reproductive biology of the cumacean *Leucon profundus* and found that vitellogenesis progressed from early to late during the period April-June to July-September. The presence of newly produced broods in the marsupium from February-April indicated oviposition early in the year. Both vitellogenesis and the release of young were shown to coincide with the seasonal peak of phytodetrital flux to the seabed. Two species of bivalve mollusc, *Ledella pustulosa* and *Yoldiella jeffreysi*, have been shown to exhibit a distinct cycle of ovarian growth, with a small oocyte size in January-February to a maximum oocyte size of 100μm in December to early January (Lightfoot *et al.*, 1979). The deep-sea anemones *Paracalliactis stephensoni* (Van-Praet, 1990) and *Amphianthus inornata* (Bronsdon *et al.*, 1993) also reproduce on a seasonal basis producing eggs of diameter 180μm and 205μm respectively.

As highlighted by the previously described studies, there is an increasing amount of evidence for the direct relationship between sinking organic material and variation in the reproductive cycles of deep-sea invertebrates. Sinking organic matter, such as phytodetritus, is thought to provide a labile food source that can be rapidly utilised for reproductive development. Boolootian *et al.* (1959, cited in Hansen, 1975) demonstrated that the echinoid *Allocentrotus fragilis*, from c.150m depth, exhibits a distinct breeding cycle that is probably correlated with planktonic larvae utilising a phytoplankton bloom. There is however a problem with this simple hypothesis, in that the majority of deep-sea invertebrates that exhibit a continuous pattern of reproduction, do not appear to respond to this seasonal influx of food. Therefore it has been suggested, by Tyler (1988), that the link between sedimentation of seasonally varying surface-derived organic matter and

seasonal patterns in the physiological processes of the deep-sea benthos must be facilitated by the animals' mode of feeding.

Lampitt (1985) used time-lapse images to show how *Echinus affinis*, a seasonal breeder (Tyler and Gage, 1984a), feeds on the carpet of organic material gathering on the seafloor. Analysis of the gut contents of *E. affinis* supports this observation (Campos-Creasey *et al.*, 1994). Billett *et al.* (1988) also used gut content analysis to show how deep-sea holothurians, from the Porcupine Seabight, will feed selectively on freshly deposited phytodetritus. Analysis of the chlorophyll content of the deposited detritus, superficial layers of the sediment, and the holothurian gut contents produced similar fluorescence chromatograms, indicating that *Benthogone rosea* and *Laetmogone violacea* feed directly on the detrital layer and the few millimetres of sediment lying directly beneath it. Data for a third species, *Paroriza pallens*, was not so comparable, indicating that it may adopt a different feeding strategy from *B. rosea* found at the same location. However, even though this and other more recent studies (Ginger *et al.*, 2000, 2001; Iken *et al.*, 2001) have shown how some holothurians feed preferentially on freshly deposited phytodetritus, there is still no record of a seasonal pattern of reproduction for any of the 400 known species of deep-sea holothurian. The discovery of seasonal cycles in the deep oceans has shown how the deep-sea community is not so isolated and different from those communities in shallower water, as had previously been thought. The direct influence of surface derived primary production has tightened the coupling between the benthic and pelagic ecosystems, providing a means for ongoing changes in climatic conditions to influence processes at the deep-sea floor.

1.6. Aims of this study.

The main objective of this study is to examine the driving forces at work behind an observed change in the mega-benthic community structure at an abyssal locality in the NE Atlantic Ocean. Major changes in abundance have been recorded for several invertebrate taxa, most notably the Holothurians. This study focuses on the patterns of change among the benthic megafauna and on the biology and ecology of the Elasipodid holothurian *Amperima rosea*. The abundance of *Amperima rosea* on the Porcupine Abyssal Plain increased dramatically by several orders of magnitude, making it the dominant species in the benthic megafaunal community. Several different techniques were employed in this study in an attempt to assess the megafaunal changes and to analyse various aspects of the ecology and biology of *A. rosea*.

The major topics covered in this study are;

1. Megafaunal changes on the Porcupine Abyssal Plain.

2. Reproductive Biology of *Amperima rosea* in relation to a variable supply in surface derived organic matter.

3. Abundance and sediment tracking. Utilising time-lapse photography to assess the scale and potential impact of the "*Amperima* Event".

4. Diet and selective feeding. Analysing chloropigments in the gut contents of *Amperima rosea* and other abyssal holothurians using HPLC methods.

5. Population genetics of *Amperima rosea*. Investigating temporal and spatial relationships between *Amperima* populations using DNA sequence analysis.

Chapter Two – Faunal change on the Porcupine Abyssal Plain

2.1. Introduction.

2.1.1. Long-term sampling programmes: case studies from the literature.

Knowledge of temporal change, on a variety of time-scales, is of fundamental importance if we are to understand how systems function and respond to environmental change. Time-series sampling is essential for the assessment of change in both deep-sea and shallow-water communities. The funding of larger, multi-disciplinary research programmes has increased our collective knowledge of the oceans. However, there are still relatively few locations that have been subjected to a continued period of sampling and monitoring. With the exception of the hydrothermal vent fields of the Pacific, Atlantic and now Indian mid-ocean ridges, the majority of longer term monitoring, both in shallow and deep water, has been focussed on variability in ocean primary productivity. The coupling of this productivity, via particle fluxes, to the processes occurring within the benthic faunal community has received a great deal of attention from numerous workers in areas as diverse as the highly productive Southern Ocean and the oligotrophic Cretan Sea.

The discovery of seasonal, annual, and possibly decadal patterns in deep-sea benthic processes has increased the need for an understanding of how systems, communities and populations behave and fluctuate naturally within time and space. This knowledge will become increasingly beneficial if we, the scientific community, are to accurately assess the impact of anthropogenic disturbance as human activity extends into deep water in search of new resources and/or a location for the dumping of unwanted waste material.

The following sections review the aims and major conclusions of some of the previous time-series sampling programmes that came before the BENGAL programme. Many deal with the study of vertical flux of organic carbon, and its subsequent influence on deep-sea benthic communities, in several of the world's major oceans and seas.

2.1.1.1 The CINCS project: pelagic-benthic Coupling IN the oligotrophic Cretan Sea.

Conducted by collaborating EU Institutes between May 1994 and June 1996 the main goal of this project was to detect and quantify the transfers of shelf and surface-derived organic matter to the benthos on a bathymetric, seasonal and inter-annual basis (Tselepides, 2000). The Cretan Sea is one of the most oligotrophic areas in the world ocean; it receives almost no terrestrial run-off and hence is relatively impoverished in terms of nutrients. Throughout the two-year duration of the CINCS project particle fluxes were monitored both in the water column and as they were deposited on the seafloor. The responses to these fluxes by various components of the benthic community were also examined along with an assessment of the benthic biodiversity on both a temporal and spatial basis. One of the most distinctive features recorded during this project was the persistent intrusion of Transition Mediterranean Water (TMW) from the east, at intermediate depths of 200-700m. These intrusions were apparent during each sampling period and they have important implications to the functioning of the system. Being an older water mass the TMW is comparatively richer in nutrients than other water masses present in the Cretan Sea (Tselepides *et al.*, 2000b). The intrusion of TMW contributed to the importation of nutrients into the euphotic zone and so enhanced productivity in the area and affected the distribution of organic matter. Annual primary production was estimated to be 80 g C m^{-2} yr^{-1} over the continental margin, but further offshore it dropped to just 20 g C m^{-2} yr^{-1}, of which more than half was supported by nutrients released by remineralisation in the upper layers of the water column.

Values of mean particulate organic carbon (POC) flux, recorded from sediment traps, were lower than those that would be expected based on observed rates of primary production. This suggests that fluxes of dissolved organic carbon (DOC) may be far more important component in the carbon export to deeper waters than those of POC/faecal pellets (Danovaro *et al.*, 2000).

A clear, though weak, signal from the surface waters to the benthos was evident throughout the late winter-early spring mixing period. This was manifested in the observed increases in the fluxes of chloroplastic pigment and faecal pellets reaching the deep benthic environment (Danovaro *et al.*, 2000). The response elicited in the benthic

ecosystem was detected as an increase in the metabolic rates and increases in the abundance and biomass of the bacterial community (Duineveld *et al.*, 2000).

In contrast to classic studies in the NW Atlantic, which show an increase with depth in the diversity of a variety of macrofaunal taxa from the shelf to upper-rise depths (Sanders, 1968; Rex, 1981), the CINCS project revealed a decreasing pattern of macrofaunal diversity, as well as abundance and biomass (Tselepides *et al.*, 2000a).

2.1.1.2. The abyssal NE Pacific.

Prior to 1989 a lack of long time-series measurements, examining the input of pelagically-derived food supply and its subsequent impact on benthic boundary layer (BBL) processes, led Ken Smith Jr and colleagues to establish a long-term, abyssal study site in the NE Pacific Ocean. Since 1989 they have continuously monitored the flux of sinking particulate matter through the BBL and have employed time-lapse photography to record dynamic benthic processes. Measurements of particulate, suspended, and dissolved organic and inorganic fractions through the water column and in the sediments have also been made along side the monitoring of sediment community oxygen consumption (SCOC). The location for Smith's Pacific site had to fit a series of principal criteria including: 1) a low-relief area at abyssal depths with no recent evidence of turbidity flows or slumping, 2) strong seasonal variability in surface water productivity, and 3) logistically convenient for seasonal occupation by research vessels. In June 1989 a site was chosen, centred at 34°50'N, 123°00'W, 4100m depth. Named Station M, it was conveniently located 220km west of Point Conception off the central Californian coast.

For the studies in this programme the BBL was defined as the sediments and the overlying 600m of the water column. The 600m altitude was above the zone of resuspension that extended from the seafloor up to ~200m above bottom (mab)(Smith *et al.*, 1994). Light transmission profiles suggested that the benthic mixed layer extended up to 80 mab with an average of 45 mab (Beaulieu and Baldwin, 1998). Fluxes of sinking particulate matter entering the BBL varied seasonally, with a primary peak in early summer and a secondary peak in late autumn (Baldwin *et al.*, 1998). Significant interannual variations in flux patterns were observed during the 1992-1993 El Niño Southern Oscillation event when sinking particulate matter fluxes were greatly reduced

(Baldwin *et al.*, 1998). With the aid of time-lapse camera systems detrital aggregates were observed on the seafloor, typically arriving in summer and remaining visible through late autumn (Lauerman and Kaufmann, 1998; Smith *et al.*, 1998). Sinking particulate matter fluxes at 50 mab were greater than at 600 mab suggesting resuspension or near-bottom lateral advection of material into the study site (Baldwin *et al.*, 1998). Phytodetritus reaching the seafloor, and collected using the submersible *Alvin* in 1994, varied temporally in composition from a dominance of chain-forming diatoms in August to phaeodarians in September (Beaulieu and Smith, 1998). During that same year a flocculent layer of detrital material carpeted the seafloor, in contrast to previous years when the detrital cover was less pronounced, further indicating substantial interannual variability in particle flux (Lauerman and Kaufmann, 1998). The concentrations of total and organic carbon, total nitrogen, chlorophyll *a*, and phaeopigments in detrital aggregates collected from the seafloor were similar to those concentrations found in sinking particulate material but were higher than those recorded for the surface sediments (Smith *et al.*, 1998). Responses to this variable flux of organic matter were observed for both the meio- and macrofaunal fractions of the benthic community. Density and biomass of protozoan meiofauna increased significantly over a four-week period following the sedimentation of detritus to the seafloor. This indicated that the response time of these organisms to organic inputs could be on the time-scale of weeks. The protozoans, dominated by agglutinated foraminifera, and five dominant metazoan macrofaunal taxa, including nematodes and polychaetes, exhibited seasonal increases in density during the winter months after detrital aggregates had disappeared from the seafloor (Drazen *et al.*, 1998). The abundance and distribution of epibenthic echinoderms were also examined using camera-sled surveys and time-lapse photographs. There were few consistent temporal patterns, abundance varied spatially and distributions were generally indistinguishable from random. In contrast, the detrital aggregates on the seafloor exhibited a clumped distribution. The lack of consistent correlation between echinoderm distribution and abundance and the presence of aggregate material was attributed to the persistence, for long periods, of extensive detrital coverage on the seafloor (Lauerman and Kaufmann, 1998).

This unique data set, which is still growing, highlights the close links between surface production and benthic processes. This bentho-pelagic coupling provides a pathway for seasonal variability in the fluxes of sinking particulate matter to have an impact on the

suspended particulate and dissolved matter in the water column and ultimately elicit a response from the sediment community.

2.1.1.3. Joint Global Ocean Flux Study (JGOFS): Northeast Atlantic.

The North Atlantic Ocean, both east and west, is of great importance to the ocean's regulation of climate and to its control of the global carbon cycle, and it was the Northeast Atlantic that provided the location for JGOFS first large and internationally co-ordinated process study. This study, referred to as the North Atlantic Bloom Experiment (NABE), was carried out in spring and summer 1989 at seven sites along a trophic gradient between 18° and 72°N. This process studies approach was repeated in 1990 and 1992, and all three studies were designed to quantify inter-annual variations of the spring bloom development. While new production over the spring season was quite similar during all three studies (Newton *et al.*, 1994), particle flux to the deep ocean varied considerably between 1989 and 1990 (Newton *et al.*, 1994) as a result of differences in dominant phytoplankton species and food web dynamics (Boyd and Newton, 1995). During spring 1992 atypical patterns of atmospheric forcing gave rise to a sequence of small phytoplankton blooms that significantly affected deep particle flux and food supply to the sediment community (Pfannkuche *et al.*, 1999).

Much of the work coming out of the JGOFS programme in the Northeast Atlantic focussed on upper ocean processes, particularly primary production (Louanchi and Najjar, 2001; Oschlies, 2001), but the vertical flux of organic carbon to the deep ocean is also of great importance. The spring phytoplankton blooms support events of 'pulsed sedimentation' (Lampitt and Antia, 1997) that are believed to export organic carbon to the deep ocean sediments via a more efficient pathway than the oligotrophic gyres (Billett *et al.*, 1983). One of the major problems associated with estimating particle-flux using trap data are the biases resulting from hydrodynamics, organisms actively entering the traps and fractional dissolution of particulate matter. During the JGOFS programme in the Northeast Atlantic Scholten *et al.* (2001) used naturally-occurring radioactive isotopes to measure the efficiency of deep-ocean sediment traps. ^{234}Th has already been used successfully to demonstrate and quantify the uncertainty related to hydrodynamic biases in drifting shallow-water sediment traps. Scholten *et al.* (2001) measured the distribution of ^{230}Th and ^{231}Pa in the water column and in traps, and by applying various particle

dynamics models they measured the ^{230}Th trapping efficiency to be between 9 and 143% for different traps. No relation to current velocities was observed. The long half-life of the ^{230}Th isotope (75,200 years compared to 24 days for ^{234}Th) make it important to consider the lateral transport and scavenging of ^{230}Th at the ocean margins when using it to calibrate particle interceptor traps.

Numerous studies have now shown how the arrival of this vertical particle flux at the deep seafloor can control both biological and chemical processes and elicit a response from the benthic fauna. During JGOFS studies in the northeast Atlantic Christiansen *et al.* (2001) compared the structure and respiratory carbon demand of the benthopelagic fauna and epibenthic megafauna at two sites in the northeast Atlantic, one in the Iceland Basin (59°N, 20°W) and one at the NABE-47 site (47°N, 20°W). The total biomass of benthopelagos was five times greater at 59°N than at 47°N. Epibenthic fishes dominated the northern site, while the majority of the biomass at the southern site resulted from the contribution of epibenthic megafauna. However, it should be noted that Christiansen's sites were at different depths (2900m at 59°N and 3800-4550m at 47°N) and varied topographically, which would undoubtedly influence the faunal communities sampled at these sites. Based on the respiratory carbon demand of the benthopelagos and a rough estimate of the demand of bacteria in the BBL, Christiansen *et al.* (2001) concluded that the sum of the total carbon demand of the epipelagos and of the sediments (e.g. Pfannkuche, 1993; Pfannkuche *et al.*, 1999) exceeds the carbon supply. This neatly provides indirect support for Scholten *et al.* (2001) who suggested trapping efficiencies in deep-traps at the NABE-47 site may be less than 100%.

Detecting possible long-term trends and changes against the background of the interannual variability of biogeochemical processes and hydrodynamics is best achieved using the time-series approach. In the North Atlantic, time-series studies of upper-ocean processes were established at the European Time Series Station (ESTOC) near the Canary Islands and at a site near Bermuda in the Northwest Atlantic (Michaels and Knap, 1996).

2.1.1.4 JGOFS: Equatorial Pacific.

The equatorial Pacific Ocean is also of great importance to the ocean's control of the global carbon cycle. It plays a major role in two aspects of this cycle: the flux of CO_2 to the atmosphere; and the export of organic carbon and $CaCO_3$ to the deep sea. Between 1990 and 1996 scientists from Australia, France, Japan and the U.S.A have conducted major field programs in the area between 12°N and 16°S, and 179°E and 180°W. The major emphasis of the equatorial Pacific programme was on carbon fluxes and their controls, but also focussed on the control of benthic processes by this biogenic particle flux (Smith *et al.*, 1997). This multi-national collaborative programme substantially improved our knowledge of carbon fluxes and their controls in the equatorial Pacific (Berelson *et al.*, 1997; Dunne *et al.*, 1997; Smith *et al.*, 1997; Wakeham *et al.*, 1997). Initial estimates were that DOC was the major form of exported new carbon production (Murray *et al.*, 1994). As more data were collected the contribution resulting from DOC has decreased. Murray *et al.* (1996) concluded that when integrated over 10°N to 10°S new production and particulate export were approximately in balance and Hansell *et al.* (1997) suggested that DOC export is less than half of the new production. This programme of study in the equatorial Pacific made great progress toward understanding the controls on carbon cycling and fluxes. It is now generally accepted that iron is the limiting nutrient for new production. Various models for this region have illustrated that iron supply controls the variability of primary production, but it is grazing that balances primary production and controls phytoplankton biomass. The reason for a high nitrate environment, such as the equatorial Pacific, having low levels of chlorophyll is because of grazing (Loukos *et al.*, 1997). Kelvin waves and tropical instability waves were both found to influence variability, possibly because they control the vertical transport of iron into the euphotic zone (Eldin *et al.*, 1997). This variability was subsequently recorded by a series of high temporal resolution measurements, of physical and bio-optical properties, collected using a moored instrument array (Foley *et al.*, 1997).

As part of the US programme in this region, the translation of these variable particle fluxes into the benthic ecosystem were studied to evaluate their control of key benthic processes. Rates of bioturbation near the equator were found to be about ten-fold greater than at 9°N, and appeared to exhibit substantial dependence on particulate-organic carbon flux, age-dependent mixing of the sediments and pulsed mixing from burrowing urchins

(Smith *et al.*, 1997). Where measured, megafaunal and macrofaunal abundances were strongly correlated with annual particulate-organic carbon flux. Sedimentary microbial biomass was also correlated with the flux of organic carbon, but less strongly than larger biota and on shorter time scales (i.e. approximately 100 days). Smith *et al.* (1997) concluded that the vertical flux of biogenic particles exerts a tight control on the nature and rates of benthic processes in the abyssal equatorial Pacific. They suggested that global changes in productivity on decadal or greater time scales could yield profound changes in deep-sea benthic ecosytems.

2.1.1.5. DEEPSEAS: Deep-Sea Seasonality in the abyssal NE Atlantic.

Two abyssal sites in the northeast Atlantic, with presumed contrasting regimes of organic carbon supply, were studied by the Institute of Oceanographic Science, Deacon Laboratory (now part of the DEEPSEAS Group, Southampton Oceanography Centre). The first of these sites, on the Porcupine Abyssal Plain (PAP), has an overlying water column with a winter mixed layer in excess of 500m and was forecast to receive a highly seasonal organic input, a significant fraction arriving in the form of rapidly sinking phytodetritus derived from the spring bloom. The winter mixed layer over the second site, on the Madeira Abyssal Plain (MAP), is much shallower at ~150m, and the resulting flux to the benthos was expected to be quantitatively less and not in the same form of aggregated phytodetritus as that observed on the PAP.

Photographic surveys, utilising both time-lapse and transect photography, were used to assess the presence or absence of phytodetritus on the abyssal seafloor (Rice *et al.*, 1994). There is considerable year-to-year variability in the timing of the main abyssal water column flux (Newton *et al.*, 1994) and the phytodetrital drop, potentially reflecting differences in the timing of algal blooms in the surface waters. The photographic results of this study support the hypothesis that the PAP site is subject to a seasonal supply of aggregated phytodetritus to the seafloor but that the MAP site is not. However, sediment trap data do not support the hypothesis that predicted a significantly higher and more seasonal flux to the seafloor at the PAP locality. It is possible that the main difference between these two sites is that a significant proportion of flux at the PAP site arrives at the seafloor in the form of large, possibly more labile, phytodetrital aggregates, while that at the MAP site is mainly in the form of small particles not easily detectable on

conventional photographs (Rice *et al.*, 1994). Whatever the true situation, the differences highlighted by the photographic surveys are supported by quantitative data for the benthic macrofauna and megafauna at the two sites (Thurston *et al.*, 1994). Results suggest that for the >1mm macrofauna the numerical abundance and biomass are 6.5-8.0 and 20-60 time greater at the PAP site compared to the MAP. However, the biomass may be affected by differences in the dominant fauna between sites. The megafaunal numerical abundance and biomass are also 1.8-3.7 and 15-40 times greater respectively. It should be noted that measures of megafaunal biomass were made using values of wet-weight. It is important that this is taken into consideration when comparing the biomass of the two sites as the structure of the communities is very different. The PAP megabenthos is dominated by large 'wet' holothurians, whereas the MAP megabenthos, although generally less abundant, is dominated by calcareous, 'dry' asteroids. Using ash-free dry weight (AFDW) would have made a better comparison (see Lampitt *et al.*, 1986). Such a contrast in abundance, and probably biomass, is hard to explain except in terms of a difference in the quantity or quality of the incoming organic matter (Thurston *et al.*, 1994). The differences in organic matter input to these two sites have now also been shown to have an influence on life-histories of the megabenthos (Ramirez-Llodra *et al.*, in press)

The work begun at the PAP site during this programme has continued and been extended to encompass a more multi-disciplinary approach to the study of seasonality in the deep ocean. The EU BENGAL time-series programme returned to the PAP site to study the impact of a seasonally variable flux of organic matter on the biology and chemistry of the abyssal seafloor.

2.1.2 The BENGAL programme: High resolution temporal and spatial study of the BENthic biology and Geochemistry of a north-eastern Atlantic abyssal Locality.

BENGAL was a three-year multidisciplinary study of how the abyssal benthic boundary layer (BBL) in the northeast Atlantic responds to, and modifies, the incoming material flux to the seafloor. The programme set out to determine how the geochemistry of abyssal sediments and the characteristics of the BBL community change seasonally in response to a highly seasonal input of organic matter from the overlying water column (Billett and Rice, 2001). The quantification and characterisation of the downward fluxes of organic matter and its arrival at the seafloor was undertaken using a range of observational techniques. These included time-series sediment traps, marine snow profilers, benthic lander systems, long-term moorings and time-lapse photography. The fate of the incoming particle flux once it had arrive in the BBL, and how the benthic community reacted and interacted with it, were addressed by analysing radioisotopes (Iken *et al.*, 2001), the organic and inorganic chemistry of core samples (Ginger *et al.*, 2001; Witbaard *et al.*, 2000), the gut contents of the fauna (Roberts *et al.*, 2001) and sediment profile images, and by conducting *in situ* incubation experiments.

BENGAL studied how the seasonal fluctuations in particle flux affected the composition and activity of all faunal size classes in the BBL food web, including microbiota, protozoan and metazoan meiofauna, macrofauna, megafauna, fish and near-bottom zooplankton, micronekton and scavengers (Billett and Rice, 2001).

During the mid-1990s, when the BENGAL project was being formulated, numerous aspects of the structure and functioning of the deep-sea benthic ecosystem were poorly understood. Rice *et al.* (1998) produced an 8-point rationale designed to address some of the outstanding gaps in our knowledge of deep-sea BBL processes. These 8 points were:

1. To monitor the quantitative and qualitative temporal and spatial variations in the particulate flux to the BBL over a full seasonal cycle at a site influenced by marked seasonal changes in organic flux from the overlying water column.
2. To monitor the hydrodynamic regime within the BBL.

3. To obtain in situ measurements of mass solute fluxes across the sediment/water interface, covering four seasons in a single year.
4. To undertake a quantitative analytical description of the composition and activities of all benthic faunal size classes of the BBL food web over a full year.
5. To describe the temporal kinetics of organic matter, both within the sediment and within the guts of benthic deposit feeders, thorough observation and experiment, and to relate these data to the abundance and distribution of micro-and meiofaunal organisms.
6. To improve our understanding of the complex relationships between the activity of the benthic fauna and its imprint on the sedimentary record using a combination of radioisotope distributions within the sediment and biological studies.
7. To establish the relationship of all the above to carbon burial using a variety of proxies, including opal and lipid biomarkers and a mix of radio-tracers covering a range of half-lives.
8. To reconcile the divergent geochemical and biological approaches to seafloor carbon cycling by developing a three-dimensional diagenetic model for the BBL system.

The BENGAL study area was located in the central region of the Porcupine Abyssal Plain (c. 4850m depth) approximately 270km southwest of Ireland. The central sampling site was at 48°50'N, 16°30'W (see Figure 2.1). This locality had been sampled between 1989 and 1994, during other EU-funded projects (Rice *et al.*, 1994). It was chosen because it is a relatively flat area, remote from both the continental slope to the east, and the mid-ocean ridge and its associated foothills to the west. This location is unlikely to be influenced by strong downslope or advective processes, making it suitable for the study of the vertical, or near vertical, deposition of detrital material and its impact on the BBL system. The BENGAL site also lies between two other important sampling sites in the northeast Atlantic; firstly 47°N 20°W, in the foothills of the mid-Atlantic Ridge, a site studied extensively during the JGOFS North Atlantic Bloom Experiment (NABE) (Ducklow and Harris, 1993), and secondly the European continental margin southwest of Ireland, which was the focus for the Ocean Margin Exchange programmes (OMEX)(van Weering *et al.*, 1998).

The BENGAL programme provided the first evidence of long-term, persistent change in the deep-sea ecosystem, not only changes in faunal abundance but in sediment chemistry and the vertical flux of organic material.

2.1.3. Faunal change on the Porcupine Abyssal Plain.

During the BENGAL programme observations were made of a dramatic change in the structure of the benthic community over an entire range of faunal taxa. The holothurians are the dominant megafauna in the majority of bathyal and abyssal samples, both numerically and in terms of biomass (Billett, 1991), and it was they that exhibited the most pronounced changes of both abundance and dominance within the benthic community. The greatest contributor to this change was the small elasipodid holothurian *Amperima rosea* Pawson 1965, previously notable only for its scarcity in earlier samples. Since its first description, *A. rosea* has been a minor component of the northeast Atlantic benthic community, and in all samples taken by recent oceanographic expeditions it has occurred in very small numbers or not at all. The appearance of large numbers of *A. rosea* in the north-east Atlantic had not previously been recorded since the early 1900s, when Hérouard noted an exceedingly high abundance of individuals on the Iberian Abyssal Plain west of Portugal (Hérouard, 1923). The sudden appearance of this holothurian prompted Hérouard to remark that the distribution of *A. rosea* must be highly localised. The inference was that its distribution was localised geographically within space, but equally it may have been localised in time.

Over a three-year period (1996-1999) the abundance of *A. rosea*, and its close relative *Ellipinion molle*, increased by two or three orders of magnitude (Billett *et al.*, 2001), leading to the overall changes in the benthic system being termed the "*Amperima* Event". The increase of a single species is not unknown in deep-sea communities. However, these events tend to be localised spatially, restricted to a single species and, in the case of the closely related holothurian *Kolga hyalina*, to have lasted no more than one or two years (Billett and Hansen, 1982). The "*Amperima* Event" encompasses significant increases in abundance of a number of different taxa and has occurred over a large area and has persisted for at least five years. It is argued that the observed changes in abundance of *A. rosea* and the overall scale of the community change are more likely to be the result of environmental forcing rather than stochastic change. In addition to changes in holothurian

abundance accompanying changes were recorded in the abundance of other megafaunal taxa (Billett *et al.*, 2001), macrofauna and meiofauna (Galéron *et al.*, 2001), organic sediment chemistry (Witbaard *et al.*, 2000; Ginger *et al.*, 2001) and flux of surface-derived organic material (Lampitt *et al.*, 2001).

This chapter examines temporal changes in megafaunal community structure during the 11-year period from September 1989 to October 2000. It also assesses potential spatial variability on the PAP by examining the additional trawl samples from 1998 and 1999.

2.2. Materials and Methods

2.2.1. Sample collection and preservation.

All trawl samples (1989-2000) were taken in the middle of the Porcupine Abyssal Plain to the southwest of Ireland (Table 2.1) (Rice *et al.*, 1991; Thurston *et al.*, 1994; Thurston *et al.*, 1998). The trawls were located within a 20 nautical mile (37km) radius of the central BENGAL station (48°50'N 16°30'W) where the associated coring programme was undertaken. Seabed depth within the sampling area varied within a few metres of 4840m (trawled range 4802-4850m).

Additional trawls in March 1998 were fished some 30 nautical miles (55km) to the east of the BENGAL area (Figure 2.1 Site A). Three further sites were added in April 1999, they were located 50km southeast, 50km northeast, and 100km to the north of the BENGAL site (Figure 2.1 Sites, B, C, D). All the additional trawls were undertaken to set the temporal samples taken at the single BENGAL locality in a wider spatial context within the PAP environment.

Sampling was undertaken with a semi-balloon otter trawl (OTSB 14) with a wing-end spread of 8.6m (Merrett and Marshall, 1981). The height of the net from the footrope to the headline was approximately 1.5m. The net was constructed of 44mm aperture stretch mesh in the main part of the net, 37mm aperture stretch mesh in the middle part, and a 13mm aperture stretch mesh liner in the cod end. During fishing, the OTSB was monitored by an acoustic beacon mounted on one of the trawl doors. Acoustic telemetry provided information on the depth of the net, the time the trawl spent in contact with the seabed, and the fishing performance of the net, as measured by the angle of the trawl door. Distance trawled and wing-end spread of the net was used to calculate an area fished for each trawl. This area was recorded in units of hectares (1ha=10,000m^2).

Samples were sorted at sea to at least family level, although most specimens could be sorted to species level. Specimens were fixed in 5% borax-buffered formaldehyde in seawater, and transferred to 80% methylated spirit after 3-5 days. The majority of samples were weighed and measured upon return to the laboratory, with the exception of some of the holothurians from 1999 that were measured and weighed at sea. These values

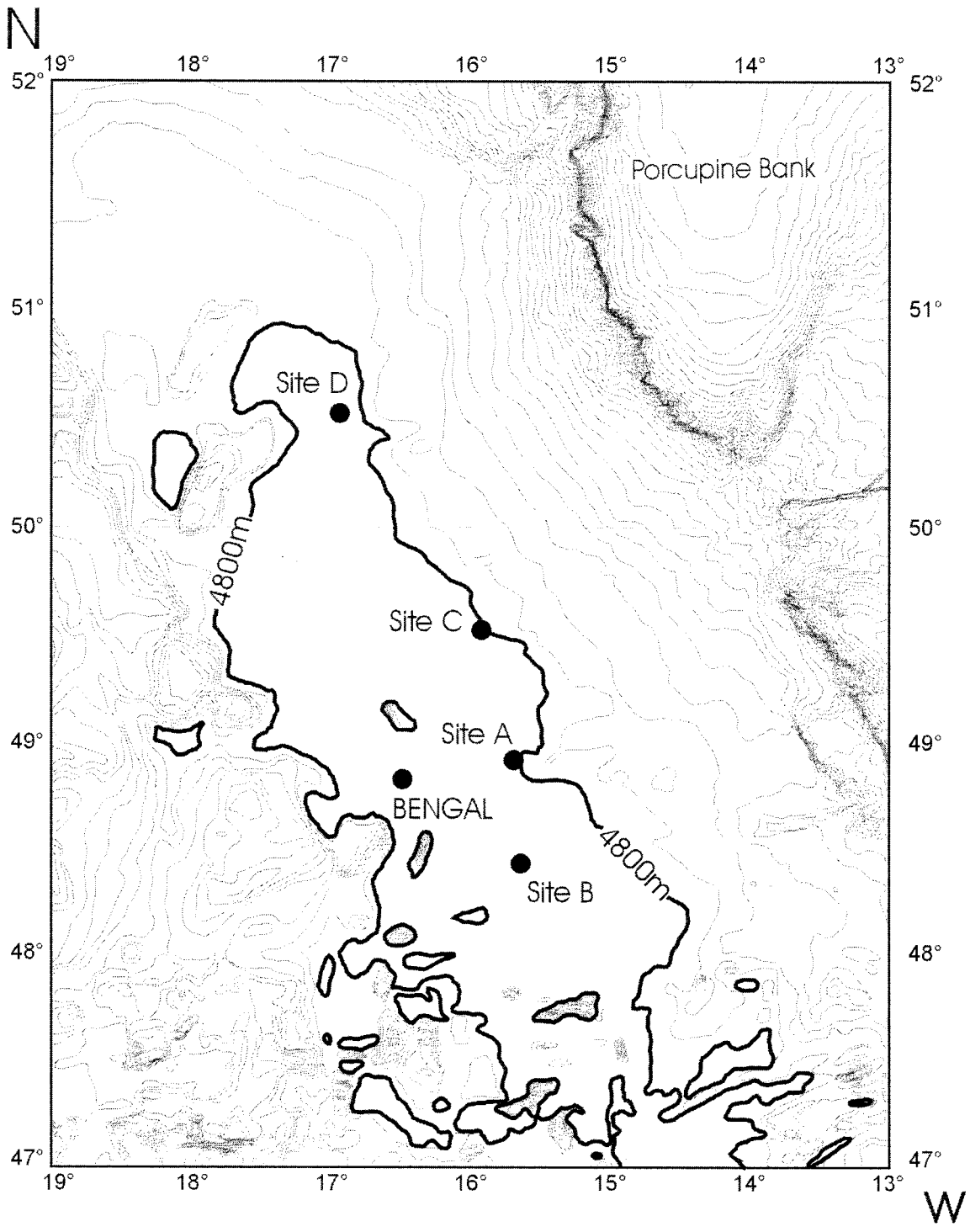

Figure 2.1. Bathymetric chart of the central Porcupine Abyssal Plain showing the location of the BENGAL sampling area and the additional trawl sites used to assess spatial variability in the distributions of invertebrate megafauna. Site A trawls were taken in March 1998 and the site B, C and D trawls were taken in April 1999. Contours every 200m.

were then adjusted for comparison with the preserved measurements made for other samples, by re-weighing after preservation.

Variations in species/taxa abundance and biomass were assessed statistically by analysis of variance and analysis of similarity (ANOVA and ANOSIM; Sokal and Rohlf, 1995) using the Minitab 12.0 and PRIMER 5 software packages. Inter-annual variability in the composition of the megafaunal community was assessed by multivariate analysis using the PRIMER 5 package (Clarke and Warwick, 1994).

For the purpose of this study the majority of the analysis was restricted to 9 major invertebrate taxa, including a more detailed focus on seven species of holothurian (see Tables 2.2 and 2.3). However the multivariate analysis of community change took into account all available data on the abundance of benthic invertebrates, including values for 29 identified taxa (see Appendix I).

2.3. Results.

2.3.1. Taxonomic composition of the PAP megabenthos.

More than 20 major taxonomic groups were recognised among the invertebrate megafauna of the PAP (see Appendix I). The holothurians were the dominant taxon in terms of both abundance and biomass, which is typical of many deep-sea communities, especially in the Northeast Atlantic (Billett, 1991). In terms of abundance, actinarians, ophiuroids, asteroids and polychaetes were also major components of the PAP community. However, during all three periods (1989-91, 1996-98 and 1999-2000) the holothurians remained the dominant taxon. During the pre-BENGAL period the dominant holothurian species, in terms of abundance, were *Oneirophanta mutabilis* Théel, 1879, *Pseudostichopus villosus* Théel, 1886 and *Peniagone diaphana* Théel, 1882. Both *O. mutabilis* and *Pseudostichopus villosus* showed no significant change in abundance during the BENGAL period, whilst *Peniagone diaphana* showed a significant decrease in abundance. During the BENGAL period it was *Amperima rosea* and *Ellipinion molle* Théel, 1879, which became the dominant megafaunal species on the PAP. The post-BENGAL period saw a decrease in the abundance of *A. rosea*, although it was still the most abundant holothurian on the PAP ahead of *O. mutabilis and Psychropotes longicauda*.

2.3.2. Megafaunal change: temporal variation in abundance and biomass.

Over the period of study (1989-2000) significant changes in invertebrate megafaunal abundance were observed at the BENGAL site on the PAP (see Figure 2.1 for location). The mean total abundance of megafauna, sampled by otter trawl between 1996-1998, varied between 193 and 379 ind. ha^{-1} (average 299 ind. ha^{-1}). These abundances were about three times greater than the mean abundances found at the BENGAL site between 1989 and 1994, and almost double the mean abundances recorded for the post-BENGAL period (1999-2000). Figure 2.2 shows the temporal variation in the abundance of invertebrate megafauna and the contribution from the holothurians (the most dominant of the nine taxa under consideration) to that total abundance. There was a sharp increase in abundance at the start of the BENGAL programme in September 1996 followed by an even more dramatic increase in April 1997. The changes in total invertebrate abundance were mirrored by changes in the abundance of the holothurians.

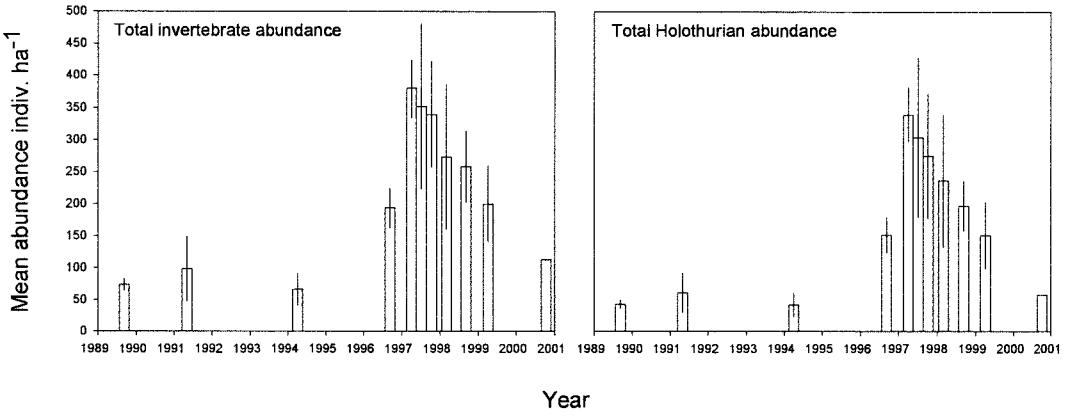

Figure 2.2. Temporal variability of mean abundance (± standard deviation) of total invertebrate and holothurian megafauna at the BENGAL site, PAP. 1989-2000.

The holothurians were a major component of the trawl catch, often accounting for 82 to 97% of the total invertebrate biomass and 51 to 93% of the total abundance. The greater variability in the holothurian contribution to total abundance is reflective of the dramatic increases in abundance observed during the "*Amperima* Event" (1996-1998). However, the contribution to the overall invertebrate wet-weight biomass does not vary to the same extent over the same period (see Figure 2.3). The large increase in abundance, accompanied by a non-significant change in biomass, results from the dramatic increase in abundance of two small holothurian species. *Amperima rosea* and *Ellipinion molle* are small (<80mm) elpidiid holothurians, previously scarce in abyssal samples from the PAP.

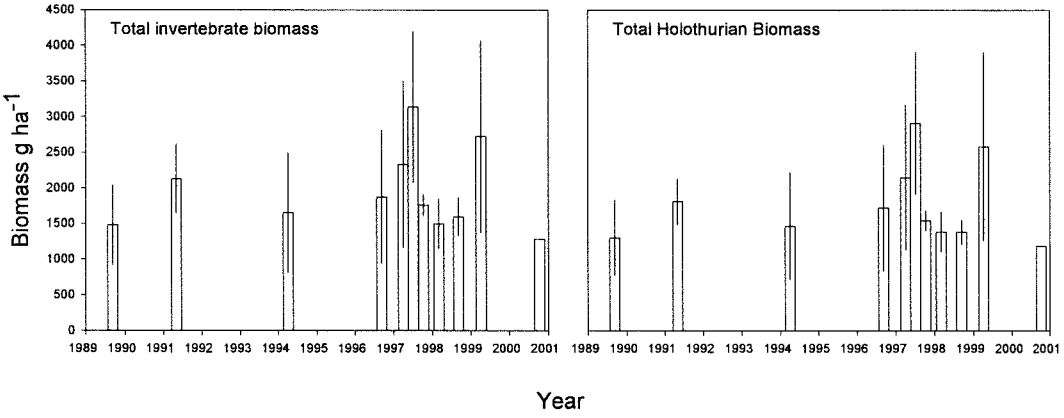

Figure 2.3. Temporal variability of mean biomass (± standard deviation) of total invertebrate and holothurian megafauna at the BENGAL site, PAP. 1989-2000.

Amperima rosea alone accounted for 3-80% of the holothurian abundance and 2-73% of the total invertebrate abundance, yet only 0.2-30% of the holothurian biomass and 0.2-28% of the total invertebrate biomass. The dramatic increase in abundance of *A. rosea* over the period of study is illustrated by Figure 2.4.

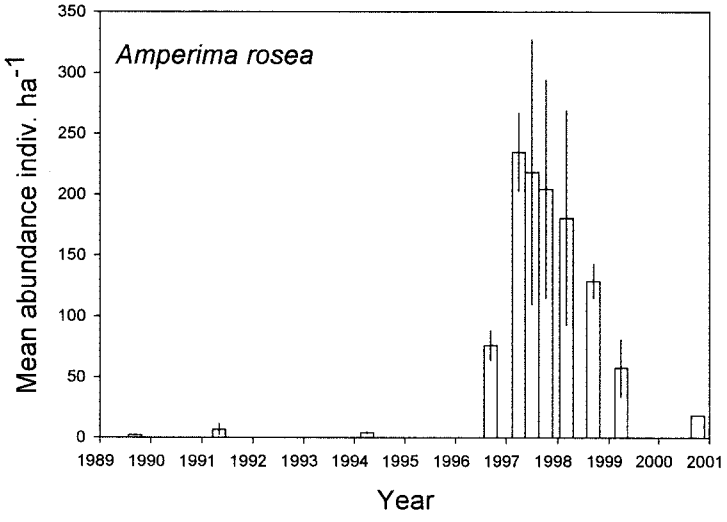

Figure 2.4. Temporal variability of mean abundance (± standard deviation) of *Amperima rosea* at the BENGAL site, PAP. 1989-2000.

Before 1996, *A. rosea* was rare, with a mean abundance of 2 to 6 ind. ha^{-1}. By September 1996 its abundance had increased to about 76 ind. ha^{-1}, and in March 1997 to about 234 ind.ha^{-1}. After March 1997 its abundance declined slightly, although it still dominated the megabenthos numerically through 1998, 1999 and into 2000.

Unlike previous reports of rapid change in deep-sea benthic communities, the "*Amperima* Event" encompassed change in a range of invertebrate taxa and species, predominantly but not restricted to, the holothurians. Table 2.2 shows the mean abundance and standard deviation for nine major invertebrate taxa sampled during 12 cruises over the 11-year period. There are significant differences between cruises, years and periods for five of the nine taxa. The decapod crustaceans and the Porifera showed no significant change in abundance, while the actinarians and asteroids only exhibited significant between cruise and between year variability. The actinarians did show an increase in abundance between the pre-BENGAL and BENGAL periods, an increase that remained constant throughout the post-BENGAL period, which may account for a lack of significant difference being

	Pre-BENGAL			BENGAL						Post-BENGAL				
Date	Sep-89	May-91	Apr-94	Sep-96	Apr-97	Jul-97	Oct-97	Mar-98	Sep-98	Apr-99	Oct-00	ANOVA Cruises	ANOVA Years	ANOVA Periods
Taxa														
PORIFERA	0.17 (0.08)	0.08 (0.02)	0.15 (0.16)	0.31 (0.16)	0.08 (0.14)	0.29 (0.23)	0.26 (0.26)	0.1 (0.07)	0.11 (0.03)	0.06 (0.07)	0.21 -	ns	ns	ns
ACTINARIA	6.21 (1.48)	9.63 (4.31)	7.23 (1.89)	10.12 (2.47)	11.95 (2.60)	12.75 (6.31)	23.33 (1.17)	11.76 (4.40)	18.23 (2.77)	17.99 (4.73)	10.99 -	P<0.005	P<0.005	ns
ZOANTHIDEA	6.46 (4.22)	3.46 (1.26)	0 -	1.59 (0.40)	1.92 (1.01)	2.54 (1.20)	2.14 (1.06)	2.39 (1.78)	6.98 (4.59)	0.51 (1.02)	0 -	P<0.01	P<0.001	P<0.001
ANNELIDA	3.6 (0.86)	4.35 (3.27)	4.58 (1.8)	11.49 (2.17)	9.78 (6.14)	12.49 (6.35)	13.49 (2.96)	8.52 (3.56)	8.36 (3.90)	11.56 (3.32)	23.11 -	P<0.001	P<0.001	P<0.05
DECAPODA	3.69 (0.87)	2.06 (1.53)	1.23 (0.52)	0.94 (0.24)	0.736 (0.44)	1.05 (0.52)	2.23 (0.35)	0.58 (0.63)	0.23 (0.40)	1.23 (0.74)	0.29 -	ns	ns	ns
ASTEROIDEA	3.69 (0.61)	8.45 (5.99)	3.78 (2.11)	3.87 (2.29)	3.15 (1.94)	3.74 (2.96)	6.38 (0.89)	2.48 (1.50)	5.35 (1.61)	4.07 (1.55)	2.43 -	P<0.005	ns	ns
OPHIUROIDEA	0.56 (0.15)	2.51 (0.38)	2.26 (1.35)	1.28 (0.52)	1.02 (0.57)	2.12 (1.24)	3.94 (1.08)	1.23 (1.40)	6.91 (0.71)	3.49 (2.04)	2.92 -	P<0.001	P<0.001	P<0.001
HOLOTHUROIDEA	42.71 (6.69)	60.65 (31.03)	41.43 (18.33)	151.165 (28.02)	339.66 (42.69)	303.92 (124.92)	274.64 (97.02)	235.79 (104.03)	196.17 (38.81)	150.34 (52.22)	57.43 -	P<0.01	P<0.005	P<0.001
TUNICATA	0.99 (0.84)	0.33 (0.19)	0.67 (0.52)	5.12 (3.06)	3.88 (4.49)	5.76 (2.85)	3.83 (1.35)	3.22 (2.76)	4.19 (3.66)	0.28 (0.19)	4.34 -	P<0.01	P<0.005	P<0.001
TOTAL INVERTEBRATES	73.47 (9.58)	98.03 (50.96)	65.88 (24.93)	193.53 (31.11)	379.71 (45.13)	351.84 (128.33)	339.56 (82.60)	272.8 (112.90)	258.02 (55.62)	200.58 (59.20)	112.34 -	P<0.001	P<0.001	P<0.001
Holothurian species.														
Amperima rosea	2.05 (0.51)	6.59 (4.95)	3.92 (0.72)	76.07 (12.22)	234.83 (32.37)	218.26 (108.33)	204.33 (90.04)	180.59 (88.56)	128.59 (14.39)	57.32 (23.79)	18.33 -	P<0.001	P<0.001	P<0.001
Ellipinion molle	0 -	0 -	0.15 (0.13)	32.94 (10.22)	46.31 (13.33)	27.94 (14.27)	12.57 (0.17)	8.86 (4.51)	0.13 (0.18)	1.03 (0.97)	1.57 -	P<0.001	P<0.001	P<0.001
Oneirophanta mutabilis	24.23 (4.89)	28.63 (14.89)	19.17 (9.84)	16.3 (5.07)	22.09 (5.30)	23.41 (0.83)	27.83 (3.68)	18.73 (4.12)	26.26 (9.48)	32.64 (12.46)	13.34 -	ns	P<0.05	ns
Peniagone diaphana	6.52 (1.5)	5.42 (0.34)	0.36 (0.20)	6.71 (3.36)	0.32 (0.51)	0.57 (0.29)	0.39 (0.43)	0.56 (0.27)	0.24 (0.21)	0.85 (0.79)	6.13 -	P<0.001	P<0.001	ns
Pseudostichopus sp.	1.06 (0.56)	5.35 (3.56)	4.35 (2.43)	3.68 (1.71)	7.11 (2.82)	6.04 (1.72)	7.55 (2.64)	6.28 (3.70)	12.07 (7.51)	9.74 (3.70)	3.85 -	P<0.01	P<0.005	P<0.05
Pseudostichopus villosus	3.81 (0.95)	8.9 (6.53)	6.39 (3.34)	6.21 (4.57)	8.63 (1.92)	9.06 (2.68)	7.68 (1.76)	8.64 (4.90)	6.75 (3.03)	13.59 (4.66)	6.13 -	ns	ns	P<0.05
Psychropotes longicauda	3.02 (0.09)	1.37 (0.49)	4.07 (1.60)	6.34 (2.24)	17.52 (3.90)	15.38 (6.45)	9.55 (1.30)	9.46 (5.07)	17.18 (7.69)	30.17 (17.98)	5.85 -	P<0.001	P<0.001	P<0.001

Table 2.2. Temporal variability in the composistion of the megafaunal community on the Porcupine Abyssal Plain, with reference to nine major taxa (upper table) and seven major holothurian species (lower table). Mean abundances (individuals per hectare) and standard deviation (in parentheses) for each cruise. Corresponding P values from ANOVA for between cruise, year and period comparisons.

recorded between periods. The asteroids showed no overall consistent pattern of increase or decrease in abundance. Overall, the invertebrate megafauna (all 29 identified taxa) showed a significant between-cruise, -year and -period change in abundance, with an overall trend for increasing abundance between the pre-BENGAL and BENGAL periods (1996-1998). Figure 2.5 shows the patterns of temporal variation in abundance for the additional eight major taxa covered in detail by this study. It is immediately apparent that the abundance of holothurians (Figure 2.2) is an order of magnitude greater than any of the other taxa, even during the period prior to the "*Amperima* Event" (1989-1994). The actinarians, annelids, ophiuroids and tunicates all show a significant increase in abundance between the pre-BENGAL and BENGAL periods, whilst the zoanthids exhibit a significant decrease in abundance over the same period. Of those taxa exhibiting an increase in abundance during 1996-98, the majority show a subsequent decrease during the post-BENGAL period.

The holothurians were, and still are, the dominant taxa of abyssal megafauna on the PAP. The significant increase in abundance during 1996-98 was greatly influenced by the dramatic increase in abundance of *A. rosea*. However, it was not the only species of holothurian to show changes in abundance over the three periods. Table 2.2 also shows the mean abundance (± standard deviation) for seven major holothurian species from the PAP. In addition to *A. rosea*, *Ellipinion molle*, *Pseudostichopus* sp. and *Psychropotes longicauda* all show a significant increase in abundance between the pre-BENGAL and BENGAL periods. In contrast, *Peniagone diaphana* shows a significant decrease in abundance between these periods, subsequently increasing again in the post-BENGAL period as the abundance of *A. rosea* decreases. Figure 2.6 illustrates the temporal variability in abundance of six additional species of common PAP holothurians.

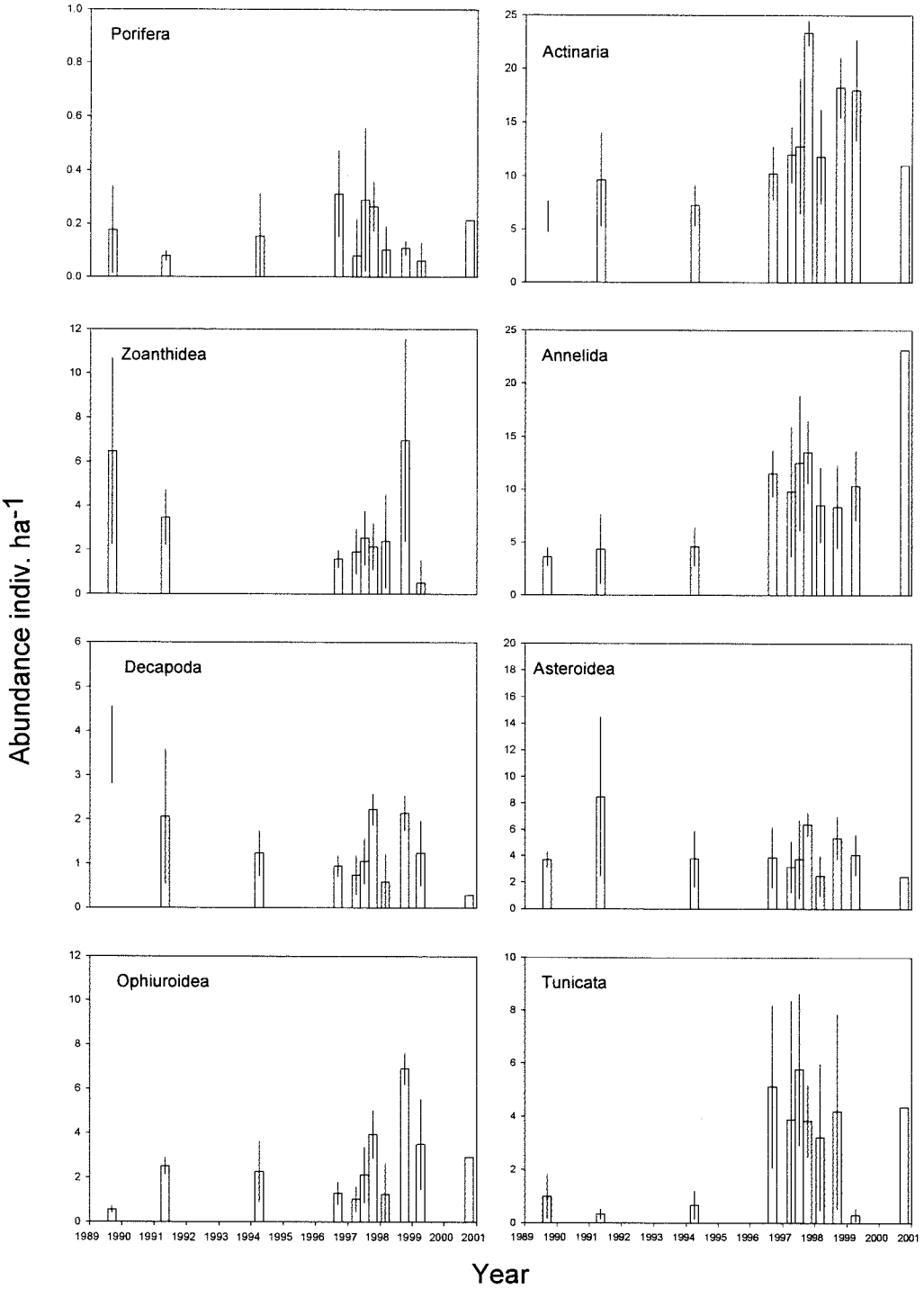

Figure 2.5. Temporal variability of mean abundance (± standard deviation) of eight megafaunal taxa sampled at the BENGAL site, PAP. 1989-2000.

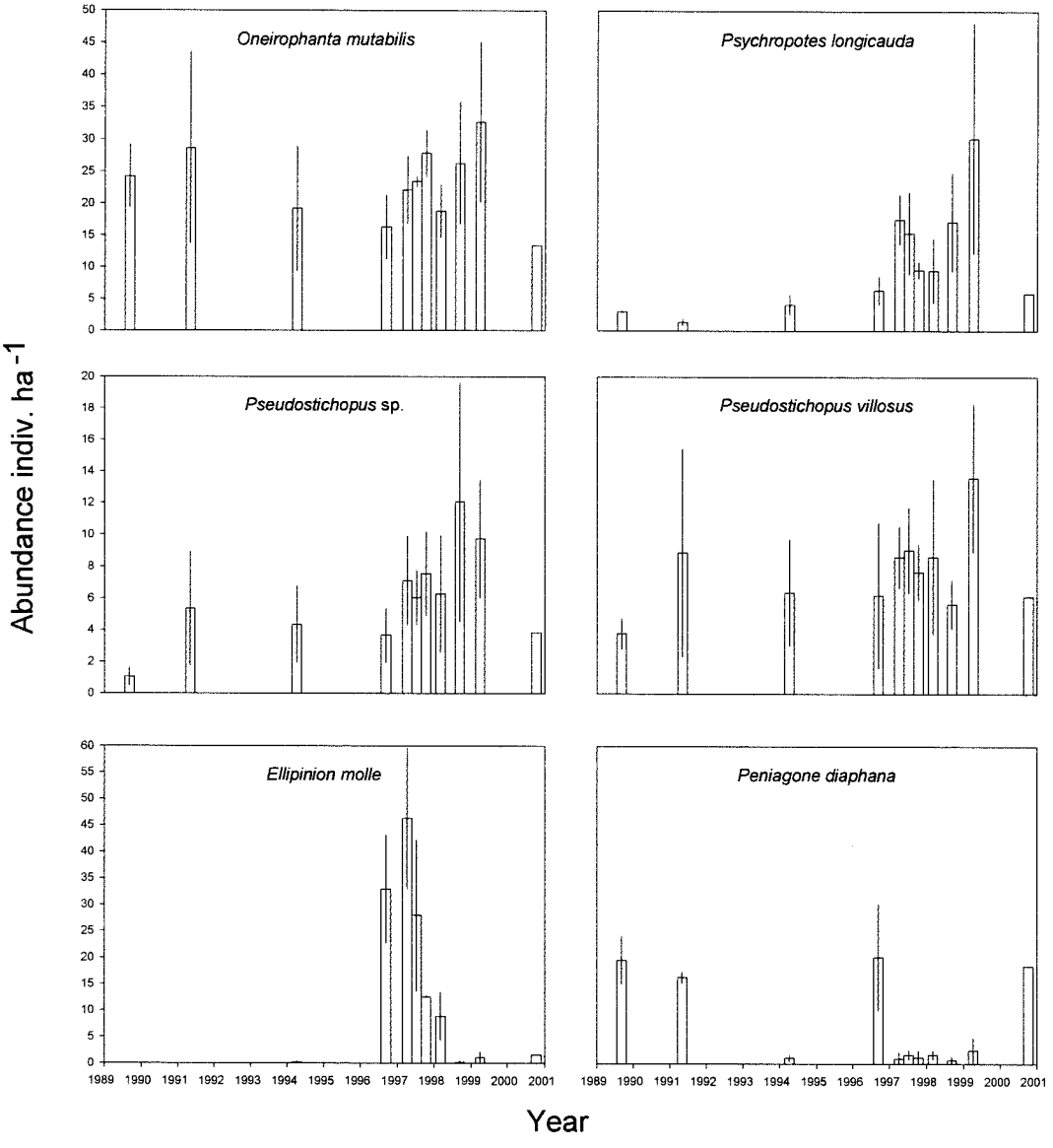

Figure 2.6. Temporal variability of mean abundance (± standard deviation) of six holothurian species sampled at the BENGAL site, PAP. 1989-2000.

2.3.2.1. Multivariate analysis of temporal community change.

Hierarchical classification of the full megafaunal temporal data set, using cluster analysis, indicates a clear distinction between the pre-, post- and BENGAL samples (Figure 2.7). The separation of the data sets is equally apparent in the corresponding non-metric multi-dimensional scaling ordination (Figure 2.8); i.e. the pre-, post- and BENGAL samples do not overlap in ordination space. The overall ordination shows no temporal trends other than separating pre-, post- and BENGAL samples.

An analysis of similarity test (ANOSIM), using abundance data for 29 identified taxa, showed a significant difference in the megafaunal community between periods and between certain cruise and year-pairs (see results of pair-wise tests in Table 2.3a and b). No significant difference was recorded between cruises within a year.

(a)	Sep-89	May-91	Apr-94	Sep-96	Apr-97	Jul-97	Oct-97	Mar-98	Sep-98	Apr-99
May-91	-									
Apr-94	0.029	-								
Sep-96	0.018	0.048	0.008							
Apr-97	-	-	0.029	0.018						
Jul-97	0.029	-	0.029	0.016	ns					
Oct-97	-	-	-	0.048	-	-				
Mar-98	0.029	0.036	0.029	0.011	ns	ns	ns			
Sep-98	-	-	-	0.048	-	-	-	ns		
Apr-99	0.029	-	0.029	0.008	ns	0.029	-	0.029	-	
Oct-00	-	-	-	-	-	-	-	-	-	-

(b)	1989	1991	1994	1996	1997	1998	1999
1991	-						
1994	0.029	-					
1996	0.018	0.048	0.008				
1997	0.005	0.036	0.001	0.001			
1998	0.012	0.036	0.005	0.004	0.001		
1999	0.029	-	0.029	0.008	0.005	0.005	
2000	-	-	-	-	-	-	-

Table 2.3. Results of the analysis of similarity (ANOSIM) of megafaunal composition (abundance of 29 identified taxa); P values are shown for: a) pair-wise comparison of cruises; and b) pair-wise comparison of years (ns, not significant; -, test not possible).

Although the ANOSIM test was not valid between 2000 and other years it should be noted that the R statistic values did indicate differences between this year and the majority of others, but as only data from one trawl could be used the significance values of each test were low (P = 0.1-0.6) and the test was not reliable. The September 1996 data set (see Table 2.1) occupies an intermediate position, i.e. it is significantly different from all pre-BENGAL, BENGAL and post-BENGAL samples. All possible pre-1996 to post-1996 comparisons indicate significant differences, whereas all possible internal

comparisons of post-1996 BENGAL sample sets are non-significant. However, the samples from 1999 (post-BENGAL) were significantly different from some post-1996 samples, namely July 1997 and March 1998. Variations in faunal composition between years were assessed in a similar manner (Table 2.3b). In this case all possible comparisons were significant, including all of the internal comparisons for the BENGAL period.

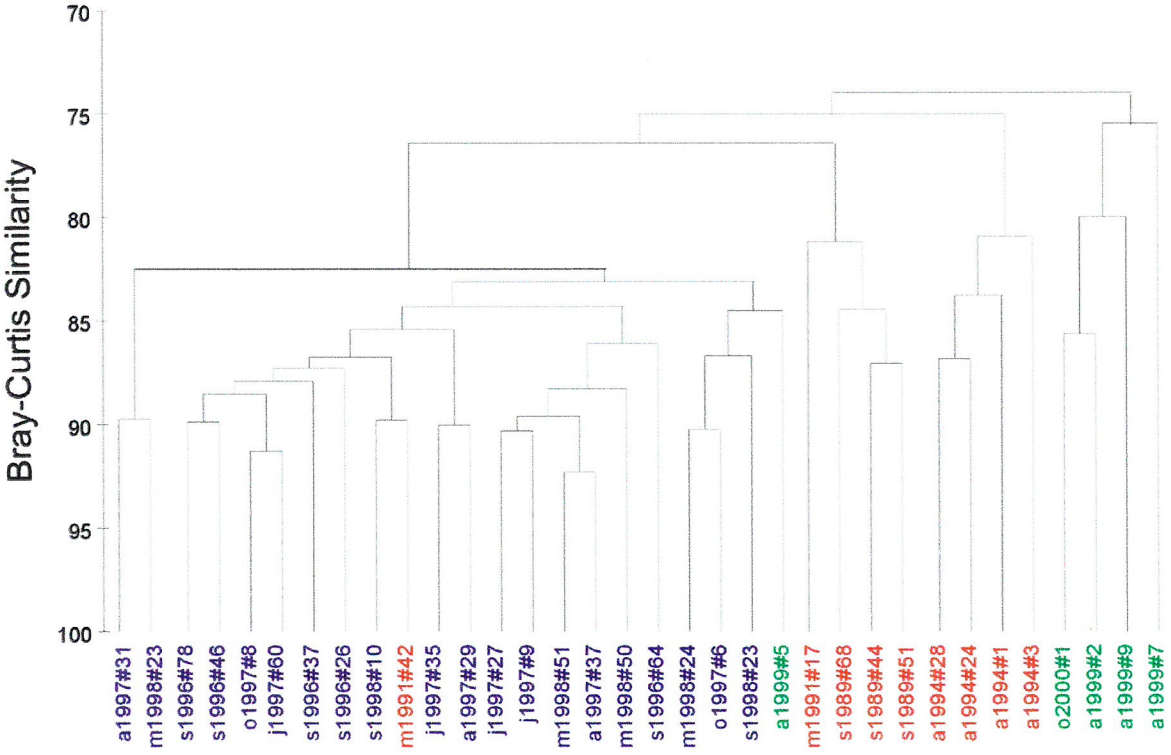

Figure 2.7. Dendrogram for hierarchical clustering of 34 individual trawl samples taken during an 11 year period between 1989 and 2000, using group-average linking of Bray-Curtis similarities calculated on √√-transformed abundance data for 29 identified megafaunal taxa. Red = pre-BENGAL samples; blue = BENGAL samples; green = post-BENGAL samples.

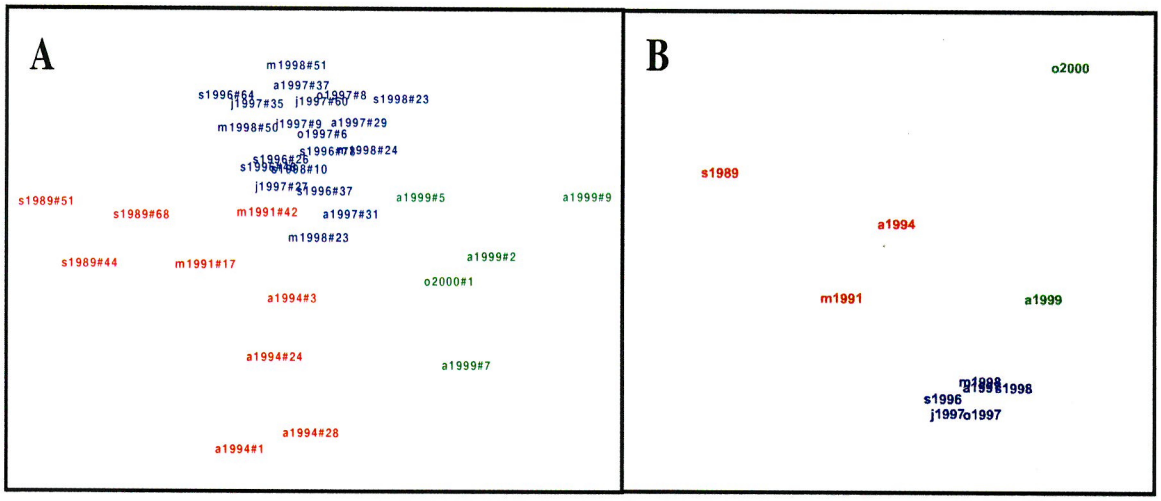

Figure 2.8. Multi-dimensional scaling (MDS) ordination of; A, 34 individual trawl samples and B, 11 mean cruise samples, taken during an 11 year period between 1989 and 2000, using group-average linking of Bray-Curtis similarities calculated on √√-transformed abundance data for 29 identified megafaunal taxa (stress A = 0.16; B = 0.06). Red = pre-BENGAL samples; blue = BENGAL samples; green = post-BENGAL samples.

As the holothurians were the dominant taxon on the PAP throughout the whole 11-year period of the study, cluster analysis and MDS were also applied to the holothurian species data set. 19 species were identified in this data set (see Appendix I) and their abundances recorded for each of the 11 cruises. Cluster analysis (Figure 2.9) shows a clear separation of the pre-BENGAL and BENGAL samples. However, the post-BENGAL samples are split, with the April 1999 samples clustering out with those from September 1998 and the single October 2000 sample clustering out with the pre-BENGAL samples. This is supported by the accompanying MDS ordinations (Figure 2.10A and B) with no overlap of samples from different periods. The post-BENGAL samples appear to occupy a 'transition' period in the holothurian community, where the make-up of that community is returning to a pre-BENGAL state.

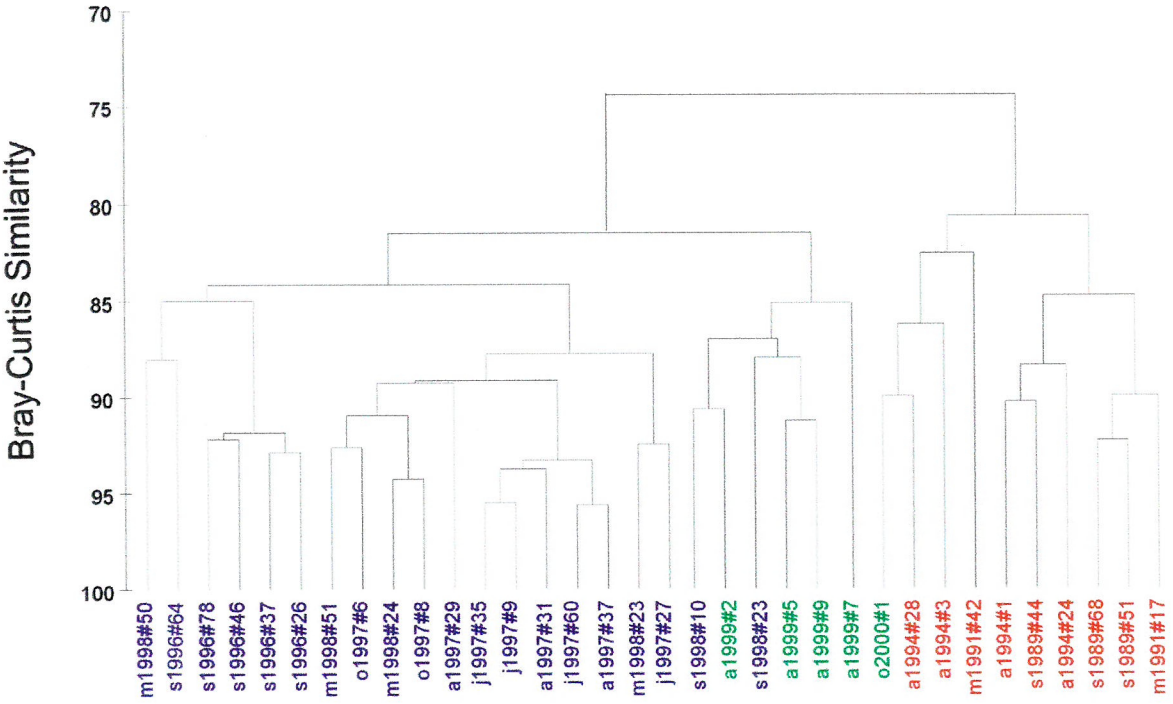

Figure 2.9. Dendrogram for hierarchical clustering of 34 individual trawl samples taken during an 11 year period between 1989 and 2000, using group-average linking of Bray-Curtis similarities calculated on √√-transformed abundance data for 19 identified holothurian species. Red = pre-BENGAL samples; blue = BENGAL samples; green = post-BENGAL samples.

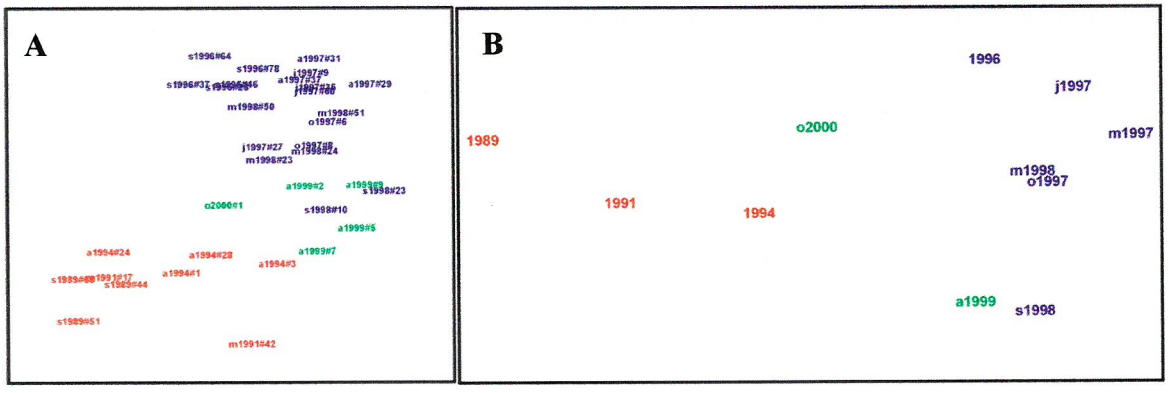

Figure 2.10. Multi-dimensional scaling (MDS) ordination of A, 34 individual trawl samples; and B, 11 mean cruise samples, taken during an 11 year period between 1989 and 2000, using group-average linking of Bray-Curtis similarities calculated on √√-transformed abundance data for 19 identified holothurian species (stress, A = 0.13; B = 0.04). Red = pre-BENGAL samples; blue = BENGAL samples; green = post-BENGAL samples.

2.3.2.2. Temporal variability in community structure.

Variability in the structure of the megafaunal community, between the three periods, was assessed by calculating the percentage contribution of each taxon to the total invertebrate abundance. Figure 2.11 shows the percentage contribution of each of the nine major invertebrate taxa, plus the combined contribution of the 'other' taxa, to the total invertebrate megafauna abundance during the pre-, post- and BENGAL periods.

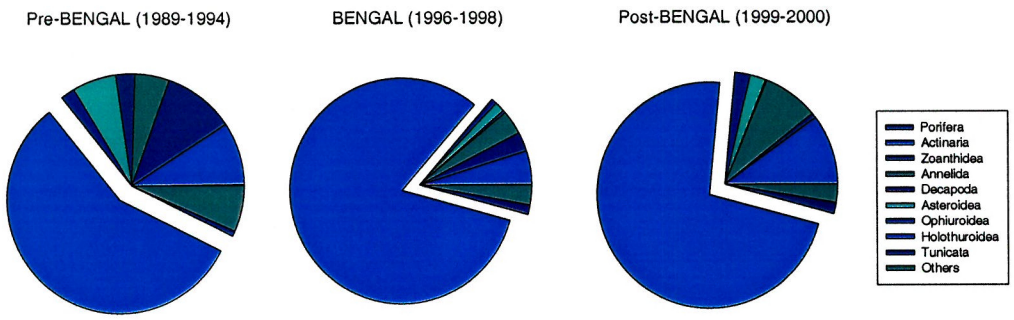

Figure 2.11. Megafaunal community structure (% contribution by major taxa) for pre-, post- and BENGAL samples.

The holothurians dominated the community during all three periods. During the pre-BENGAL period they accounted for 57% of the invertebrates on the PAP. This figure rose to 82% during the BENGAL period before declining to 72% during the post-BENGAL period. After the holothurians the next 'most abundant' taxa were the Actinaria and Zoanthidea, which together accounted for about 20% of the invertebrates in the pre-BENGAL period, 7% in the BENGAL period and 11% in the post-BENGAL period. The annelids, ophiuroids and tunicates are all minor components of the megafaunal community (as sampled by otter trawl). In addition to showing the greatest change in abundance and community dominance, the holothurians also exhibited changes in their own community structure, with the dominant species changing throughout the three periods. Figure 2.12 shows the percentage contribution of each of the seven major holothurian species, plus the combined contribution of the 'other' species to the total holothurian abundance during the pre-, post- and BENGAL periods.

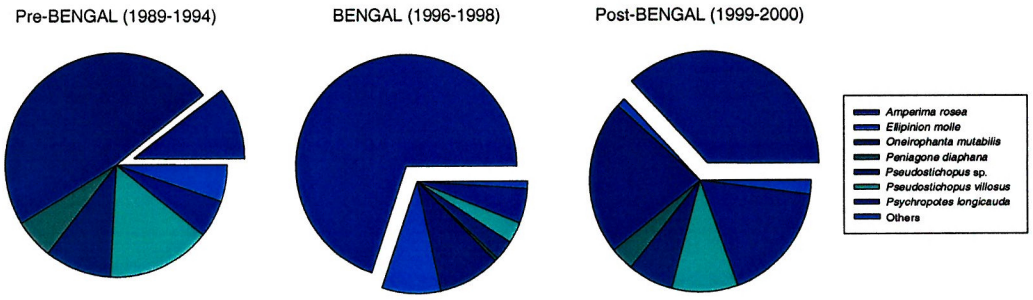

Figure 2.12. Holothurian community structure (% contribution by major species) for pre-, post- and BENGAL samples.

During the pre-BENGAL period *Amperima rosea* was a minor component of the holothurian community, accounting for just 10% of the total holothurian abundance. By the BENGAL period it had increased in number significantly to become the dominant species in the holothurian community, accounting for 70% of the total abundance. The post-BENGAL period saw a decrease in the abundance of *A. rosea* (see Figure 2.4) and its contribution to the holothurian community also decreased to about 37%. In the earlier samples taken between 1989-1994 *Oneirophanta mutabilis* was the most abundant holothurian on the PAP, accounting for nearly 50% of the total holothurian abundance. By 1996 and the start of the BENGAL period it had been displaced by *A. rosea* as the dominant holothurian species, accounting for only 9% of the total abundance. The synallactids *Pseudostichopus villosus* and *Pseudostichopus* sp. together made up 25% of the holothurian community during the pre-BENGAL period yet only 6% during BENGAL in spite of *Pseudsostichopus* sp. increasing in abundance during this period (see Figure 2.6). *Psychropotes longicauda* was another species to increase in abundance during the BENGAL period. However, its contribution to the total abundance did not change from the 5% during the pre-BENGAL period, another indication of the overwhelming dominance of *A. rosea*. Unlike many other species, and indeed many invertebrate taxa, *P. longicauda* continued to increase in abundance during the post-BENGAL period increasing its contribution to the total abundance to 17%.

The rapid increase in the abundance of *A. rosea* noticeably altered the make-up of the holothurian community, and although it now appears to be in decline at the BENGAL site it remains the dominant holothurian species of the PAP megabenthos.

2.3.3. Megafaunal change: spatial variability in abundance and biomass.

The additional trawl samples taken in March 1998 and April 1999 were designed to set the temporal changes observed at the central BENGAL site in a more spatial context on the wider Porcupine Abyssal Plain. Figure 2.13 shows the spatial variation in total invertebrate and holothurian abundance.

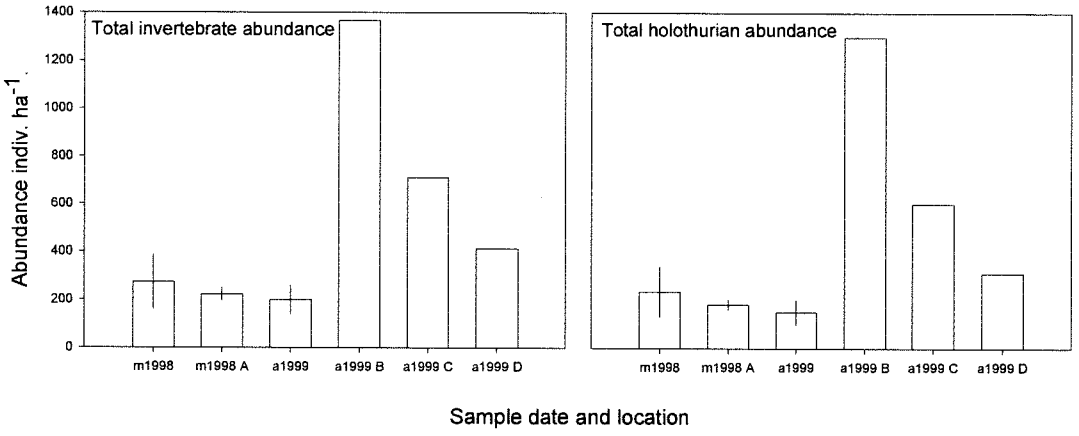

Figure 2.13. Spatial variability in the abundance of total invertebrates and total holothurians for the central BENGAL site (1998 and 1999) and four additional sites on the PAP (A, 1998; B, C, D, 1999).

As with the temporal samples the holothurian abundance closely followed the pattern of abundance for the total invertebrate megafauna. The most obvious feature was the significant spatial variability in the abundance of both the total invertebrates and the holothurians between the samples taken in 1999. This was not apparent in the two sites sampled in 1998 (see Table 2.4). The variability in total invertebrate abundance is probably highly influenced by the variability in the dominant holothurians. However, it should be noted that the values of abundance for the 1999 spatial samples are based on only one trawl at each of the three sites.

Unlike the temporal samples, where biomass was not found to increase significantly for either total invertebrates (ANOVA, F=1.36, 10df, P=0.258) or holothurians (ANOVA, F=1.51, 10df, P=0.197), the biomass did vary significantly between the central BENGAL site and site A in March 1998. Both the total invertebrates (ANOVA, F=6.48, 1df, P=0.064) and the holothurians (ANOVA, F=13.38, 1df, P=0.022) had a significantly greater biomass at site A compared to the central site.

	BENGAL			Post-BENGAL				
Date	Mar-98	Mar-98 A	ANOVA	Apr-99	Apr-99 B	Apr-99 C	Apr-99 D	ANOVA
Taxa	Central	East	Sites	Central	SouthEast	NorthEast	North	Sites
PORIFERA	0.1 (0.07)	1.62 (0.58)	ns	0.06 (0.07)	0.17 -	0.16 -	0 -	ns
ACTINARIA	11.76 (4.40)	5.95 (1.73)	ns	17.99 (4.73)	25.03 -	31.41 -	33.96 -	ns
ZOANTHIDEA	2.39 (1.78)	0.56 (0.24)	ns	0.51 (1.02)	0.5 -	0 -	0 -	ns
ANNELIDA	8.52 (3.56)	9.35 (1.16)	ns	11.56 (3.32)	10.5 -	17.56 -	23.02 -	ns
DECAPODA	0.58 (0.63)	0.23 (0.19)	ns	1.23 (0.74)	2.18 -	2.96 -	2.14 -	ns
ASTEROIDEA	2.48 (1.50)	2.16 (2.02)	ns	4.07 (1.55)	2.82 -	8.95 -	11.07 -	ns
OPHIUROIDEA	1.23 (1.40)	0.1 (0.14)	ns	3.49 (2.04)	9.28 -	9.74 -	0.6 -	ns
HOLOTHUROIDEA	235.79 (104.03)	180.98 (21.89)	ns	150.34 (52.22)	1298.37 -	600.5 -	311.47 -	P<0.05
TUNICATA	3.22 (2.76)	13.18 (6.71)	ns	0.28 (0.19)	5.3 -	9.27 -	0 -	P<0.01
TOTAL INVERTEBRATES	272.8 (112.90)	221.67 (25.16)	ns	200.58 (59.20)	1366.75 -	708.81 -	412.03 -	P<0.05
Holothurian species.								
Amperima rosea	180.59 (88.56)	117.21 (3.65)	ns	57.32 (23.79)	1231.25 -	285.33 -	181.77 -	P<0.05
Ellipinion molle	8.86 (4.51)	1.78 (1.42)	ns	1.03 (0.97)	0 -	0.31 -	1.5 -	ns
Oneirophanta mutabilis	18.73 (4.12)	19.54 (3.37)	ns	32.64 (12.46)	39.78 -	34.39 -	24.09 -	ns
Peniagone diaphana	0.56 (0.27)	1.17 (0.12)	ns	0.85 (0.79)	0.83 -	2.67 -	1.35 -	ns
Pseudostichopus sp.	6.28 (3.70)	14.17 (4.97)	ns	9.74 (3.70)	0 -	85.74 -	10.92 -	P<0.05
Pseudostichopus villosus	8.64 (4.90)	19.28 (10.41)	ns	13.59 (4.66)	0.66 -	167.09 -	58.2 -	P<0.05
Psychropotes longicauda	9.46 (5.07)	5.89 (2.50)	ns	30.17 (17.98)	24.69 -	20.73 -	20.05 -	ns

Table 2.4. Spatial variability in the composition of the megafaunal community on the PAP, with reference to nine major taxa (upper table) and seven major holothurian species (lower table). Mean abundances (individuals per hectare) and standard deviation (in parentheses) for each site during March 1998 and April 1999. Corresponding P values from ANOVA for between site comparisons.

During April 1999 there was a significant difference in biomass between site B and the other three sites, but no significant difference in biomass between the central site and sites C and D. Site B was dominated, in terms of abundance, by *Amperima rosea* (Figure 2.15), but other, larger holothurians were less abundant than at the other sites, particularly *P. villosus* and *Pseudostichopus* sp., resulting in a lower biomass for both total invertebrates and holothurians at this site. The greater biomass observed during April 1999 at the central site and both sites C and D can be attributed to the greater abundance of the larger holothurians, *Pseudostichopus* sp. and *P. villosus*.

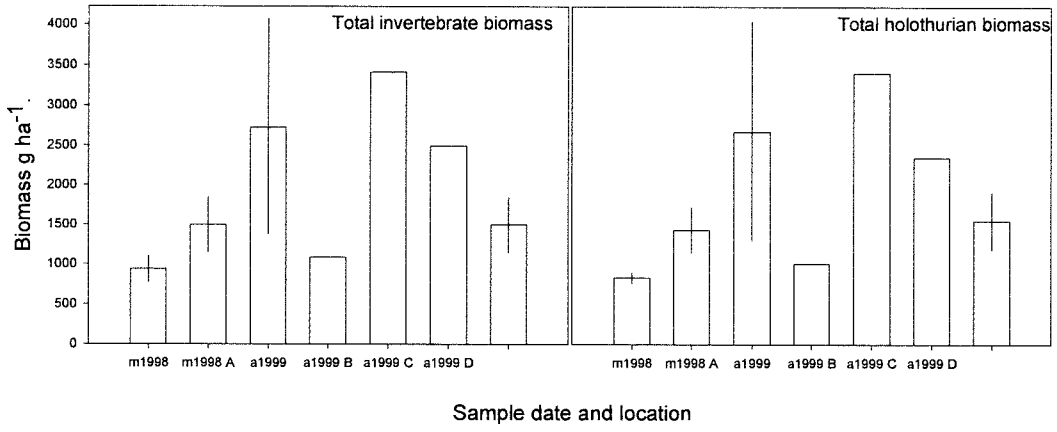

Figure 2.14. Spatial variability of biomass for total invertebrates and total holothurians from the central BENGAL site (1998 and 1999) and four additional sites on the PAP (A, 1998; B, C, D, 1999).

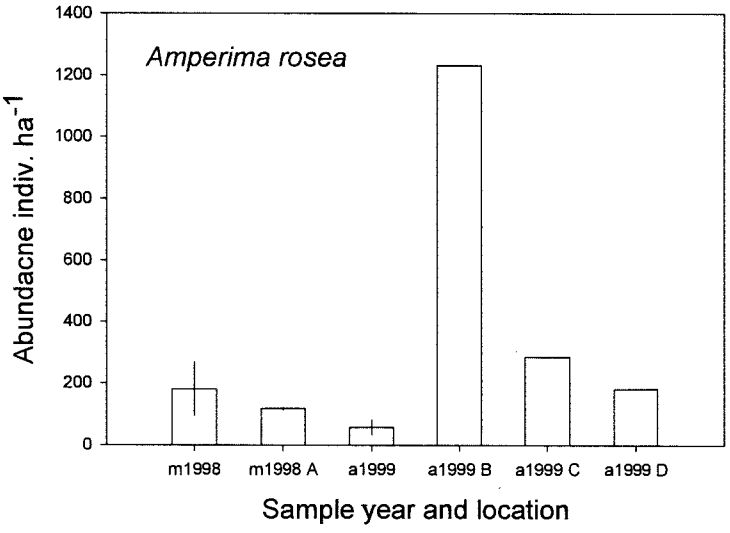

Figure 2.15. Spatial variability in the mean abundance of *Amperima rosea* for the central BENGAL site (1998 and 1999) and four additional sites on the PAP (A, 1998; B, C, D, 1999).

Three of the seven major holothurian species showed significant spatial variability in abundance (Table 2.4). *A. rosea* was significantly more abundant at site B than any of the other sites. The recorded abundance of 1231 ind. ha^{-1} was more than five times greater than any recorded during the entire BENGAL programme. The abundance of *A. rosea* at

site C was also higher than any recorded at the central site, while the abundance at site D was similar to that recorded back in early 1998 at the central site.

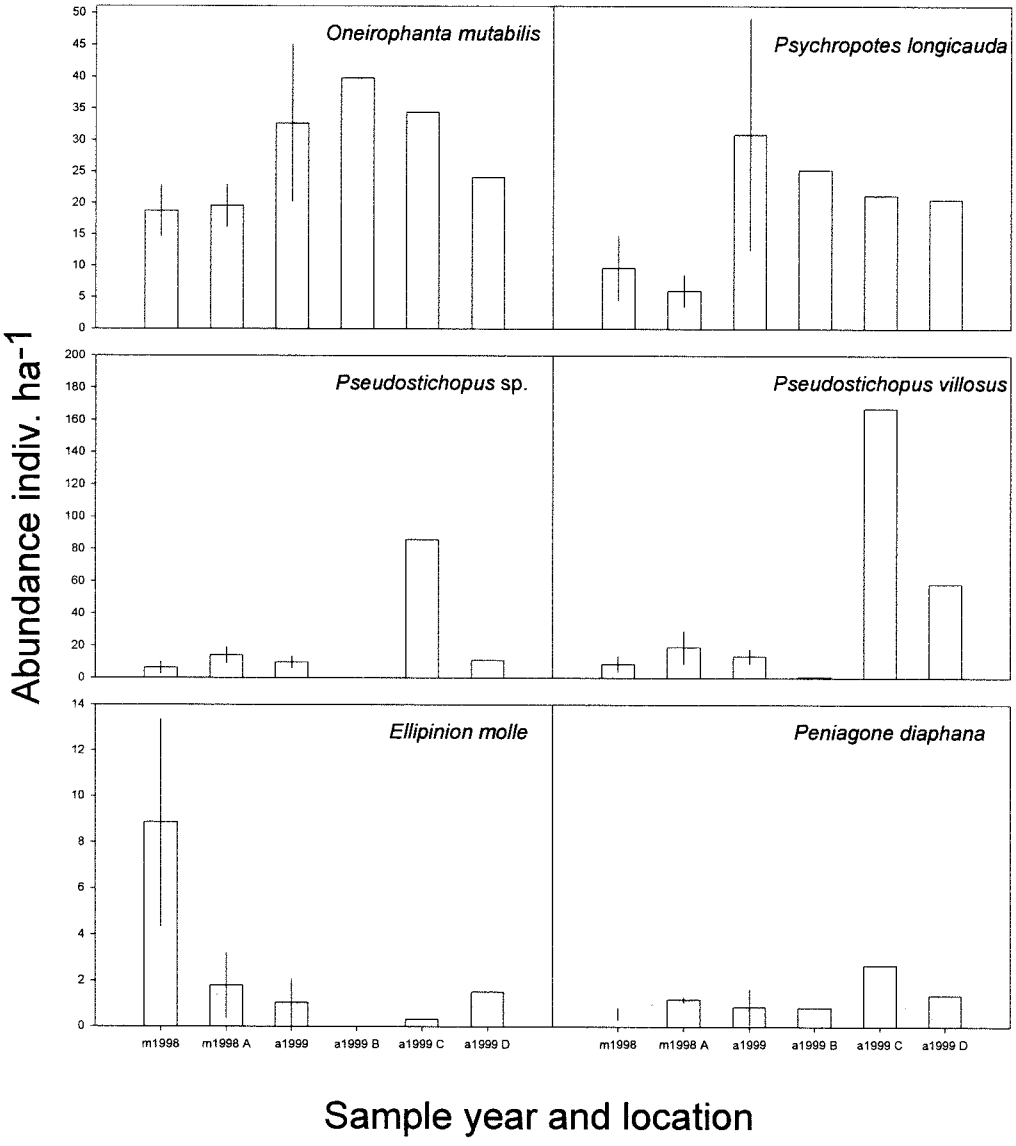

Figure 2.16. Spatial variability of mean abundance (± standard deviation) of six holothurian species sampled at the central BENGAL site (1998 and 1999) and four additional sites on the PAP (A, 1998; B, C, D, 1999).

The other two species to show significant spatial variability in abundance were *Pseudostichopus* sp. and *P. villosus*. The abundance of both species at site C was significantly higher than any previously recorded at the central site. With the exception of *A. rosea*, both *P. villosus* and *Pseudostichopus* sp. dominated the holothurian megafauna

at site C. *Pseudostichopus villosus* was again present in high numbers at site D where it was almost three-times more abundant than *O. mutabilis* and *Psychropotes longicauda*.

2.3.3.1. Multivariate analysis of spatial community variability.

Hierarchical classification of the full spatial data set, using cluster analysis, indicates a clear distinction between sample sites in 1999. The spatial distinction is not as clear for the 1998 samples with the Site A samples clustering with those from the central BENGAL site (Figure 2.17). The separation of the data sets is equally apparent in the corresponding non-metric multi-dimensional scaling ordination (Figure 2.18A); i.e. the samples do not overlap in ordination space. The overall ordination shows a clear spatial pattern for the 1999 samples but not for the 1998 samples, a pattern further emphasised by the ordination of mean cruise data (Figure 2.18B).

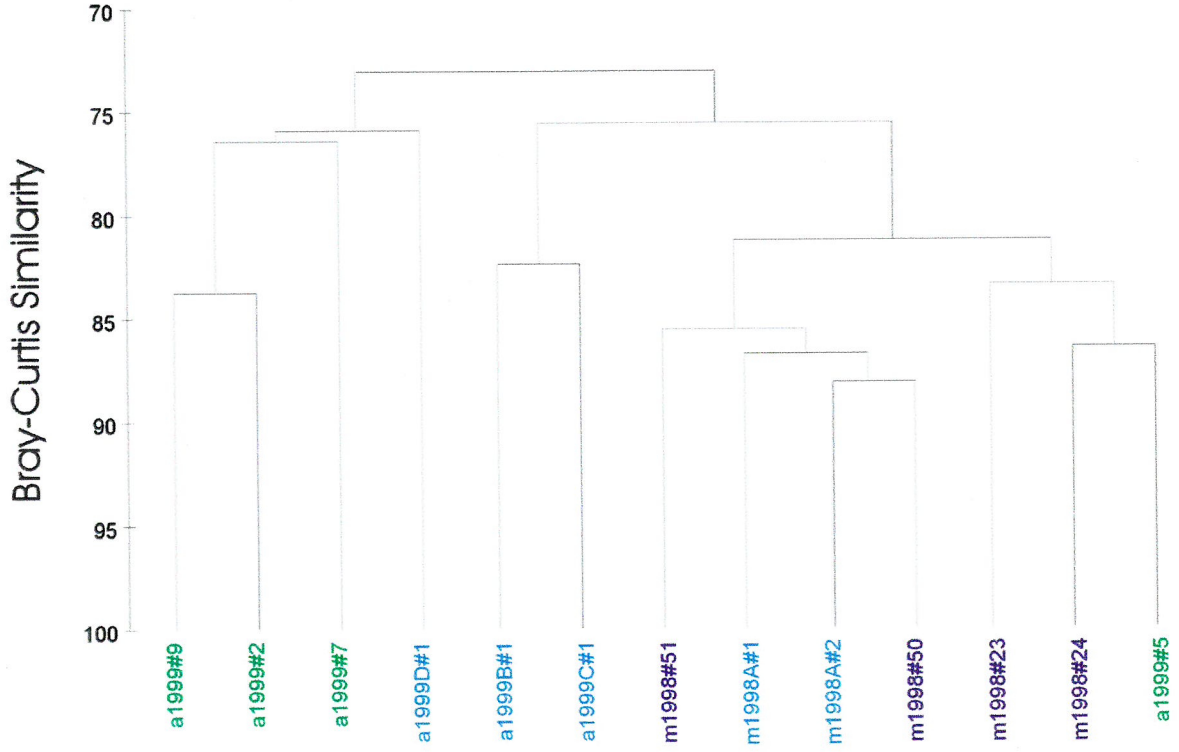

Figure 2.17. Dendrogram for hierarchical clustering of 13 individual trawl samples taken at four separate locations during March 1998 and April 1999, using group-average linking of Bray-Curtis similarities calculated on √√-transformed abundance data for 29 identified megafaunal taxa. Dark blue = central site, BENGAL; Green = central site, post-BENGAL samples; Light blue = sites A, B, C and D, additional spatial samples.

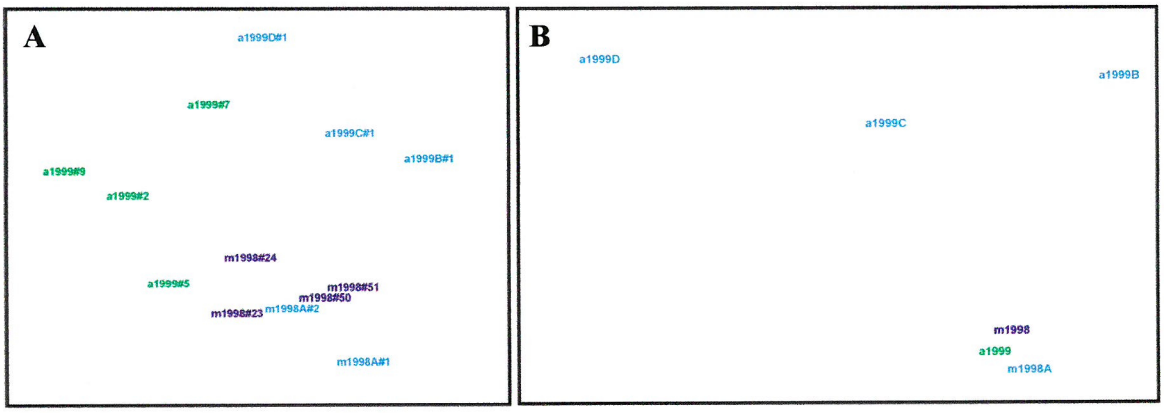

Figure 2.18. Multi-dimensional scaling (MDS) ordination of; A, 13 individual trawl samples; and B, 6 site-mean samples, taken at four separate locations during March 1998 and April 1999, using group-average linking of Bray-Curtis similarities calculated on √√-transformed abundance data for 29 identified megafaunal taxa (stress, A = 0.14; B = 0.00). Dark blue = central site, BENGAL samples; Green = central site, post-BENGAL samples; Light blue = sites A, B, C and D, additional spatial samples.

The holothurians again dominate the megafaunal community at all five sites as they did at the central site throughout the whole 11-year period of the study. Cluster analysis and MDS were also applied to the holothurian species data set. The abundances of each of the 19 identified species were recorded for each of the five sites including the central site in both March 1998 and April 1999. Cluster analysis (Figure 2.19) shows a clear separation of the central site samples from those at sites A-D, with the replicate trawls from March 1998 and April 1999 clustering together within their respective years. The sample from Site B is very distinct as the result of the unexpectedly high abundance of *A. rosea* and low abundance of both *Pseudostichopus* sp. and *P. villosus*. The Site C sample is separated, probably as a result of the unexpectedly high abundance of *Pseudostichopus* sp. and *P. villosus*. This is supported by the accompanying MDS ordinations (Figure 2.20A and B) with no overlap of samples from different periods. However, the MDS ordination shown in Figure 2.20A is calculated without the sample from Site B. The huge abundance at *A. rosea* at this site made it hard to resolve the relationships of the other sites and years. The resulting ordination plot (minus Site B) clearly shows the separation of the five sites with no-overlap in ordination space. The separation of sites is supported by Figure 2.20B which shows the sample means for each location, including Site B.

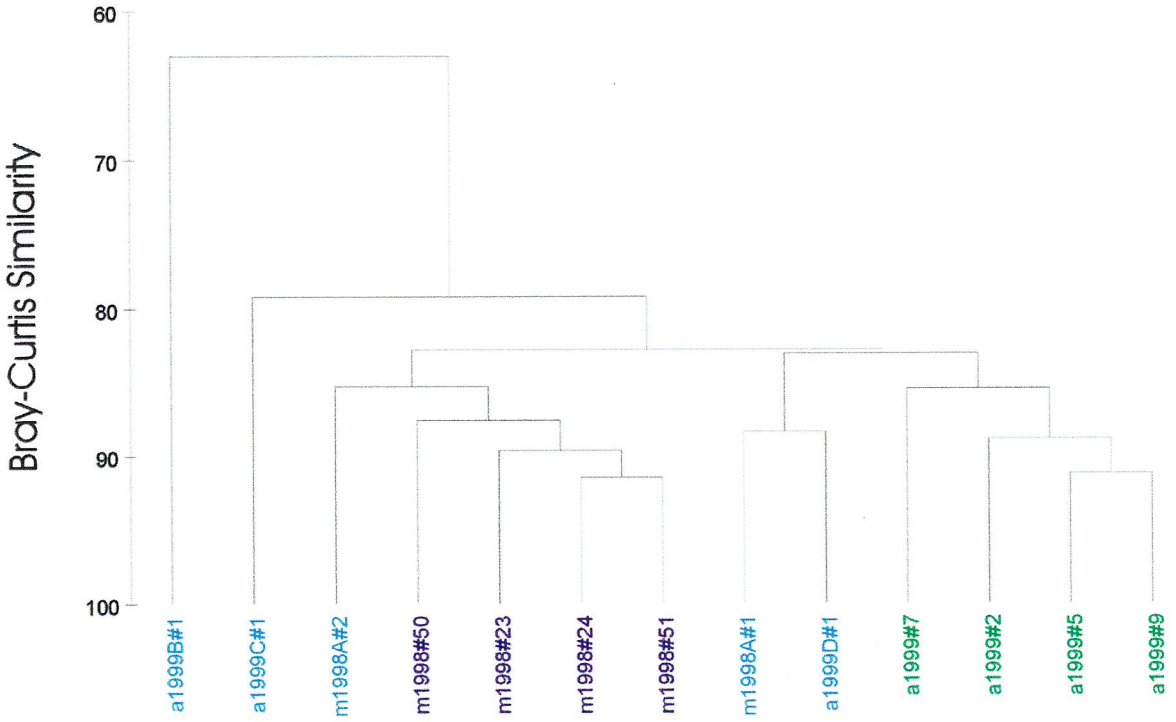

Figure 2.19. Dendrogram for hierarchical clustering of 13 individual trawl samples taken at four separate locations during March 1998 and April 1999, using group-average linking of Bray-Curtis similarities calculated on √√-transformed abundance data for 19 identified holothurian species. Dark blue = central site, BENGAL; Green = central site, post-BENGAL samples; Light blue = sites A, B, C and D, additional spatial samples.

Figure 2.20. Multi-dimensional scaling (MDS) ordination of; A, 12 individual trawl samples; and B, 6 site-mean samples, taken at four separate locations during March 1998 and April 1999, using group-average linking of Bray-Curtis similarities calculated on √√-transformed abundance data for 19 identified holothurian species (stress, A = 0.12; B = 0.00). Dark blue = central site, BENGAL samples; Green = central site, post-BENGAL samples; Light blue = sites A, B, C and D, additional spatial samples.

2.3.3.2. Spatial variability in community structure.

Variability in the structure of the megafaunal community, among the five sites, was assessed by calculating the percentage contribution of each taxon to the total invertebrate abundance. Figure 2.21 shows the percentage contribution of each of the nine major invertebrate taxa, plus the combined contribution of the 'other' taxa, to the total invertebrate megafauna abundance at the central site (1998 and 1999) and sites A-D.

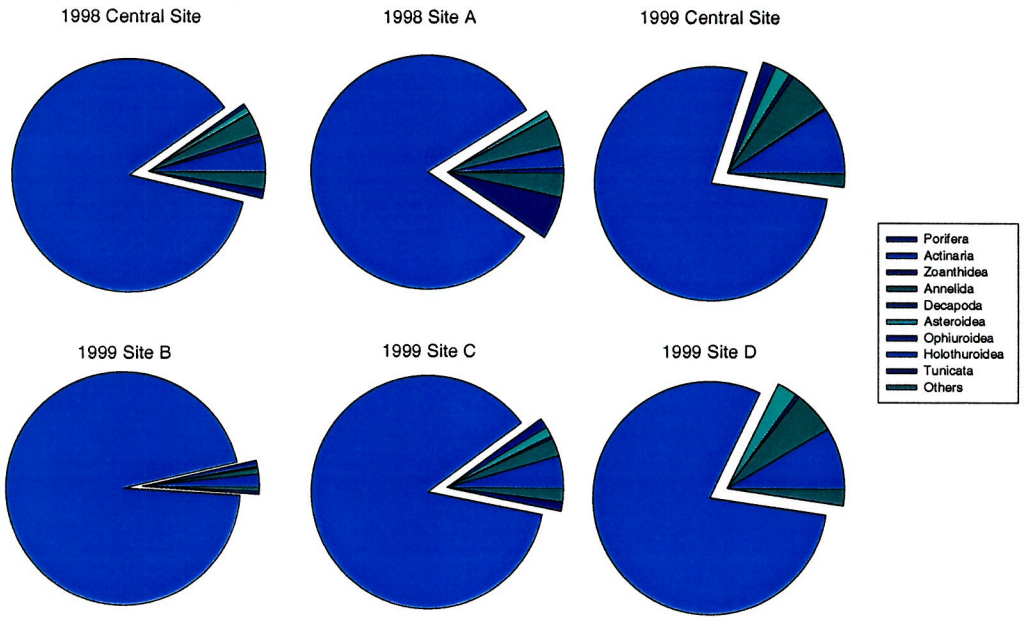

Figure 2.21. Megafaunal community structure (% contribution by major taxa) for five locations on the PAP. Central BENGAL site (1998 and 1999), Site A (1998) and Sites B-D (1999).

Again the megafaunal community at all five sites was dominated by the holothurians, as was the central site during the 11-year temporal study. In March 1998 both sites were dominated by holothurians, although slightly less so at Site A (~81% compared to ~86%). In addition to the decreased contribution of holothurians, the major differences between the central site and Site A in 1998 were a) a decreased contribution by actinarians (4.3% to 2.6%), and b) an increased contribution by tunicates (1.2% to 5.9%). In 1999 all four sites differed with respect to the percentage contribution of the holothurians. The exceptionally high abundance of *A. rosea* at Site B contributed to the holothurians accounting for 95% of the megafaunal community. Such was the dominance of this one

species, and the holothurians as a whole, that even though the actinaria were also found in greater numbers (~40% increase), their % contribution to the megafaunal community fell from 9.3% to 1.8%. The abundance of holothurians at Site C was also higher than at the central site, an increase in percentage contribution of ~9%. Their dominance masked the greater abundances of annelids, actinarians and asteroids that all showed a lower percentage contribution to the total megafauna compared to the central site community. Site D has a very similar community structure to the central site. Even though the holothurian abundance was more than double that of the central site, additional large increases in the abundance of actinarians (~100%) and annelids (~100%) contributed to an overall similar benthic community. Notable absentees were poriferans, zoanthids and tunicates, all of which were rare at the central site anyway.

As with the temporal samples (see Figure 2.12), the variability in spatial community structure was equally as evident within the holothurian community itself. Figure 2.22 shows the percentage contribution of each of the seven major holothurian species, plus the combined contribution of the 'other' species to the total holothurian abundance at each of the five locations on the PAP.

Amperima rosea was the dominant species at all five sites during both years. In 1998 it was less abundant at Site A, and coupled with an increase in the abundance of *O. mutabilis* and both *Pseudostichopus* species, the % contribution of *A. rosea* to the overall holothurian community fell by ~12%. *Pseudostichopus* sp. and *P. villosus* together accounted for nearly 20% of the holothurian community at Site A compared with a combined contribution of only 6% at the central site. In 1999 *A. rosea* accounted for only 39% of holothurian community, although it was still the dominant holothurian species. Higher abundances (compared to 1998) of *O. mutabilis* and *Psychropotes longicauda* increased their contribution to the holothurian community by more than one third. Site B was overwhelmingly dominated by *A. rosea*, increasing its contribution to 95%, even though *O. mutabilis* was also present in a higher abundance than at the central site. Notable absentees from this location were *E. molle* and *Pseudostichopus* sp., while *P. villosus* was

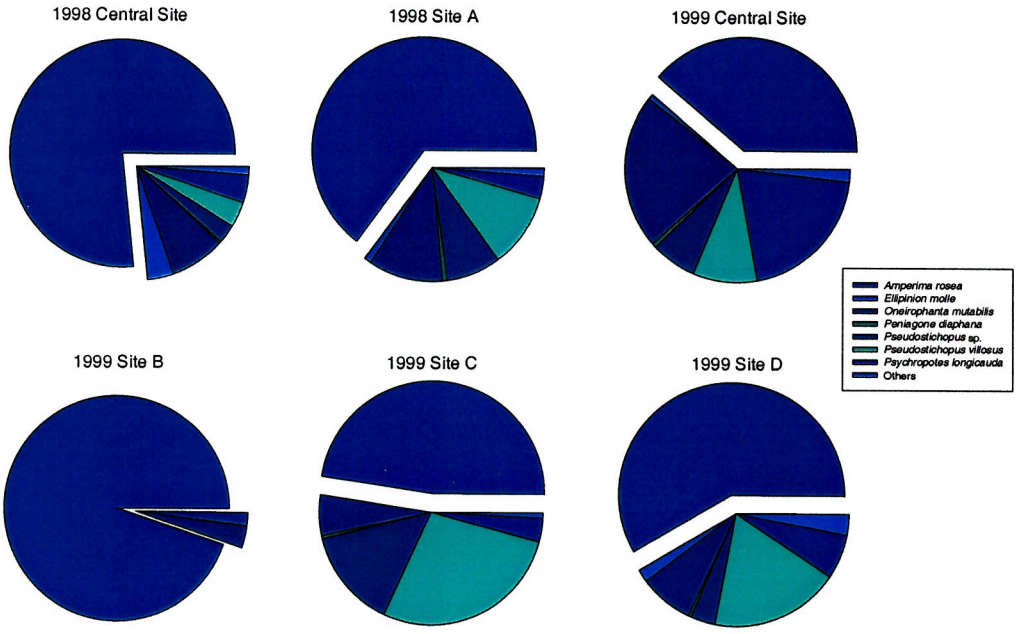

Figure 2.22. Holothurian community structure (% contribution by major species) for five locations on the PAP. Central BENGAL site (1998 and 1999), Site A (1998) and Sites B-D (1999).

very scarce. Even though the recorded abundance was more than 3 times higher than the central site, *A. rosea* accounted for <50% of the holothurian community at Site C. Unusually high abundances of both *Pseusdostichopus* sp. and *P. villosus* at this locality resulted in both species accounting for >40% of the holothurian community. *O. mutabilis* was also slightly more abundant at Site C than at the central site, yet its contribution to the overall holothurian community fell by more than 15%. Site D was more similar to the central site than either of sites B and C. *A. rosea* was again more abundant but the overall community was more balanced with *O. mutabilis* and *Psychropotes longicauda* contributing more to the total abundance than at either Site B or Site C. *Pseudostichopus villosus* was again the most abundant holothurian after *A. rosea*. The holothurian community across the wider PAP was highly variable, often dependent on the relative abundance of *A. rosea*. When the abundance of *A. rosea* is lower the community is more balanced.

2.3.4. Multivariate analysis of the entire PAP data set: temporal and spatial combined.

Hierarchical classification by cluster analysis indicates that replicate trawls from a cruise or location generally cluster together. Therefore, for a clearer graphical representation, the following multivariate tests were performed on the mean abundances for each cruise/location.

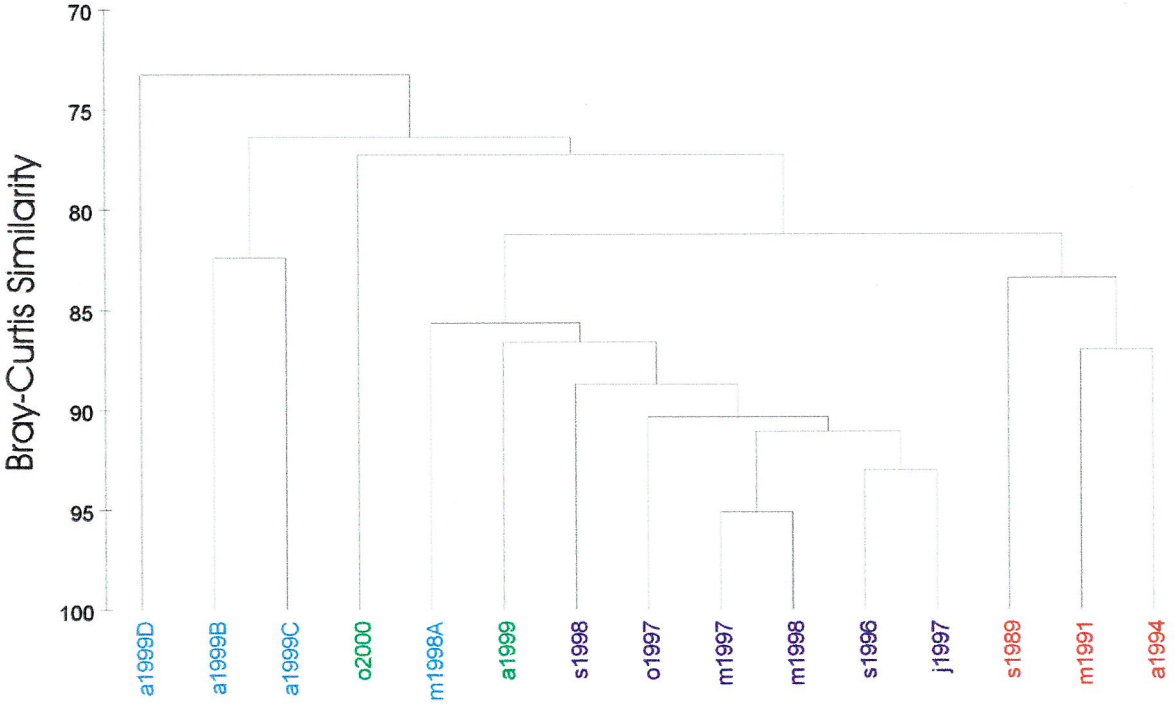

Figure 2.23. Dendrogram for hierarchical clustering of 15 mean cruise samples taken during an 11 year period between 1989 and 2000 at the central PAP site, and at four additional sites during 1998 and 1999. Using group-average linking of Bray-Curtis similarities calculated on √√-transformed abundance data for 29 identified megafaunal taxa. Red = pre-BENGAL samples; Dark blue = BENGAL samples; green = post-BENGAL samples; Light blue = sites A, B, C and D.

There is a clear distinction between pre-BENGAL and BENGAL samples (Figure 2.23). The central site samples from both March 1998 and April 1999 cluster together with the other BENGAL samples from the central site rather than with the accompanying spatial samples from the same year, implying that spatial variability was greater than temporal variability. The accompanying MDS ordination (Figure 2.24A) supports the clear separation of spatial samples from central samples along with the distinction between pre-

BENGAL and BENGAL samples. The single sample from October 2000 is somewhat anomalous, clustering independently rather than with April 1999 (the other post-BENGAL sample).

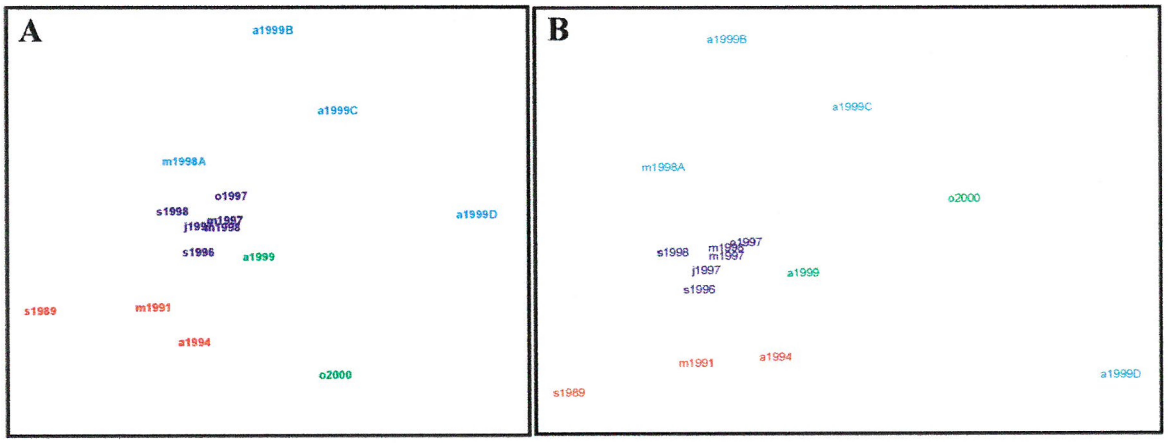

Figure 2.24. Multi-dimensional scaling (MDS) ordination of 15 mean cruise samples taken during an 11 year period between 1989 and 2000 at the central PAP site, and at four additional sites during 1998 and 1999. Using group-average linking of Bray-Curtis similarities calculated on √√-transformed abundance data for A, 29 identified megafaunal taxa (stress = 0.10); B, 28 identified megafaunal taxa (minus holothurians)(stress = 0.12) Red = pre-BENGAL samples; Dark blue = BENGAL samples; green = post-BENGAL samples; Light blue = sites A, B, C and D.

The holothurians were the dominant taxa in all samples and changes in their community structure and relative species abundances have been shown to mirror patterns observed for the entire megafauna community. In an attempt to discern whether or not it was the holothurians, and changes in their abundance and community structure, that influenced the separation of temporal and spatial megafaunal samples, multivariate analysis was performed on the total data set minus the abundance values for the holothurians. Both the dendrogram (Figure 2.25) and MDS ordination (Figure 2.24B) support the separation of pre-BENGAL and BENGAL samples and the separation of the additional four locations from the central site in both 1998 and 1999. It is also notable that spring and autumn samples cluster out together (e.g. a1997 and m1998, and o1997 and s1998). The additional location sampled in March 1998 (Site A) is closer in megafaunal composition to the samples taken at the central site than the three sites sampled in April 1999, even without the influence of exceptionally high abundances of several holothurian species.

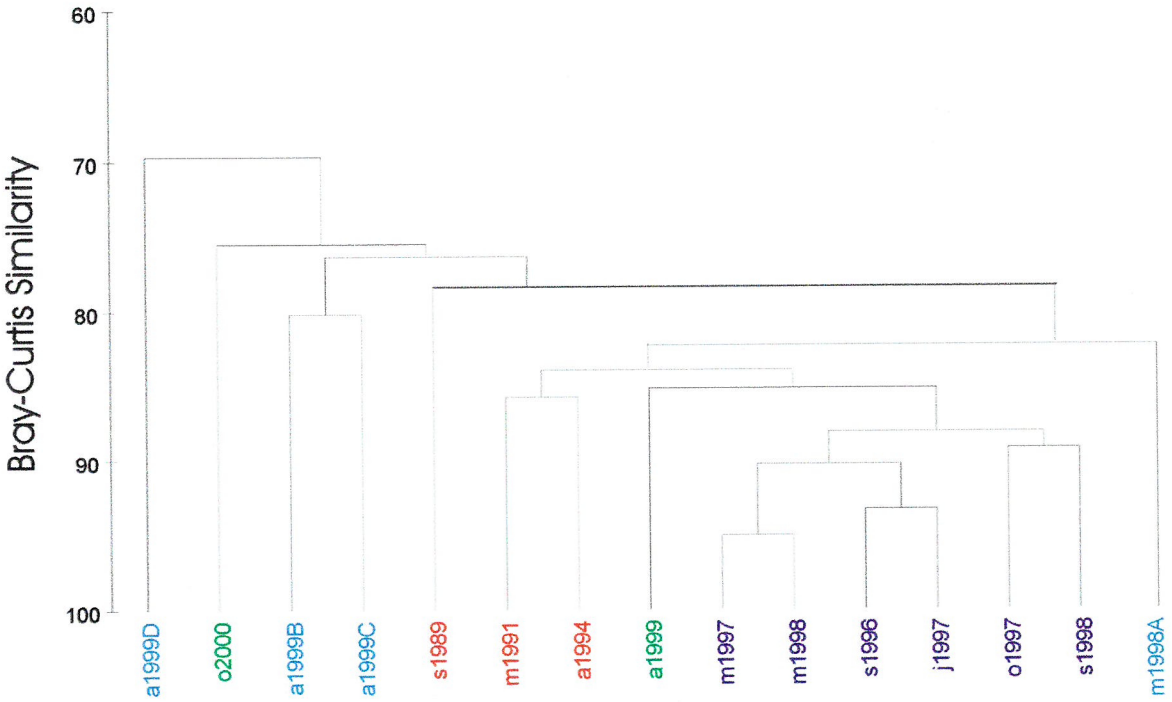

Figure 2.25. Dendrogram for hierarchical clustering of 15 mean cruise samples taken during an 11 year period between 1989 and 2000 at the central PAP site, and at four additional sites during 1998 and 1999. Using group-average linking of Bray-Curtis similarities calculated on √√-transformed abundance data for 28 identified megafaunal taxa (minus holothurians). Red = pre-BENGAL samples; Dark blue = BENGAL samples; green = post-BENGAL samples; Light blue = sites A, B, C and D.

2.4. Discussion.

Between April 1994 and September 1996 there was a sudden and significant increase in the abundance of many invertebrate megafauna taxa and species on the Porcupine Abyssal Plain (PAP), a trend that continued until April 1997 and persisted until at least late 2000. Although several taxa showed highly significant increases in abundance, overall the observed changes were dominated by the holothurians and one species in particular, *Amperima rosea*. The additional samples taken from other locations on the PAP showed that *A. rosea* occurred in high numbers across the area and was the dominant component of the epibenthic megafauna on the PAP. The sudden and sustained increase in its numbers on the PAP appears to have been a widespread and persistent event. It can be considered a 'long-lasting' event in that it appears not to have occurred previously in the last two decades, and yet has persisted for at least four years.

A sudden, apparently 'random' increase in the abundance of a single species is not unknown in deep-sea communities (e.g. Billett and Hansen, 1982). However, these apparently random changes in populations appear to have been localised spatially and restricted to a single species, and in the case of the holothurian *Kolga hyalina* (a species in the same family as *A. rosea*) to have lasted no more than one or two years (Billett and Hansen, 1982; Billett, 1991). The '*Amperima* Event', however, has occurred over a large area, has not been confined to one or two species and has persisted for at least four years. The simultaneous increases in the abundances of actinarians (notably *Actinauge abyssorum*, *Amphianthus bathybium* and *Iosactis vagabunda*), polychaetes, tunicates and the holothurians *Psychropotes longicauda* and *Pseudostichopus* sp. (see Table 2.2), indicated that community change at this scale on the PAP is more likely to be the result of environmental forcing.

Billett *et al.* (2001) found that the increase in abundance of *P. longicauda* was accompanied by a change in its body size distribution. The reproductive strategy of *P. longicauda* (see Tyler and Billett, 1987) is not suited to the rapid and large-scale colonisation of the seafloor, so an increase in its abundance may indicate a response to a longer-term trend. In addition, the *P. longicauda* populations during the BENGAL period were dominated by intermediate-sized individuals (Billett *et al.*, 2001), and the *A. rosea*

populations were dominated by small individuals (see Chapter 3), which may also point to an underlying environmental control of population dynamics, rather than to random changes in the population.

Deep-sea megafaunal communities are variable, although often in relation to geographical or bathymetric differences between localities. Thurston *et al.* (1998) present data on latitudinal variation in megafaunal abundance from three abyssal localities in the Northeast Atlantic. The mean abundance of invertebrate megafauna on the PAP was more than three times higher than that recorded for two localities on the Maderia Abyssal Plain (MAP). All three sites exhibited major differences in trophic structure, with the two MAP sites being more similar to each other than either were to the PAP site.

The pattern of temporal change on the PAP, in particular the speed at which the community structure was altered, was a previously unrecorded event in the deep sea. The availability of food has long been believed to be a major factor structuring the deep-sea benthos (Thiel, 1979). Food, in the form of phytodetritus sinking form surface waters, has already been shown to have a significant effect on the abundance of meiofauna in both the abyssal Northeast Atlantic (Gooday and Rathburn, 1999) and Northeast Pacific Oceans (Drazen *et al.*, 1998). The arrival of fresh phytodetritus at the deep seafloor and its subsequent ingestion by deposit-feeding megafauna has been suggested as an environmental cue for seasonal breeders (Tyler, 1988) and an important factor in the successful recruitment of juveniles to the benthos (Sumida *et al.*, 2000). During the BENGAL programme measurements of downward particle flux showed strong seasonal variation (Lampitt *et al.*, 2001). However, there was no discernable trend or large change in the quantity (dry-mass flux) of material sinking through the water column. The combination of the increase in abundance of several, but not all, taxa and the lack of inter-annual variability in quantitative particle flux leads to the question of whether it is the quantity or quality of food arriving at the seafloor that is the important driving force behind these changes.

One of the major questions to arise from this study is whether or not the temporal changes, observed at the central BENGAL site, were simply a reflection of wider spatial variability in megafaunal communities across the PAP. The analysis of trawl samples

from the four additional localities showed significant differences in the community structure from the central site (see Table 2.4), although only in 1999 and only between two of the nine major taxa. The majority of variation between sites appears to be attributable to changes in holothurian abundance, which may indicate that movements of these mobile organisms could influence the result of a single temporal sample. However, the sites are still separated even when the influence of the holothurians is removed from the analysis of the communities (see Figure 2.25). It is possible that large-scale changes in the abundance of mobile megafauna could be the result of immigration into the BENGAL area, but the changes observed during the '*Amperima* Event' encompassed changes in many taxa, some of them sessile or hemi-sessile (i.e. actinarians and tunicates). Spatial variability in the megafaunal community was still apparent, even without the potentially biased influence of the holothurians (see Figure 2.24B). The spatial variability on the PAP is real but it is unlikely to result in the significant temporal changes observed at the central site. In spite of the spatial variability in the megafaunal community *Amperima rosea* was the dominant species in all post-1996 samples, from all five sites. Therefore it is more likely that the changes at the central site reflect a large-scale temporal change across the wider PAP, with *A. rosea* becoming the dominant megafaunal species over the majority of the abyssal plain, as indicated by the exceptionally high abundance of this species at Site B.

When questioning the causative factors behind a sudden population explosion, such as that observed with *Amperima rosea*, scenarios such as natural or anthropogenic catastrophes, changes in the levels of intra- or interspecific competition, immigration, or changes in patterns of reproduction and recruitment are often considered as possible explanations for the changes observed.

The prolonged monitoring of this particular area of the Northeast Atlantic rules out the first of these potential causative factors. The PAP site is far enough away from the continental slope to not be affected by natural slope processes such as sediment slides and turbidity currents, and at present the potentially deleterious activity of fishing, oil, gas, mineral and waste disposal companies is non-existent in this part of the ocean.

The influence of competition can be addressed in several ways. *Amperima* rosea may be adapted physiologically for the rapid and efficient utilisation of a patchy food source. It is

possible that *A. rosea* is able to locate and exploit discrete patches of phytodetritus and that its life-history strategy is suitably adapted to rapidly utilise this input of energy and channel it into an increased reproductive effort, which may ultimately lead to a large scale recruitment event within the population.

The analysis of *in situ* images (see Chapter 4), obtained from the SOC Bathysnap system (Lampitt and Burnham, 1983), will allow a relatively detailed study of the population biology and impact of *A. rosea*, particularly seafloor coverage (sediment tracking) and variability in abundance.

Differences in patterns of reproduction and recruitment (see Chapter 3), particularly in response to an environmental cue, may also play a role in facilitating a sudden 'bloom' of one or two closely related species. Although the dominant mode of reproduction in many deep-sea invertebrates has been shown to be continuous, there is evidence for a seasonal pattern of reproduction in certain species of deep-sea invertebrate, mainly from within the echinoderms (Tyler *et al.*, 1994a) For a deep-sea species to develop a strategy of seasonal reproduction there must be some exogenous factor that controls or initiates the development of the gonad and/or spawning. Gage and Tyler (1991) suggested that the sinking from surface waters of seasonally produced organic matter forms a food source for developing larvae in the plankton; and when it reaches the seafloor provides a nutritional source to fuel vitellogenic development in the adults and the growth of newly settled post-larval juveniles.

Several studies, conducted during the BENGAL programme, have highlighted a common nutritional link between those species that have increased in abundance. The links between food availability, species-specific selection and changes in abundance are discussed further in Chapter 5, with reference to the major holothurian species. (Iken *et al.*, 2001) have shown, using stable isotope analysis, that species exhibiting the greatest increase in abundance (i.e. *Amperima rosea*, *Ellipinion molle* and *Ophiocten hastatum* (Bett *et al.*, 2001)) have $\delta^{15}N$ values close to that of phytodetritus and those meiofauna that have been shown to respond rapidly to the deposition of phytodetritus (Gooday, 1988). Data presented on the sterol chemistry of holothurians (Ginger *et al.*, 2000; 2001) and the flux of sterols and lipids through the water column (Kiriakoulakis *et al.*, 2001)

indicates that these sterols form an important nutritional component in the diet of deposit-feeding megafauna. It is suggested that some sterols are more important, and more readily metabolised, than others, particularly in the case of *A. rosea*. Species that feed preferentially on fresh phytodetritus may be particularly influenced by changes in the timing, quantity or more importantly the quality of organic matter arriving at the seafloor.

The possible immigration of *Amperima* to the PAP from other areas of the ocean basin is a problem that can hopefully be addressed by the application of molecular genetic techniques (see Chapter 6). It will be important to establish whether or not samples taken, and those still to be taken, are from the same population at the BENGAL site. It is hoped that the analysis of variability in mitochondrial DNA will provide information as to the spatial relationships between groupings of *A. rosea* collected from sites across the whole PAP.

Chapter Three – The reproductive biology of *Amperima rosea*

3.1 Introduction.

3.1.1 Reproductive patterns in marine invertebrates.

Over 99% of all invertebrates exhibit sexual reproduction at some stage in their lives and it is usually the only means by which reproduction can take place. However, many organisms can reproduce asexually. Asexual reproduction can take place either by subdivision of an existing body into two or more multicellular parts (budding and fission) or by the production of diploid eggs (parthenogenesis). Reproduction by fission is particularly common in the soft-bodied phyla such as the Porifera, Cnidaria and some Echinodermata. Fission is usually combined with the capacity for sexual reproduction in complex life cycles with asexual and sexual generations, e.g. the cladoceran *Daphnia*. Examples of both sexual and asexual reproduction can be found within the marine invertebrates. However, sexual reproduction is by far the more prevalent strategy.

The environment in which animals live may influence reproductive patterns. Marine invertebrates, reproducing sexually, are able to discharge gametes into the surrounding medium where external fertilisation takes place, frequently followed by development into planktonic larvae. The majority of animal phyla with marine representatives have most or at least some species with pelagic larvae and external fertilisation; they are described as having a pelago-benthic life cycle. In addition, all those phyla with pelagic larvae also have representatives with non-pelagic, benthic larvae, a condition usually interpreted as an 'advanced' state involving the suppression or modification of the larval phase. A smaller number of species have non-feeding pelagic larvae that are supplied with yolk by the parent organism. These different kinds of larvae are described as exhibiting planktotrophic, lecithotrophic or direct patterns of development. Although the possession of planktotrophic larvae is considered to be a 'primitive' trait, over 70% of all marine invertebrates in temperate zones have planktotrophic larval development. This seems to imply that in most cases the planktotrophic mode of development has clear advantages; possibly in some or all of the following situations:

1. Exploitation of the temporary food resource provided by phytoplankton blooms.
2. Colonisation of new habitats.
3. Expansion of geographical range.
4. Avoidance of catastrophe associated with local habitat failure.
5. Avoidance of competition.
6. Exposure of diverse offspring to the maximum degree of habitat diversity

A major correlate of the 'primitive' reproductive patterns is the mass discharge of gametes in an annual spawning event. This is made possible by the storage of gametes in body cavities, often the coelom, a common practice among many marine invertebrates. 'Advanced' reproductive traits, such as lecithotrophic larva, brooding and continuous spawning are often confined to the smaller representatives of most taxa, although these traits may also be observed in marine invertebrates living in environmental conditions such as deep sea, polar or estuarine regions. The evolution of large body size, particularly amongst deep-sea invertebrates, has made it possible for certain taxa to adapt more 'primitive' patterns of reproduction. Annual spawning events, resulting in the production of planktotrophic larvae, have been recorded for several species of deep-sea echinoderms (Tyler and Pain, 1982; Tyler *et al.*, 1984b; Tyler *et al.*, 1990).

Successful reproduction for those animals exhibiting a single, seasonal, spawning event requires a high degree of synchronisation not only of reproductive events within an individual but also between individuals within a population. Spawning has been shown to be highly synchronous for several shallow water invertebrates, most famously the colonial corals of the Great Barrier Reef. In many cases it has been shown that the spawning of males actually stimulates the females to release eggs into the water column. Eggs shed directly into a suspension of sperm from their own species will have a much greater chance of being successfully fertilised. Many invertebrates, particularly echinoderms, have been shown to form aggregations (Grassle *et al.*, 1975), or very occasionally pairs (Tyler *et al.*, 1992) presumably to increase their fertilisation success by spawning in the close vicinity of conspecifics.

The key elements in the reproductive patterns of marine invertebrates are the repeated cycles of gamete production and release. These cycles can be controlled through the cyclic production of two basic groups of hormone: those with a gonadotrophic role and

those that induce spawning or gamete maturation. In echinoderms the gonadotrophic function is associated with changes in the relative levels of progesterone and oesterone, while in asteroids, spawning is initiated by a reaction involving the secretion of a neurosecretory peptide from the radial nerves triggering the production of 1-methyl adenine by the ovary. Similar patterns of gonadotrophy and spawning cues have also been discovered in polychaetes and molluscs.

3.1.2 Gametogenesis.

Larval development and settlement are both major patterns of reproduction that play important roles in the ecology and biogeography of the adult animals. However, prior to the larval phase there is sequence of reproductive processes, including the differentiation, growth and maturation of gametes, spawning and fertilisation. All these events may be influenced by both phylogenetic and environmental factors. Many aspects of an organism's life history are constrained by gametogenesis that makes it one of the most important steps in the reproductive process. In addition the egg is one of the most valuable cells in the life history of any species, they are highly specialised, large cells, which following activation, initiate the process of embryogenesis (Eckelbarger, 1983; Eckelbarger, 1994). In sexually reproducing species the egg contributes half the genetic information to the zygote and provides the developing embryo with energy. In species where development is lecithotrophic, the egg provides energetic reserves for sustaining the larva until metamorphosis and settlement takes place (Jaeckle, 1995). Ovarian structure and vitellogenic mechanisms have been shown to play an important role in the rate of egg production, fecundity, energy content of eggs and related larval characteristics (Eckelbarger, 1994).

With the exception of the Porifera, egg production occurs in the reproductive organs of the female, the ovaries. Ovaries vary in shape and complexity, from the loose groups of germ cells found in the Cnidaria, to the distinct complex organs found in the majority of higher taxa (Eckelbarger, 1994; Jaeckle, 1995). The process of producing mature oocytes (eggs) is known as oogenesis. This involves the differentiation of germ cells (oogonia) into previtellogenic oocytes and the growth and maturation of these young oocytes by the accumulation of ooplasmic energy reserves (yolk) during vitellogenesis. Developing

oocytes are usually accompanied by accessory cells, which are involved in the process of heterosynthetic yolk production. In addition, nurse cells originate from germ cells and subsequently abort their development to provide metabolites for the developing oocytes. Nurse eggs are also abortive germ cells that, when phagocytosed, can pass their product to the healthy maturing oocytes (Eckelbarger, 1994).

Vitellogenesis, the process of synthesis and storage of energy reserves, is a long but important phase in the development of the oocyte. Vitellogenic patterns are species specific and have major implications for reproduction, influencing factors such as rate of egg production, fecundity and larval development (Eckelbarger, 1983; Eckelbarger, 1994; Eckelbarger and Watling, 1995). The energetic reserves of the oocyte are commonly referred to as the yolk, but they include a variety of products such as lipids, carbohydrates, proteins, free amino acids, nucleic acids and pigments (Eckelbarger, 1994). Within the invertebrates three distinct vitellogenic pathways can be found, each affecting the reproductive strategies of the adult animal. These pathways are referred to as autosynthetic, heterosynthetic and mixed yolk formation. The autosynthetic pathway involves the uptake of exogenous low molecular weight precursors and a subsequent synthesis of vitellin by the proteosynthetic organelles of the oocyte. In contrast, the heterosynthetic pathway involves the transport of externally synthesised yolk proteins into the oocyte. The third pattern is a combination of the first two pathways (Eckelbarger, 1994; Jaeckle, 1995). Those species exhibiting the autosynthetic pathway tend to have a slow rate of oocyte growth as a result of the time consuming processes taking place during vitellogenesis. Conversely, oocytes with rapid rates of growth are characteristic of species using a heterosynthetic pathway for yolk production, a common pattern for species of higher phyla (Eckelbarger, 1994).

Differences in the patterns of vitellogenesis can affect the breeding frequency of a species, while at the same time the vitellogenic mechanisms themselves can be affected by external environmental factors, such as food quantity and quality or habitat stability. Low food levels, or a drop in food quality, can cause a decrease, or even an interruption, in yolk synthesis, resulting in a slowing of the egg production rate and a lower fecundity (Bridges *et al.*, 1994; Eckelbarger, 1994; Levin *et al.*, 1994). Unstable environments, such as hydrothermal vents or episodic large organic food falls, would select for species with rapid egg production and a heterosynthetic pattern of vitellogenesis. Alternatively

species inhabiting the more constant and stable environment of the abyssal plains would be expected to show slower rates of egg production, with a periodic or continuous pattern of reproduction evident throughout their longer life (Eckelbarger, 1994; Scheltema, 1994).

3.1.3 Larval development in the deep sea.

It is important to remember that the majority of benthic organisms spend a large proportion of their early life in the water column as planktonic larvae. The study of the larval stages has a major role in our understanding of the life-history strategies, biogeography and community dynamics of benthic invertebrates. Larvae, and patterns of larval development, were originally classified depending on their nutritional mode and development location (Thorson, 1950). Planktotrophic larvae were described as those that feed and develop in the water column, and lecithotrophic as those that obtain their energy from yolk substances in the egg. The latter were sub-divided into pelagic lecithotrophs, which develop in the plankton, and brooded lecithotrophs, which do not have a pelagic phase in their development.

Currently it is Mileikovsky's classification of larval development that has been adopted (Mileikovsky, 1971; Mileikovsky, 1974). This classification includes four types of larval development: 1) pelagic development (in the plankton), 2) demersal development (in the near-bottom layer), 3) direct development (offspring hatch directly as juveniles) and 4) viviparity (development into juveniles occurs within the parental organism). Planktonic larvae may either be planktotrophic or lecithotrophic. In general those species with a planktotrophic strategy would produce a large number of small eggs (<150-200μm), while lecithotrophs produce a small number of large, yolk-rich eggs (Jaeckle, 1995; Levin and Bridges, 1995), although there are exceptions (see Young *et al.*, 1996b). Planktotrophic larvae feed on bacteria, phytoplankton, zooplankton or detritus; they therefore have well-developed feeding and swimming appendages and often spend a long period of their early life in the water column. Alternatively, lecithotrophic larvae depend on the parental investment in the egg, obtaining their energy from the egg yolk. A planktotrophic pattern of development favours wide dispersal, high levels of gene flow and a wide geographical range, but larval mortality is high. The lecithotrophic pattern implies a high maternal investment in the egg, which enhances survival of the larvae, but

is believed to have lower dispersal potential (Shilling and Manahan, 1994) and lower mutation rates (Gage and Tyler, 1991; Levin and Bridges, 1995; Morgan, 1995).

Scheltema (1971) proposed a different method of classification based on the dispersal potential of invertebrate larvae. This classification consists of four categories: 1) Teleplanic larvae with planktonic periods exceeding two months, 2) Actaeplanic larvae that remain in the plankton from one week to less than two months, 3) Anchiplanic larvae with planktonic phases ranging from a few hours to a few days, and 4) Aplanic, non-planktonic, larvae. For many years it was a common belief that deep-sea and polar species would reproduce continuously and have direct development as predicted by Orton (1920) and Thorson (1950). There is now evidence of seasonal reproduction in several abyssal species (Gage and Tyler, 1991) and planktotrophic and lecithotrophic larval development are the rule rather than the exception in deep-sea invertebrates (Gage *et al.*, 1986; Tyler *et al.*, 1990; Pearse, 1994; Scheltema, 1994).

It has long been accepted that planktotrophic larvae have higher dispersal capabilities than lecithotrophic larvae (Levin and Bridges, 1995). Although this is not always reflected in the geographical distribution of some deep-sea species. The holothurian *Psychropotes longicauda* possesses the largest measured egg of any deep-sea invertebrate at 4.4mm (4400μm), with eggs of this size a direct pattern of development may be inferred (Hansen, 1975; Tyler and Billett, 1987). However it exhibits one of the most cosmopolitan ranges of any deep-sea benthic species (Hansen, 1975). This may be related to the direct development of this species is occurring in the pelagic environment (Billett *et al.*, 1985). There is now evidence that the dispersal potential of larvae is not always related to larval type. Geographic distributions may also be determined by the 'age' of a species. On evolutionary time-scales a longer-lived species will have had longer to disperse and various geographic and oceanographic barriers that exist today may not have posed such a problem to dispersal in earlier periods of earth's history.

In order to fully understand the dispersal capabilities of an organism it is imperative that physical and environmental factors are taken into account. Shilling and Manahan (1994) showed that several lecithotrophic larvae of Antarctic echinoderms used up to 50% of their energetic reserves within 60 months compared to planktotrophic larvae that used the

same amount in only 10 months. In addition the survival time of larvae hatching from eggs of a similar size was much longer for polar species compared to species from temperate waters. It is possible that the uptake of dissolved organic matter by some polar larvae and the effect of low temperature, in polar and deep-sea waters, on reducing metabolic rates may allow lecithotrophic larvae to have longer pelagic phases whilst being sustained by their own nutrient reserves (Lutz, 1988; Gage and Tyler, 1991; Shilling and Manahan, 1994). There is now growing evidence for lecithotrophic larvae of hydrothermal vent invertebrates being found in plumes and dispersing in near-bottom flows. It is possible that this larval type could have a high dispersal potential by way of physical transport in water masses (Mullineaux, 1995; Mullineaux *et al.*, 1995; Kim and Mullineaux, 1998).

3.1.4 Reproduction in Echinoderms: Deep Sea v. Shallow Water.

During the early to mid 1900s two major rules governing reproductive processes in marine invertebrates were established and widely accepted for many years. The first, known as Orton's Rule, implied that breeding would be controlled by sea temperature. Species inhabiting isothermal waters, such as the deep sea or polar waters, were predicted to have a continuous pattern of reproduction (Orton, 1920). The second theory, known as Thorson's Rule, predicted that polar and deep-sea species would possess a pattern of direct development in response to the high mortality risks associated with a long larval phase (Thorson, 1950). Thorson went on to predict that polar and deep-sea species would have similar reproductive strategies with low fecundity and large eggs for direct development.

It is now known that the production of large eggs in most deep-sea species leads to a lecithotrophic larval development, and that some species seasonally produce a high number of small eggs that develop into planktotrophic larvae (Tyler, 1988). The majority of the evidence for challenging the rules of Orton and Thorson has come from the extensive studies of deep-sea representatives of the phylum Echinodermata.

Echinoderms have provided the most complete set of reproductive data for any of the deep-sea invertebrate taxa. A detailed analysis of the reproductive biology of a large number of echinoderms has been made by Tyler and colleagues (Tyler, 1986; Tyler and

Billett, 1987). From their observations it became evident that in the deep sea there are three main types of reproductive pattern exhibited within the Echinodermata.

1. Quasi-continuous reproduction.

This is the dominant pattern of reproduction in deep-sea echinoderms. In females, it is characterised by the production of relatively few large eggs (600μm to 4.4mm) that are all well invested with yolk. Analysis of oocyte size frequencies indicates that there is a large reserve of small previtellogenic (no yolk) oocytes (<300μm) present in the ovary. Some of these will undergo vitellogenesis, the process of yolk deposition, and develop to the maximum size. In many of the species examined there is evidence of recycling of unspawned oocytes and the occasional presence of 'nurse' oocytes. It is thought that these 'nurse' oocytes grow to a certain size and then break down presumably to provide energy for maturing oocytes.

2. Intermediate development.

This pattern of reproduction has been recorded for very few deep-sea echinoderms. Mature oocytes tend to be of an intermediate size (*c.*400μm) and females exhibit an intermediate fecundity (*c.* 10^4 eggs ind.$^{-1}$) (Gage and Tyler, 1982). However, an analysis of oocyte size frequencies shows no evidence of gametogenic seasonality. In those shallow water species that exhibit this particular reproductive pattern an abbreviated larva is usually found. The possession of pelagic larva by deep-sea species is rare or non-existant (Gage and Tyler, 1991). However, it has been suggested recently that the cosmopolitan deep-sea holothurian *Protankyra brychia* may produce a planktotrophic auricularia larva (Gage, pers comm.). Gage and Tyler (1982) have shown a seasonal pulse in recruitment of post-larvae to adult populations, and in the absence of any seasonality in the production of gametes they assume that the mortality of new recruits must vary at different times of year. It is possible that this variation is related to the seasonal deposition of fresh phytodetritus.

3. Seasonal reproduction.

This was an unexpected reproductive pattern for deep-sea invertebrates (Orton, 1920; Thorson, 1950) and was first proposed for echinoderms by Schoener in the late 1960s. By the early 1990s there was unequivocal evidence of seasonal reproduction in three

echinoderm species and circumstantial evidence for a further five species (Gage and Tyler, 1991). Identifying characters for species exhibiting seasonal reproduction include small eggs (*c.*100-200μm), high fecundity, synchrony of gamete development and a distinct seasonal reproductive cycle. The seasonal features of this pattern are: (i) the initiation of gametogenesis in the spring of each year (except in *Echinus affinis* where it occurs in November/December); (ii) active vitellogenesis during the summer months and early autumn; (iii) spawning in early spring of each year (Tyler, 1988).

The study of several echinoderm species from the northeast Atlantic has highlighted different pathways by which the seasonal phytodetrital drop may be coupled with patterns of synchronous reproduction

3.1.4.1 Ophiuroidea.

The majority of ophiuroid species in both the deep-sea and in shallow water, are gonochoristic. Those that are hermaphrodites are usually protandric, such as *Ophiacantha bidentata* (deep sea) (Tyler and Gage, 1982). Continuous reproduction is the most prevalent pattern amongst ophiuroids (e.g. Gage and Tyler, 1982), although there are records of patterns of seasonal reproduction for deep-sea species such as *Ophiura ljungmani* (Tyler and Gage, 1980) and shallow-water species such as *Amphipholis kochii* (Iwata and Yamashita, 1982). It was the analysis of populations of deep-sea ophiuroids, by Schoener (1968), that led to the concept of seasonal reproduction in deep-sea organisms becoming widely accepted.

3.1.4.2 Asteroidea.

Reproductive patterns of the asteroids, or seastars, have been well documented for both shallow and deep-water species. A range of reproductive strategies have been observed for deep-sea asteroids, with a large data set from the Rockall Trough provided by Pain, Tyler and co-workers. Quasi-continuous reproduction has been recorded for *Benthopecten simplex*, *Pectinaster filholi* and *Pontaster tenuispinus* at a depth of 2200m (Pain *et al.*, 1982b). All three species produce large eggs (800-950μm) and phagocytes break down any unspent oocytes. Examination of the gametogenic biology of *Bathybiaster vexillifer* from the same location also shows a continuous pattern of reproduction (Tyler *et al.*, 1982b). Large oocytes (~1000μm) may be spawned either as

groups or as a continuous slow release. From the large egg size, a pattern of direct external lecithotrophic development has been inferred for this species.

Evidence of an unexpected seasonal pattern of reproduction has been recorded for *Plutonaster bifrons* (Tyler and Pain, 1982) and *Dytaster insignis* (Tyler *et al.*, 1990). Both *P. bifrons* and *D. insignis* produce numerous small (~120um) eggs that in *P. bifrons* appear to show a distinct synchrony of production. A spawning period of March to early June has been predicted, with unspawned specimens showing evidence of internal oocyte degradation. On the basis of egg size, fecundity and gametogenic strategy indirect planktotrophic development is inferred for this species. It is believed that the transfer of seasonal surface primary production to deep water, i.e. sinking phytodetritus, provides food for the developing larvae. Tyler *et al.* (1993) suggested that diet might actually determine the different reproductive patterns found in the sympatric seastars *Bathybiaster vexillifer* and *Plutonaster bifrons*. Prey diversity was found to be significantly lower for the seasonally breeding *P. bifrons* compared to that recorded for *B. vexillifer*. Additionally the organic carbon content in the sediment residue from the stomachs of *P. bifrons* displayed a seasonal cycle, while no such seasonality was detected in *B. vexillifer*. Bathysnap observations of the feeding behaviour of both species shows *P. bifrons* feeds close to the sediment surface, where it may experience dietary changes brought about by the seasonal availability of phytodetritus and fish carcasses, where as *B. vexillifer* is an active predator feeding deeper in the sediment.

In contrast to the deep sea, most shallow-water asteroid species favour the production of large numbers of small eggs and show distinct seasonality in their patterns of reproduction (Crump, 1971; Barker, 1979). One form of reproduction that has so far not been reported for deep-sea asteroids, or any other group of echinoderms, is asexual reproduction by fission. Fissiparous seastars are usually distinctly asymmetrical, with a set of large and a set of smaller regenerating arms; they typically have greater than five arms, with six and eight-armed forms being particularly common (Emson and Wilkie, 1980). Fissiparous seastars are normally capable of reproducing sexually as well as asexually, as is the case with the hermaphroditic seastar *Nepanthia belcheri* (Ottensen and Lucas, 1982) although other species may be obligately fissiparous, such as the North American seastar *Stephanasterias albula* (Mladenov *et al.*, 1986). In many populations where both sexual and asexual reproduction is exhibited, it is fission that tends to be the

dominant pattern of reproduction and the primary mode of recruitment (Emson and Wilkie, 1980; Ottensen and Lucas, 1982).

3.1.4.3 Echinoidea.

The echinoids also exhibit a range of reproductive patterns, both in the deep sea and shallow water. Seasonal patterns of reproduction in deep-sea species have been identified in *Echinus affinis* (Tyler and Gage, 1984a) and *Echinus acutus* var. *norvegicus* and *Echinus elegans* (Gage *et al.*, 1986). All three species possess the small eggs (<200μm) from which a planktotrophic mode of larval development can be inferred. Alternatively, five species of the soft-bodied echinothuriid sea urchins were all found to have patterns of gametogenesis consistent with a strategy of continuous reproduction, as was the cidarid *Poriocidaris purpurata* (Tyler and Gage, 1984b). All these animals produced large eggs, 1100-1500μm. Utilising data from shallow-water relatives direct lecithotrophic mode of larval development was inferred.

In shallow water, *Echinus esculentus* also exhibits a seasonal pattern of gametogenesis, similar to its deep-sea congeners (Nichols *et al.*, 1985a; Nichols *et al.*, 1985b), where as shallow water cidarids exhibit a variety of reproductive strategies. Some species have been found to produce small (<110um) eggs indicative of planktotrophic development (Holland, 1967) where as other species such as *Heliocidaris erythrogramma* produce eggs up to 400μm in diameter and are known to have an abbreviated form of larval development (Williams and Anderson, 1975).

3.1.4.4 Holothuroidea.

Unlike their close relatives, the deep-sea holothurians do not, as yet, possess a species to which can be attributed a seasonal pattern of reproduction. However, holothurians do exhibit the peculiar reproductive strategy of brood-protection. Until the late 1960s, this particular trait was only known to occur in approximately 30 species of holothurian belonging to the orders Dendrochirotida and Apodida. All examples of species with brood protection were from littoral and sub-littoral animals, the majority being inhabitants of cold seas, particularly the waters around Antarctica. However, during the examination of the holothurians dredged during the Galathea Expedition, Danish

zoologist Bent Hansen found evidence of brood-protection in Panama Basin populations of the deep-sea holothurian *Oneirophanta mutabilis mutabilis* (Family Deimatidae, Order Elasipodida) (Hansen, 1968). Unlike most of the shallow-water species, which exhibit coelomic incubation of the developing young, *O. mutabilis mutabilis* protects its offspring within the ovaries. This is a rare condition only previously observed in the Antarctic synaptid *Taeniogyrus contortus* and a few species of Antarctic ophiuroids (Hansen, 1968).

The majority of shallow-water holothurians exhibit annual to semi-annual patterns of reproduction, producing planktotrophic larvae (Sewell and Bergquist, 1990 Sewell, 1992; Tuwo and Conand, 1992; Conand, 1993; Herrero-Perezrul *et al.*, 1999), although there are also records of direct development (Rutherford, 1973). Like their asteroid relatives the holothurians may also reproduce asexually by way of fission (Crozier, 1917). By the early 1980s asexual reproduction had been reported for six species of holothurian (Emson and Wilkie, 1980), with fissiparity particularly well documented for the genus *Holothuria* (Harriott, 1982; Emson and Mladenov, 1987; Chao *et al.*, 1993; Uthicke, 1997). Chao *et al.* (1993) found that fission in *Holothuria atra* occurred in small individuals living in shallow tidepools, suggesting that a stressful environment probably triggers fission. Uthicke (1997) found that asexual reproduction in populations of *H. atra* from the Great Barrier Reef displayed a distinct seasonal pattern. Rates of fission for this species have been shown to be higher in eutrified waters (Conand, 1996). It is possible that in these areas an accompanying increase in benthic productivity provides more food for the deposit feeding holothurians, therefore increasing the carrying capacity of the environment and allowing a denser holothurian population to be sustained. In populations where individuals are reproducing asexually, sexual reproduction can still be observed, but is often limited and restricted to certain times of the year, as is the case with *Holothuria parvula* (Emson and Mladenov, 1987). A lack of small individuals in a holothurian population is often an indicator that larval recruitment has been unsuccessful and that the population is probably being maintained by fission.

The reproductive biology of deep-sea holothurians has been relatively well documented, especially for cosmopolitan species and those found in the North Atlantic, although there is, as yet, no evidence for seasonal patterns of reproduction. Nor is there any evidence for asexual reproduction by fissiparous species. Reproductive patterns have been well

described for the majority of species sampled from abyssal and bathyal depths in the northeast Atlantic. The families Psychropotidae and Deimatidae (order Elasipodida) were examined by (Tyler and Billett, 1987). Psychropotid species, such as *Psychropotes longicauda* and *Benthodytes typica*, produce large oocytes (1200-3000μm) all of which undergo vitellogenesis. (Hansen, 1975) recorded a maximum egg size of 4400μm, from a specimen of *P. longicauda*, which is the largest egg known for any holothurian. Direct development of the larvae is inferred, however juveniles have been taken in rectangular mid-water trawls (RMTs) at distances of 17 to 1000m off the seabed. This suggests the possibility of a pelagic juvenile capable of dispersal by deep-ocean currents and may account for the cosmopolitan distribution of many of these species (Tyler and Billett, 1987; Gebruk *et al.*, 1997). The deimatids, such as *Oneirophanta mutabilis* and *Deima validum*, also produce relatively large eggs (c.900μm) but not all of them undergo vitellogenesis and develop to mature oocytes. Those eggs that do not fully develop are believed to act as nurse cells, providing additional nourishment for those oocytes continuing to grow. Representatives of both families have low fecundities, another indication of a more direct mode of larval development. One reproductive oddity of deep-sea holothurians, so far restricted to the Deimatidae, is apparent lack of functional males in any sample. About 50% of the specimens from the majority of populations sampled, the exception being the Panama Basin population, are female but the other half of the population are sexually inactive and presumed to be male (Tyler and Billett, 1987). Other holothurians examined include *Peniagone azorica* and *P. diaphana* (family Elpidiidae), whose maximum egg size (c.300μm) indicates abbreviated larval development with the benthopelagic lifestyle of adult *P. diaphana* suggesting a planktonic larva (Tyler *et al.*, 1985a).

The closely related bathyal holothurians, *Laetmogone violacea* and *Benthogone rosea*, show interspecific variation in their patterns of reproduction (Tyler *et al.*, 1985b). The eggs *of L. violacea* reach a maximum size of 350μm, whilst the eggs of *B. rosea* remain previtellogenic until 300μm before developing to a maximum size of c.750μm. The egg size of *L. violacea* suggests that it will give rise to an 'abbreviated' larva, which may be dispersed by near-bed current activity. The large egg produced by *B. rosea* is more typical of a direct mode of development to a juvenile, which probably occurs close to the

adult population. In spite of their differing modes of larval development, both species appear to reproduce continuously all year round, with no evidence of seasonal synchrony.

Other continuously reproducing deep-sea species include, *Ypsilothuria talismani* (Tyler and Gage, 1983), *Bathyplotes natans* (Tyler *et al.*, 1994b), *Cherbonniera utriculus* and *Molpadia blakei* (Tyler *et al.*, 1987). At present there is no evidence for seasonality of reproduction amongst the deep-sea holothurians, but this does not mean that their reproductive strategies are all straightforward. Holothurians of the genus *Paroriza* have often been observed and photographed in pairs or triplets. *Paroriza pallens* and *P. prouhoi* have both been found to be simultaneous hermaphrodites, reproducing continuously and producing intermediate to large (350-450μm) yolky eggs (Tyler *et al.*, 1992). It is suggested that the pairing behaviour of these holothurians increases the likelihood of cross fertilisation and that spawning is actually induced by the presence of a conspecific rather than seasonal cues.

3.2 Materials and Methods.

3.2.1 Collection.

All BENGAL samples (1996-99) were taken in the middle of the Porcupine Abyssal Plain to the southwest of Ireland (Billett and Rice, 2001). The trawls were located within a 20 nautical mile (37km) radius of the central BENGAL station (48° 50'N 16° 30'W) where the associated coring programme was undertaken (see Table 2.1 for details). Seabed depth within the sampling area varied within a few metres of 4840m (trawled range 4802-4850m).

Additional trawls in March 1998 were fished some 30 nautical miles (55km) to the east of the BENGAL area (see Figure 2.1 site A) Three further sites were added in April 1999, 50km southeast, 50km northeast, and 100km to the north (see Figure 2.1 sites B, C, and D). All these additional trawls were undertaken to assess spatial variability in the benthic community. Details of trawls taken prior to BENGAL (1989-1994) are presented in Table 2.2 (Rice *et al.*, 1991; Thurston *et al.*, 1994; Thurston *et al.*, 1998).

Sampling was undertaken with a semi-balloon otter trawl (OTSB 14) with a wing-end spread of 8.6m (Merrett and Marshall, 1981). The height of the net from the footrope to the headline was approximately 1.5m. The net was constructed of 44mm stretch mesh in the main part of the net, 37mm stretch mesh in the middle part, and a 13mm stretch mesh liner in the cod end. During fishing, the OTSB was monitored by an acoustic beacon mounted on one of the trawl doors. Acoustic telemetry provided information on the depth of the net, the time the trawl spent in contact with the seabed, and the fishing performance of the net, as measured by the angle of the trawl door. Distance trawled and wing-end spread of the net was used to calculate an area fished for each trawl. This area was recorded in units of hectares (1ha = 10,000m^2).

Samples for reproductive analysis were sorted at sea, fixed in 5% borax-buffered formaldehyde in seawater, and transferred to 80% methylated spirit after 3-5 days. The exception to this being in 1999, when samples were dissected at sea and the gonads were independently fixed in Bouin's Solution prior to being transferred into 80% methylated spirit.

3.2.2 Processing.

30 intact adult specimens of *Amperima* were selected from each cruise during the period 1994-1999; this covers the period of the BENGAL programme and one pre-BENGAL sample.

All dissected holothurians had their guts and gut contents removed and were then weighed (gut-free wet weight) along with the gonad, to the nearest 0.01g, to allow the calculation of gonad indices (Conand, 1989; Hamel *et al.*, 1993; Morgan, 2000). The dissected specimens were then labelled and placed in individual bags, containing 80% alcohol, for storage. The samples of gonad tissue were then placed in a correspondingly labelled tube, containing Bouin's solution, for 48 hrs. From this point onwards the samples from the 1999 cruise were subjected to an identical protocol. The tissue samples were then dehydrated through a series of graded alcohols before being placed in Histoclear™ solution (see appendix II for full protocol). Upon removal from Histoclear™ the samples of gonad tissue were immersed in hot paraffin wax and incubated at 70°C for at least 8hrs. Each individual sample of tissue was then imbedded in a block of paraffin wax, along with its label, and allowed to set. Fine sections of each gonad sample (5μm) were taken from the wax block using a microtome. With the aid of a water bath (40°C) each section was placed onto a labelled slide before being left to dry.

3.2.3 Staining.

To facilitate analysis of the gonad sections they have to be immersed in a series of histological stains, which reveal specific structures within the gonad. The two most commonly used stains are Mayer's Haemalum and Eosin.

The haematoxylin in Mayer's Haemalum stains the nuclei and basophilic tissues, such as the basophilic cytoplasm of previtellogenic oocytes. These features are resolved as a dark blue-purple colour. Eosin stains for muscle fibres, keratin, connective tissue, blood cells and acidophilic cytoplasm (found in vitellogenic oocytes). These features are resolved as a bright pink colour (Culling, 1974). Other stains that may be employed to resolve additional useful features include Masson's trichrome and Sudan Black.

Masson's trichrome technique can be used to identify connective tissue and nuclei stained with Mayer's haemalum. Nuclei are stained blue-black while the cytoplasm, muscle and

acidophilic granules appear red. Cartilage, mucin and basophilic cytoplasm are all resolved as a blue-green colour (Culling, 1974).

Sudan Black is a fat-soluble dye commonly used to stain phospholipids. Lipids will be stained blue or black if they are present in the tissue in enough quantity. If lipids are not abundant the tissue will be stained brownish-black. Any cell nuclei present in the tissue will be counterstained red (Pearse, 1961)

3.2.4 Image analysis.

The external and internal morphology of the gonads were described using dissecting (Leica MZ-8) and compound (Olympus BH-2) microscopes. Selected compound images of each female section were transferred to bitmap image files using a JVC Video camera (TK 1280E) and Rainbow Runner/Matrox PC-VCR software. The patterns of oogenesis were then further analysed using Jandel Scientific SigmaScanPro 4 image analysis software.

Only those oocytes that had been sectioned through the nucleus were measured to ensure that the maximum diameter possible was recorded. The feret diameter of each oocyte was selected as an appropriate measure of size, as the oocytes of *Amperima* are not uniform in their shape. The measure of feret diameter gives the diameter of a fictitious circular object that has the same area as the object being measured. Feret diameter is calculated as;

$$\text{Feret Diameter} = \sqrt{((4 \times \text{area})/ \pi)}$$

The female gametes were divided into two recognisable classes, (i) previtellogenic oocytes and (ii) vitellogenic oocytes. When stained with Haemalum and Eosin the previtellogenic oocytes are characterised by a large, dark stained nucleus that occupies most of the blue stained basophilic cytoplasm. In contrast, the vitellogenic oocytes are resolved as large cells with pink stained acidophilic cytoplasm and a smaller nucleus/cytoplasm ratio.

Measurements of oocyte feret diameter were used to estimate the minimum, maximum, and mean size for each phase of development. Measurements from each individual were pooled and measurements for each sample were grouped into size classes and oocyte size frequency diagrams were constructed for each of the 14 samples e.g. (Grant and Tyler, 1983b).

3.2.5 Gonad Indices.

Measures of gut-free body weight and gonad weight were used to calculate gonad indices (e.g. Grant and Tyler, 1983a). Gonad Indices were pooled for each sample and again for arbitrary categories of season. In their simplest form gonad indices represent a percentage contribution relationship between gonad weight and total body weight. For the purpose of this study gonad indices were calculated as follows;

$$\text{Gonad Index} = (\text{Gonad wet weight} / \text{Total gut-free wet weight}) \times 100$$

Gonad Indices for each sample are represented graphically as mean index ± one standard deviation.

3.2.6 Fecundity.

A basic estimate of fecundity was completed for 10 of the larger animals from the 1996 sample. By immersing dissected gonads in Histoclear™ and then staining the entire tissue with Eosin, larger oocytes became visible through the gonad wall when viewing the tissue under a dissecting microscope. Using a digital image analysis procedure, similar to that described above, a simple measure of fecundity was made by counting the number of oocytes in a tubule cluster. This was repeated for 10 clusters to give a mean number of oocytes per cluster. This mean value was then multiplied by the total number of clusters on the gonad, and when added to the number of oocytes observed in the central gonoduct structure, it gives a basic estimate of minimum fecundity.

3.2.7 Population structure and reproductive effort.

For the determination of adult population size-structure the length of every individual in the sample was measured along its ventral longitudinal axis. Measurements were made to the nearest millimetre from the mouth to the rounded posterior end. Owing to the gelatinous nature of the body and the fact that many specimens are somewhat damaged, these measurements lack some precision but it was deemed that a ventral longitudinal measurement along the sole of an individual was less prone to errors brought about by sampling and preservation.

Variations of size-frequency distributions, both for oocytes and body lengths, were assessed statistically. The Kolmogorov-Smirnov test (Sokal and Rohlf, 1995) provides a convenient method for testing differences between cumulative frequency distributions. This test was applied to the pooled oocyte size data. To take account of variations between replicate hauls within a cruise sample, body length data were subjected to a more elaborate statistical technique, such as that developed by Thurston *et al.* (1994) for the study of megabenthos body size distributions. A measure of 'dissimilarity' between pairs of cumulative frequency distributions was calculated based on the summed absolute deviations between the two distributions. A dissimilarity half-matrix was constructed for all possible comparisons between hauls. A 'distinctiveness' test statistic was then calculated for between-cruise comparisons and its value was based on the levels of within-cruise dissimilarity and between-cruise dissimilarity; see Sokal and Rohlf (1995) and Clarke and Green (1988) for further details (though note the examples given are typically for similarity measures). The significance of the test statistic was then assessed by a 'randomisation procedure' (Sokal and Rohlf, 1995); this was done for individual 'pair-wise tests' (i.e. all possible cruise-to-cruise comparisons). The randomisation procedure was completed using the ANOSIM function of the PRIMER 5 software package (Clarke and Warwick, 1994).

Variations in gonad index, mean oocyte size and mean body length were assessed by analysis of variance (ANOVA, (Sokal and Rohlf, 1995)) using the Minitab 12.0 software package.

3.3 Results.

3.3.1 External Gonad Morphology

The gonad of *Amperima rosea* is a single structure lying in the dorsal interradius (CD *sensu* (Hyman, 1955), often running the entire length of the coelomic cavity. The ovary consists of a single gonoduct, opening to the exterior via a gonopore at the anterior end of the animal. Off this gonoduct branch numerous compact tubules (Figure 3.1 A, B) that are roughly ovoid in shape. The ovary wall is opaque and mature oocytes are only visible after treating the tissue with Histoclear (Figure 3.1C). The testis is also a relatively large structure (Figure 3.2 A). It also has a single large gonoduct but appears to have additional branching ducts, connecting to the tubules (Figure 3.2B). The ducts continue to branch as they move away from the central gonoduct producing clusters of highly digitate tubules (Figure 3.2C). The testis has a much lighter 'feathery' appearance compared to the more compact 'nodular' ovary. In both the male and female specimens the gonad is closely associated with the digestive tract and stomach, often becoming tightly wrapped around it (e.g. Tyler *et al.*, 1985a), presumably a result of tight packing of organs within the small coelomic cavity.

3.3.2 Gametogenesis

Amperima rosea is very delicate semi-gelatinous animal and consequently its gonads are prone to damage during sampling. The gonads of many specimens were found to have been forced outside the body cavity as a result of the destructive nature of the trawl mesh. Consequently, when examined microscopically, many sections showed extensive evidence of tissue damage making the interpretation of internal structures more difficult.

3.3.2.1 Oogenesis

Developing and mature oocytes were present in all specimens from all samples, generally occurring in low numbers. The ovary wall of *Amperima rosea* consists of an outer coelomic epithelium overlying a thin muscle layer. Directly beneath this is a thin, densely stained, tissue layer (ca. 3-5μm wide) followed by a cellular connective tissue layer 25-40μm wide (Figure 3.3A). Oocyte development takes place along the germinal epithelium where the connective tissue layer is thin, as observed in other species of the Family Elpidiidae (Tyler *et al.*, 1985a) and more generally for the Order Elasipodida

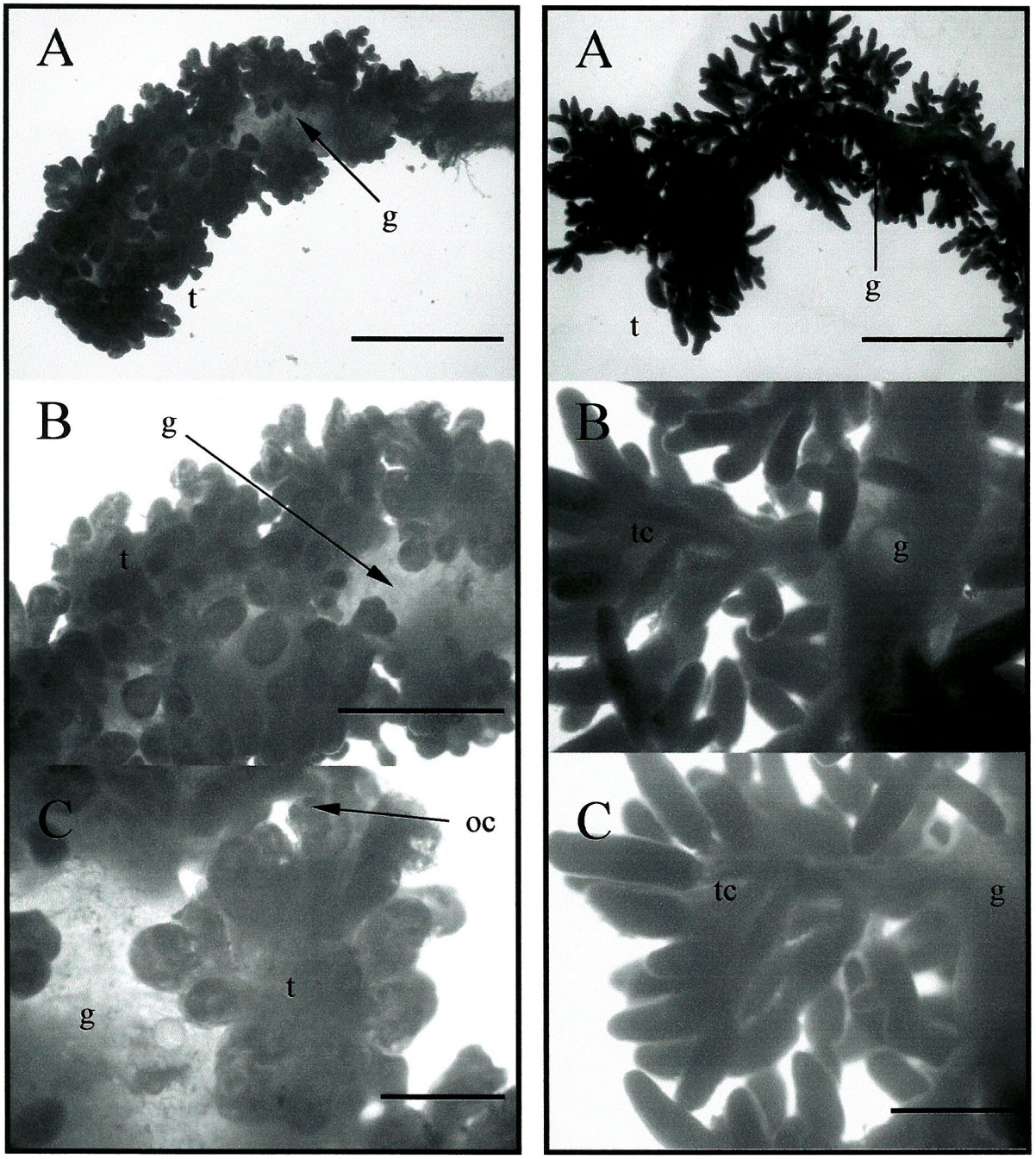

Figure 3.1. (left). External gonad morphology of ovary from *Amperima rosea*. A, bar = 5mm; B, bar = 2mm; C, bar = 1mm. G = gonoduct; tc = tubule cluster; oc = oocyte.

Figure 3.2. (right). External gonad morphology of testis from *Amperima rosea*. A, bar = 5mm; B, bar = 2mm; C = 1mm. G = gonoduct; tc = tubule cluster; oc = oocyte.

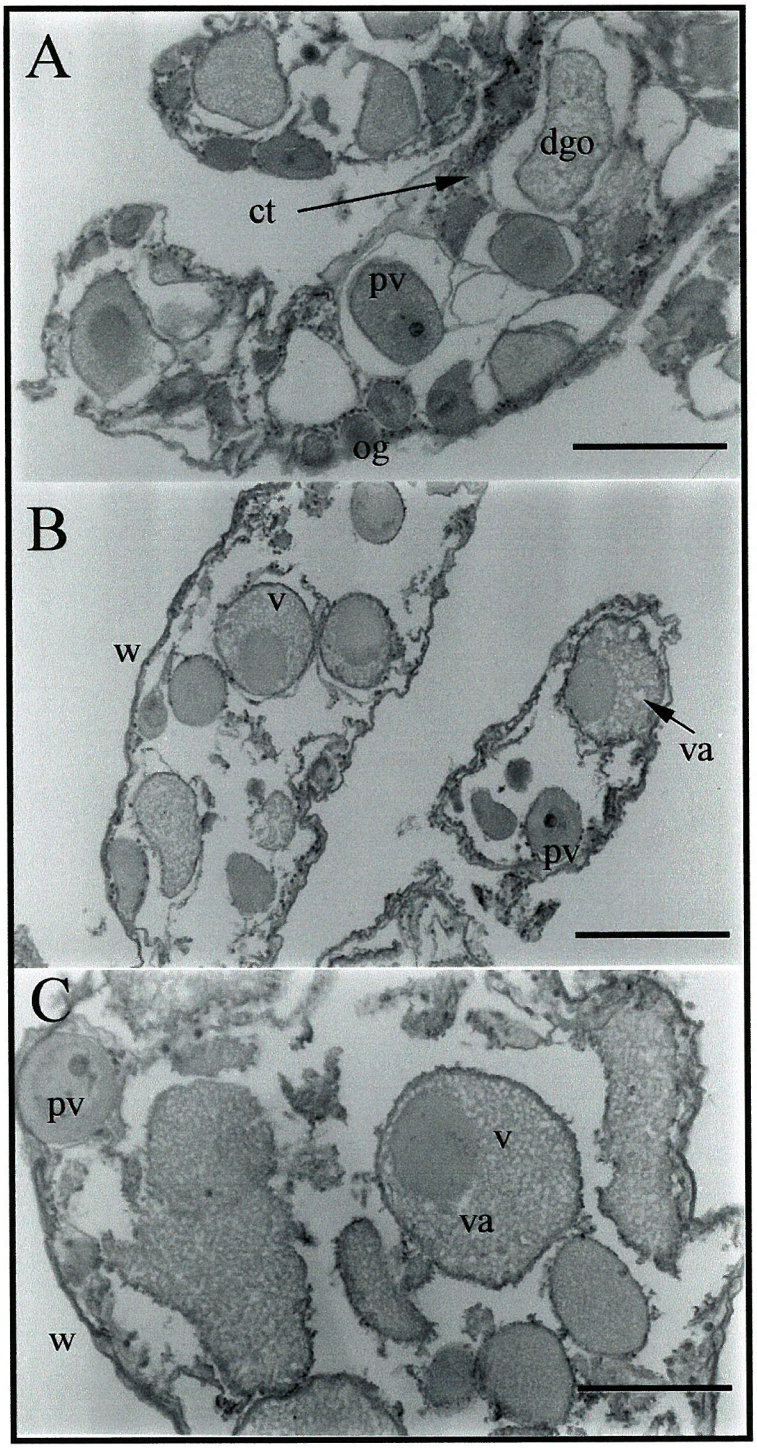

Figure 3.3. Light histology of female gonad stained with haematoxylin and eosin. w = wall; og = oogonia; pv = previtellogenic oocyte; v = vitellogenic oocyte; ct = connective tissue; va = vacuole; dgo = degenerating oocyte. Bar = 200µm.

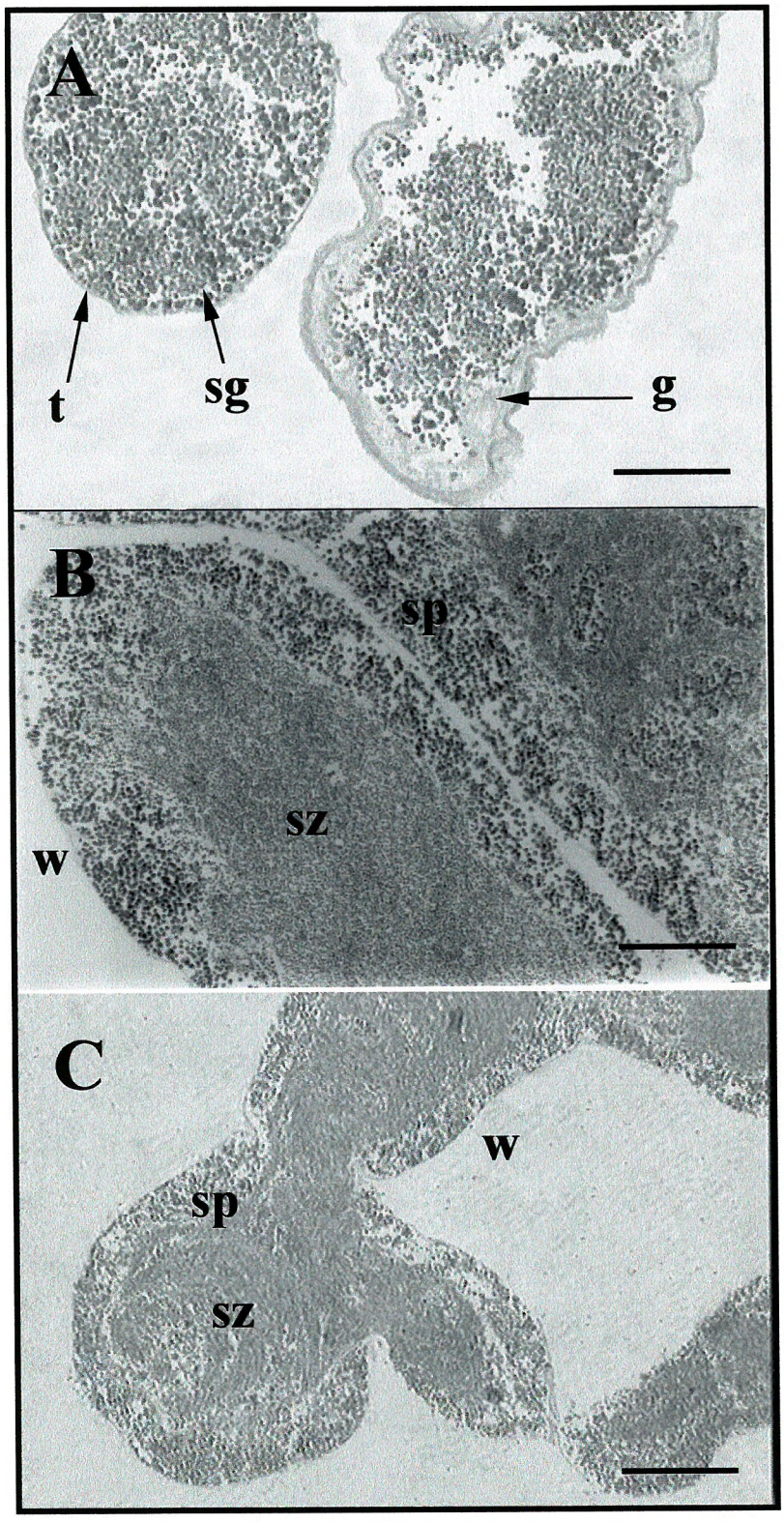

Figure 3.4. Light histology of male gonad stained with haematoxylin and eosin. w = wall; t = tubule; g = gonoduct; sg = spermatogonia; sp = spermatocytes; sz = spermatozoa. Bar = 50μm.

(Tyler and Billett, 1987). Primary oocytes of ~40μm diameter are roughly spherical with a basophilic cytoplasm (Figure 3.3A). As they grow, most remain spherical and close to the ovary wall, whilst some become elongate or misshapen, possibly as a result of spatial constraints within the ovary. At 80μm diameter, they begin vitellogenesis and are surrounded by a layer of accessory cells. At ~110μm, the oocyte cytoplasm takes on a highly vacuolated appearance (Figure 3.3B,C). Vacuoles appear close to the nucleus at first, and then spread outwards to the periphery of the cytoplasm. When the animal is alive it is possible that these vacuoles would be full of neutral lipid. Oocytes grow to size of ~200μm. At this point, if the eggs are not spawned they undergo a phagocytic breakdown. There is evidence of spent tubules in the autumn samples, with visible "gaps" where oocytes have been and the presence of phagocytes around degenerative, presumably unspawned, oocytes (Figure 3.3A).

3.3.2.2 Spermatogenesis

The wall structure of the testis is similar to that of the ovary. The testicular ducts have thick connective tissue layers, compared to the thin walled tubules (Figure 3.4A). Two size classes of male gametes were observed in the testis (Figure 3.5). Spermatocytes, with a mean diameter of 3.9 ±0.4 μm, line the germinal epithelium of the tubules (Figure 3.4B). Spermatogonia develop by mitotic divisions into the spermatocytes, which then undergo meiosis to produce secondary spermatocytes, then spermatids. Spermatids undergo spermiogenesis to give spermatozoa (2.1 ±0.2μm in diameter). The spermatozoa accumulate as dense, swirling masses of gametes in the lumen of the testes (Figure 3.4C).

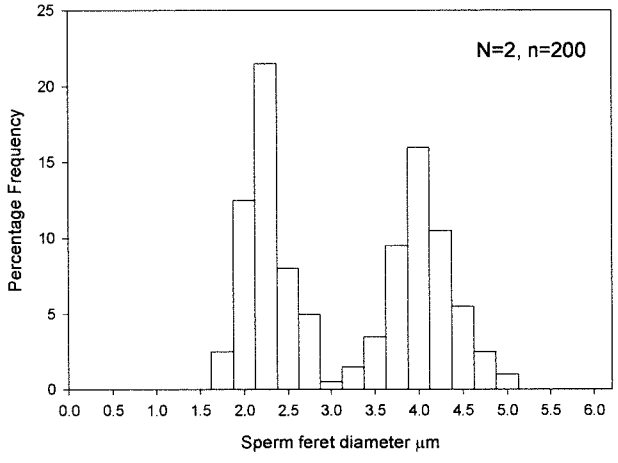

Figure 3.5. Male gamete size distribution. N = number of individuals; n = number of gametes measured.

3.3.3 Sex Ratio

The sex ratio of *Amperima rosea* from the BENGAL site did not differ significantly from an expected 1:1 ratio. The total number of sexed specimens from this locality was 256, with 133 males and 131 females ($\chi^2 = 0.008$, 1df, P= 0.931)

In addition the sex ratio of *Amperima rosea* from the four PAP sites, sampled in 1999, did not differ significantly from an expected 1:1 ratio. The total number of sexed specimens from these sites was 54, with 22 males and 32 females ($\chi^2 = 0.934$, 1df, P= 0.334)

3.3.4 Size at maturity

Samples taken by OTSB trawls tend to be biased towards the larger size classes of benthic invertebrates as a result of the mesh size used in the net and cod-end (see *Materials and Methods*). The smallest measured specimens of *A. rosea* were ~2.0 to 8.0mm in length. The smallest specimens dissected, and found to have mature oocytes were ~10mm in length. Three smaller specimens 8 and 7mm in length were dissected, and when sectioned there was no evidence of any developing gametes. The maximum recorded length of *A. rosea* is 80mm.

3.3.5 Reproductive output

3.3.5.1 Fecundity

An estimate of minimum fecundity was made from the larger (44-68mm) female specimens in the September 1996 samples. A mean fecundity of 12,800 (±1030) oocytes per individual was calculated. This is a minimum estimate as the method used could only resolve larger, mature oocytes and was not effective at resolving the 3-dimensional arrangement of oocytes in the tubule clusters and gonoduct.

3.3.5.2 Gonad Index - Temporal Patterns: BENGAL 1989-99

There was great variability in gonad index between specimens and between samples (Figure 3.6A). There is a significant difference in mean female gonad index over the study period (ANOVA, F=8.89, 9df, P<0.05). Males also exhibited within- and between-cruise variability (Figure 3.6B), and an overall significant difference in male gonad index was also observed during the period 1989-1999 (ANOVA, F=6.96, 9df, P<0.05). The

peak values for both male and female gonad indices tended to be in samples from the autumn/winter months.

Both male and female gonad indices were assigned an arbitrary seasonal rank of either Spring/Summer (March-Aug) or Autumn/Winter (Sept-Feb). Within the Spring/Summer samples males had a mean gonad index of 2.16 ±0.89 and females of 2.48 ±1.03. Autumn/Winter males had a mean of 3.28 ±1.82, while females had a mean of 3.36 ±1.45. For both sexes there was a significant difference in mean gonad index between seasons, irrespective of sampling year (male ANOVA, $F=21.84$, 1df, $P<0.05$; female ANOVA, $F=15.99$, 1df, $P<0.05$). There is no significant difference in the gonad indices of male and female *A. rosea* at the BENGAL locality (ANOVA, $F= 1.29$, 1 df, $P=0.256$).

3.3.5.3 Gonad Index - Spatial Patterns: PAP 1999

Mean gonad indices for the four 1999 sites (Figure 3.6C) showed little variability, for both male and female specimens (male ANOVA, $F=0.25$, 3df, $P=0.862$; female ANOVA, $F=0.22$, 3df, $P=0.884$).

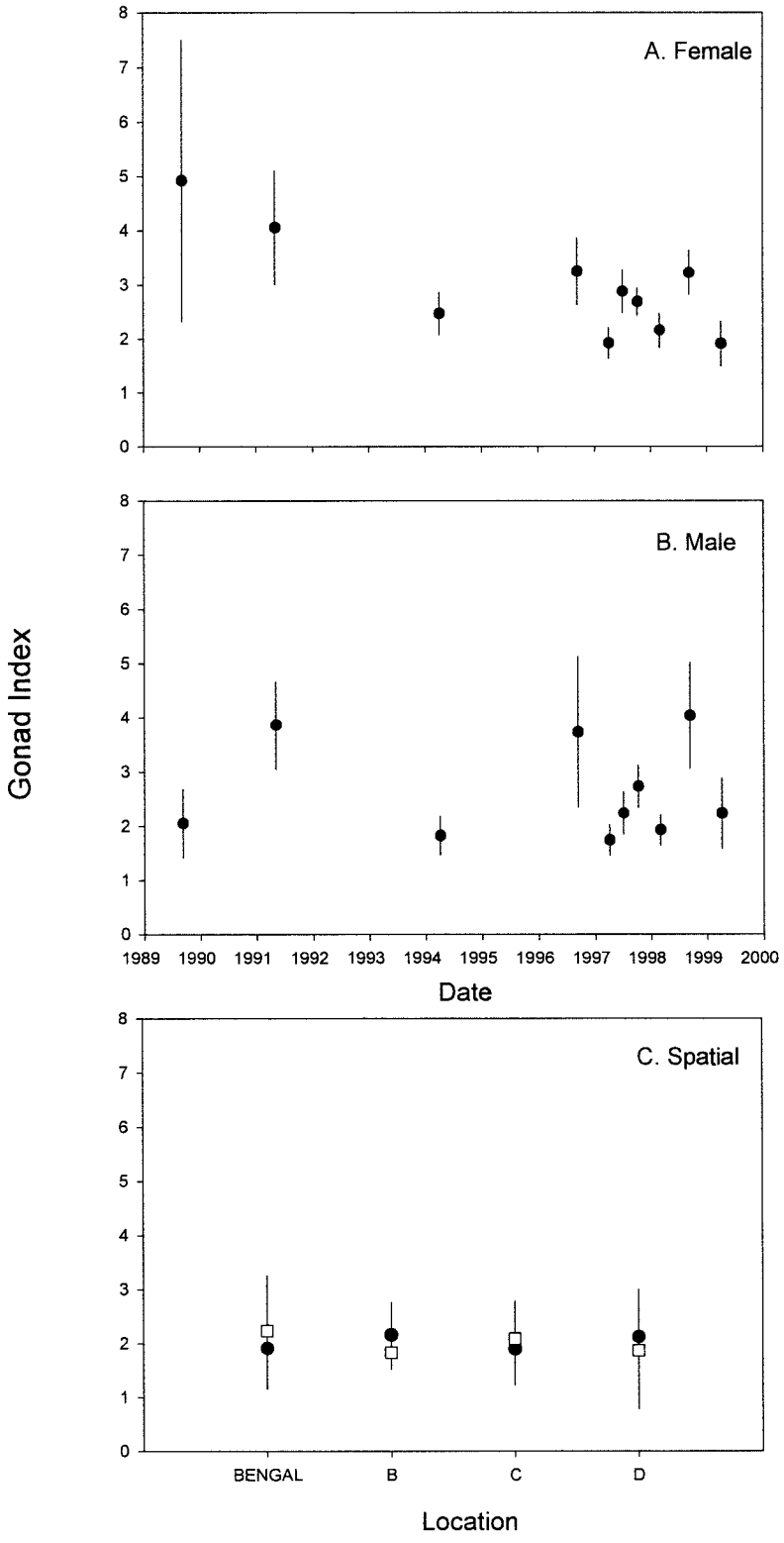

Figure 3.6. Mean Gonad Indices with standard deviation. A = females; B = males; C = spatial samples from April 1999. Male = open square; Female = closed circle.

3.3.6 Mean oocyte sizes.

The mean oocyte size (± standard deviation) for the 10 temporal samples at the BENGAL locality are shown in Figure 3.7A and for the four spatial samples taken in April 1999 in Figure 3.7B. Statistically there were significant differences between both temporal and spatial samples (temporal ANOVA, F= 80.88, 9df, P<0.005; spatial ANOVA, F= 163.69, 3df, P<0.005). However, it is uncertain as to how much biological significance can be assigned to this result as the difference between the minimum (75.9μm ±23.5) and maximum (114.1μm ±26.2) mean oocyte sizes, for the temporal samples, overlap. This results in the majority of oocytes from all samples being about the late previtellogenic (70-90μm) and early vitellogenic (100-120μm) stage of development.

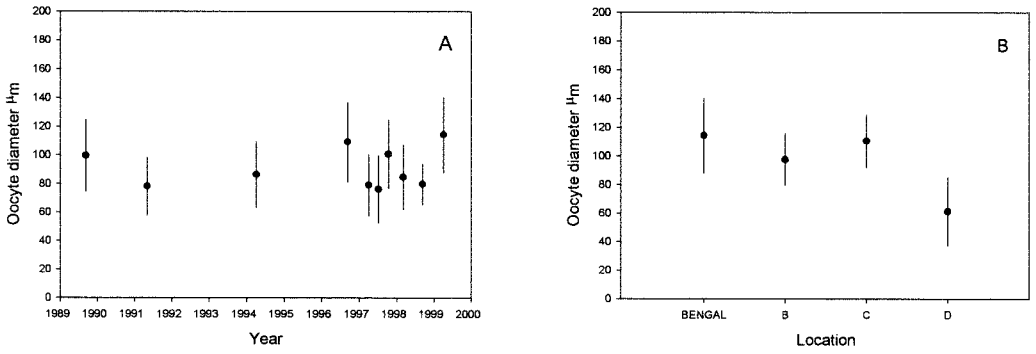

Figure 3.7. Mean oocyte diameters. A = BENGAL site 1989-1999; B = 1999 spatial samples, BENGAL and sites B, C, and D.

3.3.7 Oocyte size-frequency distribution

3.3.7.1 Temporal patterns – 1989 to 1999.

Oogenesis in *Amperima rosea* was asynchronous, with the production of vitellogenic oocytes throughout the year. Samples from 1989 to 1999 all showed a broad range of oocyte development at any one time. Significant differences were apparent when pooled data of oocyte size distributions for each sampling period (Figure 3.8) were compared using the Kolmogorov-Smirnov test statistic (Table 3.1).

At the height of the "*Amperima* Event" (1996-1998) there were not only significant differences in the distributions of oocytes throughout the year of 1997, but also between spring and autumn samples. This can be seen more clearly by looking at the cumulative

frequency distributions (Figure 3.9). Although a large range of oocyte sizes is apparent for all samples, there is great variability in the ratio of pre-vitellogenic (≤ 80μm) to vitellogenic (>80μm) oocytes. The proportion of vitellogenic oocytes in the ovary is significantly greater in autumn samples (~85%) than in those taken during the spring months (~50%). This pattern is also reflected by the gonad indices for this period.

Although significant differences between distributions have been recorded, the oocyte-size frequency distributions for the BENGAL samples (Figure 3.8) exhibit a similar general pattern. There was a wide range of oocyte sizes with a high proportion (~30%) of pre-vitellogenic oocytes and a peak percentage (~50-75%) of early vitellogenic oocytes, mainly in the 90 and 100μm size classes. The percentage of oocytes in the largest size classes (160+μm) was low and accounted for only ~5% in most samples. These data suggest that there is no synchrony in oogenesis within and amongst samples. The presence of a small percentage of oocytes in the largest size classes would imply that spawning is a slow continuous release of eggs. However the evidence from gonad indices, cumulative oocyte distributions and the appearance of spent tubules in the autumn samples indicates an increased spawning effort in the autumn months (i.e. a greater number of eggs spawned compared to other times of year).

In male specimens, after reaching first maturity the testes had a continuous supply of spermatozoa, although the ratio of spermatogonia/spermatocytes to spermatozoa did vary between individuals and between samples.

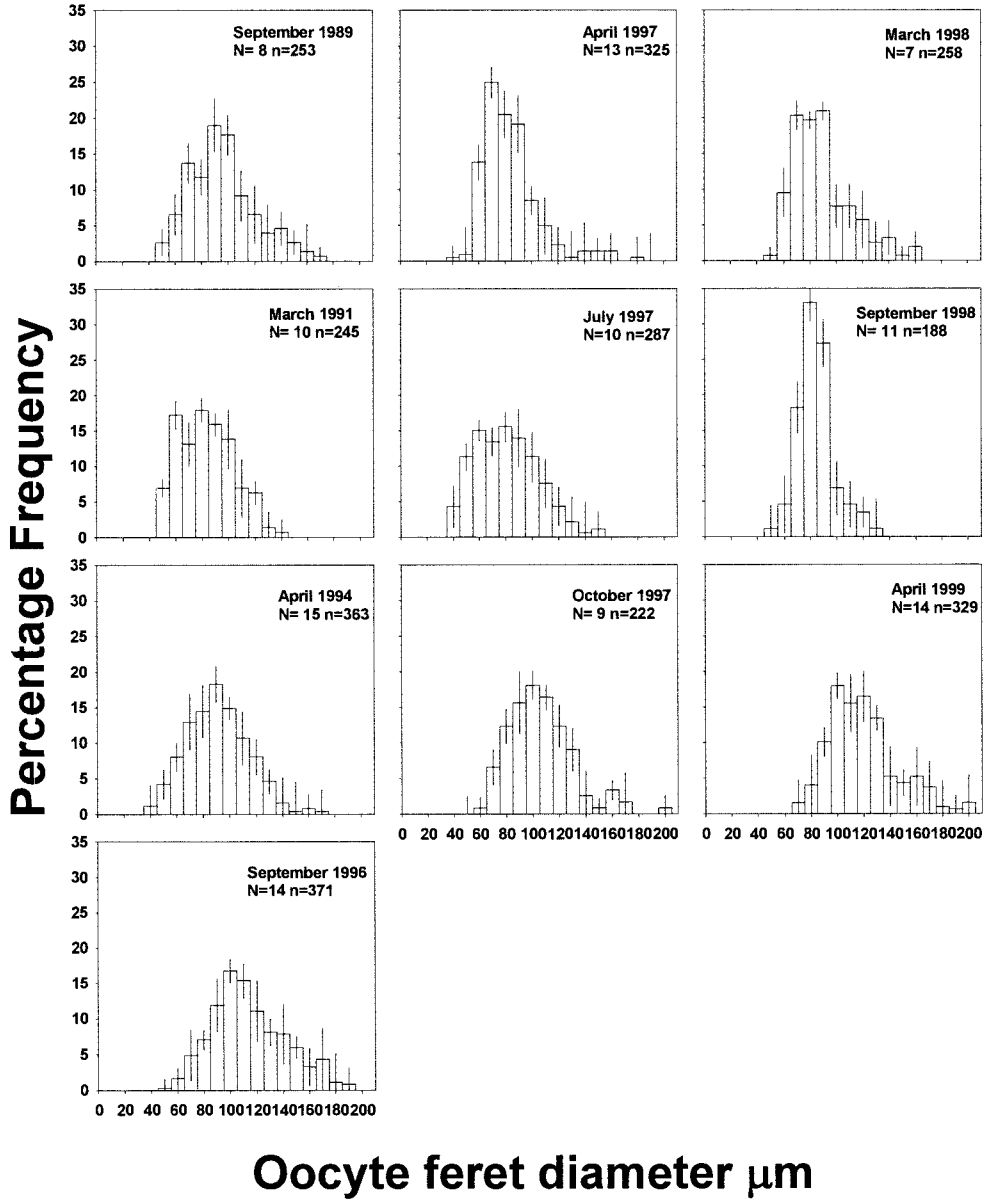

Figure 3.8. Oocyte size-frequency distributions, BENGAL site 1989-1999. Mean percentage frequency ± 1SD. N = number of females; n = number of oocytes measured.

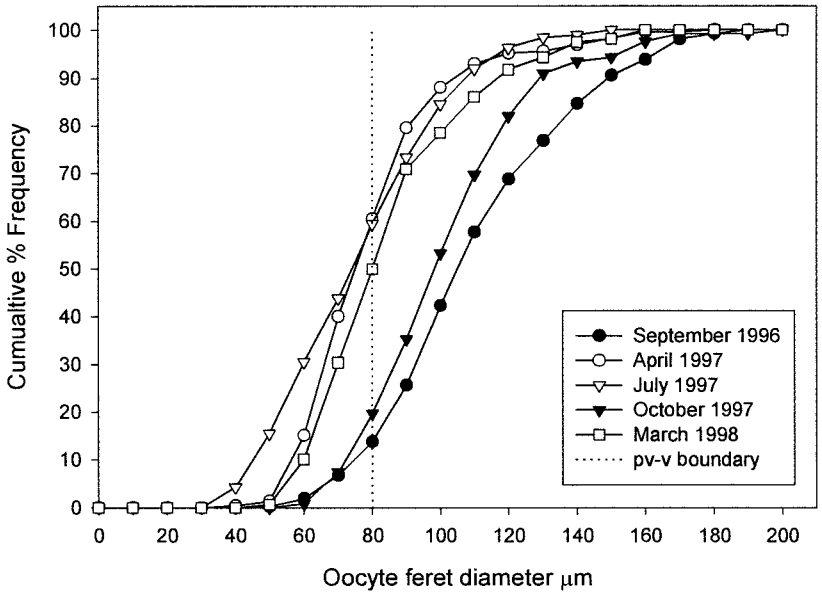

Figure 3.9. Cumulative oocyte size-frequency distributions September 1996 – March 1998. Dashed line represents transition from pre-vitellogenic to vitellogenic oocytes.

3.3.7.2 Spatial patterns – March 1998 and April 1999.

Pooled oocyte-size frequency distributions for the two spatial samples from March 1998 (site A and BENGAL) are compared with four spatial samples taken in April 1999 (sites B,C,D and BENGAL) (Figure 3.10). The March 1998 oocyte-size distributions showed significant differences between the BENGAL sample and that from site A (Table 3.1). There is a greater range of oocyte sizes present in the BENGAL sample and the distribution peaks in the 100-110μm size class, compared to 70-80μm in the site A sample. The distribution of oocytes in the site A sample is heavily skewed toward the pre-vitellogenic size classes, with >90% of the oocytes ≤80μm. The BENGAL sample exhibits a more balanced distribution with 50% pre-vitellogenic oocytes and 50% vitellogenic oocytes.

There are also significant differences between the April 1999 BENGAL sample and the samples from sites B and D (Table 3.1). There is an absence of oocytes ≤70μm in the BENGAL sample and those from sites B and C. The distributions for BENGAL and site

B are not significantly different from each other and both exhibit a large range of oocyte sizes. The distribution for the site D sample is skewed toward the smaller size classes with >80% of the oocytes ≤80μm in diameter, however it does display a large range of oocyte sizes (20-160μm). The site B sample is dominated by oocytes in the 90-100 and 100-110μm size classes and 72% of all the measured oocytes fall within these two size classes. It would appear that individuals are not in reproductive synchrony across the wider Porcupine Abyssal Plain.

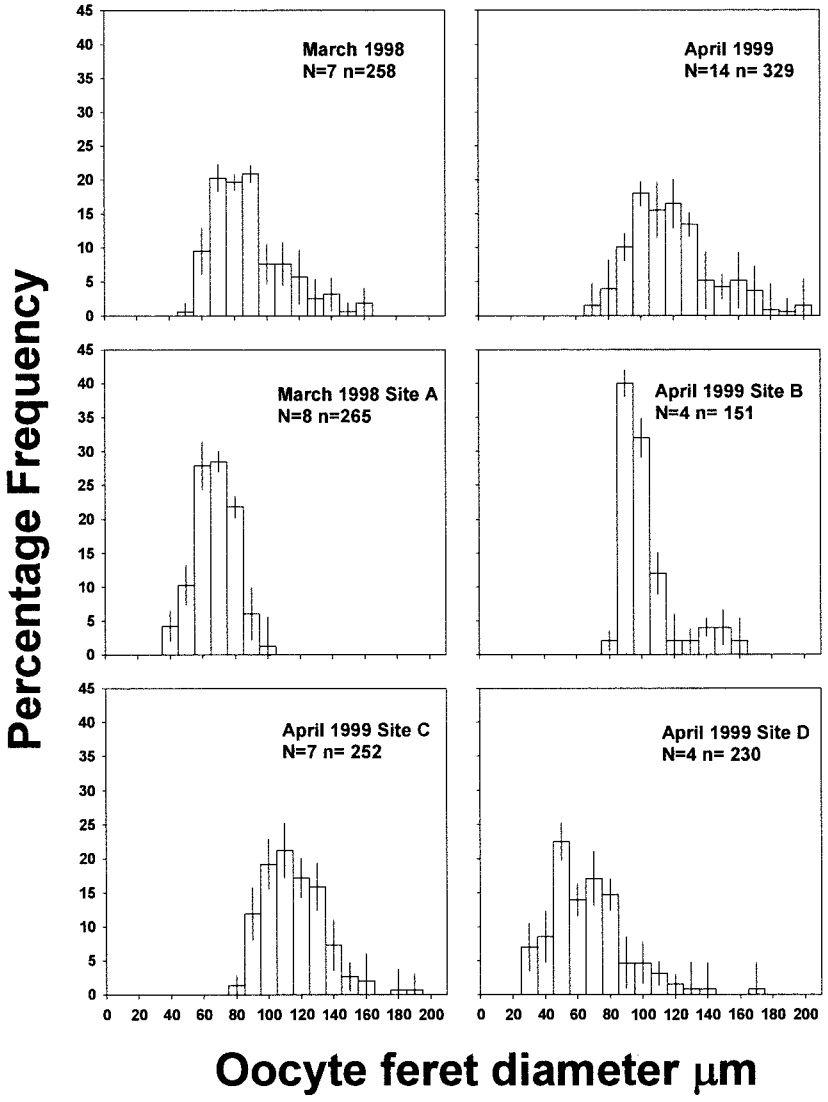

Figure 3.10. Oocyte size-frequency distributions. Spatial variability in March 1998 and April 1999. Mean percentage frequency ± ISD. N = number of females; n = number of oocytes.

	September 1989	May 1991	April 1994	September 1996	April 1997	June 1997	October 1997	March 1998	March 1998 A	September 1998	April 1999	April 1999 B	April 1999 C	April 1999 D
September 1989														
May 1991	*													
April 1994	*	*												
September 1996	5.0	1.0	5.0											
April 1997	*	*	*	1.0										
June 1997	*	*	*	1.0	*									
October 1997	*	1.0	*	*	1.0	1.0								
March 1998	*	*	*	1.0	*	*	1.0							
March 1998 A	1.0	1.0	1.0	1.0	5.0	5.0	1.0	1.0						
September 1998	5.0	*	*	1.0	*	*	1.0	*	1.0					
April 1999	1.0	1.0	1.0	*	1.0	1.0	*	1.0	1.0	1.0				
April 1999 B	5.0	1.0	1.0	5.0	1.0	1.0	*	1.0	1.0	1.0	1.0			
April 1999 C	1.0	1.0	1.0	*	1.0	1.0	*	1.0	1.0	1.0	*	1.0		
April 1999 D	1.0	5.0	1.0	1.0	1.0	*	1.0	1.0	*	1.0	1.0	1.0	1.0	

Table 3.1. Temporal and spatial variability in oocyte size distribution. Lower half-matrix tabulates significance values (p%) for cruise-cruise comparisons based on the Kolmogorov-Smirnov test. * = not significant.

3.3.8 Population size distributions

3.3.8.1 Size-frequency distributions – Temporal patterns 1989-2000

The differences in sample size of pooled size-frequency plots for each sampling period immediately show the scale and rapid onset of the *Amperima* 'bloom' (Figure 3.11). Specimens ranged in size from 6.0 to 80.0mm, with significant differences in mean size between the 11 temporal samples (ANOVA, $F=1030.97$, 10df, $P<0.05$). Tables 3.2 and 3.3 show a pair-wise comparison of the sampled distributions that allows a more detailed assessment of changes recorded over the 10-year period. The Kolmogorov-Smirnov test (Table 3.2) treats each cruise as one sample, using a pooled distribution of all individual trawl samples, whilst the ANOSIM 'dissimilarity' test (Table 3.3) takes into account within-cruise variation in size distributions but is not valid for all sampling periods as a result of low sample sizes (number of trawls per cruise).

The sampled population in September 1989 is dominated by larger individuals with a peak in the 40-45mm size class and >50% of the sample are ≥45mm in length. In May 1991 the sampled population exhibits a classic bimodal distribution with two potential cohorts, one peaking at the 20-25mm size class and the other at the 55-60mm size class. The absence of any individuals in the 20-25mm size class in the 1989 sample would suggest that *A. rosea* sampled in 1991 are either new recruits to the population, or that the individuals sampled are in fact an entirely different population from that sampled in 1989. The presence of larger individuals may be a result of growth of 1989 individuals, an approximate maximum increase of 15mm in a 19-month period.

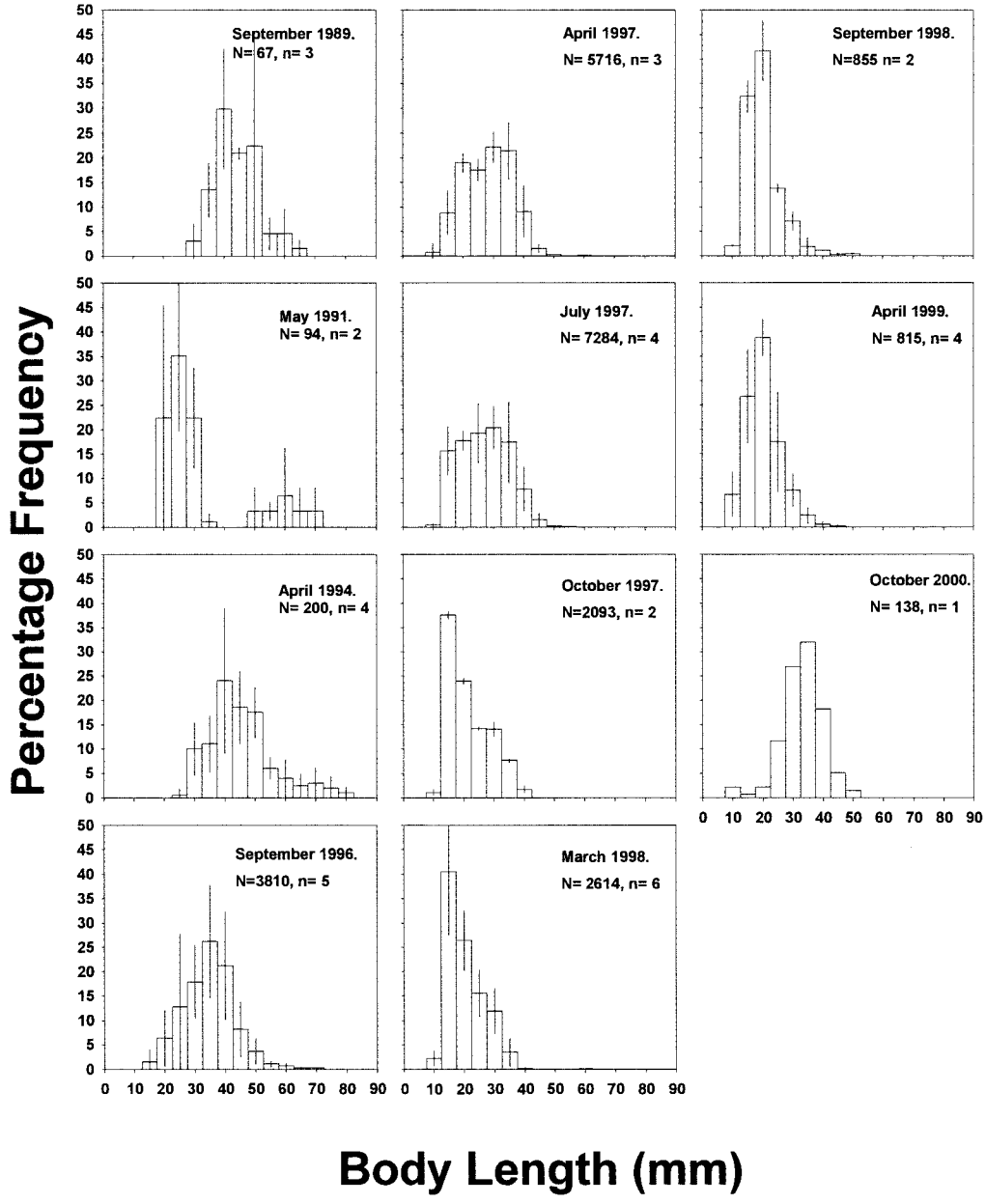

Figure 3.11. Body length size-frequency distributions 1989-2000, mean ± standard deviation. N = number of specimens measured; n = number of trawls.

The frequency distribution for 1994 shows a large reduction in individuals in the 20-25mm size class, but the peak is now in the previously unrepresented size classes of 40-50mm. The bimodal pattern is absent as the tail of the distribution extends to include larger individuals in the 75-85mm size classes. This distribution is heavily skewed toward the larger size classes with 55% of individuals >45mm in length. By September 1996 the majority of large individuals were absent from the sampled population whilst at the same

time the abundance notably increased. There is a peak at the 35-40mm size class and 65% of the sample is made up of individuals <40mm in length. The following April (1997) there is an absence of individuals >50mm present in the sample. There is a peak in the 25-30mm size class and ~68% are smaller than 30mm. The abundance has increased further, a possible indication of a successful recruitment event. In July of the same year the number of specimens being caught has increased further and the sampled population is still dominated by the smaller size classes, with ~90% of individuals ≤35mm in length.

The distribution for the October 1997 sample shows a large influx of small individuals into the sampled population. There is a marked peak in the 15-20mm size class and the distribution is heavily skewed toward the smaller size classes with ~75% of the sample ≤25mm in length. This situation would appear to be the obvious candidate for a large recruitment event with an apparent influx of small individuals. However, the corresponding abundance of animals has fallen and larger individuals are absent from the October 1997 samples and it is possible that the same population is not being sampled from year to year.

The population structure of the March 1998 samples shows a similar pattern to that for October 1997. The abundance of *A. rosea* has increased slightly as has the percentage of individuals in the peak 15-20mm size class (~3% increase). There is also a reduction (~8%) in the number of larger, ≥30mm in length, individuals in the sampled population. By September 1998 the abundance of *A. rosea* has dropped although the size distribution shows a similar pattern to that of March. There is a shift in the peak size class from 15-20mm to 20-25mm, possibly resulting from a period of growth in the major cohort. This ~5mm shift over a 6-7month period is of a similar scale to the 15mm shift observed between 1989 and 1991, although over a longer (~19 month) period. It is not known if this is a reasonable assumption as records of growth in deep-sea holothurians are non-existent and those for shallow-water animals tend to be from commercially cultured species such as *Holothuria scabra* (Battaglene *et al.*, 1999).

By 1999 the size distribution has not changed significantly and the majority of individuals are still in the 20-25mm size class, there has also been no appreciable change in abundance between September 1998 and the April 1999 samples. The one trawl sample

taken in October 2000 shows a shift in the peak size class to individuals in the 35-40mm size range. Although only one trawl sample was taken the abundance of *A. rosea* has dropped and appears to be returning to those levels recorded prior to the BENGAL programme. If we assume that this is the same population of animals as was sampled in 1999 then there is evidence to support growth of individuals, within the population, of ~15mm in the peak size class, over an 18month period. This increase corresponds well with the previous ~5mm increments recorded between March and September 1998.

	September 1989	May 1991	April 1994	September 1996	April 1997	June 1997	October 1997	March 1998	March 1998 A	September 1998	April 1999	April 1999 B	April 1999 C	April 1999 D	October 2000
September 1989															
May 1991	1.0														
April 1994	*	1.0													
September 1996	1.0	1.0	1.0												
April 1997	1.0	*	1.0	*											
June 1997	1.0	*	1.0	5.0	*										
October 1997	1.0	1.0	1.0	1.0	5.0	*									
March 1998	1.0	1.0	1.0	1.0	1.0	5.0	*								
March 1998 A	1.0	1.0	1.0	1.0	1.0	1.0	*	*							
September 1998	1.0	1.0	1.0	1.0	1.0	1.0	*	*	*						
April 1999	1.0	1.0	1.0	1.0	1.0	1.0	*	*	*	*					
April 1999 B	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	5.0	1.0	1.0				
April 1999 C	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	*	1.0	1.0	*			
April 1999 D	1.0	1.0	1.0	1.0	1.0	1.0	*	*	*	*	*	1.0	1.0		
October 2000	1.0	1.0	1.0	*	*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	

Table 3.2. Temporal and spatial variability of body length size distributions. Lower half-matrix tabulates significance values (p%) for cruise-cruise comparisons based on the Kolmogorov-Smirnov test. * = not significant

	September 1989	May 1991	April 1994	September 1996	April 1997	June 1997	October 1997	March 1998	March 1998 A	September 1998	April 1999	April 1999 B	April 1999 C	April 1999 D	October 2000
September 1989															
May 1991	-														
April 1994	*	-													
September 1996	1.8	*	0.8												
April 1997	-	-	2.9	*											
June 1997	2.9	-	2.9	4.0	*										
October 1997	-	-	-	4.8	-	-									
March 1998	2.9	-	2.9	1.6	2.9	2.9	-								
March 1998 A	-	-	-	4.8	-	-	-	-							
September 1998	-	-	-	4.8	-	-	-	-	-						
April 1999	2.9	-	2.9	1.6	2.9	*	-	*	-	-					
April 1999 B	-	-	-	-	-	-	-	-	-	-	-				
April 1999 C	-	-	-	-	-	-	-	-	-	-	-	-			
April 1999 D	-	-	-	-	-	-	-	-	-	-	-	-	-		
October 2000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

Table 3.3. Temporal and spatial variability of body length size distributions. Lower half-matrix tabulates corresponding results (to Table 3.2) from ANOSIM 'randomisation' tests. * = not significant; - = not applicable (i.e. insufficient replicates to achieve 5% level)

3.3.8.2 Size-frequency distributions – Spatial patterns March 1998 and April 1999.

The additional trawls made in March 1998 and April 1999 at sites A-D (see Figure 2.1) allowed assessment of any spatial variability in the population size distribution of *Amperima rosea* on the Porcupine Abyssal Plain. Pooled size-frequency plots for each sampling site are shown in Figure 3.12. Specimens ranged in size from 2.0 to 58.0mm, with significant differences in mean size between the 6 spatial samples (ANOVA, $F=467.74$, 5df, $P<0.05$). Results of pair-wise comparisons between sample distributions can again be seen in Tables 3 and 4. The March 1998 samples have similar distributions and are not significantly different from each other. Both samples were dominated by smaller individuals (≤25mm). The population structure of the 1999 BENGAL sample is significantly different from those samples taken at sites B and C. Animals at sites B and C were present in greater abundance than at BENGAL, although the majority of individuals were ≤20mm in length. The sample taken from site D showed a similar distribution to the BENGAL sample with individuals in the 20-25mm size class dominating. The two distributions were not significantly different (Tables 3) with both samples containing 15-18% of animals ≥20mm, compared to 1-5% in the samples from sites B and C.

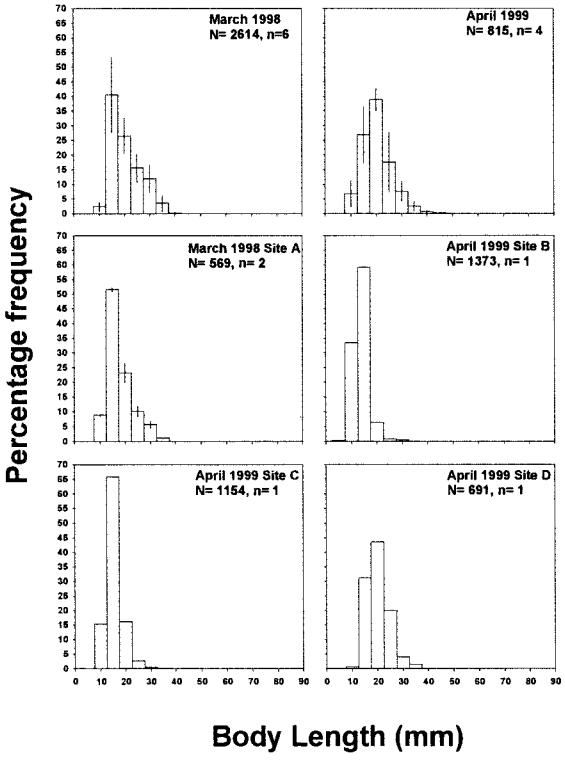

Figure 3.12. Body length size-frequency distributions. Spatial variation in March 1998 and April 1999. Mean ± standard deviation. N = number of specimens measured; n = number of trawls.

3.3.9 Parasites

During the dissection of adult specimens of *A. rosea*, and the microscopic analysis of the gonad, the presence of two separate species of parasite was noted.

The first species, *Exspina typica* Lang 1968 (Figure 3.13A), a rare example of a parasitic tanaidacean, inhabits the body cavity of *A. rosea*. The parasitised animals were taken from eight separate trawls, two from May 1991, one from March 1998 site A, three from April 1999 BENGAL site, and one each from April 1999 sites B and D. A total of ten specimens of *E. typica* were removed from eight individual specimens of *A. rosea*, with two having a parasitic load of two. All specimens of *A. rosea* found to contain the parasite were undamaged, eliminating the possibility that this association is an artefact of collection and fixation. This association between *E. typica* and *A. rosea* has only previously been recorded once from a single specimen (Thurston *et al.*, 1987).

The second parasite associated with *A. rosea* was discovered during the microscopic analysis of the gonad. Several parasitic inclusions were identified within the gonad tubules of both male and female specimens from several different samples (Figure 3.13B, C). A preliminary classification of this parasite as the sporozoan *Ixoreis psychropotae*, or other congener, was made based on comparisons made with the earlier observations and the descriptions of Massin *et al.* (1978) and Tyler and Billett (1987).

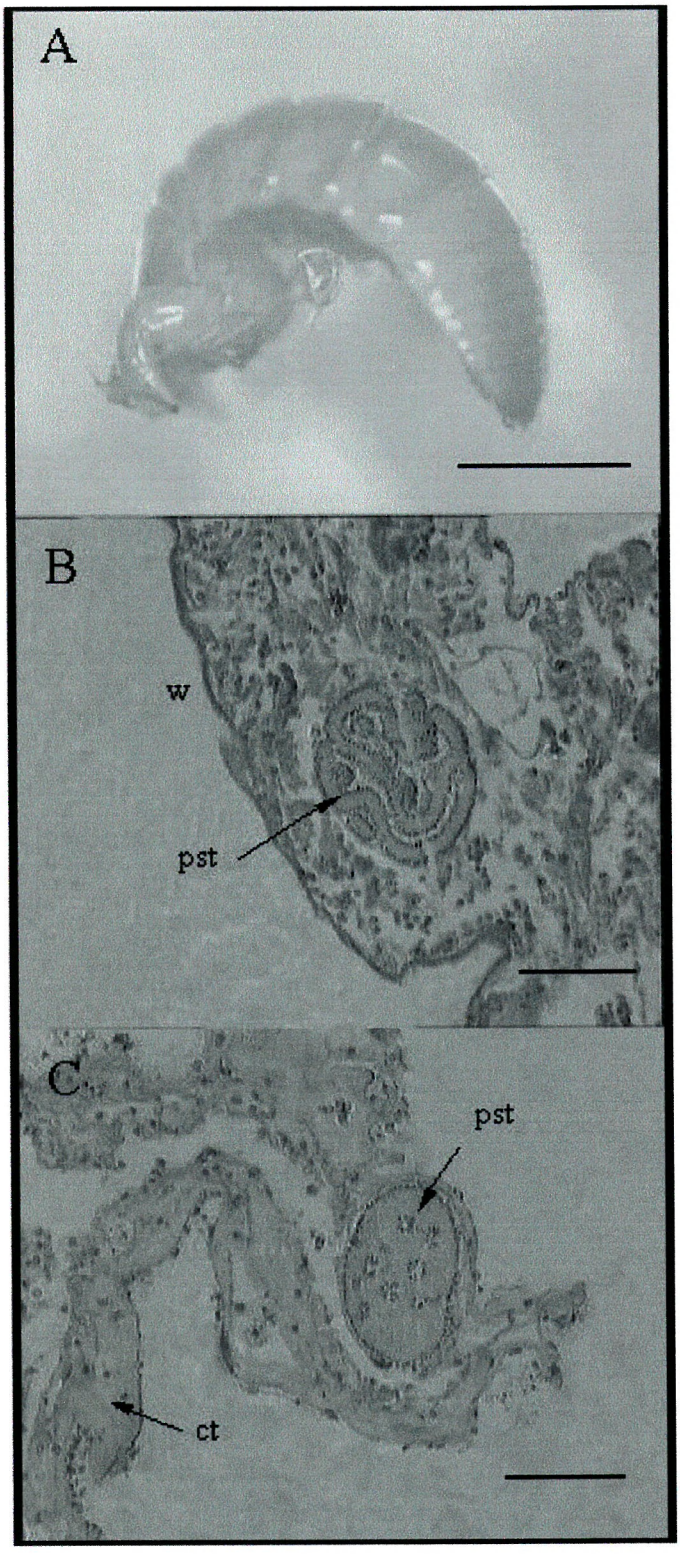

Figure 3.13. A. The tanaidacean *Exspina typica*, found in the coelomic cavity of *Amperima rosea*. Bar=1mm. B and C. Parasitic inclusions from the gonad of *Amperima rosea*. w= wall; ct= connective tissue; pst= parasitic inclusion. Bar= 200µm.

3.4 Discussion.

Holothurians are the dominant epibenthic invertebrate taxon in many areas of the deep-sea. They are one of the few faunal groups that can penetrate the deepest parts of the oceans, overwhelmingly dominating the biomass at hadal (>6000m) depths (Belyaev, 1972; Hansen, 1975). Holothurians play an important role in structuring the deep-sea ecosystem and in recycling carbon supplied by the vertical flux of phytodetritus. It is therefore important to understand how their biology and in particular their reproductive strategies might be adapted to bring about rapid, large scale increases in abundance. Reproduction, gametogenesis and the maturation and spawning of mature gametes, are energetically expensive processes that can all be affected by changes in the input of energy (in the form of organic matter) to the deep-sea ecosystem.

The abyssal community in the northeast Atlantic is subject to a variable flux of organic matter, and flux to the seafloor of the PAP may vary both seasonally and inter-annually (Lampitt *et al.*, 2000; 2001). In addition, it is clear that fresh organic matter forms a major component of the diet of both *Amperima rosea* and *Ellipinion molle* (Iken *et al.*, 2001). Although the inter-annual variability in the supply of organic matter shows no apparent long-term trend that may account for the rapid increase in abundance of *A. rosea* and some other fauna on the PAP (Billett *et al.*, 2001) it is possible that inter-annual variability in the quality of organic matter supply (Kiriakoulakis *et al.*, 2001) may lead to rapid and persistent changes in the benthic community.

Ginger *et al* (2000; 2001) have highlighted the importance of sterols in the diet of abyssal holothurians. They argue that sterols in deep-sea holothurians are produced by the transformation of dietary sterols rather than *de novo* synthesis. *Amperima rosea* has a distinctive sterol composition and is able to metabolise 4α-methylcholestenol unlike the other dominant, large holothurian species such as *Psychropotes longicauda*, *Oneirophanta mutabilis* and *Pseudostichopus villosus*. 4α-methylcholestenol is a major lipid component of the particulate organic matter arriving at the seafloor [Kiriakoulakis, in press #823] and the presence of metabolites of this sterol in the body tissue of *A. rosea* indicates that a major component of the nutritive input must come from phytodetritus. Based on estimates of sterol requirement by *A. rosea*, Ginger *et al.* (2000) indicated that

the depletion of phytosterols in the surficial sediments observed between July and October 1997, resulted from the selective uptake of fresh phytodetritus by *A. rosea* and other holothurians.

The reproductive patterns of *Amperima rosea* are consistent with those observed for other species of the Family Elpidiidae (Tyler *et al.*, 1985a). Elpidiid species have some of the smallest recorded eggs of all deep-sea holothurians. A maximum oocyte diameter of ~200μm is similar to that recorded for *Kolga hyalina* (Figure 3.14) *Ellipinion molle* (Figure 3.14) and *Elpidia glacialis* (Hansen, 1975). In addition to species of the genus *Peniagone*, individuals of *Kolga, Ellipinion* and *Elpidia* can all occur in dense populations (Barnham *et al.*, 1967; Heezen and Hollister, 1971; Rowe, 1971; Hansen, 1975; Billett and Hansen, 1982; Ohta, 1983; Smith and Hamilton, 1983; Sibuet *et al.*, 1988; Billett, 1991; Gutt and Piepenburg, 1991). Elpidiid holothurians appear to be able to respond rapidly to the deposition of organic matter. In addition, the geographical distribution of the genus *Elpidia* appears to be restricted to areas under high surface primary productivity where the flux of organic matter to the seafloor is episodic (Hansen, 1975).

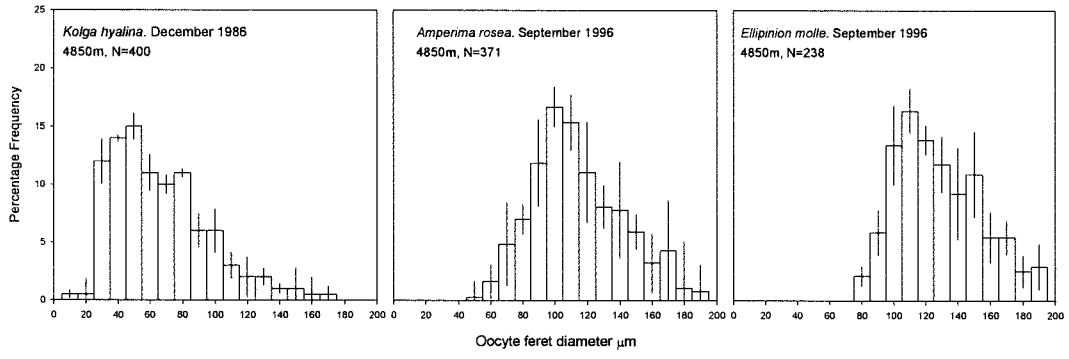

Figure 3.14. Mean oocyte size-frequency distributions (± standard deviation) for *Kolga hyalina*, *Amperima rosea*, and *Ellipinion molle*, from the Porcupine Abyssal Plain.

The links between phytodetritus as a food source and the effects variability in supply may have on the reproductive processes of benthic invertebrates has been well documented for many deep-sea species, including seasonally reproducing asteroids (Tyler and Pain, 1982b; Tyler *et al.*, 1990; Tyler *et al.*, 1993) and echinoids (Tyler and Gage, 1984a; Gage

et al., 1986; Campos-Creasey *et al.*, 1994), and also some asynchronously reproducing species e.g *Phormosoma placenta* (Campos-Creasey, 1992).

In spite of its small oocyte size (≤200μm diameter) *Amperima rosea* does not exhibit any of the classic patterns of synchronous development associated with seasonally breeding, free-spawning invertebrates. It does, however, exhibit some temporal trends that may indicate a more opportunistic reproductive strategy than those found in other elasipodid holothurians (Tyler and Billett, 1987). The differences in gonad index between spring and autumn samples may represent a period of gonad growth and maturation. Evidence provided by cumulative frequency plots of oocyte distribution shows an increase in the proportion of vitellogenic oocytes present in the ovaries of females from the autumn samples. It is possible that a period of gonad growth may be associated with an increase in fecundity. However, this pattern is not repeated in April 1999, when there was a much higher proportion of large vitellogenic oocytes present in the ovary. It is possible that these were unspawned oocytes.

Fecundity is not a highly conservative parameter within species, and can vary in response to nutrition, population density and adult age and size (Eckelbarger, 1986). It has been predicted that, given a constant allocation of energy, species will produce either a small number of large eggs or a high number of small eggs (Thorson, 1950; Tyler and Pain, 1982b; Jaeckle, 1995; Podolsky and Strathmann, 1996). If the supply of available energy entering the system changes then an opportunistic species may be able to allocate more energy to reproductive processes, therefore increasing its reproductive output during periods when the quality of food supply is nutritionally beneficial to that species. Ramirez-Llodra *et al.* (in press) have shown how variable food supply can affect the reproductive output of abyssal asteroids. Individuals of the porcellanasterid *Styracaster horridus*, taken from the Maderia Abyssal Plain (MAP), had a significantly lower fecundity than those individuals taken from the PAP. The MAP is more oligotrophic than the PAP, with no evidence of a seasonal deposition of phytodetritus (Rice *et al.*, 1994). Data presented here suggest that *A. rosea* may produce batches of oocytes and then effectively 'hold off' further maturation of the majority of those previtellogenic oocytes until conditions are energetically favourable for the vitellogenesis and spawning of large numbers of eggs.

The oogenic patterns of development in invertebrates are phylogenetically constrained, and are therefore intrinsic to the species and independent of environmental variability (Eckelbarger, 1994; Eckelbarger and Watling, 1995). *Amperima rosea*, as a species in the Family Elpidiidae, would be predicted to produce a lecithotrophic larvae, possibly with an abbreviated stage as in *Penaigone azorica* and *P. diaphana* (Tyler *et al.*, 1985a) and *Elpidia glacialis* (Hansen, 1975), but very little is known about larval development in deep-sea holothurians (Gebruk *et al.*, 1997). The maximum egg size of 200μm is right on the borderline between proposed planktotrophic and lecithotrophic development. Smiley *et al.* (1991) note that shallow-water species in the families Holothuriidae, Stichopodidae (order Aspidochirotida), and Synaptidae (order Apodida), which have relatively small eggs (85-250μm), have indirect development with a planktotrophic auricularia larva. The cosmopolitan deep-sea synaptid *Protankyra brychia* also has small (200μm) eggs (Billett, 1991), and is thought to have a planktotrophic developmental stage. Gage (pers. comm.) notes similarities in the calcareous deposits between *P. brychia* and the giant holothurian auricularia *Auricularia nudibranchia* (Pawson, 1971) that suggests that the latter could be the larval form of the former. Small egg sizes and planktotrophy in deep-sea echinoderms have so far all been associated with a distinct seasonal pattern of reproduction. Although there is no clear seasonal pattern for *A. rosea*, the small size of egg may be accompanied by higher fecundities than those of the other dominant holothurians on the PAP (Tyler and Billett, 1987). The estimate of potential fecundity calculated in this study (12,800 ±1030) is already larger than the ~5000 eggs per individual recorded for *P. azorica* (Tyler *et al.*, 1985a). It is the potential for increased reproductive output under favourable nutritional conditions that may account for the observed increase in abundance of *A. rosea*, rather than a predisposition for seasonal reproduction in response to the variable flux of organic matter.

Invertebrate life histories contain many stages where a variable food supply could impact on reproductive success. Apart from energy requirements for vitellogenesis, and perhaps for the requirement of specific lipids, the role of the larval stages and the effects a variable food supply may have on recruitment success should not be overlooked. *Amperima rosea* may not produce a feeding planktotrophic larva, but successful settlement and growth of the early post-larval stages may be dependent on the timing and

magnitude of the flux of organic matter from surface waters. Gage and Tyler (1981a) and Sumida *et al.* (2000) have shown that high fecundities, together with high population densities, in the bathyal ophiuroid *Ophiocten glacialis* result in regular settlement of large numbers of post-larvae, maintaining high population densities, even though juvenile mortality is high. Dramatic increases in the abundance of the abyssal congener *Ophiocten hastatum* have been recorded at the BENGAL station on the PAP (Bett *et al.*, 2001). *O. hastatum* has also been shown to feed preferentially on fresh phytodetritus to a similar extent to *A. rosea* and *E. molle* (Iken *et al.*, 2001). A subtle change in the quality of organic matter (i.e. sterols) may provide the nutritional cue for opportunistic species, such as *A. rosea* and *E. molle*, to increase their reproductive output and subsequently lead to a greater number of successful post-larval recruits settling out of the water column (see Table 4 in Sumida *et al.*, 2000).

The potential of *Amperima rosea* to have an opportunistic reproductive strategy may be further enhanced by rapid growth and onset of sexual maturity in the juvenile stages. Rates of growth for deep-sea holothurians have not yet been measured, and only measured indirectly in other echinoderm species (Gage and Tyler, 1981b; Gage and Tyler, 1982b; Gage and Tyler, 1985; Gage *et al.*, 1986; Gage, 1987; Gage, 1990). Direct records of holothurian growth rates tend to be restricted to shallow-water, commercially exploitable species. Battaglene *et al.* (1999) recorded growth rates of ~15mm month^{-1} for juvenile specimens of *Holothuria scabra*, a tropical aspidochirotid sea cucumber that continually produces eggs of ~200μm in diameter and spawns bi-annually in response to an abundant food supply (Morgan, 2000).

The relatively small size (~10mm) of *A. rosea* at first maturation may be an additional indication of their opportunistic reproductive strategy. This can be put into context by examining the elpidid *Peniagone azorica* (Tyler *et al.*, 1985a), which also attains a length of ~80-90mm, but shows no sign of gonad development at lengths of <30mm. However, the same authors report how gonad development in the benthopelagic congener *P. diaphana* starts at body lengths of ~10mm. From tentative estimates of growth rates (~ 1mm month^{-1}) it is possible that *A. rosea* could be sexually mature within one year after settlement. This could be advantageous to *A. rosea*, as a species, if it is to take advantage of a subtle change in food supply. *P. diaphana* also attains sexual maturity at a relatively

small size (~10mm, ~10% of maximum recorded size) and along with *P. azorica* is believed to have rapid growth up to the size of maturity (Tyler *et al.*, 1985a). Successful recruitment in these two species is thought to be temporally variable. As with *A. rosea* the lack of evidence for periodic egg production points to the survival of post-larvae as being an important contributing factor to variability in population structure and density. Survival of post-larvae in the water column and post-recruitment juveniles in the benthos is thought to vary temporally and spatially in response to variability in the seasonal flux of phytodetritus, as suggested by Gage and Tyler (1982b) for the ophiuroid *Ophiomusium lymani*. Similar results of enhanced growth and reproductive output with accompanying high food quantity or quality have been found in shallow-water asteroids and echinoids. Specimens of *Lepasterias epichlora*, taken from an area of high food availability, were found to be larger and produced a greater number of larger eggs than those specimens taken from a less nutritionally favourable area (George, 1994a; 1994b). In addition, specimens of the echinoid *Abacia lixula*, from a site containing a high abundance of high nutritive quality algae, were found to produce eggs with a higher protein content than those animals from a site with less food availability (George *et al.*, 1990).

The constant presence of mature spermatozoa in the male specimens, irrespective of sample season or year, may be an adaptation to life in the deep-sea benthos, where a chance encounter with a spawning female would stimulate a spawning response from the male.

The unimodal distributions observed for *A. rosea* populations on the PAP may represent progeny of a single cohort (Tyler *et al.*, 1985a). Unimodal peaks in size structure, similar to those observed for *A. rosea*, can be found in populations of *Kolga hyalina* from the Porcupine Seabight, NE Atlantic (Billett and Hansen, 1982). The authors interpreted the unimodal peaks as the progeny of a single, highly synchronised breeding event. The absence of polymodality was thought to reflect rarely occurring recruitment to the population.

Analysis of the size structure of *A. rosea* on the PAP provides conflicting results. The large increase in abundance recorded between September 1996 and April 1997 and again between April and July 1997 was not accompanied by a large increase in the smaller size classes as may be expected in the wake of a large successful recruitment event. Evidence

from time-lapse photographs does indicate the presence of small (<10mm) individuals during the 1996-1998 period (Wigham, unpublished data). The lack of these specimens in OTSB samples can be explained by sampling bias. Many small specimens may be lost through the coarse (44mm) outer mesh of the trawl net, and the smallest animals may even pass through the 13mm mesh of the cod-end liner. Secondly, evidence from epibenthic sledge video observations indicate that *A. rosea*, as a neutrally buoyant animal, is very easily displaced by the bow wave formed ahead of the towed sledge (pers. obs.). It is expected that the OTSB trawl will produce a similar bow wave.

The data obtained suggest that the arrival of surface-derived organic matter to the seafloor can have an important impact on the life history of *Amperima rosea*, and possibly other elpidiid holothurians. The ability of *A. rosea* to metabolise phytosterols (Ginger *et al.*, 2000; 2001) that are not available to other abyssal holothurians may provide them with the means to allocate more energy to increasing their reproductive output during nutritionally favourable periods. While current evidence favours this interpretation, the possibility that the observed bloom of *A. rosea* on the Porcupine Abyssal Plain may turn out to be the result of a cyclic reproductive event occurring over >1 year time scales cannot be dismissed. However, such a pattern cannot currently be resolved without continued long-term time-series sampling, of a higher temporal resolution, of the abyssal ecosystem.

Chapter Four – Time-lapse photography: temporal variability in the activity of abyssal megafauna

4.1. Introduction.

4.1.1 Photography

Photographs give us a permanent visual representation of the deep-sea bed better than can be obtained by any other technique. Analysis of oblique photographs of the seabed can resolve surface structures and small organisms more clearly than those taken directly downwards. However, downward orientated photographs may prove better suited for the analysis of spatial patterns, movement and feeding behaviours.

4.1.1.1. Time-lapse photography.

Time-lapse photography has been used in scientific applications for over 100 years, and has been used in deep-sea operations for the last three decades. In its 'purest' form time-lapse photography is intended to make slow (biological and geological) phenomena visible, from the growth of a deep-sea barnacle (Lampitt, 1990) to the migrations of sediment ripples across the deep-sea floor (Wimbush *et al.*, 1982). In the case of biological observations in the deep sea, this pure application of time-lapse photography is often fortuitous. Although with increased use of Remotely Operated Vehicles (ROVs) and submersibles, directed studies are certainly possible (e.g. Tunnicliffe *et al.*, 1990). Time-lapse photography is a powerful tool for monitoring animal behaviour on the deep-sea floor (e.g. Rowe *et al.*, 1974; Paul *et al.*, 1978; Lampitt and Burnham, 1983; Smith, 1985; Bett and Rice, 1993; Smith *et al.*, 1993). The development of moored camera systems, such as the SOC Bathysnap (Lampitt and Burnham, 1983), Scripps' Tripod Camera (Kaufmann and Smith, 1997) and Aberdeen University's AUDOS (Smith *et al.*, 1997), has enabled *in situ* studies of benthic communities to be made over time-scales that are not physically feasible for submersibles or ROVs. These camera systems can be deployed on the seafloor for long periods, often up to one year, during which time they can take a series of still photographs at pre-set intervals.

The results of studies utilising this technology have greatly increased our understanding of benthic processes in the deep sea. Data from time-lapse cameras has been used in a variety of ways to investigate the ecology and behaviour of deep-sea animals. Time-lapse films have allowed the measurement of previously inaccessible patterns of growth, locomotion and

feeding of both errant and sessile benthic organisms. Lampitt and Paterson (1987) used a combination of photographic and current data from the Bathysnap system to study the feeding behaviour of the abyssal anemone *Sicyonis tuberculata*. They were able to contradict the findings of previous reports and show how this animal actively orientates its oral disk into the prevailing current and feeds approximately 150 times per day on a wide range of material. The Bathysnap system has also been successfully used to study the feeding behaviour of other deep-sea animals, including decapod shrimps (Lampitt and Burnham, 1983), echiuran worms (Bett and Rice, 1993), amphipods (Lampitt *et al.*, 1983) and echinoids (Campos-Creasey *et al.*, 1994).

Curiously the majority of these studies have resulted from a certain degree of serendipity, especially those cases involving sessile organisms. It is impossible to pre-determine the landing site of a free-fall camera system, so if the camera happens to land near a sessile organism, such as an anemone or crinoid, or burrow, then the photographs taken can provide a large accurate data set for the behaviour of a particular animal. A particularly fortuitous event led to the direct measurement of growth in a deep-sea barnacle (Lampitt, 1990). During a deployment of the Bathysnap camera system a cyprid, of the barnacle *Poecilasma kaempferi*, settled on a plastic current-direction vane. The vane was in the field of view of the camera, enabling its growth to be measured over the next 228 days. Additional equipment is often added to these camera systems to accomplish specific scientific goals. The most common addition is that of bait, the so called 'monster camera' approach, which is often deployed during studies of organic decomposition rates, foraging and feeding behaviour, and for the trapping of invertebrate megafauna (Hessler *et al.*, 1978; Hargrave, 1985; Armstrong *et al.*, 1991; Jones *et al.*, 1998; Priede and Merrett, 1998).

4.1.1.2. Towed camera systems.

Stills cameras are often integrated with a video system and other instruments within a towed frame. This type of hardware is towed behind a research vessel either several metres above the seabed utilising downward facing cameras, e.g. the SOC WASP (Wide-Angle Seafloor Photography) and Woods Hole's ARGO II, or actually in contact with the seafloor utilising cameras facing obliquely downwards and forwards, e.g. the SOC (IOS) Epibenthic Sledge

(Rice *et al.*, 1982). Downward facing photographs can cover large areas of seafloor, but limited resolution of smaller deep-sea organisms makes identification difficult. This type of photo-transect is often useful in broad scale surveys of the nature of the deep-sea bed. Oblique images taken from cameras on epibenthic sleds tend to provide photo-transects with more information on seabed topography and images of organisms that are considerably easier to identify. Photographic surveys can provide information on the spatial relationships between the macro- and megabenthos and their environment (Fujita *et al.*, 1987). Photographic surveys have been used in the past for assessing the density and spatial distribution of the deep-sea fauna.

4.1.2 Video.

Video holds an advantage over conventional stills photography in that it provides an unbroken record of events occurring at the deep-sea floor. High-resolution video cameras and recorders attached to towed vehicles or ROVs have proved to be a useful tool for recording the behaviour of deep-sea organisms (e.g. Laver *et al.*, 1985). Static rigs, incorporating video cameras, may also be left on the seafloor, recording changing events at a single site over time (e.g. Wilson and Smith Jr, 1984; Copley *et al.*, 1997) and video transects made using cameras mounted on submersibles can also be used to make estimates of animal biomass and behaviour (Chevaldonne and Jollivet, 1993) and temporal change in communities (Shank *et al.*, 1998).

4.2 Materials and Methods.

4.2.1 The 'Bathysnap' system.

Southampton Oceanography Centre's '*Bathysnap*' is an autonomous free-fall camera system that can be launched from a research vessel. It is designed to have a minimal effect on near-bed currents and can be deployed for short (2-3 days) or long (≥ year) periods. The basic seabed unit consists of a conical plastic frame, to which are attached the camera, flashgun, recording current meter and an acoustic release system, this is in turn attached to a 76kg ballast weight. Once activated the camera and flashgun fire at preset intervals (15 seconds to 8+ hours) for the duration of the deployment (up to 12+ months), limited by the film load of some 2500 half-frame 35mm stills. On recovery the ballast weight is jettisoned via an acoustic command from the ship and the camera frame rises under the support of the buoyancy spheres. Should the acoustic signal fail to trigger the release then there is a backup contingency. The frame is attached to the ballast weight by magnesium bolts which, when immersed in seawater, will slowly corrode allowing the camera frame to float to the surface.

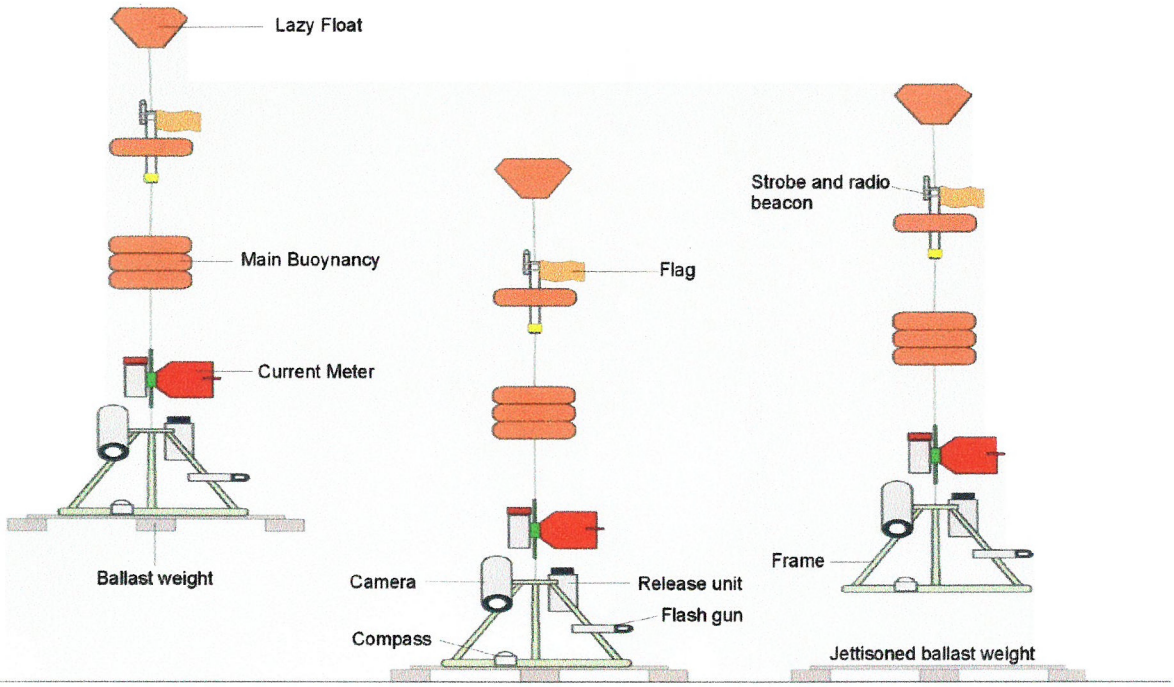

Figure 4.1. Schematic representation of deployment and recovery of the SOC '*Bathysnap*' system.

4.2.2. Bathysnap deployments.

Four successful, long-term *Bathysnap* deployments were made on the Porcupine Abyssal Plain between April 1997 and April 2000 (see Table 4.1 and Figure 4.2). The deployment sites were located within the 15 nautical miles (28km) of the central BENGAL position (48°50'N 16°30'W) at c.4844m water depth (with the exception of deployment 13370#8, which landed on a slight rise: 4823m). The time interval between consecutive photographs for each deployment is specified in Table 4.1.

Station	Position	Depth (m)	First Frame	Last Frame	Frame Interval	Deployment Cruise	Recovery Cruise
13078#7	48°54.6'N 16°34.9'W	4844	08/04/97 08:30	10/07/97 15:43	20/day	RRS *Discovery* 226[a]	RRS *Discovery* 229[b]
13200#95	48°57.9'N 16°11.6'W	4842	26/07/97 11:42	12/03/98 11:42	5/day	RRS *Discovery* 229[b]	RRS *Discovery* 231[c]
13370#8	48°59.7'N 16°13.0'W	4823	27/03/98 15:39	11/02/99 01:09	5/day	RRS *Discovery* 231[c]	RRS *Challenger* 142[d]
54904#2	48°58.9'N 16°24.7'W	4843	03/05/99 18:57	25/04/00 04:33	5/day	RRS *Challenger* 142[d]	RRS *Charles Darwin* 121[e]

Table 4.1. Station and photographic data for *Bathysnap* deployments on the PAP. a; Rice, (1997), b; Bett, (1998), c; Rice, (1998), d; Billett, (2000), e; Campbell, (2000).

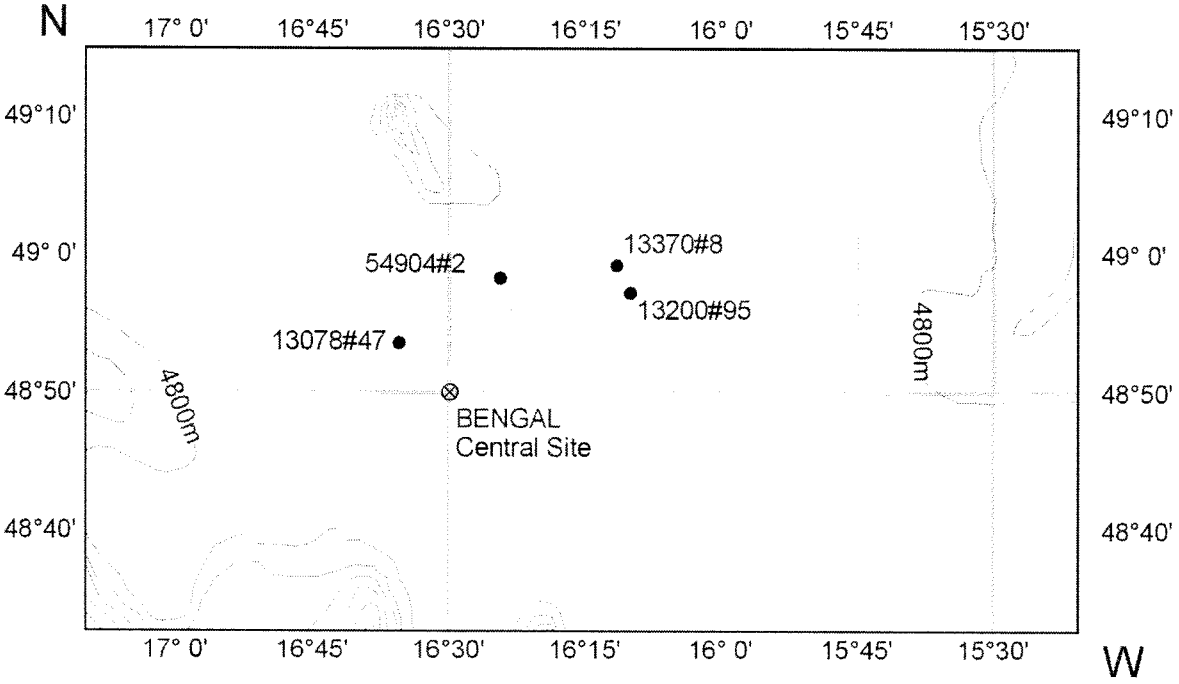

Figure 4.2. Bathymetric chart showing location of *Bathysnap* deployment sites on the Porcupine Abyssal Plain. Bathymetry 4300-4800m in 100m intervals.

4.2.3 Film Analysis.

All photographs were taken in half-frame cine format on 35mm colour negative film (AGFA XT320 or Kodak E5297). Four films, one from each deployment, combined to give nearly 36 months temporal coverage of activity on the abyssal seafloor of the PAP. Following retrieval from the camera, each film was processed and a Carl Zeiss Jena DLZ Dokumator film reader was used to view positive copies of each film (see Figure 4.3 for example of Bathysnap stills). Quantitative analysis of the stills is possible through knowledge of the optical geometry of the system, which enables the preparation of a perspective (or 'Canadian') grid (see Wakefield and Genin, 1987). In practice, a perspective grid was not used, and scaling and quantification were carried out by a combined use of commercially available image analysis packages and custom written software (see detailed methodology below). Each film was assessed for the presence of *Amperima rosea,* and additional components of the errant megafauna, and measurements of each individual were made.

4.2.3.1 Measuring the megabenthos.

Projecting an image of each frame onto a 215 x 165 mm piece of blank paper enabled the rough dimensions of each individual to be recorded. Each animal was recorded on paper as a sequence of four points forming a diamond (Figure 4.4A), each point corresponding to either the head, tail, back or sole of the animal. The direction in which the animal was facing was recorded by shading the head end of the diamond. Each point of the animal diamond represents a set of X,Y co-ordinates. These co-ordinates are categorised as being part of the major or minor axis of the animal. The major axis being the length (head-tail) and the minor axis being the width (back-sole) (Figure 4.4A).

MAx_1y_1 = Tail

MAx_2y_2 = Head

mAx_1y_1 = Back

mAx_2y_2 = Sole

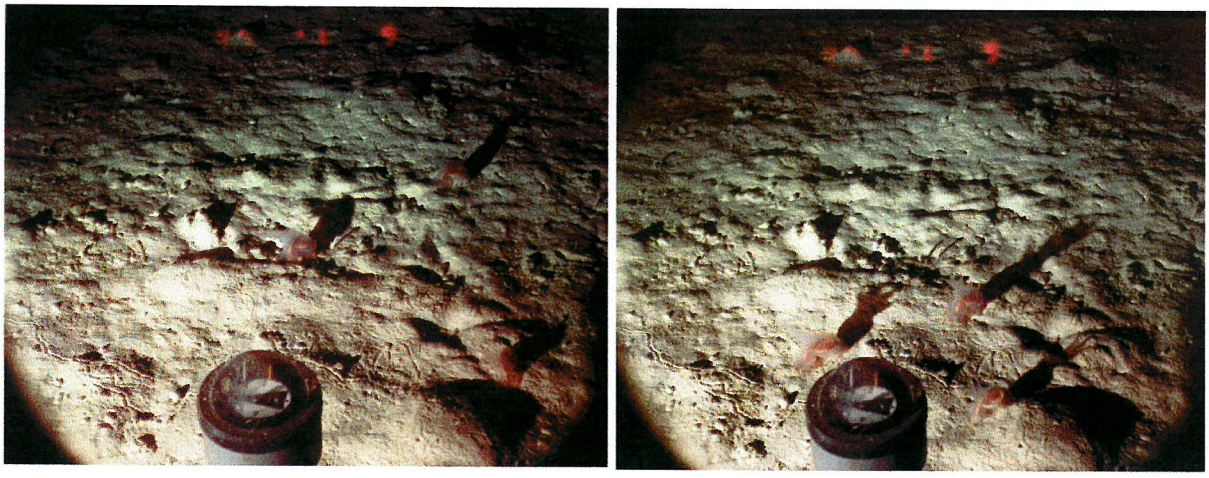

Figure 4.3. Example of consecutive frame still images obtained from the *Bathysnap* system (frame interval = 4.8 hrs; frame area = 2.02m^2) showing three specimens of *Amperima rosea*.

Measuring the difference between the head and tail X and Y co-ordinates of the major axis gives the dimensions for two sides of a right-angled triangle, the hypotenuse of which represents the length of the animal. Conversely the same principle can be applied to the co-ordinates of the minor axis to give the width of the animal. This procedure allows for the measurement of animals that are not lying in a true X or Y plane. The location of each animal within each frame is given by the co-ordinates of the sole (mAx_2y_2), or the right hand point of the minor axis for animals lying in the Y-plane. In addition to these measurements an estimate of the direction of travel can be made. The angle made between the line of the animal's sole and the horizontal can be calculated using basic trigonometry (Figure 4.3B). This angle can then be converted to a bearing between 0° and 360°, which indicates the animals heading. All the basic measurements made from the initial analysis of the film are summarised in Figure 4.4

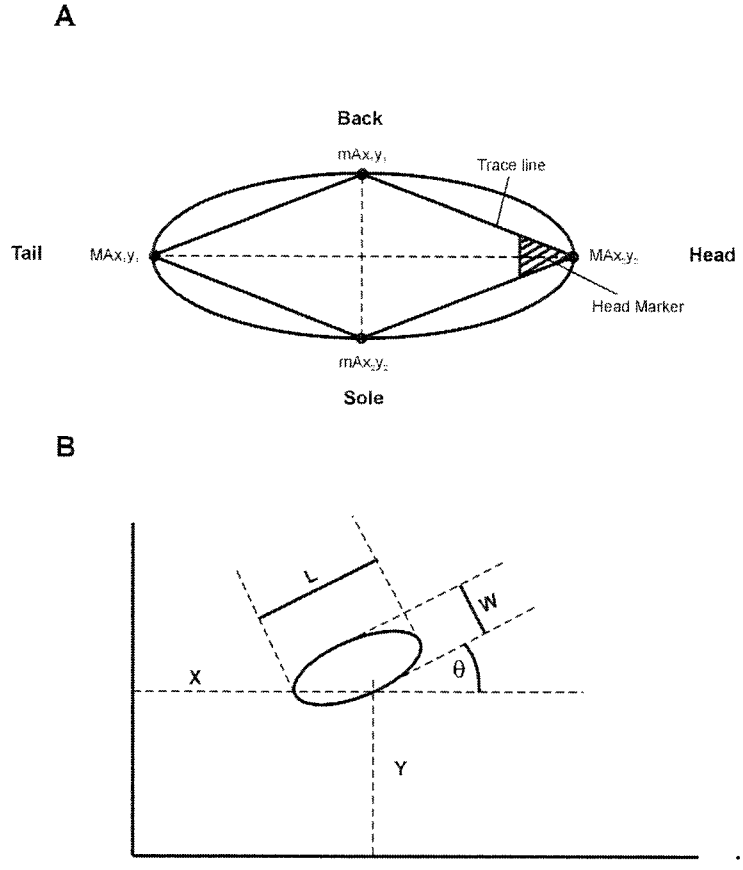

Figure 4.4. Measurements obtained from *Bathysnap* stills. A, diamond trace and major x,y reference points. B, major measurements obtained from each individual; L = length, W = width; θ = heading (degrees).

The extraction of the X-Y co-ordinates and subsequent conversion of each recorded point to real 'seafloor' values required the use of SigmaScan-Pro™ image analysis software and a light tablet. By tracing around each diamond with the light-tablet mouse the computer recorded an X,Y position for each of the four points. After completing this procedure for each diamond measurement the raw data were converted into real 'seafloor' units, taking into account the oblique nature of the photographs. This was achieved by entering the raw data into a spreadsheet incorporating formulas modified from Wakefield and Genin (1987). The number of megabenthos present in each photographic frame was recorded and the location and size of each individual was calculated.

4.2.3.2. Analysis of megabenthic activity.

Megabenthic activity was quantified as 'tracking' (or 'area traversed', (Smith *et al.*, 1993)), which was the area of seabed moved over (or tracked) by each individual megabenthic organism. Tracking was quantified as the product of the distance travelled between consecutive photographs and the body width (estimated from photographs) of each individual. The body width was averaged from all the available measurements for each film. Linear motion was assumed between consecutive observations. For specimens entering or leaving the field of view between consecutive observations, the distance travelled was assumed to be the shortest distance required to enter or leave the field of view. Thus estimates of tracking and rates of movement are minimum values, which underestimate the true distances travelled.

4.3. Results.

4.3.1 Abundance of Amperima rosea and 'other' holothurians.

In estimating the abundance of *Amperima* and 'other' holothurians, a number of precautions and corrections were applied to ensure the data were as representative as possible:

(a) Individuals of the galatheid *Munidopsis* commonly appear to 'adopt' the Bathysnap frame as some form of refuge or perch. Consequently, the frequent observations of *Munidopsis* reflect localised foraging of one or two individuals rather than a true measure of local population abundance. All observations of *Munidopsis* have therefore been excluded from abundance calculations.

(b) The numerous observations of actinarians (during deployments 13078#47 and 54904#2) are not a reliable measure of abundance, but reflect the sessile or hemi-sessile habit of the species (see Riemann-Zurneck, 1997). Only distinct observations, i.e. the first observation at any one location, of actinarians have been included in abundance calculations.

(c) For the 'other' holothurians (i.e. all holothurians except *Amperima rosea*) the abundance estimates have been based simply on total observations and the total area of seabed effectively observed (i.e. the product of seabed area observed, 2.02m^2, and the number of frames analysed).

(d) There is reason to believe these sample estimates may under-represent the true abundance of small species, such as *A. rosea*. Smith *et al.* (1993) noted that oblique seabed photographs have to be interpreted carefully with respect to the detection of small individuals across the full area of the frame. They adopted a probability density function approach to take into account this potential bias in abundance estimates. For this study a related approach, based on line transect methods for the estimation of abundance was adopted (see Krebs, 1998). A 'detection function' was estimated for *A. rosea* that indicated that estimates of their density were only reliable for those specimens appearing in 0-60cm band from the proximal edge of the field of view (see Figure 4.5).

(e) Abundance data have been expressed as numbers per hectare (i.e per 10,000 m^2), to be consistent with the megabenthos abundance data from the trawls presented in Chapter 2. These values should not be taken as an indication of the precision of the abundance estimates, as they relate to repeated observations of a seabed area of just 1-2 m^2.

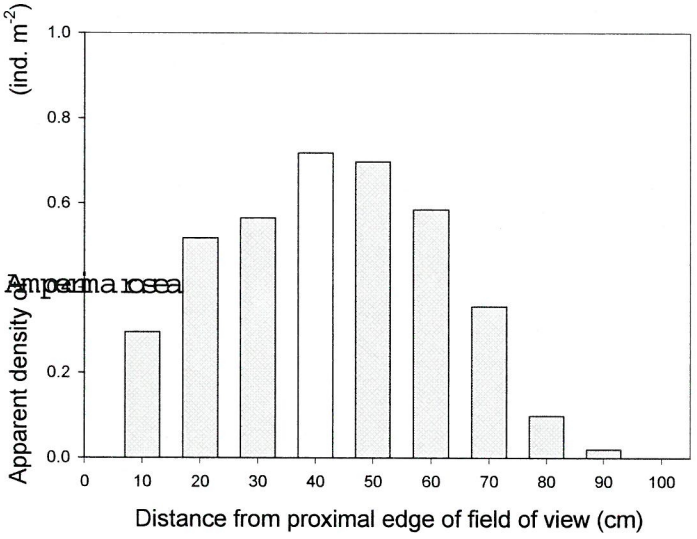

Figure 4.5. Apparent density of *Amperima rosea* as determined at increasing ranges from the *Bathysnap* camera system deployed on the PAP. Note rapid reduction in density beyond 60cm.

A major assumption of both the simple and line transect methods for estimating density is that the various observations made are independent, an assumption that is unlikely to be met in the case of the sequential Bathysnap observations. This is readily illustrated in a frame-to-frame comparison of the presence or absence of *Amperima rosea*. Table 4.2 details the observed and expected (under random/independent conditions) presence/absence of *A. rosea* in consecutive pairs of Bathysnap frames.

Station	13078#47		13200#95		13370#8		54904#2	
	Observed Frequency	Expected Frequency	Observed Frequency	Expected Frequency	Observed Frequency	Expected Frequency	Observed Frequency	Expected Frequency
Absent-absent	1284	937.4	577	366.8	1161	915.0	1444	1213.0
Absent-present	36	382.6	71	281.6	53	299.2	30	261.0
Present-present	504	157.2	427	216.0	337	91.4	286	55.0
Present-absent	36	382.8	71	281.6	54	299.4	30	261.0
Total	1860	1860	1146	1146	1605	1605	1790	1790

Table 4.2. Observed and expected frequencies of presence or absence of *Amperima rosea* in consecutive pairs of *Bathysnap* frames, for all four deployments.

Its is immediately apparent that the observed present-present category is substantially over-represented with respect to the random expectation for each of the four deployments. Consequently, a G-test (Sokal and Rohlf, 1995) of goodness of fit to the random distribution is highly significant ($p<0.005$) in each case. Therefore the null hypothesis that the observed frequencies are drawn from a random distribution can be confidently rejected. These results suggest that for each deployment the *Amperima* observations are significantly aggregated in time.

Figure 4.6 illustrates the temporal variations in *Amperima rosea* observations during deployment 13078#47. This deployment was over a shorter period (April-July 1997) and had a shorter frame interval (20 d^{-1}) compared to the subsequent three deployments. The values shown are day totals with a seven-day running mean superimposed. Marked temporal variation is apparent and it is tempting to speculate on the occurrence of some pattern in these data. However, statistical analysis of the does not appear to reveal any consistent pattern. Figure 4.7 shows a TTLQV (two-term local quadrat variance) plot of the total daily data. The TTLQV method (Krebs, 1998) is designed to assess spatial variation in data from contiguous quadrats, but is here employed to assess temporal variation in consecutive photographs. TTLQV plots are effective in detecting and quantifying the size and spacing of 'clumps' of observations. The plot shown in Figure 4.7 does not indicate the existence of coherent clumps in the data, there is no distinct peak in the variance values. This result seems to suggest that the temporal aggregations of *A. rosea* are not particularly coherent with respect to their temporal extent or temporal spacing.

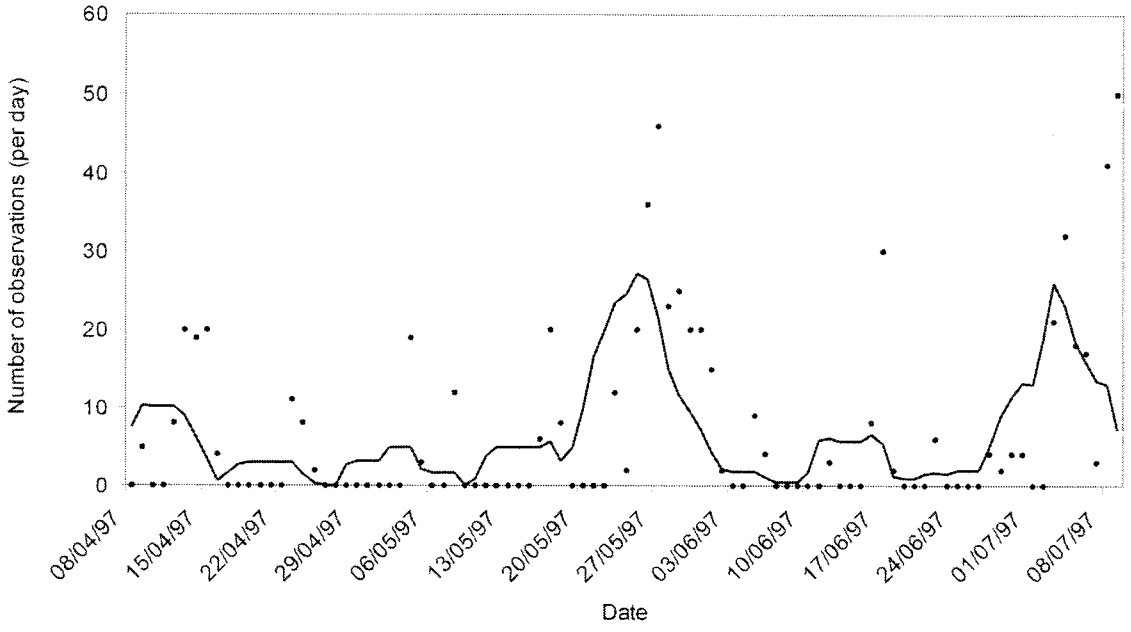

Figure 4.6. Temporal variation in the abundance of *Amperima rosea* at the PAP BENGAL site. Plotted points are the total number of individuals observed per day (total of twenty frames), the field of view being 2.02m^2. The solid line is a seven-day running mean of plotted daily total data.

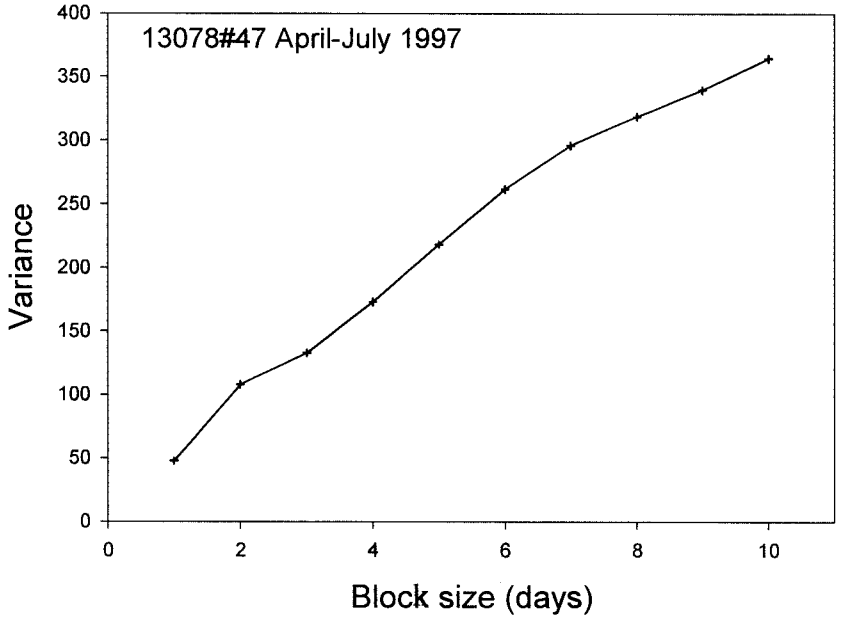

Figure 4.7. Two-term local quadrat variance (TTLQV) plot of *Amperima* abundance, data grouped into blocks of 1 to 10 days in duration. Deployment 13078#47, April-July 1997.

Accepting the lack of independence of observations and aggregated distribution of the data, Figure 4.8 provides the best estimate of *A. rosea* density. These results are based on the number of *A. rosea* specimens observed within 60cm of the camera during each day (i.e. 20 frames). A mean and 95% confidence limit are shown for each consecutive week of observation, together with the grand mean of the weekly observation results (0.6 individuals per m^2 equivalent to 6,295 individuals per hectare). Although significant variations (ANOVA, F=2.35, 13df, P=0.01) do occur week-to-week there is no indication of any pattern to these variations.

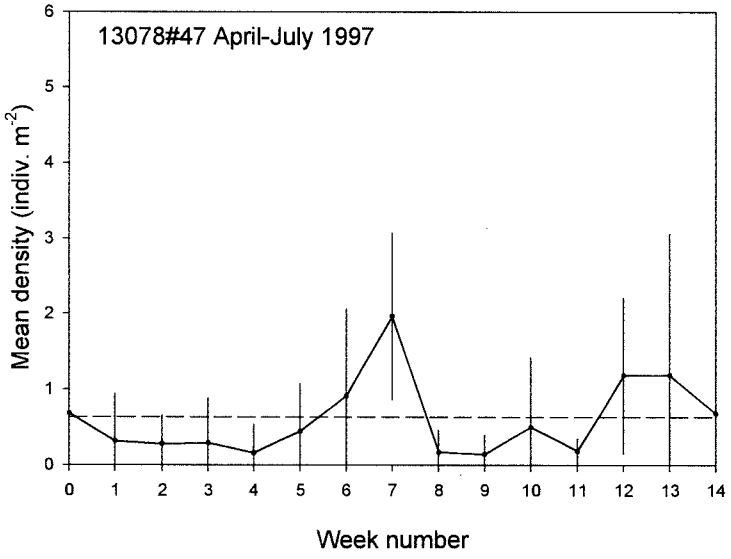

Figure 4.8. Temporal variation in the density of *Amperima rosea* at the PAP BENGAL site. Weekly mean density and 95% confidence limits are shown together with grand mean of the weekly means (horizontal dashed line).

Figure 4.9 illustrates the temporal variations in *Amperima rosea* observations during three deployments, 13200#95, 13370#8, and 54904#2, covering the period July 1997-April 2000. All three deployments had a longer frame interval (5 d^{-1}) compared to deployment 13078#47 and were therefore more comparable to each other. Again the values shown are day totals with a seven-day running mean superimposed. Marked temporal variation is again apparent, especially during the latter deployment (May 1999-April 2000).

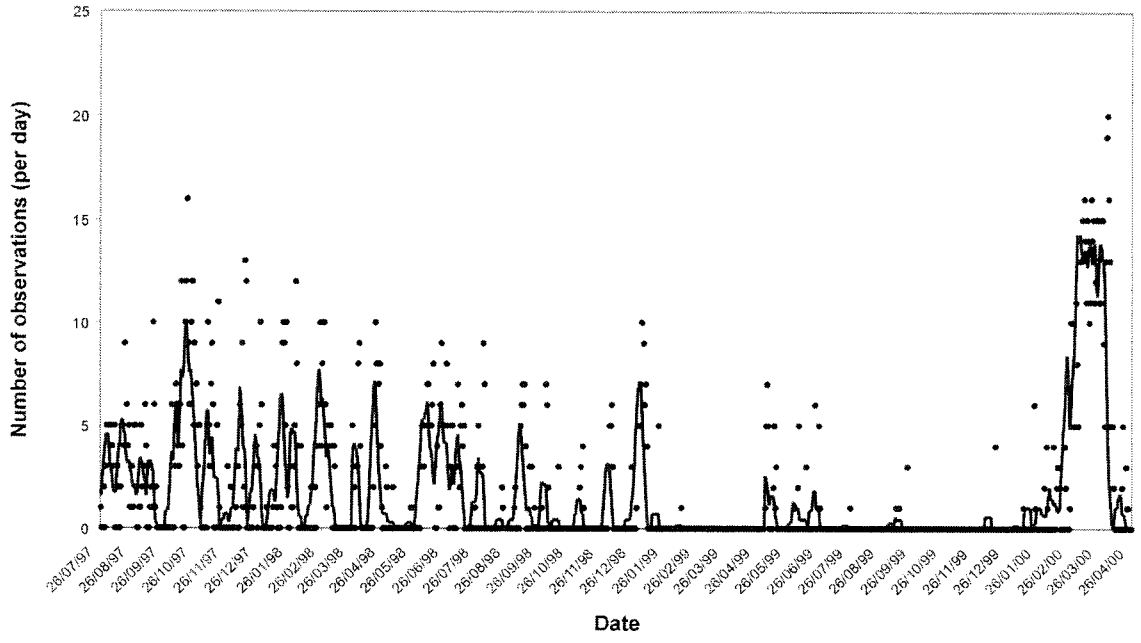

Figure 4.9. Temporal variation in the abundance of *Amperima rosea* at the PAP BENGAL site. Deployments 13200#95, 13370#8 and 54904#2. Plotted points are the total number of individuals observed per day (total of five frames), the field of view being 2.02m^2. The solid line is a seven-day running mean of the plotted daily total data.

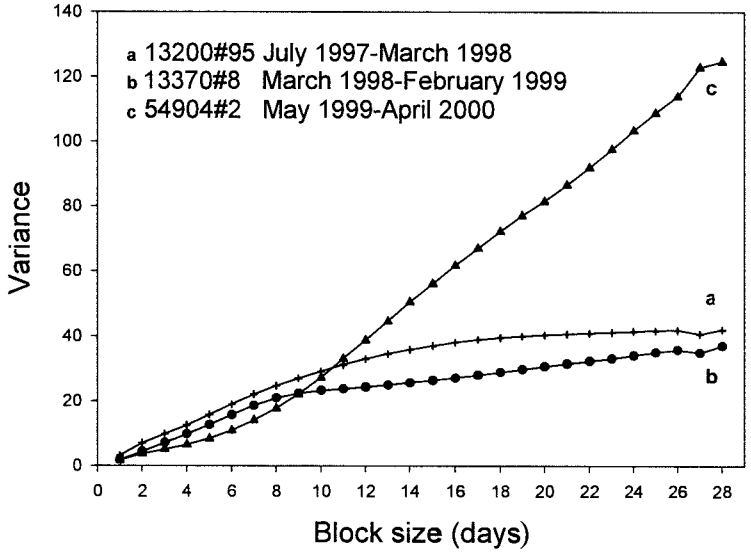

Figure 4.10. Two-term local quadrat variance (TTLQV) plots of *Amperima* abundance, data grouped into blocks of 1 to 28 days in duration. Deployments 13200#95, 13370#8, and 54904#2.

Given the results presented in Table 4.2 and the highly significant G-test values, TTLQV plots for the three longer-term deployments were also calculated to check for the existence of 'clumps' in the data. Figure 4.10 shows the TTLQV plots for deployments 13200#95, 13370#8, and 54904#2. These plots, like that for the shorter deployment, do not indicate the existence of coherent clumps in the data. As above this suggests that the temporal aggregations of *Amperima* are not particularly coherent in extent or spacing for any of the three deployments.

Figure 4.11A-C provides the best estimate of *A. rosea* density for each of the three deployments. Again the results are based on the number of *A. rosea* specimens observed within 60cm of the camera during each day (i.e. 5 frames). A mean and 95% confidence limit are shown for each consecutive week of observation, together with the grand mean of the weekly observation results (A, 0.8 indiv.m^{-2} ≅ 7,781 indiv.ha^{-1}; B, 0.5 indiv.m^{-2} ≅ 4,517 indiv.ha^{-1}; C, 0.6 indiv.m^{-2} ≅ 6,077 indiv.ha^{-1}). Although significant variations (ANOVA; A, $F=3.62$, 32df, $P<0.000$; B, $F=6.08$, 45df, $P<0.000$; C, $F=38.28$, 51df, $P<0.000$) do occur week-to-week there is no indication of any pattern to these variations.

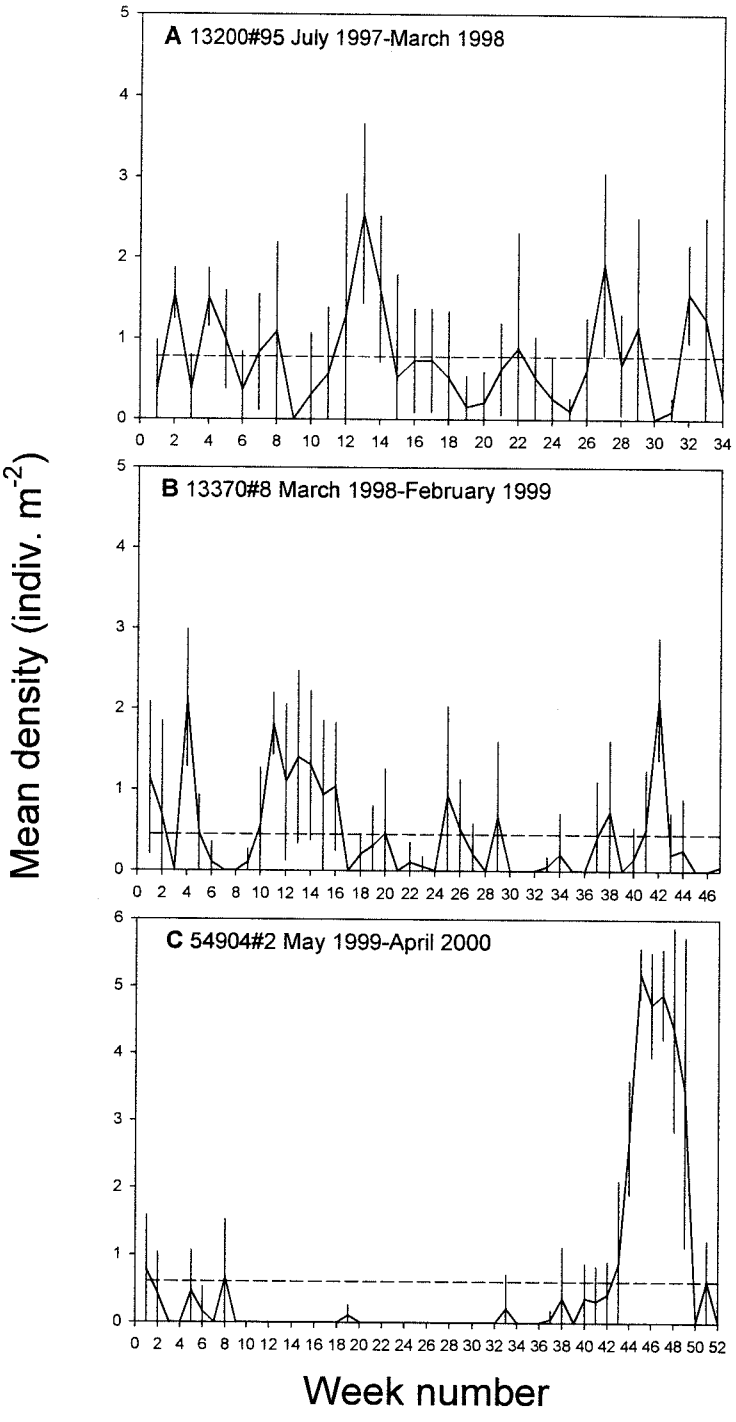

Figure 4.11. Temporal variation in the density of *Amperima rosea* at the PAP BENGAL site. 3 deployments covering the period July 1997-April 2000. Weekly mean density and 95% confidence limits are shown together with grand mean of the weekly means (horizontal dashed line).

Abundance data from the 1997-2000 period (Table 4.3) were consistent with the relative abundance of larger megabenthic taxa as determined from the corresponding trawl catches (see Chapter 2 and Billett *et al.*, 2001), i.e. holothurians dominated the megabenthos and *Amperima rosea* totally dominated the holothurian populations.

Station number	13078#47	13200#95	13370#8	54904#2
Frames analysed	1860	1146	1605	1790
Number of observations				
Amperima rosea	692	690	476	614
Other holothurians	23	38	315	50
Other megabenthos*	2	0	6	8
Abundance (/ha)				
Amperima rosea	6695	8286	4782	6552
Other holothurians	61	164	972	138
Other megabenthos*	5	0	19	22
Total megabenthos*	6761	8450	5773	6712

* Excludes observations of *Munidopsis* and repeat observations of actinarians

Table 4.3. Observations, and corresponding abundance estimates, of megabenthos from four consecutive *Bathysnap* deployments on the PAP (April 1997-April 2000).

The comparison of mean abundances of the megabenthos as estimated from *Bathysnap* observations and trawl catches (Table 4.4) indicates that for 'other holothurians' and the larger megabenthos the estimates are of a similar order. However, *A. rosea* abundances (and consequently total megabenthos) are two orders of magnitude higher in the *Bathysnap* records than in the trawl samples. Total megabenthos over the four deployments averaged 287 indiv. ha^{-1} in the trawl samples, compared with 6924 indiv. ha^{-1} from *Bathysnap* – a 24-fold difference.

	13078#47			13200#95			13370#8			54904#2		
Taxon	Photo	Trawl [a]	F% [e]	Photo	Trawl [b]	F% [e]	Photo	Trawl [c]	F% [e]	Photo	Trawl [d]	F% [e]
Amperima rosea	6695	225.4	3.4	8286	200.4	2.4	4782	163.3	3.4	6552	57.3	0.9
Other holothurians	61	93.9	153.9	164	70.4	42.9	972	59.3	6.1	138	93.0	67.4
Other megabenthos	5	44.5	890.0	0	46.9	-	19	45.3	238.4	22	32.7	148.6
Total megabenthos	6761	363.8	5.4	8450	317.8	3.8	5773	267.9	4.6	6712	199.4	3.0

[a] Mean of trawls 13078#29,31,37 (April 1997) and 13200#9,27,35,60 (July 1997)

[b] Mean of trawls 13200#,9,27,35,60 (July 1997), 54301#6,8 (October 1997) and 13368#23,24,50,51 (March 1998)

[c] Mean of trawls 13368#23,24,50,51 (March 1998) and 13627#10,23 (September 1998)

[d] Mean of trawls 54901#2,5,7,9 (April 1999)

[e] Fishing efficiency (trawl/photo %)

Table 4.4. Comparison of Bathysnap and trawl caught estimates of megabenthos abundance on the Porcupine Abyssal Plain.

4.3.2. Disturbance (seafloor tracking) rate estimates.

Table 4.5 details the tracking of *Amperima rosea* as measured from the Bathysnap observations. Tracking rates for *A. rosea* alone are high, but show a decrease in activity over time. Estimates of seafloor tracking indicate that *A. rosea* alone can track 100% of the seafloor in less than one year (range 2.6-11.0 months). The large increase in abundance of *A. rosea* during the BENGAL period is likely to have a significant impact on the benthic community as a whole. Tracking rates increase with abundance and deposit feeders like *A. rosea*, therefore, can have a significant impact on energy flow through the rest of the benthic community.

Station	13078#47	13200#95	13370#8	54904#2
Frame area assessed (m^2)	0.64	0.87	0.87	0.62
Frame interval (hrs)	1.2	4.8	4.8	4.8
Time elapsed (d)	93	229.2	321	358
Total area tracked (m^2)	0.74	2.19	1.25	0.66
Tracking ($cm^2/d/m^2$)	125.2	109.6	44.8	29.8
Tracking (%/yr)	457	400	163.5	108.7
Tracking (months/100%)*	2.6	3.0	7.3	11.0

* The number of months to track over 100% of the seafloor

Table 4.5. Seafloor tracking by *Amperima rosea*, as measured from *Bathysnap* deployments on the PAP (April 1997-April 2000).

The tracking of other mobile deposit-feeders will also contribute to the recycling and redistribution of organic matter. Bett *et al.* (2001) calculated tracking rates for ophiuroids and the total megabenthos for these four deployments in addition to three deployments in the pre-BENGAL period (1991-1994). Other taxa observed during the course of the deployments were either suspension feeders/sestonivores (actinarians, brisingiid asteroids), scavenger-omnivores (*Munidopsis*, *Plesiopenaeus*) or predators (pycnogonids). Sediment surface-feeding activity by echiurans (Bett and Rice, 1993) was observed during a number of the *Bathysnap* deployments. These 'mega-infaunal' echiurans may make a significant

contribution to total megabenthic tracking (see Bett *et al.*, 1995). For the purpose of the present study, however, the observed echiuran activity is discounted.

Amperima rosea also exhibited temporal variation in seafloor tracking during both the short-term deployment (13078#47; Figure 4.12) and the three longer deployments (13200#95, 13370#8, 54904#2; Figure 4.13).

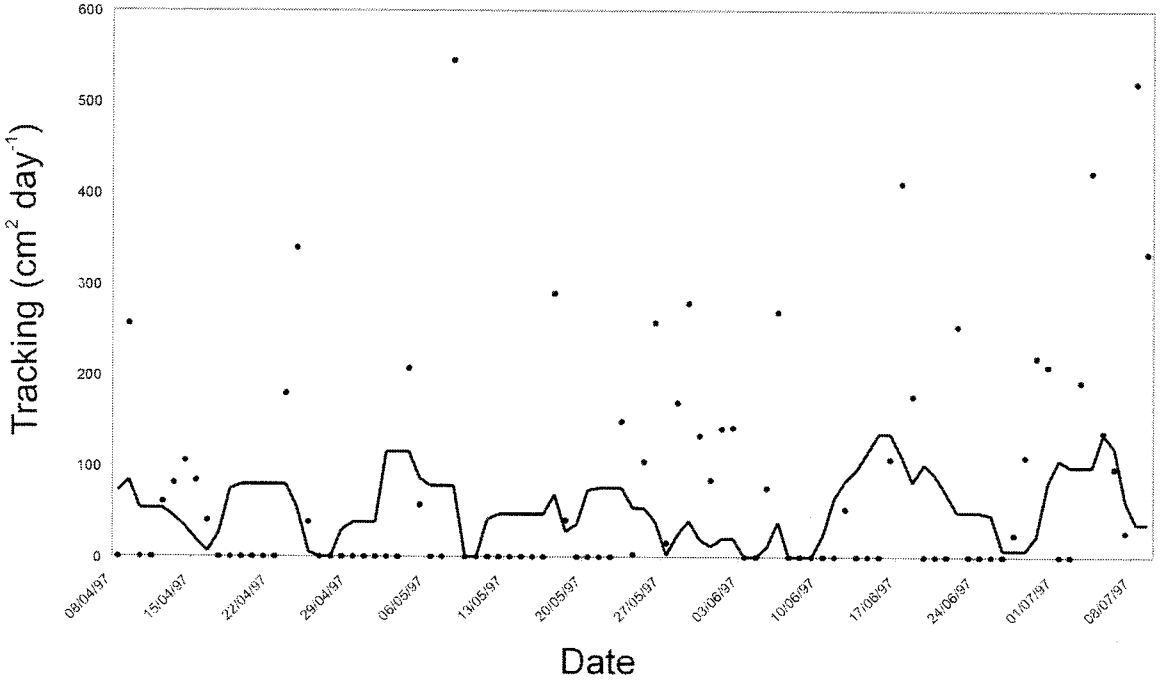

Figure 4.12. Temporal variation in seafloor tracking by *Amperima rosea* on the PAP during deployment 13078#47 (April-July 1997). Plotted points show total tracking per day, and the solid line is a seven-day running mean of the plotted daily total data.

However, the variation in tracking rates is probably related to the patchy distribution and varying abundance of *Amperima rosea*, rather than a seasonal response to a changing environmental factor (i.e. phytodetritus flux)

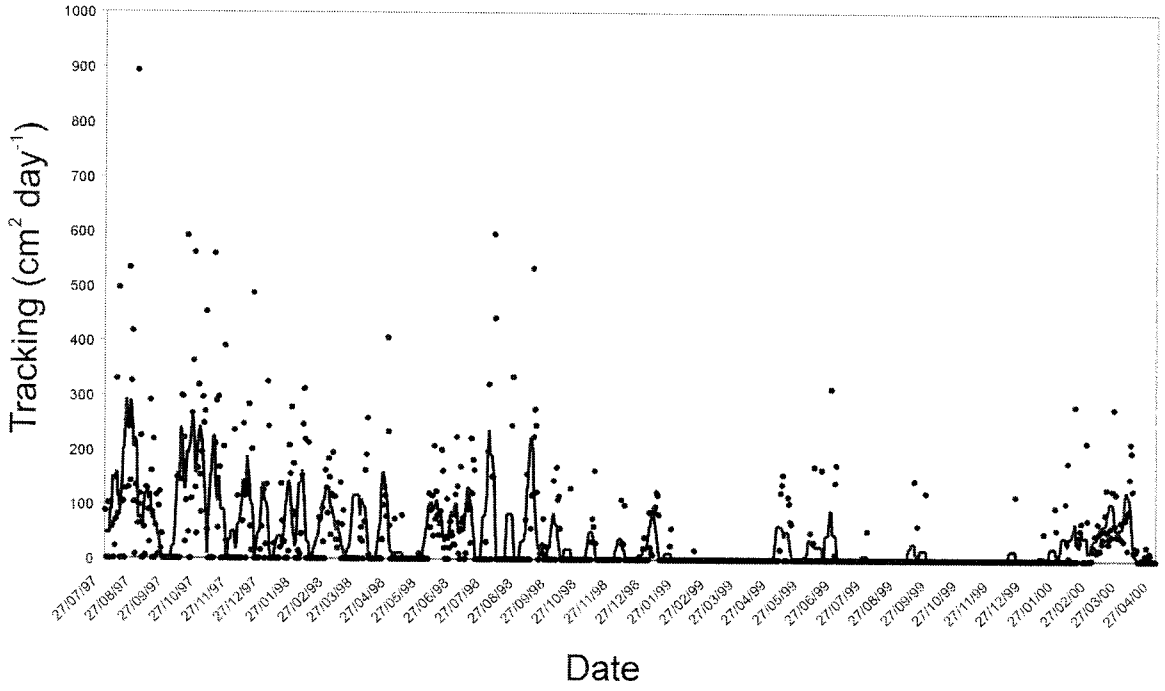

Figure 4.13. Temporal variation in seafloor tracking by *Amperima rosea* on the PAP during deployments 13200#95, 13370#8, and 54904#2 (July 1997-April 200). Plotted points show total tracking per day, and the solid line is a seven-day running mean of the plotted daily total data.

The day totals are all highly variable, from zero to in excess of 800cm^2 day^{-1}; not surprisingly the overall pattern for all four deployments is very similar to that of *Amperima rosea* abundance (Figures 4.6 and 4.9). The exception being during March and April 2000 when abundance is high yet tracking remains low. The significance of these temporal variations was assessed by analysis of variance of total daily tracking grouped by week (deployment 13078#47) and month (deployments 13200#95, 13370#8, and 54904#2). The ANOVA result was non-significant (F=0.66, 13df, P=0.791) for the week-to-week comparison of tracking during the short deployment (13078#47), which can be clearly seen in Figure 4.14.

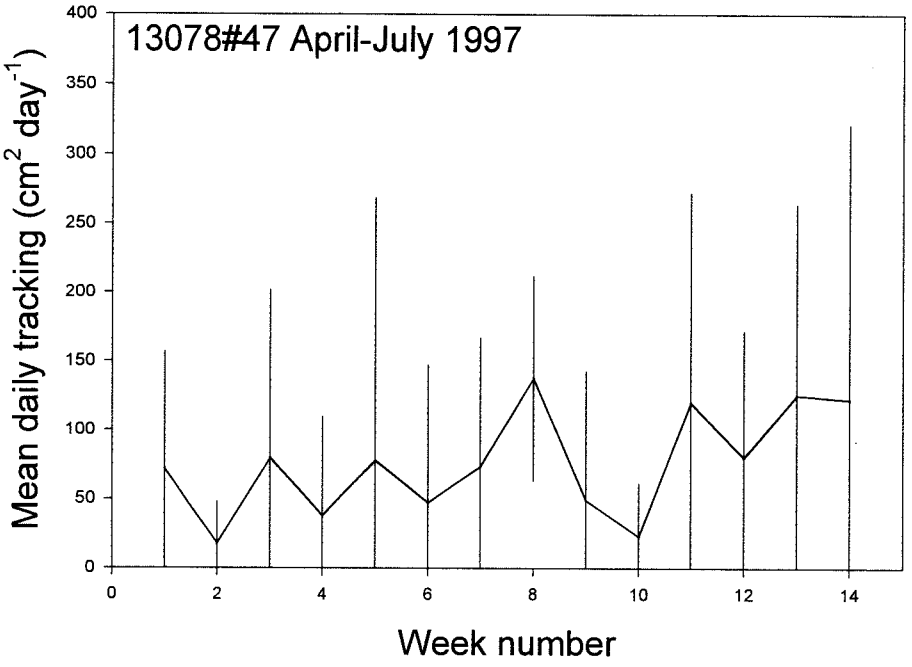

Figure 4.14. Temporal variation in seafloor tracking by *Amperima rosea* on the PAP, deployment 13078#47 (April-July 1997). Seafloor tracking is expressed as a weekly mean and 95% confidence limits.

The ANOVA results, based on monthly means, for deployments 13200#95, 13370#8, and 54904#2 were all highly significant (A, $F=5.27$, 8df, $P<0.001$; B, $F=3.77$, 11df, $P<0.001$; C, $F=7.62$, 11df, $P<0.001$). However, there is no obvious consistent trend in the data (see Figure 4.15A-C). Daily tracking will obviously be related to the patchy distribution and abundance of *Amperima*, and the plots shown in Figure 4.15 reflect the variability in densities shown in Figure 4.11.

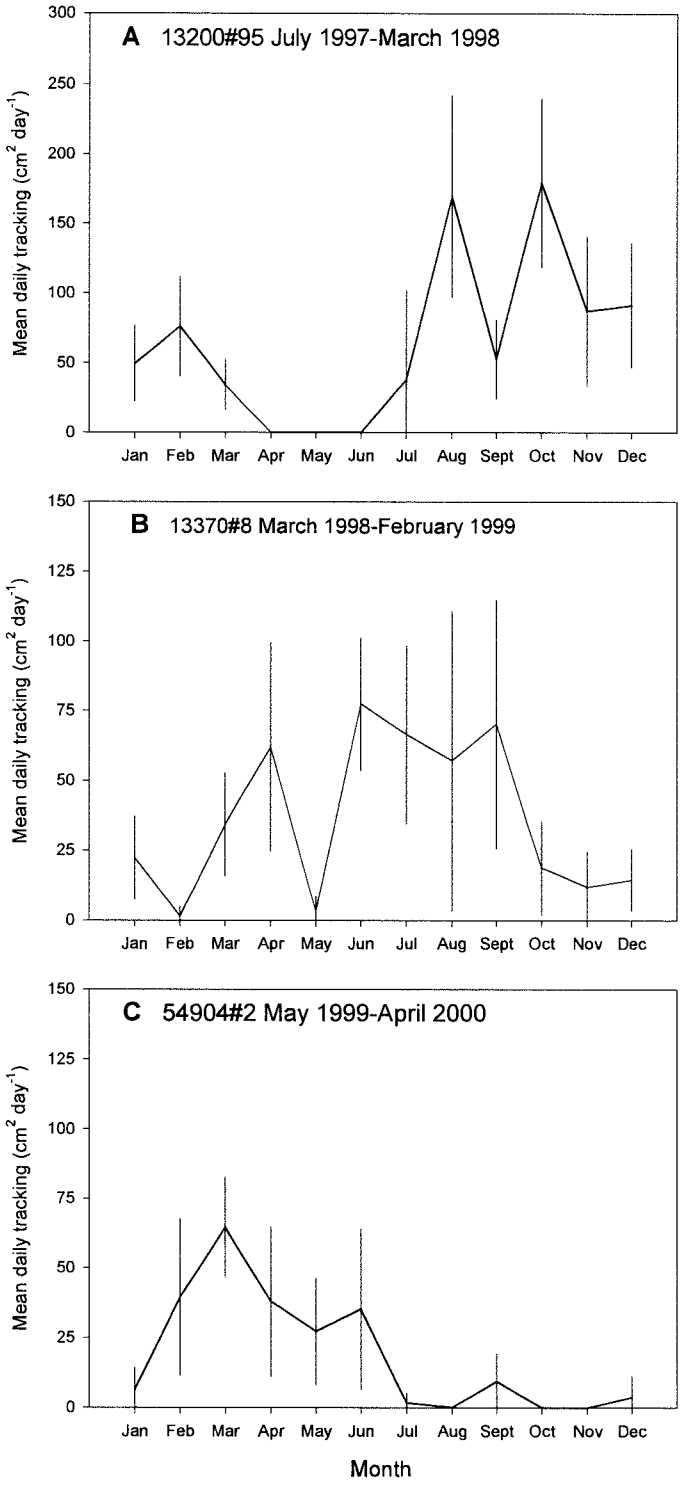

Figure 4.15. Temporal variation in seafloor tracking by *Amperima rosea* on the PAP, deployments 13200#95 (A), 13370#8 (B), and 54904#2 (C). Seafloor tracking is expressed as a monthly mean and 95% confidence limits.

4.3.3. Movement rate estimates.

Rates of holothurian movement were calculated assuming straight-line translocation between consecutive observations or from minimum distances required to enter or exit the field of view between consecutive frames. Consequently, all estimates of movement rates represent minimum values. The distribution of observed *Amperima rosea* speeds from each deployment are shown in Figure 4.16.

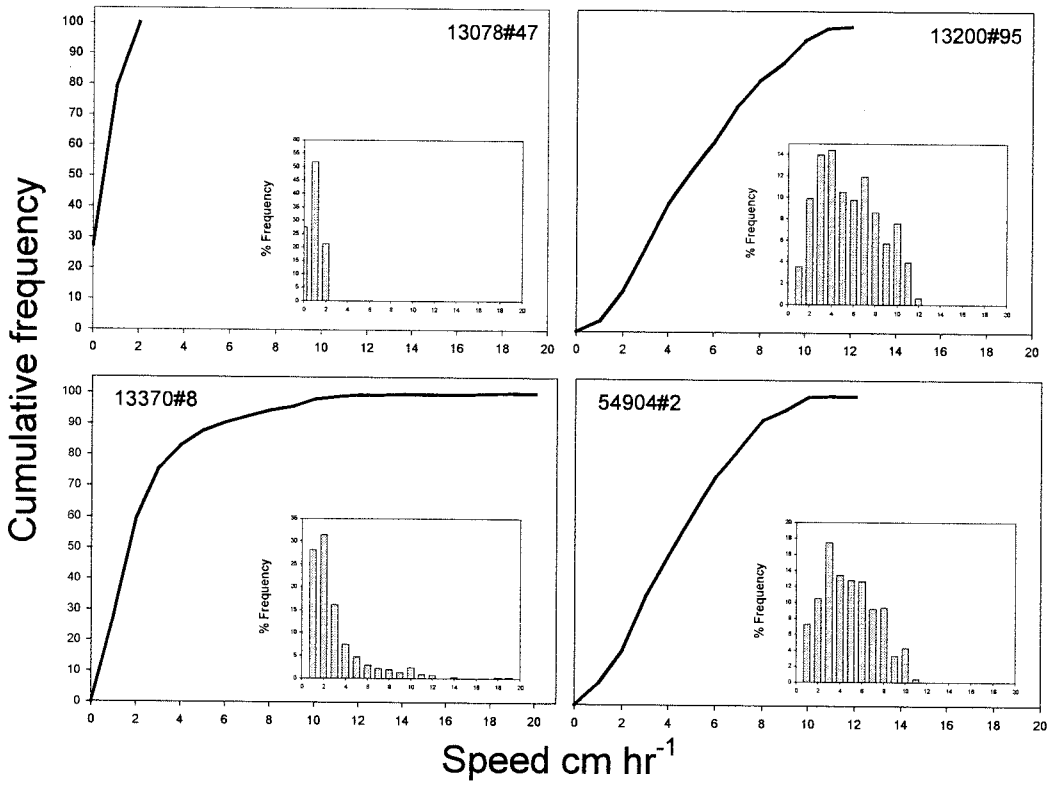

Figure 4.16. Estimated rates of movement of the holothurian *Amperima rosea*, observed by time-lapse photography, on the PAP during deployments 13078#47 (April-July 1997), 13200#95 (July 1997-March 1998), 13370#8 (March 1998-February 1999) and 54904#2 (May 1999-April 2000). The speed data are presented as a cumulative frequency distribution in the main plot and as an aggregated percentage frequency in the inset graph.

The distribution of observed *Amperima* speeds is skewed to the left in both the short-term deployment (13078#47; April-July 1997) and the three longer-term deployments, most

notably during deployment 13370#8 (March 1998-February 1999). Given the relatively long frame interval (1.2-4.8 hrs) between observations in each of these deployments, it is difficult to interpret the distribution of speeds in ecological terms, rather it may mostly reflect the fact that the recorded speeds are minimum values. Considering only speeds determined from consecutive observations the following general statistics are derived for each deployment.

	Station			
	13078#47	13200#95	13370#8	54904#2
Mean speed cm hr^{-1}	1.2	5.1	2.5	4.4
Median speed cm hr^{-1}	1.2	4.8	1.7	4.1
Maximum speed cm hr^{-1}	2.7	11.9	18.2	10.1
Maximum speed body lengths hr^{-1}	0.8	3.4	5.8	4.2
Frequency of 'stationary' specimens (speed <0.1 body lengths hr^{-1})	3.5%	7.0%	20.8%	4.9%
Frequency of 'slow' specimens (speed <1.0 body lengths hr^{-1})	65.6%	84.0%	95.2%	57.1%

Table 4.6. Speeds and frequency of slow and stationary specimens of *Amperima rosea* on the PAP during deployments 13078#47, 13200#95, 13370#8, and 54904#2.

Mean speeds are within the same order for all four deployments and *A. rosea* would appear to be a fairly slow-moving holothurian. There were more 'slow' specimens observed during the short-term deployment that may more accurately observe the foraging behaviour of the species. Figure 4.17 examines temporal variations in the observed rates of movement of *Amperima rosea* during deployment 13078#47. The upper graph (4.17A) shows the running average (21 observations) of *A. rosea* speed in cm hr^{-1}. Speed is variable over time yet more or less comparable throughout the entire period of observation. Given the likely influence of body size on speed it is, however, necessary to also consider variation in body length when assessing any trend. The central graph (4.17B) shows the temporal variation in *A. rosea* body length presented in the same manner. Major fluctuations in mean body length are apparent and there is a tendency for larger mean values in the latter one third of the record.

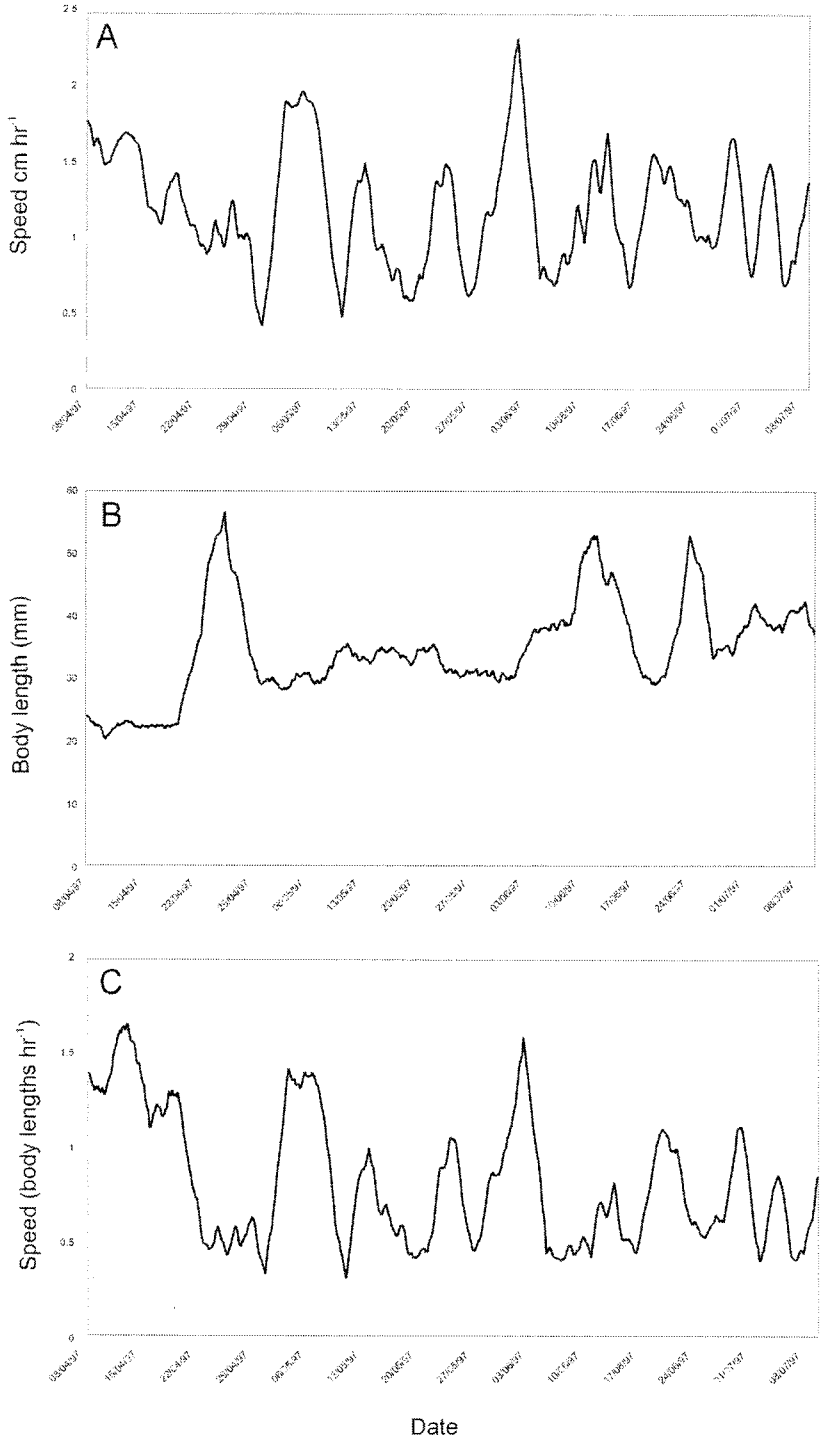

Figure 4.17. Temporal variation in the rates of motion of *Amperima rosea* on the PAP during deployment 13078#47. A, Plot of running mean (21 observations) of speed in units of cm hr^{-1}. B, Plot of running mean (21 observations) of body length. C, Plot of running mean (21 observations) of speed in units of body lengths hr^{-1}.

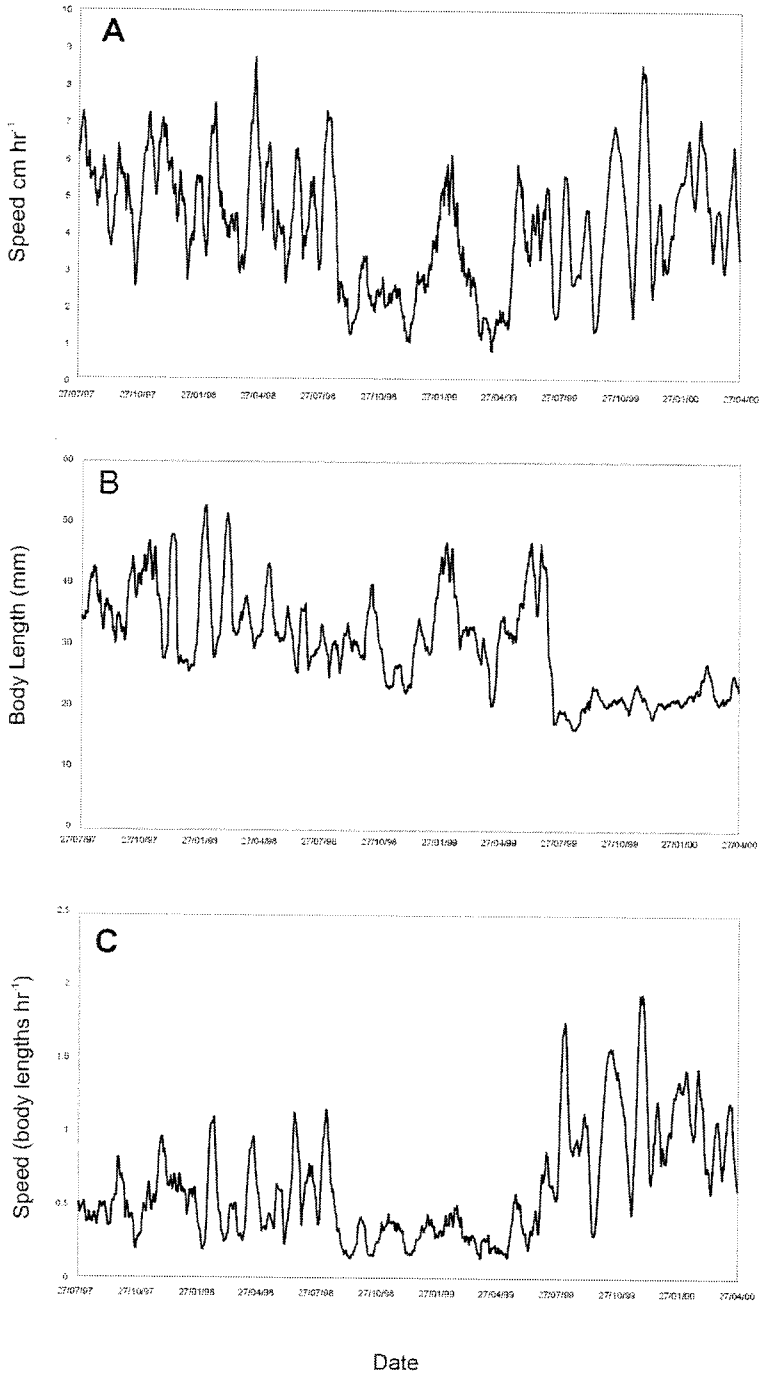

Figure 4.18. Temporal variation in the rates of motion of *Amperima rosea* on the PAP, during deployments 13200#95, 13370#8, and 54904#2 (July 1997-April 2000). A, Plot of running mean (21 observations) of speed in units of cm hr^{-1}. B, Plot of running mean (21 observations) of body length. C, Plot of running mean (21 observations) of speed in units of body lengths hr^{-1}.

When speed is presented in units of body length per hour (see 4.17C), there is an apparent decline in speed as the individual holothurians increase in size.

Figure 4.18 presents data for speed and body length, for *Amperima rosea* during deployments 13200#95, 13370#8 and 54904#2, in a similar manner to the previous, shorter, deployment. Again, speed in units of cm hr^{-1} (4.18A) was temporally variable yet more or less comparable across the entire period of the observation, with no obvious trend of increase or decrease in speed. The central graph (4.18B) shows the temporal variation in body length of *Amperima*. Individuals are considerably smaller during the latter part of the period of observation, a trend reflected in the plot of speed in units of body lengths hr^{-1} (4.18C). During the corresponding latter period of observation there was a slight increase in speed (body lengths hr^{-1}) as the specimens of *A. rosea* decrease in size.

Figure 4.19 shows the distribution of *Amperima rosea* body lengths for each of the four deployments. Small individuals (<40mm) dominate the sample from each deployment and the decrease in size during the last deployment (54904#2) is evident from these plots with almost 90% of individuals ≤ 40mm.

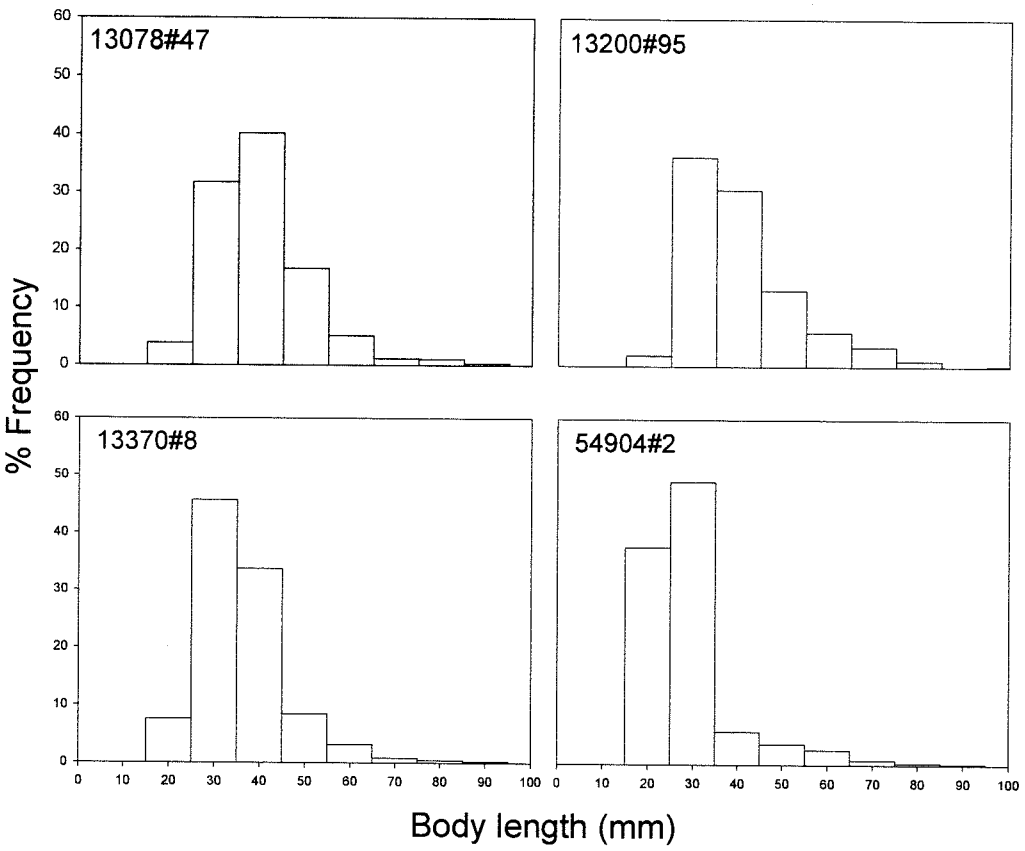

Figure 4.19. Distribution of body lengths (mm) for *Amperima rosea* observed during four deployments, 13078#47, 13200#95, 13370#8, and 54904#2, on the PAP.

4.3.4. Orientation and direction of movement.

The heading (in degrees) of each observation of *Amperima rosea* was assessed in order to determine whether or not there was any uniform direction of movement for groups of individuals. During the short-term deployment (13078#47) the majority of individuals appear to have been orientated in two main directions, approximately 180° apart. Throughout the second deployment (13200#95) the majority of individuals are now orientated in two general directions, northwards and southwards, again approximately 180° apart. During deployment 13370#8 there is a similar pattern with individuals observed moving in most directions, however, there are gain two main groupings moving in approximately opposite directions.

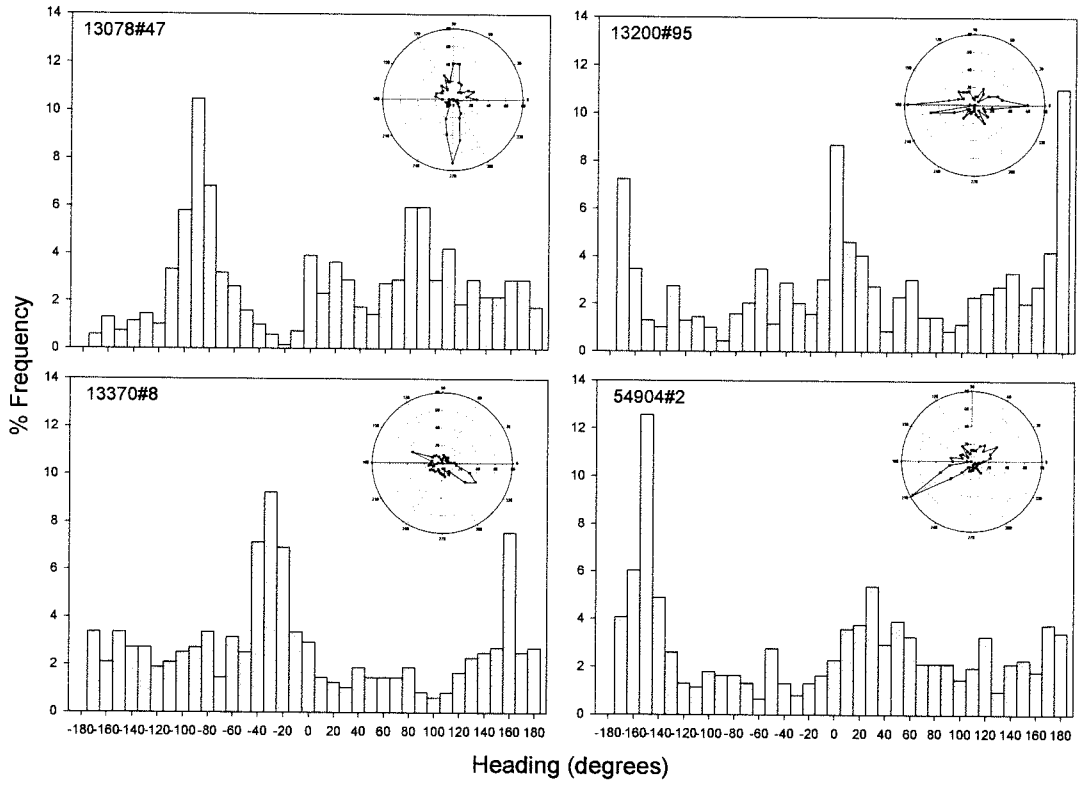

Figure 4.20. Distribution of the orientation (-180° to +180°) of *Amperima rosea*, during four deployments on the PAP. 13078#47 (April-July 1997), 13200#95 (July-March 1998), 13370#8 (March 1998-February 1999), and 54904#2 (May 1999-April 2000). Insert plots show frequency of observations for headings 0° to 360°.

The final deployment, 54904#2, also produces a distribution with two main groupings, although less pronounced in one direction (see insert plot). There is no consistent orientation of *A. rosea* and the results appear to be the result of a systematic error discussed in section 4.4.

4.4. Discussion.

During the period of the *Bathysnap* deployments (April 1997-April 2000) otter trawl estimates of megabenthos density (see Chapter 2 and Billett *et al.*, 2001) indicate that *Amperima rosea* was, by almost an order of magnitude, the most abundant taxon present. Subsequent analysis of ophiuroid abundance (also calculated from *Bathysnap* observations) by Bett *et al.* (2001) has shown that this taxon (principally *Ophiocten hastatum*) was in fact the most abundant fraction of the epibenthic megafauna during the BENGAL and post-BENGAL periods. There is, however, a mismatch between trawl and *Bathysnap* estimates of *A. rosea* density.

The *Bathysnap* observations confirm the dominance of *A. rosea*, yet estimates of density for both this species (6,457 ind. ha^{-1}) and the ophiuroids (53,539 ind. ha^{-1} (Bett *et al.*, 2001)) are considerably greater than those recorded from trawl samples. An assessment of 'fishing efficiency' (the proportion of the 'true' population caught by trawling) was made by comparing the abundances of *Amperima rosea* estimated from *Bathysnap* observations to those recorded from the trawl samples (Table 4.4). Estimated fishing efficiency for *A. rosea* ranges from 0.9-3.4%. However, it is considerably higher for the larger megabenthos. As expected, fishing efficiency appears to be broadly related to the mean taxon body size (fishing efficiency for ophiuroids calculated at 0.01% (Bett *et al., 2001*)) i.e. ophiuroids < *Amperima rosea* < 'other' holothurians. The fishing efficiency of the semi-balloon otter trawl (OTSB 14) for 'other' holothurians and larger megabenthos is surprisingly high. The fishing performance of the trawl, as operated during the BENGAL programme and all subsequent samples taken since, is greatly improved by monitoring in real-time the otter door angle and bottom contact using an acoustic telemetry system; this enables a far more accurate assessment of the area of seafloor fished than would be possible with a 'blindly' fished net. Although it is not usually regarded as a quantitative sampler, the estimated fishing efficiency of the OTSB for larger megabenthos (mean 68% (Bett *et al., 2001*)) exceeds that of the USNEL-type box corer (c. 50% see Bett *et al.*, 1994; Bett, 2001), an instrument widely regarded as being quantitative (Hessler and Jumars, 1974). The OTSB14 and box corer both suffer from a common problem, the bow-wave effect. Direct video observations of the response of megabenthos, on the PAP, to the approach of an epibenthic sledge showed that

smaller megabenthos (specifically *Amperima rosea*) tend to be either 'blown aside' or even thrown into suspension ahead of the sledge (pers. obs.). The bow wave appears to precede the sledge by at least a metre. The low (2.5%) and extremely low (0.01%) fishing efficiency of the OTSB 14 for *A. rosea* and ophiuroids respectively, probably reflects both the bow-wave effect and the direct loss of these small organisms through the meshes of the net (body mesh 44-37mm stretch, cod-end mesh 13mm stretch).

Significant temporal variations were recorded in the density of *A. rosea* during all four deployments. There were no obvious trends or patterns in these variations (see Figures 4.7, 4.8, 4.10, and 4.11) and they may instead reflect the aggregated spatial distribution of this species. Dense aggregations, or 'herds', of small, abundant holothurians have been observed previously (*Scotoplanes globosa*, (Barnham *et al.*, 1967); *Kolga hyalina*, (Billett and Hansen, 1982; see figures 6.2 and 8.8 in Gage and Tyler, 1991). The TTLQV (see Figures 4.7 and 4.10) assessment of temporal variations in density did not detect any particular size or spacing in the likely aggregations of *A. rosea*. However, there is no reason to believe that such aggregations or herds should have a common size or a regular temporal/spatial spacing. Given the probable importance of *A. rosea* to the ecology of the PAP, further study of its spatial distribution patterns would be particularly valuable. We know from OTSB14 trawl samples that *A. rosea* is found across the wider PAP basin, in variable densities (see Chapter 2 and Billett *et al.*, 2001). However, the potential of an aggregated distribution of large numbers of this species is hard to discern from these data. It is clear that using towed samplers is not the most effective method for such studies, and given the possible bow-wave effect, bottom towed cameras may also prove problematic. Off-bottom towed cameras, such as the SOC WASP system, may prove more effective. However, problems encountered in resolving small, amorphous organisms such as *A. rosea* from 'high' altitude photographs and video needs to be addressed.

Bett *et al.* (2001) compared these data on *Amperima* density to those calculated from the previous analysis of films from the pre-BENGAL period (1991-1994) (Narayanaswamy, unpublished data). They observed a dramatic longer-term change in the abundance of both *A. rosea* and the ophiuroids between the two periods of observation (1991-1994 versus 1997-

2000). The observed changes are also notable for the fact that the rest of the megabenthos also appeared to have increased in abundance. Data from trawl samples (see Chapter 2 and Billett *et al.*, 2001) show that a number of megabenthic taxa from the PAP had significantly increased in abundance over this period, including one of the largest species, the holothurian *Psychropotes longicauda*. This general increase in the abundance of the megabenthos, which spans a range of taxonomic groups would seem to suggest an increase in food supply. However, the predicted and observed fluxes of organic matter to the BENGAL locality did not increase between 1991-1994 and 1997-2000, remaining at 1.5 and 1.0g org. C. $m^{-2}yr^{-1}$ at 3000m (data derived from Lampitt *et al.*, 2001). Despite the significant change in the megabenthos community of the BENGAL site there is no obvious parallel change in the flux of organic matter to the seafloor. The change has influenced various components of the biological community, and seems to be detectable over a wide area of the PAP (to a 100km-scale at least; see Chapter 2). It is possible that despite the lack of any obvious trend in flux to the seafloor, some variation in timing, or quality or nutritional value of the organic matter supply may have been responsible for the observed community changes. Support for this suggestion comes from studies of the nutrition of the two 'bloom' species, *Ophiocten hastatum* (the dominant ophiuroid) and *Amperima rosea* (to be discussed further in Chapter 5).

Time-lapse photography has previously been used to study the rates of motion of abyssal holothurians both at the BENGAL site (Smith *et al.*, 1997; Narayanaswamy, unpublished data) and at a site in the eastern North Pacific, at 4100m water depth off the Californian coast (Smith *et al.*, 1993; Kaufmann and Smith, 1997). Table 4.7 compares the various holothurian speeds estimated during these studies with those calculated in the present study.

Species	Speed	Porcupine Abyssal Plain
Amperima rosea	3.3 cm hr^{-1}	This study
Pseudostichopus sp.	5.7 cm hr^{-1}	This study
Pseudostichopus villosus	1.9 cm hr^{-1}	This study
Oneirophanta mutabilis	6.3 cm hr^{-1}	Narayanaswamy (unpubl.)
Mesothuria candelabri	4.1 cm hr^{-1}	Narayanaswamy (unpubl.)
Psychropotes longicauda	2.6 cm hr^{-1}	Narayanaswamy (unpubl.)
Paroriza prouhoi	1.9 cm hr^{-1}	Narayanaswamy (unpubl.)
Oneirophanta mutabilis	128.9 cm hr^{-1}	Smith, Matthiopoulos and Priede (1997)
Laetmagone violacea	102.6 cm hr^{-1}	Smith, Matthiopoulos and Priede (1997)
		Eastern North Pacific
Oneirophanta mutabilis	84.8, 64.6 cm hr^{-1}	Smith, Kaufmann and Wakefield (1993), Kaufmann and Smith (1997)
Abyssocucumis abyssorum	17.8, 12.8 cm hr^{-1}	Smith, Kaufmann and Wakefield (1993), Kaufmann and Smith (1997)
Peniagone vitrea	8.1, 10.1 cm hr^{-1}	Smith, Kaufmann and Wakefield (1993), Kaufmann and Smith (1997)
Elpidia minutissima	14.8, 18.0 cm hr^{-1}	Smith, Kaufmann and Wakefield (1993), Kaufmann and Smith (1997)
Scotoplanes globosa	12.5 cm hr^{-1}	Kaufmann and Smith, (1997)
Synallactes profundi	16.7 cm hr^{-1}	Kaufmann and Smith, (1997)

Table 4.7. Holothurian speeds as estimated from time-lapse photography in studies at the Porcupine Abyssal Plain and the Eastern North Pacific.

The speeds of motion estimated in the present study are among the lowest recorded. All of the rates estimated from *Bathysnap* studies on the Porcupine Abyssal Plain (this study and that of Narayanaswamy (unpublished data)) are lower than those recorded by other authors. Aside from the likely true differences in the rates of motion in different species it is probable that some of the differences are of a systematic nature. The results presented by Smith *et al.* (1997) are based on photographs taken at one-minute intervals and those of Smith *et al.* (1993) and Kaufmann and Smith (1997) at one hour intervals, whereas all of the *Bathysnap* studies employed longer frame intervals. All speed estimates based on time-lapse photography are likely to represent minimum values, the longer the frame interval the greater the potential underestimate of speed. This problem is exemplified by the speeds given for the large holothurian *Oneirophanta mutabilis*:

At one minute intervals	129 cm hr-1	Smith, Matthiopoulos and Priede (1997)
At one hour intervals	74.5 cm hr-1	mean of Smith *et al.*, (1993) and Kaufmann and Smith (1997)
At eight hour intervals	6.3 cm hr-1	Narayanaswamy (unpubl.)

Consequently it is difficult to make reliable comparisons between these various studies. However, it is during the shorter deployment (13078#47) with a frame interval of only 1.2 hrs that the lowest average *Amperima* speeds (range 0.005-2.65cm hr^{-1}) were recorded. It is possible that these low speeds, observed during deployment 13078#47, represent patterns of locomotion associated with an increase in feeding activity of *A. rosea*. The movement patterns of *Amperima rosea* were examined by plotting the tracklines of individuals observed on several consecutive frames. The tracks of two individual animals from deployment 13078#47 (lowest mean speed) and deployment 13200#95 (greatest mean speed) were plotted (Figure 4.21). It appears that individual *A. rosea* observed during deployment 13078#47 exhibit similar movement patterns with short bursts of movement followed by periods of small movements concentrated in a small area. Kaufmann and Smith (1997) used time-lapse photography to plot the movement patterns of deep-sea holothurians from the abyssal Northeast Pacific, including *Elpidia minutissima*, a similarly-sized relative of *Amperima*. They categorised different movement patterns into three groups, two of which were commonly observed for *E. minutissima*. These two patterns, called the 'loop' and the 'run and mill', are very similar to those patterns observed for *A. rosea*. The frame interval (1hr) used by Kaufmann and Smith (1997) was similar to that used for *Bathysnap* deployment 13078#47 (1.2hrs). However, the patterns recorded for individuals from deployment 13200#95 are different with longer straight-line movements and less re-tracking of the same area of seafloor. It should be noted that the frame interval of later deployments was longer (4.8hrs), therefore the patterns obtained for *Amperima* are likely to be less accurate as a result of poorer resolution of movement between consecutive frames, yet it is still a possibility that these differences in locomotion patterns may explain the lower speeds recorded during the supposedly 'more accurate' short-frame interval deployment.

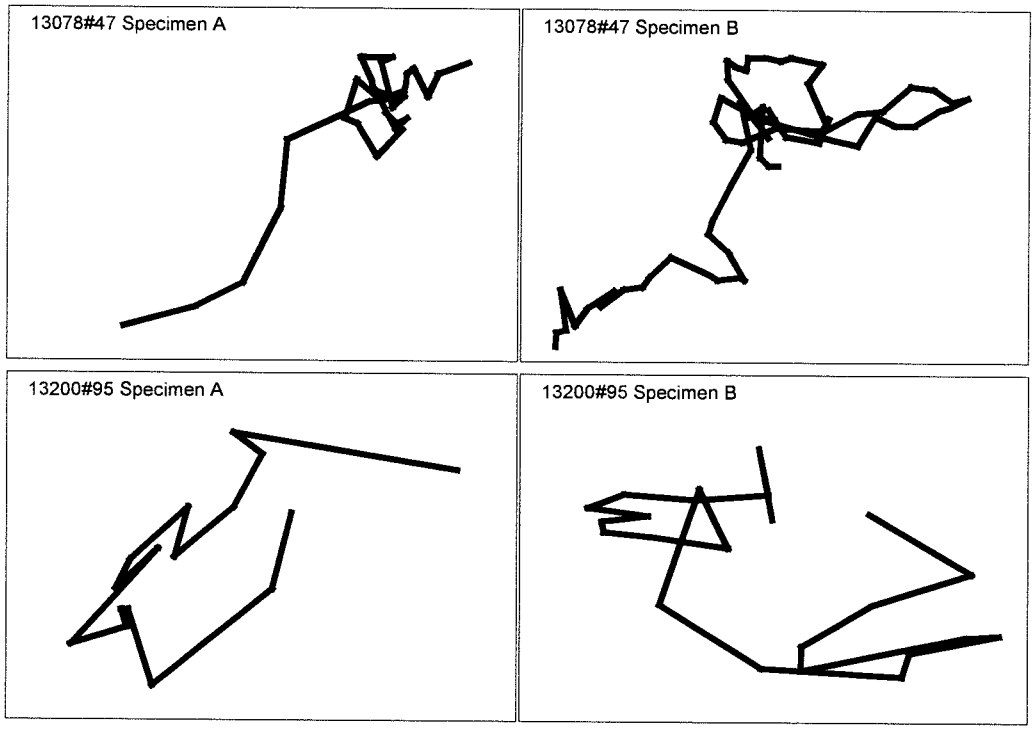

Figure 4.21. Patterns of locomotion, based on consecutive observations, for four individual specimens of *Amperima rosea* on the PAP. 13078#47 A, 31 observations over 1.5 days; 13078#47 B, 64 observations over 3.2 days; 13200#95 A, 23 observations over 4.6 days; 13200#95 B, 28 observations over 5.6 days.

In the case of *Amperima rosea* it is clear that it is a slow-moving species, with the vast majority of *Bathysnap* observations indicating a speed of less than one body length per hour (see Table 4.6). Mean rates of motion in *A. rosea* varied substantially during the period of *Bathysnap* observations. However, there was no obvious trend or pattern to these variations that could be ascribed to either seasonal or inter-annual change.

The analysis of the directional heading of *Amperima* specimens indicated that, for each deployment, the majority of individuals were moving in either in one direction or its reciprocal opposite direction (approximately 180° apart; see Figure 4.20). The directions varied among deployments even though the patterns remained similar. It would be interesting to speculate on a possible link between these patterns of orientation and the direction of residual currents on the PAP. Records of preferred orientation into a prevailing current have been made for other holothurian species, mostly elpidiids, including *Kolga hyalina* (Porcupine Seabight, Billett and Hansen, (1982)), *Ellipinion delagei* (Bahamas, Pawson, (1982)) and *Scotoplanes* sp. (San Diego Trough, Barnham *et al.*, (1967)). The direction and velocity of near-bottom currents on the PAP have been recorded during several studies, including the BENGAL programme. Based on long-term current measurements of 1996-97, the current regime at the BENGAL site is characterised by semi-diurnal oscillations generated by the internal tide (Vangriesheim *et al.*, 2001). The semi-diurnal tidal oscillations were orientated north-south (based on data from a 15mab current meter). The residual current was very low and mainly northeastwards or northwards, but there was a period of westerly residual currents in early 1997. Current speeds recorded at 0.5mab (Vangriesheim *et al.*, 2001) and 3mab (Lampitt *et al.*, 2001) showed low, but variable speeds. Lampitt *et al.* (2001) recorded higher current velocities (15-20cm s^{-1}) during late 1997 and early 1998 but in general current speeds rarely exceeded 10cm s^{-1}, with Vangriesheim *et al.* (2001) recording a mean velocity of 3.5cm s^{-1} at 0.5mab. Records of current direction and velocity from a current meter attached to the *Bathysnap* mooring have also been recorded at the PAP site (Rice *et al.*, 1994). During the one-month deployment, current velocity was again generally low but variable (range 1.8-10cm s^{-1}). The direction was also shown to vary substantially over this short period from northwest to southeast over the duration of the deployment (see Figure 5 in Rice *et al.*, (1994)).

Based on the observations from the current *Bathysnap* deployments it would appear that the majority of *Amperima* individuals are moving in the plane, or perpendicular to the plane, of the residual northward/northeastward residual current. However, as with all measurements derived from the analysis of time-lapse photographs it is important to consider sampling induced bias in the results. Certainly it appears interesting that in each deployment the

majority of individuals are moving in one general direction or in the opposite reciprocal direction. However, these headings differ among deployments and when we consider the angle in which the camera is pointing it is clear that in each case the majority of animals are moving across the field of view at approximately 90° to the camera. It is therefore possible that animals moving in this plane are more easily resolved than those moving in the same plane as the camera view.

When considering the implications of the sudden and significant increase in abundance of *Amperima rosea* (and other taxa i.e. ophiuroids (Bett *et al.*, 2001)) it is important to take into account their impact on the benthic community in terms of the removal, 'repackaging', and redistribution of organic matter and disturbance of the sediment, which may be important in the context of structuring macro- and meiofaunal communities. In this study holothurian tracking is used as a measure of the rate of disturbance of superficial sediments. Given the overwhelming dominance of holothurians at the BENGAL study site this measure is likely to be a good proxy of the overall rate of sediment disturbance at the site. Over the four periods of observation tracking by *A. rosea* ranged from 29.8 to 125.2 $cm^2day^{-1}m^{-2}$, equivalent to tracking 100% of the seafloor in 2.6 to 11.0 months. There are few studies with which to set these figures in context. For their abyssal site in the eastern North Pacific, Kaufmann and Smith (1997) calculated that the total mobile megabenthos (>95% holothurians) tracked 180% of the seafloor annually, equivalent to a tracking rate of 49.3 $cm^2day^{-1}m^{-2}$. The potential of *Amperima rosea* to track and remove organic matter from the seafloor is immediately apparent when they alone could be responsible for tracking a similar area in less than half the time (or >400% of the seafloor annually).

Significant temporal variations in tracking occurred at the PAP site during the period of *Bathysnap* observations. However, no obvious seasonal or inter-annual variation is apparent in the data. Studies of the megabenthos at the BENGAL site suggest that longer-term change in the megabenthos populations may be more significant than any of the seasonal or inter-annual changes apparent during the period of study itself. Bett *et al.*, (2001) calculated tracking rates for holothurians during the pre-BENGAL *Bathysnap* deployments (1991-1994). During this period, unlike the present four deployments, there was evidence of

phytodetrital aggregates on the seafloor. Figure 4.22 shows the results of the analysis of phytodetritus coverage of the seafloor, as estimated from Bathysnap observations and multi-core samples.

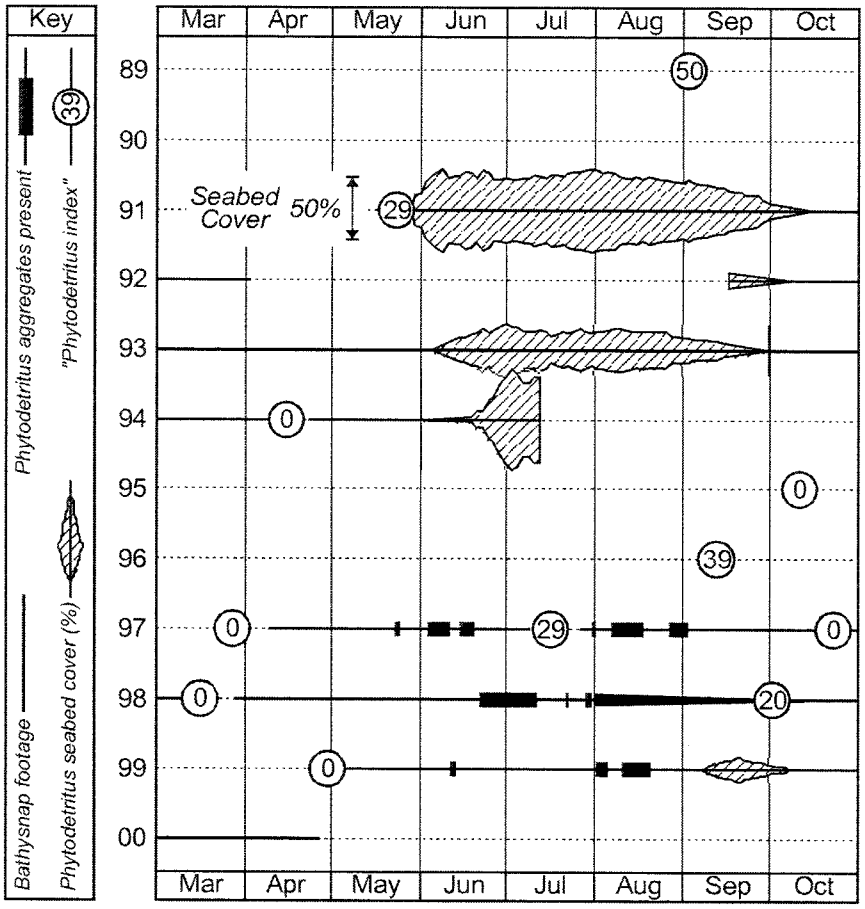

Figure 4.22. Calendar presentation of phytodetritus observations at the seafloor on the Porcupine Abyssal Plain 1989-2000 (see key for details of data presented)(taken from Bett *et al.*, (2001)).

The timing of the occurrence of phytodetritus on the seafloor of the PAP is generally consistent across the years studied, initially arriving in late May or early June, increasing to a maximum before decreasing through September and finally disappearing from view in the early part of October. The maximum extent of seafloor coverage was 96% in July 1994, whereas during the summer months of 1997-1999, phytodetritus was normally 'absent'. The 'phytodetritus index' scores (i.e. occurrence of phytodetritus on recovered multi-core

samples) are broadly in agreement with the *Bathysnap* observations. As the amount of phytodetritus visible on the seafloor deceased substantially between the pre-BENGAL and BENGAL periods, the megabenthos tracking rates (as estimated by Bett *et al.*, (2001)) increased. During the earlier deployments (1991-1994) tracking averaged 61% of the seafloor (range 27-118%) whereas during the later deployments (1997-2000) it averaged 900% (range 533-1,331%). This is equivalent to 100% of the seafloor being tracked in about 29 months during 1991-1994, and the same area in only 1.6 months during 1997-2000. There appears to be an inverse relationship between megabenthos abundance and the occurrence and extent of phytodetritus. The correlation between megabenthic tracking and maximum seafloor phytodetritus cover is also statistically significant (Bett *et al.*, 2001).

Lampitt *et al.* (2001) assessed surface ocean primary production and subsequent water column flux over the PAP for the period 1989-1999. They found that neither parameter exhibited a systematic trend over that period. Therefore it would appear that factors operating in the pelagic ecosystem do not appear to have been responsible for the non-appearance of mass deposits of phytodetritus during the four *Bathysnap* deployments studied here. It is more likely that benthic processes, i.e. the feeding activity of large number of *Amperima rosea* and ophiuroids, were responsible for the apparent absence of phytodetritus. From the flux data of Lampitt *et al.* (2001) and gut content analysis of the major 'bloom' species (Chapter 5; Iken *et al.*, 2001) we know that phytodetritus was reaching the abyssal seafloor and being consumed by the megabenthos. Bett *et al.* (2001) suggest that the vastly increased megabenthos tracking rates estimated for the 1997-2000 period, relative to the 1991-1994 period, could have been responsible for removing (by consumption, disaggregation, burial etc.) most, if not all, of the incoming phytodetrital flux.

The potential ability of just a few species of surface deposit feeding megabenthos to remove, repackage and redistribute the vast bulk of the incoming flux of phytodetritus may well have a significant impact on energy flow through the rest of the benthic community. Bioturbation and impoverishment of the surficial sediment layers has been shown to affect the vertical distribution of abyssal meiofauna and macrofauna at the BENGAL site (Galéron *et al.*, 2001). Populations of foraminifera and opheliid polychaetes that feed on phytodetritus have

been shown to respond fairly rapidly to pulses of organic matter (Gooday and Lambshead, 1989; Gooday and Turley, 1990; Galéron *et al.*, 2001; Vanreusel *et al.*, 2001), if this pulse signal is removed by increased feeding activity of the megabenthos, then the population structure of these meiofaunal organisms may be significantly affected. However, given the number of assumptions made during these analyses, it may be safer to note that the megabenthos (for the 1997-2000 period) track 2.5% of the seafloor per day and so may have been consuming the incoming flux at an equivalent rate. This level of tracking does nevertheless cover 100% of the seafloor in six weeks, which may be more significant in terms of disturbances and bioturbation of the surficial sediment layer. Recent studies by Miller *et al.* (2000) have shown that some deep-sea deposit feeding holothurians can selectively ingest fresh phytodetrital material. Using ^{234}Th as a tracer of fresh material they estimated that just three of the dominant megabenthic species were capable of consuming some 40-50% of the daily flux of ^{234}Th. Results have shown that *Amperima rosea* is able to selectively ingest not only fresh phytodetritus, but certain fractions of that phytodetritus (see Chapter 5). Thus the potential of the 1997-2000 'bloom' species on the PAP, *Amperima rosea* and *Ophiocten hastatum*, to consume and repackage significant quantities of the incoming flux should not be discounted. Indeed, Ginger *et al.* (2001) have indicated that, at 1997-2000 population levels, *A. rosea* alone may consume the total incoming phytosterols flux, depleting the phytosterols content of the surficial sediments.

Chapter Five – Diet and selective feeding in deep-sea holothurians

5.1. Introduction.

5.1.1. Deposit feeding.

In deep-ocean basins, as in shallow-water soft-bottom communities, deposit feeding is common, becoming the dominant mode of feeding as water depth increases (Lampitt *et al.*, 1986). A variety of annelids, molluscs, echinoderms and crustaceans obtain their nourishment by ingesting accumulated detritus and digesting its organic material. Some deposit feeders indiscriminately ingest any available sediment, whilst others will preferentially select organically-rich particles for consumption.

Deep-sea sediments comprise a complex mixture dominated by inert mineral grains of both terrigenous and pelagic origin. Unlike shallow-water sediments they tend to be poor in terms of organic content, both in dissolved and particulate organic matter (Gage and Tyler, 1991). Much of the particulate organic matter (POM) reaching the seafloor has often already been recycled by mid-water animals, and is likely to be highly refractory. Despite what would appear to be an exceptionally poor food source, deep-sea deposit feeders flourish, albeit at lower densities than their shallow-water counterparts.

For many years it was thought that all particulate material would sink very slowly to the deep-sea floor, and that food for deposit feeders resulted solely from a slow and continuous 'rain' of residual, refractory particles. However, McCave (1975) suggested that the sinking of particles could be more rapid owing to the formation of aggregates. It has now been shown that the supply of POM to the seafloor may be pulsed into large falls of labile material sinking rapidly from the surface layers (Deuser, 1986; Alldredge and Silver, 1988). The use of sediment traps (Honjo *et al.*, 1980; Honjo and Doherty, 1988; Lampitt *et al.*, 2000) and deep-sea bed photography (Billett *et al.*, 1983; Lampitt and Burnham, 1983) has shown how this labile material can reach abyssal depths of 4km and greater; and in some areas of the world it appears as a seasonally predictable pulse.

It is now widely accepted that approximately 1-3% of surface organic primary production reaches the abyssal seabed by a variety of pathways (see Chapter 1). Cole *et al.* (1987) suggest that 50 to 85% of the organic carbon reaching the deep-sea floor is remineralised within one year. The remainder is believed to have a residence time of between 15 and

150 years in the surficial sediments compared to 0.3 to 3.0 years in the water column (Gage and Tyler, 1991).

Most abyssal taxa are deposit feeders, which can be subdivided into subsurface and surface-deposit feeders. All deposit feeders bulk process sediment through the alimentary tract and evidence from shallow-water studies suggests that sediment feeders can process an amount of sediment equivalent to its own body weight at any one time (Lopez and Levinton, 1987). Data on weight specific feeding rates amongst deep-sea species are not available, although for the holothurians at least, gut residence times are thought to be longer than those of their shallow-water relatives (Sibuet *et al.*, 1982). The distance between faeces ejected by large, deep-sea epifaunal holothurians, and the absence of any tracks or sediment removal led Heezen and Hollister (1971) to suggest that only the very surface film of sediment is ingested by such deposit feeders. This is supported by the analysis of gut contents (Sibuet *et al.*, 1984; Billett *et al.*, 1988). Similarly the spoke-like feeding traces made by echiuran worms (Bett and Rice, 1993), and the morphology of the feeding palps of polychaetes, suggests that only the most recently sedimented material is taken by the more sedentary deposit feeders. As in shallow water, the foraging patterns of many deep-sea deposit feeders seem to reflect a dependence on sediment transport (Jumars *et al.*, 1990). Horizontal transport may particularly favour the utilisation of newly deposited detrital material. Selection of this material by burrowing macrofaunal deposit feeders may be achieved by means of shallow feeding pits rather than by the extension of feeding tentacles or palps. These 'floc-pits' effectively capture detrital material in bedload transport and enhance the rates of local deposition of suspended particles. In those areas where the flow regime is much quieter, large epifaunal deposit feeders, such as holothurians, may be favoured. The motility of holothurians allows them to feed on the most nutritious surface layers of the sediment, while their size provides a large gut capacity and deters predation. The distributions and amounts of chloropigments in the guts of bathyal holothurians and in the surrounding sediment suggest that holothurians are effective in feeding on patches of new detritus (Billett *et al.*, 1988). Inter-species variability in pigment profiles may also indicate that holothurians feed selectively on the detrital material accumulating on the deep seafloor.

5.1.2 Holothurian feeding strategies

Holothurians are particulate feeders ranging in size from 1.5mm (*Parvotrochus belyaevi*) to >3m (*Synapta maculata*) and occur from the high intertidal zone (*Polycheira rufescens*) to the greatest ocean depths (*Elpidia glacialis*).

Holothurians capture suspended and deposited food particles and transfer them to the pharynx by means of circumoral tentacles. During feeding, tentacles are expanded into the water column or onto or into the substratum and, when loaded with particles, contract, bend into the mouth and pass food into the pharynx. The number, size and arrangement of holothurian tentacles is different for various groups. Holothurian tentacles can be grouped into five types: dendritic, peltate, pinnate, digitate and peltodendritic (Massin, 1982a). All dendrochirotid holothurian possess dendritic tentacles and all molpadiids possess digitate tentacles. The remaining orders include species with different tentacle types. The majority of Aspidochirotida have peltate tentacles; whilst a few, including some deep-sea species, have digitate tentacles (Massin, 1982a). Species in the order Apodida possess either a pinnate or, like the Dactylochirotida, a digitate tentacle structure. The Elasipodida, which are an exclusively deep-sea order, have peltate, digitate or dendritic tentacles (Hansen, 1975). The high degree of adaptive radiation in tentacular structure, which facilitates habitat and feeding specialisation of different species, has been known for a long time (Theel, 1882). In deep-sea species, mode of feeding may be inferred from the tentacle structure (Hansen, 1975; Roberts *et al.*, 1991)(Table 5.1).

Species	Bathymetric distribution	Tentacle structure type	Feeding
Pseudostichopus villosus	2850-4845	Pelto-digitate	Raker
Oneirophanta mutabilis	2850-4845	Digitate	Picker
Psychropotes longicauda	3310-4845	Peltate	Sweeper
Amperima rosea	4012-4845	Pelto-digitate	Picker
Molpadia blakei	2470-4845	Digitate	Infaunal

Table 5.1. Tentacle structure and inferred feeding type of 5 major holothurian species from the PAP. Data adapted from Roberts *et al.* (2000).

Holothurians, like other deposit feeding taxa, can be divided into surface and subsurface feeders. In addition there are also species that can be categorised as 'funnel feeders', which create funnel-shaped depressions in soft sediments and feed on material slumping into the funnel (Rhoads and Young, 1971). A number of suspension feeding holothurians may also shift opportunistically to deposit-feeding, both in shallow-water (Fankboner, 1981) and deep-sea (Billett, 1991) ecosystems. Pelagic holothurians are another group in which some species feed on suspended particles in the water column. However, analyses of the stomach contents of some pelagic holothurians show that only specimens caught close to the seabed have material in their guts and that this material comes from the sediment surface (Billett *et al.*, 1985). In addition, two of these species, *Enypniastes eximia* and *Peniagone diaphana*, have been seen feeding on the sediment surface (Ohta, 1985; Billett, 1986).

Most surface deposit-feeding holothurians feed by ingesting large quantities of particulate matter of varying organic content and typically show greater diversity at shallow and abyssal locations than at intermediate depths (Billett, 1991). Deep-sea holothurians, particularly the elasipodids, exhibit a remarkable variety of ecological forms falling into three main groups of truly pelagic, benthopelagic and benthic. Billett (1991) recognises five different feeding groups within the deep-sea holothurians: mobile superficial-sediment feeders, sedentary superficial-sediment feeders, pelagic superficial-sediment feeders, infaunal species and opportunistic species. Of these, >70% of species are mobile superficial-sediment feeders. Burrowing forms and those partly submerged in the sediment are not represented in the elasipodids, but are common in other orders, e.g. *Molpadia blakei* (Young *et al.*, 1985; Tyler *et al.*, 1987). Different feeding strategies and the activity of deep-sea holothurians are likely to depend on various environmental factors; the most important is likely to be the well-documented pulses of organic material to the deep seafloor. When phytodetrital material first arrives on the seafloor in the Northeast Atlantic it is more or less evenly distributed and its composition matches that of the foregut contents of holothurians (Billett *et al.*, 1988). Although newly fallen detritus is redistributed within hours, it becomes concentrated in depressions and grooves (Santos *et al.*, 1994), and the epibenthic holothurians are effective at locating these patches of new detritus (Billett *et al.*, 1988). These patches of detrital material may retain their integrity over several months and resuspension of the material was thought to be rare, at least in the northeast Atlantic (Santos *et al.*, 1994). However, increased near-bottom flux on the

PAP has been attributed to resuspension events with a significant correlation between current speed and nephelometry (Vangriesheim *et al.*, 2001). Near-bottom resuspension was found to increase after the large peaks of particle flux, some of which may be attributable to the feeding activity and sediment reworking by the benthic and benthopelagic megafauna.

Various theories have been put forward to explain how abyssal holothurians subsist on temporally variable but predominantly refractory food resources. These include: selective ingestion of organic rich components of the sediment (Khripounoff and Sibuet, 1980; Billett *et al.*, 1988; Billett, 1991) and/or the utilisation of either bacteria transient within the gut lumen (Deming and Colwell, 1982; Billett, 1991) or commensal gut bacteria that are associated with the digestive epithelium (Feral and Massin, 1982). Relationships between food quality, gut residence time and digestion reaction kinetics in deposit feeders, including holothurians, have been modelled extensively (Penry and Jumars, 1987; Jumars, 2000a; 2000b). Models based on the chemical reactions and the physical and energetic constraints of digestion include;

1. Batch reactors (BRs), in which food and enzymes (reagents) are mixed then allowed to react.
2. Plug flow reactors (PFRs), in which there is a continuous, orderly flow of reagents with no axial mixing along the tube (gut).
3. Continuous flow stirred tank reactors (CSTRs), in which reagents undergo continuous thorough mixing.

Gut morphologies have also been used to infer digestive strategies (Moore *et al.*, 1995).
It has long been a common belief that biological processes in the deep sea proceed at slower rates than in shallow water. Although this may be true for some pelagic groups, such as fishes and crustaceans, benthic groups show less or no decline in metabolic rates when compared with shallow-water species (Childress, 1995). Locomotory activity of deep-sea holothurians is comparable to shallow-water species, although it should be noted that calculated speeds for deep-sea species are often made from time-lapse photographic surveys (Chapter 4; Smith *et al.*, 1993; Smith *et al.*, 1997; Bett *et al.*, 2001) and accurate comparisons of speeds are difficult as a result of varying frame intervals, the length of which are often determined by the duration of deployment. It is possible that inter-species differences in the locomotory activities of deep-sea holothurians may be related to different feeding behaviours. It has been suggested that the arrival of food in pulses,

patchiness of food and the dominance of vertical over the horizontal transport of food to megafaunal deposit feeders in the deep sea favours highly mobile megafauna capable of rapid processing of ingested material. *Oneirophanta mutabilis* is one such species that may be successful under these conditions (see Khrinpounoff and Sibuet, 1980; Smith *et al.*, 1994; Moore *et al.*, 1995; Lauerman *et al.*, 1997; Lauerman and Kaufmann, 1998; Witbaard *et al.*, 2001).

5.1.3. Selective feeding by deep-sea holothurians

It has been suggested that some deep-sea epibenthic holothurians are able to locate patches enriched with organic matter (Billett *et al.*, 1988). This is certainly the case in laboratory and field studies of sediment patch selectivity by shallow-water tropical holothurians (Uthicke and Karez, 1999).

Deep-sea elasipodid holothurians of the Family Elpidiidae have been observed around baited traps and fish remains (Massin, 1982a). However, it is more likely that the holothurians are attracted to bacteria developing on the remains or it may be a serendipitous observation. However, the swimming holothurian *Peniagone* sp. has been observed feeding on the bacterial mats associated with the clam *Calyptogena phaseoliformis* at 5640m depth in the Japan Trench (Juniper and Sibuet, 1987). Another elpidiid, *Scotoplanes* sp., has been recorded in great numbers in the Peruvian subduction zone at ~ 4000m depth, also in association with *Calyptogena* sp. and bacterial mats (Olu *et al.*, 1996). Bacteria and their products are believed to be an important source of organic matter in the deep ocean (Richardson and Young, 1987; Jumars *et al.*, 1990). Estimates of bacterial biomass in deep-sea sediments give values up to 7.6 mg C m^{-2} (for the abyssal northeast Atlantic) (Pfannkuche, 1992), which are comparable to those, reported from shallow waters (Lochte, 1992). Bacterial biomass varies significantly between areas with different trophic conditions and strongly depends on surface primary production (Patching and Eardly, 1997). A substantial increase in microbial activity is caused by the seasonal input of organic matter, such as phytodetritus (Lochte and Turley, 1988; Turley and Lochte, 1990; Pfannkuche, 1992) and sinking zooplankton faecal pellets (Pfannkuche and Lochte, 1993). It is unlikely that bacteria are a major source of carbon for deposit feeders but they may be important in supplying nitrogen. The role of bacteria as gut symbionts in deposit feeders is probably more important than their potential nutritional

value. Gut bacteria may mediate the breakdown of refractory substances ingested by holothurians. Most marine deposits are poor in organic matter and a high proportion of this organic matter is refractory (Rowe *et al.*, 1991). The transformation of ingested detrital material by gut-associated microflora in deep-sea holothurians has been suggested by many authors (reviewed by Billett, 1991). The subsequent evidence for bacterial numbers remaining elevated along the guts of a number of deep-sea deposit-feeding holothurians (Moore *et al.*, 1995) and a contrasting decline of bacterial numbers in the guts of shallow-water holothurians (Manship, 1995) lends weight to this suggestion.

Various authors (reviewed by Billett, 1991) have described how deep-sea holothurians can select a wide range of items including, detritus, foraminiferans and faecal pellets. Faecal material has an important role in the nutrition of deep-sea deposit feeders in general and holothurians often use faecal pellets as a food source. Selectivity towards faecal pellets has been recorded for several species of holothurian, especially elasipodids (Khripounoff and Sibuet, 1980).

When the organic content of sediments from holothurian guts are compared to that of the surrounding sediment there is usually an increase in total organic matter by a factor of 2 to 8, and this is referred to as the "selection coefficient" (Billett *et al.*, 1988). When analysing the gut contents of holothurians it is important to distinguish between the organic matter taken from the surrounding sediment and that attributed to the holothurian's own digestive secretions. Massin (1980) estimated that only 4 to 8% of the increase in organic matter within the oesophagus of the shallow-water species *Holothuria tubulosa* could be attributed to its own pharyngeal secretions. In addition, data from analyses of the gut contents of two deep-sea species, *Deima validum* and *Pseudostichopus atlanticus* (Sibuet *et al.*, 1982) show that, at most, the holothurians provide only 6 to 12% of the organic matter ingested, assuming that all the holothurians secretions are contained in the water soluble protein and carbohydrate fractions. To assess accurately feeding selectively in holothurians, it is necessary to analyse a marker compound that is associated with the holothurian's detrital food but cannot be synthesised by the holothurian itself. Chlorophyll and its breakdown products, referred to as the chloropigments, and certain carotenoid pigments have been used successfully as phytoplankton biomarkers, both in the water column (Wright *et al.*, 1991) and in the sediments (Brotas and PlanteCuny, 1996). Chloropigments and carotenoids are associated

with many of the detrital particles that are deposited on the seafloor. These particles, such as faecal pellets and amorphous aggregates of 'marine snow', account for the majority of the downward vertical flux in the oceans. Data on gut contents indicate that holothurians feed directly on freshly deposited detritus, some more so than others (Iken *et al.*, 2001). It is clear that chloropigments provide a valuable marker for examining holothurian feeding behaviour. The use of chloropigments to study selectivity in deep-sea detritus feeders has already been undertaken to good effect by Billett *et al.* (1988) for bathyal holothurians in the Porcupine Seabight. Significant differences between the chloropigment profiles of holothurian gut contents and the surrounding sediment indicated some degree of selectivity by three species of bathyal holothurian. Further inter-species differences in the chloropigments content of gut contents suggest that different species of holothurian may be feeding on different fractions of the sediment organic matter, be it fresh phytodetrital particles or older, more degraded compounds in the sediment. It is therefore possible that observed differences in holothurian diet and their associated feeding strategies (Roberts *et al.*, 1995) may be related to morphological differences in tentacle structure and/or the relative mobility of each individual species (Roberts *et al.*, 1988; Moore *et al.*, 1995). However, they may also be related to varying nutritional requirements of the individual holothurian species.

5.1.4 Phytoplankton pigments as markers

Phytoplankton pigments are routinely used as biogenous markers for plant debris in the marine environment. Measuring the amount and distribution of chlorophyll a in surface sediments has been used as an indicator of plant biomass in numerous shallow-water studies (Brotas and Plante-Cuny, 1998). Pigment degradation products may also be used to infer availability of source material to consumers. The degradation product phaeophorbide has been found to be closely associated with metazoan grazing activity (Bianchi *et al.*, 1988). The most common use of pigment biomarkers is in oceanographic studies of surface-water phytoplankton blooms (e.g. Barlow *et al.*, 1993a), although the technique has been used to study variability in invertebrate deposit feeders (Riaux-Gobin *et al.*, 2000). The photosynthetic function of chlorophyll makes it a unique indicator of oceanic plant biomass and productivity. It is probably the most frequently measured biochemical parameter in oceanography.

Carotenoid pigments are useful markers for the identification of phytoplankton groups. The carotenoid fucoxanthin is a characteristic marker of diatoms while zeaxanthin serves as a marker for the presence of cyanobacteria (blue-green algae). Carotenoids can only be synthesised *de novo* by prokaryotes, fungi, algae and higher plants. They are useful for studying selection by deposit feeders as they relate to different phytoplankton sources and therefore, potentially, the different fractions of the phytodetritus that reach the seabed. 600 different carotenoid pigments are known and those found in animals are derived from ingested pigments, often chemically modified after digestion (Porra *et al.*, 1997).

5.2. Materials and Methods.

5.2.1. Sample collection and preservation.

Samples for pigment analysis were all collected in October 2000 during RRS *Discovery* cruise 250. Holothurians were collected using a semi-balloon otter trawl (see description in 2.2.1). Unfortunately, as a result of bad weather, only one trawl was undertaken and the coring programme was cancelled, therefore no sediment cores could be collected for use as a background reference. The one successful trawl was located on the Porcupine Abyssal Plain at 48°53'N, 16°45'W (see table 2.1) and specimens of *Amperima rosea*, *Molpadia blakei*, *Pseudostichopus villosus*, *Pseudostichopus* sp., *Oneirophanta mutabilis* and *Psychropotes longicauda* were selected for study. Once on deck the selected holothurians (see table 5.2) were placed in cold (4°C) water and removed to the CT lab to preserve the gut pigments.

	A. rosea	*M. blakei*	*Pseudostichopus* sp.	*P. villosus*	*O. mutabilis*	*P. longicauda*
Anterior gut	-	-	-	10	10	10
Posterior gut	-	-	-	10	10	10
Whole gut	17	3	10	-	-	-

Table 5.2. Holothurian gut samples collected during cruise D250 to the PAP, October 2000.

The guts of each animal were dissected out, and the contents were removed and individually frozen at –80°C. Only the three larger holothurian species had their guts separated into anterior and posterior halves before removing the sediment and freezing (see Table 5.2).

5.2.2. Pigment extraction

Frozen sediment samples were freeze-dried prior to extracting the pigments. For the larger sediment samples a sub-sample of 0.5g was weighed out into a centrifuge tube. All other samples were weighed and the total sediment was placed into the tubes. Pigments were extracted in 5ml of 90% HPLC grade acetone. All extracts were ultrasonicated for 30 seconds and centrifuged at 3000rpm for 10 minutes. The supernatant was filtered through a 0.2μm Nyalo membrane filter (Gelman).

5.2.3 HPLC analyses.

HPLC (High-Performance Liquid Chromatography) provides a powerful tool for accurately calculating chlorophyll *a*, and for the identification and quantification of accessory pigments and degradation products (Millie *et al.*, 1993). Pigments were separated by ion pairing reverse phase HPLC, as described by (Mantoura and Llewellyn, 1983) and modified by (Barlow *et al.*, 1993a), using a Perkin Elmer C18 column and a thermoseparation HPLC system with an online vacuum degasser, a dual solvent pump (P2000), an autosampler (AS3000), a UV detector (UV1000), a fluorometer (FL3000), integrator (SN4000) and integration software PC1000. Ammonium acetate was used as an ion-pairing reagent, because separation of the acidic compounds in the pigment mixture is poor under normal conditions as a result of the anionic character of the carboxyl group, which is dissociated at neutral pH. The ion-pairing reagent prevents dissociation, allowing separation of pigments not possessing a phytol group (i.e. chlorophylls and phaeophorbides) at pH 7 (Zapata *et al.*, 1987). Filtered pigment extracts were loaded into the autosampler that retained a temperature of 0°C. 500µl sample aliquots were mixed with 500µl 1M ammonium acetate and 100µl of the mixture was injected onto the column. The mobile phase consisted of a binary elutant system with solvent A (80% methanol and 20% 1M ammonium acetate) and solvent B (60% methanol and 40% acetone). A linear gradient at a flow rate of 1ml per minute was run from 0% to 100% B for 10 minutes and was followed by an isocratic stop at 100% B for 7.5 minutes. A second gradient of 2.5 minutes was used to return to the initial condition of 100% solvent A. Carotenoids were detected by absorbance at 440nm, chlorophylls and degradation products were detected by absorption at 440nm as well as by fluorescence with excitation at 410nm and emission at wavelengths >670nm.

5.2.4 Identification and quantification of pigments.

Phytoplankton pigments are identified based on their retention times in the HPLC column. Different calculations have to be made depending on whether pigments are being identified from absorption or fluorescence chromatograms.

5.2.4.1 Identification of chlorophylls and carotenoids.

Chlorophyll *a* and carotenoid peaks were identified by comparing retention times to those produced by commercial standards and extractions of known phytoplankton species.

Table 5.3 shows pigment retention times and the reference algal cultures used in this study.

Species	Algal Group	Pigments	Retention Time in this study	Reference
All species		Chlorophyll a	10.1min	standard injection
Phaeodactylum tricornutum	Diatom	Chlorophyll c1c2 Fucoxanthin Diadinoxanthin Diatoxanthin b carotene	2.6min 4.1min 5.4min 6.5min 11.9min	Jeffery and Vesk, 1997
Synechoccus sp.	Cyanobacteria	Zeaxanthin b carotene	6.8min 12.1min	Wright *et al., 1991*
Amphidinium carterae	Dinoflagellate	Chlorophyll c1c2 Peridinin Diadinoxanthin Diatoxanthin b carotene	2.7min 3.2min 5.4min 6.3min 11.9min	Wright *et al.*, 1991
Tetraselmis suecica	Green algae	Diadinoxanthin b carotene	5.3min 11.9min	Jeffery and Vesk, 1997
Emiliania huxleyi	Coccolithophore	Chlorophyll c3 Fucoxanthin 19' hexanoyloxyfucoxanthin b carotene	2.0min 4.3min 4.8min 12.1min	Barlow *et al.*, 1993

Table 5.3. Identification of the main pigments, their retention times and reference cultures in which they occur. Chlorophyll *a* was obtained as a commercial standard (Sigma).

Cultures of the five phytoplankton species, listed above, were grown from commercially obtained stock. 1ml of each culture was filtered through Whatman N°.5 filter paper. The filter paper was then placed in a centrifuge tube and the pigments were extracted using the same protocol as that used for the sediment samples (see section 5.2.2). Retention times are were not exact for each sample run, but the relationships between the elution times of the pigments remain constant, allowing for identification of the absorbance peaks (i.e. diatoxanthin always elutes ~1min after diadinoxanthin).

5.2.4.2 Identification of phaeopigments.

Phaeopigments are the degradation products of chlorophyll *a*. There are two main classes, phaeophorbides and phaeophytins. Phaeophorbides are usually associated with the breakdown of chlorophyll *a* by metazoan grazing activity (i.e. copepods) and phaeophytins are often associated with the breakdown of chlorophyll *a* by senescence and/or bacterial action. When analysed by HPLC and detected by online fluorescence phaeophorbides eluted prior to chlorophyll *a* and phaeophytins after chlorophyll *a* (Barlow *et al.*, 1993b).

5.2.4.3. Quantification of chlorophylls and carotenoids.

After the analysis of samples with HPLC pigment concentrations in μg per gram of dry-weight sediment were calculated using the following equation:

$$C = (A_p V)/(W R_f B 100)$$

Where A_p is the peak area detected at 440nm, V is the extract volume in ml, W is the dry weight of sediment in grams, R_f is the response factors and B is the buffer dilution factor (0.5). The response factors for each pigment are calculated by converting the standard concentrations of each pigment from mgl^{-1} to ng per column and then plotting them against peak area. The response factors for each pigment used in the present study are given below in Table 5.4.

Pigment	**Response Factor (R_f)**
Chlorophyll *c*1 *c*2	12378.69
Peridinin	6761.49
Fucoxanthin	10258.69
19'hexanoyloxyfucoxanthin	10258.70
Diadinoxanthin	16963.81
Diatoxanthin	10637.60
Zeaxanthin	14388.95
Chlorophyll *a*	3514
β-carotene	8818.79

Table 5.4. Response factors used in the quantification of pigments identified from HPLC analyses.

5.2.4.4. Quantification of phaeopigments.

The concentration of phaeopigments, in μg per gram of dry sediment, identified from the fluorescence chromatogram can be quantified using the following equation:

$$C = (A_f V f_i)/(WBE_{670})$$

Where A_f is the fluorescence peak area divided by the response factor of each pigment, V is the extract volume in ml, f_i is the response factor of the UV detector in the instrument (0.00187), W is the weight of dry sediment in grams, B is the dilution factor (0.5) and E_{670} is the specific extinction coefficient for each pigment. Table 5.5 shows the response factors and extinction coefficients (taken from Barlow *et al.*, 1993b) for the phaeophorbides and phaeophytins identified in this study.

Phaeopigment	Response Factor for fluorometer	E_{670}
Phaeophorbide a1	15.531	69.8
Phaeophorbide a2	12.501	69.8
Phaeophytin a1	14.831	49.5
Phaeophytin a2	13.203	49.5

Table 5.5. Response factors and extinction coefficients for the quantification of phaeopigments (taken from Barlow *et al.*, (1993b)).

5.2.5. Analysis of pigment profiles.

Qualitative differences in the pigment profiles of holothurian gut contents can be made by viewing the pattern of the absorption and fluorescence chromatograms produced from the HPLC analysis (e.g. Billett *et al.*, 1988).

Quantitative analyses of the concentrations of individual pigments in the gut sediments were undertaken using various methods. Direct between species, and within species comparisons were assessed statistically using analyses of variance and similarity (ANOVA, ANOSIM (Sokal and Rohlf, 1995)). Variations in total pigment distributions were analysed using multivariate statistical analyses on the PRIMER 5 software package (Clarke and Warwick, 1994). Selection of fresh chloropigments was assessed by

calculating a chlorophyll *a*: phaeophorbide (C:P) ratio for each species (e.g. Witbaard *et al.*, 2001).

As result of the loss of the accompanying coring programme, references to the pigment concentrations of the surrounding sediment had to be made from examples in the literature. It is important to note that although these reference data are from a similar region of the PAP they are often from different years and/or seasons. This is likely to influence the records of pigments present in the sediment as there is considerable inter-annual variability in the flux of material to the deep seafloor (Lampitt and Antia, 1997; Lampitt *et al.*, 2001) and in the composition and timing of the phytoplankton bloom in surface waters (Barlow *et al.*, 1993a; Boyd and Newton, 1995). However, data from the PAP, such as that provided by Witbaard *et al.* (2000), can be used to make basic estimates of the selection coefficient (e.g. Billett *et al.*, 1988) of each species, at least for the chloropigments. Selection coefficients were calculated by dividing the concentration of pigment in the gut by the concentration of pigment in the sediment.

5.3. Results

5.3.1 Pigment profiles: qualitative between species comparisons.

5.3.1.1. Chlorophyll and carotenoid pigments.

Examples of the absorption chromatograms produced for each of the six species under investigation are shown in Figure 5.1. The patterns observed were consistent within each species, but between-species differences were apparent. The most striking difference was the lack of any pigment signature on the chromatogram for *Molpadia blakei*. *M. blakei* is an infaunal feeder compared to the other five species, which are surface deposit feeders (possibly shallow sub-surface in the case of *Pseudostichopus* sp. and *Pseudostichopus villosus*). *Pseudostichopus* sp. itself also has a different pigment profile from the other species. Pigments identified include fucoxanthin, 19'hexanoyloxyfucoxanthin, diatoxanthin, zeaxanthin, chlorophyll *a* and β-carotene. These pigments could be from a range of phytoplankton particles, although all are in an undegraded form. The pigment profiles of the three larger species, *P. villosus*, *Oneirophanta mutabilis* and *Psychropotes longicauda*, were similar to each other with the majority of the main pigments represented. Zeaxanthin was the only pigment not present in the gut sediments of *P. villosus*, and was only a minor constituent of the total pigments in the guts of *O. mutabilis* and *P. longicauda*. The profiles of these three species differed from those of *Pseudostichopus* sp. and *Amperima rosea* with respect to the presence of chlorophylls *c*1*c*2 and *c*3 and peridinin and in the relative proportions of fucoxanthin and 19'hexanoyloxyfucoxanthin, the last two being the major pigment fractions in the gut samples of the three larger holothurian species.

The chromatogram of *A. rosea* has a similar pigment profile to that of *Pseudostichopus* sp. but they differ vastly in the relative contribution of each pigment. Zeaxanthin and chlorophyll *a* were the major pigments present in the gut contents of *A. rosea*, whereas they were only very minor components of the total pigments in the gut contents of *Pseudostichopus* sp. The pigment signature of *A. rosea* differed greatly from all the other five species, especially in the relative contribution of three main pigments, zeaxanthin, chlorophyll *a* and also β-carotene. It also lacked any trace of chlorophylls *c*1*c*2 and *c*3, and peridinin, signature pigments of dinoflagellate phytoplankton.

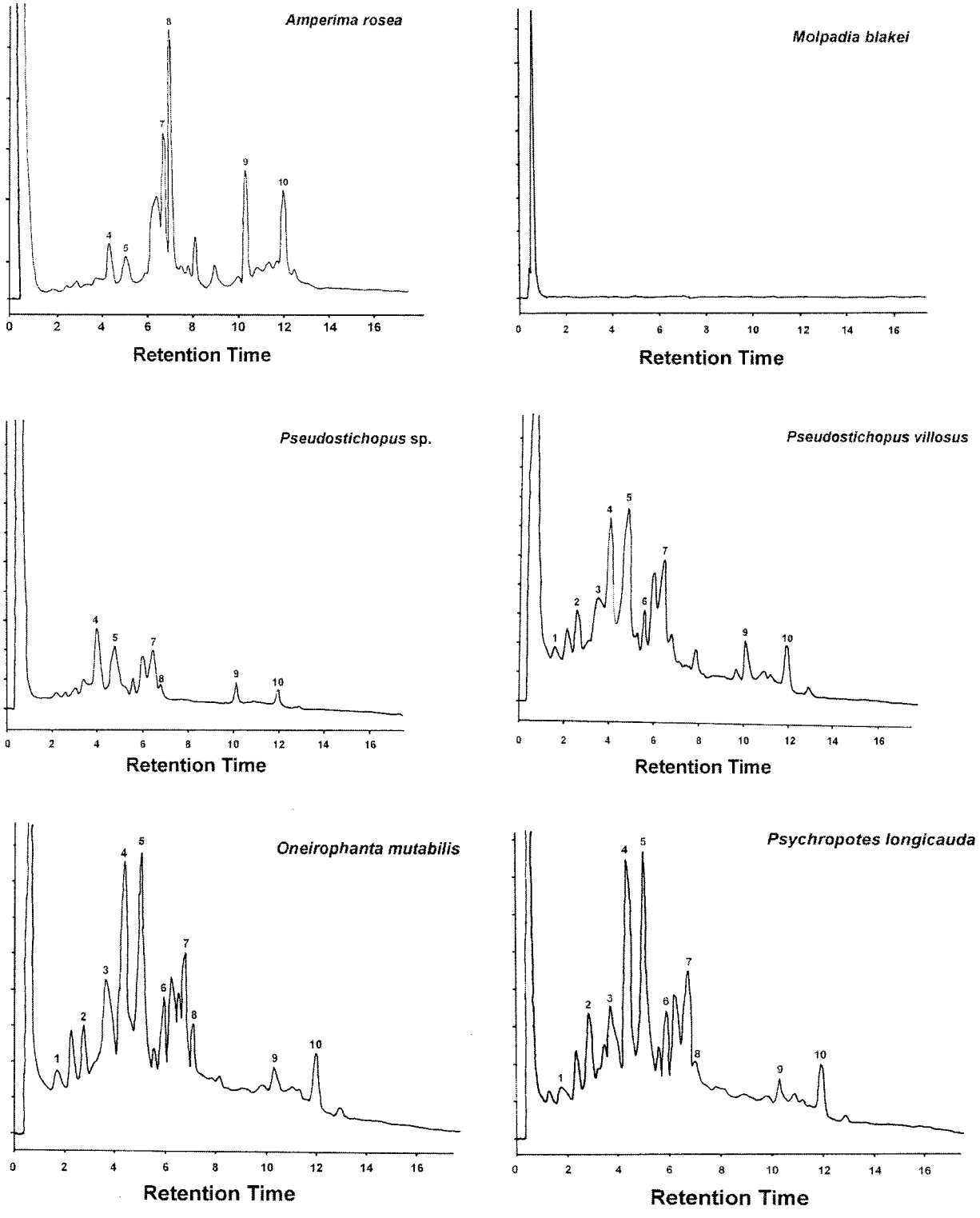

Figure 5.1. Absorption chromatograms of chlorophyll and carotenoids pigments in the gut content sediment of six species of abyssal holothurian. 1, chlorophyll *c*3; 2, chlorophyll *c*1*c*2; 3, peridinin; 4, fucoxanthin; 5, 19'hexanoyloxyfucoxanthin; 6, diadinoxanthin; 7, diatoxanthin; 8, zeaxanthin; 9, chlorophyll *a*; 10, β-carotene

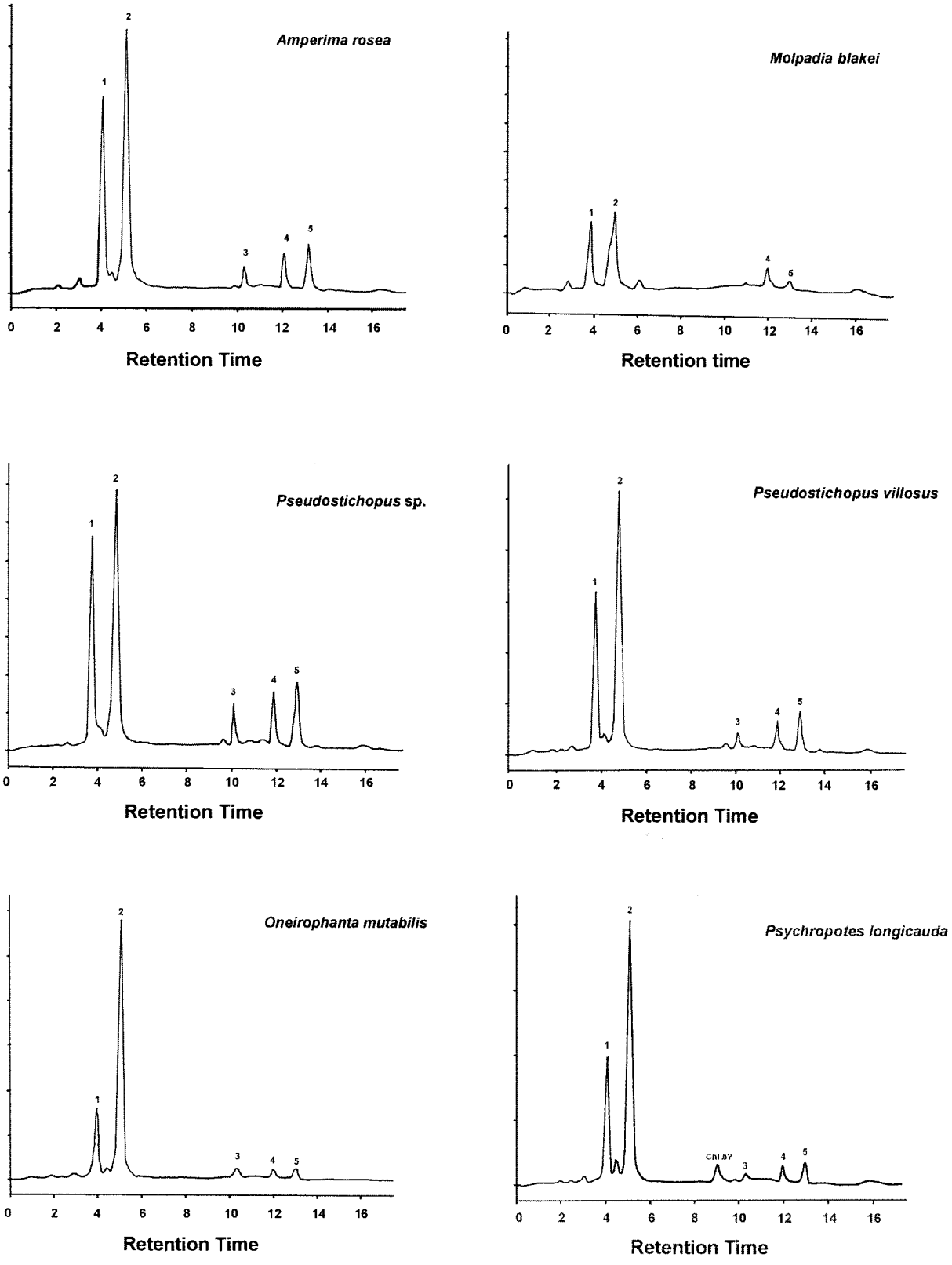

Figure 5.2. Fluorescence chromatograms for chlorophyll *a* and associated refractory phaeopigments from the gut content sediment of six species of abyssal holothurian. 1, phaeophorbide *a*1; 2, phaeophorbide *a*2; 3, chlorophyll *a*; 4, phaeophytin *a*1; 5, phaeophytin *a*2.

5.3.1.2. Refractory phaeopigments.

The fluorescence chromatograms for each species are similar in appearance, with easily identifiable peaks for phaeophorbides a1 and a2, phaeophytins a1 and a2, and chlorophyll *a* (Figure 5.2). The pigment profiles differ between species in respect of the varying contribution of the phaeopigments to the total chloropigment pool. The fluorescence chromatogram for *M. blakei* reveals the presence of refractory chloropigments in the gut sediments but no discernable peak for chlorophyll *a*. This is not entirely unexpected considering where *M. blakei* feeds, coupled with the short half-life of chlorophyll *a* (Graf, 1989). For all six species, the phaeophorbides were a much larger component of the total chloropigments than the phaeophytins. The profiles obtained from the anterior and posterior portions of the guts of *O. mutabilis, P. longicauda, P. villosus* did not differ significantly, so the examples of the fluorescence chromatograms shown in Figure 5.2 are representative of both the anterior and posterior gut contents.

5.3.2. Pigment profiles: quantitative between species comparisons.

5.3.2.1 Chlorophyll and carotenoid pigments.

Significant between-species differences in the concentration of nine shared carotenoid and chlorophyll pigments were observed (Table 5.6). *Molpadia blakei* has no carotenoid or undegraded chlorophyll pigments in its gut contents, as a result subsequent references to "all species" correspond to all species except for *M. blakei*. As highlighted by the absorption chromatograms (Figure 5.1) all the species under investigation share a basic suite of six identifiable carotenoid pigments, with a further two pigments shared between the three larger species (*P. villosus, O. mutabilis* and *P. longicauda*) The relative contribution of each pigment to the total profile differs in terms of their individual concentrations, highlighting the potential of certain species to positively select particles to ingest. Figure 5.3 shows the concentration of each pigment ($\mu g\ gDW^{-1}$) found in the gut content sediment of each species. The three larger species all have traces of chlorophyll *c*1*c*2 and peridinin in their gut contents unlike *A. rosea*, *Pseudostichopus* sp. and *M. blakei*. However, both *O. mutabilis* and *P. longicauda* had significantly more chlorophyll *c*1*c*2 and peridinin in their guts than *P. villosus*. Fucoxanthin and 19'hexanoyloxyfucoxanthin were present in all species but again it is in the gut contents of *O. mutabilis* and *P. longicauda* that significantly higher pigment concentrations could

Species / Pigment	*Amperima rosea*	*Molpadia blakei*	*Pseudostichopus* sp.	*Pseudostichopus villosus*	*Oneirophanta mutabilis*	*Psychropotes longicauda*	ANOVA species	ANOVA no *Molpadia*
CHLOROPHYLL *c*1*c*2	0.00 -	0.00 -	0.00 -	0.39 (0.14)	0.81 (0.18)	0.89 (0.34)	P<0.001	P<0.001
PERIDININ	0.00 -	0.00 -	0.00 -	0.79 (0.14)	3.65 (1.51)	3.11 (0.68)	P<0.001	P<0.001
FUCOXANTHIN	2.24 (1.82)	0.00 -	1.52 (1.15)	1.06 (0.28)	4.70 (0.94)	3.83 (1.14)	P<0.001	P<0.001
19' HEXANOYLOXYFUCOXANTHIN	0.93 (1.18)	0.00 -	1.43 (0.89)	1.49 (0.33)	5.13 (0.83)	5.29 (0.81)	P<0.001	P<0.001
DIADINOXANTHIN	0.00 -	0.00 -	0.10 (0.15)	0.13 (0.04)	0.54 (0.21)	0.47 (0.12)	P<0.001	P<0.001
DIATOXANTHIN	18.50 (11.24)	0.00 -	0.95 (0.73)	0.43 (0.19)	2.27 (1.00)	1.43 (0.23)	P<0.001	P<0.001
ZEAXANTHIN	29.77 (29.70)	0.00 -	0.07 (0.14)	0.05 (0.03)	0.37 (0.32)	0.18 (0.04)	P<0.001	P<0.001
CHLOROPHYLL *a*	45.94 (40.41)	0.00 -	0.68 (0.52)	0.11 (0.08)	1.08 (1.05)	0.71 (0.34)	P<0.001	P<0.001
B-CAROTENE	20.37 (18.55)	0.00 -	0.35 (0.27)	0.15 (0.03)	0.85 (0.47)	0.67 (0.19)	P<0.001	P<0.001
PHAEOPHORBIDE *a*1	19.75 (8.11)	0.97 (0.40)	31.04 (20.42)	18.36 (2.25)	44.34 (7.80)	42.29 (3.94)	P<0.001	P<0.001
PHAEOPHORBIDE *a*2	72.54 (32.02)	2.16 (1.00)	67.48 (40.65)	58.28 (6.23)	177.07 (24.73)	169.49 (11.82)	P<0.001	P<0.001
PHAEOPHYTIN *a*1	3.03 (2.32)	0.16 (0.04)	8.22 (5.39)	3.20 (0.77)	12.34 (3.80)	7.35 (2.87)	P<0.001	P<0.001
PHAEOPHYTIN *a*2	5.95 (4.48)	0.18 (0.16)	15.76 (9.00)	4.54 (1.07)	18.39 (4.73)	8.73 (3.04)	P<0.001	P<0.001

Table 5.6. Among-species variability in mean gut pigment concentration (μg gDW^{-1})(standard deviation in parathenseses) with results of among-species analysis of variance (ANOVA).

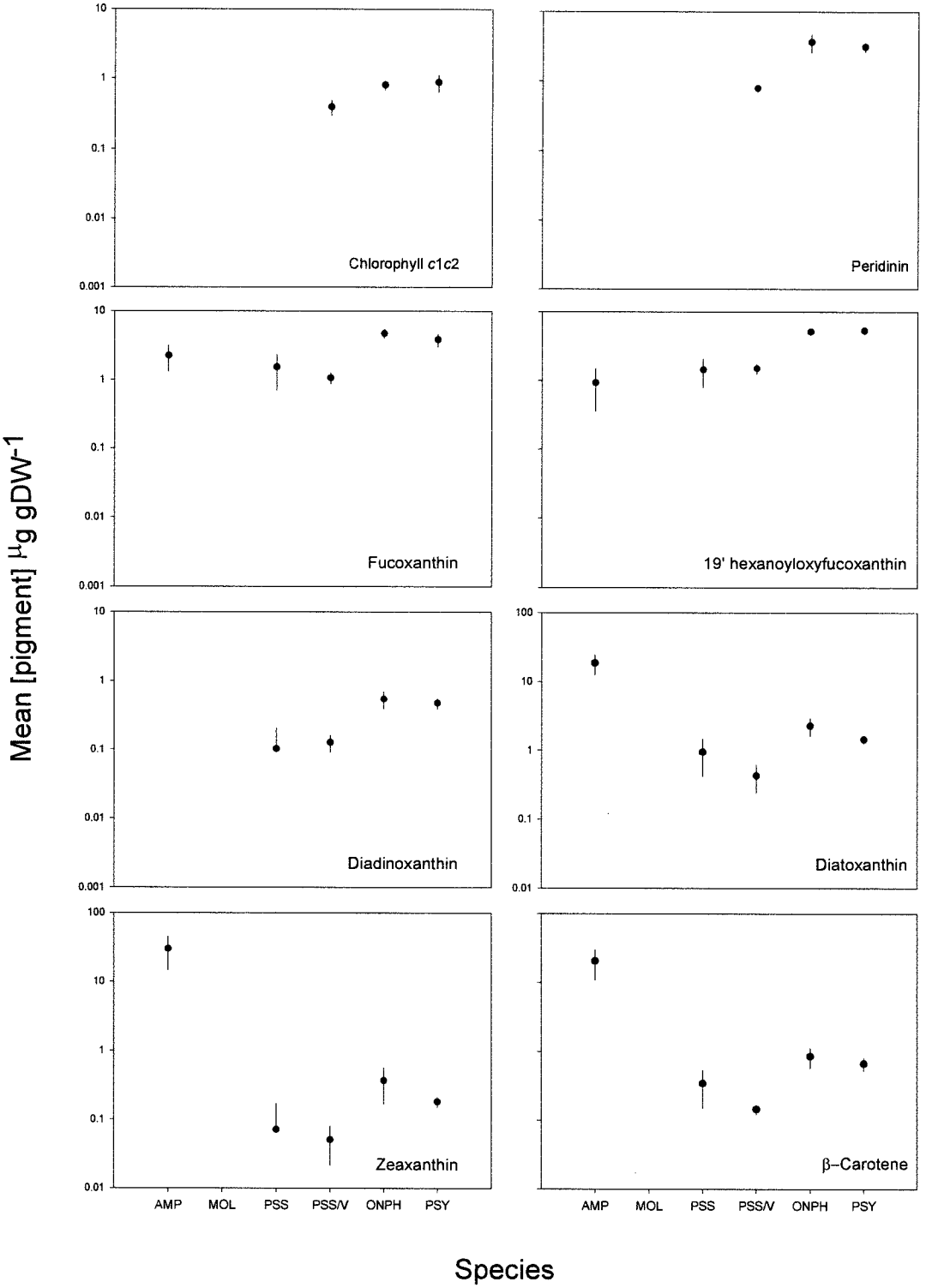

Figure 5.3. Variability in the concentration of pigments found in the gut contents of six abyssal holothurians. Mean concentration (µg gDW^{-1} ± 95% confidence) for 11 identified pigments. AMP, *Amperima rosea*; MOL, *Molpadia blakei*; PSS, *Pseudostichopus* sp.; PSS/V, *Pseudostichopus villosus*; ONPH, *Oneirophanta mutabilis*; PSY, *Psychropotes longicauda*.

be found. These two species also had higher concentrations of diadinoxanthin compared to the two *Pseudostichopus* species. *A. rosea* had no diadinoxanthin in its gut contents. All species had the pigments diatoxanthin, zeaxanthin and β-carotene in their gut contents. However, *A. rosea* has significantly higher, by an order of magnitude, concentrations of these three pigments compared to the other species (see table 5.6, figure 5.3). All species also had chlorophyll *a* present in their gut contents, although *A. rosea* again stands out as having significantly more of this pigment in its gut contents than the other species (see figure 5.4)

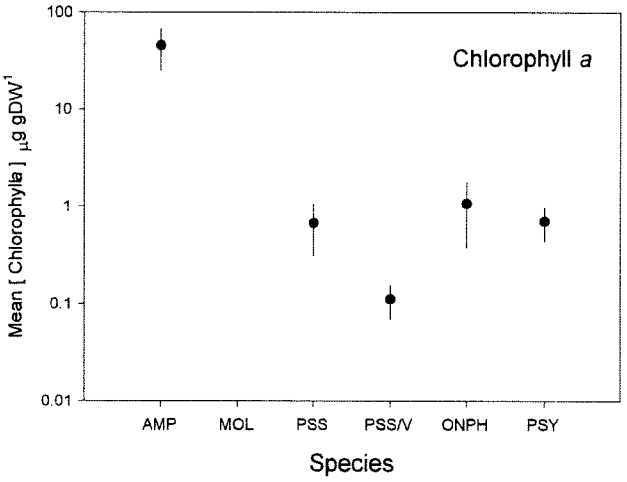

Figure 5.4. Mean concentration (µg gDW^{-1} ± 95% confidence) of chlorophyll *a* in the gut contents of six species of abyssal holothurian. AMP, *Amperima rosea*; MOL, *Molpadia blakei*; PSS, *Pseudostichopus* sp.; PSS/V, *Pseudostichopus villosus*; ONPH, *Oneirophanta mutabilis*; PSY, *Psychropotes longicauda*.

The quantitative pigment profiles for all six species (including *M. blakei*) re-emphasise the similarities and differences between the presence/absence and concentrations of each pigment in their gut contents (see figure 5.5). The pigments present in the lowest concentrations in the guts of *A. rosea* (chlorophyll *c*1*c*2, peridinin, fucoxanthin and 19'hexanoyloxyfucoxanthin) were the more prevalent pigments in the guts of the other species (not including *M. blakei*). For the three larger species, *P. villosus*, *O. mutabilis* and *P. longicauda*, mean pigment concentrations are given for both the anterior and posterior gut samples. For the majority of pigments the mean concentration in the anterior gut was always greater than that in the posterior gut, although not always significantly greater. All six species had the breakdown product pigments phaeophorbide and phaeophytin in their guts.

5.3.2.2. Chlorophyll a and its breakdown products: the 'chloropigments'

Chlorophyll *a* was present in the gut contents of all the species, with the exception of *M. blakei*. *A. rosea* had the highest concentrations of chlorophyll *a* of all the species under investigation, as shown in Figure 5.4. All six species were found to have phaeophorbides and phaeophytins in their guts (see Figure 5.5) and the concentration of total phaeophorbide in the gut was always significantly greater than the concentration of total phaeophytin (with the exception of *M. blakei*, which may be the result of low detection levels, see Table 5.7).

Species	Mean (± SD) phaeophorbide	Mean (± SD) phaeophytin	T-test
AMP	92.30 ± 35.70	8.97 ± 6.76	T = 9.45, P<0.001
MOL	3.13 ± 1.40	0.34 ± 0.19	T = 3.42, P = 0.076
PSS	98.50 ± 60.90	24.0 ± 14.30	T = 3.77, P = 0.004
PSS/V	76.63 ± 8.17	7.74 ± 1.71	T = 26.08, P<0.001
ONPH	221.40 ± 32.00	30.73 ± 8.41	T = 18.20, P<0.001
PSY	211.80 ± 13.90	16.08 ± 5.72	T = 41.07, P<0.001

Table 5.7. Results of paired t-tests for concentration of phaeophorbides and phaeophytins (μg gDW^{-1}) found in the gut sediments of six species of abyssal holothurian.

Four separate phaeopigments were identified in each species, phaeophorbides a1 and a2 and phaeophytins a1 and a2. In all species the guts always contained more phaeophorbide a2 than a1, but the proportions of phaeophytins a1 and a2 were more or less equal for all species, except for *M. blakei* that had more a1 than a2 (see Figures 5.2 and 5.6). *O. mutabilis* and *P. longicauda* had much higher concentrations of phaeophorbides in their guts than the other four species. *A. rosea* and *Pseudostichopus* sp. also had relatively high concentrations (compared to other pigments) of phaeophorbide although there was more variability between individuals. For all species (except *A. rosea*) the phaeopigments were the most abundant pigment group in the gut sediments (see Figure 5.5).

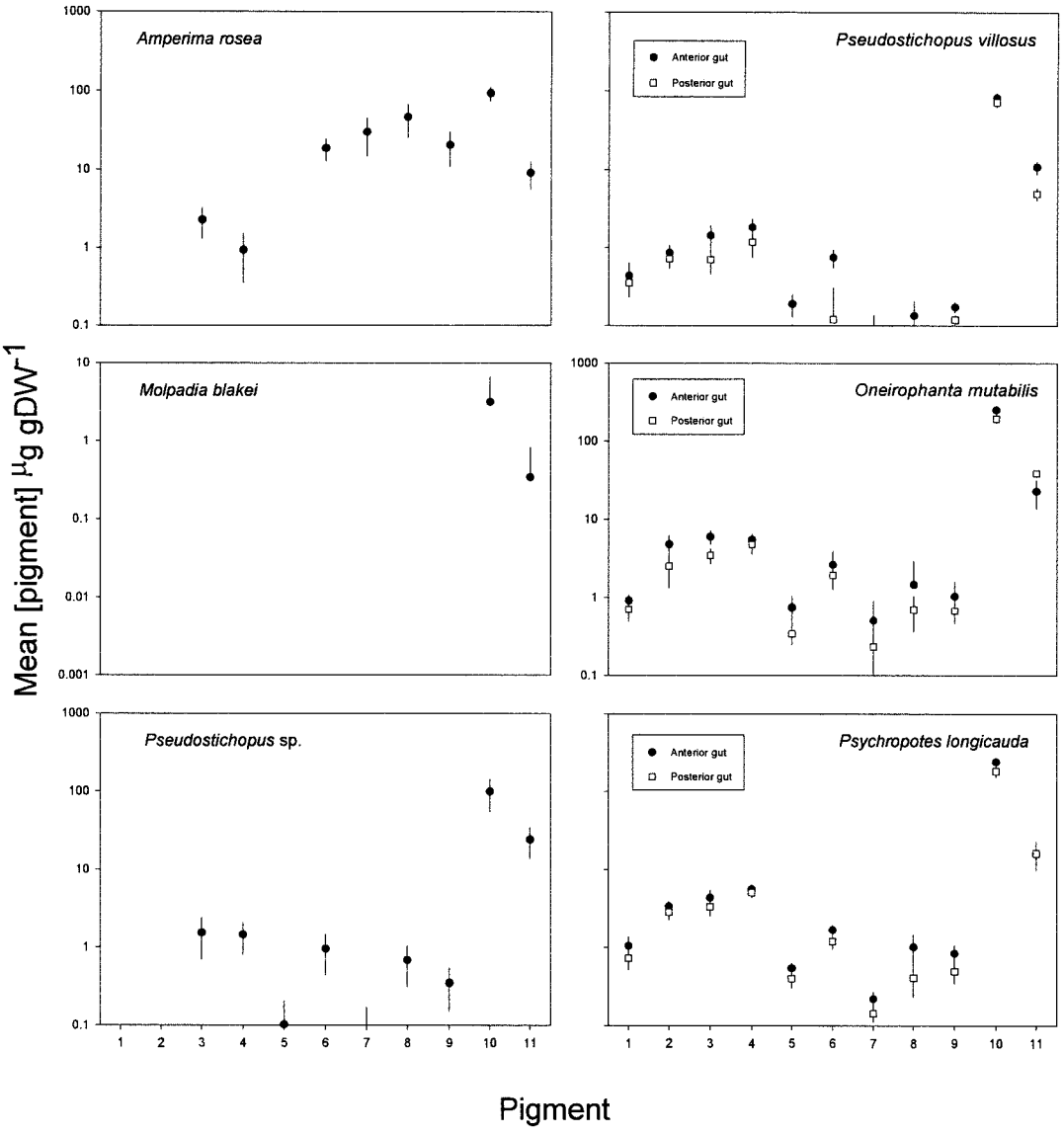

Figure 5.5. Quantitative pigment profiles for the gut contents of six species of abyssal holothurian. Mean concentration (µg gDW^{-1} ± 95% confidence) of 11 identified pigments (combined phaeophorbides and phaeophytins). 1, chlorophyll *c*1*c*2; 2, Peridinin; 3, Fucoxanthin; 4, 19' hexanoyloxyfucoxanthin; 5, Diadinoxanthin; 6, Diatoxanthin; 7, Zeaxanthin; 8, chlorophyll *a*; 9, β-carotene; 10, Total Phaeophorbide; 11, Total Phaeophytin.

5.3.2.3. Chlorophyll a: phaeophorbide ratios

Ratios of chlorophyll *a* to phaeophorbides have often been used as an indication of 'freshness' for phytodetrital material (Thiel *et al.*, 1989). Calculating a ratio for each holothurian species gave an indication of their individual selectivity for fresher, undegraded detrital material. Figure 5.6 shows the chlorophyll *a*:phaeophorbide ratio for each species along with the ratios for trap material sampled in September 1996 and July 1997. There is a strong indication that *A. rosea* feeds only on the freshest fraction of the phytodetritus.

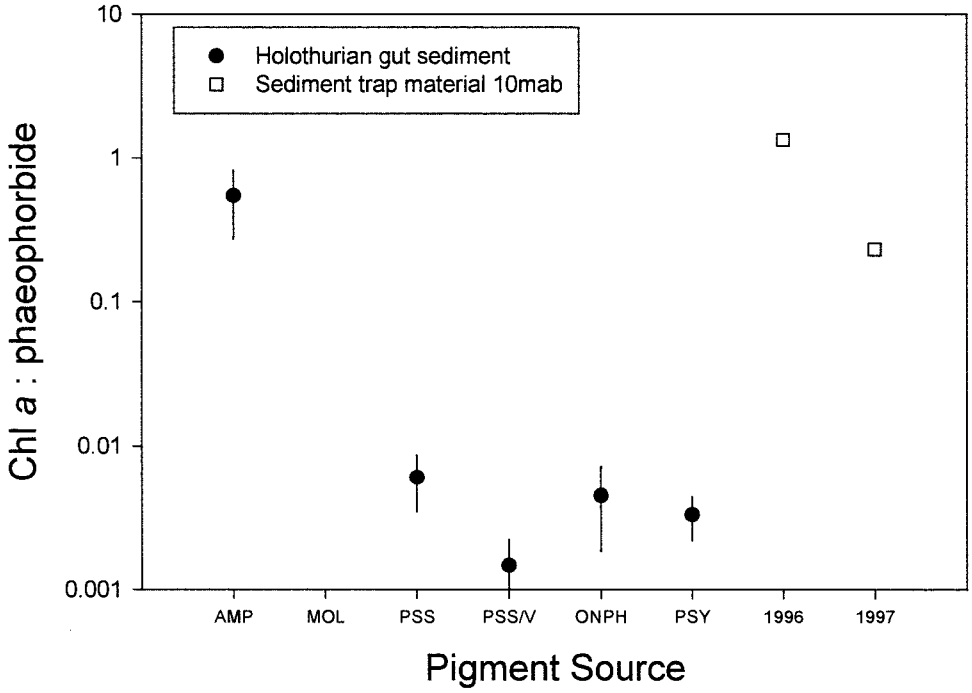

Figure 5.6. Mean chlorophyll *a*:phaeophorbide ratios (± 95% confidence) as an indicator of phytodetrital 'freshness'. Holothurian gut sediments compared to sediment trap material (data taken from Witbaard *et al.* (2001)). AMP, *Amperima rosea*; MOL, *Molpadia blakei*; PSS, *Pseudostichopus* sp.; PSS/V, *Pseudostichopus villosus*; ONPH, *Oneirophanta mutabilis*; PSY, *Psychropotes longicauda*.

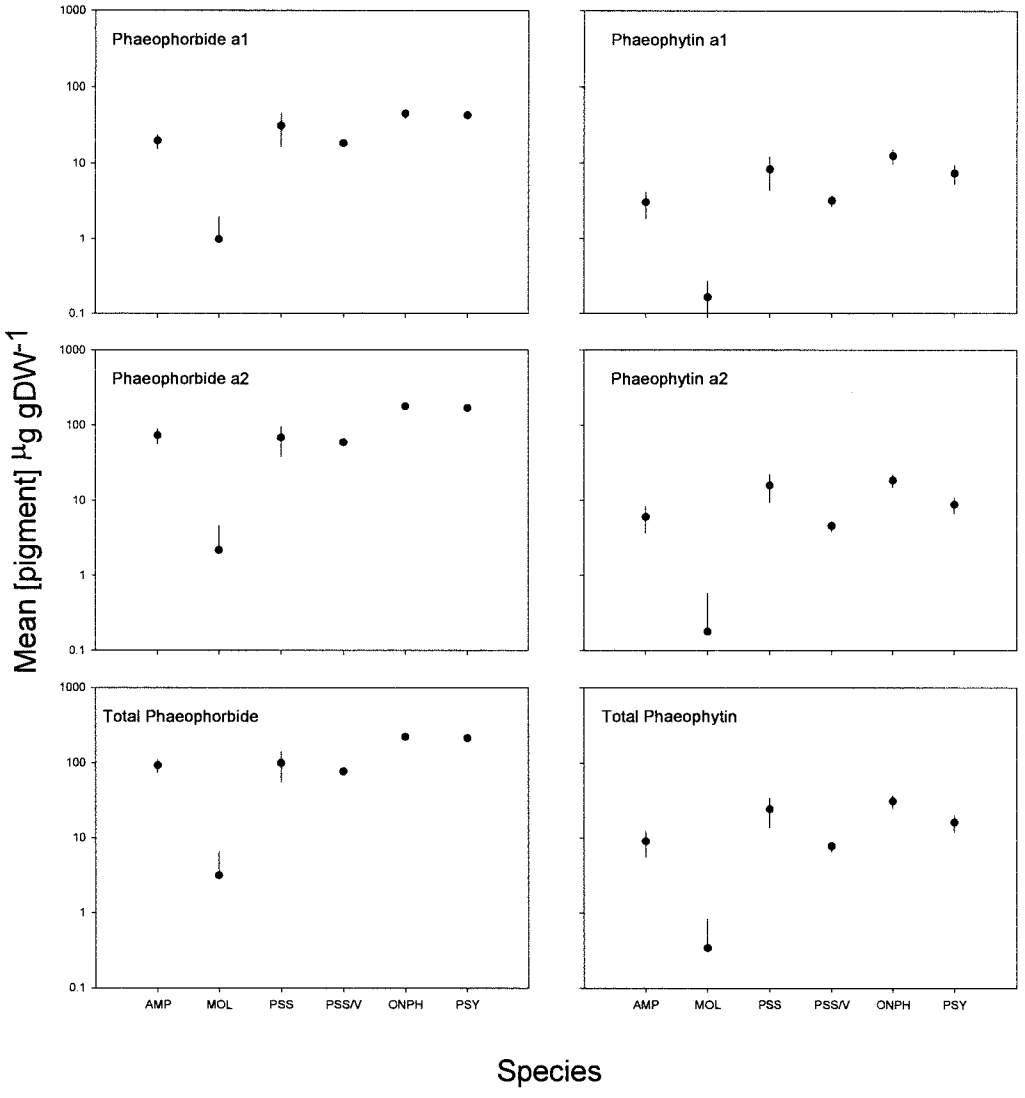

Figure 5.7. Variability in the concentration of phaeopigments found in the gut contents of six species of abyssal holothurian. Mean concentration (µg gDW^{-1} ± 95% confidence) for 4 identified pigments plus total phaeophorbide and total phaeophytin. AMP, *Amperima rosea*; MOL, *Molpadia blakei*; PSS, *Pseudostichopus* sp.; PSS/V, *Pseudostichopus villosus*; ONPH, *Oneirophanta mutabilis*; PSY, *Psychropotes longicauda*.

5.3.2.4. Inter-species variability in total pigment profiles: a multivariate approach

The variability between species and between individuals of a species, based on quantitative values of each pigment, was assessed using analysis of similarity (ANOSIM). There were significant differences between all possible combinations of species pairs (see table 5.8)

Groups	*R* statistic	Significance level
AMP v MOL	1.000	P = 0.002
AMP v PSS	0.706	P = 0.001
AMP v PSS/V	0.887	P = 0.001
AMP v ONPH	0.843	P = 0.001
AMP v PSY	0.847	P = 0.001
MOL v PSS	0.956	P = 0.003
MOL v PSS/V	1.000	P = 0.003
MOL v ONPH	1.000	P = 0.003
MOL v PSY	1.000	P = 0.003
PSS v PSS/V	0.641	P = 0.001
PSS v ONPH	0.662	P = 0.001
PSS v PSY	0.663	P = 0.001
PSS/V v ONPH	1.000	P = 0.001
PSS/V v PSY	0.999	P = 0.001
ONPH v PSY	0.281	P = 0.001

Table 5.8. Results of ANOSIM test for between species variability based on total pigment profiles. *R* statistic = 1 only if all replicates within a sample are more similar to each other than any other replicates from different samples. *R* will usually fall between 0 and 1.

Cluster analysis and multi-dimensional scaling (MDS), based on Bray-Curtis similarities, revealed individual samples clustering out in species groups (see Figures 5.8 and 5.9). *A. rosea* and *M. blakei* formed two distinct groups. Individuals of *Pseudostichopus* sp. and *P. villosus* also tended to cluster together. However, two individuals of *Pseudostichopus* sp. clustered with *M. blakei*. These two specimens had very pigment-poor profiles, with only the phaeopigments and small amounts of fucoxanthin and chlorophyll *a* present in the guts. Individuals of *O. mutabilis* and *P. longicauda* could not be resolved into species groups by either cluster analysis or MDS, however the results of ANOSIM show a small but significant difference in their quantitative profiles. An additional MDS ordination (Figure 5.10) performed on *O. mutabilis* and *P. longicauda* data alone did resolve two species clusters.

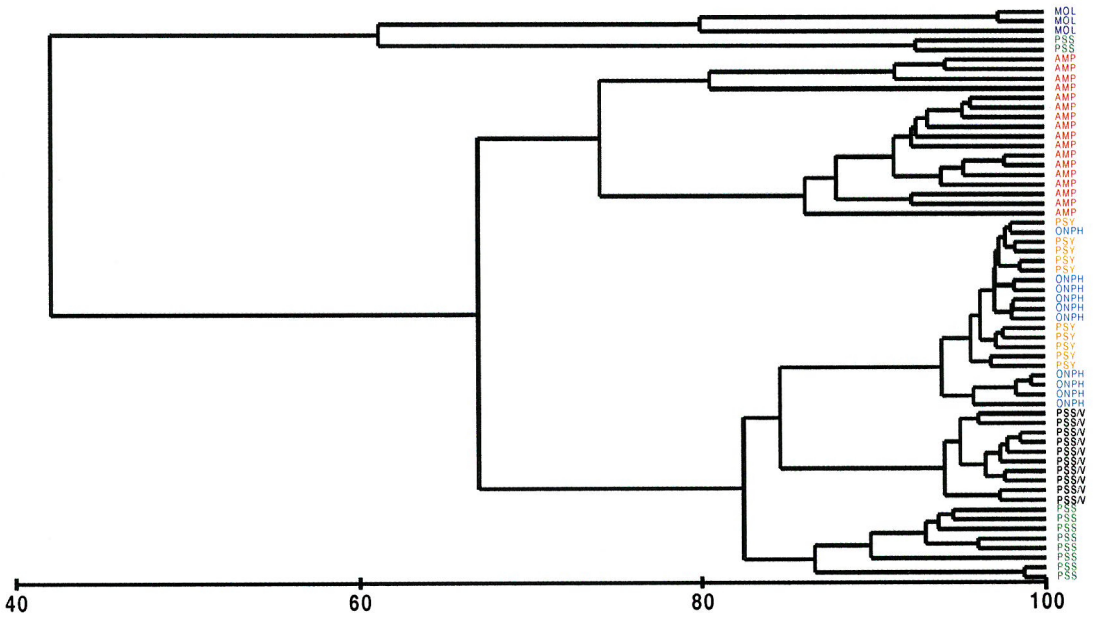

Figure 5.8. Dendrogram for hierarchical clustering of 60 individual holothurian specimens, using group-average linking of Bray-Curtis similarities calculated on √√-transformed pigment concentration data.

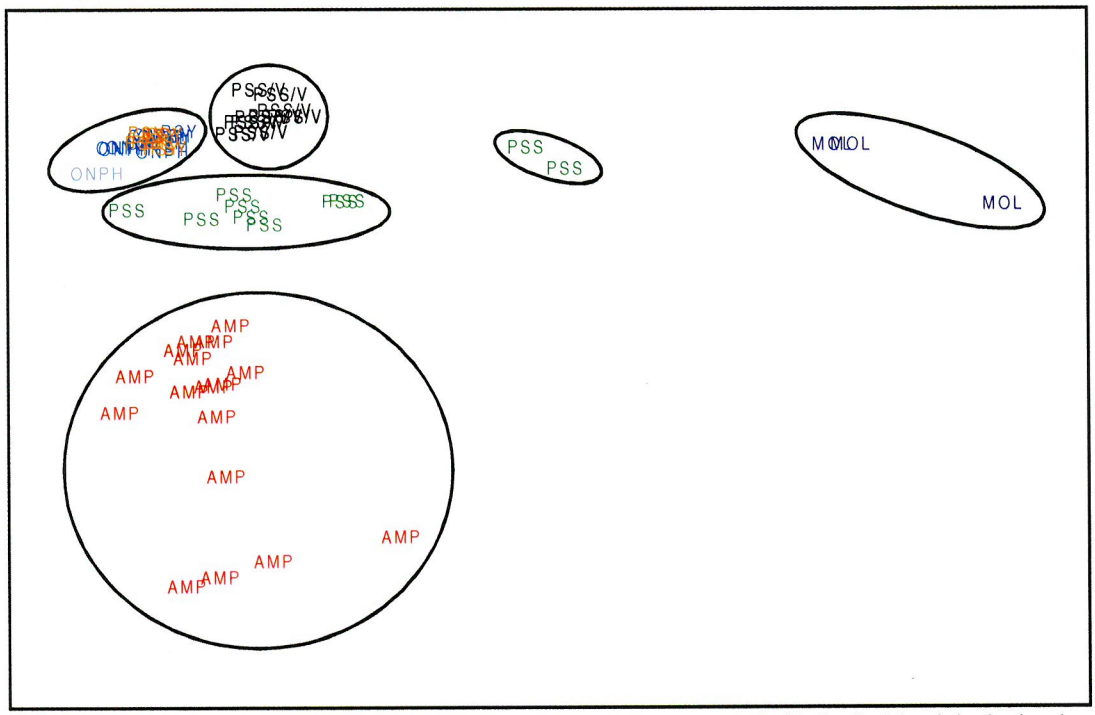

Figure 5.9. Multi-dimensional scaling (MDS) ordination of 60 individual holothurian specimens, based on √√-transformed pigment concentrations and Bray-Curtis similarities (stress = 0.05).

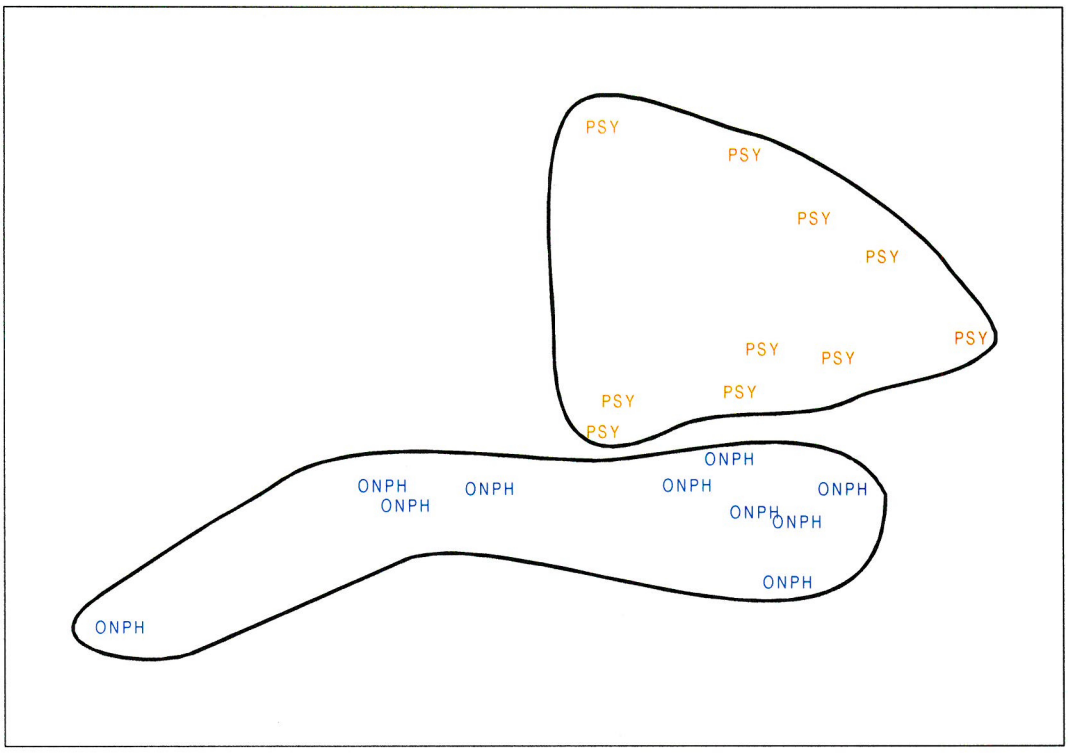

Figure 5.10. Multi-dimensional scaling (MDS) ordination of 20 individual specimens of *O. mutabilis* and *P. longicauda*, based on √√-transformed pigment concentrations and Bray-Curtis similarities (stress = 0.1).

5.3.3. Selection coefficients.

Data for chloropigment concentrations in the background sediment were adapted from values given in Witbaard *et al.* (2000). Firstly, for calculation of selection coefficients, it was necessary to convert the units used by Witbaard *et al.* (2000) to comparable units for use with the gut pigment concentrations used in this study. Concentrations of chlorophyll *a* and phaeophorbide in the sediment were given as ng cm^{-3}, whereas gut pigment concentrations were given as μg gDW^{-1}. For the top 1cm of PAP sediments $1cm^3$ of wet sediment will dry to ~0.5g (Thomson pers. comm.). Therefore, values of ng cm^{-3} can be converted into values of μg gDW^{-1} by following the steps set out below.

ng cm^{-3} = ng $0.5gDW^{-1}$

ng/1000 = μg

$2(ng\ cm^{-3}/1000) = \mu g\ gDW^{-1}$

Working at the BENGAL site Witbaard *et al.* calculated the concentration of chlorophyll *a* and phaeophorbide in the PAP sediments during five separate cruises between September 1996 and September 1998. As the samples for gut pigment analysis were collected during RRS *Discovery* cruise D250 in September/October 2000, the most reasonable comparisons could be made using September/October data from Witbaard *et al.*, who presented values for three autumn samples, September 1996, October 1997 and September 1998. Table 5.9 gives values for the concentration of pigments in the sediment for these three cruises, in both ng cm^{-3} (from Witbaard *et al.*, 2000) and the converted values in μg gDW^{-1}. The mean 'autumn' value was then used in the calculation of selection coefficients.

	September 1996	October 1997	September 1998	Mean 'autumn'
Chlorophyll *a* ng cm^{-3}	16.3	6.8	4.3	9.13 ± 6.33
Chlorophyll *a* μg gDW^{-1}	0.0326	0.0136	0.0086	**0.0183 ± 0.0126**
Phaeophorbide ng cm^{-3}	211	105	121	146 ± 57
Phaeophorbide μg gDW^{-1}	0.4220	0.2100	0.2420	**0.2913 ± 0.1143**

Table 5.9. Concentration of chlorophyll *a* and phaeophorbides in the top 1cm of PAP sediments. Data taken and adapted from Witbaard *et al.* (2000). Mean values (± standard deviation) used in the calculation of selection coefficients are shown in bold.

Figure 5.10 shows the calculated selection coefficients for chlorophyll *a* and phaeophorbides for each of the six species under investigation. All six species exhibit greater variability in their chlorophyll *a* selection coefficient compared to the phaeophorbides. *A. rosea* has a significantly greater chlorophyll *a* selection coefficient than the other five species. It is also the only species to have a greater chlorophyll *a* selection coefficient than its corresponding phaeophorbide one.

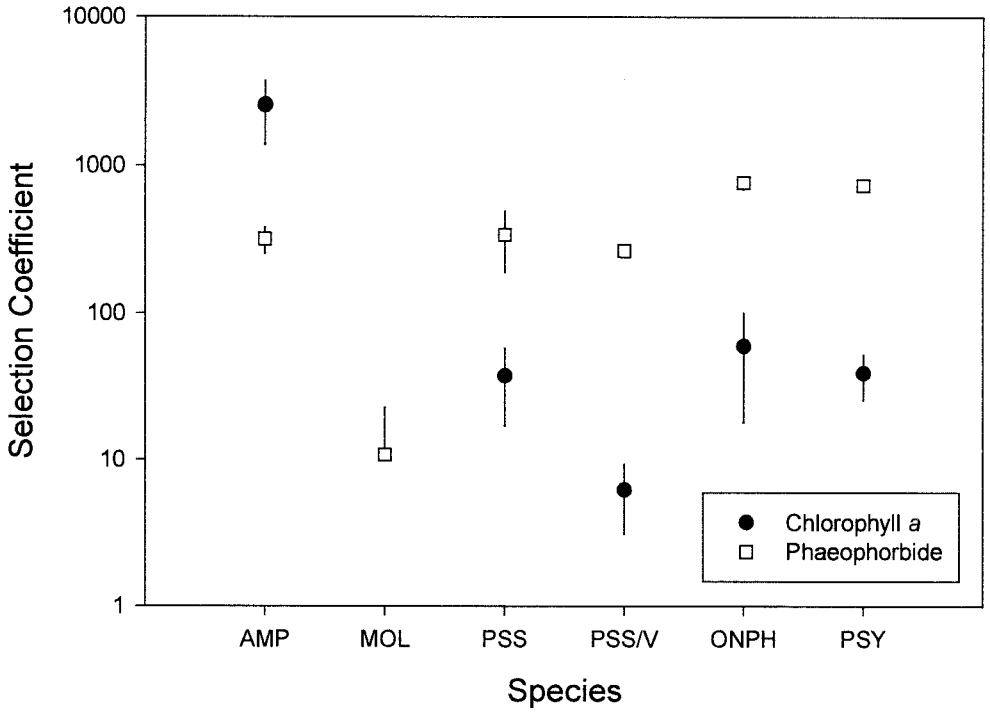

Figure 5.11. Mean selection coefficients (± 95% confidence) for chlorophyll *a* and phaeophorbides for six species of abyssal holothurian. AMP, *Amperima rosea*; MOL, *Molpadia blakei*; PSS, *Pseudostichopus* sp.; PSS/V, *Pseudostichopus villosus*; ONPH, *Oneirophanta mutabilis*; PSY, *Psychropotes longicauda.*

Two-sample T-tests showed no significant difference in the chlorophyll *a* selection coefficients of *Pseudostichopus* sp., *O. mutabilis* and *P. longicauda.* However, all three species did have significantly higher selection coefficients than *M. blakei* and *P. villosus* and were all significantly lower than the selection coefficient for *A. rosea. M. blakei* had a significantly lower selection coefficient for phaeophorbide than any of the other five species. *O. mutabilis* and *P. longicauda* both had significantly higher selection coefficients than the other four species, but there was no significant difference between the two species. There was also no significant difference in phaeophorbide selection coefficient between *A. rosea*, *Pseudostichopus* sp. and *P. villosus.*

5.4 Discussion

The PAP site is thought to receive a large phytodetritus fall every year with the main pulse arriving at the seafloor between May and June, where it remains recognisable until October (Rice *et al.*, 1994). However, this flux has been shown to be variable both in timing (Lampitt *et al.*, 2001) and in the qualitative characteristics of the phytodetritus (Fabiano *et al.*, 2001; Kiriakoulakis *et al.*, 2001; Witbaard *et al.*, 2001). It is likely that the seasonal and inter-annual variability in the quality and availability of food will influence the benthic fauna, affecting its composition as well as the feeding and life-history strategies of the individual species. The pulsed and unpredictable character of the food supply to the deep-sea benthos appears to favour large, mobile organisms such as the holothurians that seem to exploit rapidly any patches of deposited phytodetritus.

The presence of chlorophyll *a* in the gut sediments of five out of the six species indicates a shared preference for the selection of 'fresh' phytodetrital material. *Amperima rosea* had, by an order of magnitude, the greatest concentration of chlorophyll *a* in its gut. The chlorophyll *a* :phaeophorbide ratio for the gut contents of each species (Figure 5.6) shows a significant selection for fresh material by *A. rosea*. Its ratio value is more similar to that recorded for material in sediment traps than to the other holothurian species. The selection co-efficients (calculated using sediment data from Witbaard *et al.*, 2000) again highlight the difference between *A. rosea* and the other species in terms of selection for fresh phytodetrital material. Selection co-efficients calculated in this study were significantly greater than those quoted for other deep-sea holothurians (Khripounoff and Sibuet, 1980; Sibuet *et al.*, 1982; Billett *et al.*, 1988; Witbaard *et al.*, 2001). The previously highest recorded selection coefficient is that for *Pannychia moseleyi*, a bathyal elasipodid from the Santa Catalina Basin, NW Pacific. Miller *et al.* (2000) calculated a 500-fold chlorophyll *a* 'enrichment factor' for this species, with accompanying chlorophyll *a* concentrations in the gut of 200.77 ±71.90 μg gDW^{-1}.

Witbaard *et al.* (2001) calculated a mean 16-fold chlorophyll *a* 'concentration factor' for *Oneirophanta mutabilis* on the PAP. Their value, based on samples taken during the '*Amperima* Event' (1996-1998), is considerably less than the selection coefficient of 60 calculated during this study. Witbaard *et al.* claim that the 'magnitude' of their

'concentration factors' demonstrate that *O. mutabilis* is a selective deposit feeder. Additional seasonal fluctuations in this value suggest that its selectivity is related to the 'richness' of the surficial sediment. In general, if a surface deposit-feeder actively searches out enriched patches on an otherwise nutritionally-poor sediment surface, the phytopigment concentrations in its gut contents would be higher than the mean sediment concentrations, and the observed selection coefficients would be expected to rise. Conversely, when phytodetritus is fresh and more evenly distributed across the seafloor, it would be expected that concentrations in the gut and in the surficial sediments would converge, decreasing the value of the selection coefficient. Data presented by (Witbaard *et al.*, 2001) show a reverse trend with low selection coefficients during food-poor periods. This led them to conclude that *O. mutabilis* is feeding continuously without searching for phytodetritus 'hotspots'. Billett *et al.* (1988) also noted how the selection coefficient for *Benthogone rosea*, and presumably other epibenthic holothurians, changes on a seasonal basis related to the annual cycle in the deposition of phytodetritus.

If this were true for *O. mutabilis*, then a high selection coefficient would appear to indicate the presence of large quantities of 'fresh' phytodetritus on the seafloor during late 2000. Even allowing for the fact that the selection coefficients calculated in this study used sediment data from the 1996-98 period, the value is still double that recorded during the 'food-rich' period of late 1996. If we subsequently use the value given by Witbaard *et al.* (2001) for chlorophyll *a* concentrations in the surficial sediment during September 1996 (0.0326μg gDW^{-1}), we get a selection coefficient for *O. mutabilis* of 36, which is similar to the 'concentration factor' of 30 calculated using Witbaard *et al.* (2001) gut values from 1996. Based on this comparison of selection coefficients for *O. mutabilis* it is possible to consider that the benthic environment in which the holothurians were feeding in October 2000 was similar, in terms of 'food-richness', to that of September 1996. If this were true we could expect the selection coefficients shown in Figure 5.11 to be reduced by approximately one half.

Even allowing for this adjustment the chlorophyll *a* selection coefficient of *A. rosea* would still be approximately 1500. This figure is still significantly different higher than the 558 recorded for *P. moseleyi* (Miller *et al.*, 2000). It is clear that *A. rosea* is highly selective for fresh phytodetritus compared to other deep-sea holothurians.

With the exception of *A. rosea*, all the species studied showed greater selection for phaeophorbides than chlorophyll *a*. Chlorophyll *a*:phaeophorbide ratios for all six species surprisingly show *Pseudostichopus* sp. to have a higher value than either *O. mutabilis* or *P. longicauda*. However, when this result is considered in the context of the selection coefficient and mean gut pigment concentrations of each species, it is clear that this value is influenced by a reduced selection for phaeophorbides, rather than an increased selection for 'chlorophyll *a* rich' fresh material by *Pseudostichopus* sp. Unlike the findings of Witbaard *et al.* (2001), the presence of chlorophyll *c*1 and *c*2 was recorded in the guts of not only *O. mutabilis*, but also *P. longicauda* and *P. villosus*. Chlorophyll *c*1*c*2 is seen as an indicator of fresh material, yet *A. rosea* showed no trace of this pigment in its gut contents, in spite of high concentrations of chlorophyll *a*. All the recorded values of chlorophyll *c*1*c*2 from all three species were low ($\leq 1.2\mu g\ gDW^{-1}$), yet its presence in fresh phytodetritus and subsequent absence in the gut of *A. rosea* may indicate an even finer level of selection among species.

Both *O. mutabilis* and *P. longicauda* exhibited the greatest selectivity for phaeophorbides and, in addition, both species had the highest concentrations of chlorophyll degradation products in their guts (see figure 5.7). *A. rosea* has been shown to be highly selective for chlorophyll *a*, possibly 'enforcing' a greater selection for its degradation products by other species as a result of their high abundance and ability to track large areas of seafloor in a relatively short time (Chapter 4; Bett *et al.*, 2001). There is certainly significant variation in the flux of chlorophyll *a* to the PAP, on a seasonal basis, and also inter-annually (at least between 1996-97 and 1997-98 (Fabiano *et al.*, 2001)). The qualitative and quantitative among-species differences in gut chloropigments indicate a degree of selectivity by each individual species that could be related to their specific mode of feeding and arrangement and structure of the tentacles, as suggested by Roberts *et al.* (1988) and Moore *et al.* (1995). Figures 5.12 and 5.13 show the arrangement of the oral tentacles of each of the species under consideration.

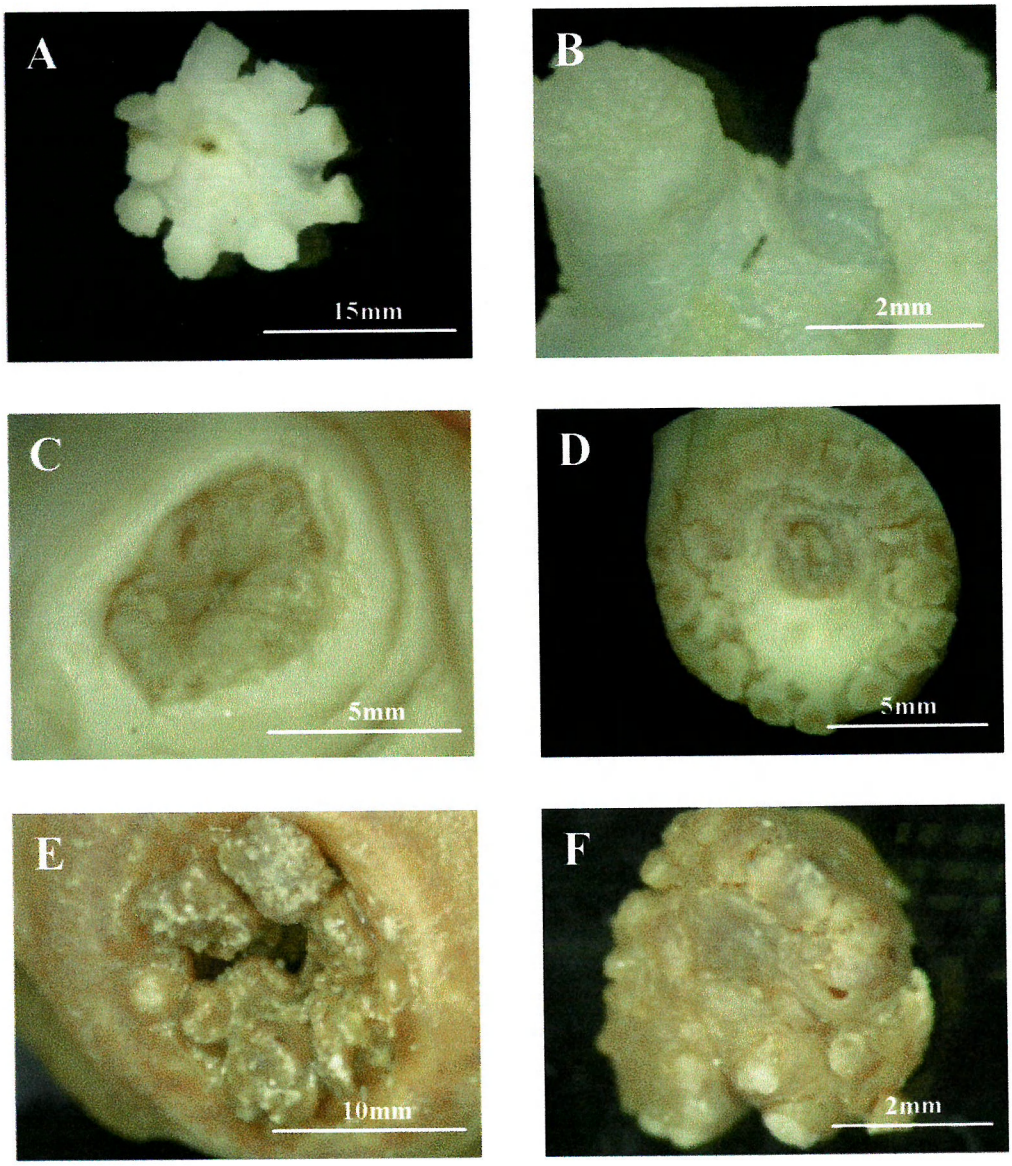

Figure 5.12. Arrangement of oral tentacles in abyssal holothurians. A. *Amperima rosea*, crown of oral tentacles; B. *A. rosea*, enlargement of tentacle surface; C. *Molpadia blakei*, retracted crown of oral tentacles; D. *Pseudostichopus* sp., everted crown of oral tentacles; E. *Pseudostichopus villosus*, partially retracted crown of oral tentacles; F. *P. villosus*, enlargement of tentacle surface.

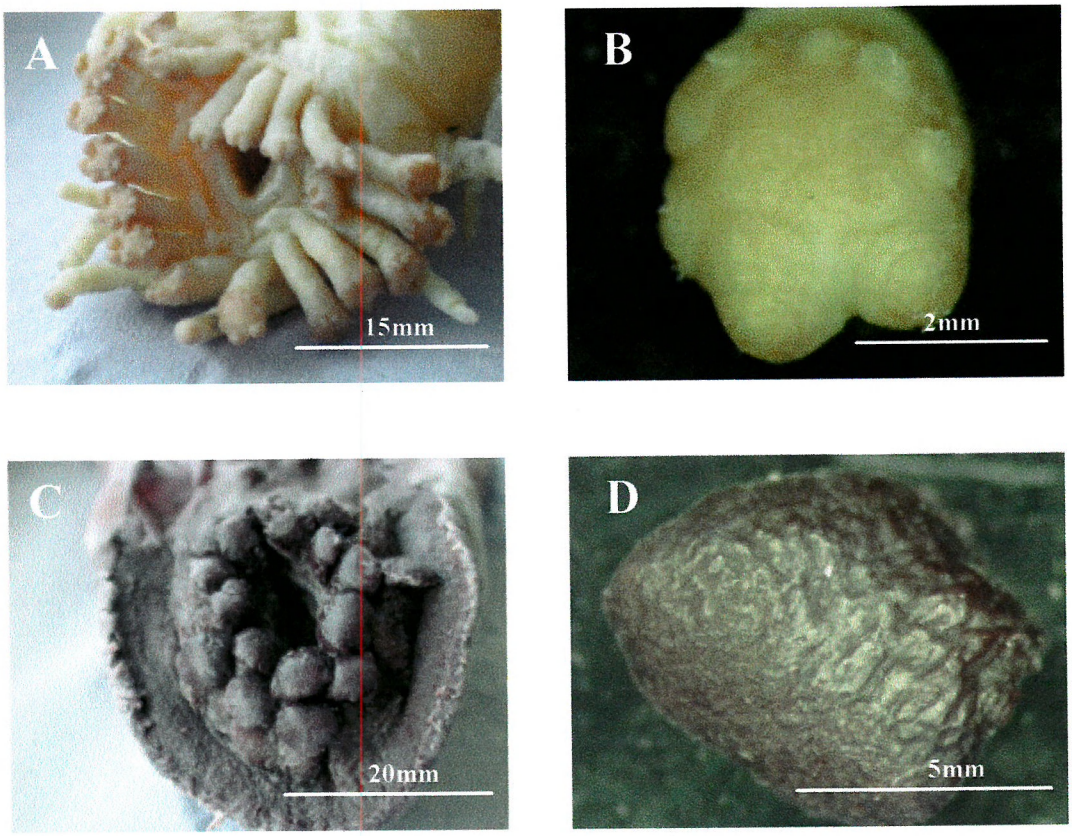

Figure 5.13. Arrangement of oral tentacles in abyssal holothurians. A. *Oneirophanta mutabilis*, crown of oral tentacles; B. *O. mutabilis*, enlargement of tentacle surface; C. *Psychropotes longicauda*, crown of oral tentacles; D. *P. longicauda*, enlargement of tentacle surface.

Amperima rosea possesses a crown of ten, peltate, oral tentacles (figure 5.12 A, B). *Mopladia blakei* possesses digitate tentacles whilst *Pseudostichopus* sp. and *P. villosus* have a more intermediate pelto-digitate tentacle (figure 5.12 C-F). *Oneirophanta mutabilis* has twenty, large, digitate and non-retractable tentacles with which it is thought to 'rake' the sediment surface for finer particles. In contrast *P. longicauda* has eighteen, large peltate tentacles with which it 'sweeps' the surficial sediment for organic material (Roberts *et al.*, 2000). *M. blakei* is assumed to be a head-down infaunal feeder, inhabiting a burrow and feeding approximately 6-7cm down in the sediment, as has been observed for *Molpadia oolitica* in shallow-water (Rhoads and Young, 1971). At that depth in the sediment it ingests mainly refractory chloropigments (see figure 5.5). It is probably important to mention that it is difficult to accurately assess the selectivity of infaunal deposit feeders, such as *M. blakei*, when we are comparing their gut contents to surficial

sediments. Miller *et al.* (2000) found that the mean gut ^{234}Th activity of a molpadiid holothurian from the Santa Catalina Basin increased significantly when compared to the sediments at 6-7cm depth where it would have actually been feeding.

Recalculating the phaeophorbide selection coefficient for *M. blakei*, using pigment concentrations from the sediment at 5cm depth (Witbaard *et al.*, 2001), increases the value from 11 to 87. Therefore, *M. blakei* shows a greater selectivity for phaeophorbides when comparisons are made using sediment concentrations from a depth at which it would actually be feeding, Diversity in tentacle structure and mode of feeding may allow a degree of selectivity, but as yet there is no correlation between tentacle structure and the gut contents of deep-sea holothurians. Both *Pseudostichopus* sp. and *P. villosus* possess pelto-digitate tentacles yet their pigment chromatograms had remarkably different profiles. *P. villosus* had a lower concentration of chlorophyll *a* indicating ingestion of less fresh material. This may be related to the almost sub-surface mode of deposit feeding observed in *P. villosus*. Time-lapse photography shows *P. villosus* 'ploughing' a deep furrow through the sediment (Billett, 1991; pers. obs), possibly disturbing much of the fresher phytodetritus in the surficial sediments and ingesting sediment from 1-2cm depth. Roberts *et al.* (1995) found an increased number of harpaticoids in the guts of *P. villosus*, compared to other species, a further indication of sub-surface deposit feeding. The 'longer', 'finer' tentacles of *A. rosea* and *O. mutabilis* may allow them to 'select' and manipulate larger aggregates of phytodetritus which the tentacles of the other surface deposit feeders would find hard to pick up. The variability in activity rates of different holothurian species may also influence their particle selection. Those species exhibiting higher rates of activity may be able to utilise a freshly deposited resource before it becomes mixed into the deeper surface sediments by the activity of other benthic fauna.

The use of carotenoid pigments as biomarkers for different algal groups has been well exploited in studies of upper ocean algal diversity and variability of biomass (Gibb *et al.*, 2000), but its potential for assessing selectivity in deep-sea deposit feeders has not currently been exploited. Quantitative and qualitative among-species differences in carotenoid pigments, calculated during this study, show a similar pattern to that of the chloropigments. Of the nine identifiable pigments, only *P. villosus*, *O. mutabilis* and *P. longicauda* had all of them present in their gut. In these three species the major pigments were peridinin, fucoxanthin and 19' hexanoyloxyfucoxanthin (19'hex), all major

taxonomic pigments of dinoflagellates (Jeffrey *et al.*, 1997). In contrast, fucoxanthin and 19'hex are minor pigments (low concentrations) in the gut of *A. rosea*, while peridinin is absent. As peridinin is only found in dinoflagellates (Jeffrey *et al.*, 1997) it is unlikely that *A. rosea* is selecting this fraction of the phytodetritus. Fucoxanthin and 19'hex can both be found in the prymnesiophytes (coccolithophores) and chlorophylls *c*1 and *c*2 are major diatom pigments. The absence of chlorophyll *c*1*c*2 from the guts of *A. rosea* and *Pseudostichopus* sp. indicates an additional lack of selection for this fraction of the phytodetritus. The lower concentrations of chlorophyll *c*1*c*2 found in the gut of *P. villosus*, compared to *O. mutabilis* and *P. longicauda*, may reflect feeding on particles mixed deeper in the sediment and therefore less abundant and readily available.

The dominant pigment in the gut of *A. rosea* is zeaxanthin. This pigment is present only in minute quantities in the guts of other species. Zeaxanthin is the major taxonomic pigment of cyanobacteria. Cyanobacteria are important primary producers in the surface waters of the northeast Atlantic, mostly during the summer months and not at high latitudes. Cyanobacteria have been found in the phytodetritus sinking and settling in the abyssal northeast Atlantic, often attached to aggregates (Lochte and Turley, 1988) or incorporated into salp faeces (Pfannkuche and Lochte, 1993). The cyanobacteria found on the abyssal seafloor of the northeast Atlantic resemble *Synechococcus* sp., a common species in the surface waters of the northeast Atlantic (Zubkov *et al.*, 2000). Results from the 20°W northeast Atlantic transect, between 62 and 37°N, show that in colder, northern waters, prymnesiophytes and diatoms dominate the algal biomass, where as to the south the cyanobacteria dominate (Gibb *et al.*, 2001). Pfannkuche and Lochte (1993) also reported a decrease in cyanobacteria biomass at high latitudes.

Pfannkuche and Lochte (1993) noted how the sedimenting detritus after a salp swarm contained high levels of cyanobacteria and its accompanying pigment zeaxanthin. They noted that the detritus was different in appearance to the amorphous 'fluffy' detritus typical of a sedimenting algal bloom. Cyanobacterial cells do not readily sink on their own, therefore the activity of salps in the surface waters could feasibly affect the composition of phytodetritus reaching the abyssal seafloor. Salp blooms will exert grazing pressure on phytoplankton, preventing large blooms and therefore large fluxes of amorphous aggregates ('marine snow'). If salps bloom, then the amount of cyanobacteria reaching the seafloor may outweigh the flux of diatoms, dinoflagellates and

prymnesiophytes. This would alter the composition of the food available to deposit feeders and thus the pigment profiles of their gut contents would also differ.

The possibility of *A. rosea* feeding on a mainly bacterial food source is not unreasonable. Pfannkuche and Lochte (1993) found cyanobacterial cells in the gut contents of both *O. mutabilis* and *P. longicauda*, and both species have small amounts of zeaxanthin present in their guts. Therefore cyanobacteria is ingested by abyssal holothurians on the PAP. Other elpidiid holothurians have also been observed feeding on bacterial mats at abyssal depths (Juniper and Sibuet, 1987; Olu *et al.*, 1996). *A. rosea* has been shown to have significant qualitative and quantitative differences in pigment profile from the other major abyssal holothurian species on the PAP. The fact that the concentration of chlorophyll a in its gut was significantly greater than any other species (so far recorded) is a clear indication of its selection for freshly deposited phytodetritus. This is supported by the work of Iken *et al.* (2001) that shows close isotopic links ($\delta^{15}N$) between phytodetritus and *A. rosea* (see figure 5.14). Microscopic analysis also showed a high proportion of fresh material in the guts of *A. rosea*.

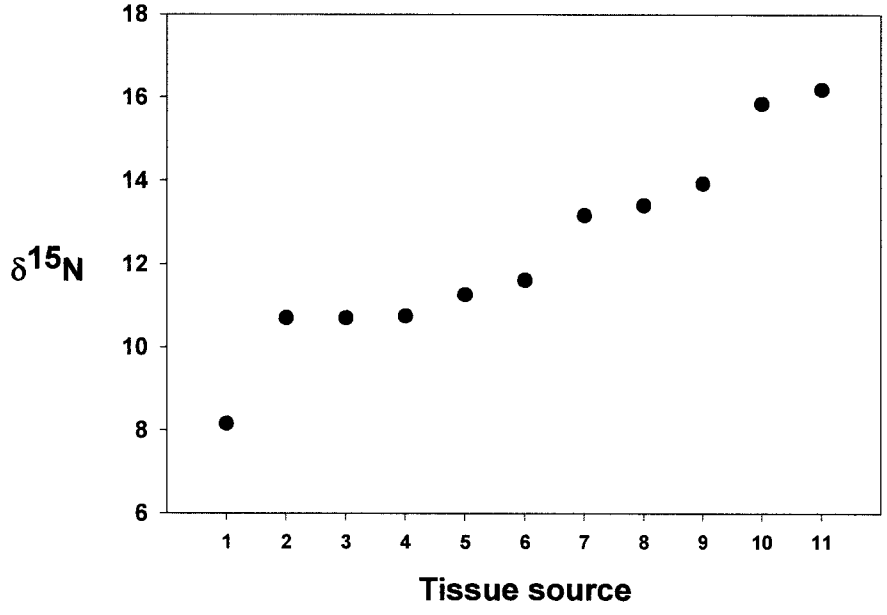

Figure 5.14. Stable isotope analysis ($\delta^{15}N$) of phytodetritus and fauna from the PAP, Northeast Atlantic (drawn using data from Iken *et al.* (2001)). 1, phytodetritus; 2, foraminifera; 3, *Ophiocten hastatum*; 4, *Amperima rosea*; 5, *Ellipinion molle*; 6, *Peniagone diaphana*; 7, *Oneirophanta mutabilis*; 8, *Pseudostichopus* sp.; 9, *Psychropotes longicauda*; 10, *Paroriza prouhoi*; 11, *Pseudostichopus villosus*.

Amperima rosea is grouped closely (isotopically) with both the foraminifera and the ophiuroid *Ophicoten hastatum*, of which the foraminifera have been shown to respond rapidly to the deposition of fresh phytodetritus (Gooday, 1988) and *Ophiocten hastatum* has also increased dramatically in abundance during the '*Amperima* Event'(1996-98) (Bett *et al.*, 2001).

A. rosea also has significantly greater concentrations of β-carotene, another important carotenoid pigment, in its gut than the other five species. β-carotene can be found in most algae, but along with chlorophyll *a* and zeaxanthin it is a major pigment of cyanobacteria (Jeffrey *et al.*, 1997). This is an additional indication of the potential importance of cyanobacteria in the diet of *A. rosea*. β-carotene has been shown to be a common carotenoid pigment in oocytes, of both invertebrates and vertebrates. Bandaranayake and Des Rocher (1999) found that the shallow-water holothurian, *Holothuria atra*, may provide specific carotenoids to the ovaries by metabolising β-carotene and/or zeaxanthin to the ketocarotenoids canthaxanthin and astaxanthin. The authors also reported that ~90% of the total carotenoids in the body wall and ovaries of *H. atra* are highly oxidised, the main components being astaxanthin and canthaxanthin. Interestingly the analysis of carotenoids, in the body wall and gut of *A. rosea*, has shown the presence of astaxanthin, canthaxanthin and zeaxanthin (Neto pers. comm.). A more detailed analysis by Neto and co-workers has shown that the dominant pigments in *A. rosea* are carotenoid esters. To date one of these has been identified as 6-hydroxysiphonixanthin-trans-Δ2-dodecenoate on the basis of its mass spectrum and collision induced fragmentation. This particular compound has so far only been found in prasinophytes (Egeland *et al.*, 1997). In addition, the major pigments of the prasinophyceae (green-algae) are chlorophyll *a*, β-carotene and zeaxanthin (Jeffrey *et al.*, 1997), again the three pigments found in high concentrations in the gut of *A. rosea*. *Amperima* may be feeding on the detrital remains of both cyanobacteria and prasinophytes, although it is cyanobacteria that is the more frequently recorded class in the waters of the north Atlantic (Zubkov *et al.*, 2000) and therefore more likely to provide the majority of organic material for *A. rosea* feeding on the abyssal seafloor. Although the presence of dominant pigments from the prasinophyceae may also provide a link between variability of *A. rosea* abundance and variability in the composition of the micro- and picophytoplankton of the surface waters.

Variability in the latitudinal distribution of cyanobacteria (particularly *Synechococcus* sp.) has been shown by Zubkov *et al.* (2000). During spring 1997 there was a high abundance of this species between 40 and 50°N in the northeast Atlantic (see figure 5.15). A May peak was also recorded at 44°N with an additional July peak at 47°N.

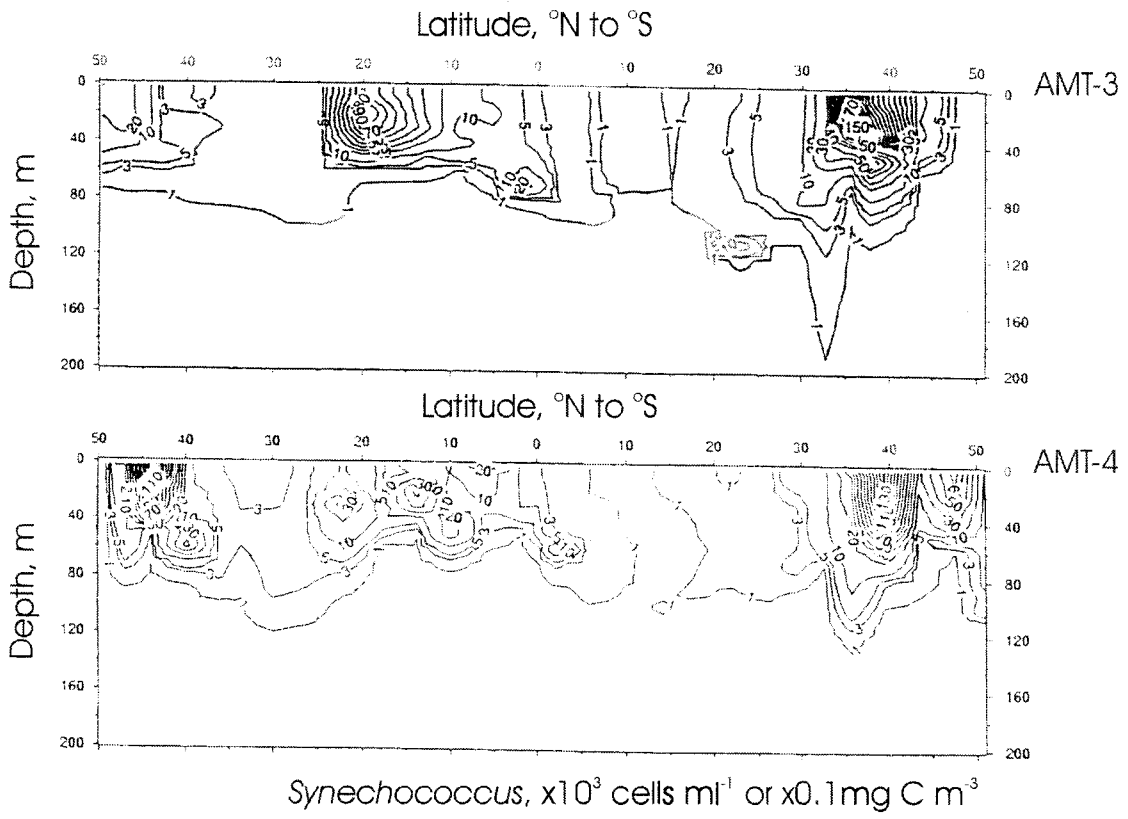

Figure 5.15. Latitudinal distribution of the cell concentrations of *Synechococcus* sp. (cyanobacteria) derived from counts in samples from AMT-3 (Sept-Oct 1996) and AMT-4 (April-May 1997). In each case the contour lines also represent biomass values, since the cells of each type are regarded as having a characteristic carbon content. Figure adapted from Fig. 4 in Zubkov *et al.* (2000).

The predominance of cyanobacteria pigments in the gut of *A. rosea*, coupled with the evidence for their metabolites being incorporated into body tissues and the potential for those metabolites to have a reproductive role in the ovaries, highlights a potential link between changes in *A. rosea* abundance and the variability in surface water biomass of cyanobacteria. The marine phytoplankton of the Northeast Atlantic, between 40-50°N, is usually dominated by diatoms and dinoflagellates, this being particularly obvious during the period May 1996 to October 1997 (Gibb *et al.*, 2000). The pigment biomarkers of these algae, fucoxanthin, 19'hex and peridinin, were found in the guts of the majority of

species and, with the exception of *A. rosea*, they are the dominant pigments in the guts of the surface deposit feeding holothurians. If we are to believe that the differences in gut pigment profiles observed in October 2000 are representative of differences among species in other years, then the increased biomass of *Synechococcus* sp. observed in spring 1997 may have provided a nutritionally beneficial food source to both the adults and new recruits of *A. rosea* on the PAP.

Ultimately the observed among-species differences in selectivity for both chloropigments and carotenoids may go some way to explaining, or at least helping to interpret, the observed diversity of both holothurians and other abyssal deposit feeders. The ability of different species to feed selectively on separate fractions of the same phytodetrital food source may allow large numbers of species to co-exist in the same food-limited ecosystem.

Chapter Six – Genetic variability of *Amperima rosea* on the Porcupine Abyssal Plain

6.1. Introduction

6.1.1. Genetic variation in deep-sea organisms.

Current genetic data on deep-sea organisms are based mainly on allozyme studies (see section 6.1.2 for explanation of method), as other types of genetic data are still rare for deep-sea organisms. The subsequent use of 16S mitochondrial DNA sequences to analyse genetic variability in *Amperima rosea* is one of the few studies of a deep-sea invertebrate to utilise this more complex technique, and is the first for a deep-sea holothurian.

The neutral theory of molecular evolution (Kimura, 1983) states that high levels of genetic variability should be related either to high mutation rates or temporally-stable population sizes. Redfield *et al.* (1980) predicted that deep-sea organisms would have heterozygosity similar to that of shallow-water, low latitude organisms, and a higher heterozygosity than high latitude species. Creasey and Rogers (1999) examined the relationship between heterozygosity and protein polymorphism and concluded that it could be explained by the neutral theory. Therefore, for deep-sea organisms the majority of evolutionary changes and variability at the molecular level are the result of random genetic drift of neutral or nearly neutral alleles.

The life-history strategies of deep-sea organisms may also be important in the level of heterozygosity in a population. Smith and Fujio (1982) reported that observed heterozygosities in deep-sea fish were lower than in shallow-water species and were typically of an intermediate value for marine teleosts. This lead to the hypothesis that deep-sea fish exhibited a habitat generalist life history. This hypothesis states that low heterozygosities would be observed in habitat generalists (i.e. the rat-tail *Coryphaenoides rupestris*), and higher heterozygosities in habitat specialists (i.e. hydrothermal vent endemic species). Small effective population size has also been cited as a potential cause of low heterozygosity in populations (Nei *et al.*, 1975; Gilpin, 1991; McCauley, 1991). If a population is subject to frequent bottlenecks or extinctions, then the number of alleles

within the population may be reduced in proportion to the severity of the bottleneck (Nei *et al.*, 1975). Even where re-colonisation from other populations is immediate, the probability of successful establishment of a persisting population may be low (i.e. high immigration does not guarantee successful recolonisation; (McCauley, 1991)). Smith and Fujio (1982) stated that heterozygosity varied with egg type in teleost fish, but because of low sample sizes no significant comparisons could be made between reproductive strategy and heterozygosity. Crisp (1978) examined the relationship between reproductive strategy and observable genetic consequences, and concluded that species with temporally and spatially large populations, which exhibited high fecundity and planktonic dispersal, should exhibit high genetic variability. Conversely, species with lower dispersal and fecundity should exhibit reduced genetic diversity.

There have been numerous studies of spatial patterns of genetic variation (differentiation) in shallow-water marine environments (Nishida and Lucas, 1988; Fevolden, 1992; Saavedra *et al.*, 1993). In contrast, there are still very few corresponding studies on deep-sea organisms (e.g. Doyle, 1972; Hensley *et al.*, 1995; Creasey *et al.*, 1997; Rogers *et al.*, 1998). The studies to date are mainly on commercially important vertebrates from less than 1000m depth (e.g. Smith, 1986). However, recent studies have begun to focus on the degree of spatial genetic variation in hydrothermal vent organisms (reviewed by Creasey and Rogers, 1999). Hydrothermal vents are effectively islands of suitable habitat, for vent-endemic species, organised along linear ridge systems with geographic barriers in the form of transform faults on a scale of hundreds of kilometres and major geographic barriers between different ridge systems. They are therefore ideal for the study of the relationships between genetic differentiation and geographic separation of populations.

One of the first population genetic studies on a deep-sea species was conducted on the ophiuroid *Ophiomusium lymani* from the Hatteras submarine canyon in the northwest Atlantic (Doyle, 1972). This study utilised a single enzyme system (esterase) to examine genetic differentiation between populations both along and among different isobaths. The author concluded that genotype frequencies for esterase in *O. lymani* were heterogenous between bathymetrically segregated (range 1700 to 2700m depth) populations separated

by a horizontal distance of 8km. In contrast, genotype frequencies were homogenous between populations separated by over 200km along isobaths. Interpretations of these genotype frequencies are equivocal, as individuals from the deepest station were sampled in a different year to those at the other three stations. *Ophiomusium lymani* is known to show seasonal recruitment in some regions (Gage and Tyler, 1982), and the individuals in deep samples may represent a different cohort from those taken at the shallower sites. Subsequent allozyme studies by Hensley *et al.* (1995) revealed little genetic differentiation between populations of *O. lymani*. This is despite the fact that samples were taken from a similar range of depths to that of Doyle (1972). *Ophiomusium lymani* has abbreviated lecithotrophic development (Gage and Tyler, 1982; Hensley *et al.*, 1995), and in both studies there is a sufficient level of gene flow to maintain panmixia over hundreds of kilometres.

Molluscs have proved a popular focus of many deep-sea studies, often providing a model for increased diversity in the deep sea through the discovery of cryptic species (Chase *et al.* 1998; Etter *et al.*, 1999). Quattro *et al.* (2001) have used 16S sequence analysis to discover three phylogenetically distinct clades of the deep-sea gastropod *Frigidoalvania brychia* at stations within an 80km radius. It was suggested that this could result from high levels of intraspecific divergence or from the presence of morphologically cryptic species. Etter *et al.* (1997) reported strong genetic differentiation, using DNA sequencing, between populations of gastropods and bivalves from slope and abyssal depths. This genetic differentiation is reflected in morphological variation in gastropods along a similar depth gradient (Etter and Rex, 1990). Whether this variation is intra- or interspecific is uncertain at the present time. In some species of deep-sea invertebrates inhabiting slope and abyssal habitats, it appears that high levels of gene flow are maintained between populations (e.g. Hensley *et al.*, 1995). However, unexpected levels of genetic and morphological differentiation have been found between populations separated by depth (see Creasey *et al.*, 1997), and in most of these cases it is probable that different depths are inhabited by morphologically similar but genetically distinct species. This would imply that depth, in terms of its accompanying physicochemical and ecological variables, might play a significant role in speciation through bathymetric

allopatry (White, 1987). In some cases it is possible that horizontal allopatric speciation has occurred with species distributions later extending to occupy the same geographic area, though they remain separated bathymetrically. The unexpectedly high levels of genetic differentiation, over small geographic distances, between conspecific populations, located on the continental slope (e.g. Creasey *et al.*, 1997) may be relevant in terms of microevolutionary processes.

In the spider crab *Encephaloides armstrongi*, which was sampled from the oxygen-minimum zone in the Arabian Sea, high levels of genetic differentiation were explained by extreme topography, gradients in physiochemical variables, life history and behaviour (Creasey *et al.*, 1997). Also, in the deep sea, varying scales of temporal and spatial patchiness occur. These may range from the local sphere of influence of an organism up to large-scale disturbances such as food falls and benthic storms (Hollister and McCave, 1984; Gage, 1996). This patchiness occurs with random low-intensity recruitment from a system with few apparent barriers to dispersal (Grassle and Maciolek, 1992; Gage, 1996), which in itself may lead to unexpected patterns of spatial genetic variation in deep-sea species.

6.1.2. Methods of determining population structure and genetic differentiation.

There are a number of techniques that can be employed to determine levels of population and genetic variation. These include: allozyme electrophoresis; DNA fingerprinting (multilocus and single-locus microsatellite analysis); random amplified polymorphic DNA (RAPD) analysis and DNA sequencing. For this study of *Amperima rosea* it was initially planned to use allozyme electrophoresis but when this failed to produce any results the more complex, DNA sequencing approach was undertaken. Therefore the basic principles of each technique are described below.

6.1.2.1. Allozyme electrophoresis.

During the past 30 years, electrophoresis has been the most widely used tool in determining the population structure and level of genetic differentiation in deep-sea organisms. This is largely because of its ease of use, relatively inexpensive cost and the reliability of the method for the wide variety of different organisms and enzymes screened. Allozyme electrophoresis allows differentiation of intra- and interspecific populations. The major problem of this technique, as discussed by Thorpe and Sole-Cava (1994), is that it requires fresh or recently frozen tissue.

Allozyme electrophoresis relies upon the selective migration of colloidal particles (e.g. proteins) under an electric potential. Different alleles of an enzyme will move across a gel (either starch or cellulose acetate) according to electric charge and molecular size. This will allow mutations (rare alleles) to be observed at different distances along the gel to the more common alleles. The correct application of allozyme data requires that banding patterns observed on gels are correctly interpreted. The most basic assumption made is that changes in the mobility of enzymes in an electric field reflect changes in the encoding DNA sequence. Therefore, if the banding patterns of two individuals differ, it is assumed that these differences are genetically based and heritable (Hillis *et al.*, 1996). Other main assumptions, as stated by Thorpe and Sole-Cava (1994), are that gene frequencies are not altered by postzygotic selection and are approximately in Hardy-Weinberg equilibrium. Given these two criteria, any degree of significant differentiation at a gene locus may represent some degree of reproductive isolation.

Population geneticists have developed many statistical models for interpreting genetic population structure (see review in Hillis *et al.*, 1996). The most relevant of these to gel interpretation is the Hardy-Weinberg equilbrium principle. This states that in the absence of selection, drift, and migration, the frequencies of alleles in a randomly mating population will maintain a stable equilibrium with genotype frequencies of $AA = p^2$, $Aa = 2pq$, and $aa = q^2$, where p is the frequency of allele A, and q the frequency of the alternative allele a (Hillis *et al.*, 1996). Nonconformity to the prediction of Hardy-Weinberg equilibrium indicates that the phenotypic variation has a nongenetic basis or

that one of the Hardy-Weinberg assumptions is not met within the population. For example, the individuals may not be randomly mating, some natural selective force may be acting on the species, or genes from neighbouring populations may be migrating into the study site.

The first recorded use of allozyme electrophoresis on a deep-sea species was on the pogonophoran *Siboglinum atlanticum* (Manwell and Baker, 1970). It has subsequently been used in numerous studies of deep-sea taxa, including deep-sea holothurians (Bisol *et al.*, 1984).

6.1.2.2. DNA Sequencing.

Nucleic acid sequencing can be carried out by three possible methods: cloning, *in vitro* amplification and RNA isolation (see Hillis *et al.*, 1996). Of these three methods, *in vitro* amplification of mitochondrial DNA by the Polymerase Chain Reaction (PCR) technique is the most frequently used in population genetic studies. Starting with virtually any amount of DNA, it is possible to amplify a target sequence, which (if the specimen was homozygous at the target locus and the amplified fragment is relatively short) can be sequenced directly. Under the PCR procedure, DNA is denatured into its respective strands by heating, and oligonucleotide primers, that are complementary to opposite strands of the target sequence, are annealed (Avise, 1994). Primer extension then occurs, whereby complementary strands to the DNA between the primers are synthesised by a thermostable DNA polymerase from the thermophilic bacterium *Thermus aquaticus* (*Taq* polymerase). Repeated heating and cooling, in the thermal block of a PCR machine (e.g. Perkin Elmer 480 Thermal Cycler), allow the process to be repeated and relatively large amounts of DNA to be replicated.

The PCR reaction involves the addition of certain buffers, in addition to the *Taq* polymerase, to the DNA template. Among the parameters that can be varied to optimise amplification are the concentrations of DNA template (10ng μl^{-1} is often a good concentration to aim for), primers, dNTPs, $MgCl_2$, *Taq* polymerase, and the addition of

Qiagen 'Q-solution'™. The length and temperature of the annealing cycle can also be varied to optimise the PCR reaction.

DNA sequencing provides a resolution appropriate to phylogenetic and population-level studies (depending on the variability of the region being used (Hillis *et al.*, 1996)) on deep-sea organisms. The availability of universal primers, especially for the frequently used 16S and Cytochrome oxidase (CO1) regions of mitochondrial DNA (mtDNA), are particularly useful as they may be applied to studies of deep-sea taxa for which there are no previous molecular genetic data (e.g. Shank *et al.*, 1998b). Studies which have been undertaken using sequencing upon deep-sea organisms include: the Orange Roughy (*Hoplostethus atlanticus*; (Baker *et al.*, 1995)); bresiliid shrimps (*Rimicaris exoculata*; (Shank *et al.*, 1998b)); vestimentiferans (*Lamellibranchia* sp., *Ridgeia* sp., and *Tevnia jerichonana*; (Williams *et al.*, 1993; Black *et al.*, 1997; Kojima *et al.*, 1997; Black *et al.*, 1998)); and molluscs (*Calyptogena* spp., *Ectenagena extenta* and *Vesicomya* spp.; (Peek *et al.*, 1997)).

6.2. Brief explanation of methods.

6.2.1. Allozyme (starch-gel) electrophoresis.

See appendix III for starch-gel electrophoresis protocol.

6.2.2. DNA extraction, amplification and sequencing.

DNA is usually extracted from tissue, stored in 100% molecular grade Ethanol, using the Phenol-Chloroform technique. This is a long procedure designed to extract, precipitate and elute the DNA, leaving a 'pellet' of DNA that is then re-suspended and stored in a buffer solution. Unfortunately this method was not successful in extracting DNA from the *Amperima rosea* tissue collected during April 1999 and October 2000. A more expensive method was employed, using commercially available kits (DNeasy™ Tissue Kit; Qiagen) to extract the DNA from the tissue. Although much more successful than the

standard phenol-chloroform protocol the DNA extracted from the *Amperima* tissue was still fairly degraded.

Extracted DNA can be visualised and quantified by running the template on an agarose gel. The gel is impregnated with Ethidium Bromide, which binds with the DNA and then fluoresces under UV illumination. The DNA extracted from each individual is quantified by comparing it to a known DNA standard or 'ladder' that appears on the gel as a series of bands of varying DNA concentrations (see Figure 6.1). The DNA is quantified using the UviDoc system in conjunction with Uvisoft DNA quantification software.

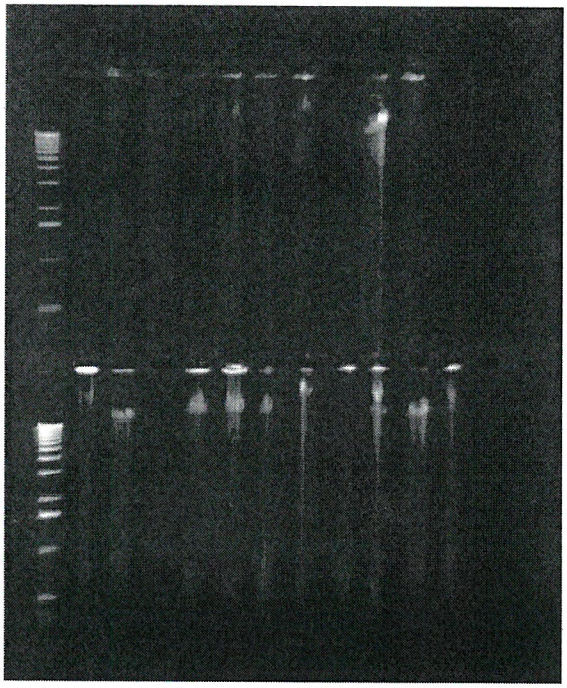

Figure 6.1. Gel image of DNA extractions from *Amperima rosea* using the DNeasy™ extraction kit (Qiagen). Streaky nature of DNA in each lane is an indication of degradation. 1 kb ladder visible in far left lane.

A reasonable amount of DNA for amplification is 10ng μl^{-1} (Hillis *et al.*, 1996). Following quantification it maybe necessary to dilute or concentrate the DNA template before adding it to the PCR reaction mix. To optimise the PCR step it is necessary to try different annealing temperatures, concentrations of dNTPs, *Taq* polymerase, $MgCl_2$ and Q-solution™. The reaction can also be carried out without Q-solution but in the majority

of cases it is found to work better with Q-solution than without. The protocol and optimal PCR reaction mix and thermal cycle program for *Amperima rosea* was eventually finalised and can be found in full in appendix IV.

Following successful amplification of the target DNA sequence the product is run on a gel for quantification (see Figure 6.2) before being cleaned using the QIAquick™ PCR purification kit (Qiagen). The PCR product is rinsed through a number of buffers in a centrifuge tube filter chamber before being eluted. Direct sequencing of the purified products was carried out in both directions using the ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer). The quantities and cycle-sequence program settings are also listed in appendix IV.

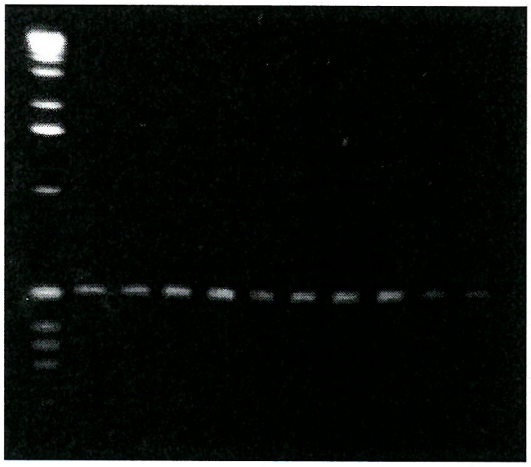

Figure 6.2. Gel image of amplified PCR products from 10 individual specimens of *Amperima rosea*. Amplified sequence of DNA is approximately 350 base-pairs in length.

The cycle sequence product also has to be cleaned and is filtered through an angled gel bed (Dye-Ex Spin Kit™ (Qiagen)) and centrifuged to remove excess dye. The dried DNA pellet is then stored at –20°C before it is re-suspended in loading dye prior to sequencing. Sequencing was carried out on a Perkin Elmer automated sequencer. A laser follows the progress of each sequence as it migrates through the gel plate and identifies the individual nucleotide bases, which are dyed different colours. (Adenine = green; Thymine = red; Cytosine = blue; Guanine = black).

Following successful sequencing of both the forward (5'-3') and reverse-compliment (3'-5') of the target 16S region the sequence has to be manually checked to minimise 'uncalled' bases. The forward and reverse-compliment sequences can be used to produce one clear sequence with the effects of 'noise' reduced. The homologus sequences from each individual specimen then have to be aligned.

6.3. Aims for the molecular study.

At the outset of this project it was hoped that starch-gel allozyme electrophoresis would provide the data necessary to assess the spatial and temporal relationships between samples of *A. rosea* taken from the Porcupine Abyssal Plain at four sites during April 1999 and at the BENGAL site during October 2000. Allozyme data would have allowed the assessment of whether *Amperima rosea* was sexually reproducing and outbreeding by comparison to the Hardy-Weinberg equilibrium. However, the allozyme study was wholly unsuccessful, despite attempting various different methods in an attempt to optimise the procedure (see section 6.4.1).

The decision to focus on a mitochondrial DNA sequencing approach significantly increased the cost of this study but it was hoped that sequence data for the 16S region of *Amperima rosea* mitochondrial DNA would provide data for the analysis of the sampled population(s). Sequence data, from approximately 20 individuals from each of the five samples, will be aligned with each other using the program Clustal X (Thompson *et al.*, 1997). This program can also be used to estimate similarity between sequences. It is often useful to indicate, say, that one sequence 93% identical to that of the homologous molecule from a second sample or species and that none of the other known sequences are more than 78% identical (Hillis *et al.*, 1996). In general, it is the relationships between sequences that are of interest in molecular systematics, not the sequences themselves. Fractional (percentage) similarity is by far the most common method of summarising the relationship between two or more sequences. In its simplest form, the

sequence similarity is equal to the number of aligned sequence positions containing identical residues divided by the number of sequence positions compared.

Phylogenetic trees can be constructed from the sequence data using the Neighbour-Joining method of Saitou and Nei (1987). Trees can be graphically presented using the programme Treeview (Page, 1996). A single specimen of *Peniagone vignoni*, from the West Antarctic Peninsula, has been sequenced to use as an out-group.

It is hoped that molecular data will provide information on whether the same breeding population of *Amperima rosea* is being sampled from year to year. In addition, are samples separated by 50-100km part of that same larger population and what level of gene flow is evident within a large population or among smaller ones. Comparing molecular data to size-class analysis of the sample may give an indication as to whether or not smaller individuals are new recruits, and if so are they from the larger adult population or have they migrated in from another locality on the abyssal Northeast Atlantic basin.

6.4. Preliminary results.

6.4.1. Allozyme electrophoresis.

As previously mentioned the allozyme-based section of this study produced no results. However, I feel it is important to mention the various protocols attempted during this study in the hope that they may prove a useful source of information for anyone attempting a similar study on deep-sea animals (particularly holothurians) in the future. Table 6.1 lists the various combinations of buffer solutions and enzyme stains used in this study, and the reference to their use in other studies on echinoderms.

The lack of results from the allozyme study could be the result of a number of factors, the most likely being protein degradation resulting from the changes in temperature, pressure and exposure to UV radiation. The allozyme study also used the frozen tissue dissected at

sea, and it is possible that the delay between collection, dissection and freezing at –80°C was too long and is something that has to be considered when sampling deep-sea organisms for molecular work.

ECHINODERM ELECTROPHORESIS: ENZYMES AND BUFFERS				
Enzyme	**Abbreviation**	**Buffer**	**Species**	**Reference**
Acid phosphotase	Acph	TRIS-Citric Acid pH 7.0	*Benthogone rosea*	Bisol *et al.*(1984)
			Benthodytes typica	Bisol *et al.*(1984)
Alkaline phosphotase	Aph	TRIS-Boric Acid pH 8.5	*Benthogone rosea*	Bisol *et al.*(1984)
			Benthodytes typica	Bisol *et al.*(1984)
Amylase	Amy	TRIS-Citric Acid pH 7.0	*Benthodytes typica*	Bisol *et al.*(1984)
Aminopeptidase	Ap	TRIS-Boric Acid pH 8.5	*Benthogone rosea*	Bisol *et al.*(1984)
			Benthodytes typica	Bisol *et al.*(1984)
Esterase	Est	TRIS-EDTA-Boric Acid pH 9.1	*Benthogone rosea*	Bisol *et al.*(1984)
			Benthodytes typica	Bisol *et al.*(1984)
		Lithium Hydroxide pH	*Coscinasterias calamaria*	Johnson & Threlfall (1987)
Esterase D	Est D	TRIS-EDTA-Boric Acid pH 9.1	*Benthogone rosea*	Bisol *et al.*(1984)
			Benthodytes typica	Bisol *et al.*(1984)
Glucose-6-phosphateisomerase	Gpi	TRIS-Boric Acid pH 8.5	*Benthogone rosea*	Bisol *et al.*(1984)
			Benthodytes typica	Bisol *et al.*(1984)
		TRIS-Glycine pH 8.4	*Holothuria atra*	Uthicke *et al.* (1998)
			Stichopus chloronotus	Ballment *et al.* (1997)
Glutamatedehydrogenase	Gdh	TRIS-Citric Acid pH 7.0	*Benthodytes typica*	Bisol *et al.*(1984)
Hexokinase	Hk	TRIS-Glycine pH 8.4	*Holothuria atra*	Uthicke *et al.* (1998)
Leucineaminopeptidase	Lap	Lithium Hydroxide-Boric Acid pH 8.2	*Benthogone rosea*	Bisol *et al.*(1984)
			Benthodytes typica	Bisol *et al.*(1984)
Leucylglycylglycinepeptidase	Lgg	TRIS-Glycine pH 8.4	*Holothuria atra*	Ballment *et al.* (1997)
			Stichopus chloronotus	Ballment *et al.* (1997)
Leucyltyrosinepeptidase	Ltp	Lithium Hydroxide pH	*Coscinasterias calamaria*	Johnson & Threlfall (1987)
		TRIS-Glycine pH 8.4	*Holothuria atra*	Ballment *et al.* (1997)
			Stichopus chloronotus	Ballment *et al.* (1997)
Malate dehydrogenase	Mdh	TRIS-Maleate	*Coscinasterias calamaria*	Johnson & Threlfall (1987)
		TRIS-Glycine pH 8.4	*Holothuria atra*	Ballment *et al.* (1997)
			Stichopus chloronotus	Ballment *et al.* (1997)
Mannosephosphateisomerase	Mpi	TRIS-Boric Acid pH 8.5	*Benthodytes typica*	Bisol *et al.*(1984)
Peptidase (Leu-Pro)	Lp	TRIS-Glycine pH 8.4	*Holothuria atra*	Uthicke *et al.* (1998)
		TRIS-Glycine pH 8.4	*Stichopus chloronotus*	Ballment *et al.* (1997)
Phosphogluconatedehydrogenase	Pgd	TRIS-EDTA-Citric Acid pH 7.9	*Holothuria atra*	Uthicke *et al.* (1998)
Phosphoglucomutase	Pgm	TRIS-EDTA-Citric Acid pH 7.9	*Holothuria atra*	Uthicke *et al.* (1998)
		TRIS-Glycine pH 8.4	*Holothuria atra*	Uthicke *et al.* (1998)
			Stichopus chloronotus	Ballment *et al.* (1997)
		TRIS-Maleate	*Coscinasterias calamaria*	Johnson & Threlfall (1987)
Phosphoglucoseisomerase	Pgi	TRIS-EDTA-Boric Acid pH 9.1	*Coscinasterias calamaria*	Johnson & Threlfall (1987)
Superoxidedismutase	Sod	TRIS-EDTA-Boric Acid pH 9.1	*Coscinasterias calamaria*	Johnson & Threlfall (1987)
		TRIS-Boric Acid pH 8.5	*Benthogone rosea*	Bisol *et al.*(1984)
			Benthodytes typica	Bisol *et al.*(1984)

Table 6.1. Enzyme stains and buffer solutions used during the allozyme electrophoresis study of *Amperima rosea* from the Porcupine Abyssal Plain. The enzyme stains and buffer solutions had been used successfully on the species noted.

6.4.2. DNA Sequencing.

Extraction of the DNA proved difficult at first, probably as a result of the tissue being used. The gelatinous nature of the body tissue created problems during extraction, even when using the commercial kits. Extraction with CTAB buffer and β Mercaptoethanol was also tried, after it had been previously used for extracting DNA from gastropod molluscs with a high mucus content (Preston pers. comm.). However, this also proved unsuccessful.

Following the optimisation of the PCR protocol (see appendix IV) a product of approximately 350bp in length was obtained using universal 16S primers (16SAR and 16SBR). This product was sequenced and then checked for comparison with other mtDNA sequences using the NCBI BLAST facility on the internet (www.ncbi.nlm.nih.gov/blast/Blast.cgi). BLAST searches showed that the 16S region of the mtDNA had been successfully amplified and sequenced, although gaps in the base-pair sequence were apparent. Good matches were made with sequenced 16S regions of the shallow-water holothurians *Psolus fabricii*, *Cucumaria frondosa*, and *Chirotida laevis*. However, having successfully amplified the target sequence for approximately 20 individuals from each sample, only 12 were successfully sequenced by the automated sequencer.

After finding additional funds to repeat the process of amplification and sequencing it was decided to refine the technique further by designing a specific pair of primers for the *Amperima* 16S region. The Clustal X program was used to align one of the *Amperima* sequences, obtained with the universal 16S primers, with the complimentary 16S sequences from *Psolus fabricii*, *Cucumaria frondosa*, and *Chirotida laevis*. Figure 6.3 shows the alignment of the four sequences and the location of the regions chosen as primers for *Amperima rosea*. These regions were selected from the many 'picked' by the Primer oligonucleotide selection program, also available as a web-based facility (www.genome.wi.mit.edu/cgi-bin/primer). Both selected primers are of the same length (20 base-pairs) and have similar melting temperatures (60 and 58°C respectively). Having similar melting temperatures is beneficial for setting a suitable annealing temperature for the PCR protocol. The aligned sequences in Figure 6.3 show that the location of both primers is in a highly conserved region of the sequence, with shared bases across the four species. However, the region between the primers, which will form the *Amperima* sequence to be analysed, is highly variable between the four species. Therefore, it is possible that there will also be some degree of variability between *Amperima* individuals at this site on the mtDNA. The selected primers are then manufactured and provided in a concentrated form by Sigma-Genosys.

These primers provided a significantly greater PCR product of over 50ng µl-1, even after the purification process (see figure 6.4), and produced a clean sequence of approximately 310 base-pairs (Figure 6.5).

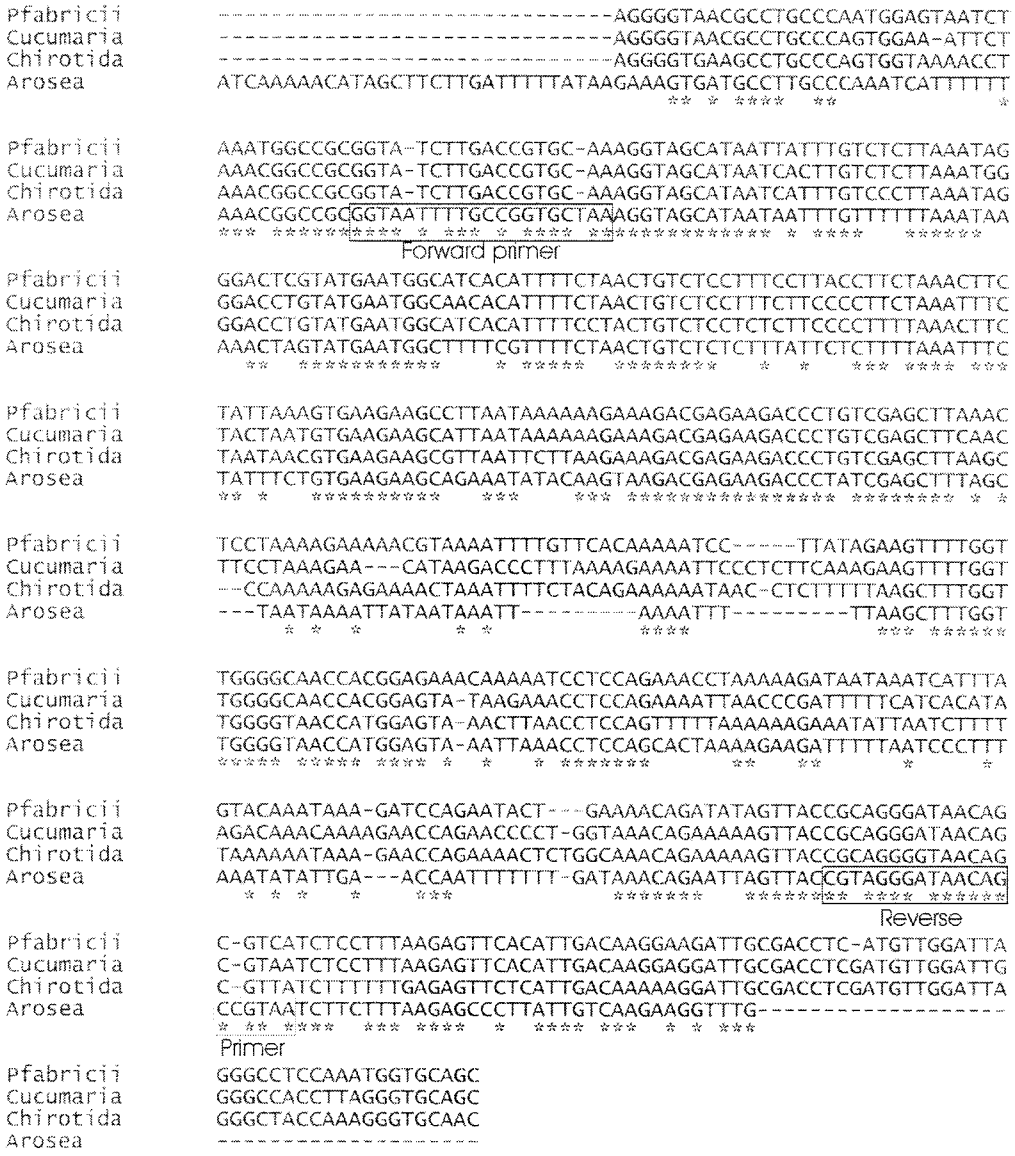

Figure 6.3. Alignment of partial sequences from the 16S mitochondrial DNA region of four species of holothurian. The locations of the forward and reverse primers, selected for *Amperima rosea*, are indicated by boxes. Asterisks indicate conserved regions of shared bases across all four species.

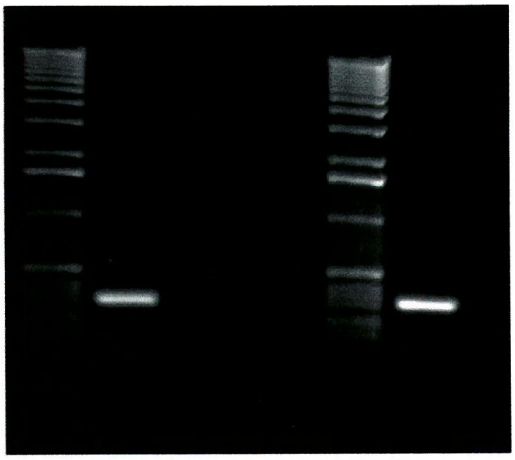

Figure 6.4. Purified PCR products from two individual specimens of *Amperima rosea*. Both products were amplified using the new *A. rosea* 16S primers. 1kb ladder is visible on the left of each product band.

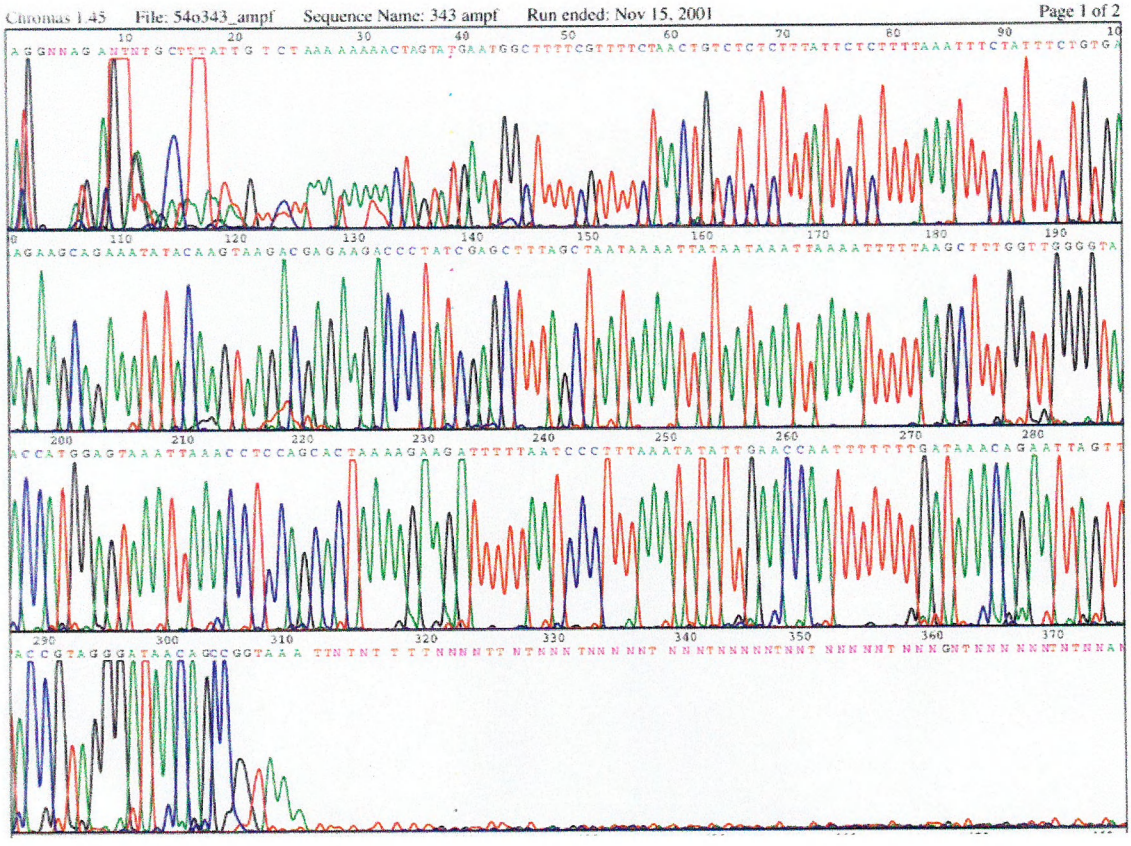

Figure 6.5. Partial sequence (approx. 310bp) from the 16S region of *Amperima rosea* mtDNA. Sequence displayed using Chromas1.45 software.

6.5. Further work.

With additional financial support it is now hoped that the extracted DNA from the remaining specimens of *Amperima rosea* (~20 per sample) can be amplified, cleaned and sequenced using the newly designed primers, which appear to yield a good consistent product of the target sequence.

It is hoped that analysis of the molecular data, using the methods described in section 6.3, will provide additional information as to the functioning of this species in the abyssal community and perhaps how it came to appear so suddenly in such large numbers. Analysis of 16S sequence data may provide information as to the mechanism of recruitment of individuals into the area and the levels of gene flow between groups (populations?) of *A. rosea* at different geographic localities on the Porcupine Abyssal Plain.

7. Summary and conclusions

The rapid changes in abundance of both megafauna and macrofauna on the Porcupine Abyssal Plain represent a previously unrecorded phenomenon in the deep sea. The deep-sea has been perceived as a stable environment, but recent discoveries of episodic pulses of organic matter and the response it elicits in the fauna of the megabenthos has changed that perception. However, this long-term and widespread event alters our view of the dynamics of deep-sea communities. The '*Amperima* Event' encompasses significant increases in abundance of a number of different taxa and has occurred over a large area and has persisted for at least five years. It is increasingly likely that environmental forcing, in the form of the arrival of phytodetritus at the seafloor, rather than stochastic change is responsible for the observed changes in the composition and abundance of the megafauna.

The seasonal flux of phytodetritus to the deep seafloor has already been shown to have a significant influence on the abundance, dominance and timing of reproduction of many benthic invertebrates and protozoans. Subtle changes in the benthic environment have often been attributed to changes in the timing and size of the phytodetrital flux. However, in this case it appears to be a change in the quality or composition of the phytodetritus that is responsible for these changes. We now know that *Amperima* has a different sterol signature in its body tissues compared to other PAP species and that the depletion of sterols in the surficial sediments could be attributed to the feeding activity of *Amperima* during periods of peak abundance (Ginger *et al.*, 2000; 2001). It is clear that *Amperima* is feeding on the freshest phytodetritus but it now appears that there is an even finer level of selection between species. *Amperima* favours fresh, zeaxanthin and β-carotene-rich particles, while at the same time it appears to be selecting against fresh phytodetritus of dinoflagellate origin. The hypothesis is presented that bloom composition in the surface waters can influence community structure at abyssal depths. From the pigment signatures found in the gut contents of *Amperima* it is likely that this species is preferentially ingesting phytodetritus with a high cyanobacterial content. The latitudinal distribution of phytoplankton in the surface waters can be affected by numerous factors, including sea

surface temperature and surface irradiance. The influence of the North Atlantic Oscillation (NAO) has already been observed in the abundance of *Calanus* species. Changes in temperature and wind stress are both directly driven by the NAO and fluctuations in abundance of *Calanus finmarchicus* mainly result form the combination of these two factors (Fromentin and Planque, 1996). Large aggregations of cyanobacterial cells are common at equatorial latitudes. However, it has already been shown that there can be years where peak abundances of cyanobacteria are found at higher latitudes than usual (Zubkov *et al.*, 2000). Winter sea surface temperatures were recorded as being warmer than usual during the late 1990s.

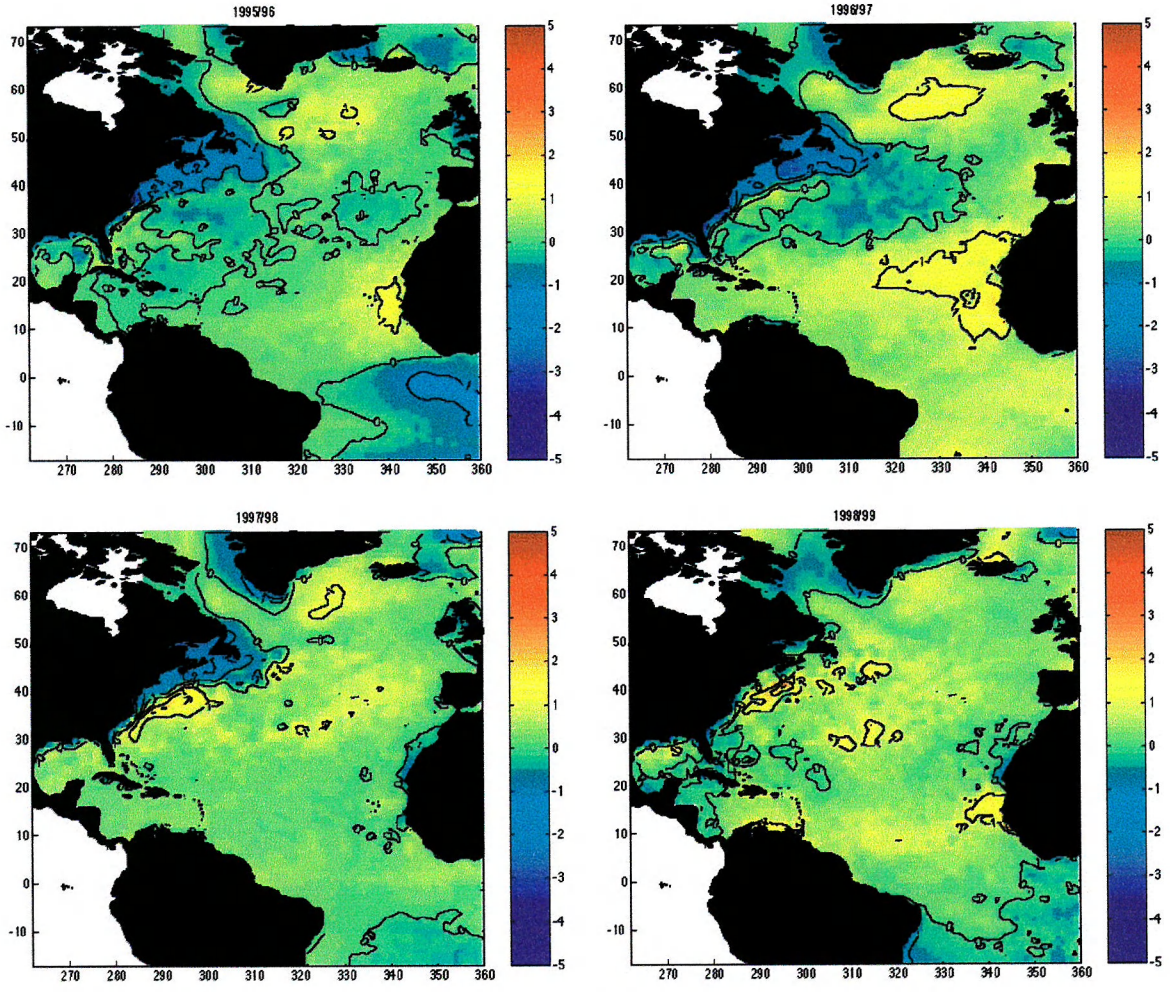

Figure 1. Winter (November-April) sea surface temperatures in the North Atlantic, 1995-1999, taken from AVHRR satellite data. Colour bars and isolines represent relative change in degrees Celsius.

If changes in sea surface temperature can extend the latitudinal range of peak abundance of phytoplankton then the bloom composition over the PAP may be altered. Cyanobacteria are small cells that under normal conditions will sink relatively slowly through the water column. However, the speed at which cyanobacterial cells reach the deep seafloor can be affected by the grazing activity of gelatinous zooplankton, in particular salps (Pfannkuche and Lochte, 1993). The packaging of cyanobacteria in sedimenting salp faeces provides a fast and direct link between the epipelagic and benthic ecosystems.

The abundance and distribution of gelatinous zooplankton, in particular many species of jellyfish, is often episodic with spring and summer blooms when planktonic food is available in greater abundance. Beyond the basic life-cycle driven seasonal changes in abundance there are several other events that appear to be increasing the numbers of gelatinous organisms in some ecosystems. Some blooms appear to be long-term increase in native populations, but there are other species whose populations regularly fluctuate, apparently with changes in climate (Mills, 2001). The famed El Niño event has also been cited as a cause of changes in abundance and dominance in populations of mesopelagic hydromedusae. During two separate El Niño events seldom-seen species were observed in high numbers, while historically common species became rare (Raskoff, 2001). Changes in climate in the Northeast Atlantic may alter the distribution and abundance of not only the cyanobacteria cells but also the gelatinous zooplankton that feed on them. An increase in cyanobacteria coupled with a bloom of grazing salps could provide a link for the rapid transfer of these particles to the deep seafloor where they provide the required nutritional cue for species such as *Amperima* and *Ellipinion* to successfully reproduce and recruit within the existing population.

The lack of photographic evidence for the arrival of phytodetritus can be attributed to the feeding activity of not only large numbers of *Amperima* but also ophiuroids (Bett *et al.*, 2001). Estimates of sterol requirement by *Amperima* indicate that they could have been responsible for consuming all of the incoming sterol flux during the BENGAL sampling period (Ginger *et al.*, 2001; Kiriakoulakis *et al.*, 2001). The rapid removal of phytodetritus, as indicated by both Ginger *et al.* (2001) and the high tracking rates

estimated in this study, is likely to have a significant impact on not only the other epibenthic megafauna but infaunal macro- and meiofauna in the sediment. Fatty acid profiles of key species of abyssal holothurian indicate that while *Amperima* was present in high numbers it was out-competing *Oneirophanta mutabilis* for resources, whereas *Psychropotes longicauda* was able to exist on whatever resources remain in the sediment (Neto, unpublished data). Meiofaunal and macrofaunal densities can change rapidly in response to the flux of phytodetritus to the seafloor. Depending on the response time of each individual species the rapid removal of phytodetritus by large numbers of epibenthic deposit-feeders may affect the resources available to this fraction of the fauna. The feeding activity of holothurians and ophiuroids would not only remove fresh phytodetritus from the sediment surface but would 'repackage' it in their faeces, creating an additional resource which may be suitable for certain species to take advantage of and bloom.

Amperima rosea has a life-history strategy that appears to be suited to the rapid exploitation of an episodic food supply. Understanding the life history strategies of species within a community is essential to fully determine the structure and functioning of the ecosystem (Eckelbarger, 1994). The process of reproduction is the most sensitive stage of a life cycle and plays an important role in the establishment of a species in a certain environment. The ability of *Amperima* to produce large numbers of small eggs would allow for large-scale recruitment when conditions are optimal. The presence of a suitable food source on the deep seafloor may not only be important for the adult animals, in terms of maturation of gonads and egg production, but also for the successful settlement of post-larvae from the water column. Gonad indices indicate that *Amperima* increases gamete production following periods when phytodetritus has been present on the seafloor. *Amperima* may also be able to develop its gametes to pre-vitellogenic stage before holding off maturation and spawning of the egg until conditions are energetically favourable, for both and adult and post-larvae. This opportunistic reproductive strategy, where the species waits for optimal conditions before converting its slowly accumulated energy into reproduction, has been suggested for opheliid polychaetes, at the PAP site, which show evidence of episodic recruitment (Vanreusel *et al.*, 2001).

The only previous record of *Amperima* occurring in such high numbers was that of Hérouard. The sample was collected further south between Portugal and the Azores. The subsequent lack of consistent sampling in this region may actually mean that *Amperima rosea* does regularly occur in greater abundances at more southerly latitudes. The sudden increase in abundance of this species at 48°N may be a reflection of range extension resulting from a successful recruitment event. The presence of a suitable food resource that is usually in short supply at this latitude (i.e. cyanobacteria), may have allowed the successful settlement and growth of juveniles produced by a more southerly breeding population.

One of the major problems with a study of this type is that it can never be 100% certain that the same population is being sampled from year to year. It is hoped that the molecular study of genetic variation in the *Amperima* population sampled in 1999 and 2000 will provide some indication as to the temporal and spatial relationships of groups of *Amperima* on the PAP.

This study has highlighted the fact that the deep ocean is not an isolated system, but interacts with global circulation and climate. In addition there is an increasing anthropogenic pressure affecting deep oceanic ecosystems. The effects that global climate change, oil exploration and deep-sea fisheries have on deep-sea communities are little understood. It is important that time-series studies of this type continue and are not considering as simple monitoring programmes. The understanding of natural fluctuations and processes in the deep-sea is important if we are to accurately assess and predict the potential effects of anthropogenic disturbance on deep-sea communities. At present the level of anthropogenic disturbance on deep-sea fauna is increasing at a much faster rate than that of our understanding of these processes that drive not only change but persistence in these deep-sea benthic environments. Although it is separated from the surface by over 4km of water, the deep ecosystem of the Porcupine Abyssal Plain is closely coupled to processes occurring in the upper ocean. It is now entirely possible that changes in climate, so long linked to change in shallow-water marine systems

(Southward *et al.*, 1995), can have an affect on the communities living on the abyssal seafloor.

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Appendix I: Taxon/Species list for the Porcupine Abyssal Plain 1989-2000.

Taxa	Family/Species
PORIFERA	
PENNATULACEA	*Umbellula* sp.
GORGONACEA	
ACTINIARIA	*Actinauge abyssorum*
	Amphianthus bathybium
	Doantesia porcupina
	Iosactis vagabunda
	Kadosactis commensalis
	Misc
	Monactis vestita
	Segonzactis platypus
	Sicyonis biotrans
	Sicyonis sp.
MADREPORARIA	
ZOANTHIDEA	
VERMES	
ECHIURA	
NEMERTINA	
SIPUNCULA	
ANNELIDA	Polynoidae
	Misc
	Annelida - A
	Annelida - B
	Worm tubes
CIRRIPEDIA	
Mysidacea	
Amphipoda	
Isopoda	
Copepoda	
DECAPODA	*Benthesicymus* sp.
	B. brasiliensis
	B. irridescens
	Glyphocrangon atlantica
	Munidopsis crassa
	Munidopsis parfaiti
	Munidopsis antonii
	Munidopsis misc
	Natantia
	A. microphthalmus
	Ehpyrina bifida
	Hymenodora glacialis
	Parapasiphae sulcatifrons
	Plesiopenaeus armatus
	Stereomastis sp.

	Eryonidea sp.
	Misc
ECTOPROCTA	
PYCNOGONIDA	
GASTROPODA	
SCAPHOPODA	
BIVALVIA	
CEPHALOPODA	
ASTEROIDEA	
	Dytaster grandis grandis
	Freyella elegans
	Freyastera tuberculata
	Freyastera sexradiata
	Freyastera benthophila
	Hyphalaster inermis
	Pythonaster atlantidis
	Styracaster horridus
	Styracaster elongatus
	Stryacaster chuni
OPHIUROIDEA	
ECHINOIDEA	
HOLOTHURIOIDEA	***Amperima rosea****
	Benthodytes sordida
	Deima validum
	Ellipinion molle
	Kolga hyalina
	Mesothuria candelabri
	Misc
	*Molpadia blakei**
	Oneirophanta mutabilis*
	Paroriza prouhoi
	Peniagone diaphana
	Peniagone azorica
	Protankyra brychia
	Pseudostichopus* sp.
	Pseudostichopus villosus*
	Psychropotes longicauda*
	Psychropotes semperiana
CRINOIDEA	
TUNICATA	

Taxa/species in bold are those used in analysis of community change (Chapter 2).

Holothurian species marked with an asterisk are those used in the study of selective feeding (Chapter 5).

Appendix II: Procedure for the preparation of specimens for histological analysis.

A. Dehydration and sectioning.

1. Place dissected gonads in glass tubes and immerse in Bouin's solution for a period of approx. 24hrs (Bouin's contains Picric acid, Glacial acetic acid and Formaldehyde). Seal the tubes with plastic stoppers.
2. Transfer specimens to 70% alcohol. Remove waste Bouin's solution to an appropriately labelled receptacle for proper disposal.
3. After approx. 24hrs in 70% alcohol transfer the specimens to 95% alcohol. After ~12 hours transfer specimens to Absolute alcohol (100%) and then process them through three periods of immersion, each of 2hrs.
4. Transfer specimens to Histoclear™ for a minimum period of 24hrs.
5. Upon removal from the Histoclear™ dry the specimens on paper and immerse them in a tube of hot wax for at least 8hrs, prior to being embedded in wax blocks.
6. Each specimen, now encased in a wax block, is sectioned (5-7μm) for microscopic analysis. Float the thin sections onto glass slides in a water bath before placing the slides on a heated drying rack.
7. The slides are then stained, usually with Haematoxylin and Eosin but other stains are available (see Culling, 1974).

B. Staining.

Place dried slides back-to-back in an appropriate staining rack (most hold 10-20 slides). The slides are then processed through the following staining protocol;

1.	Histoclear	5 mins
2.	100% Alcohol	2 mins
3.	70% Alcohol	2 mins
4.	Haematoxylin (Mayer's Haemalum)	5 mins
5.	Cold running water	15mins
6.	Eosin	2 –3 mins
7.	Rinse in tap water	
8.	70% Alcohol	2 mins
9.	100% Alcohol	2 mins
10.	Histoclear	2 mins

The stained slides should then be pat-dried, removing any excess stain. The section is then fixed on the slide with DPX mounting solution and a coverslip put in place.

Appendix III: Allozyme (starch-gel) electrophoresis.

A. Preparing the gel.

1. Weigh out 47.5g of molecular grade potato starch and place in a 1000ml side-arm flask with 380ml of gel buffer.

2. Heat the flask over a Bunsen burner while continuously swirling the mixture. The mixture will go through two phase changes before nearly boiling. First it will go very viscous then it will become less opaque and less viscous. Beware of the pyrex flask cracking at this point if it becomes too hot.

3. When gel has passed through its second phase change and is beginning to boil remove from the Bunsen flame and place on the bench. Attach the vacuum pump hose to the side-arm flask, place a bung over the flask and turn on the vacuum pump for de-gassing. When the gel has degassed (about 30 secs – 1 minute) slowly remove the bung. When the vacuum is released turn off the pump and detach the hose from the flask.

4. Pour the molten starch into the gel plate former and remove any bubbles with a spatula. Before the gel sets, place the top glass plate onto the gel without trapping any bubbles. Wrap the gel mould in cling-film and place a weight on the top plate to prevent any air being drawn into the gel as it cools and contracts.

5. Place the gel in a cool place and leave to set overnight.

B. Preparing the tissue samples

6. Remove frozen tissue samples from the –80°C freezer and place in a container of liquid N_2. it is usually possible to run 20 specimens on one gel.

7. Take a small (3mm x 3mm) cube of tissue and place in a 2ml eppendorf tube with 100µl of grinding buffer. Macerate the tissue with a glass pestle. If the grinding buffer conatins β-Mercaptoethanol then this step must be performed in a fume hood.

8. Centrifuge the sample at 14,000 rpm in a refridgerated centrifuge at 4°C for 10 minutes.

9. Absorb the supernatant onto a 3mm x 12mm filter paper wick (Whatman No. 1). Tracker dye may also be absorbed onto the wick at this stage.

C. Loading and running the gel.

10. Take the gel and carefully lever off the top glass plate. Use a scalpel to cut around the gel and cut a straight line across the gel 2cm from the end. Also cut a small corner off the opposite end of the gel to aid identification of specimens order later in the process.

11. Remove the gel sides of the gel mould and gently move the 2cm slice away from the rest of the gel. Place the filter paper wicks on the end of the gel being careful to record the order of specimens and being careful not to have too much extract on each wick.

12. Place the gel slice back against the gel trapping the filter paper wicks. Replace the sides of the gel mould with a couple of extra perspex spacers in case of gel shrinkage.

13. Pour 900ml of electrode buffer into the gel tank making sure that the level is the same for both sides of the tank.

14. Place the loaded starch-gel onto the gel tank and place the cloth electrodes onto each side (about 1-2cm onto the gel) of the gel making sure that the edge of the electrode is straight.

15. Place the lid on the gel tank and move the entire unit into the fridge. The tank is then connected to the power supply and the fridge door closed. The power supply is then switched on, the voltage set (150v, 48mA) and the time for the gel set (7-8 hrs). Do not run the equipment with the lid off the gel tank. Any malfunction with the equipment should be denoted by an audible alarm from the power pack, and the run will have to be aborted and abandoned unless there is a spare gel tank available.

C. Enzyme stains: preparation and use.

16. Weigh out the dry components of each stain to be used in 100ml beakers using an analytical balance placed in a fume hood. It is advisable to prepare and use stain recipe cards for this step. When the stain is complete place parafilm over the beaker and leave in the fume hood.

17. When the electrophoresis run has ended, switch off the power and disconnect the power supply from the tank. Carefully remove the gel from the tank.

18. Trim the gel to as small a size as possible given the number of samples on the gel and the length the gel has run (often indicated by an indentation in the gel surface). Remove the sides of the gel mould and place a second glass plate on top of the gel. Remove the glass plate the gel was run with and replace with a clean dry plate.

19. Slice the gel on a gel slicing apparatus on alternate sides with two glass plates on the bottom side of the gel. This involves turning the gel over in the hands with the glass plates, take care not to drop the plates or damage the gel.

20. Remove each slice, as it is cut, and place it in a staining tray. When slicing is complete stain the gels. Remove the parafilm from the beakers containing the stains and add the appropriate buffer solutions. Add a stirring bar and mix the stain. Add any enzyme suspensions using a pipette and add agar if necessary using a small measuring cylinder.

21. Pour the stain onto the gel slice. If the stain is volatile this must be carried out in a fume hood. Place a cover over each tray as light can react with many of the stains.

22. Allow the gel to develop (1 minute – 1 hour) and then use graph paper to record the positions of the alleles for each specimen on each slice.

Appendix IV: Optimised PCR protocol for DNA amplification.

For 20µl reactions:

1. Label an appropriate amount of UVed 0.5ml PCR tubes (or 96-well plate for larger runs)

2. Remove all reagents and DNA samples from freezer and keep over ice during the PCR set-up.

3. Once thawed, vortex and touch-spin centrifuge all reagents. DO NOT vortex DNA sample, simply touch-spin.

4. In a 2ml Eppendorf tube prepare the PCR reagent mix. This should include the molecular grade dH_2O, dNTPs, 10x buffer, $MgCl_2$, and the Qiagen 'Q-solution'.

5. If the PCR is to be run immediately the *Taq* polymerase can be added to the mix last, but should not be added if the reaction mix is being prepared for a later run.

6. When preparing the reagent mix add enough of each reagent for n+1 samples.

7. For the amplification of DNA from *Amperima rosea*, the following reaction mix was optimised for each 20µl reaction.

dH_2O	6.5µl
dNTPs	1.6µl
10x buffer	2.0µl
Q-solution	4.0µl
$MgCl_2$	2.4µl
Primer 1	1.0µl
Primer 2	1.0µl
Taq polymerase	0.5µl

8. After a quick touch-spin of the reaction mix, pipette 19.5µl of the mix into each labelled 0.5ml PCR tube (or plate well), remembering to include a tube for a 'blank' reaction (no sample DNA).

9. Pipette sample DNA into the appropriate tubes, taking care to deliver the DNA slowly.

10. Place the PCR tubes into the thermal cycler machine and, if it has one, ensure that the machine's thermostat tube is connected and in place.

11. Close the lid of the machine and start the appropriate PCR step-cycle programme. This can often be stored in the machine's memory.

12. For the successful amplification of DNA from the 16s region of *Amperima rosea* mtDNA the following step-cycle programme was optimised.

Step One	95°C	15 minutes	
Step Two	94°C	1 minute	Repeat for 35 cycles
	56°C	1 minute	
	72°C	1 minute	
Step Three	72°C	10 minutes	
	4°C	hold	

13. Following completion of the PCR cycle, run the PCR product on a 1% agarose gel to check for the presence of an amplified DNA product. Check that the band is of a similar length (base-pairs) to that expected from the gap between the two primers used.

14. Photograph the gel under UV illumination and use the UviDoc programme to quantify the amount of product from each individual.

15. Successfully amplified products can then be cleaned in preparation for sequencing.

The following published paper was included in the bound thesis. This has not been digitised due to copyright restrictions, but the doi is provided.

Brian J Bett, M.Gabriella Malzone, Bhavani E Narayanaswamy, Benjamin D Wigham (2001) **Temporal variability in phytodetritus and megabenthic activity at the seabed in the deep Northeast Atlantic** Progress in Oceanography: 50 (1-4), 349-368 http://dx.doi.org/10.1016/S0079-6611(01)00066-0