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ABSTRACT

**Sexual Biochemistry in the Deep Sea – The Link between
Phytoplankton and Abyssal Holothurians**
by Tania Smith

Holothurians play an important role in carbon cycling. They dominate the abyssal oceanic megabenthos, reworking large amounts of organic matter. Holothurians require essential organic nutrients, such as carotenoids for their reproduction. Enhanced carotenoid concentration in the ovaries of echinoderms increases reproductive output and larval survival. Carotenoids cannot be synthesised *de novo* by holothurians, only by phytoplankton. To examine the link between diet and reproduction in deep-sea holothurians, the pigment biochemistry of holothurians, sediment and particulate organic matter from three abyssal sites as investigated.

A temporal comparison at the Porcupine Abyssal Plain (PAP), NE Atlantic, has shown 1) the supply of organic material (OM) can affect the diet of holothurians, depending on their feeding adaptations and 2) holothurian reproductive biochemistry can be affected by compositional differences in the OM reaching the seafloor, although the extent of this influence appears to differ between species. Two abyssal sites around the Crozet Islands, Southern Ocean, were investigated to compare contrasting OM supply on the diet and reproductive biochemistry of holothurians. The sites are only 460 km apart, with no topographic boundary to separate them. However, they are subject to differing overlying primary productivity regimes and therefore biochemical differences can be ascribed to the composition and amount of organic matter reaching the sea floor at each site. The results showed that 1) the quantity of OM reaching the seafloor at each site differed, mirroring the overlying primary productivity regimes. This was also reflected in the diet of some holothurian species, depending on their ability to take advantage of the fresh material. 2) The reproductive biochemistry of the holothurians sampled at both sites showed quantitative differences, mirroring the supply of OM to each benthic site.

The present study has shown that changes in the composition and quantity of the supply of OM to the deep-sea floor can affect holothurian diet and ovarian biochemistry. This may lead to large community changes as seen at the PAP in the NE Atlantic, which alters the reworking rate of the sediment, ultimately affecting the sequestration of carbon.

Sexual Biochemistry in the Deep Sea – The Link between Phytoplankton and Abyssal Holothurians

Chapter 1 – Introduction

1.1 Rationale and aim of this study

Holothurians dominate the abyssal megabenthos in terms of abundance and biomass. They form a key part of the deep-sea benthic ecosystem because they rework large amounts of organic matter (OM), playing an important role in carbon cycling (Billett, 1991). Recent studies have shown that abyssal and bathyal holothurians assimilate specific organic compounds, such as carotenoids, into their ovaries (Hudson et al., 2003; Wigham et al., 2003a; Hudson, 2004). Carotenoids are photosynthetic pigments that can only be synthesised *de novo* by plants and fungi (Goodwin, 1980). Studies on shallow-water echinoderms have shown that carotenoids can enhance gonad production, fecundity and larval survival (George et al., 2001; George and Lawrence, 2002; Plank et al., 2002). Abyssal and bathyal holothurians show interspecific differences in their ovarian carotenoid profiles. Some species have identical gut sediment and ovarian profiles, leading to the conclusion that they selectively feed on specific carotenoids (Wigham et al., 2003a; Hudson, 2004). It is therefore possible that a change in the composition of the OM reaching the seafloor may influence the reproductive biology of specific species, leading to community change as seen at an abyssal site in the NE Atlantic (Billett et al., 2001; Wigham et al., 2003a; Hudson, 2004).

The aim of this study is to examine the link between the diet and reproductive carotenoid biochemistry of abyssal holothurians. Two particular questions are addressed. Will changes in the quantity and composition of the OM reaching the seafloor influence the carotenoid biochemistry of the holothurians? If there is an influence, are there interspecific differences?

1.2 Holothuroidea

1.2.1 Holothurians in the deep sea

The class Holothuroidea belong to the Phylum Echinodermata, which also includes the Classes Asteroidea, Ophiuroidea and Crinoidea. At bathyal (<3000m) and abyssal (>3000m) depths, holothurians dominate the abyssal benthic megafauna both numerically and in terms of biomass (Billett, 1991). They are one of the few faunal groups that have penetrated the deepest regions of the ocean and their dominance at hadal depths (>6000m) led Belyaev (1972) to describe this habitat as 'the kingdom of the holothurian'.

Deep-sea holothurians can range in size from a length of half a metre, e.g. *Benthodytes* spp., to a few millimetres, e.g. *Kolga* spp. and *Cherbonniera utriculus* (Sibuet, 1974; Billett and Hansen, 1982; Billett et al., 1988). The majority of holothurians feed on the top few millimetres of sediment, although some species are infaunal, e.g. *Molpadia* spp. (Pawson, 1982) or have developed the ability to swim and live in the water column above the sediment, e.g. *Enypniastes eximia* (Miller and Pawson, 1990). Photographs of the deep-sea floor have shown holothurians to be important bioturbators of the surficial sediment; their characteristic tracks and faecal pellets provide evidence of this activity (Mauviel and Sibuet, 1985; Billett, 1991). Six Orders of holothurians are recognised (Dendrochirotida, Dactylochirotida, Elasipodida, Aspidochirotida, Molpadida and Apodida) and all are represented to a greater or lesser extent in the deep sea (Billett, 1991). The main diagnostic features of each order is summarised in Table 1.1.

Order	Description
Dendrochirotida	Most species in this order live in shallow water. They are suspension-feeding holothurians, with highly branched tentacles. Respiratory trees are present and they have muscles for retracting the oral introvert.
Dactylochirotida	Species in this Order have digitiform or digitate tentacles and a testaceous body wall. Respiratory trees are present and they have muscles for retracting the oral introvert.
Elasipodida	Exclusively deep-sea species. Digitate tentacles are used to 'shovel' sediment. Some species have peltate tentacles. Respiratory trees are present. The calcareous ring is without posterior projections. With the exception of one family, Deimatidae, the body wall is soft to gelatinous.
Aspidochirotida	Pelto-dendritic or peltate tentacles. Respiratory trees are present. The calcareous ring is without posterior projections. The body wall is generally soft and pliant. Large contrast in ossicle form in comparison to Elasipodida.
Molpadida	Tentacles digitate to simple. Respiratory trees are present. The calcareous ring may have short posterior projections. The body wall is generally soft and pliant. Most species live in relatively shallow water, although one family is restricted to the deep sea.
Apodida	Without papillae (tube feet). Tentacles are digitate, pinnate, or, in some small species, simple. Respiratory trees are absent. The calcareous ring is low and band-like, without posterior projections.

Table 1.1 The six holothurian Orders and their distinguishing features (compiled from Hyman, 1955; Hansen, 1975; Pawson, 1982; Kerr and Junhyong, 2001)

1.2.2   Body morphology

The body morphology of deep-sea holothurians is essentially the same as that of shallow-water species (Hyman, 1955). Generally, they have elongated cylindrical bodies with a mouth at one end and an anus at the other (Fig. 1.1). Variously-shaped tentacles encircle the mouth, the shape and morphology of which depends on the Order and feeding guild (Roberts et al., 2000). The tentacles and tube feet, or podia (if present), are controlled by the water vascular system. Five longitudinal muscles are attached to the body wall,

calcareous ossicles are embedded in the body wall in many species. The single gonad found only in the mid-dorsal interradius, discharges through a gonopore near to the oral crown. A few species are hermaphrodites. Coelomic fluid surrounds the gut. The gut is often long and terminates in a posterior cloaca (Hyman, 1955).
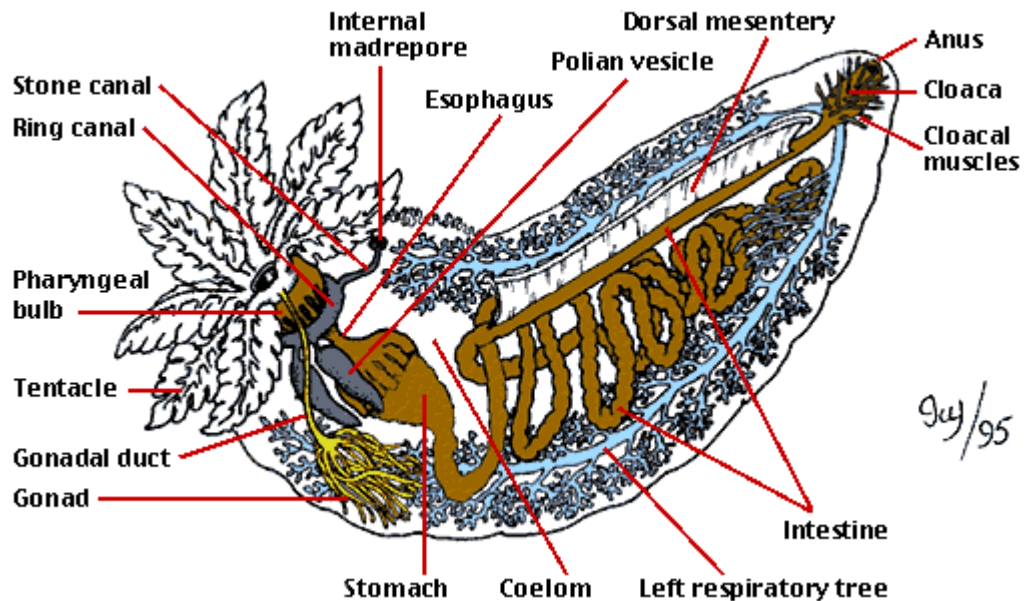
Figure 1.1 Main internal anatomical features of a cucumariid sea cucumber (Dendrochirotida). Drawing by Ivy Livingstone. Copyright © 1995 BIODIDAC

Holothurians have morphological adaptations that reflect their lifestyle in the deep sea. Elasipodid holothurians have distinct papillae that are believed to aid respiration and have tube feet to 'walk' on the sediment allowing them to move between food patches. Some species, e.g. *Pseudostichopus* spp., have reduced tube feet and use their bodies to 'plough' though the sediment (Billett, 1991). Species of *Pelopatides* have body morphologies that allow the holothurian to 'swim' through the water. Molpadiid holothurians live 'head down' in the sediment and have a modified anus that connects with the sediment surface for respiration and excretion (Hyman, 1955; Billett, 1991).

1.2.3   Holothurian feeding adaptations

Holothurians feed by moving their tentacles through or across the sediment surface to collect food particles. Holothurian tentacles can be grouped into five types: dendritic, peltate, pinnate, digitate and pelto-dendritic (Massin, 1982). Feeding guilds have been inferred from the tentacle structure (Moore and Roberts, 1994). Simple forms with little or no branching are described as digitate and those which have complex branching are dendritic; the three other tentacle types are graduations between the two extremes (Roberts and Moore, 1997). Peltate tentacles are thought to be used to 'sweep' the sediment into the mouth, whereas digitate tentacles 'rake' through the sediment (Roberts et al., 2000). Adhesive secretions are thought to aid particle capture and selection (Roberts and Bryce, 1982). The tentacle types of some abyssal holothurians are given in Table 1.2.

The tubular digestive tract consists of the pharynx, oesophagus, intestine, rectum and cloaca (Roberts et al., 2000). Holothurian gut structure differs between species and has been used to define feeding guilds. Species with fermentor guts are characterised by expanded chambers which allow increased mixing of food and bacteria and extended gut residence times (Penry and Jumars, 1990). A model by Alexander (1991) predicts that foregut fermentors do better than hindgut fermentors on poorer foods. The deep-sea holothurian *Pseudostichopus* spp. is found submerged in the sediment and feeds on relatively poor sediment just below the sediment/water interface. This species moves slowly across the sediment surface and has peltate tentacles that may be more suited to sediment particle handling than raking up phytodetritus (Moore and Roberts, 1994). The feeding adaptation of this species would be favoured by foregut fermentation, which is supported by the presence of an enlarged foregut with elevated numbers of bacteria (Roberts et al., 1994). Conversely, *Oneirophanta mutabilis* has relatively rapid locomotion and rake-like digitate tentacles exploiting richer food sources (Moore and Roberts, 1994). Its gut

structure reflects this richer diet, although the elevated bacterial numbers in its hindgut offers the potential for microbial digestive processes (Roberts et al., 1994).

Microbes can play a role in the nutrition and digestion of holothurians. High bacterial abundances have been observed in the oesophageal gut contents of deep-sea holothurians. Abundances decline in the anterior intestine, but bacteria proliferate in the hindgut area of some species (Deming and Colwell, 1982; Sibuet et al., 1982; Roberts et al., 2001). The marked decrease in bacterial numbers after the oesophageal region may suggest the capability of deep-sea holothurians to utilise ingested bacteria as a food source. However, the decrease in bacterial numbers may be related to sub-optimal conditions for bacterial survival. Experiments with ^{14}C labelled food have shown the shallow-water holothurian *Parastichopus parvimensis* to have assimilation efficiencies of the microbes associated with detrital material of greater than 40% (Yingst, 1976). Some of the ingested bacterial population appears to survive the initial digestion and flourishes in the hindgut/cloaca. Elevated bacterial numbers have been found in the enlarged gut chambers of some deep-sea species (Roberts et al., 1994). Subcuticular bacteria are also associated with holothurians. All of the bathyal and abyssal holothurians in the orders Elasipodida and Aspidochirotida studied by Roberts et al. (1991) had bacteria confined in the subcuticular space among apical folds and microvilli in their tentacles. These bacteria appear to be regulated by phagocytosis. It is postulated that bacterial metabolites provide a source of dietary material in the food-limiting deep sea (Roberts et al., 1991). Transmission electron micrographs have revealed rod-shaped gram-negative bacteria directly associated with the gut wall epithelial tissue of the deep-sea holothurian *Psychropotes*. These bacteria were only found in the hindgut section of the gut wall and it has been suggested that they are carried as resident gut flora (Deming and Colwell, 1982).

1.2.4 Holothurian reproduction

The egg size and fecundity of holothurians can be used as broad indicators of the developmental mode of a species, but some caution in the interpretation is necessary because eggs of a similar size can develop in different ways (Billett et al., 2001). Eggs with a diameter of 100 μm are usually numerous and are considered to lead to planktotrophic development. Eggs greater than 1000um are produced in low numbers and lead to direct development of a juvenile without an intermediary stage. Intermediate egg sizes are usually produced in moderately abundances and undergo lecithotrophic development (Tyler et al., 1982). The egg size and inferred developmental mode of some abyssal holothurians is given in Table 1.2.

Species	Tentacle structure	Egg diameter (μm)	Fecundity	Inferred developmental mode	Reference
Molpadia blakei	unknnown	200	100	planktotrophic or lecithotrophic with abbreviated larval stage	Tyler et al., 1987
Peniagone diaphana	simple peltate	300	5000	lecithotrophic with abbreviated larval stage	Tyler et al., 1985; Roberts et al., 2000
Pseudostichopus aemulatus	peltate	225	50,000	planktotrophic	Roberts et al., 1991; Watson, 2004
Paroriza Prouhoi	digitate	450		lecithotrophic	Tyler et al., 1992b; Roberts and Moore, 1997
Oneirophanta mutabilis	digitate	950	500-1500 (affected by available resources)	lecithotrophic	Tyler and Billett, 1987; Roberts and Moore, 1997; Ramirez-Llodra et al., 2005
Psychropotes longicauda	peltate	3000	>10	direct development	Tyler and Billett, 1987; Moore and Roberts, 1994
Amperima rosea	peltate	200	12,800	planktotrophic or lecithotrophic with abbreviated larval stage	Roberts et al., 2000; Wigham et al., 2003b

Table 1.2 Tentacle structure, egg diameter, fecundity and inferred developmental mode of some abyssal holothurians

1.2.5   Change in deep-sea holothurian communities

Shifts in community structure have been reported at two deep-sea benthic time-series stations. In the NE Pacific, a major change in the dominant epibenthic abyssal megafauna has occurred and has been correlated to climatic fluctuations dominated by El Niño/La Niña (Ruhl and Smith, 2004). Specifically, the holothurians *Elpidia minutissima* and *Peniagone vitrea* decreased in abundance after 2000; prior to this they were relatively high in abundance. In contrast, *P. diaphana*, *Abyssocucumis abyssorum*, *Scotoplanes globosa* and *Psychropotes longicauda* all increased substantially in abundance during 2001 and 2002.

At the Porcupine Abyssal Plain (PAP) in the NE Atlantic, long-term change has also been observed in the invertebrate megafauna over a period of 10 years (Billett et al., 2001). This change has been termed the '*Amperima* Event', characterised by the holothurians *Amperima rosea* and *Ellipinion molle*, which increased in abundance by more than two orders of magnitude between 1996 and 1999 (Billett et al., 2001). In addition, the metazoan meiofauna and macrofauna showed a response over this time period. Organisms living at the sediment surface showed an increased in abundance, and several of the infaunal species showed vertical movement correlating with variations in the burying of labile organic matter (Galeron et al., 2001). The increase in abundance of *A. rosea* was also thought to affect the reproduction of a small opheliid polychaete (Vanreusel et al., 2001). Temporal changes in the abundance of certain foraminiferal species was also observed at the PAP site (Gooday and Rathburn, 1999).

There was no apparent long-term trend in the total OM flux that might have accounted for the *"Amperima* Event" (Billett et al., 2001; Lampitt et al., 2001). The change has since been related to the selective feeding of some species (Ginger et al., 2001; Wigham et al., 2003a) and the resources (lipids and carotenoids) available to the animals (Hudson et al., 2003; Neto et al., 2006).

This has highlighted the need to study the resource allocation between species, the ways in which deep-sea animals procure their food, and the subsequent allocation of these resources into reproductive tissue.

1.3 Organic material flux to deep-sea sediments

Food availability is one of the most important limiting factors in deep-sea ecosystems. Excluding hydrothermal vents and cold seeps, all production is fuelled directly, or indirectly, by the input of primary production from the upper ocean (Gage and Tyler, 1991; Lampitt et al., 2001). The deep sea is an allochtonous, heterotrophic ecosystem i.e. the organic food is largely imported from a different environment and broken down there (Gage, 2003).

1.3.1 Controls on the supply of organic material to the deep-sea floor

Following the discovery of seasonal export flux of organic material from the upper ocean to bathyal and abyssal depths (Billett et al., 1983), it is now thought that most, if not all of the organic flux from the epipelagic environment to the abyss results from the sedimentation of aggregated phytoplankton (marine snow). This flux is tied closely to the annual cycle of primary production in the surface waters of the region (Deuser et al., 1981). The majority of organisms found in the benthic boundary layer are strongly linked to the sediment surface when they are adults and so are dependent on OM 'raining' down from the (often spatially remote) surface waters.

Organic material from the upper ocean may be repackaged and recycled during its descent with pelagic organisms taking advantage of the fresher material as it sinks through the water column (Turley et al., 1995). The vertical flux of marine snow depends on various factors, including the timing and make-up of the phytoplankton bloom, zooplankton interaction and physical dynamics of the water column (Turner, 2002). Large, rapidly-sinking particles are formed from the aggregation of small, slowly-sinking particles. This aggregation can occur through the biological activities of zooplankton, i.e. transformed into faecal pellets or trapped in the feeding webs of gelatinous zooplankton (Caron et al., 1989; Lampitt et al., 1993; Yoon et al., 2001). Studies have revealed that

salp faecal pellets play an important role in supplying labile material to the deep sea (Matsueda et al., 1986; Kawaguchi et al., 2004) especially as their fine webbed mucus nets can retain picoplankton (Silver and Bruland, 1981; Matsueda et al., 1986; Pfannkuche and Lochte, 1993; Kawaguchi et al., 2004). Intact cyanobacteria have been found in salp faeces recovered from the surface of a sediment sample taken at 4500 m (Pfannkuche and Lochte, 1993). Salps have characteristically large faecal pellets that can have fast sinking rates of 43 to 1167 m day^{-1} (Yoon et al., 2001). They have a patchy distribution and can be limited by high phytoplankton abundance (Kawaguchi et al., 2004). However, their high filtering rates means they can be serious competitors for other herbivorous zooplankton (Kawaguchi et al., 2004). They are also efficient in retaining particles within a relatively large size range (Pakhomov, 2004). Some zooplankton species are diel vertical migrators, a process which can positively influence the downward flux of their faecal pellets (Andersen, 1998). Aggregation of OM can also occur through collisions between particles to form progressively larger, faster sinking aggregates (Alldredge, 2001). This is important in phytoplankton bloom periods, where rapid aggregation and mass settlement of the primary production removes carbon from the upper ocean to the midwater and benthos before it is consumed and recycled by the near-surface grazers (Alldredge and Jackson, 1995). Sinking particles are of key importance to the global carbon cycle – the removal of carbon from the upper ocean to deeper parts of the ocean is referred to as the 'carbon pump'. Most of the organic carbon that reaches the benthos is respired, but the refractory residue is buried in the sediment and removed from circulation for centuries to millions of years (Turley, 2000).

Not all biological activity associated with sedimenting particles increases the downward flux of carbon. Attached bacteria remineralise and solubilise the aggregates before they reach the deep ocean. The degree of solubilisation is affected by processes controlling enzyme activity and production, such as temperature and pressure. Zooplankton can also disaggregate some particles

(Turley et al., 1995). Smaller zooplankton – particularly cyclopoid copepods – can have a detrimental effect on the downward flux of organic material (Gonzalez and Smetacek, 1994; Viitasalo et al., 1999; Svensen and Nejstgaard, 2003). These copepods are coprophagus – consuming the faecal pellets of larger copepods. The vertical flux of faecal pellet carbon has been shown to have a significant negative correlation with the biomass of the cyclopoid copepod *Oithona* sp. (Svensen and Nejstgaard, 2003).

Strong seasonal patterns and interannual variability in the flux of material to the seabed has been observed at the PAP between 1989 and 1999. This was evident in the material caught in sediment traps (Lampitt et al., 2001) and in photographic (Bathysnap) records of phytodetritus reaching the seafloor (Bett et al., 2001). Bathysnap recorded a maximum coverage of phytodetritus on the sea floor of 96% in 1994, whereas no mass deposition of phytodetritus was observed between 1997 and 1999, presumably because of the high feeding rate of the megafauna (Bett et al., 2001). Interannual variability has also been seen in the flux of particulate organic material (POM) during a time series study in the NE Pacific (Baldwin, 1998). The OM food source available to deep-sea organisms at this site changes dramatically over short time periods; in some cases in a matter of weeks (Beaulieu and Smith, 1998). This may have implications for the foraging and handling of food by abyssal megafauna and may also influence the dynamics of the deep-sea community (Billett et al., 2001). However, the quantity of OM reaching the deep-sea floor alone cannot always explain community shifts; the quality of OM must therefore be investigated (Billett et al., 2001).

1.3.2   *Changes in the chemical composition of the organic material flux*

The chemical composition of sinking and sedimentary OM can reflect the degradation, scavenging and the source of the material. Composition of OM caught in sediment traps from four different depths at the PAP showed that this

material changed with depth, reflecting the processes of dissolution and remineralisation (Lampitt et al., 2001). Labile compounds such as amino acids and Poly Unsaturated Fatty Acids (PUFAs) are degraded or scavenged quickly in the upper part of the water column (Wakeham et al., 1984). Intermittent appearances of large amounts of wax esters, steryl esters and sterols indicate biological activity on OM deeper in the water column (Wakeham et al., 1984). De Baar et al. (1983) also found PUFAs and total lipids decreased with water depth; the flux gradients of fatty acids increasing with the number of double bonds and decreasing in the number of carbon atoms.

The loss of fatty acids through the water column changes with the particle flux in the Arabian Sea (Reemsta et al., 1990). Degradation occurs higher up in the water column during low flux events; during high fluxes degradation occurs deeper in the water column. At the PAP, the lipid component of settling particles during high fluxes of OM is richer in labile compounds, specifically PUFAs and low molecular weight alcohols. During low flux events, other compounds such as sterols, steroidal ketones and trisnorhopan-21-one are more abundant (Kiriakoulakis et al., 2001). Neto (2006) observed similar temporal changes in the lipid biochemistry of the sediments at the PAP. Spatial variability in lipid biochemistry at the PAP has been linked to the patchiness of the phytodetritus (Santos et al., 1994).

Photosynthetic pigments are labile compounds that can also be used to elucidate the degradation and the source of OM. In a study in the Bellingshausen Sea, Antarctica, Fileman et al. (1998) found that at least some undegraded material of photosynthetic origin reaches the deep ocean. Xanthophyll, fucoxanthin and some labile fatty acids were found at depths of 3900m. The distribution of this labile material horizontally and vertically reflected the planktonic species composition and the physical environment (Fileman et al., 1998). Considerable diagenesis of chlorophyll with depth has been observed in the equatorial Pacific, with suspended particles in the northern hemisphere seemingly more degraded than in the southern, according

to the pigment signature. It is suggested that differences in the food chain could account for this i.e. zooplankton grazing creates chlorophyll breakdown products (Lee et al., 2000). Unaltered pigments have been detected at 1500 m in the Peru upwelling region. Approximately half of the carotenoids transported out of the euphotic zone by large particles in the Peru Upwelling region were not metabolised, but this only happened in productive areas where the higher trophic levels were the major consumers of phytoplankton; shipboard experiments indicated that zooplankton recycle carotenoids to non-carotenoids very quickly (Repeta and Gagosian, 1984). Therefore, where zooplankton are major contributors to the flux of large particles to the sediment, transformation products may be present in high concentrations.

Strong seasonal variations have been found in the fluxes of carbohydrate, protein and phytopigments through the water column at the PAP in the NE Atlantic. Pulses of labile organic material occur in spring and early summer, coinciding with the phytoplankton bloom (Fabiano et al., 2001). The freshness of OM caught in a sediment trap (as determined by its chlorophyll *a*: phaeophorbide ratio (Thiel et al., 1989)) was greater in September 1996 than the other sampling periods (March 1997, July 1997 and October 1997) (Witbaard et al., 2000; Witbaard et al., 2001). The chlorophyll *a*: phaeophorbide ratio of sediment samples taken from sediment cores also showed the same seasonal and interannual patterns (Witbaard et al., 2000; Witbaard et al., 2001).

1.3.3 *Benthic community and population response to the flux of organic material*

Organisms living close to the sediment surface have the strongest impact on the decomposition of phytodetritus (Aberle and Witte, 2003). Mobile megafauna are able to repackage and concentrate the phytodetrital material, depositing their faecal pellets in different locations. This material may be altered in the

animal's gut, thus changing the horizontal distribution and composition of the settled material on the sediment surface (Lauermann et al., 1997). Movement patterns of mobile epibenthic deep-sea fauna may also facilitate the redistribution of patchy resources.

Temporal changes in food quantity and quality lead to changes in potential food sources for benthic organisms. This may affect the way the benthic organisms handle and forage for their food (Beaulieu and Smith, 1998). In particular, organisms may have adapted to deal with seasonal variation in the fluxes of OM. In shallow-water ecosystems, the seasonal input of labile OM derived from primary production creates a burst of activity in benthic organisms. The response to this seasonal flux is more pronounced in species feeding directly on it than in species feeding on the meiofauna and sub-surface sediments (Graf et al., 1982).

Benthic responses to organic input can differ from year to year depending on the quality and quantity of food available (Pfannkuche et al., 1999). The nature of the response to the seasonal OM flux differs depending on the trophic environment of the region (Gooday, 2002a). In abyssal oligotrophic areas, the response to seasonal OM supply is primarily seen in small organisms such as bacteria and protozoa (Soltwedel, 2000). Bacteria and meiofauna in a food-limited environment have been shown to be more efficient in exploiting particulate organic fluxes (Danovaro et al., 1999). Food limitation may even influence bacteria (Turley, 2000), and such conditions are insufficient to fuel population or reproductive responses in larger animals (Gooday, 2002a). Simulated falls of detrital aggregates on deep-sea microbial populations found rapid colonisation, growth and decomposition rates of microbes to inputs of organic carbon (Turley and Lochte, 1990). The rapid bacterial response may result in transforming detritus into nutritious bacterial biomass that would otherwise be unavailable to higher consumer organisms (Turley and Lochte, 1990).

Meiofauna are generally less responsive to seasonal OM fluxes than protists such as foraminifera, although seasonal increases in abundance and body size have been reported (Soltwedel, 2000). A difference in benthic response (in terms of biomass and density) by meiofauna has been observed in two different trophic environments in the Mediterranean (Danovaro et al., 1999). Meiofauna in oligotrophic areas showed no seasonal response to organic input, whereas meiofauna found in areas of high primary productivity reacted to the temporal pulses in organic material (Danovaro et al., 1999). Drazen et al. (1998), observed a significant increase in protozoan (foraminiferal) density over a short time scale (4 weeks) in response to phytodetrital input at an abyssal site in the NE Pacific. Obvious macro- and mega-faunal population responses to pulsed food inputs are difficult to establish because of their larger size and longer life histories (Gooday, 2002a). Macrofaunal responses have been observed during an *in situ* experiment at an abyssal station, using ^{13}C as a tracer. Macrofauna were seen to be more important than bacteria and foraminifera in initial carbon degradation; after 2.5 days, 77% of the macrofauna had ingested ^{13}C-labelled organic material (Witte et al., 2003). This contrasts with the results from a bathyal continental margin site, where foraminifera were rapid consumers of fresh OM, suggesting they may play a central role in the initial processing of fresh OM arriving at the seafloor (Moodley et al., 2002). Two holothurian species studied at a NE Pacific site have shown responses to food input. These megafaunal species had varying rates of movement across the sediment, depending on the seasonal organic input to the sea floor (Kaufmann and Smith, 1997).

Measurements of the supply of organic material to the benthos and sediment community oxygen consumption (SCOC) can be used to approximate the supply and demand for carbon in the deep sea (Smith and Kaufmann, 1999). During an *in situ* pulse-chase experiment, the sediment community oxygen consumption doubled after simulated phytodetrital input (Witte et al., 2003). Seasonality has been observed in the SCOC and is related to the vertical flux of the seasonal phytoplankton blooms in the upper ocean (Smith and Baldwin,

1984), although this seasonality has not always been observed (Lampitt et al., 1995). Smith et al. (1998) showed highly variable SCOC depending on the quality of the aggregates arriving at the deep-sea floor. The quality of the material may explain the lack of a SCOC peak during the highest flux event (September 1996) at the PAP in a sample period (September 1996, March/April 1997, July 1997 and September 1998), i.e. the most labile fraction of the aggregates may have been lost, despite relatively enhanced chlorophyll *a*: phaeophorbide ratios (Witbaard et al., 2000). Although the flux of material was high compared to the other sample periods in the study, it is suggested that the seasonal difference in the quantity or quality of organic matter was too small to provoke bacterial activity and hence no difference in the SCOC was observed (Witbaard et al., 2000).

There is some evidence for a discrepancy between supply and demand for carbon in the deep sea; the vertical flux of food supply does not always meet the energy requirements (Smith and Kaufmann, 1999; Thomsen, 1999; Smith et al., 2001). In particular, a deficit in food supply has been reported at an abyssal station in the NE Pacific. The sediment community cannot be sustained under these conditions without ultimately affecting the structural and functional characteristics of the community (Smith et al., 2001). Several reasons have been put forward to explain this deficit. It could be linked to increasing surface water temperature and reduced plankton biomass. Sediment traps are also likely to under-sample large sinking aggregates, and hence underestimate the accumulation and composition of detritus found on the sea floor (Beaulieu and Smith, 1998). Lateral advection of POM and dissolved organic carbon may also fuel the sediment community. Longer time-series and improved sampling methods are needed to cover cyclical events, such as El Niño, that may affect faunal patterns over time.

Some deep-sea species show a response to the vertical flux of primary production by reproducing seasonally. However, the vast majority of deep sea animals (particularly at abyssal depths) reproduce aperiodically or continuously

(Young, 2003). Species found to reproduce seasonally are the ophiuroids *Ophiura ljungmani, Ophiocten hastatum* (an abyssal species) and *Ophiocten gracilis* (a bathyal species), the asteroids *Plutonaster bifrons* and *Dytaster grandis* (an abyssal species), and the echinoid *Echinus affinis* (Tyler et al., 1982; Tyler, 1986; Tyler, 1988; Suminda et al., 2000; Gooday, 2002a; Gage et al., 2004). As well as other features, these species spawn in the early spring of each year (Gage and Tyler, 1991). It is suggested that the seasonal flux of primary production fuels vitellogenesis in the adults and provides food for the planktotrophic larvae.

1.3.4 *Benthic response to the quality of organic material*

Recent studies have shown a link between the quality of OM reaching the deep-sea floor and the effect this can have on the biochemistry of the organisms. However, this response can also be dependent on the feeding ecology and reproductive adaptations of the organisms (Ginger et al., 2001; Hudson et al., 2003; Wigham et al., 2003a; Hudson et al., 2004; Neto et al., 2006). A study at the PAP in the NE Atlantic, suggested that a bloom of *Amperima rosea, Ellipinion molle* and other megafauna selectively removed phytosterols from the fresh flux of phytodetritus in less than four months (Ginger et al., 2001). This will have an impact on the food resource to other animals. Deep-sea megafauna rely on the supply of phyto-derived sterols for their metabolism; the supply of these compounds effectively controls their population. Phytosterols are an important resource because they cannot be biosynthesised *de novo* (Ginger et al., 2001). *Amperima rosea* is the only deep-sea holothurian that clearly assimilates 4α-methylsterols (Ginger et al., 2000), which are diagnostic of dinoflagellates (Brassel and Eglington, 1986). *Amperima rosea* has been observed to increase rapidly in population size, and colonise large areas quickly, with full vitellogenetic development dependent on environmental stimuli such as long-term variations in food supply. Its

requirement for specific sterols indicates that the quality of food can have an affect on the population dynamics of this species (Wigham et al., 2003a).

Temporal variations have been observed in the fatty acid composition of holothurians found at abyssal and bathyal depths (Hudson et al., 2004; Neto et al., 2006). *Amperima rosea*, *Bathyplotes natans* and *Laemogone violacea* all show significant fatty acid changes concurrent with the seasonal deposition of organic material (Hudson et al., 2004). It is suggested that these species may allocate PUFAs and reproduce at times when the availability of fresh organic material is high (Hudson et al., 2004), although *A. rosea* has shown no clear evidence of seasonal or episodic reproductive events (Wigham et al., 2003b). *Psychropotes longicauda* and *Benthogone rosea* have the opposite trend – their PUFA levels are higher before the deposition of phytodetritus (Hudson et al., 2004). These holothurians have large eggs (Billett, 1991) that develop into a juvenile without a larval stage (Tyler and Young, 1992). It is proposed they release a greater number of eggs during the arrival of fresh phytodetritus, when nutritional resources for juveniles are high (Hudson et al., 2004), although no seasonal changes in fecundity have been recorded (Tyler and Billett, 1987). Other deep-sea holothurians, *Oneirophanta mutabilis,* and *Deima validum,* show little temporal change in PUFA proportions (Hudson et al., 2004). They have similar egg sizes of 950 and 800 μm (Billett, 1991). Hudson et al. (2004) suggest they produce a continuous supply of eggs throughout the year. Both species contained high levels of 18:1(n-7) monoene – a bacterial fatty acid marker – during the pre-bloom period, indicating they utilise bacteria as a source of carbon at this time (Hudson et al., 2004). Variations in body wall fatty acid composition have also been observed in response to a changing supply of lipids, dependent on the feeding guild of the species (Neto et al., 2006). *Oneirophanta mutabilis* tissues show an increase in sterols concurrent with an increase in sterols in POM reaching the sea floor. However, *O. mutabilis* feeds on the same material as *Psychropotes longicauda* when rich organic matter is scarce (Neto et al., 2006).

1.4 Carotenoids

1.4.1 Biochemistry of carotenoids

Carotenoids represent one of the most widespread groups of natural pigments (Goodwin, 1980) and range in colour from yellow to red (Davis, 1991). They can be synthesised *de novo* by plants, fungi, algae and bacteria (Goodwin, 1980). All carotenoids are based on the hydrocarbons α-carotene, β-carotene and ε-carotene (Goodwin, 1980). There are two types of carotenoids: the oxygen containing xanthophylls, e.g. diatoxanthin and zeaxanthin and the hydrocarbon carotenes e.g. β-carotene (Olson and Owens, 1998) (Fig. 1). They are accumulated in the diet by animals and are sometimes modified further. These modifications can be significantly different between Classes and Families in a Phylum, as well as at species level. Novel carotenoids can be found in different tissue types such as the gonads where the animal modifies carotenoids from its diet and accumulates the new forms where it is required (Tsushima et al., 1996). Specific carotenoids can play different functional roles in the same animal; esterified astaxanthin was found in the cuticle, whereas no esterified astaxanthin was found in the ovary of the crayfish *Cherax quadricarinatus* (Sagi et al., 1995). Figure 1.2 shows the structure of some carotenoids found in echinoids (Matsuno, 2001) and in the gut sediment and ovaries of deep-sea holothurians (Hudson et al., 2003; Wigham et al., 2003a).
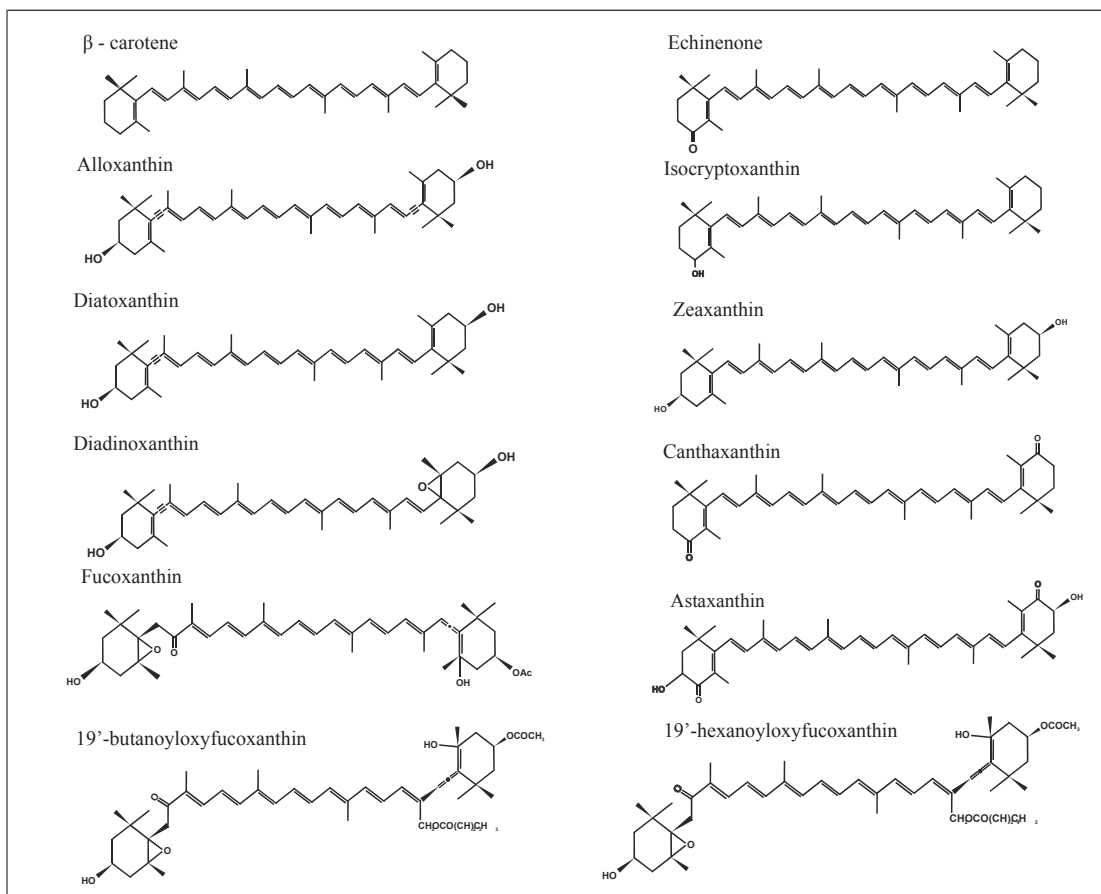
Figure 1.2 The structure of the main carotenoids found in echinoids and deep-sea holothurians (Matsuno, 2001; Hudson, 2004 and the present study). (Diagrams from (Goodwin, 1980))

Carotenoids stabilise proteins and membranes, and deactivate reactive chemical species that may otherwise induce harmful processes in biological systems (Krinsky, 1994; Britton, 1995; Matsuno, 2001). Much of the literature on the functions of carotenoids in marine systems discusses their photoprotective functions. The deep sea is a dark environment, therefore carotenoids must play functional roles other than photoprotection in the deep-sea biota that accumulate and modify them. They can play a beneficial role in modifying cell membrane structure, properties and stability. Britton (1995) described how carotenoids can be found in precise orientations and locations in subcellular structures and that their properties can be strongly influenced by other molecules, particularly proteins and lipids, in their near vicinity. In a

review of the functions of carotenoids in Mollusca, Vershinin (1996) suggested their most probable function is that of stabilising the fluidity of cell membranes.

Carotenoids also act as a good donor and acceptor of electrons in chemical reactions. Oxygen free-radicals produced during aerobic respiration can damage DNA, proteins and carbohydrates. Therefore carotenoids are able to reduce the undesirable effects of aerobic respiration (Bendich and Olson, 1989; Di Mascio et al., 1991; Olson, 1996). Carotenoids may also contribute to cellular immuno-protection at critical stages, for example oocyte differentiation, that pose a high potential for free radical production (Linan-Cabello et al., 2003). Matsuno and Tsushima (1995) have suggested that novel carotenoids are especially abundant in the eggs of shallow-water sea cucumbers and play an important role in preventing oxygen toxicity.

1.4.2 Carotenoids and reproduction

Carotenoids are accumulated from the diet into the eggs of many taxa in the animal kingdom. Ovarian maturation is characterised by an accumulation of carotenoids in crustaceans (Linan-Cabello et al., 2002). Carotenoids give the characteristic yellow yolk of chicken eggs and the orange roe of scallops. They are also accumulated into the eggs of echinoderms (Matsuno and Tsushima, 1995; Matsuno and Tsushima, 2001). It is believed maternally-derived carotenoids in eggs protect the developing embryo from elevated reactive oxygen species released by the metabolism of lipids used for nourishment (Blount et al., 2000; 2004; Lotocka et al., 2004).

Carotenoid profiles and concentrations in the gonads of echinoderms can be affected by various factors. They can vary between orders (Tsushima et al., 1993a; Matsuno and Tsushima, 1995; Tsushima et al., 1995), between species (Borisovets et al., 2002; Hudson et al., 2003; Wigham et al., 2003a; Pantazis, 2006) as well as between sexes and stage of gonadal maturity, which may

explain variance between samples in a species (Borisovets et al., 2002; Lawrence et al., 2004). Diet can also have an effect on the carotenoid profiles and concentrations in eggs (Kawakami et al., 1998). Studies of carotenoids found in marine biota have focused mainly on economically important species because of the need to gain the greatest quantity and most viable offspring, as well as obtaining the desirable deep-coloured roe. Feeding sea urchins a diet containing no pigment results in light, beige coloured gonads (Robinson et al., 2002). The synthesis of a feed in aquaculture that produces high quality gonads improves broodstock quality and quantity as well as the aesthetic quality of the roe.

Shallow-water echinoderm feeding experiments have shown carotenoids can enhance the colour of the roe to increase commercial viability, but more importantly, increase fecundity, larval maturation and survival (George and Young, 1998; George et al., 2001; Mclaughlin and Kelly, 2001; George and Lawrence, 2002). Increased food quality (a rich, algal diet) can increase green sea urchins gonad size, body mass and total mass, demonstrating the importance of the quality of diet (Lemire and Himmelman, 1996). However, Plank et al. (2002) observed that the growth of the gonad was independent of carotenoids in the diet of the sea urchin *Lytechinus variegatus*. This study also found that the carotenoid profile of the gut varied with diet, but was not identical to the composition of the diet (Plank et al., 2002).

Favourable conditions for adults are translated into the production of high quality eggs. The bathyal echinoid *Stylocidaris lineata* requires fresh algal input to maintain the production of high quality eggs, although some 'fresh' diets were preferable to others. The quality of eggs was maintained, although the number of eggs decreased on a diet of *Thalassia testudinium* compared to a *Sargassum* spp. diet (George and Young, 1998). Larvae of the sea urchin *Lytechinus variegatus* from parents fed on xanthophylls (20-25% lutein, 60% zeaxanthin and the rest orange-red xanthophylls) were larger throughout development, developed faster, had higher survival rates and attained

metamorphic competence faster than those fed just β-carotene. The numbers of juveniles originating from parents fed xanthophylls were also significantly higher (George et al., 2001). Survival rates increased further for all maternal feeding experiments, when the larvae were then fed a mixed algal diet (George and Lawrence, 2002). This study shows that maternal diet is very important, especially for species with lecithotrophic larvae (i.e. many deep-sea species) where the development of the offspring relies on the maternally derived nutriment. The availability of certain essential carotenoids in the deep sea may determine the viability of offspring and thus influence population dynamics, especially for species that are able to take advantage of higher 'quality' food patches either by selective feeding or by increased mobility between patchy food sources.

Evidence of carotenoid metabolism has been obtained through echinoderm feeding experiments. The metabolism of β-carotene to echinenone (for deposition into the gonad) has been observed in *Lytechinus variegatus* (Plank et al., 2002). There are several pathways of metabolising dietary carotenoids and some of these occur in the gut. Individuals fed zeaxanthin had the lowest percentage of zeaxanthin in their gonads and highest percentage in their test. It was postulated that zeaxanthin was either immediately metabolised or not deposited in the gonad. β-carotene was metabolised to echinenone (the major carotenoid constituent in the gonad accounting for up to 82% of the total) and assimilated into the gonad (Plank et al., 2002). Echinenone is found in many echinoderm species, especially in their ovaries (Matsuno, 2001) and its metabolism from β-carotene has been shown in other studies (Tsushima and Matsuno, 1990a; Tsushima et al., 1993b; Matsuno and Tsushima, 1995). The conversion of β-carotene to echinenone occurs in the gut wall via its precursor β-isocryptoxanthin (Tsushima et al., 1993b). Matsuno and Tsushima (1995) showed that echinenone can be converted to canthaxanthin and further to astaxanthin. Bandaranayake and Des Rocher (1999) found approximately 90% of the total carotenoids of the gut wall and gonad of the sea cucumber *Holothuria atra* to be highly oxidised (with the main carotenoids being

astaxanthin and canthaxanthin). They postulated this species provides specific carotenoids to the ovaries either by concentrating very small amounts of oxygenated carotenoids from the diet or by the more probable means of metabolising β-carotene and/or xanthophylls. The presence of β-carotene in significant amounts in the diet and gut contents but not in the gut tissues and ovaries supports the modification theory (Bandaranayake and Des Rocher, 1999).

The molecular structure of astaxanthin and canthaxanthin makes them significantly more efficient antioxidants than β-carotene. This may explain why animals modify carotenoids from their diet. Astaxanthin and canthaxanthin have thirteen conjugated double bonds, in contrast to eleven in β-carotene (see Fig. 1), giving them significantly greater antioxidant capacity. Superior singlet oxygen quenching ability of astaxanthin has been demonstrated over β-carotene, zeaxanthin and canthaxanthin (Miki, 1991; Shimidzu et al., 1996; Naguib, 2000). A hydroxyl group on each cyclohexane ring (see Fig. 1) makes astaxanthin highly polar, enhancing its membrane protection ability. The polar end groups allow astaxanthin to sit near the lipid/water interface, where free radical attack first occurs (Kurashige et al., 1990).

The decreasing quality of OM with increasing depth results in reduced availability of carotenoids essential for reproduction in echinoderms. The bathyal echinoid *Stylocidaris lineata* is a deposit feeder that ingests sediment, animal remains and pieces of macroalgae that infrequently settle from the euphotic zone. A feeding experiment has shown *S. lineata* can survive but not reproduce solely on a sediment diet, and that it requires macroalgae to produce eggs (George and Young, 1998). Food-driven environmental forcing has been suggested as a cause for the increase in abundance of the abyssal holothurian *Amperima rosea* on the Porcupine Abyssal Plain (Wigham et al., 2003a). The gut content and ovarian carotenoid profiles of this species were identical and dominated by the pigments zeaxanthin, chlorophyll *a*, echinenone and β-

carotene. The gut pigment profiles of *A. rosea* lacked pigments characteristic of other phytoplankton groups, which were observed in the other abyssal species. Relatively high concentrations of the carotenoid zeaxanthin (a biomarker for cyanobacteria (Jeffrey et al., 1997)) were found in the gut sediment and ovaries of *A. rosea*, compared to other holothurian species. It was postulated that elevated levels of cyanobacteria in the organic matter flux to the deep sea-floor may have given *A. rosea* a reproductive competitive advantage, possibly leading to major community changes like the "*Amperima* Event" (Wigham et al., 2003a). It is postulated that resource partitioning of the phytodetrital flux at abyssal depths (and less pronounced at bathyal depths; Hudson et al., 2003), may explain the mechanism for maintaining high diversity of deposit feeders in the deep sea. Resource partitioning may have given *A. rosea* a reproductive advantage in utilising any change in the composition of the food source (Hudson et al., 2003; Wigham et al., 2003a). This is highlighted by a study that indicates *A. rosea* has an opportunistic reproductive pattern, with an apparent hold on vitellogenesis until resources are favourable (Wigham et al., 2003b).

1.4.3 Carotenoids as biomarkers

Chemotaxonomy of water column communities using phytopigments has been adapted in order to characterise the phytoplankton community reaching the benthos (Repeta and Gagosian, 1982; Riaux-Gobin et al., 1987; Bianchi et al., 2000a). Examples of some of the main chemotaxonomic pigments are given in Table 1.2.

Light, oxygen and temperature affects the degradation rates of carotenoids (Leavitt, 1988; Abele-Oeschger, 1991). Oxygen is an essential factor for light and metabolic diagenesis (Leavitt, 1988), suggesting pigments degrade faster in oxic sediments. Low oxygen concentrations in the water column and sediment are therefore an important factor for carotenoid preservation

(Sinninghe Damsté and Koopmans, 1997). An experimental approach introducing phytoplankton to mesocosms found the fastest pigment decay and build-up of chlorophyll breakdown products occurred under oxic conditions with deposit feeding macrofauna. Bioturbation (the biogenic mixing of sediment and porewater; sensu Richter, 1952) stimulates diagenesis by increasing oxygen availability and mechanical fragmentation through feeding activities (Bianchi et al., 2000b).

The lability of pigments and their transformation products - resulting from chemical and metabolic processes - can cause problems when diagnosing which phytoplankton group dominates or contributes to the vertical flux of organic matter (Lotocka, 1998). Pigments exhibit different degrees of degradation, making interpretations of the phytoplankton classes contributing to the organic matter reaching the sea floor difficult. The ratios of pigment concentrations should therefore not be used as an indicator of the relative biomass of certain classes of phytoplankton when investigating sediment samples (Rabalais et al., 2004). The transformation products resulting from chemical and metabolic processes have not been studied in detail. Therefore, there are many carotenoids in sediments that remain unidentified (Repeta and Gagosian, 1987). These breakdown products can also mask small concentrations of 'pristine' pigments on HPLC chromatograms (Howell et al., 2004)

Phytoplankton Group/Pigment Source	Pigment Biomarkers
Cyanophyta (Cyanobacteria)	Zeaxanthin, β-carotene, echinenone (in filamentous Cyanobacteria with gas vacuoles)
Prochlorophyta (*Prochlorococcus* spp.)	Zeaxanthin, Divinyl chlorophyll *a* and *b*, β-carotene
Cryptophyta (nanoplanktonic flagellates)	Alloxanthin, chlorophyll c_2
<u>**Chlorophyta**</u> (green flagellates) - Chlorophyceae (naked flagellates *Chlorella* and *Dunaliella* spp.) - Prasinophyceae (flagella and body covered in organic scales)	Chlorophyll *b*, β-carotene, lutein, neoxanthin, violaxanthin Zeaxanthin Prasinoxanthin
Euglenophyta (fusiform flagellates, can be indicators of organic pollution)	Chlorophyll *b*, β-carotene, diadinoxanthin, fucoxanthin
Eustigamatophyta (yellow-green algae *Nannochloropsis* spp., often used in aquaculture)	β-carotene, violaxanthin
Bacillariophyta Diatoms	Chlorophyll c_1 and c_2, fucoxanthin, diadinoxanthin, diatoxanthin
Dinophyta Dinoflagellates	Chlorophyll c_2, peridinin, diadinoxanthin, diatoxanthin, dinoxanthin
<u>**Haptophyta and Chrysophyta**</u> (golden-brown flagellates) - Prymnesiophyceae (coccolithophores and *Phaeocystis*) - Chrysopyceae (most freshwater) - Raphidophyceae (most freshwater, some coastal blooms)	Chlorophyll c_2, β-carotene, fucoxanthin Chlorophyll c_1 and c_3, 19-butanoyloxyfucoxanthin, 19-hexanoloxyfucoxanthin Chlorophyll c_3, 19-butanoyloxyfucoxanthin Chlorophyll c_1
Zooplankton	Astaxanthin
Grazing – digestion by herbivores (breakdown product of Chlorophyll *a*)	Phaeophorbide *a*
General breakdown product of Chlorophyll *a*, *b* and *c* respectively	Phaeophytins *a*, *b* and *c*

Table 1.2 Phytoplankton groups and their biomarkers (compiled from Repeta and Gagosian, 1987; Leavitt, 1993; Strom, 1993; Jeffrey et al., 1997; Lotocka, 1998; Jeffrey et al., 1999; Bianchi et al., 2002; Hansen and Josefson, 2003)

Chlorophyll *a* is more labile than many of the carotenoid pigments (Hodgson et al., 1997; Rabalais et al., 2004). The proportion of zeaxanthin in salp faeces found on the sediment surface at a depth of 4500m in the Northeast Atlantic was higher than that in fresh salp faeces, suggesting zeaxanthin is more stable than chlorophyll *a* related pigments (Pfannkuche and Lochte, 1993). Zeaxanthin is generally more stable than chlorophyll *a* as it acts as its

photoprotectant in cyanobacteria (Bianchi et al., 2002). It has also been used as a marker to study past changes in deposition in sediments in the Baltic Sea (Bianchi et al., 2000a). The carotenoid fucoxanthin degrades faster than chlorophyll a (Klein and Riaux-Gobin, 1991). Ester hydrolysis and dehydration of fucoxanthin has been observed with particles collected in sediment traps, and it is proposed that carotenoids structurally similar to fucoxanthin (peridinin and diadinoxanthin) will have analogous transformations (Repeta and Gagosian, 1982). Selective degradation occurs in the sediment of fucoxanthin, peridinin and diadinoxanthin over diatoxanthin, which differs from the other 3 structurally (Repeta and Gagosian, 1987). Fucoxanthin and diadinoxanthin have an epoxide function and/or the presence of an ester group which makes them more sensitive to acidification, oxidation and hydrolysis (Britton, 1983). Descy et al. (1999) suggested that these processes occur in zooplankton guts. Fucoxanthinol, which is slightly more polar than fucoxanthin may be a zooplankton-derived metabolite (Repeta and Gagosian, 1982). Descy et al. (1999) also proposed that alloxanthin is found regularly in copepod guts because it is resistant to degradation processes. However, a recent study by Antajan and Gasparini (2004) showed that zooplankton store alloxanthin (as a possible precursor to astaxanthin) in their bodies, which makes it unsuitable as a biomarker for cryptophytes in their diet. They proposed that this pigment could have been obtained from their diet or by transformations of other dietary carotenoids.

Phytoplankton and zooplankton community structure can affect the quantity and quality of 'pristine' pigments arriving at the deep-sea floor. Astaxanthin is the main active carotenoid in copepod metabolism (Lotocka et al., 2004). β-carotene, zeaxanthin, alloxanthin, diatoxanthin and lutein have been suggested as astaxanthin precursors (Katayama et al., 1973; Sagi et al., 1995; Ohkubo et al., 1999). In a mesocosm experiment, astaxanthin production in copepods was low when copepods were fed with low phytoplankton biomass or heavily silicified diatoms (the diatoms were not consumed and grazing was mainly on prymnesiophytes). Astaxanthin production was highest when the copepods

were grazing on a diverse phytoplankton community of high biomass dominated by chlorophytes, dinoflagellates and diatoms with thin silica frustules (Andersson et al., 2003).

Cyanobacteria have been used to indicate benthic-pelagic coupling between salps and deep-sea holothurians in the Northeast Atlantic (Pfannkuche and Lochte, 1993). The salps concentrated the small cells and they were quickly removed from the water column by the fast sedimentation of their faecal pellets. This suggests one way of 'pristine' carotenoids (in this case zeaxanthin associated with the Cyanobacteria) reaching the deep-sea benthos. Pfannkuche and Lochte (1993) found no significant decrease in cyanobacteria between stomach and hindgut of two deep sea holothurians *Oneirophanta mutabilis* and *Psychropotes longicauda*. This suggests the lack of efficient degradation of cyanobacteria by these holothurians. However, only three specimens of each species were examined and no corresponding faecal pellets were examined.

1.5 The approach of this study

Hudson et al. (2003) and Wigham et al. (2003a) suggest that the supply of specific carotenoids, and the selective feeding on these carotenoids, may give certain deep-sea holothurians a reproductive advantage. However, a comparison has yet to be made between the supply of carotenoids in the flux of OM to the seafloor and the influence this may have on the ovarian biochemistry of deep-sea holothurians. The principal objective of the present study is to examine the link between diet and the ovarian carotenoid biochemistry of abyssal holothurians, which potentially has implications for their reproductive output. This will be approached in three ways – a temporal study at an abyssal site in the NE Atlantic, a spatial study in the Southern Ocean and a comparison between all three sites.

Temporal study

The supply of phytopigments (in the phytodetritus and sediment) and carotenoid biochemistry (gut wall and ovaries) of abyssal holothurians from the PAP will be determined from two consecutive years. Shallow-water echinoderm studies have shown the metabolism of β-carotene through to echinenone occurs (Tsushima et al., 1993b; Plank et al., 2002) in the gut wall and that this is a site of elevated carotenoid concentration (Griffiths and Perrott, 1976). The present study examines the carotenoid biochemistry of the gut wall in order to establish whether this is true for abyssal holothurians. Holothurian species were chosen to include differing feeding guilds and reproductive adaptations. Comparisons were made between years and between species comparisons were be made, and the possible influence of organic matter composition and quantity on the reproductive and feeding adaptations of each species assessed

Spatial study

Two deep-sea sites around the Crozet Islands, Southern Ocean, were sampled to determine the supply of phytopigments to the sea floor. These two sites are

similar in benthic topography and depth, but differ in their overlying primary productivity regimes; each site is thought to receive contrasting abundance and composition of OM. Holothurians, chosen for their abundance and co-occurrence at each site, were sampled and analysed for their gut wall and ovarian carotenoid biochemistry. The supply of the phytopigments and holothurian biochemistry were compared between sites and related to the feeding guild and reproductive adaptations of the holothurians.

Among-site comparison

This project provides an opportunity to compare the influence of OM supply on the biochemistry of holothurians at spatially remote abyssal sites. The PAP is situated in the Northern hemisphere, whereas M5 and M6 are located in the Southern hemisphere. The PAP and M5 both exhibit a seasonal phytoplankton bloom. Primary productivity values are similar (~2 $mgC/m^2/d$) between these two sites during their respective bloom periods (Lampitt et al., 2001; Seeyave et al., 2007). While it is known that the seasonal bloom at the PAP creates a seasonal flux of OM to the seafloor on an annual basis (Lampitt et al., 2001), the flux of OM to the seafloor has only been recorded at M5 over a period of a year (Salter, 2007). An extended flux of phytodetritus was observed at M5 after the seasonal phytoplankton bloom in the Austral Summer of January 2005, the flux then declined until the start of the next bloom in December 2005 (Salter, 2007). Unliike the PAP and M5, M6 by exhibits little or no seasonal phytoplankton bloom (SeaWIFS data) (Pollard et al., 2002). Productivity values at this site (taken during the bloom period at M5 – December 2004) were 0.4 $mgC/m^2/d$. The flux of phytodetrital material at M6 has only been measured over a one-year period. A short, high flux of material was observed at the start of a sediment trap deployment in December 2004; after this, the flux was negligible (Salter, 2007).

The supply of OM, the sediment biochemistry, as well as the holothurian feeding selectivity and carotenoid biochemistry of species common to the three sites, will be compared. Differences and/or similarities will be discussed with

regard to the biogeochemistry of each site, as well as the feeding guild and reproductive adaptations of the holothurians.

Finally, the project also aims to refine the approach used by Wigham et al. (2003a), Hudson et al. (2003) and Howell (2004) for determining feeding selectivity using phytopigments as biomarkers. A new analytical method to improve the resolution and confidence of identification of pigment peaks will be described.

Chapter 2 – Methods

Three abyssal sites have been chosen for this study. The first, at the Porcupine Abyssal Plain (PAP) in the NE Atlantic is the focus of a temporal (time-series) study. Two nearly adjacent sites (M5 and M6) around the Crozet Islands, Southern Ocean, are the focus of a spatial comparison. Data from the three sites will be compared to ascertain differences and similarities in the influence of OM supply on the carotenoid biochemistry of the holothurians.

Phytodetritus, sediment and holothurians were sampled at the PAP in the NE Atlantic during two research cruises in June 2004 and July 2005. The sediment at stations, M5 and M6 around the Crozet Islands in Southern Ocean, was sampled during a research cruise in December 2004 and January 2005. The following year (December 2005 and January 2006) the phytodetritus, sediment and holothurians were sampled at M5 and M6.

2.1 Porcupine Abyssal Plain, Northeast Atlantic – a Temporal Study

2.1.1 The study area

The Porcupine Abyssal Plain (PAP) is situated 270km southwest of Ireland (Fig. 2.1) at a depth of c. 4850m (Fig. 2.5). Since 1989, a number of European Union-funded projects have generated a time-series of benthic observations at this site with the aim of determining how the seabed community and geochemistry of the sediments change in response to a highly seasonal input of organic matter from the overlying waters (Billett and Rice, 2001). The site was chosen for its distance from the continental slope and mid-Atlantic ridge, making it relatively free of any downslope sediment transport. The seabed is very flat, facilitating the use of many types of benthic sampling equipment.
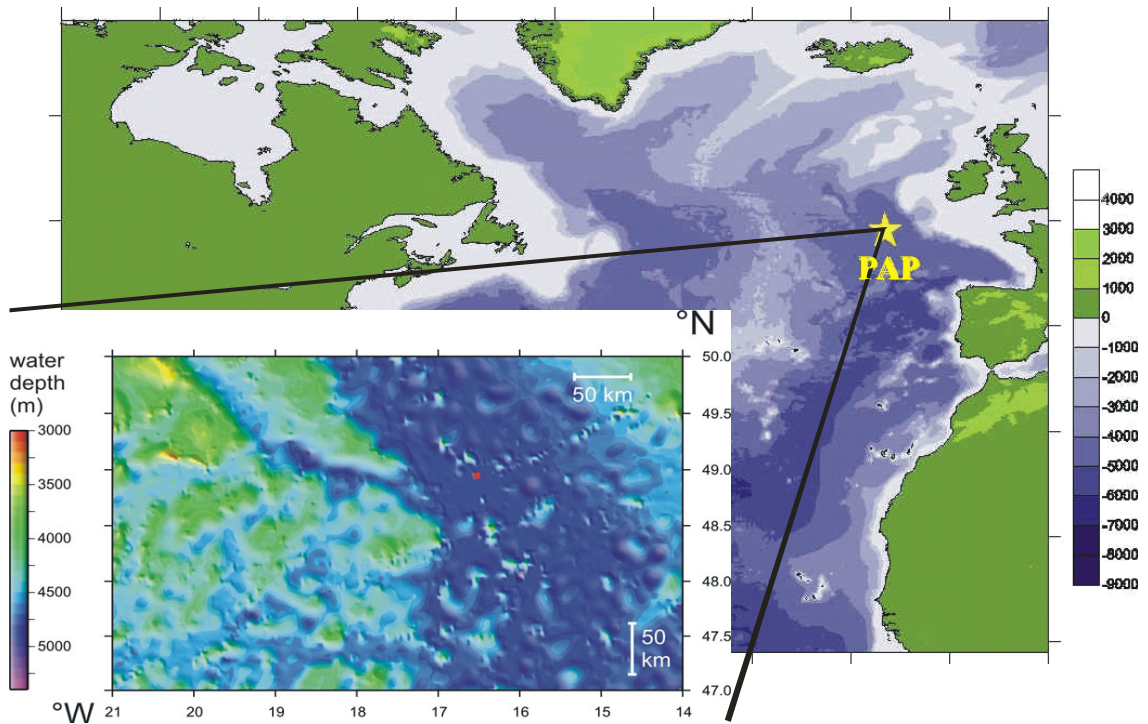
Figure 2.1 Location of Porcupine Abyssal Plain (PAP)

2.1.2 Hydrography

Northeast Atlantic Deep Water (NEADW), formed by the waters from the Iceland/Scotland overflow and Labrador Sea, overlies the PAP. It is characterised by a deep salinity maximum and high oxygen levels (Tomczak and Godfrey, 1994; Van Aken, 2000). Over a 10 year study, currents within 150m of the seabed at the PAP remained <15cm s^{-1} and there was no evidence of benthic storms (Lampitt et al., 2001). The winter mixed layer in the upper ocean over the PAP is approximately 500m (Rice et al., 1994), although there can be large interannual variations (Lampitt et al., 2001). According to the Kraus-Turner model, upper layer mixing in winter is controlled primarily by changes in air-sea flux and in summer by wind (Bleck et al., 1989).

2.1.3 Sediment composition

The sediment at the PAP is a calcareous ooze, although clinker deposits from burnt coal and shale litter the seabed. This material, dumped overboard during the steamship era, is more widespread on abyssal plains than debris deposited by geologic agents such as icebergs (Kidd and Huggett, 1981). The soft sediment of this area is an ideal substratum for deposit feeding organisms, and the clinker deposits may provide hard surfaces for sessile organisms that would not otherwise be able to settle. The C:N ratio of surficial sediments at the PAP sampled in September 1989 ranged from 4.8 to 7.8, with total organic carbon (TOC) between 0.27-0.32% (Santos et al., 1994). TOC values of PAP surficial sediment were ~0.35%, between March an October 1997, although this increased to 0.45% in September 1996 after a large deposition of phytodetritus (Rabouille et al., 2001). Sedimentary proteins, carbohydrates and lipids showed significant temporal changes (Danovaro et al., 2001). Variability in the distributions of lipids in the surface sediments is consistent with photographic evidence of the patchiness of the PAP sediments, which in turn is strongly influenced by the benthic fauna (Santos et al., 1994).

2.1.4 Flux of organic matter to the PAP

The timing and composition of the phytoplankton bloom, zooplankton interaction (repackaging and recycling) and the physical dynamics of the water column can all affect the quality and quantity of the seasonal POM flux to the seafloor (Hurley and Armstrong, 1990; Turner, 2002). Upper ocean phyto- and pico-plankton community structure changes temporally over the PAP (Gibb et al., 2000; Zubkov et al., 2000), which in turn will affect the quantity and composition of carotenoids in the flux of OM.

Upper ocean surface chlorophyll concentrations over the PAP from SeaWIFS data show that the spring bloom occurs from mid to late May. During the

spring bloom in 1997 to 1999, chlorophyll *a* concentration (measured by SeaWIFS) were 1.5 to 2.5 mg/m^3 and in 1997 productivity measured 1.9 g/C/m^2/d (Lampitt et al., 2001). The seasonal primary productivity bloom creates a strong seasonal pattern of flux to the PAP seafloor, reaching a maximum in mid-summer (Lampitt et al., 2001). Downward particle flux has been measured at the PAP at depths of 1000, 3000 and 4700m since 1989 using time-series sediment traps. The data have shown there is a strong seasonal signal, but also significant interannual variations, in both the timing and magnitude of the flux (Fig. 2.2) (Lampitt et al., 2001; Lampitt, 2008). The compositional spectrum of protein amino acids has been used to quantify the degradation state of settling particulate material at the PAP. Of the phytodetrital flux samples (3000m sediment trap) analysed between 1998 and 2004, the least degraded material was found in association with high POC and lithogenic fluxes observed in 2001 (Salter, 2007).

Temporal variation in the flux of OM has also been observed through time-lapse camera footage (Bathysnap) of phytodetritus reaching the seafloor at the PAP. Mass deposition of phytodetritus was recorded during the summer months of 1991, 1993 and 1994, covering between 56% and 90% of the seafloor. This coverage was not seen during the summers of 1997-1999 (Bett et al., 2001). There was no decrease in surface productivity or export flux that could account for the apparent absence of observable phytodetritus between 1997-1999 (Lampitt et al., 2001). The absence has been attributed to changes in the abundance of some species of megabenthos, which in turn changed rates of phytodetritus re-working at the seafloor (Bett et al., 2001).
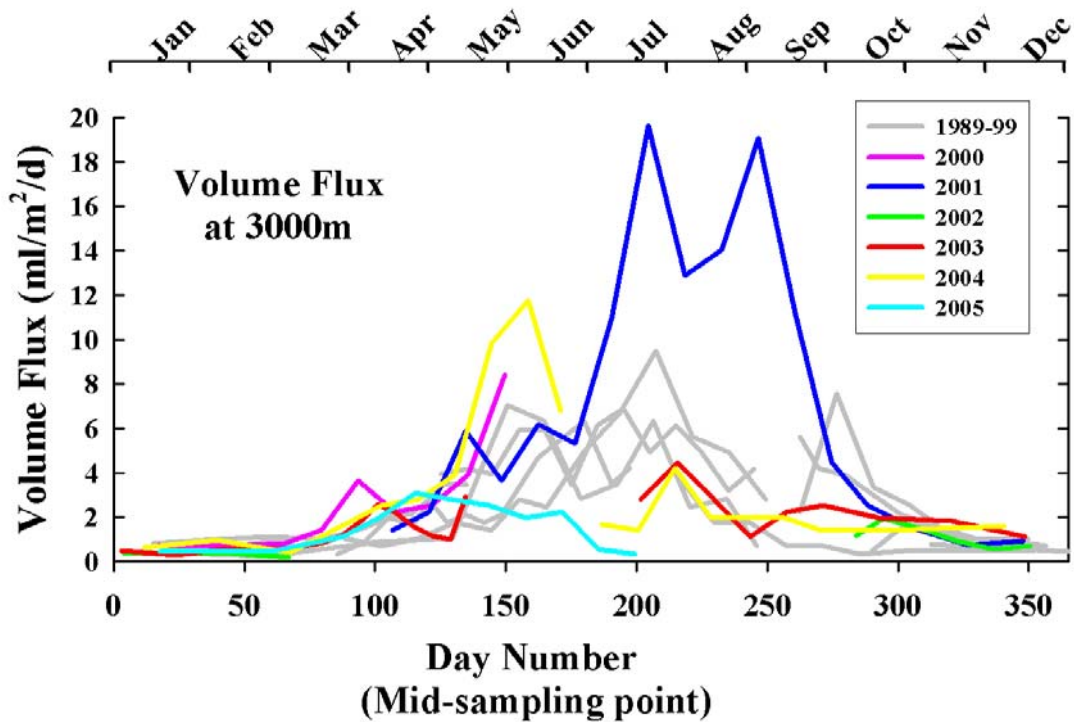
Figure 2.2 Downward flux of the dry mass of particulate material at 3000m over the PAP. Each data point represents the average flux over a predetermined period of time which may be as short as a week. During periods of constant flux such as in the winter, longer sampling intervals were selected (Lampitt et al., 2001; Lampitt, 2008)

2.1.5 Changing benthic community at the PAP

Between 1989 and 1999 a radical change occurred in the megabenthic community composition at the PAP. Actinarians, annelids, pycnogonids, tunicates, ophiuroids and holothurians all increased significantly in abundance (Billett et al., 2001). Prior to 1996, the small holothurian *Amperima rosea* (Fig. 2.3) was always a minor component of the megafauna. After this date it increased in abundance by more than two orders of magnitude from 4 indv/hectare (April 1994) to 193 indv/hectare (April 1997) (Bett et al., 2001; Billett et al., 2001). *Amperima rosea* and the other species that increased in abundance (the holothurian *Ellipinion molle* and the ophiuroid *Ophiocten hastatum*) are all primary consumers of phytodetrital material, as shown by ^{15}N analysis (Iken et al., 2001). The time period over which the entire sediment

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surface was reworked by megafauna increased from two and a half years, prior to 1996, to six weeks following the faunal change community change. The change was attributed to the increase in abundance of these species (Bett et al., 2001). The change in community could not be attributed to the quantity of OM reaching the PAP, rather it is postulated a change in the quality of the OM initiated the community change (Billett et al., 2001).

Figure 2.3 *Amperima rosea* **on the sea-floor at the PAP (photo taken with Bathysnap) (Bett et al., 2001)**

2.2 Crozet Islands, Southern Ocean – a Spatial Study

2.2.1 The study area

The Crozet Islands are situated 2585km southeast of South Africa at 51.0^{o}E, 46.5^{o}S (Fig. 2.4). The present study forms part of the Benthic CROZEX programme. This programme aims to examine how the variability in both the quality and quantity of primary productivity and fluxes of OM to the deep-sea floor influences benthic structure, dynamics and diversity. The focus is on two sites, M5 and M6 (Fig. 2.4). These sites were chosen because of their similar depth (~4200m), topography and close proximity (460km) (Fig. 2.6). The two sites differ by having contrasting upper ocean primary productivity regimes. Benthic station M5, east of the Crozet Islands, is located beneath an area with an enduring seasonal phytoplankton bloom, as deduced by satellite surface chlorophyll (SeaWIFS) data (Fig. 2.4). To the south of the islands, M6 is located in a High Nutrient Low Chlorophyll (HNLC) area (Fig. 2.4). Organic fluxes to the seafloor at these two sites reflect the productivity regimes: benthic station M5 receives a greater quantity of OM (Pollard et al., 2007a). There is no topographical boundary between the sites, so differences in benthic community, diversity and dynamics can be attributed to the quantity and quality of OM reaching the seafloor.
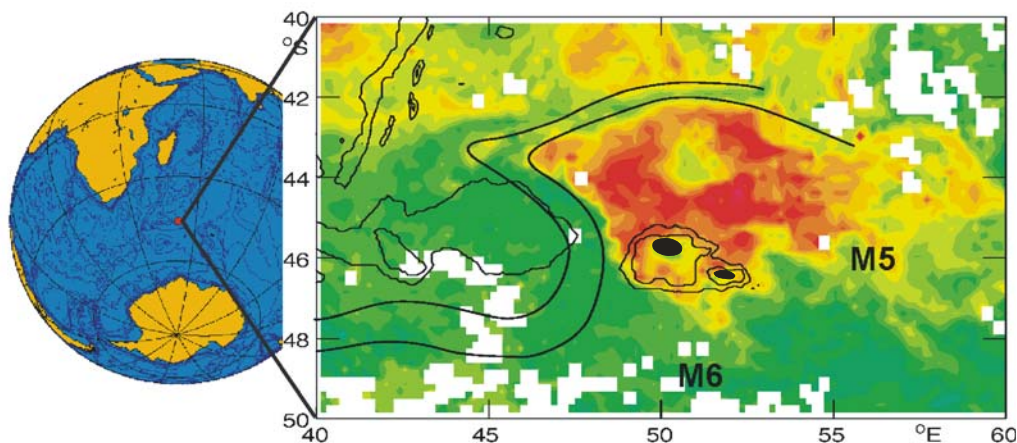
Figure 2.4 Location of the Crozet Islands (black) and SeaWIFS image showing Chlorophyll constrained by the S-shaped Antarctic Circumpolar Current pathway (Pollard and Read, 2001)

2.2.2 Hydrography

The Crozet Islands are located in an area in the Southern Ocean called the Polar Frontal Zone (PFZ), which is situated between the Subantarctic Front (SAF) to their north and the Polar Front (PF) to the south (Park and Gamberoni, 1997). The Crozet Basin, east of the Crozet Islands, plays a major role in the exchange of water masses between the Southern and Indian Oceans (Park et al., 1993; Park and Gamberoni, 1997). The bottom waters are guided by the bottom topography, with the Antarctic Bottom Water (AABW) penetrating into the Crozet Basin through the Crozet-Kerguelan gap. In the upper ocean, a confluence of three fronts occurs above the Crozet Islands; Aghulus Return Current Front (AF), Subtropical Front (STF) and SAF, creating the Antarctic Circumpolar Current (ACC) (Fig. 2.4) (Pollard and Read, 2001). Satellite sea surface temperature data from the period 1997-1999 shows the position and latitudinal changes in these fronts to be strongly constrained by the bottom topography (Kostianoy et al., 2004).

2.2.3 Upper ocean primary productivity and flux of OM

The Southern Ocean is considered to be a HNLC region (Treguer and Jacques, 1992). Few studies have investigated primary production in the Crozet area. In the austral summer of 1999, chlorophyll *a* concentrations (measured directly from seawater) across the Crozet basin were shown to increase from <0.3μg l^{-1} in subantarctic waters to 0.8μg l^{-1} in the subtropical waters (Fiala et al., 2003). Highest chlorophyll *a* concentrations occur when meandering fronts are in close proximity to each other (Fiala et al., 2004). In the frontal regions (AF, STF and SAF), factors such as nutrient renewal in the convergence zone, iron availability, water stability and water temperature control variations of cell concentrations and distributions of major species (Kopczynska and Fiala, 2003). The most comprehensive study of upper ocean primary production around the Crozet Islands found the phytoplankton assemblage in the north to be

dominated by *Phaeocystis* sp. and microplankton (comprising 50% of the total chlorophyll *a*) and to the south a community composed of microflagellates, cyanobacteria and some diatoms. Productivity derived from satellite sensors (SeaWIFS) has measured a maximum of 1.9g C m^{-2} d^{-1} in the north of the Island (late October 2004), 0.6g C m^{-2} d^{-1} over the Crozet plateau (late March 2005) and 0.4g C m^{-2} d^{-1} South of the Islands (early December 2004) (Seeyave et al., 2007).

An anti-cyclonic flow around the Crozet plateau advects a small filament of primary production (determined though satellite chlorophyll *a* data) southwards around the western side of the plateau (Pollard et al., 2007b; Venables et al., 2007). It has been suggested that this filament of high chlorophyll observed in the surface waters between October 2004 and end of November 2004 was received in a sediment trap at M6 (Fig. 2.4) in late December 2004 early January 2005 (assuming phytodetritus settles at 100-200m d^{-1} (Diercks and Asper, 1997)) (Venebles, H., pers. comm.). This hypothesis is not supported by the phytoplankton community found in the deep sediment trap at M5 and M6 (Fig. 2.4). If the short, high flux of material at M6 had originated from a filament of chlorophyll *a* enhanced water from the North, both sites would have similar dominant phytoplankton species in their respective sediment traps. This was not the case. The OM flux at M6 was dominated by the diatom *Fragilariopsis kerguelensis*, and at M5 by another diatom *Eucampia antarctica*, although towards the end of the flux profile at M5, *E. antarctica* became less important and *F. kerguelensis* began to increase in abundance (Salter, I., pers. comm.). It is important to note that although *Phaeocystis* spp. may have contributed to the flux of organic matter, this genus is difficult to enumerate and quantify, as there is no mineralised component to the cell. It is probable that the short, high-mass flux event at M6 corresponded to the small chlorophyll *a* peak observed in the surface waters in the HNLC region in December (Pollard et al., 2002; Venables et al., 2007)

Iron addition experiments have shown that primary production in most regions of the Southern Ocean is iron limited (Boyd, 2002; Cochlan et al., 2002;

Sedwick et al., 2002). The response of the phytoplankton community in this region to the addition of iron can be variable, depending on differences in diatom composition, availability of light or silicic acid (Blain et al., 2002; Fiala et al., 2004). SeaWIFS images show three regions in the Southern Ocean where regular annual phytoplankton blooms are observed downstream of an Island – South Georgia, Crozet and Kerguelan. The bloom around the Crozet Islands is constrained to the west and north by the hydrography around the Islands (Fig. 2.4) (Pollard et al., 2002). Iron from lithogenic inputs and sediments around the islands initiate the blooms around the Kerguelan Islands and Crozet Islands (Blain et al., 2001; Bucciarelli et al., 2001; Planquette et al., 2007).

The different 'zones' in the Southern Ocean have different productivity regimes which exert a control on the flux of phytodetritus to the sea bed (Jacques and Minas, 1981). The general view is that the flux rate of organic matter in the PFZ is high, although studies do not always agree. Wefer and Fischer (1991) suggested the PFZ had high annual production (83-170g C m^{-2}) and flux rates when compared to the average annual primary production (26g C m^{-2}) of all the areas of the Southern Ocean. Tsunogani et al. (1986) observed particle fluxes in the PFZ of 1g m^{-1} d^{-2}. Low sedimentation rates of 1 to 5cm kyear^{-1} were reported by Raboullie et al. (1997, 2002). Contrasting flux rates are most likely a result of temporal variability and differences in upper ocean productivity. For example, annually integrated POC and PON fluxes 3000m below an iron fertilised region to the east of the Crozet Islands were 5 times higher than at an adjacent HNLC area (stations M5 and M6, as described in the present study) (Salter, 2007). In the Iron enriched region, POC flux at 3000 m peaked in late December - early January (10.8 mg C m^2 d^{-1}), and gradually reduced towards zero six months later. In the –Fe region POC flux comprised one short peak of lower magnitude in late January and was zero at all other times (Pollard et al., 2007a).

2.2.4 Sediment composition

Siliceous oozes characterise the deep-sea sediments of the Southern Ocean
(Demaster et al., 1991). Few deep-sea sediment studies have been carried out
in the Crozet area. During the ANTARES I cruise (April-May 1993), no 'fluff'
(indicating 'freshly deposited phytodetritus/marine snow') was found at the
sediment-water interface in deep (~4000m) stations east of the Crozet Islands.
It is suggested this illustrates stronger advection (deep geostrophic currents)
and higher grazing in the PFZ region than in the Permanently Open Ocean
Zone (POOZ) region to the South, where a consistent bioclastic 'fluffy layer'
was found (Riaux-Gobin et al., 1997), although the differences may reflect
contrasting upper ocean production and ecosystem dynamics. Intact
phytoplankton cells made a minor contribution to the settled material in the
PFZ area, reflecting high degradation in the water column. Sediment samples
taken in March-May 1993, had low chlorophyll levels and absence of revivable
cells from samples taken from the sediment/water interface (Pinturier-Geiss et
al., 2001). Sampling stations closer to the Crozet Islands were enriched in
pigments compared to those stations also in the PFZ further away from the
Islands (Riaux-Gobin et al., 1997).

2.2.5 Biology of the benthos

There are very few deep-sea benthic fauna studies in the Southern Ocean,
especially around the Crozet Islands. The Challenger expedition completed
sample trawls close to the Crozet Islands on their journey around the world
between 1873 and 1876. To the east of the Crozet Islands, at a depth of 2926m
they found the holothurians *Peniagone purpurea*, *Peniagone affinis*,
Achlyonice lactea and *Laetmogone wyville-thomsoni* (Théel, 1882; Théel,
1886). A study of macrobenthic fauna on the Western Antarctic Peninsula
collected one deep-sea (1019m) sample with an Agassiz Trawl south of the
Crozet Islands. It revealed muddy substratum, with the most abundant fauna

being pycnogonids, isopods, ophiuroids, asteroids, sponges and prosobranch gastropods. There were a few rare occurrences of holothurians, echinoids (irregular), crinoids, amphipods, mysids, cephalopods and nematodes (Arnaud et al., 1998).

2.3 Carotenoids in the gut wall and ovaries of holothurians

2.3.1 Holothurian collection

A semi-balloon otter trawl (OTSB; Rice et al., 1990) was used to collect abyssal holothurians from the PAP (Table 2.1 and Fig. 2.5) and at two sites (M5 and M6) around the Crozet Islands (Table 2.2 and Fig. 2.6). Intact holothurians were chosen in order to represent differing feeding modes (i.e. selectivity for fresh material as shown by the studies of Iken et al. 2001 and Wigham et al. 2003a), morphologies, abundance and in order to compare with previous studies (Billett et al., 2001; Wigham et al., 2003a).

Station	Date	Lat (N)	Long (W)	Depth	Samples Collected
56515#1	21/06/04	48°58.30'	16°18.50'	4845m	*Amperima rosea* (n = 4), *Oneirophanta mutabilis* (n = 5), *Peniagone diaphana* (n = 4)
56523#1	24/06/04	48°52.90'	16°30.20'	4844m	*Amperima rosea* (n = 10), *Oneirophanta mutabilis* (n = 4), *Psychropotes longicauda* (n = 5) , *Paroriza prouhoi* (n = 5)
15711#1	17/07/05	48°54.00'	16°20.00'	4840m	*Oneirophanta mutabilis* (n = 4), *Paroriza prouhoi* (n = 4), *Psychropotes longicauda* (n = 2), *Pseudostichopus aemulatus*, *Pseudostichopus villosus* (n = 3), *Molpadia blakei* (n = 1)
15717#1	19/07/05	48°46.60'	16°29.80'	4842m	*Oneirophanta mutabilis* (n = 1), *Molpadia blakei* (n = 4), *Psychropotes longicauda* (n = 2), *Pseudostichopus villosus* (n = 4)

Table 2.1 Holothurian species collected during RRS *Charles Darwin* cruise CD158, June 2004 and RRS *Discovery* cruise D296, July 2005 to the Porcupine Abyssal Plain, Northeast Atlantic. (Location given is the start of trawl activity (ships position)). (thick line separates samples taken from separate cruises)
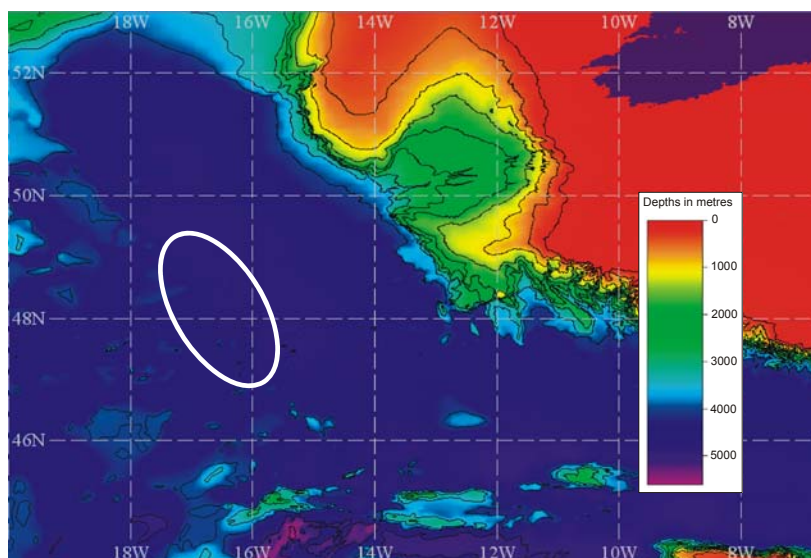
Figure 2.5 Bathymetric map of the, NE Atlantic showing sampling area (ringed in white) on PAP

Site	Station	Date	Lat (S)	Long (E)	Depth	Samples Collected
M5	15773#8	12/12/05	45°43.06'	56°32.16'	4290m	*Abyssocucumis abyssorum* (n = 3), *Peniagone* spp. (n = 2), *Scotoplanes globosa* (n = 3), *Oneirophanta mutabilis* (n = 3), *Psychropotes* aff. *longicauda* (n = 2), *Amperima robustrum* (n = 2)
M5	15773#17	15/12/05	45°43.47'	56°36.66'	4283m	*Oneirophanta mutabilis* (n = 4), *Abyssocucumis abyssorum* (n = 2), *Pseudostichopus* villosus (n = 4)
M5	15773#23	16/12/05	45°40.05'	56°35.27'	4275m	*Psychropotes longicauda* (n = 3), *Pseudostichopus* villosus (n = 3), *Amperima robustrum* (n = 2)
M5	15773#32	20/12/05	45°40.45'	56°33.70'	4270m	*Benthodytes sp.* (n = 2), *Psychropotes* aff. *longicauda* (n = 1), *Peniagone* spp (n = 3), *Abyssocucumis abyssorum* (n = 2), *Pelopatides* sp. (n = 1)
M6	15775#4	27/12/05	48°56.21'	51°03.90'	4195m	*Abyssocucumis abyssorum* (n = 4), *Peniagone* spp. (n = 4), *Psychropotes* aff. *longicauda* (n = 1)
M6	15775#13	29/12/05	49°01.15'	51°04.52'	4191m	*Molpadia blakei* (n = 2), *Peniagone* spp. (n = 4), *Psychropotes longicauda* (n = 1), *Benthodytes* sp. (n = 2)

Table 2.2 Holothurian species collected during RRS *Discovery* cruise D300, December 2005 at two abyssal sites (M5 and M6) around the Crozet Islands. (Location given is the start of trawl activity (ships position))
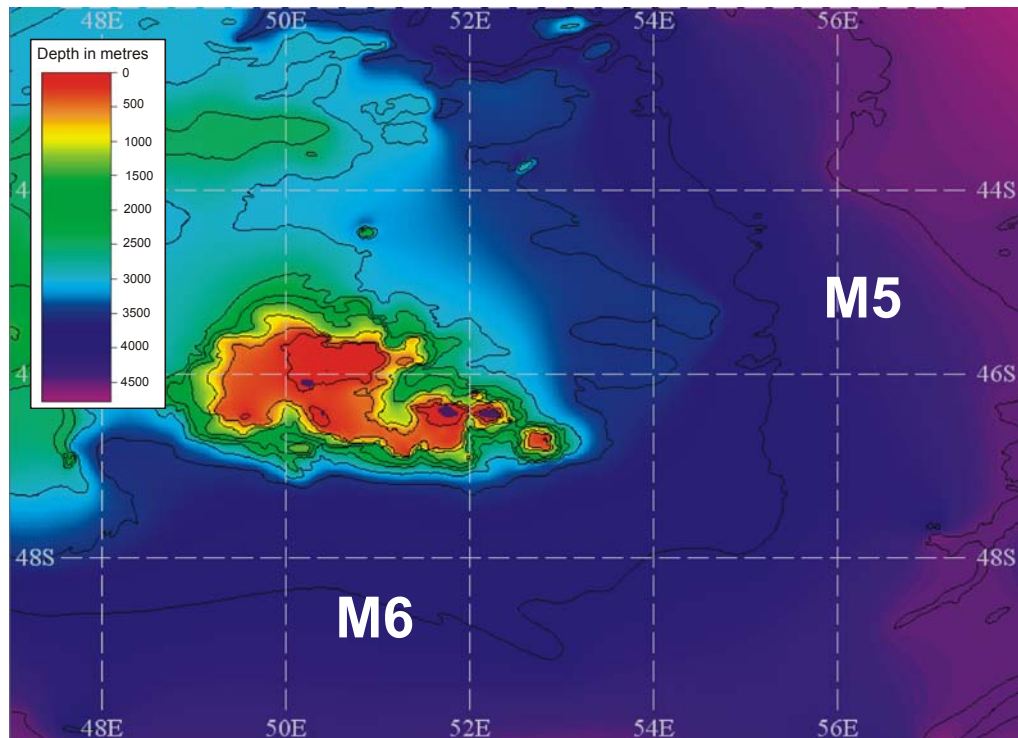
Figure 2.6 Bathymetric map of the sampling sites, M5 and M6, around the Crozet Isalnds, Southern Ocean.

Immediately after trawl recovery, the selected holothurians were put in pre-chilled seawater (4°C) and transferred to a constant temperature lab (4°C) for dissection. Holothurians were dissected individually. Dissection tools were washed between specimens to eliminate cross contamination. Holothurians with burst guts were rejected (this was often the case for the infaunal species *Molpadia blakei*). Specimens were dissected along the dorsal surface from the anus to the oral crown. Coelomic fluid was drained away and the whole gut tract was either dissected out or, if there was a risk of contamination during gut removal, sampled *in situ*. Ovarian and gut wall samples were also taken from each specimen (Fig. 2.7). The samples were transferred to separate cryovials and immediately frozen (-80°C).

At the National Oceanographic Centre, Southampton, holothurian identifications were first checked. *Peniagone* specimens were found to include

different species at each site around the Crozet Islands. *Peniagone affinis* and *P. willemeösi* were dominant species at M6; *P. challengeri* and *Peniagone* sp. nov. were dominant at M5 (Ian Cross, pers comm.). Therefore, *Peniagone* sampled at both sites are collectively referred to as *Peniagone* spp., because species level identification could not be made before the specimens were dissected. The gut wall of *Abyssocucumis abyssorum* was very thin, which prohibited samples to be taken.
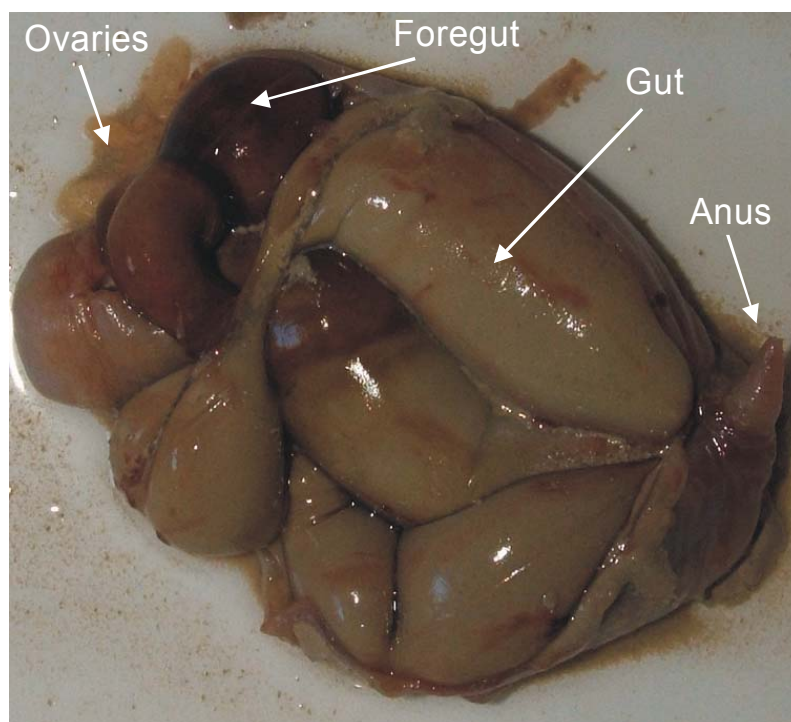
Figure 2.7 *Molpadia blakei* dissected to show the gut, foregut, ovaries and anus.

2.3.2 High performance liquid chromatography (HPLC) – method optimisation

Holothurian ovarian and gut wall carotenoids were determined using high performance liquid chromatography (HPLC). Holothurian samples were analysed by using either the method of Barlow et al. (1993) (as described by Wigham et al. (2003a) and Hudson et al. (2003)), or the method of Barlow et al. (1997). The method of Barlow et al. (1993) was used for the June 2004 PAP

samples, before it was decided to improve the analytical procedure. Barlow et al. (1997) method was used for July 2005 PAP and all Crozet samples. Excluding divinyl chlorophyll *a* and lutein, for which the method of Barlow et al. (1997) is used, both methods can identify the same suite of pigments. The Barlow et al. (1997) method is preferable however, because it enhances the resolution of the pigment peaks of diadinoxanthin, diatoxanthin, alloxanthin, and zeaxanthin (Fig. 2.7), improving confidence in identification. Additional standards increased the number of quantifiable pigments in comparison to the studies of Wigham (2002) and Hudson (2004).
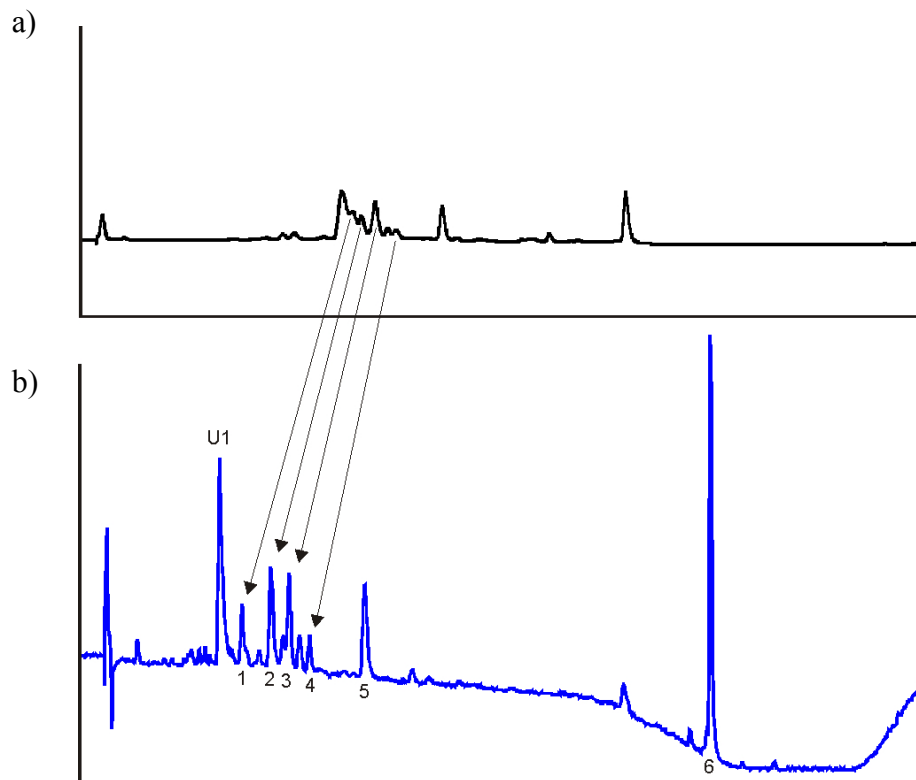
Figure 2.7 *Oneirophanta mutabilis* ovarian pigment chromatograms using the Barlow et al. (1993) method (a) and Barlow et al. (1997) method (b). U1 = unidentified peak, 1 = diadinoxanthin, 2 = alloxanthin, 3 = diatoxanthin, 4 = zeaxanthin, 5 = canthaxanthin and 6 = β-carotene.

2.3.3 Carotenoid determination in the gut wall and ovaries

Frozen gut wall and ovarian tissue samples were lypholised (-60°C; 10^{-2}T) and weighed. Pigments were extracted in 3 mL 90% HPLC grade acetone. Samples were ultrasonicated for 30 seconds then centrifuged for 10 mins at 3000 rpm. The extract was passed through a (0.2 μm) Nyalo membrane filter (Gelman) prior to analysis to remove any small particles. Samples were transferred to amber vials and loaded into the chilled (0°C) HPLC autosampler tray. Aliquots of sample (500 µL) were mixed with 1 M-ammonium acetate (500 µL) and 100 µL of this mixture injected onto the HPLC column.

The HPLC was controlled by the ChromQuest software system. It consisted of either a Perkin Elmer C18 column (Barlow et al., 1993) or Perkin Elmer C8 column (Barlow et al., 1997), a Thermoseparation HPLC system with an online vacuum degasser, a dual solvent pump (P2000), autosampler (AS3000), a UV photodiode array detector (UV6000), and a Spectra System fluorescence detector (FL3000). Chlorophylls and carotenoids were detected by absorbance at 440 nm; phaeopigments were monitored with the fluorescence detector using excitation and emission wavelengths of 410 and 670 nm, respectively. Pigments were identified by comparison of relative retention times with pigment standards. Supporting identification was gained by comparison of spectral data with known standards as well as by reference to Jeffery et al. (1997).

Pigment concentrations (µg g^{-1} dry weight sediment/tissue sample) were calculated as follows (Barlow et al., 1993):

$$C = (A_pV)/(WR_fB100)$$

Where A_p is the peak area detected at 440nm, *V* is the extract volume (mL), *W* is the dry weight of material (g), R_f is the response factor and *B* is the buffer dilution factor (0.5). Response factors for each of the pigments were calculated

by plotting concentrations of the standards against peak area. Reproducability of the analytical technique was better than ±10% and the analytical precision < ±5%.

2.3.4 Data analysis

Gut wall and ovary carotenoid concentrations are analysed for between year and between site statistical differences. Data were tested for their distribution using the Ryan-Joiner test (Ryan et al., 1976). Statistical analysis of pigment concentrations were implemented with Minitab software (Version 12.21). The means of normally distributed data were compared using the t-test; the Mann-Whitney test was applied to non-normally distributed data to compare their medians.

Pigment concentrations in the gut wall and ovaries of the holothurians were transformed to their percentage contributions to the total carotenoids in each sample, in order to diagnose differences in pigment biochemistry between species. This approach removes differences that might be related to the pigment load in each specimen. Differences seen in the MDS plots of holothurian ovarian carotenoid biochemistry in previous studies (Hudson et al., 2003; Wigham et al., 2003a) are dictated by the concentration of carotenoids in the ovaries as well as the contribution of specific carotenoids to the sample. Direct between-species, between-year and between-site comparisons are made using ANOSIM. The R-values are interpreted as >0.75 = well separated; R>0.5 = overlapping, but clearly different and R<0.25 = barely separable, in accordance with the PRIMER-manual (Clarke and Gorley, 2001). Multivariate statistical analysis on square-root transformed data was performed using the PRIMER 6 software package (Clarke and Warwick, 1994).

Clearer intra and interspecific differences will be made for some data by using square root transformed raw data – differences on the plot can be attributed to concentrations of carotenoids as well as the pigment composition of samples.

2.4 Quantifying feeding ecology and selectivity – the gut contamination problem

Comparisons between gut sediment and ovarian carotenoid profiles of deep-sea holothurians led Hudson et al. (2003) and Wigham et al. (2003a) to infer that some species selectively feed on OM enriched in specific carotenoids required for their reproduction. Ginger et al. (2001) reported that gut sediment samples of *Oneirophanta mutabilis* collected from the PAP were contaminated with holothurian-derived lipids ($>C_{20}$ fatty acids and Δ^7 sterols). They suggested this contamination derived from unregulated lipolysis of phospholipid within the digestive tissue resulting from the death of organisms on recovery (Ginger et al., 2001). Presumably if carotenoids are present and/or stored in gut wall cells (Griffiths and Perrott, 1976) reproductively important carotenoids may leach into the gut sediment upon recovery. This may explain why Witbaard et al. (2001) found canthaxanthin and high nucleic acid levels in the gut sediment of *Oneirophanta mutabilis*, but not in the surrounding sediment or sediment trap material. Canthaxanthin is a metabolite of β-carotene, found in many invertebrates (Tsushima et al., 1993b), as well as a minor pigment in some diatoms and prymnesiophytes (Jeffrey et al., 1997).

To examine the contamination of the gut sediment by the leaching of compounds from the gut wall, two different holothurians - *Amperima rosea* and *Psychropotes longicauda* were investigated. *Amperima rosea* has a fragile gut, making it difficult to sample without contamination from the gut wall. Carotenoid profiles of the gut sediment and ovary of *A. rosea* are very similar (Wigham et al., 2003a). *Psychropotes longicauda* in contrast to *A. rosea* has a large compact gut, which facilitates easier sampling of the gut sediment away from the gut wall. This species has differing gut sediment and ovarian carotenoid profiles (Wigham et al., 2003a). Lipid analysis of the gut sediment and gut wall will be used to assess the contribution of holothurian derived lipids from the gut wall into the gut sediment.

2.4.1 Sample collection

Holothurians were collected from the PAP during RRS *Charles Darwin* cruise 158 (see Table 2.1) in June 2004 and dissected as described in section 2.3.1. Gut sediment samples were removed from the centre of the gut, to reduce risks of contamination from the gut wall and coelomic fluid (Ginger et al., 2001). Five *A. rosea* specimens were dissected for gut sediment and muscle tissue and five specimens of *P. longicauda* were dissected for gut sediment.

2.4.1 Lipid analysis

Sample preparation and analysis followed the methods described by Neto et al. (2006). Lypholised (-60°C; 10^{-2}T) gut sediment or muscle tissue lipids were extracted in dichloromethane (DCM): methanol; 9:1 v/v by sonification (30 mins, x3). Known amounts of two internal standards (5α(H)-cholestane, 2.008 μg and 5β(H)-cholanic acid, 3.015 μg, in DCM) were added before extraction. The extract was transferred to a pre-weighed vial, and the solvents were removed under a stream of N_2. The extract was re-dissolved in DCM and dried by passing through a column of anhydrous sodium sulphate. The sample was then methylated using the method of Chambaz and Horning (1969) and silylated by treatment with *bis*-trimethylsilyltrifluoroacetamide (60°C; 2 h). Derivatised fractions were dissolved in DCM and analysed using a ThermoQuest CE gas chromatograph (Trace 2000 series) coupled with ThermoFinnigan TSQ-7000 mass spectrometer. The GC was fitted with an on-column injector and a capillary column (DB5-MS; 60 m x 0.25 mm i.d., 0.10 μm film thickness, J&W). The oven was held initially at 60°C for 1 min, then heated from 60°C to 180°C at 12°C min^{-1} and from 180°C to 315°C at 2.5°C min^{-1}, and held for 10 min at 315°C. Helium was used as carrier gas at a constant flow (1.6 mL min^{-1}, with vacuum compensation). A stream of air was used to cool the injector prior to, and for 1 min after each injection. Typical operating conditions for the mass spectrometer were: electron energy at 70eV,

scanning from 50 to 600 Thomsons, scan time of 1s, ion source temperature at 230°C, interface temperature at 320°C. Xcalibur Software (Version 1.0) was used to acquire and process the data. Fatty acids and sterols were identified by comparison of their relative retention times and mass spectra with those of authentic standards and/or by comparison with the literature. Concentrations of individual compounds were determined by comparison of their peak areas with those of the internal standards and were corrected after calculation of their relative response factors (Kiriakoulakis et al., 2004).

2.4.2 Data analysis

The contribution of holothurian derived choles-7-*en*-3β-ol to the gut sediment and muscle tissue was determined by calculating the percentage of choles-7-*en*-3β-ol as a total of 24-ethylcholest-5-*en*-3β-ol (dietary derived) and choles-7-*en*-3β-ol. Choles-7-*en*-3β-ol is not found in the surrounding sediment (Santos, 1993) or in sediment trap material (Kiriakoulakis et al., 2001). If the percentage contribution of choles-7-*en*-3β-ol in the gut sediment is the same as that of the muscle tissue, it indicates the gut sediment is fully contaminated with holothurian derived material. Statistical analysis between the percentage contribution of choles-7-*en*-3β-ol to the gut sediment and gut wall was implemented with Minitab software (Version 12.21). These data were tested for their distribution using the Ryan-Joiner test (Ryan et al., 1976). The means of normally distributed data were compared using the t-test; the Mann-Whitney test was applied to non-normally distributed data.

2.5 Chlorophyll *a* in the gut sediment of holothurians

2.5.1 Gut sediment chlorophyll a analysis

Holothurians were collected and dissected as in section 2.3.1. Gut sediment samples were taken from holothurians prior to the removal of gut wall and ovary tissue samples. Gut sediment was removed from the centre of the gut, to reduce risks of contamination from the gut wall and coelomic fluid (Ginger et al., 2001). These samples were transferred to separate cryovials and frozen immediately (-80°C). They were prepared and analysed by HPLC as for the gut wall and ovary samples (see section 2.3.3 for details).

2.5.2 Data analysis

Chlorophyll *a* concentrations (μg gDW^{-1}) in the holothurian gut sediment were compared for between species, year and site differences. Statistical analysis of pigment concentrations were implemented with Minitab software (Version 12.21). Data were tested for their distribution using the Ryan-Joiner test (Ryan et al., 1976). The means of normally distributed data were compared using the t-test; the Mann-Whitney test was applied to non-normally distributed data to compare their medians. If variables had a normal distribution and a homogenous variance, ANOVA was applied to find any statistical variation in species gut chlorophyll *a* concentration.

2.6 Quantifying the supply of carotenoids to the deep-sea benthos

Holothurians display a range of feeding adaptations, that range from feeding on the freshly deposited phytodetritus to the deeper layers of sediment (Billett, 1991; Roberts et al., 2000). To assess the phytopigments available to the holothurians in their diet, phytodetritus and sediment samples were collected and analysed for phytopigment analysis. To prepare sediment for analysis, water needs to be extracted to obtain pigment concentration per dry weight of sample. Freeze-drying of sediment significantly improves the extraction of pigments from sediment samples (Buffan-Dubau and Carman, 2000). This method has been used successfully by Hansen and Josefson (2003) to study the accumulation of algal pigments in the aphotic zone. Acetone and methanol do not significantly differ in their capability of extracting pigments from the freeze dried sediment (Buffan-Dubau and Carman, 2000). Stand alone pump systems (SAPS, Challenger Oceanic) were used during the Crozet study to quantify the phytopigments associated with POM in the upper and lower sections of the water column at each station.

2.6.1 Sediment collection

Sediment samples were collected with either a Barnett-Watson multiple corer (Barnett et al., 1984) (June 2004) or Bowers-Connelly mega-corer (Barnett, 1998-1999) (July 2005 and Crozet). These samplers collect undisturbed cores of 58mm (multicore) or 100mm (megacore) diameter. Sediment samples were taken from the PAP in June 2004 and July 2005 (Table 4 and Fig. 2.4) and from M5 and M6 around the Crozet Islands in December 2004 – January 2005 (hereafter called Crozet cruise 1) and December 2005 – January 2006 (hereafter called Crozet cruise 2) (Table 2.4 and Fig. 2.5). On recovery, the cores were taken to a constant temperature laboratory (4°C) for sectioning into 5mm horizontal sections. Phytodetritus present in the depressions at the

sediment surface was carefully removed by pipette and frozen (-80°C) separately from the top 5 mm of sediment.

Station	Date	Lat (N)	Long (W)	Depth	Samples Collected
56502 #1	19/06/04	48°51.20'	16°29.20'	4835m	Sediment core and phytodetritus (n = 1)
56508 #1	20/06/04	48°51.00'	16°30.00'	4838m	Sediment core (n = 1) and phytodetritus (n = 2)
56519 #1	22/06/04	48°51.00'	16°29.90'	4833m	Sediment core and phytodetritus (n = 1)
15720 #1	19/07/05	48°52.10'	16°29.80'	4838m	Sediment cores and phytodetritus (n = 1)
15724 #1	20/07/05	48°52.00'	16°29.70'	4836m	Sediment cores and phytodetritus (n = 2)

Table 2.3 Sediment samples taken from the PAP in June 2004 and July 2005. (thick line separates samples taken from separate cruises)

Site	Station	Date	Lat (S)	Long (E)	Depth	Samples Collected
M5	15582#6	28/12/04	45°59.91'	56°08.94'	4270m	Sediment core
M5	15582#9	28/12/04	46°00.00'	56°09.07'	4269m	Sediment core
M6	15587#1	04/01/05	49°00.01'	51°20.00'	4221m	Sediment core
M6	15599#4	05/01/05	49°00.01'	51°20.00'	4221m	Sediment core
M5	15773#20	15/12/05	45°53.34'	56°24.24'	4189m	Sediment core
M5	15773#31	20/12/05	45°53.56'	56°25.77'	4200m	Sediment core
M6	15775#6	28/12/05	49°03.55'	51°15.73'	4202m	Sediment core
M6	15775#19	31/12/05	49°04.59'	51°13.49'	4202m	Sediment core
M6	15775#25	03/01/06	49°04.53'	51°13.12'	4202m	Sediment core
M6	15775#36	05/01/06	49°01.99'	51°14.01'	4192m	Sediment core

Table 2.4 Sediment samples taken from sites (M5 and M6) around the Crozet Islands in December 2004 – January 2005, and December 2005 – January 2006 (thick line separates samples taken from separate cruises)

2.6.2 SAPS sampling – phytopigments associated with particulate organic matter

Pre-ashed GF/F filters (22cm; 400°C; 24h) were used in each SAPS deployment (Table 2.5). In order to assess the potential source of the POM, SAPS were deployed in the upper water column at a depth that would collect sinking particles falling out of the biologically productive surface layers. This

was achieved by deploying the SAPS below the thermal mixed layer as determined by a preliminary CTD cast. Additionally, bottom water material was collected with the SAPS in order to capture the 'phytodetritus' which was evident on the seafloor (Wolff, 2006), but was not successfully sampled by sediment coring. After deployment, filters were wrapped in aluminium foil and immediately frozen (-80°C). The filter papers were divided for analysis of lipids (not for present study) and phytopigments.

Site	Station	Date	Lat (S)	Long (E)	Depth	Vol. filtered
M5	15773#3	11/12/05	46°00.17'	56°14.95'	80m	55.75 L
M5	15773#16	14/12/05	45°55.07'	56°25.65'	80m	285.50 L
M5	15773#26	17/12/05	45°54.10'	56°25.42'	4241m	175.38 L
M5	15773#41	24/12/05	45°56.41'	56°25.49'	80m	33.80 L
M6	15775#9	28/12/05	49°04.43'	51°14.89'	60m	64.13 L
M6	15775#15	30/12/05	49°11.41'	51°09.56'	4200m	183.63 L
M6	15775#29	04/01/06	49°06.43'	51°12.56'	50m	99.75 L

Table 2.6 Stand alone pump samples taken at sites M5 and M6 around the Crozet Islands in December 2005 – January 2006

2.6.2 Extraction and quantification of phytopigments

Abyssal sediment samples were lypholised (-60°C; 10^{-2}T) and weighed prior to extraction. Acetone (90%) was added (6mL) to the lypholised sediment or SAPS filter, sonicated for 30 seconds and centrifuged at 3000rpm for ten minutes. The extract was passed through a (0.2 μm) Nyalo membrane filter (Gelman) prior to analysis to remove any remaining small particles. Phytopigments were quantified using either the method of Barlow et al. (1993) or Barlow et al. (1997), as described in section 2.3.2.

Phytopigments in the POM from the SAPS samples were quantified (ng/l^{-1}) using the following equation (Barlow et al., 1993):

$$C = (A_p V_{ex} 1000)/(V_{fil} R_f B V_{inj})$$

Where A_p is the peak area detected at 440nm, V_{ex} is the extract volume (mL), V_{filt} is the volume filtered through the SAPS (L), R_f is the response factor, B is the buffer dilution factor (0.5) and V_{inj} is the volume injected (100μL).

2.6.3 Analysis of data

Pigment concentrations ($\mu g\ gDW^{-1}$) in the phytodetritus and sediment were compared for between years and/or between site differences. Pigment concentrations ($\mu g\ L^{-1}$) in the SAPS samples were compared for between site differences. Statistical analysis of pigment concentrations were implemented with Minitab software (Version 12.21). Data were tested for their distribution using the Ryan-Joiner test (Ryan et al., 1976). The means of normally distributed data are compared using a two-way ANOVA; Friedmans method of randomised blocks was applied to data that do not meet the assumptions of normality or homogeneity of variance. The ratio of chlorophyll *a* to phaeophorbide is used to indicate freshness of phytodetrital material (Thiel et al., 1989). Sediment pigment biochemistry was finally compared to the holothurian gut wall and ovarian pigment profiles to examine the link between diet and holothurian ovarian biochemistry (which may exert a control on reproductive output); species gut sediment chlorophyll *a* concentrations were compared to assess their responses to a differing food supply.

Chapter 3 - Quantifying feeding ecology and selectivity – the gut contamination problem

3.1 Introduction

The diets of many deep-sea animals are unknown because of the difficulties of sampling and observing deep sea fauna (Howell et al., 2004). Stomach content analysis (by scanning electron microscope and light microscopy) has been used to determine diet and trophic interactions of deep-sea fauna (Carey, 1972; Khripounoff, 1979; Khripounoff and Sibuet, 1980; Jangoux, 1982; Tyler et al., 1990; Tyler et al., 1992a; Tyler et al., 1992b; Pfannkuche and Lochte, 1993; Tyler et al., 1993; Campos-Creasey et al., 1994), but there are limitations in the method. Deep-sea animals can suffer loss of their ingesta or feed on other material in the trawl cod-end during capture and retrieval (Feller et al., 1985). Many deep-sea asteroid species feed extra-orally and so are rarely observed with any material in their stomachs (Carey, 1972). The identification of partially ingested material can also be problematic (Carey, 1972; Feller et al., 1985; Tyler et al., 1992a; Tyler et al., 1992b) and there is variable resistance to degradation of different food items (Fukuda and Naganuma, 2001).

New biochemical and isotopic approaches overcome the limitations associated with stomach content analysis and have aimed to determine diet in order to answer key ecological questions. For example, isotopes, such as ^{234}Th, ^{13}C and ^{15}N have been used successfully in the deep-sea to infer feeding selectivity and relative trophic status of organisms (Lauermann et al., 1997; Miller and Smith, 2000; Iken et al., 2001). Lipid (fatty acids and sterols) analysis of tissue can be used to identify biomarkers of bacteria and phytoplankton species and their contribution to animal and protistan diets (Sargent et al., 1987; Ginger et al., 2001; Gooday, 2002b; Suhr et al., 2003; Suhr and Pond, 2006). The feeding ecology of abyssal echinoderms has been determined using the lipid biomarker approach (Ginger et al., 2000; Ginger et al., 2001; Howell et al., 2003; Hudson

et al., 2004; Neto et al., 2006). Phytopigment biomarkers have frequently been used as a tracers in pelagic food webs (Kleppel and Pieper, 1984; Kleppel et al., 1988; Breton et al., 1999; Gasparini et al., 2000; Cotonnec et al., 2001) and this technique has been transferred to deep-sea benthic fauna (Billett et al., 1988; Hudson et al., 2003; Wigham et al., 2003a; Howell et al., 2004). Chlorophyll *a* comprises a large fraction of algal photosynthetic pigments and is therefore widely used as a measure of marine algal biomass and productivity (Jeffrey et al., 1997). There is a wide range of accessory pigments (carotenoids and chlorophylls) found in algae, some of which are unique to specific algal taxa (Jeffrey et al., 1997). Carotenoids, essential for echinoderm reproduction (Matsuno and Tsushima, 1995; Matsuno and Tsushima, 2001) can be synthesised *de novo* only by plants, fungi, algae and bacteria (Goodwin, 1980) and it is thought that these essential compounds are in short supply in the deep sea (George and Young, 1998; Hudson et al., 2003; Wigham et al., 2003a).

Comparisons between gut sediment and ovarian carotenoid profiles of deep-sea holothurians led Hudson et al. (2003) and Wigham et al. (2003a) to infer that some species feed selectively on organic matter enriched in specific carotenoids required for their reproduction. However, gut sediment samples of *Oneirophanta mutabilis* collected from the Porcupine Abyssal Plain (PAP) have been shown to be contaminated with holothurian-derived lipids ($>C_{20}$ fatty acids and Δ^7sterols; Ginger et al., 2001). This contamination probably derived from unregulated lipolysis of phospholipid within the digestive tissue resulting from the death of organisms on recovery (Ginger et al., 2001). Presumably, if carotenoids are present and/or stored in the gut wall cells of the abyssal holothurians (as in the shallow water echinoid *Strongylocentrotus dröbachiensis*; Griffiths and Perrott, 1976) then they may also leach into the gut sediment upon recovery. This may explain why Witbaard et al. (2001) found canthaxanthin and high nucleic acid levels in the gut sediment of *Oneirophanta mutabilis*, but not in the surrounding sediment or sediment trap material. Canthaxanthin is a minor pigment in some diatoms and prymnesiophytes (Jeffrey et al., 1997). It is also a metabolite of β-carotene

(Tsushima et al., 1995). The use of pigments as biomarkers in zooplankton diet has recently been questioned by Antajan and Gasparini (2004). Zooplankton store alloxanthin (as a possible precursor to astaxanthin) in their bodies, making it an unsuitable biomarker for cryptophytes in their diet (Antajan and Gasparini, 2004). They proposed that this pigment could have been obtained from their diet and/or by transformations of other dietary carotenoids.

To examine the potential contamination of the gut sediment, by the leaching of compounds from the gut wall, two different holothurians - *Amperima rosea* and *Psychropotes longicauda* were investigated. *Amperima rosea* has a fragile gut, making it difficult to sample without contamination from the gut wall. Carotenoid profiles of the gut sediment and ovary of *A. rosea* are very similar (Wigham et al., 2003a). In contrast, *P. longicauda* has a large compact gut, which facilitates easier sampling of the gut sediment away from the gut wall. This species has different gut sediment and ovarian carotenoid profiles (Wigham et al., 2003a). Lipid analysis of the gut sediment and muscle tissue is used to assess if there is contamination of holothurian gut sediment. If the distinctive holothurian-derived compounds, $C_{23:1}$ and $C_{24:1}$ fatty acids (Ginger et al., 2000), are found in the holothurian gut sediment, contamination has occurred. The contribution of the holothurian-derived sterol choles-7-*en*-3β-ol relative to 24-ethylcholest-5-en-3ß-ol (ascribed to phytoplankton and is a dominant sterol in PAP sediments; Santos et al., 1994) is then used to assess the degree of contamination.

3.2 Lipid contamination results

Amperima rosea and *Psychropotes longicauda* gut sediment samples were contaminated with holothurian-derived fatty acids, namely $C_{23:1}$ and $C_{24:1}$ (Table 3.1).

	$C_{23:1}$	$C_{24:1}$
***Amperima rosea* gut sediment (n =4)**	892.99	1130.94
	266.69	423.65
	1331.83	4251.73
	177.41	301.92
Average	**667.23**	**1527.06**
	545.59	*1852.85*
***Psychropotes longicauda* gut sediment (n = 4)**	107.95	42.73
	320.28	53.29
	263.24	105.33
	186.72	48.38
Average	**219.55**	**62.43**
	92.35	*28.92*

`

Table 3.1. Holothurian derived $C_{23:1}$ and $C_{24:1}$ lipids (µg/gDW) in the gut sediment of *Amperima rosea* and *Psychropotes longicauda*. Standard deviation in italics.

The presence of the holothurian derived Δ^7 sterol, choles-7-en-3β-ol (Ginger et al., 2000) (NB shorthand notation, first number = carbon number, second number = degree of unsaturation, Δ^x = position of double bond) also suggests contamination from holothurian gut wall tissue. The contribution of choles-7-en-3β-ol relative to 24-ethylcholest-5-en-3ß-ol in *A. rosea* gut sediment samples (Table 3.2) was not statistically different to its contribution to the muscle tissue (t (6) = 0.3, P>0.05). *Psychropotes longicauda* muscle tissue lipid composition was taken from Neto (2002). The contribution of choles-7-en-3β-ol to the gut sediment of *P. longicauda* (Table 3.2) is significantly lower than its contribution to the muscle tissue (t (25) = 10.13, P<0.05).

	24-ethylcholest-5-en-3ß-ol	choles-7-en-3ß-ol	% ratio choles-7-en-3ß-ol
Amperima rosea **gut sediment** **(n = 4)**	3033	394	11.5
	11089	1357	10.9
	304	105	25.8
	108	9	7.4
			13.9 (*8.1*)
Amperima rosea **muscle tissue** **(n = 4)**	356	57	13.9
	242	39	13.9
	6733	837	11.1
	7695	1021	11.7
			12.8 (*1.46*)
Psychropotes longicauda **gut sediment** **(n = 4)**	575	22	3.66
	446	72	13.93
	594	32	5.1
	328	28	7.84
			7.6 (*4.5*)
Psychropotes longicauda **muscle tissue** **(n = 23)**	130	90	41.4
	110	50	29.6
	140	20	12
	130	50	29.2
	90	330	79.4
	0	90	100
	40	210	83.1
	80	90	53.6
	470	1330	74.1
	0	90	100
	0	160	100
	0	90	100
	110	350	76.4
	0	900	100
	180	360	66.2
	570	210	27.2
	250	490	66
	240	240	50.4
	0	70	100
	0	770	100
	380	1180	75.6
	150	570	78.6
	190	510	73
			70.2 (*27.6*)

Table 3.2. Holothurian derived choles-7-en-3ß-ol and`24-ethylcholest-5-en-3ß-ol (μg/gDW) in the gut sediment and muscle tissue of *Amperima rosea* and *Psychropotes longicauda* (muscle tissue data from Neto (2002). % ratio choles-7-en-3ß-ol = percentage contribution of choles-7-en-3ß-ol to the choles-7-en-3ß-ol and 24-ethylcholest-5-en-3ß-ol total. (Average in bold, standard deviation in italics)

3.3 Discussion - the use of biomarkers to determine feeding ecology and selectivity

Similar % ratios of the holothurian derived choles-7-en-3β-ol to the gut sediment and muscle tissue suggests the gut sediment of *Amperima rosea* is fully contaminated by holothurian derived material from the lysis of the gut wall during recovery, i.e. the same proportion of holothurian derived material is found in the gut sediment and muscle tissue. This result is not surprising because of the fragile nature of the gut wall and the 'sloppy' gut content of *A. rosea*. It is hard to sample the gut sediment without an orange/pink mucus-like contamination from the gut wall (personal observation). High concentrations of carotenoids found in the gut sediments of *A. rosea* (present study, Chapter 4, Wigham et al., 2003a) are likely to derive from their gut walls. The concentration of carotenoids in the gut wall of *A. rosea* is the highest of all holothurians sampled in the present study (Chapter 2); two orders of magnitude greater than that of *Psychropotes longicauda*. If lysis of the gut wall occurs, leading to contamination of the gut sediment, holothurian derived high carotenoid concentrations will mask the phytopigment composition of the sediment ingested by the holothurian. Although *P. longicauda* had significantly less holothurian-derived contamination in its gut sediment than found in the muscle tissue, the presence of choles-7-*en*-3β-ol and $C_{23:1}$ and $C_{24:1}$ fatty acids in the gut sediment suggest there is nonetheless some degree of contamination.

The gut sediment of *A. rosea* from the PAP was also analysed for phytopigment content (Appendix 1). Ten out of fourteen samples contained no chlorophyll *a* but they did contain high concentrations of carotenoids. Of these carotenoids present in the gut sediment of *A. rosea*, echinenone and canthaxanthin (known metabolites of echinoderms (Tsushima et al., 1993b), were not observed in the surrounding phytodetritus or sediment (Chapter 4 Fig. 4.1) (Appendix 1). Echinenone and Ccanthaxanthin were observed in the gut wall of *A. rosea* (Chapter 4) further suggesting that the carotenoids in the gut sediment in this species were holothurian derived and not only ingested

selectively. This has been observed before; canthaxanthin was one of the principal pigments found in the gut sediment of *Oneirophanta mutabilis*, but the pigment was absent from the sediment and sediment trap material (Witbaard et al., 2001).

The results of this study suggest that the use of biomarkers in the gut content of deep-sea organisms should be used with caution when determining feeding ecology. If compounds are assimilated and/or stored by gut wall cells, these compounds may leach back into the gut sediment through the lysis of cells during recovery, and can lead to a misleading conclusion of selective feeding. The findings of the present study may therefore invalidate the inferences of previous studies that have used biomarkers present in the gut sediment as proxies for feeding ecology.

Wigham (2003a) and Hudson (2004) inferred that the high carotenoid concentrations, as well as similar gut sediment and ovarian biochemical profiles, were indicative of *A. rosea* selectively feeding on specific carotenoids that are assimilated directly into its ovaries. The present study shows *A. rosea* gut sediment is contaminated by holothurian-derived compounds from the gut wall. *Amperima rosea* is a selective feeder on fresh OM (Iken et al., 2001), but contamination from the carotenoid enhanced gut wall will mask the pigment composition of the ingested OM. Seasonal variations in the diet of bathyal holothurians, inferred by the pigment composition of their gut sediment (Hudson et al., 2003), may have reflected seasonal changes in the holothurians gut wall carotenoid composition rather than changes in diet. Gut sediment contamination from the gut wall may also invalidate the inference of the study by Howell et al. (2004), which suggested specific phytoplankton groups contributed to the diet of two deep-sea asteroids.

Biomarkers for feeding ecology other than pigments will also be subject to gut sediment contamination, if the compounds are also present in the gut wall of the organism. It is likely that the values given for the selectivity, concentration

in the gut and assimilation efficiency of lipids and proteins by the abyssal holothurians *Deima validum* and *Pseudostichopus villosus* (Sibuet et al., 1982) were affected by contamination from the gut wall. Selectivity values (i.e. concentration in the gut sediment compared to the surrounding sediment) may be exaggerated if compounds leach back into the gut sediment from the gut wall. Assimilation by the holothurian may therefore be underestimated. A separate study of total organic carbon and nitrogen in the gut contents of *P. villosus*, *Oneirophanta mutabilis* and *Psychropotes longicauda* (Moore and Roberts, 1994) may also be compromised.

The selection of 'fresh' material can be inferred from biomarkers in the gut sediment that are not assimilated by the animals, for example, chlorophyll *a*. This method has been used successfully to infer the feeding strategies of abyssal holothurians through gut sediment chlorophyll *a* content (Moore and Roberts, 1994; Duineveld et al., 1997; Witbaard et al., 2001). Furthermore, temporal variability in biomarker distributions in tissue samples (gut wall and ovaries of a particular species) can be related to the selectivity of the species for specific compounds (Neto et al., 2006). For example, consistent biomarker profiles may suggest the compounds are assimilated selectively and therefore the species may rely on the supply of these compounds in the diet.

The main implication of this gut contamination study on the remainder of the present project is that the selectivity of species for specific carotenoids cannot be established by examining the carotenoid biochemistry of the gut sediment. This should be taken into account when planning future deep-sea feeding studies. Only chlorophyll *a* is used to determine the selectivity of fresh OM by the holothurians in the present study. The intraspecific consistency of the gut wall and ovarian carotenoid profiles will provide evidence for the requirement of species for specific carotenoids. A consistent profile suggests the species may benefit if there is an enhanced supply in the OM reaching the seafloor of the specific carotenoids found in the tissue samples.

Chapter 4 – The link between diet and abyssal holothurian ovarian biochemistry; a temporal study at the Porcupine Abyssal Plain

4.1 Introduction – aim of the Porcupine Abyssal Plain study

Understanding how climate can affect Particulate Organic Carbon (POC) quantity and quality, and the different ways in which abyssal taxa utilise POC, are important areas of investigation (Ruhl, 2007). Changes in the community structure over time at abyssal depths, as seen at the PAP (Billett et al., 2001) have substantial ecological implications; for example, they affect the rates and intensity of bioturbation (Bett et al., 2001). Comprehending these changes and their effects on the processing of organic matter arriving at the sediment surface is critical for understanding the carbon cycle. The influence of climatic variation on the resources available to terrestrial, freshwater and marine flora and fauna has highlighted the impact such changes can have on community structure (Weltzin and Mcpherson, 1997; Harrington et al., 1999; Walther et al., 2002). The dominance (abundance and biomass) of holothurians at abyssal depth (Billett, 1991), and their apparent response to food supply (Ruhl and Smith, 2004), supports their use as an indicator group for understanding the effects of climate variation on abyssal benthos and the long-term sequestration of carbon (Ruhl, 2007).

Feeding selectivity has been demonstrated in holothurians from the PAP using various methods. Iken et al. (2001) used stable isotope analysis and gut content analysis to study the trophic positions of holothurians at the PAP. Seasonal differences in holothurian muscle lipid biochemistry has been related to the feeding mode of the species (Hudson et al., 2004) and changes in food supply (Neto et al., 2006). Witbaard et al. (2001) used chlorophyll *a* as a biomarker for the feeding selectivity of *Oneirophanta mutabilis*. Wigham et al. (2003a) and

Hudson et al. (2003) quantified the carotenoids in the gut sediment of the holothurians and related interspecies differences to feeding selectivity.

The pigment biochemistry of the ovaries of abyssal holothurians from the PAP show intraspecies consistency, but interspecies differences (Wigham et al., 2003a; Hudson, 2004). Carotenoids are reproductively important compounds that can only be made *de novo* by phytoplankton; it is believed they cannot be synthesised by holothurians. Comparisons between the gut sediment and ovarian pigment profiles led Wigham et al. (2003a) and Hudson et al. (2003) to suggest that *Amperima rosea* feeds selectively on reproductively important compounds. It is postulated that the supply of specific compounds to the deep-sea floor may favour some species, initiating community change. An increase in the supply of zeaxanthin (a pigment associated with cyanobacteria) to the PAP is suggested to favour *A. rosea* because of the high concentration and percentage contribution of this carotenoid over others in its ovaries.

High concentrations of nucleic acids in the gut sediment of *O. mutabilis* suggests that this species contains high numbers of actively-dividing bacteria in its gut, although these compounds may also have been derived from cell lysis of the gut epithelium (Witbaard et al., 2001). Ginger et al. (2001) and the present study (Chapter 3) indicates holothurian gut sediment is contaminated with holothurian derived lipids from the lysis of gut wall cells during recovery. This contamination has inhibited the reliable determination of organic matter in the gut sediment of abyssal holothurians (Ginger et al., 2001) and may also preclude the use of carotenoids as biomarkers of feeding selectivity, if they are found in gut wall tissue.

The aim of the present PAP temporal study is to examine the influence of a changing food supply on the diet and ovarian biochemistry of abyssal holothurians at the PAP. This was approached by analysing holothurian gut sediment for chlorophyll *a* to assess selective feeding, analysing holothurian gut wall tissue and ovaries for their pigment biochemistry and comparing these

data with the phytodetritus and sediment pigment composition. Details of the methods and a general introduction to the study site can be found in Chapter 2. Within year interspecies differences and between-year differences will be discussed and related to the feeding mode and reproductive adaptations of the species.

4.2 Results

4.2.1 Phytopigments in the phytodetritus and sediment - June 2004 and July 2005

Ten pigments were identified in the phytodetritus and sediments sampled in June 2004, and twelve in July 2005 (Tables 4.1 and 4.2; Fig. 4.1). Phytodetritus consistently had higher concentrations of phytopigments compared to the top 5mm section of the sediment. Furthermore, the top 5mm of sediment consistently yielded greater concentrations of phytopigments compared to the 5 to 10mm section of sediment. Phaeophorbide *a* was a dominant pigment in the phytodetritus in both years, although in July 2005, 19'-butanoyloxyfucoxanthin was present in similar concentrations to phaeophorbide (Table 4.2). In June 2004, chlorophyll *c2*, diatoxanthin and β-carotene were present in the phytodetritus, but were absent in surficial sediment samples (0 to 5mm). Diatoxanthin and zeaxanthin were present in the phytodetritus but not the sediment in 2005 (Fig. 4.1). Chlorophyll *c2*, a marker for a range of algal groups (Cryptophyta, Bacillariophyta, Dinophyta, Chrysophyta and Haptophytes) (Jeffrey et al., 1997), was present in the phytodetritus in June 2004 but not July 2005. 19'-butanoyloxyfucoxanthin and 19'-hexanoyloxyfucoxanthin (both markers of prymnesiophytes and some dinoflagellates) were absent in the phytodetritus and surficial sediment in June 2004; together they contributed a large percentage of the total in 2005 (Table 4.2).

Nine pigments co-occurred in surficial sediments from 2004 and 2005. All were found in higher concentrations in 2004 (Fig. 4.1). The concentration and percentage contribution of chlorophyll *a* was greater in the phytodetritus in 2004 (0.26 μg gDW^{-1}; 20%) than in 2005 (0.006 μg gDW^{-1}; 1%) (Tables 4.1 and 4.2). Concentrations of chlorophyll *a*, which is associated with intact phytoplankton cells and is an indicator of the contribution of fresh organic matter (Stephens et al., 1997), was 9 times greater in the phytodetritus than in

the surficial sediment in June 2004, but present in similar concentrations in the phytodetritus and surficial sediment in July 2005. The percentage contribution of phaeophytin was much greater in the surficial sediment than in the phytodetritus. Phaeophytin was the greatest contributor to the total pigment load in surficial sediment in 2004 and 2005 (Tables 4.1 and 4.2).

	phytodetritus (n = 4)	phytodetritus % total	0 to 0.5cm sediment (n = 3)	0 to 0.5cm sediment % total
Chlorophyll *c2*	0.005	0.4%	0	
	0.006	*0.4%*		
Fucoxanthin	0.204	15.7%	0.024	19.24%
	0.215	*16.5%*	*0.008*	*6.02%*
Diadinoxanthin	0.038	2.9%	0.002	1.26%
	0.044	*3.4%*	*0.003*	*2.18%*
Alloxanthin	0.126	9.7%	0.006	4.51%
	0.139	*10.7%*	*0.006*	*4.92%*
Diatoxanthin	0.013	1.0%	0	
	0.014	*1.1%*		
Zeaxanthin	0.153	11.8%	0.014	10.82%
	0.148	*11.4%*	*0.004*	*3.38%*
Chlorophyll *a*	0.263	20.2%	0.029	22.83%
	0.279	*21.4%*	*0.010*	*7.62%*
β-carotene	0.056	4.3%	0	
	0.053	*4.1%*		
Phaeophorbide	0.345	26.5%	0.020	15.70%
	0.292	*22.4%*	*0.015*	*12.01%*
Phaeophytin	0.099	7.6%	0.032	25.64%
	0.069	*5.3%*	*0.001*	*0.91%*

Table 4.1 Average concentration (μg gDW^{-1}) of pigments and average percentage contribution to the total identified pigment load in the sediment and phytodetritus sampled in June 2004. (Standard deviation in italics)

	phytodetritus (n = 3)	phytodetritus % total	0 to 0.5cm sediment (n = 3)	0 to 0.5cm sediment % total	0.5 to 1cm sediment (n = 3)	0.5 to 1cm sediment % total
19'-butanoyloxyfucoxanthin	0.111	26.6%	0.012	13.1%	0.004	15.3%
	0.080	*19.3%*	*0.016*	*16.9%*	*0.005*	*18.5%*
Fucoxanthin	0.026	6.2%	0.003	3.5%	0.001	3.7%
	0.020	*4.8%*	*0.004*	*4.1%*	*0.002*	*6.4%*
19'-hexanoyloxyfucoxanthin	0.031	7.3%	0.014	14.9%	0.005	20.3%
	0.024	*5.8%*	*0.010*	*10.3%*	*0.005*	*19.2%*
Violaxanthin	0.009	*2.1%*	0.001	*1.6%*	0	
	0.006	*1.4%*	*0.002*	*1.7%*		
Diadinoxanthin	0.036	8.7%	0.006	6.0%	0.001	5.5%
	0.025	*6.1%*	*0.006*	*6.0%*	*0.002*	*6.0%*
Alloxanthin	0.020	4.9%	0.010	11.3%	0	
	0.018	*4.2%*	*0.014*	*14.8%*		
Diatoxanthin	0.002	0.4%	0.0004	0.4%	0	
	0.002	*0.4%*	*0.0007*	*0.7%*		
Zeaxanthin	0.002	0.4%	0		0	
	0.003	*0.6%*				
Chlorophyll *a*	0.006	1.4%	0.005	5.7%	0	
	0.006	*1.5%*	*0.005*	*5.0%*		
ß-carotene	0.010	2.4%	0.002	2.1%	0	
	0.009	*2.2%*	*0.003*	*3.7%*		
Phaeophorbide	0.119	28.7%	0.015	16.1%	0.004	16.1%
	0.121	*29.0%*	*0.014*	*15.4%*	*0.004*	*15.3%*
Phaeophytin	0.045	10.9%	0.023	25.2%	0.010	39.1%
	0.030	*7.2%*	*0.011*	*11.7%*	*0.009*	*33.0%*

Table 4.2 Average concentration (µg gDW^{-1}) of pigments and average percentage contribution to the total identified pigment load in the sediment and phytodetritus sampled in July 2005. (Standard deviation in italics)
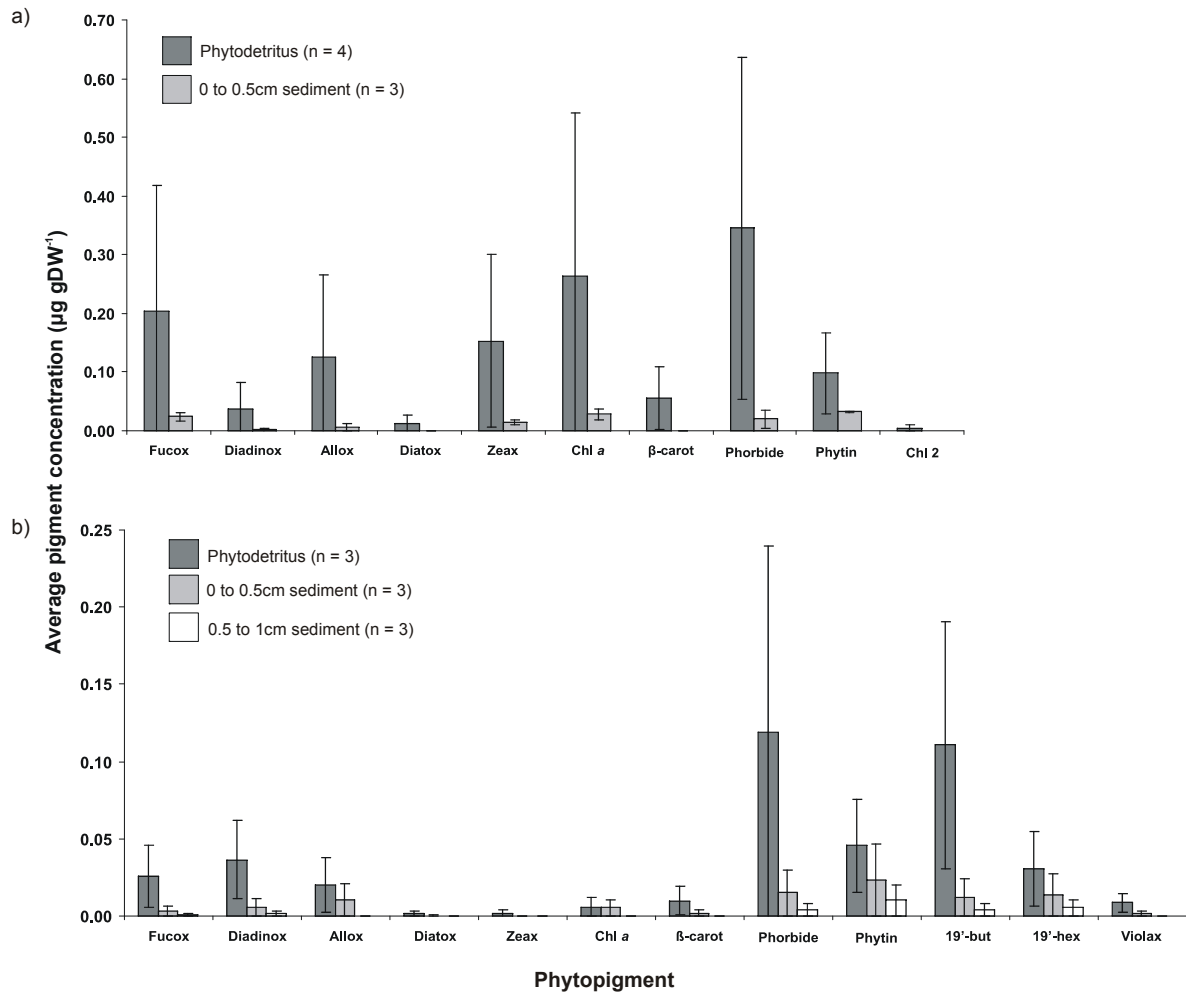
Figure 4.1 Phytopigments found in 1) the phytodetritus, 2) 0 to 0.5cm and 3) 0.5 to 1cm of sediment from (a) June 2004 and (b) July 2005 (mean μg gDW^{-1} ± SD). Fucox = fucoxanthin; Diad = diadinoxanthin; Allox = alloxanthin; Diatox = diatoxanthin; Zeax = zeaxanthin; Chl a = chlorophyll *a*; β-carot = β-carotene; Phorbide = phaeophorbide; Phytin = phaeophytin; 19'but = 19'-butanoyloxyfucoxanthin; 19'-hex = 19'-hexanoyloxyfucoxanthin; Violax = violaxanthin; Chl 2 = chlorophyll c2. (note different scales on y-axis).

Table 4.3 gives the ratios of chlorophyll *a* to phaeophorbide for each phytodetritus sample. The phytodetritus in 2004 had an average chlorophyll *a*: phaeophorbide ratio of 0.67 (S.D ± 0.15) compared to 0.29 (S.D. ± 0.49) in 2005. Because of the high variability in 2005, there was no significant difference (t(5) = 1.84, P>0.05) in this ratio between the years.

	Chlorophyll *a*	Phaeophorbide	Chl:Phorb ratio	Average	Standard deviation
Phytodetritus June 2004 (n = 4)	0.058	0.110	0.525		
	0.142	0.209	0.680		
	0.176	0.292	0.604		
	0.674	0.768	0.878	**0.672**	**0.151**
Phytodetritus July 2005 (n = 3)	0.012	0.014	0.856		
	0.005	0.251	0.021		
	0	0.093	0	**0.292**	**0.488**

Table 4.3 Chlorophyll *a* and phaeophorbide concentrations (µg gDW^{-1}) and chlorophyll *a* to phaeophorbide ratios (Chl:Phorb ratio).

4.2.2 Chlorophyll a *in holothurian gut sediment*

All species had chlorophyll *a* gut sediment concentrations (µg gDW^{-1}) higher than that of the sediment or phytodetritus, with the exception of *Molpadia blakei* (Tables 4.2, 4.3 and 4.4). Chlorophyll *a* was absent in the gut sediment of *M. blakei* sampled in 2005. Chlorophyll *a* was absent in ten of the fourteen samples of *Amperima rosea* gut sediment (only sampled in 2004). Average gut sediment chlorophyll *a* concentration was 1.19 µg gDW^{-1} when all *A. rosea* samples are included. The average concentration of the four *A. rosea* specimens containing chlorophyll *a* was 4.16 µg gDW^{-1}; this is greater than the average chlorophyll *a* gut sediment concentration of the other species sampled (Table 4.4; Figure 4.2).

Chlorophyll *a* concentrations in the gut sediment of *Oneirophanta mutabilis* and *Psychropotes longicauda* were significantly greater in 2004 than in 2005 (t(12) = 3.58, P<0.05; t(11) = 2.19, P<0.05, respectively). Variability in *O. mutabilis* gut sediment chlorophyll *a* concentration was high in 2004. There was no significant between-year difference in chlorophyll *a* gut sediment concentration in *Paroriza prouhoi* (t(5) = 0, P>0.05). The gut sediment chlorophyll *a* concentration of *O. mutabilis* was significantly greater than that of *P. longicauda* in 2004 (t(12) = 2.24, P<0.05), but was the same in 2005 (t(11) = 0.71, P>0.05). Although gut chlorophyll *a* concentrations were similar for all

holothurians (with the exception of *m. blakei*) in 2005, statistical analysis suggests there were interspecific differences ($F_{4,25}$ = 1.42, P>0.05), which can be attributed to the lower concentration of *Pseudostichopus villosus* and higher concentration of *P. aemulatus* (Fig. 4.2).

	June 2004	July 2005
Amperima rosea (n = 14)	1.19 (4.16) *2.02 (1.06)*	Nc
Peniagone diaphana (n = 5)	1.88 *1.58*	Nc
Oneirophanta mutabilis (n = 9; 2004, 5; 2005)	2.60 *1.81*	0.40 *0.24*
Psychropotes longicauda (n = 5; 2004, 8; 2005)	0.72 *0.48*	0.32 *0.16*
Paroriza prouhoi (n = 4; 2004, 3; 2005)	0.45 *0.45*	0.45 *0.33*
Pseudostichopus aemulatus (n = 5)	Nc	0.63 *0.64*
Pseudostichopus villosus (n = 9)	Nc	0.20 *0.27*
Molpadia blakei (n = 4)	Nc	0

Table 4.4 Chlorophyll *a* (μg gDW^{-1}) in the gut sediment of holothurians sampled in June 2004 and July 2005. Average concentration of *A. rosea* specimens containing chlorophyll *a* in their gut sediment (i.e. excluding zero values) is given in brackets – see text for details. Standard deviation in italics. (Nc = not collected)
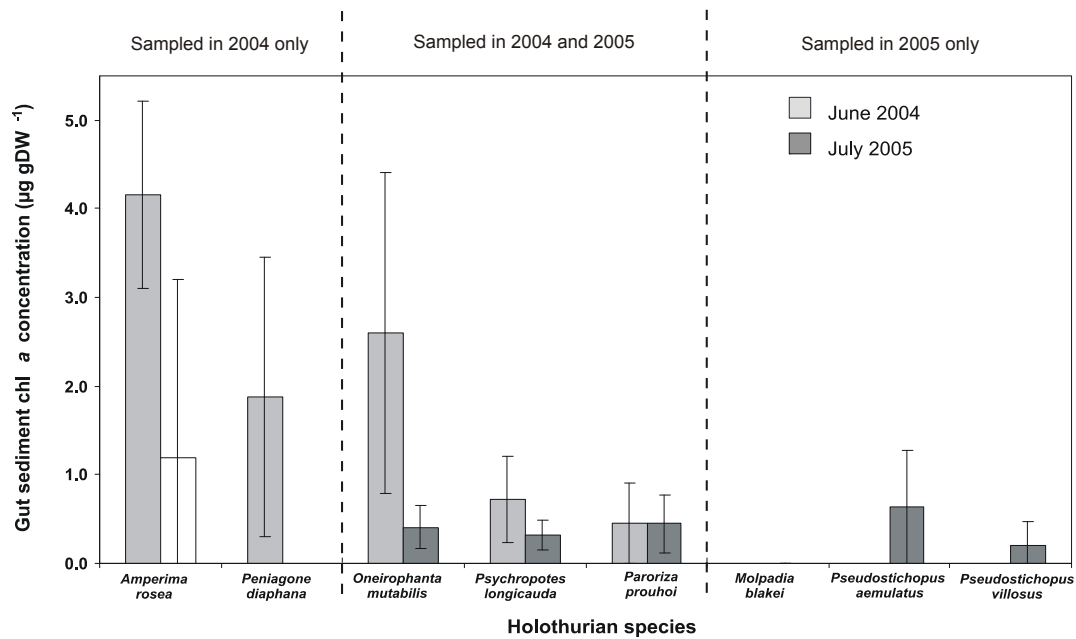
Figure 4.2 Chlorophyll a concentration (mean μg gDW-1 ± SD) in the gut sediment of different holothurian species sampled at PAP in June 2004 (light grey) and July 2005 (dark grey). (*Amperima rosea* mean gut chlorophyll *a* concentration based on all samples, including samples with empty guts, indicated by white histogram - see text).

4.2.3 Interspecies comparisons of gut wall and ovarian carotenoid biochemistry – June 2004

Quantitative comparisons of the gut walls and ovaries of animals sampled in June 2004 show that they contain carotenoids (Tables 4.5 & 4.6; Fig. 4.3). The total concentration of carotenoid pigments (μg gDW^{-1}) in different species varied considerably. For example, *A. rosea* had pigment concentrations an order of magnitude greater than *O. mutabilis* and *Peniagone diaphana*, and two orders of magnitude greater than *Psychropotes longicauda* and *Paroriza prouhoi* (Fig. 4.3). Variability of the pigment concentrations in the gut wall and ovary was often high. This was particularly apparent for *Psychropotes longicauda* and *Paroriza prouhoi*, and the gut wall of *O. mutabilis*. Carotenoids found in the greatest concentrations in the gut wall and ovaries of *A. rosea* and *O. mutabilis* were zeaxanthin and β-carotene respectively; they contributed >24% of the total identified pigment load (Tables 4.5 and 4.6).

These two carotenoids are also the most variable in their concentration in these species.

MDS ordinations of the square root transformed pigment percentage contributions of the gut wall and ovary samples show some species clustering (Fig. 4.4 a and b). *Amperima rosea* shows the tightest species-specific clustering in both the gut wall and ovarian tissues, indicating the specimens had a consistent biochemical profile. Species clustering was more defined for all species within the ovarian tissues. *Psychropotes longicauda* and *Paroriza prouhoi* gut wall samples showed the least species specific clustering. The outlying *P. prouhoi* sample to the top right of the MDS plot contained no β-carotene and a high proportion of zeaxanthin; the outlying sample bottom right had relatively high proportion of canthaxanthin and low proportion of diadinoxanthin (Fig. 4.5 a). *Psychropotes longicauda* samples were spread from the left to the right of the plot; samples to the left contained higher proportions of echinenone; low proportions of alloxanthin and diadinoxanthin were evident in specimens placed to the right of the plot. Diadinoxanthin also accounted for the spread of samples of *Peniagone diaphana* on the MDS plot. The spread of *O. mutabilis* ovary samples from the centre to the top right reflected the relative contribution of β-carotene to the total pigment load; other carotenoids showed little variation in concentration (Fig. 4.3). *Oneirophanta mutabilis* appears to assimilate β-carotene selectively (Fig. 4.3); its contribution to the total pigment load in the ovarian samples ranged between 30% and 70%. *Paroriza prouhoi* ovarian samples were spread from top right to centre of the MDS plot because of the presence or absence of alloxanthin and the increasing contribution of diadinoxanthin.

	Amperima rosea (n=14)	Amperima rosea % of total	Oneirophanta mutabilis (n=8)	Oneirophanta mutabilis % of total	Peniagone diaphana (n=5)	Peniagone diaphana % of total	Psychropotes longicauda (n=5)	Psychropotes longicauda % of total	Paroriza prouhoi (n=5)	Paroriza prouhoi % of total
Diadinoxanthin	22.02 *15.62*	0.1 *0.0*	4.81 *2.69*	0.1 *0.0*	5.03 *6.12*	0.1 *0.1*	0.95 *0.49*	0.2 *0.1*	1.27 *1.45*	0.4 *0.1*
Alloxanthin	34.90 *21.18*	0.1 *0.0*	8.05 *5.05*	0.2 *0.0*	3.87 *4.09*	0.1 *0.0*	1.05 *0.67*	0.2 *0.1*	0.86 *0.93*	0.3 *0.1*
Diatoxanthin	27.62 *18.45*	0.1 *0.0*	3.38 *2.16*	0.1 *0.0*	2.19 *2.09*	0.1 *0.0*	0.43 *0.23*	0.1 *0.0*	0.22 *0.24*	0.1 *0.0*
Zeaxanthin	124.92 *100.13*	0.3 *0.1*	5.80 *3.84*	0.2 *0.0*	5.25 *2.71*	0.2 *0.0*	0.34 *0.25*	0.1 *0.0*	0.32 *0.29*	0.1 *0.1*
Canthaxanthin	13.55 *8.63*	0.0 *0.0*	2.17 *1.48*	0.1 *0.0*	1.17 *0.51*	0.1 *0.0*	1.22 *0.95*	0.2 *0.2*	0.16 *0.17*	0.1 *0.1*
Echinenone	68.89 *37.63*	0.2 *0.0*	3.15 *2.69*	0.1 *0.0*	5.79 *2.24*	0.3 *0.1*	1.19 *1.54*	0.2 *0.2*	0.00 *0.00*	0.0
ß-carotene	36.59 *19.67*	0.1 *0.0*	7.62 *4.18*	0.2 *0.1*	6.47 *5.66*	0.2 *0.0*	0.56 *0.38*	0.1 *0.1*	0.43 *0.46*	0.1 *0.1*

Table 4.5 Pigment concentrations (μg gDW^{-1}) and percentage contribution of pigments to the total pigment load in the gut wall of holothurians sampled in June 2004. (Standard deviation in italics)

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	Amperima rosea (n=14)	Amperima rosea % of total	Oneirophanta mutabilis (n=8)	Oneirophanta mutabilis % of total	Peniagone diaphana (n=4)	Peniagone diaphana % of total	Psychropotes longicauda (n=5)	Psychropotes longicauda % of total	Paroriza prouhoi (n=5)	Paroriza prouhoi % of total
Diadinoxanthin	16.78	7.6%	0.95	4.6%	1.13	4.0%	1.11	29.3%	0.46	12.9%
	9.56	*1.3%*	*0.49*	*3.2%*	*1.38*	*4.0%*	*1.62*	*8.0%*	*0.67*	*9.8%*
Alloxanthin	24.56	11.2%	1.05	7.7%	1.65	5.0%	0.55	16.9%	0.22	4.4%
	12.43	*1.3%*	*0.67*	*6.3%*	*2.20*	*4.5%*	*0.69*	*2.4%*	*0.28*	*4.4%*
Diatoxanthin	19.27	8.5%	0.43	4.4%	1.04	3.2%	0.35	9.3%	0.07	1.9%
	10.25	*1.4%*	*0.23*	*3.5%*	*1.37*	*2.9%*	*0.53*	*2.1%*	*0.08*	*2.0%*
Zeaxanthin	63.43	26.6%	0.34	5.4%	2.86	17.1%	0.35	14.3%	0.45	9.4%
	39.15	*8.1%*	*0.25*	*5.6%*	*2.62*	*8.4%*	*0.40*	*5.4%*	*0.54*	*8.5%*
Canthaxanthin	14.05	6.8%	1.61	11.8%	2.81	14.1%	0.63	14.9%	1.22	51.3%
	6.31	*1.8%*	*0.93*	*5.3%*	*2.97*	*4.3%*	*0.98*	*4.3%*	*1.43*	*18.7%*
Echinenone	48.79	22.1%	0.87	9.3%	7.23	29.7%	0.10	2.4%	0.47	19.0%
	28.04	*4.1%*	*0.77*	*13.1%*	*9.05*	*6.0%*	*0.18*	*2.2%*	*0.57*	*5.4%*
ß-carotene	36.60	17.1%	8.66	56.8%	9.62	26.8%	0.37	13.0%	0.07	1.2%
	18.94	*5.0%*	*5.45*	*18.8%*	*14.24*	*11.4%*	*0.43*	*5.2%*	*0.11*	*1.7%*

Table 4.6 Pigment concentrations (μg gDW^{-1}) and percentage contribution of pigments to the total pigment load in the ovaries of holothurians sampled in June 2004. (Standard deviation in italics)

Figure 4.3. Pigment concentrations (mean µg gDW^{-1} ± SD) in the gut wall (light grey) and ovary (dark grey) of holothurians sampled at PAP in June 2004. Diad = diadinoxanthin; Allox = alloxanthin; Diatox = diatoxanthin; Zeax = zeaxanthin; Canthax = canthaxanthin; Echin = echinenone; β-carot = β-carotene. (note different scales on y-axis)
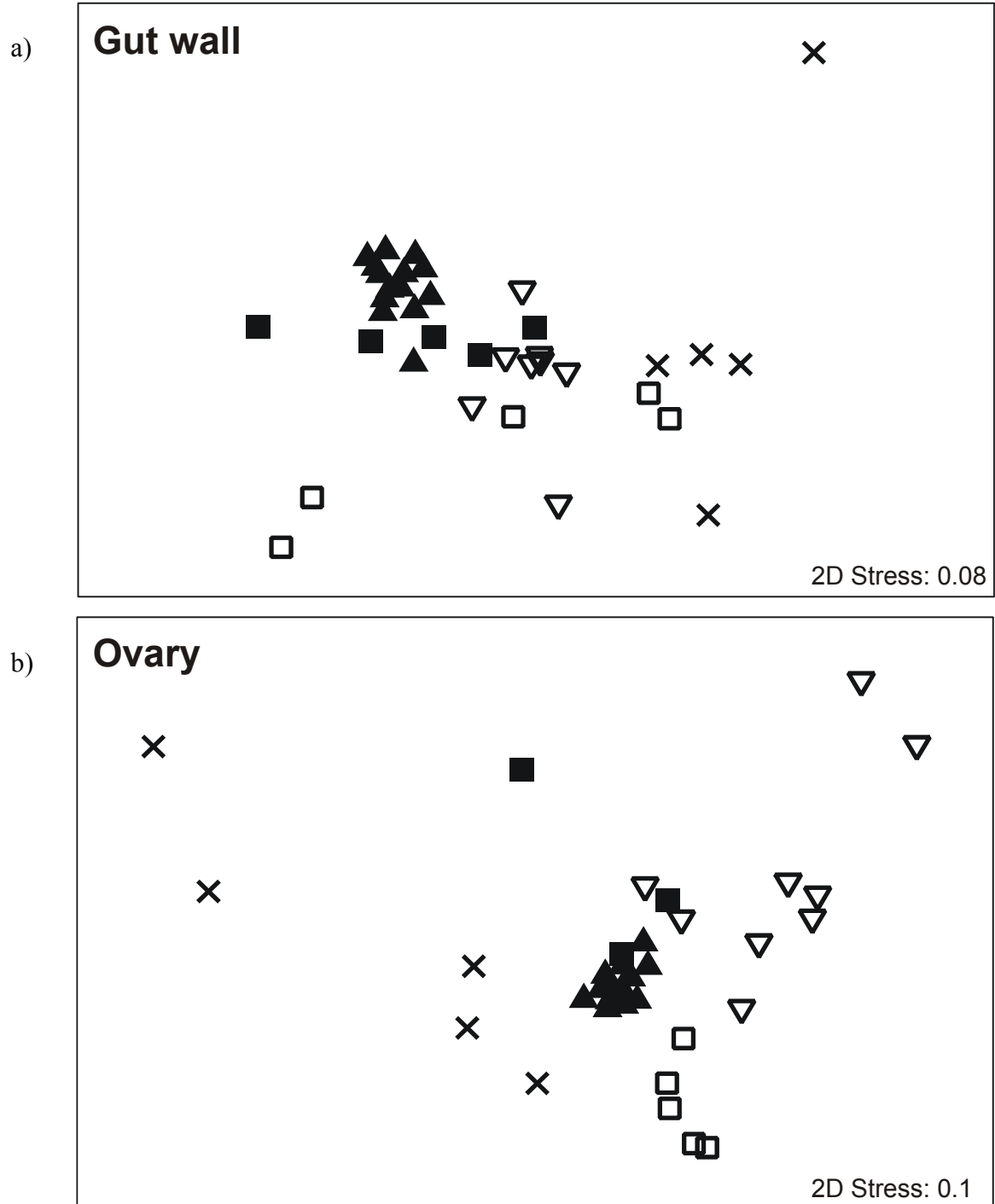
Figure 4.4 MDS ordination of individual holothurian gut wall (a) and ovary (b) samples from PAP June 2004, based on √-transformed pigment percentage contributions and Bray-Curtis similarities. Key: Filled triangles = *Amperima rosea***; open triangles =** *Oneirophanta mutabilis***; filled squares =** *Peniagone diaphana***; open squares =** *Psychropotes longicauda***; crosses =** *Paroriza prouhoi***.**

4.2.4 Interspecies comparisons of gut wall and ovarian carotenoid biochemistry – July 2005

Oneirophanta mutabilis had the highest concentration of carotenoids in the gut wall and ovaries (μg gDW^{-1}) of all holothurians sampled in July 2005; *Psychropotes longicauda* and *Pseudostichopus aemulatus* the least (Fig. 4.5). Pigment concentrations were very variable in the gut wall of all species but less so in their ovaries. 19'-butanoyloxyfucoxanthin was present in the gut wall and ovaries of all species sampled, while 19'-hexanoyloxyfucoxanthin was not found in the ovaries of *Molpadia blakei* and *Pseudostichopus aemulatus* (Fig. 4.5). Both 19'-butanoyloxyfucoxanthin and 19'-hexanoyloxyfucoxanthin were found in relatively lower concentrations compared to the other carotenoids present in the gut wall and ovary in *O. mutabilis*. β-carotene contributed to 25% and 51% of the total pigments in *O. mutabilis* gut wall and ovary, respectively (Table 4.7 and 4.8).

Species-specific clustering is evident only for gut wall samples of *O. mutabilis* and *M. blakei*, and less so for *Pseudostichopus villosus* in the MDS ordination plot of percentage contribution (Fig. 4.6 a). Species specific clustering can be observed for *O. mutabilis, Psychropotes longicauda* and *Paroriza prouhoi* on the MDS ordination plot of ovarian samples. The clusters of these three species are also very close to each other (Fig. 4.6 b). *Molpadia blakei* shows species specific clustering with the exception of one sample, which contained no 19'-butanoyloxyfucoxanthin. *Pseudostichopus aemulatus* and *P. villosus* showed little ovarian species specific clustering.

	M. blakei (n=3)	M. blakei % of total	O. mutabilis (n=4)	O. mutabilis % of total	P. emulatus (n=4)	P. emulatus % of total	P. longicauda (n=6)	P. longicauda % of total	P. prohoui (n=4)	P. prohoui % of total	P. villosus (n=5)	P. villosus % of total
19'-butanoyloxyfucoxanthin	0.38	21.2%	0.99	3.6%	0.38	23.2%	0.26	16.4%	0.23	19.5%	0.48	14.5%
	0.44	*16.0%*	*0.46*	*2.0%*	*0.34*	*19.4%*	*0.37*	*30.5%*	*0.29*	*23.3%*	*0.80*	*9.5%*
19'-hexanoyloxyfucoxanthin	0.00	0.0%	0.56	1.4%	0.24	14.5%	0.16	6.9%	0.21	12.3%	0.54	13.8%
			0.08	*1.2%*	*0.28*	*16.7%*	*0.24*	*9.6%*	*0.28*	*9.1%*	*1.09*	*12.1%*
Diadinoxanthin	0.00	0.0%	4.41	13.1%	0.14	9.6%	0.22	6.8%	0.24	18.2%	0.11	1.9%
			1.86	*1.8%*	*0.17*	*11.0%*	*0.38*	*10.7%*	*0.35*	*9.4%*	*0.22*	*3.1%*
Alloxanthin	0.48	18.0%	7.58	22.1%	0.33	20.9%	0.13	4.1%	0.46	31.3%	0.68	13.8%
	0.77	*14.1%*	*3.58*	*2.2%*	*0.13*	*8.8%*	*0.24*	*6.6%*	*0.78*	*17.7%*	*1.24*	*11.8%*
Diatoxanthin	0.00	0.0%	3.31	9.4%	0.16	10.5%	0.04	1.2%	0.09	7.3%	0.11	2.2%
			1.61	*1.3%*	*0.15*	*9.6%*	*0.07*	*2.0%*	*0.14*	*5.8%*	*0.22*	*2.4%*
Zeaxanthin	0.22	4.4%	4.42	11.5%	0.17	11.1%	0.02	0.8%	0.06	4.5%	0.06	1.5%
	0.44	*8.9%*	*2.64*	*3.8%*	*0.17*	*11.2%*	*0.04*	*1.2%*	*0.10*	*6.5%*	*0.10*	*3.1%*
Canthaxanthin	0.48	28.5%	2.40	7.5%	0.00	0.0%	0.18	20.7%	0.05	1.2%	0.50	15.2%
	0.55	*21.3%*	*1.70*	*2.3%*			*0.16*	*21.2%*	*0.12*	*2.4%*	*0.71*	*7.8%*
Echinenone	0.00	0.0%	1.93	5.9%	0.00	0.0%	0.27	14.3%	0.01	0.3%	0.21	6.3%
			0.81	*0.7%*			*0.32*	*14.6%*	*0.03*	*0.7%*	*0.32*	*9.1%*
ß-carotene	0.06	2.8%	6.86	25.3%	0.16	10.3%	0.21	28.8%	0.12	5.4%	0.34	14.1%
	0.08	*3.7%*	*2.36*	*7.0%*	*0.20*	*13.7%*	*0.20*	*29.5%*	*0.26*	*6.3%*	*0.38*	*12.2%*

Table 4.7 Pigment concentrations ($\mu g\ gDW^{-1}$) and percentage contribution of pigments to the total pigment load in the gut wall of holothurians sampled in July 2005 (Standard deviation in italics). 19'but = 19'-butanoyloxyfucoxanthin; 19'-hex = 19'-hexanoyloxyfucoxanthin.

Table 4.8 Pigment concentrations (μg gDW^{-1}) and percentage contribution of pigments to the total pigment load in the ovaries of holot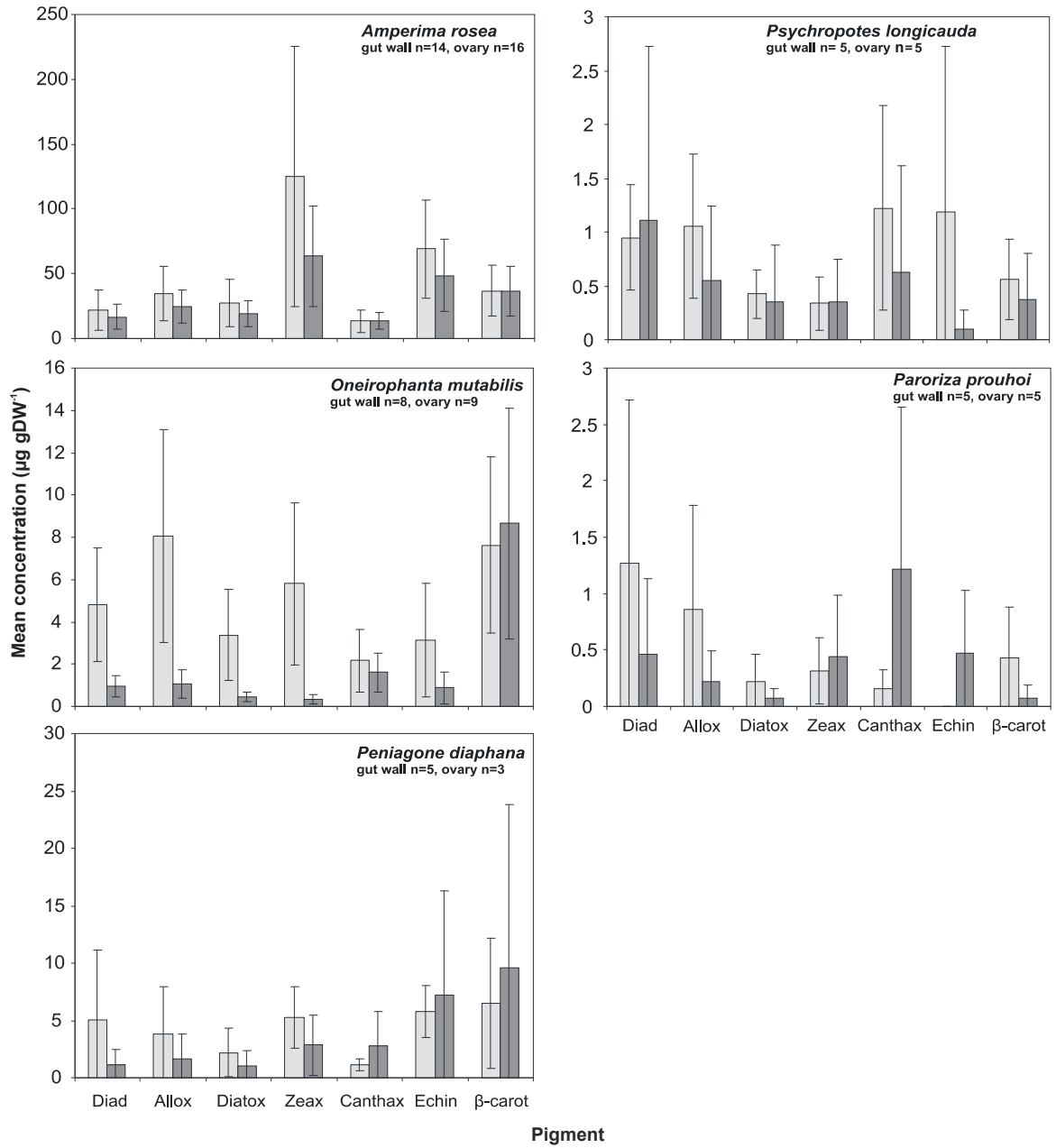hurians sampled in July 2005 (Standard deviation in italics). 19'but = 19'-butanoyloxyfucoxanthin; 19'-hex = 19'-hexanoyloxyfucoxanthin.

	M. blakei (n=5)	M. blakei % of total	O. mutabilis (n=3)	O. mutabilis % of total	P. aemulatus (n=3)	P. aemulatus % of total	P. longicauda (n=2)	P. longicauda % of total	Paroriza prohoui (n=3)	Paroriza prohoui % of total	P. villosus (n=7)	P. villosus % of total
19'-but	0.44 *0.28*	18.2% *11.1%*	0.17 *0.11*	1.6% *0.9%*	0.09 *0.08*	8.0% *10.1%*	0.16 *0.07*	17.9% *15.9%*	0.07 *0.06*	4.1% *4.2%*	0.07 *0.09*	2.9% *2.8%*
19'-hex	0.00	0.0%	0.19 *0.14*	1.8% *0.6%*	0.00	0.0%	0.05 *0.02*	5.7% *4.6%*	0.14 *0.09*	5.8% *1.3%*	0.04 *0.06*	1.5% *2.2%*
Diadinoxanthin	0.19 *0.11*	7.8% *4.6%*	0.86 *0.41*	8.6% *0.7%*	0.19 *0.26*	10.7% *10.8%*	0.22 *0.21*	16.5% *9.1%*	0.63 *0.33*	26.6% *3.0%*	0.12 *0.17*	4.8% *5.2%*
Alloxanthin	0.35 *0.16*	17.6% *2.7%*	1.45 *0.71*	14.5% *3.1%*	0.14 *0.15*	9.7% *8.6%*	0.19 *0.17*	14.6% *6.4%*	0.44 *0.29*	17.7% *2.9%*	0.17 *0.20*	9.2% *6.9%*
Diatoxanthin	0.06 *0.04*	2.5% *1.4%*	0.42 *0.19*	4.3% *0.5%*	0.08 *0.10*	5.3% *4.6%*	0.04 *0.04*	3.2% *1.3%*	0.12 *0.11*	4.7% *1.2%*	0.04 *0.05*	1.7% *1.7%*
Zeaxanthin	0.21 *0.13*	8.5% *5.1%*	0.34 *0.17*	3.4% *0.8%*	0.06 *0.06*	4.4% *4.0%*	0.05 *0.07*	3.0% *4.2%*	0.13 *0.07*	5.5% *2.0%*	0.06 *0.10*	2.2% *2.8%*
Canthaxanthin	0.57 *0.21*	33.0% *14.6%*	1.01 *0.43*	10.4% *0.8%*	0.11 *0.19*	4.8% *8.3%*	0.30 *0.16*	26.6% *0.3%*	0.40 *0.30*	15.7% *2.8%*	0.40 *0.43*	37.5% *28.5%*
Echinenone	0.00	0.0%	0.40 *0.11*	4.3% *0.9%*	0.00	0.0% *0.0%*	0.11 *0.08*	9.1% *2.4%*	0.04 *0.05*	1.4% *1.2%*	0.15 *0.19*	6.5% *6.5%*
ß-carotene	0.22 *0.16*	12.4% *7.1%*	5.04 *2.51*	51.2% *5.3%*	0.45 *0.25*	57.1% *37.3%*	0.03 *0.01*	3.4% *2.5%*	0.45 *0.33*	18.5% *1.9%*	0.38 *0.40*	33.5% *25.2%*

Figure 4.5 Pigment concentrations (mean μg gDW-1 ± SD) in the gut wall (light grey) and ovary (dark grey) of holothurians sampled at PAP in July 2005. 19'-but = 19'-butanoyloxyfucoxanthin; 19'-hex = 19'-hexanoyloxyfucoxanthin; Diad = diadinoxanthin; Allox = alloxanthin; Diatox = diatoxanthin; Zeax = zeaxanthin; Canthax = canthaxanthin; Echin = echinenone; β-carot = β-carotene. (note different scales on y-axis)
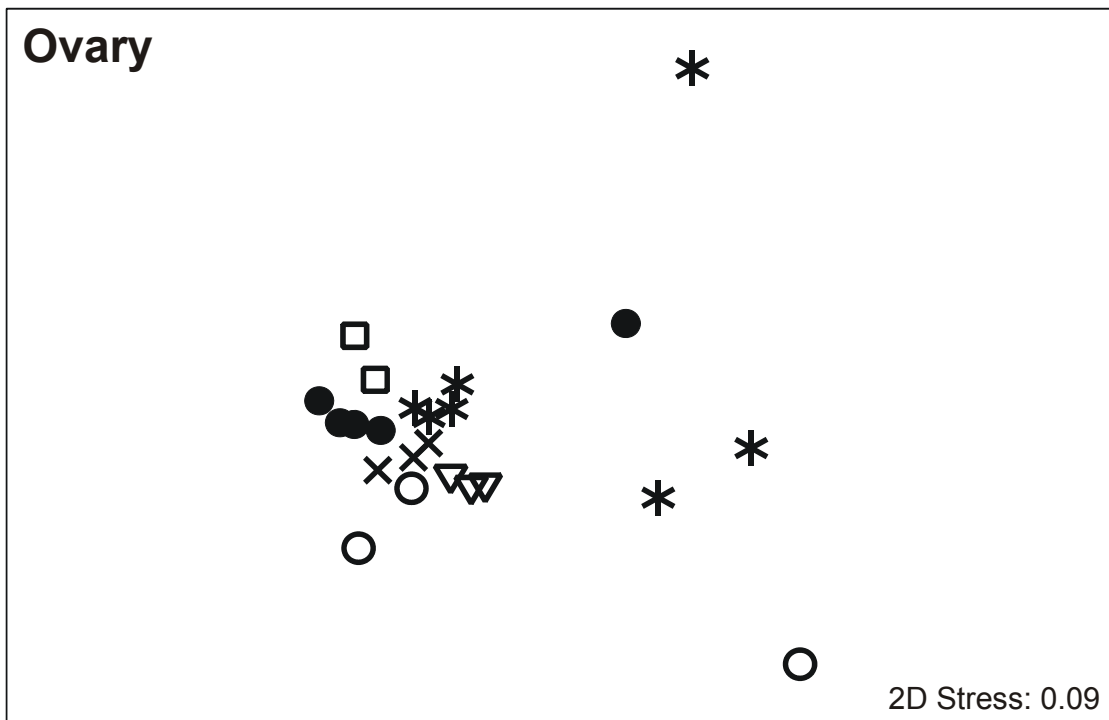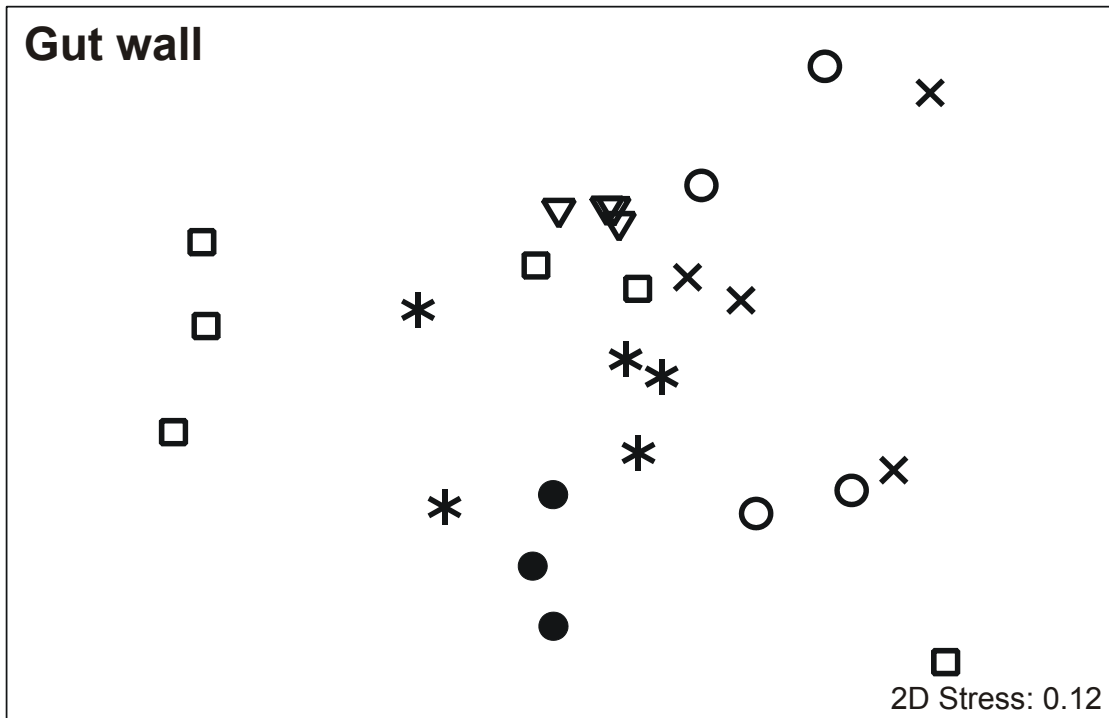
Figure 4.6 MDS ordination of 26 individual holothurian gut wall (a) and 23 individual holothurian ovary (b) samples from PAP July 2005, based on √-transformed pigment percentage contributions and Bray-Curtis similarities. Key: Open triangles = *Oneirophanta mutabilis*; filled circles = *Molpadia blakei*; open circles = *Pseudostichopus aemulatus*; open squares = *Psychropotes longicauda*; crosses = *Paroriza prouhoi*; stars = *Pseudostichopus villosus*.

4.2.5 Between-year intraspecies comparison

Oneirophanta mutabilis, *Psychropotes longicauda* and *Paroriza prouhoi* were sampled June 2004 and in July 2005. Comparison of the mean concentrations of carotenoids in their gut wall samples show that all carotenoids (with the exception of 19'-butanoyloxyfucoxanthin and 19-hexanoyloxyfucoxanthin) were present in greater concentrations in 2004 (Fig. 4.3 and 4.5). Diadinoxanthin, alloxanthin, diatoxanthin, zeaxanthin and canthaxanthin were found in significantly greater concentrations in the gut wall of *Psychropotes longicauda* in 2004 (t-test, P<0.05 – Appendix 2). Because of their high variability, the other carotenoids in this species and in the gut walls of *O. mutabilis* and *Paroriza prouhoi* did not differ significantly (t-test, P>0.05 - Appendix 2) between years.

Average concentrations of carotenoids in the ovaries of *Psychropotes longicauda* and *O. mutabilis* were greater in 2004 with the exception of 19'-butanoyloxyfucoxanthin and 19'-hexanoyloxyfucoxanthin (absent from samples taken in 2004) as well as alloxanthin and diadinoxanthin in *O. mutabilis*. There was no significant differences in the concentration of the carotenoids between the years (t-test, P>0.05 – Appendix 2) because of high variability. *Paroriza prouhoi* ovarian samples contained greater average concentrations of all carotenoids in 2005, with the exception of zeaxanthin, canthaxanthin and echinenone, although this was not significantly different (t-test, P>0.05).

MDS ordination plot of gut wall pigment percentage contribution shows that *O. mutabilis* has the tightest interspecies and between year clustering (Fig. 4.7 a). *Oneirophanta mutabilis* samples taken from 2005 and 2004 cluster together because of the low relative contribution of 19'-butanoyloxyfucoxanthin and 19'-hexanoyloxyfucoxanthin. These carotenoids only contribute a small amount (less than 5%) to *O. mutabilis* gut wall total pigment loads, whereas for *Psychropotes longicauda* and *Paroriza prouhoi* they contribute to an average of 40% of the total load (Table 4.6). ANOSIM analysis indicates that *P. prouhoi* has significantly similar gut wall pigment composition between the years (ANOSIM R = 0.297, P<0.05; Table 4.8). This is

because the samples are widely spread and variable within each year – the sample replicates within each year are as similar to other replicates from the other year as they are to each other; there is no consistent biochemical pigment profile. Samples of *Paroriza prouhoi* samples from 2004 and 2005 show differences because of the contribution of 19'-butanoyloxyfucoxanthin and 19'-hexanoyloxyfucoxanthin in samples taken in 2005. *Psychropotes longicauda* has similar between year pigment gut wall composition, but the R-statistic is not significant (ANOSIM R = 0.143, P>0.05; Table 4.8). *Psychropotes longicauda* 2005 samples are widely spread on the MDS plot and therefore variable in pigment percentage composition, which influences the probability of the ANOSIM result; the MDS ordination plot shows this species does not have a consistent biochemical profile in its gut wall between the years (Fig. 4.7 a). The *P. longicauda* 2005 outlying samples to the right of the plot contain only canthaxanthin, echinenone and β-carotene. Those to the left also contain varying proportions of 19'-butanoyloxyfucoxanthin and 19'-hexanoyloxyfucoxanthin, diatoxanthin, alloxanthin and diadinoxanthin. *Oneirophanta mutabilis* gut wall samples for the two years are found close together on the plot, but samples from the two sites are closely grouped, giving some degree of separation, which influences the probability of the low R-statistic suggesting samples from the two years are similar (ANOSIM R = 0.086, P = 0.244; Table 4.8).

MDS ordination of the pigment percentage contributions in the ovaries show close clustering between years for *O. mutabilis* samples; the slight between year separation is caused by the small percentage (together less than 5%) contribution of 19'-butanoyloxyfucoxanthin and 19'-hexanoyloxyfucoxanthin in samples taken in 2005 (Fig. 4.7 b). *Oneirophanta mutabilis* PAP 2004 samples are spread out on the plot because of the varying contribution of β-carotene ranging from 30% (samples in middle of MDS plot) to 70% (samples to top right of plot). ANOSIM analysis indicated *Oneirophanta mutabilis* samples showed no difference in composition between the years, but the R-statistic was not significant (ANOSIM R = 0.143, P>0.05; Table 4.9), because of the wide spread (variability) of the PAP 2004 samples (Fig. 4.7b). *Psychropotes longicauda* and *Paroriza prouhoi* samples were significantly different in their ovarian pigment biochemistry between years (ANOSIM R = 0.891 (*Psychropotes*

longicauda) R = 0.877 (*Paroriza prouhoi*), P<0.05; Table 4.9). Differences between the years for *Psychropotes longicauda* and *Paroriza prouhoi* can be attributed to the high percentage contribution of 19'-butanoyloxyfucoxanthin and 19'-hexanoyloxyfucoxanthin and the decreased percentage contribution and concentration of zeaxanthin (Figs 4.3 and 4.5) in their ovaries.
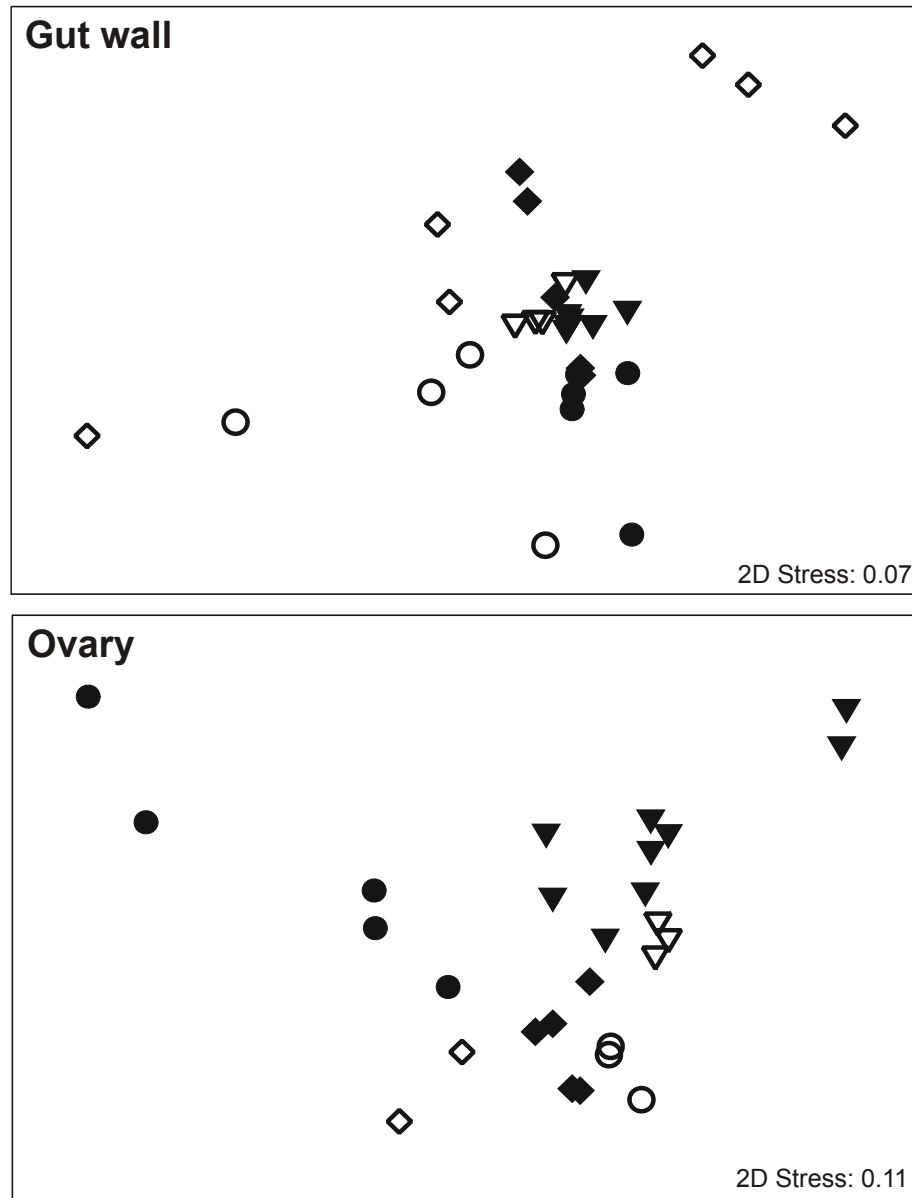
Figure 4.7 MDS ordination of 32 individual holothurian gut wall (a) and 23 individual holothurian ovary (b) samples from PAP June 2004 and July 2005, based on √-transformed pigment percentage contributions and Bray-Curtis similarities. Key: filled triangles = *Oneirophanta muta*bilis **June 2004; open triangles =** *Oneirophanta mutabilis* **July 2005; filled diamonds =** *Psychropotes longicauda* **June 2004; open diamonds =** *Psychropotes longicauda* **July 2005; filled circles =** *Paroriza prouhoi* **June 2004; open circles =** *Paroriza prouhoi* **July 2005**

Group	R-statistic	Significance level
O. mutabilis 2004 v *O. mutabilis* 2005 gut wall	0.086	P = 0.244
O. mutabilis 2004 v *O. mutabilis* 2005 ovary	-0.143	P = 0.732
P. longicauda 2004 v *P. longicauda* 2005 gut wall	0.143	P = 0.123
P. longicauda 2004 v *P. longicauda* 2005 ovary	0.891	P = 0.048*
P. prouhoi 2004 v *P. prouhoi* 2005 gut wall	0.297	P = 0.032*
P. prouhoi 2004 v *P. prouhoi* 2005 ovary	0.877	P = 0.018*

Table 4.9 Results of similarity test (ANOSIM) comparing holothurian gut wall and ovarian pigment percentage contribution to the total load between June 2004 and July 2005. R-statistic = 1 only if all replicates within a sample are more similar to each other than any other replicates from different samples. * = significant

4.3 Discussion

4.3.1 Supply of material to the sea-floor at the PAP

The present study supports previous observations that in many oceanic areas there is no consistent food supply to the deep-sea benthos; compounds essential to the deep-sea benthic community vary temporally in their availability (Danovaro et al., 2001; Kiriakoulakis et al., 2001; Neto et al., 2006). For example, 19'-butanoyloxyfucoxanthin, 19'-hexanoyloxyfucoxanthin and violaxanthin were present in the phytodetritus and sediment in July 2005 but were absent in June 2004, suggesting different phytoplankton groups contributed to the flux of OM to the seafloor. 19'-butanoyloxyfucoxanthin and 19'-hexanoyloxyfucoxanthin are biomarkers of prymnesiophytes (e.g. *Phaeocystis*, a colony species that can form large blooms) and some dinoflagellates; violaxanthin is considered a biomarker for green algae: chlorophytes, prasinophytes and eustigmatophytes (small pico or nano-phytoplankton; Jeffrey et al., 1997).

Temporal variability in the freshness of the phytodetritus and sediment was apparent. Chlorophyll *a* is associated with intact phytoplankton cells and phaeophorbide is apparently produced exclusively as a result digestion by herbivores (Lorenzen, 1967; Daley and Brown, 1973; Hendry et al., 1987). Chlorophyll *a* to phaeophorbide *a* ratios have been used to indicate freshness of phytodetrital material (Thiel et al., 1989). Greater chlorophyll *a* concentrations and chlorophyll *a* to phaeophorbide *a* ratios in the phytodetritus and sediment in June 2004 (0.26 μg gDW^{-1}; average ratio of 0.67 in phytodetritus) indicate the material at the seafloor was fresher than in July 2005 (0.006 μg gDW^{-1}; average ratio of 0.29 in phytodetritus), at least at the time of sampling. These results are similar to those seen before at the PAP. A chlorophyll *a* to phaeophorbide *a* ratio of 0.23 was recorded at the PAP in July 1997, while a high ratio of 1.33 was recorded in September 1996 after a large flux of relatively fresh phytodetritus (Witbaard et al., 2000). The compositional spectrum of amino acids can be used to quantify degradation and have shown high flux events are linked to fresher OM reaching the seabed at the PAP (Salter, 2007). A sediment trap moored at 3000 m over the PAP site has showed between year differences in the mass flux of material (Lampitt et al., 2001;

Lampitt, 2008). The timing of the flux to the seafloor at PAP was late May/early June in 2004 and early May in 2005 (Lampitt, 2008). Mass flux of material in June 2004 was between 150-200 mg m^{-2}d^{-1}, over double that of 20-50 mg m^{-2}d^{-1} in June and July 2005. This contrast in the mass flux to the seafloor was mirrored in the amount of carbon in the flux of OM (Lampitt, 2008). Spatial variability in the freshness of the phytodetritus was also more pronounced in 2005 than in 2004, as indicated by the high variability (greater than the mean) in the chlorophyll *a* to phaeophorbide *a* ratio in 2005. The higher concentrations of phaeophorbide in the phytodetritus and, conversely, phaeophorbide in the sediment may be related to their degradation rates. Phaeophorbide are preferentially degraded at the sediment surface in comparison to phaeophytin (Keely and Brereton, 1986; Hurley and Armstrong, 1990).

The chemical composition of OM is known to vary temporally and spatially at the PAP seafloor (Santos et al., 1994; Kiriakoulakis et al., 2001; Witbaard et al., 2001; Neto et al., 2006). For example, chlorophyll *a* concentrations in the top 1mm of sediment at the PAP ranged from 0.009 to 0.033 µg gDW^{-1} (converted from ng cm^{-3} by Wigham, 2002 in samples collected between September 1996 to September 1998 (Witbaard et al., 2001)). In the present study, chlorophyll *a* concentrations in the sediment and phytodetritus were 0.03 and 0.26 µg gDW^{-1}, respectively in June 2004 and 0.005 and 0.006 µg gDW^{-1} respectively in July 2005. Hudson (2004) measured both carotenoids and chloropigments in PAP sediments in October 2002 and reported a chlorophyll *a* concentration of 0.12 µg gDW^{-1} in the top 1mm sediment. Large differences in the percentage contributions of individual pigments are apparent when compared to the present study. β-carotene made up the highest percentage (> 18% of the total pigments) in October 2002 (Hudson, 2004) whereas in the present study it contributed only 4% in 2004 and 2% in 2005 to the total pigment load in the phytodetritus, and was absent (2004) or a minor component (2%; 2005) of the total pigment load in the surficial sediment. These differences may be attributed to variability in supply, but they may also be a function of the thickness of the sections of sediment analysed in each study – 1mm (Hudson, 2004) compared to 5mm (present study). This highlights the need to standardise future sampling protocols.

Concentrations and the variety of pigments were greater in the phytodetritus than in the sediment in both years. This suggests that species with the capibility to locate and feed selectively on phytodetrital material, would have been exposed to a greater concentration and range of pigments. Some carotenoids (diatoxanthin and β-carotene – June 2004; diatoxanthin and zeaxanthin – July 2005) utilised by the holothurians were only available in the phytodetritus. As in previous studies of intact proteins and individual lipids in PAP sediments (Kiriakoulakis et al., 2001) phytopigment concentrations in the present study decreased with sediment depth. The increased percentage contribution of phaeophytin in the deeper strata of sediment result in its formation from the breakdown of chlorophyll a (Hendry et al., 1987) and/or its greater stability in comparison to the other pigments. Degradation rates of pigments in the deep sea are not known. Chlorophyll a to phaeophorbide ratios cannot be used to determine the freshness of the sediment because of the selective degradation of phaeophorbide in relation to chlorophyll and phaeophytin in sediments (Hendry et al., 1987). Such selective degradation would give misleading chlorophyll a to phaeophorbide ratios.

4.3.2 Chlorophyll a *in the gut sediments of holothurians*

Concentrations of chlorophyll a in the guts of holothurians differed greatly between species suggesting some species are more capable of utilising fresh organic matter. *Amperima rosea* had a high chlorophyll a concentration in its gut sediment. However, the variability of concentration was high because of the absence of chlorophyll in some samples. The contrast between 1) the absence and 2) high concentrations of chlorophyll a in *A. rosea* gut sediment suggests this species feeds selectively on the freshest material when it can find it. Between 1997-1998 at the PAP, when abundance of *Amperima rosea* was high, the species exhibited a high tracking rate of 110 $cm^2 d^{-1} m^{-2}$, which was 20 times greater than the other holothurians during the same period (Bett et al., 2001). Stable isotope and microscope analysis of *A. rosea* gut contents have shown that it selects fresh material (Iken et al., 2001). Wigham et al. (2003a) and Hudson (2004) reported high chlorophyll a concentrations in the gut sediment of *A. rosea* relative to the other species sampled, but did not note any specimens lacking

chlorophyll *a*. *Amperima rosea* gut sediment chlorophyll *a* concentrations were 45.94 (± 40.41) and 30.85 (± 3.63) µg gDW^{-1} in October 2000 and March 2002, respectively (Wigham et al., 2003a). The gut sediment chlorophyll *a* concentrations were much lower in October 2002 (Hudson, 2004) and June 2004 (present study) with values of 3.45 (± 3.23) and 1.18 (± 2.01) µg gDW^{-1}, respectively. The lower concentrations in more recent samples (and the absence of chlorophyll *a* from some specimens in June 2004) may be a function of the interannual variability in the amount of fresh phytodetritus reaching the seafloor at the PAP (Salter, 2007; Lampitt, 2008). Bathysnap (time-lapse camera) and sediment trap records were incomplete for the years sampled in the times series. However, sediment trap records in 2001 show enhanced OM flux producing an order of magnitude more organic carbon at the sediment surface (Lampitt et al., 2001; Lampitt, 2008).

Peniagone diaphana and *O. mutabilis* also show greater selection for fresh material, (although greater variability is evident in *O. mutabilis*) compared with *Psychropotes longicauda* and *Paroriza prouhoi* in June 2004. These differences probably reflect their feeding modes. *Oneirophanta mutabilis* is a picker, using its digitate tentacles to transfer sediment into its mouth (Roberts et al., 2000). It also has high rate of locomotion for a holothurian (Roberts et al., 2000). *Peniagone diaphana* is a benthopelagic holothurian but feeds at the sediment surface (Billett, 1991) on fresh OM (Iken et al., 2001). *Psychropotes longicauda* also feeds on the sediment surface, but its peltate tentacle structure (sweeping sediment into the mouth) suggests it is less selective (Roberts et al., 2000). This is supported by the fact that the body tissue of *P. longicauda* is relatively enriched in the heavy isotope of nitrogen, ^{15}N, suggesting it feeds on more refractory (less fresh) material (Iken et al., 2001). *Paroriza prouhoi* has similar heavy isotope ^{15}N values to that of *Molpadia blakei* (a known subsurface feeder), which indicates both species feed on the same refractory material (Iken et al., 2001). Direct observations of *Paroriza pallens*, a closely related species found at bathyal depths, have shown it moves very slowly through the sediment (Paul Tyler, pers comm.) feeding on sub-surface sediment fractions (Roberts et al., 2000).

Chlorophyll *a* concentrations in the gut sediments of holothurians sampled in 2005 were similar among species, with the exception of *M. blakei*, an unselective subsurface feeder (Khripounoff and Sibuet, 1980; Roberts et al., 2000). In the present study, no chlorophyll *a* was found in the sediment below 5mm, which explains the absence of chlorophyll *a* from the gut sediment of *M. blakei*.

Oneirophanta mutabilis and *Psychropotes longicauda* have a greater concentration of chlorophyll *a* gut sediments in 2004 than in 2005, which probably reflects fresher and less patchy phytodetritus in 2004. Gut sediment chlorophyll *a* concentrations in *O. mutabilis* were not significantly different to *P. longicauda* in 2005, suggesting they had similar encounter rates with fresh material, despite the greater selectivity for fresher material by *O. mutabilis*. The concentrations of chlorophyll *a* in the gut sediments of *O. mutabilis* have been shown to correlate with those in the top 1mm of sediment at PAP (Witbaard et al., 2001). The present study also supports the suggestion of Neto et al. (2006) that *O. mutabilis* feeds on the same material as *P. longicauda* when fresh organic matter is scarce. Concentrations of chlorophyll *a* in the gut sediments of *Paroriza prouhoi* were not significantly different between years, suggesting it is not a selective forager or feeder on fresh OM, a conclusion consistent with previous studies (Khripounoff and Sibuet, 1980; Billett et al., 1988). Species of the genus *Paroriza* leave 'plough tracks' in their wake, suggesting that they move through, and ingest the top few millimetres of sediment (Tyler et al., 1992b). Chlorophyll *a* concentrations in the top 5mm sediment were only slightly higher in 2004 than in 2005 (0.029 µg gDW^{-1} ± 0.010 and 0.005 µg gDW^{-1} ± 0.005 respectively), but showed high variability; this may have contributed to the similar between-year *P. prouhoi* gut sediment chlorophyll *a* concentrations. *Pseudostichopus aemulatus* has a slightly higher, but variable gut sediment chlorophyll *a* concentration to that of its congener *P. villosus* and other holothurian species sampled in 2005, although this is not statistically significant because of the variability of the samples. *Pseudostichopus villosus* 'ploughs' slowly through the sediment, probably ingesting sediments from 1-2cm depth (Billett, 1991; Moore and Roberts, 1994). Therefore, *P. villosus* will only be able to exploit the deeper carotenoid-depleted sediment (Fig. 4.1 b). *Pseudostichopus aemulatus*, however, is smaller than *P. villosus* and has a different foraging behaviour, feeding on the

superficial sediment rich in OM (Billett, 1988). The relatively low concentrations of chlorophyll *a* in the phytodetritus and sediment, would had led to a lower encounter rate of holothurians with fresh material in 2005.

4.3.3 Carotenoids in holothurian gut walls and ovaries – June 2004

Canthaxanthin and echinenone were present in the gut wall and ovaries of the holothurians studied, but were absent from the phytodetritus and sediment. This suggests that the holothurians have metabolised these carotenoids from those obtained in their diet, as has been shown in shallow-water echinoderms (Tsushima et al., 1993b).

Tight species-specific clustering of *A. rosea* gut wall and ovary samples in the 2004 MDS ordination plot from June 2004 reflects the similarity of the carotenoid profiles between samples. This suggests selectivity and/or requirement for specific carotenoids – particularly zeaxanthin which constitutes >28% of the total tissue pigment. No other holothurian has such a high percentage contribution of zeaxanthin in gut wall and ovary tissues. *Amperima rosea* also had very high carotenoid concentrations in comparison with the other species. To obtain the highest dietary concentration of carotenoids it would be beneficial to feed on the freshest organic matter − as supported by the high chlorophyll *a* concentration (indicating selectivity of fresh phytodetrital material) found in some *A. rosea* gut sediment samples. *Oneirophanta mutabilis* and *Peniagone diaphana* also had high carotenoid concentrations in their gut wall and ovaries in 2004, although the carotenoid profiles are less consistent than in *A. rosea*. The contribution of only one or two carotenoids, (β-carotene in *O. mutabilis*, β-carotene and echinenone in *P. diaphana*) caused the differences between the gut wall and ovary samples. *Psychropotes longicauda* and *Paroriza prouhoi* show less species specific clustering in the gut wall in 2004 because of inconsistent carotenoid profiles between samples. A change in the relative contributions of more than one carotenoid accounted for the spread of samples on the MDS ordination plot. This suggests *Psychropotes longicauda* and *Paroriza prouhoi* are not selective in their pigment biochemistry.

4.3.4 Carotenoids in holothurian gut walls and ovaries – July 2005

The occurrence of 19'-butanoyloxyfucoxanthin and 19'-hexanoyloxyfucoxanthin in the carotenoid profiles of the gut wall and ovaries of the abyssal holothurians sampled in 2005 contrasts with the findings of Hudson et al. (2003), who reported that 19'-hexanoyloxyfucoxanthin was absent in the ovaries of bathyal holothurians (*Laetmogone violacea, Paroriza pallens, Benthogone rosea Bathyplotes natans* and *Benthothuria funebris*), despite the dominance of this carotenoid in the gut sediments. It should be noted that two of the six species sampled in the present study in 2005 (*Molpadia blakei* and *Pseudostichopus aemulatus*) did not contain any 19'-hexanoyloxyfucoxanthin in their ovaries. Hudson et al. (2003) may have sampled species that either do not assimilate this carotenoid or biosynthesise it immediately to another carotenoid/compound. The relative contributions of 19'-butanoyloxyfucoxanthin and 19'-hexanoyloxyfucoxanthin differ greatly among species. Concentrations of both are low in *O. mutabilis*. The occurrence and relative contribution of these carotenoids in the other holothurian species, drive the spread of samples in the MDS plot (Figure 6 a & b), suggesting they do not discriminate between carotenoids in the same way when assimilating these organic compounds.

Molpadia blakei feeds on refractory material as seen by the absence of chlorophyll *a* in its gut and having body tissue relatively enriched in the ^{15}N (Iken et al., 2001). Nevertheless, it has carotenoids in its gut wall and ovaries at concentrations higher or equal to those of species that feed on less refractory material - *Psychropotes longicauda, Paroriza prouhoi* and *Pseudostichopus aemulatus*. *Molpadia* spp. buries itself vertically in the sediment, with the anus extending above the sediment-water interface. The ingestion of sediment at the mouth and subsequent voiding of faeces at the surface, leads to depressions surrounding the mound of faecal material around the anus (Rhoads and Young, 1971). This mode of feeding may be a way of bringing carotenoids from the sediment surface to the mouth of *Molpadia*. Canthaxanthin was a dominant carotenoid in the gut wall and ovaries of the specimens, it is likely that *M. blakei* metabolised this carotenoid from carotenoids obtained in its diet, as observed in shallow-water

echinoderms (Tsushima et al., 1993b). This bioconversion of carotenoids increases their antioxidant properties (Foote et al., 1970; Lee and Min, 1990). The lack of chlorophyll *a* in the gut sediment of this species suggests that the material it ingests is not fresh and that the species is efficient at carotenoid assimilation and/or accumulates carotenoids over long time-scales.

4.3.5 Ovarian carotenoid concentration – a reproductive adaptation?

Large differences in the concentrations of carotenoids in the ovaries of the different species may be attributed to their reproductive adaptations. Survival of post-larvae is an important factor in response to the seasonal flux of phytodetritus, contributing to population structure and density (Wigham et al., 2003b). *Amperima rosea* has the highest carotenoid ovarian concentrations of all the species sampled. Wigham et al. (2003a) also found *A. rosea* had high carotenoid concentrations in its ovaries in comparison to *O. mutabilis*, *Psychropotes longicauda*, *Pseudostichopus villosus* and *Paroriza prouhoi*. Experiments on shallow water echinoderms have shown that the larvae of adults fed on a carotenoid-rich diet were larger throughout development, developed faster and had higher survival rates (Tsushima et al., 1997; George et al., 2001; George and Lawrence, 2002). Assimilating a high carotenoid load into its ovaries may give *A. rosea* an additional reproductive advantage. Carotenoids reduce the harmful effects of reactive oxygen species given off during the rapid metabolism of lipids in the egg, increasing larval survival (Blount et al., 2000; 2004; Lotocka et al., 2004). *Amperima rosea* reaches maturity at a small size and has a high fecundity (Wigham et al., 2003b). Therefore, *A. rosea* may produce many viable offspring during favourable conditions, leaving a large cohort either to exploit the remaining favourable OM, or to wait until the next favourable conditions occur.

Concentrations of pigments in the ovaries of *O. mutabilis* and *Peniagone diaphana*, although not as high as *A. rosea*, were still an order of magnitude greater than in *Psychropotes longicauda*, *Pseudostichopus* spp. and *Paroriza prouhoi*. Enhanced carotenoid concentrations could be one reproductive adaptation to increase the number

of offspring into the next generation. Their ability to exploit the richer sources of OM means that they have access to greater concentrations and types of carotenoids, which are then concentrated into their ovaries. The reproductive adaptations of species with low ovarian carotenoid pigment concentrations may increase the number of offspring surviving to adulthood in other ways. *Psychropotes longicauda* only produce a few eggs at a time, but these undergo direct development in the pelagic realm, where predation is thought to be less (Tyler and Billett, 1987). *Paroriza prouhoi* is hermaphroditic and exhibits pairing behaviour, increasing the likelihood of external fertilisation (Tyler et al., 1992b). *Pseudostichopus aemulatus* has small eggs and a high fecundity ranging from tens of thousands to a hundred thousand eggs per individual, suggesting it is opportunistic in its reproduction (Watson, 2004). *Pseudostichopus aemulatus* feeds on the superficial sediment rich in OM (Billett et al., 1988) and is able to convert the seasonal flux of OM arriving at the seafloor into reproductive growth rapidly. This is demonstrated in possible intra-annual cycles of reproduction – larger oocytes appear to be present after the seasonal flux of OM to the seafloor (Watson, 2004).

There appears to be no clear relationship between the developmental mode of the eggs and larvae and the concentration of carotenoids in the ovaries. *Amperima rosea* and *P. aemulatus* are both thought to be opportunistic, with small egg sizes (~210µm) and high fecundity (Wigham et al., 2003b; Watson, 2004). *Amperima rosea* has ovarian carotenoid concentrations (µg gDW^{-1}) two orders of magnitude greater than *P. aemulatus*. This is not surprising as *A. rosea* feeds selectively on the freshest OM, giving it access to the greatest range and concentration of carotenoids. *Pseudostichopus aemulatus* does not appear to discriminate between carotenoids when assimilating organic compounds allowing it to take advantage of a flux of OM regardless of its quality, whereas *A. rosea* is constrained in the carotenoids it requires – specifically zeaxanthin. Therefore, *A. rosea* can only take advantage of fluxes of OM when the quality of the material is favourable to its needs. *O. mutabilis* and *Peniagone diaphana* have relatively high ovarian carotenoid concentrations but have differing reproductive and developmental adaptations. *Oneirophanta mutabilis* has larger eggs (950µm), spawning all large oocytes at once, with direct development on the seabed or immediately above it (Tyler and Billett, 1987; Ramirez-Llodra et al., 2005). *Peniagone*

diaphana has a smaller egg size (300µm) a fecundity of 5000 and lecithotrophic larvae. This species is sexually mature at a small size with rapid growth up to maturity (Tyler et al., 1985). The reproductive adaptation of elevated concentrations of carotenoids in the ovaries is most likely related to the feeding guild of the species.

4.3.6 Carotenoid supply, feeding guild and selective assimilation

Between year comparisons show *Psychropotes longicauda* and *Paroriza prouhoi* appear not to discriminate between carotenoids during feeding and assimilation into body tissues. 19'-butanoyloxyfucoxanthin and 19'-hexanoyloxyfucoxanthin were absent from the sediment and their gut wall and ovarian tissue in June 2004, but were present in sediment and tissue samples in July 2005. The feeding guild of these species may dictate this; selectivity for specific carotenoids does not occur, as the compounds in the sediment fractions on which they feed are not abundant and are temporally variable. In contrast, *O. mutabilis* shows greater temporal consistency in its gut wall and ovary carotenoid biochemical profiles. The pigment profiles in the gut wall and ovaries show some similarity, although there is a degree of similarity between samples within each year. β-carotene contributes a large proportion of the gut wall and ovarian pigment biochemistry of *O. mutabilis*. The ability of *O. mutabilis* to exploit fresher OM, containing greater concentrations and types of carotenoids, allows for compound specific selectivity. This inference is also supported by the consistent biochemical profile of *A. rosea*, which feeds on fresh OM when it can, and the inconsistent profiles of *Pseudostichopus aemulatus* and *P. villosus*, which feed on deeper sediments and presumably assimilate carotenoids with less selectivity from deeper sediments. The consistency of the carotenoid profiles in the tissue samples of *Amperima rosea* is not related to all the specimens from June 2004 having similar numbers of vitellogenic oocytes; a study of the reproductive biology of *Amperima rosea* found there was no synchrony in oogenesis between samples taken over ten sample periods between 1989 and 1996 (Wigham et al., 2003b).

Wigham et al. (2003a) suggested that the supply of certain carotenoids may favour particular species. This was based on the occurrence of specific carotenoids in both the gut sediments and ovaries, which would infer selective feeding, e.g. zeaxanthin in *A. rosea*. Although the present study shows that zeaxanthin in *A. rosea* gut sediment may arise from the lysis of the gut wall (Chapter 3), the hypothesis of Wigham et al. (2003a) still stands because of the consistent biochemical profile in *A. rosea* gut wall and ovary tissue and its requirement for zeaxanthin in large concentrations. In addition, the enhanced supply or availability of β-carotene may favour *O. mutabilis*; β-carotene is consistently the dominant carotenoid in *O. mutabilis* ovarian tissue.

Significant temporal intraspecies differences in the gut wall pigment concentrations of *Psychropotes longicauda* may reflect the lower quantity and composition of OM in 2005. Carotenoids in the ovaries of *O. mutabilis* and *P. longicauda* were greater in concentration in 2004, but were too variable to be significant. Studies of shallow water echinoderms have shown that increased supply of carotenoids enhances the colour of the roe indicating carotenoid concentrations in the ovaries are increased. (George and Young, 1998; George et al., 2001; Mclaughlin and Kelly, 2001; George and Lawrence, 2002; Robinson et al., 2002). An increase in carotenoid concentration increases fecundity, larval maturation and survival (George and Young, 1998; George et al., 2001; Mclaughlin and Kelly, 2001; George and Lawrence, 2002).

Other controlling variables may affect the ovarian biochemistry of abyssal holothurians. As previously discussed, between-species competition for resources during times of limited supply may detrimentally affect species that are less selective and/or efficient at assimilating carotenoids. The ability of abyssal holothurians to modify dietary derived carotenoids to carotenoids other than echinenone, canthaxanthin and astaxanthin is not presently known. Metabolic pathways from β-carotene to echinenone and canthaxanthin have been demonstrated in shallow water echinoderms (Tsushima et al., 1993b; Tsushima et al., 1995; Plank et al., 2002). The occurrence of 19'-butanoyloxyfucoxanthin and 19'-hexanoyloxyfucoxanthin (found in the phytodetritus and sediment in 2005 but not 2004) in the gut wall and ovaries of holothurians in 2005, but not 2004, suggests these holothurians do assimilate carotenoids directly from their

diet into their ovaries. Further temporal studies of intraspecies pigment biochemistry response to changing food supplies are needed. This may be achieved by comparing two contrasting sites with differing food supply (see Chapter 5), or by experimental methods.

4.4 Conclusions

The composition and amount of the OM reaching the PAP varies both temporally and spatially. This can affect the diet of some abyssal holothurian species, depending on their feeding guild. *Amperima rosea*, *Peniagone diaphana* and *Oneirophanta mutabilis* selectively feed on high quality OM. They can exploit the freshest OM when it is available. When fresh OM is scarce *O. mutabilis* feeds on the same sediment as other large species (*Psychropotes longicauda*, *Paroriza prouhoi*, *Pseudostichopus aemulatus* and *P. villosus*) that are less selective in their diet. This response has previously been reported in a study of holothurian and sediment lipid biochemistry at the PAP (Neto et al., 2006).

The ovarian carotenoid biochemistry of the abyssal holothurians sampled at the PAP is a complex function of the feeding guild and reproductive adaptation of each species. *Amperima rosea*, *Peniagone diaphana* and *O. mutabilis* display consistent ovarian carotenoid profiles and have higher concentrations of carotenoids in their gut wall and ovaries than do other species. Favourable conditions may give these species a reproductive advantage, supplying specific carotenoids required for their reproduction. Enhanced carotenoid concentrations in the ovaries of some species may be a reproductive adaptation to increase larval survival. *Psychropotes longicauda, Paroriza prouhoi, Pseudostichopus aemulatus*, *P. villosus* and *M. blakei* feed on poor (in terms of concentration and number of carotenoids) OM and do not appear to discriminate between carotenoids when assimilating organic compounds. They also have low concentrations of carotenoids in their gut wall and ovaries. Their reproductive adaptations may increase cohort survival in other ways. Temporal intraspecies variation in gut wall and ovary carotenoid concentration reflects the quantity of carotenoids in the OM reaching the seafloor. Reduced supply may result in lower fecundity and larval survival.

The results from this PAP study suggests that diet and ovarian biochemistry in deep-sea holothurians are intimately linked. If shallow water echinoderms studies are true for deep-sea echinoderms, this study suggests that changes in the quantity and composition

of the diet will affect abyssal holothurian reproductive output and larval survival and maturation. Changes in upper ocean biogeochemistry, altering the quality and quantity of organic matter reaching the deep-sea floor may control holothurian reproductive output and favour certain species, which can have a subsequent effect on the surrounding biota, as seen during the '*Amperima* Event' at the PAP.

Chapter 5 – The link between diet and abyssal holothurian ovarian biochemistry; a spatial study at two contrasting sites around the Crozet Islands, Southern Ocean

5.1 Introduction

Changes in upper ocean productivity have been proposed as important drivers for variation in the biodiversity of deep-sea sediments (Levin et al., 2001; Lambshead et al., 2002; Snelgrove and Smith, 2002; Johnson et al., 2007). Fluctuations in the abundance and community structure of abyssal megafauna have been observed in both the deep northeast Atlantic and northeast Pacific since 1989 (Billett et al., 2001; Ruhl and Smith, 2004). Community changes in the northeast Pacific have been ascribed to carbon flux regulated by climatic forcing by El Niňo/La Niňa (Ruhl and Smith, 2004). This relationship is not observed in the northeast Atlantic – carbon flux data over a long time-series period (1989-2001) shows no direct relationship with the North Atlantic Oscillation Index (Lampitt, pers. comm). It is postulated the recent dramatic changes in species dominance at the Porcupine Abyssal Plain (PAP) (Billett et al., 2001) are related to the composition of OM arriving at the seafloor (Billett and Rice, 2001; Wigham et al., 2003a).

The Benthic Crozet programme was set up with the aim "To assess how biogeochemical composition and flux of OM to the deep-sea floor drives benthic community structure, dynamics and diversity at two sites with contrasting primary productivity regimes" (Wolff, 2006). The benthic site to the east of the Islands (M5) underlies waters with high surface primary productivity and is thought to receive a greater flux of material to the sea floor. The benthic site to the south (M6) underlies high nutrient, low chlorophyll (HNLC) waters. The sites are only 460km apart with no topographical boundary to separate them; therefore differences in the benthic community can be ascribed to the composition and amount of OM reaching the sea-floor at each site (general introduction to the two sites can be found in Chapter 2).

The aim of the present Crozet study is to examine how spatial differences in the composition and quantity of OM may influence the diet and ovarian biochemistry of abyssal holothurians. This differs from the PAP study, which was a temporal comparison of the influence of the composition and quantity of OM on the diet and ovarian biochemistry of abyssal holothurians.

Specific hypotheses to be tested are:

1. The variability in phytopigment composition, *as well as* the total flux of OM reaching the abyssal seafloor, is dependent on the productivity of overlying surface waters.
2. The composition and quantity of carotenoids associated with the OM supply is reflected in the biochemistry of abyssal holothurians

The Crozet study was approached by sampling sediment (in two consecutive years) holothurians and particulate organic matter (POM) (only in the second year) from two adjacent sites around the Crozet Islands (See Chapter 2 for methods). Holothurian gut contents were analysed to quantify chlorophyll *a* (to indicate selective feeding), and gut wall tissue and ovaries were analysed for their pigment biochemistry. These data were compared with the phytopigments in the POM and sediments at each site. Between site differences in the composition and concentration of pigments in the POM and sediment are discussed and are compared to the feeding selectivity and reproductive requirements for carotenoids by the holothurians at each site. Interspecies comparisons in feeding selectivity and pigment biochemistry are made within sites, as well as intraspecies comparisons between sites.

5.2 Results

5.2.1 Phytopigments in the POM of surface and deep waters

POM (particulate organic matter) was sampled using a stand alone pump system in 2005 only, beneath the chlorophyll maximum layer (80m; M5, 55m; M6) and above the seafloor (4241m; M5, 4200m; M6). POM below the thermal mixed layer (chlorophyll *a* maximum) had similar phytopigment concentrations and composition at sites M5 and M6 (Fig. 1 a). Three pigments showed between-site differences in concentration and percentage contribution – fucoxanthin, 19'-hexanoylofucoxanthin and violaxanthin. Violaxanthin was found in the POM at M6, but not M5. Fucoxanthin was found in greater concentrations at M6. The carotenoid 19'-hexanoylofucoxanthin was found in greater, but variable concentration at M5 and its percentage contribution to the total identified pigment load was >60% (Fig. 1 a; Table 5.1). The freshness of the POM, as indicated by the chlorophyll *a* to phaeophorbide ratio (Thiel et al., 1989), was greater at M5 (15.4 ± 3.2) than at M6 (7.3 ±2.0).

The difference in composition and concentrations of phytopigments in the single POM samples collected close to the sea floor by the deep-water SAPS above the sea bed at each site is large (Fig. 1 b). Only four pigments (fucoxanthin, 19'-hexanoylofucoxanthin, alloxanthin and phaeophorbide) were found at M6, all in low concentrations. 19'-butanoyloxyfucoxanthin contributed 50% to the total identified pigment load at M5. The chlorophyll *a* to phaeophorbide ratio of the POM above the seabed at M5 was 0.81; this ratio was not calculated for the M6 sample because of the absence of chlorophyll *a*.
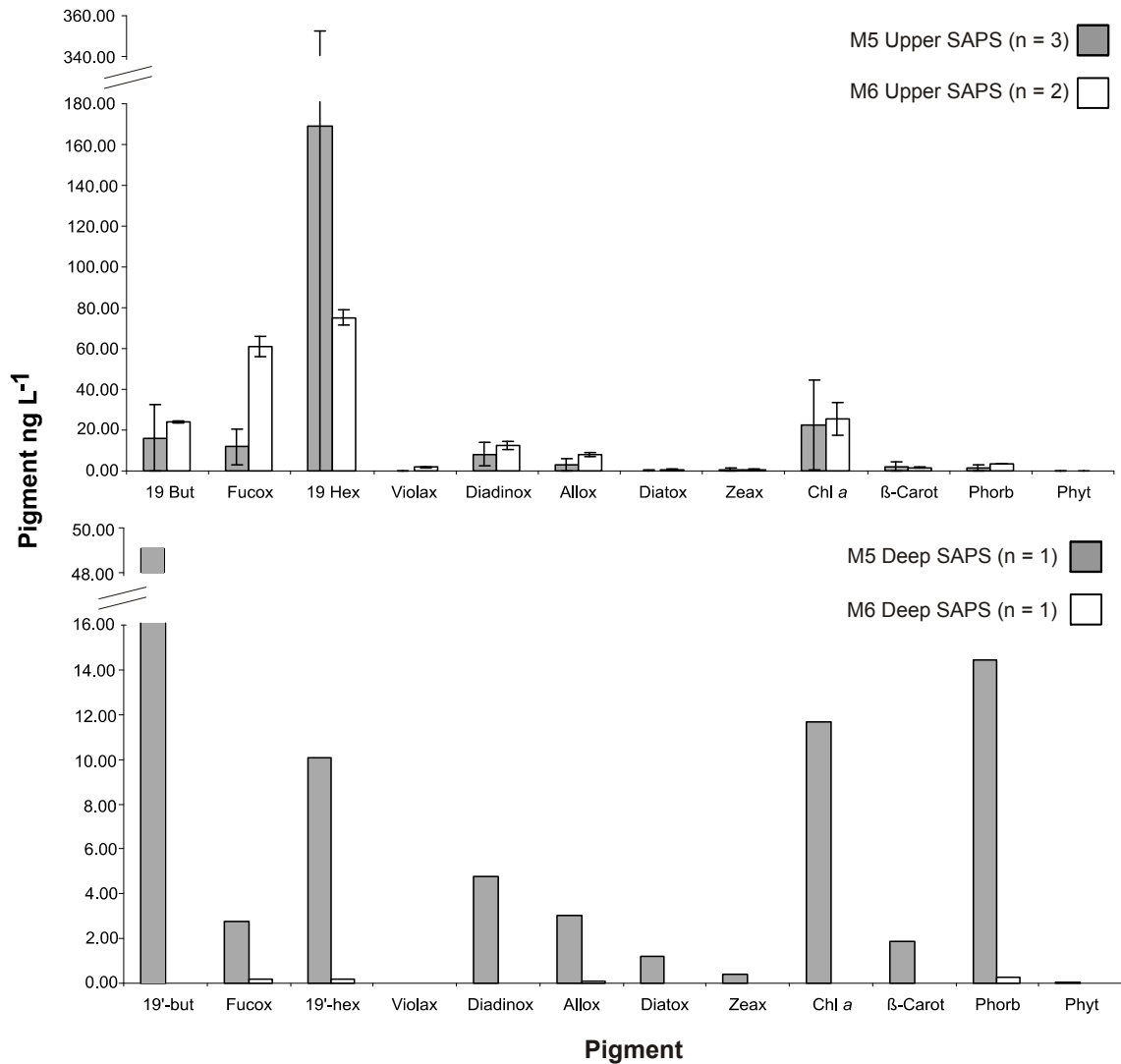
Figure 5.1 Phytopigments (ng L⁻¹) found in the POM in the upper water column (a) and above the seafloor (b) at M5 and M6. Fucox = fucoxanthin; 19'-but = 19'-butanoyloxyfucoxanthin; 19'-hex = 19'-hexanoyloxyfucoxanthin; violax = violaxanthin;   Diad = diadinoxanthin; Allox = alloxanthin; Diatox = diatoxanthin; Zeax = zeaxanthin; Chl a = chlorophyll *a*; β-carot = β-carotene; Phorb = phaeophorbide; Phytin = phaeophytin. (note break in y-axis)

	M5 Upper SAPS (n=3)	M5 Upper SAPS % of total	M6 Upper SAPS (n=2)	M6 Upper SAPS % of total	M5 deep SAPS (n=1)	M5 deep SAPS % of total	M6 deep SAPS (n=1)	M6 deep SAPS % of total
19'-but	16.1	6.1%	24.13	11.3%	49.08	49.4%	0	
	16.3	*1.9%*	*0.42*	*0.9%*				
Fucox	11.8	10.5%	61.07	28.4%	2.77	2.8%	0.19	26.3%
	8.63	*8.3%*	*5.07*	*0.3%*				
19'-hex	169.1	66.1%	75.12	35.0%	10.07	10.1%	0.18	25.8%
	181.32	*8.9%*	*3.69*	*1.6%*				
Violax	0		1.96	0.9%	0		0	
			0.29	*0.2%*				
Diadinox	8.11	4.6%	12.58	5.8%	4.78	4.8%	0	
	5.71	*1.8%*	*1.88*	*0.3%*				
Allox	2.94	*1.2%*	7.98	3.7%	3.03	*3.1%*	0.08	*11.2%*
	3.03	*0.1%*	*1.06*	*0.1%*				
Diatox	0.23	0.1%	0.68	0.3%	1.22	1.2%	0	
	0.18	*0.0%*	*0.27*	*0.1%*				
Zeax	0.65	0.3%	0.66	0.3%	0.38	0.4%	0	
	0.85	*0.1%*	*0.14*	*0.1%*				
Chl *a*	22.52	9.7%	25.6	11.8%	11.69	11.8%	0	
	21.78	*0.2%*	*8.03*	*2.6%*				
ß-carot	2.13	0.9%	1.63	0.8%	1.86	1.9%	0	
	2.18	*0.1%*	*0.14*	*0.0%*				
Phorbide	1.53	0.7%	3.5	1.6%	14.46	14.6%	0.26	36.7%
	1.36	*0.2%*	*0.16*	*0.1%*				
Phytin	0.08	0.0%	0.06	0.0%	0.03	0.0%	0	
	0.08	*0.0%*	*0.01*	*0.0%*				

Table 5.1 Pigment concentrations (ng L^{-1}) and average percentage contribution to the total in the POM sampled by the Stand Alone Pump System in the upper water column and above the seabed at M5 and M6, sampled during cruise 2 – Dec 2005-Jan 2006. Fucox = fucoxanthin; 19'-but = 19'-butanoyloxyfucoxanthin; 19'-hex = 19'-hexanoyloxyfucoxanthin; violax = violaxanthin; Diad = diadinoxanthin; Allox = alloxanthin; Diatox = diatoxanthin; Zeax = zeaxanthin; Chl a = chlorophyll a; β-carot = β-carotene; Phorb = phaeophorbide; Phytin = phaeophytin. (Standard deviation in italics)

5.2.2 Phytopigments in the phytodetritus and sediment

No phytodetritus was observed on the sediment core surfaces from either sampling period (i.e. cruise 1 or cruise 2). Eight pigments were identified in the top 5mm of sediment during cruise 1 (Dec 2004 – Jan 2005) and twelve in the top 5 mm and 5 to 10mm sediment during cruise 2 (Dec 2005 – Jan 2006) at both stations, M5 and M6 (Fig. 5.2; Tables 5.2 and 5.3). Diatoxanthin, 19'-butanoylofucoxanthin, β-carotene and

violaxanthin were found in the sediments at M5 and M6 sampled during cruise 2, but not cruise 1. These pigments were found in concentrations of less than 0.013µg gDW^{-1}, apart from 19'-butanoylofucoxanthin which had a mean concentration of 0.07 ± 0.015 µg gDW^{-1} at M5 and 0.05 ± 0.029µg gDW^{-1} at M6 (Fig. 5.2).

Chlorophyll *a* was one of the most abundant pigments in the sediment at M5 during both cruises (19% cruise 1; 41% cruise 2) (Tables 5.2 and 5.3). Zeaxanthin (17%), alloxanthin (15%) and phaeophorbide (19%) were also abundant in the top 5mm sediments at M5 during cruise 1, while diadinoxanthin (11%), 19'-butanoylofucoxanthin (10%), phaeophorbide (11%) and phaeophytin (10%) were abundant pigments in the top 5mm of sediment from M5 during cruise 2 (Tables 5.2 and 5.3). Zeaxanthin (23%), alloxanthin (15%) and phaeophytin (21%) were the most abundant pigments in M6 sediments during cruise 1, while chlorophyll *a* (19%) and phaeophytin (28%) were most abundant in the top 5mm of M6 sediments during cruise 2 (Tables 5.2 and 5.3).

Six phytopigments (fucoxanthin, 19'-hexanoylofucoxanthin, diadinoxanthin, alloxanthin, zeaxanthin, and chlorophyll *a*) had higher concentrations at M5 than at M6, in sediments collected during cruise 1 (Fig. 5.1a); diadinoxanthin and phaeophytin were present in similar concentrations. The concentration of chlorophyll *a* was three times greater at M5 than at M6 in the top 5mm sediment sampled during cruise 1 (Fig. 5.2a). Chlorophyll *a* was similar in concentration between cruise 1 and 2 (Dec 2004 – Jan 2005 and Dec 2005 – Jan 2006) in the top 5mm sediment at M5 (0.25 µg gDW^{-1}) and M6 (0.8 µg gDW^{-1}; Fig 5.2 a and b).

During cruise 2, only three pigments (chlorophyll *a*, diadinoxanthin and phaeophorbide) had greater concentrations at M5 than at M6 in the top 5mm sediment. Fucoxanthin, alloxanthin and phaeophytin were more abundant at M6 than at M5 (Fig 5.2b). All other pigments (19'-butanoylofucoxanthin, 19'-hexanoylofucoxanthin, violaxanthin, diatoxanthin, zeaxanthin and β-carotene) had similar concentrations at the two sites (Fig. 5.2b). In the 5 to 10mm sediment sections sampled during cruise 2, 19'-butanoylofucoxanthin, diadinoxanthin and chlorophyll *a* had greater concentrations at

M5, phaeophytin had a greater concentration at M6 and the other pigments were similar in concentration.
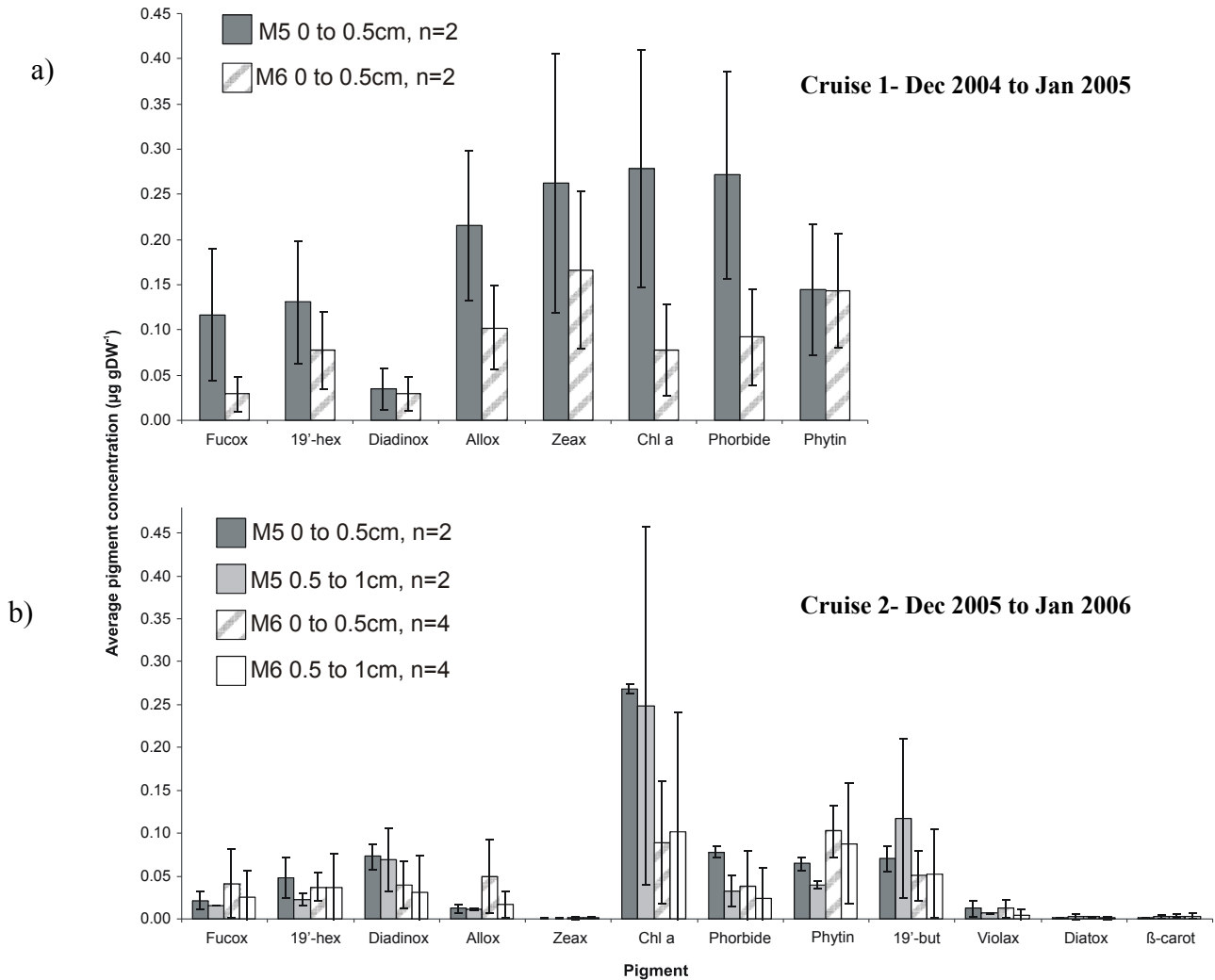
Figure 5.2 Phytopigments found in the 0 to 0.5cm and 0.5 to 1cm of sediment from (a) Cruise 1, December 2004 – January 2005 (b) Cruise 2 December 2005 – January 2006 (mean µg gDW^{-1} ± SD). Fucox = fucoxanthin; 19'-but = 19'-butanoyloxyfucoxanthin; 19'-hex = 19'-hexanoyloxyfucoxanthin; Diad = diadinoxanthin; Allox = alloxanthin; Zeax = zeaxanthin; Chl a = chlorophyll *a*; Phorbide = phaeophorbide; Phytin = phaeophytin; violax = violaxanthin; Diatox = diatoxanthin; β-carot = β-carotene.

	M5 0 to 0.5cm (n=2)	M5 0 to 0.5cm % of total	M6 0 to 0.5cm (n=2)	M6 0 to 0.5cm % of total
Fucoxanthin	0.12	7.6%	0.03	3.4%
	0.07	*2.0%*	*0.02*	*2.4%*
19'-hexanoyloxyfucoxanthin	0.13	8.7%	0.08	10.3%
	0.07	*1.5%*	*0.04*	*1.7%*
Diadinoxanthin	0.04	2.3%	0.03	3.8%
	0.02	*0.6%*	*0.02*	*1.2%*
Alloxanthin	0.22	*15.1%*	0.1	*14.8%*
	0.08	*0.8%*	*0.05*	*1.7%*
Zeaxanthin	0.26	17.4%	0.17	22.9%
	0.14	*2.1%*	*0.09*	*1.0%*
Chlorophyll *a*	0.28	19.1%	0.08	11.8%
	0.13	*1.7%*	*0.05*	*5.1%*
Phaeophorbide	0.27	18.8%	0.09	12.3%
	0.11	*3.4%*	*0.05*	*2.0%*
Phaeophytin	0.14	11.0%	0.14	20.9%
	0.07	*6.3%*	*0.06*	*2.9%*

Table 5.2 Pigment concentrations (μg gDW^{-1}) and average percentage contribution to the total in the sediment sampled during cruise 1 – Dec 2004-Jan 2005. (Standard deviation in italics)

	M5 0 to 0.5cm (n=2)	M5 0 to 0.5cm % of total	M5 0.5 to 1cm (n=2)	M5 0.5 to 1cm % of total	M6 0 to 0.5cm (n=4)	M6 0 to 0.5cm % of total	M6 0.5 to 1cm (n=4)	M6 0.5 to 1cm % of total
Fucox	0.022	3.3%	0.016	3.3%	0.041	7.5%	0.026	5.8%
	0.01	*1.3%*	*0.001*	*1.9%*	*0.04*	*3.5%*	*0.03*	*3.5%*
19'-hex	0.048	7.2%	0.023	5.3%	0.037	8.5%	0.036	8.7%
	0.024	*3.1%*	*0.006*	*4.4%*	*0.016*	*1.3%*	*0.04*	*3.4%*
Diadinox	0.073	11.2%	0.069	12.6%	0.04	8.2%	0.032	6.5%
	0.014	*1.2%*	*0.036*	*1.3%*	*0.027*	*1.7%*	*0.041*	*3.4%*
Allox	0.012	*1.8%*	0.011	2.5%	0.05	9.7%	0.017	5.2%
	0.005	*0.6%*	*0.002*	*1.8%*	*0.043*	*6.7%*	*0.016*	*2.3%*
Zeax	0.001	0.1%	0.001	0.1%	0.001	0.1%	0.001	0.3%
	0.001	*0.1%*	*0.001*	*0.1%*	*0.002*	*0.2%*	*0.002*	*0.3%*
Chl *a*	0.268	41.5%	0.249	40.0%	0.09	17.8%	0.102	19.4%
	0.006	*4.5%*	*0.209*	*12.5%*	*0.072*	*6.8%*	*0.14*	*12.5%*
Phorbide	0.078	12.0%	0.033	5.9%	0.038	6.8%	0.024	4.8%
	0.006	*0.1%*	*0.018*	*0.4%*	*0.041*	*4.9%*	*0.035*	*2.2%*
Phytin	0.064	9.9%	0.039	8.8%	0.103	26.5%	0.088	28.2%
	0.008	*0.4%*	*0.004*	*6.2%*	*0.03*	*14.9%*	*0.069*	*13.3%*
19'-but	0.07	10.9%	0.117	19.1%	0.05	11.4%	0.053	19.5%
	0.015	*3.3%*	*0.093*	*4.7%*	*0.029*	*5.5%*	*0.051*	*16.7%*
Violax	0.012	1.8%	0.007	1.6%	0.001	2.4%	0.004	0.7%
	0.009	*1.2%*	*0.001*	*1.1%*	*0.012*	*2.0%*	*0.007*	*0.8%*
Diatox	0.001	0.1%	0.002	0.3%	0.003	0.5%	0.001	0.2%
	0.001	*0.1%*	*0.003*	*0.4%*	*0.002*	*0.2%*	*0.002*	*0.2%*
ß-carot	0.001	0.2%	0.002	0.6%	0.002	0.7%	0.003	0.9%
	0	*0.1%*	*0.002*	*0.6%*	*0.003*	*0.6%*	*0.004*	*0.6%*

Table 5.3 Pigment concentrations (µg gDW⁻¹) and average percentage contribution to the total in the sediment sampled during cruise 2 – Dec 2005-Jan 2006. Fucox = fucoxanthin; 19'-but = 19'-butanoyloxyfucoxanthin; 19'-hex = 19'-hexanoyloxyfucoxanthin; violax = violaxanthin; Diad = diadinoxanthin; Allox = alloxanthin; Diatox = diatoxanthin; Zeax = zeaxanthin; Chl a = chlorophyll *a*; β-carot = β-carotene; Phorb = phaeophorbide; Phytin = phaeophytin. (Standard deviation in italics)

5.2.3 Chlorophyll a *in holothurian gut sediment*

All species sampled at M5 had chlorophyll *a* gut sediment concentrations greater than that of the surrounding top 5mm sediment (0.27 µg gDW⁻¹) (Figs. 5.2b and 5.3). *Abyssocucumis abyssorum* had an average gut sediment chlorophyll *a* concentration of 98.11 ± 20.03 µg gDW⁻¹, almost an order of magnitude higher than the other species sampled (Table 5.4; Fig. 5.3). Of the holothurians sampled at M6, only *Peniagone* spp.

and *A. abyssorum* had gut sediment chlorophyll *a* concentrations (1.40 and 3.72 μg gDW^{-1}, respectively) higher than that of the surrounding top 5mm sediment (0.08 μg gDW^{-1}), whereas *Benthodytes* sp. and *Psychropotes longicauda* did not (Figs. 2b and 3). Chlorophyll *a* was absent in the gut sediment of *Molpadia blakei* (Table 5.4).

Species sampled at both sites (*Benthodytes* sp., *Peniagone* spp., *Psychropotes longicauda* and *A. abyssorum*) had greatest average gut sediment chlorophyll *a* concentrations at M5. *Psychropotes longicauda* (U(5,4) = 0.066, P>0.05), *Peniagone* spp. (t(7) = 1.66, P>0.05) and *Benthodytes* sp. (U(2,2,) = 7, P>0.05) gut sediment chlorophyll *a* concentrations were not found to differ significantly between sites, probably because of a small sample size (*Benthodytes* sp.) and high variability (*Peniagone* spp., *Psychropotes longicauda*). *Abyssocucumis abyssorum* gut sediment chlorophyll *a* concentration was significantly different between sites (t(4) = 6.65, P<0.05).
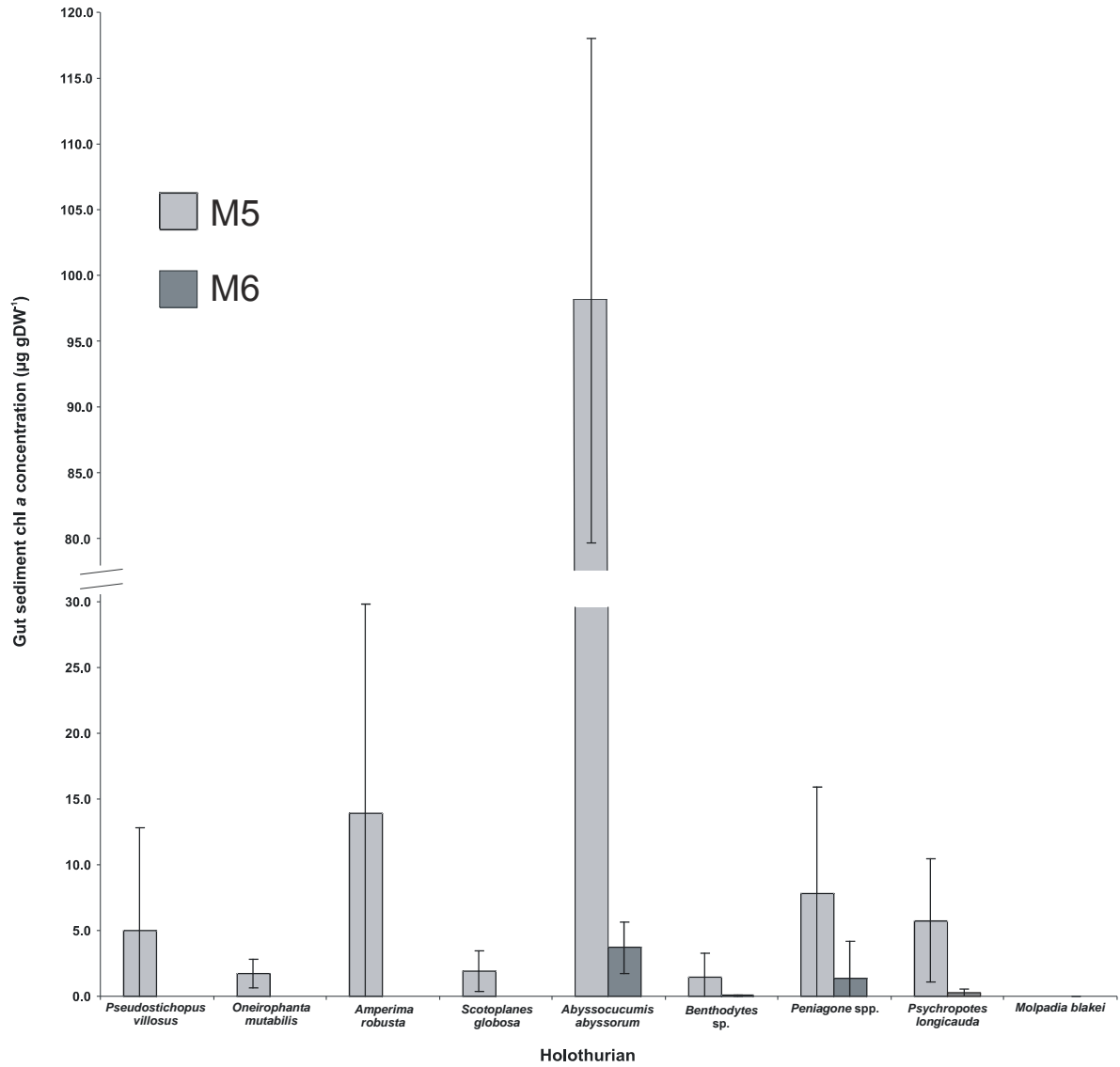
Figure 5.3 Chlorophyll *a* concentration (mean μg gDW⁻¹ ± SD) in the gut sediment of holothurians sampled at M5 (light grey) and M6 (dark grey) between December 2005 and January 2006. (note break in y-axis to accommodate *A. abyssorum* data).

	M5	M6
Pseudostichopus villosus (n = 5)	4.96 *7.86*	Nc
Oneirophanta mutabilis (n = 5)	1.69 *1.09*	Nc
Amperima robusta (n = 3)	13.88 *15.94*	Nc
Scotoplanes globosa (n = 3)	1.90 *1.53*	Nc
Benthodytes sp. (n = 2; M5, 2; M6)	1.47 *1.81*	0.05 *0.07*
Peniagone spp. (n = 5; M5, 4; M6)	7.82 *8.04*	1.40 *2.80*
Psychropotes longicauda (n = 3; M5, 4; M6)	5.76 *4.69*	0.22 *0.22*
Abyssocucumis abyssorum (n = 2; M5, 4; M6)	98.11 *20.03*	3.72 *1.95*
Molpadia blakei (n = 2)	Nc	0.00 *0.00*

Table 5.4 Chlorophyll *a* (µg gDW^{-1}) in the gut sediment of holothurians sampled at M5 and M6. (Standard deviation in italics). Nc = Not collected

5.2.4 Interspecies comparison of gut wall and ovarian carotenoid biochemistry – M5

The gut wall of *Abyssocucumis abyssorum* was very thin, which prohibited it being sampled. The gut walls and ovaries of the holothurians contained carotenoids (Fig. 5.4; Table 5.5); *Amperima robusta* and *Peniagone* spp. had the greatest gut wall and ovarian carotenoid concentrations and *Benthodytes* sp. the least. All pigments had high variability, although standard deviation relative to the mean was lowest in the ovaries of *Abyssocucumis abyssorum* (Fig. 5.4). β-carotene was often the most abundant or equally dominant carotenoid in the gut wall and ovary of all species sampled (Fig. 5.4; Table 5.6). 19'-butanoyloxyfucoxanthin was a minor carotenoid in the gut wall of all species excluding *Benthodytes* sp. and *Psychropotes longicauda* where it contributed an average of 18% and 32% respectively to the total identified pigment load (Table 5.5). 19'-butanoyloxyfucoxanthin contributed an average of 23% to the total ovarian pigment

load of *A. abyssorum*; this carotenoid was a minor contributer to the total ovarian load of all the other species sampled. β-carotene had a relatively high average percentage contribution (61%) to the gut wall in *O. mutabilis*, whereas 19'-hexanoyloxyfucoxanthin and 19'-butanoyloxyfucoxanthin were absent. Zeaxanthin had a relatively high average percentage contribution to the total identified pigment load, the gut wall (39%) and ovary (21%) of *Peniagone* spp. (Fig. 5.4; Table 5.6).
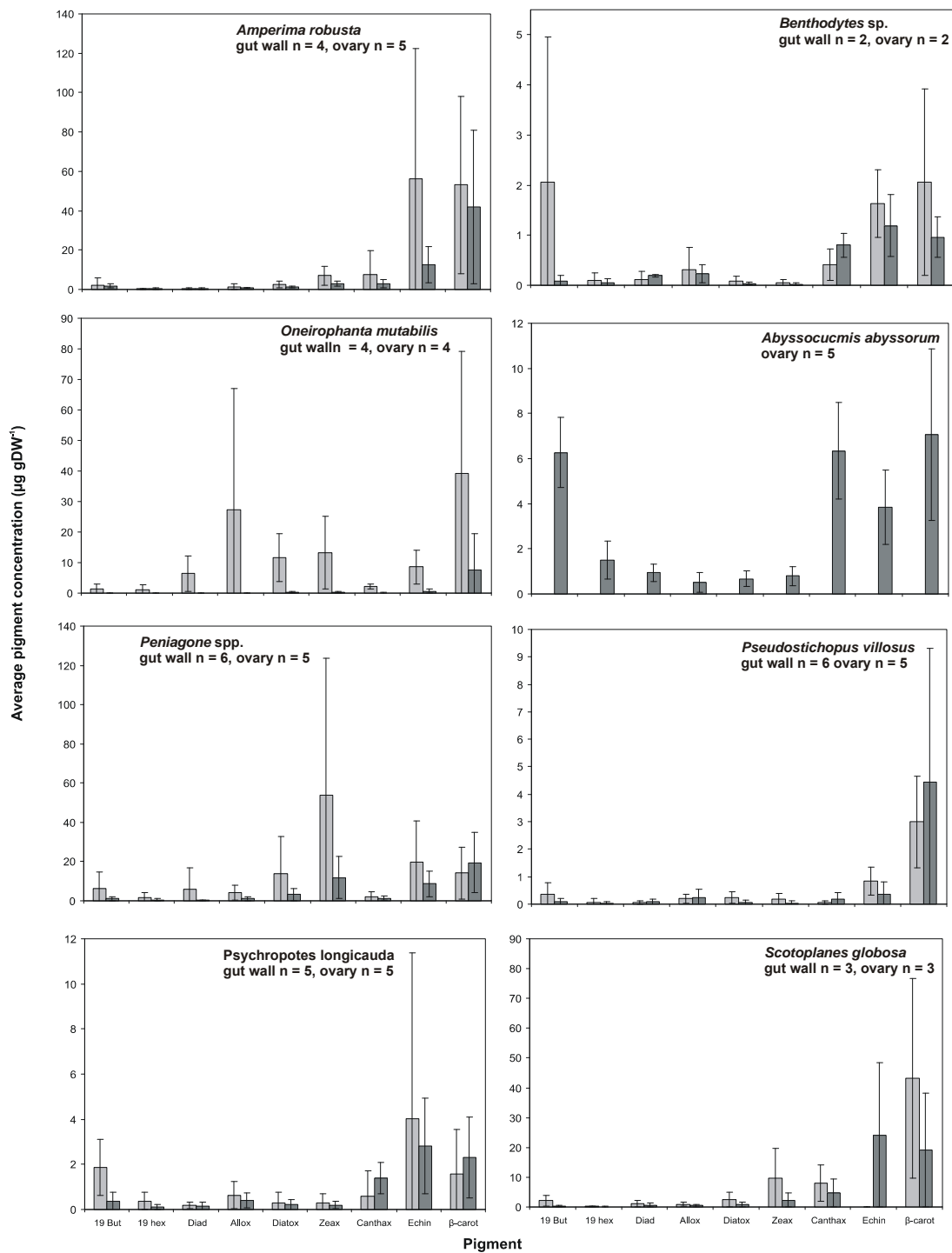
Figure 5.4. Pigment concentrations (mean μg gDW-1 ± SD) in the gut wall (light grey) and ovary (dark grey) of holothurians sampled at M5 during cruise 2 (Dec 2005-Jan 2006). 19'-but = 19'-butanoyloxyfucoxanthin; 19'-hex = 19'-hexanoyloxyfucoxanthin; Diad = diadinoxanthin; Allox = alloxanthin; Diatox = diatoxanthin; Zeax = zeaxanthin; Canthax = canthaxanthin; Echin = echinenone; β-carot = β-carotene. (note different scales on y-axis)

Table 5.5 data:

Pigment	P. villosus (n=6)	P. villosus % of total	O. mutabilis (n=4)	O. mutabilis % of total	Amperima robustrum (n=6)	Amperima robustrum total	S. globosa (n=3)	S. globosa % of total	Benthodytes sp. (n=2)	Benthodytes sp. % of total	Peniagone spp. (n=6)	Peniagone spp. % of total	P. longicauda (n=5)	P. longicauda % of total
19'-but	0.37 / *0.42*	7.1% / *9.1%*	1.33 / *1.58*	1.6% / *1.8%*	2.04 / *3.71*	1.8% / *2.3%*	2.10 / *1.90*	7.6% / *10.5%*	2.06 / *2.91*	17.8% / *25.2%*	6.46 / *8.10*	12.9% / *23.7%*	1.85 / *1.25*	32.0% / *18.3%*
19'-hex	0.07 / *0.14*	2.1% / *4.4%*	1.18 / *1.59*	2.6% / *4.2%*	0.22 / *0.36*	0.3% / *0.3%*	0.16 / *0.20*	1.0% / *1.6%*	0.10 / *0.14*	0.9% / *1.2%*	1.76 / *2.65*	1.7% / *2.5%*	0.38 / *0.39*	5.1% / *2.7%*
Diadinox	0.06 / *0.07*	1.2% / *1.3%*	6.40 / *5.81*	5.4% / *3.8%*	0.47 / *0.53*	0.3% / *0.3%*	1.12 / *1.10*	2.0% / *1.4%*	0.12 / *0.16*	1.0% / *1.4%*	5.82 / *10.84*	3.3% / *2.8%*	0.18 / *0.13*	2.9% / *1.2%*
Allox	0.20 / *0.18*	4.9% / *4.6%*	27.18 / *39.96*	16.4% / *14.0%*	1.42 / *1.54*	1.0% / *0.7%*	0.88 / *0.69*	1.3% / *0.1%*	0.32 / *0.45*	2.7% / *3.9%*	4.24 / *3.82*	4.2% / *2.0%*	0.64 / *0.61*	8.1% / *3.3%*
Diatox	0.24 / *0.20*	4.5% / *2.7%*	11.54 / *7.80*	10.8% / *7.4%*	2.44 / *1.65*	1.7% / *0.3%*	2.50 / *2.42*	3.0% / *1.4%*	0.08 / *0.11*	0.7% / *0.9%*	13.92 / *18.84*	9.6% / *3.6%*	0.31 / *0.47*	2.7% / *0.8%*
Zeax	0.18 / *0.22*	3.0% / *2.9%*	13.16 / *11.89*	14.8% / *13.8%*	7.06 / *4.77*	5.0% / *1.0%*	9.64 / *10.11*	12.1% / *4.8%*	0.05 / *0.07*	0.4% / *0.6%*	53.93 / *69.85*	38.9% / *18.1%*	0.28 / *0.43*	2.4% / *0.8%*
Canthax	0.05 / *0.06*	1.2% / *1.6%*	2.10 / *0.83*	3.2% / *3.3%*	7.71 / *11.97*	4.5% / *5.0%*	7.97 / *6.10*	10.9% / *4.5%*	0.42 / *0.31*	7.5% / *2.8%*	2.05 / *2.42*	1.6% / *1.0%*	0.59 / *1.12*	4.1% / *3.0%*
Echine	0.83 / *0.51*	16.9% / *6.7%*	8.54 / *5.54*	12.9% / *16.3%*	56.16 / *66.08*	36.8% / *23.4%*	0.00 / *0.00*	0.0% / *0.0%*	1.62 / *0.68*	36.7% / *26.1%*	19.63 / *21.01*	16.2% / *5.5%*	4.04 / *7.33*	26.4% / *15.9%*
ß-carot	2.99 / *1.67*	59.1% / *12.7%*	39.14 / *39.99*	32.4% / *8.2%*	53.03 / *45.00*	48.6% / *27.9%*	43.13 / *33.54*	62.1% / *5.2%*	2.06 / *1.87*	32.3% / *4.3%*	14.12 / *13.42*	11.6% / *7.7%*	1.57 / *1.99*	16.2% / *5.5%*

Table 5.5 Pigment concentrations (µg gDW^{-1}) and percentage contribution of pigments to the total pigment load in the gut wall of holothurians sampled at M5. 19'-but = 19'-butanoyloxyfucoxanthin; 19'-hex = 19'-hexanoyloxyfucoxanthin; Diadinox = diadinoxanthin; Allox = alloxanthin; Diatox = diatoxanthin; Zeax = zeaxanthin; Canthax = canthaxanthin; Echin = echinenone; β-carot = β-carotene. (Standard deviation in italics)

Table 5.6 (pigment data)

Pigment	P. villosus (n=5)	% of total	O. mutabilis (n=4)	% of total	Amperima robustrum (n=5)	% of total	S. globosa (n=3)	% of total	Benthodytes sp. (n=2)	% of total	Peniagone spp. (n=5)	% of total	P. longicauda (n=5)	% of total	A. abyssorum (n=5)	% of total
19'-but	0.08	1.4%	0.00	0.0%	1.88	5.3%	0.23	0.6%	0.08	3.1%	1.11	2.4%	0.36	6.2%	6.27	23.2%
19'-but SD	*0.12*	*2.0%*	*0.00*		*1.12*	*5.7%*	*0.23*	*0.6%*	*0.12*	*4.4%*	*1.12*	*2.8%*	*0.41*	*7.9%*	*1.55*	*3.8%*
19'-hex	0.03	0.5%	0.00	0.0%	0.43	1.4%	0.12	0.3%	0.05	2.0%	0.62	7.2%	0.12	1.7%	1.50	5.9%
19'-hex SD	*0.07*	*1.1%*			*0.35*	*1.8%*	*0.12*	*0.3%*	*0.08*	*2.8%*	*0.56*	*14.4%*	*0.11*	*1.8%*	*0.83*	*3.4%*
Diadinox	0.08	1.2%	0.03	1.1%	0.50	1.1%	0.68	1.1%	0.20	5.7%	0.21	1.6%	0.14	3.0%	0.95	3.4%
Diadinox SD	*0.10*	*1.8%*	*0.04*	*1.5%*	*0.35*	*0.8%*	*0.68*	*1.1%*	*0.03*	*1.2%*	*0.24*	*2.6%*	*0.19*	*4.2%*	*0.38*	*0.7%*
Allox	0.23	6.1%	0.04	1.2%	0.86	1.9%	0.46	0.9%	0.23	6.0%	1.19	3.7%	0.42	4.9%	0.51	1.7%
Allox SD	*0.30*	*6.8%*	*0.06*	*1.5%*	*0.18*	*1.6%*	*0.46*	*0.9%*	*0.19*	*3.1%*	*0.98*	*3.0%*	*0.33*	*2.8%*	*0.44*	*1.3%*
Diatox	0.07	1.3%	0.24	4.9%	1.35	2.7%	0.77	1.4%	0.04	1.3%	3.19	6.0%	0.22	2.5%	0.67	2.4%
Diatox SD	*0.10*	*1.4%*	*0.19*	*5.3%*	*0.42*	*1.4%*	*0.77*	*1.4%*	*0.03*	*1.2%*	*3.13*	*2.1%*	*0.24*	*1.3%*	*0.34*	*0.9%*
Zeax	0.04	0.2%	0.32	6.6%	3.09	5.8%	2.32	3.9%	0.02	0.6%	11.89	20.6%	0.20	2.3%	0.80	2.7%
Zeax SD	*0.08*	*0.5%*	*0.24*	*7.2%*	*1.21*	*2.5%*	*2.32*	*3.9%*	*0.02*	*0.9%*	*10.75*	*10.2%*	*0.16*	*0.7%*	*0.43*	*1.1%*
Canthax	0.19	6.2%	0.13	3.9%	2.88	6.1%	4.74	10.1%	0.80	22.6%	1.43	4.7%	1.39	19.0%	6.34	24.2%
Canthax SD	*0.22*	*7.4%*	*0.16*	*5.0%*	*2.20*	*4.7%*	*4.74*	*10.1%*	*0.24*	*1.0%*	*1.01*	*4.4%*	*0.68*	*8.1%*	*2.15*	*8.6%*
Echine	0.37	4.9%	0.63	20.9%	12.48	22.8%	24.18	46.4%	1.19	32.2%	8.62	15.6%	2.83	33.6%	3.84	13.3%
Echine SD	*0.44*	*3.1%*	*0.69*	*27.4%*	*9.32*	*15.5%*	*24.18*	*46.4%*	*0.62*	*6.2%*	*6.35*	*6.8%*	*2.12*	*7.5%*	*1.64*	*2.2%*
ß-carot	4.44	78.2%	7.51	61.3%	42.00	52.8%	19.07	35.2%	0.96	26.5%	19.46	38.2%	2.31	26.9%	7.05	23.3%
ß-carot SD	*4.88*	*14.9%*	*11.88*	*26.3%*	*38.89*	*29.0%*	*19.07*	*35.2%*	*0.41*	*2.3%*	*15.29*	*12.9%*	*1.80*	*7.9%*	*3.81*	*10.5%*

Table 5.6 Pigment concentrations (μg gDW^{-1}) and percentage contribution of pigments to the total pigment load in the ovaries of holothurians sampled at M5. 19'-but = 19'-butanoyloxyfucoxanthin; 19'-hex = 19'-hexanoyloxyfucoxanthin; Diadinox = diadinoxanthin; Allox = alloxanthin; Diatox = diatoxanthin; Zeax = zeaxanthin; Canthax = canthaxanthin; Echin = echinenone; β-carot = β-carotene. (Standard deviation in italics)

Figure 5.5 MDS ordination of individual holothurian gut wall (a) and ovary (b) samples from M5 cruise 2 (Dec 2005-Jan 2006), based on √-transformed pigment percentage contributions and Bray-Curtis similarities. Key: Filled triangles = *Amperima robusta***; open triangles =** *Oneirophanta mutabilis***; filled squares =** *Peniagone* **spp.; open squares =** *Psychropotes longicauda***; open circles =** *Abyssocucumis abyssorum***; crosses =** *Benthodytes* **spp.; stars =** *Pseudostichopus villosus***; filled diamonds =** *Scotoplanes globosa.*

Psychropotes longicauda showed the tightest species specific clustering on the gut wall MDS ordination plot of square root transformed pigment percentage contributions. The slight spread of *Psychropotes longicauda* samples is attributed to the variation in the contribution of 19'-butanoyloxyfucoxanthin. *Peniagone* spp. showed tight clustering apart from one sample that differed from the others in having relatively low zeaxanthin percentage contribution and relatively high 19'-butanoyloxyfucoxanthin contribution. *Oneirophanta mutabilis* and *Amperima robusta* both had one outlying sample from their gut wall species clusters (Fig. 5.5a), which is attributed to a relatively high contribution of echinenone. *Pseudostichopus villosus* and *Benthodytes* sp. showed the least species specific clustering, with samples spread across the middle of the MDS ordination plot (Fig. 5.5a).

Abyssocucumis abyssorum showed the tightest species specific clustering on the ovarian MDS ordination plot of square root transformed pigment percentage contributions (Fig 5.5b). *Peniagone* spp. samples also showed tight clustering. The slight spread of samples can be attributed to the variation in contribution of β-carotene; the sample to the right of the plot had a relatively high percentage contribution of 19'-hexanoyloxyfucoxanthin. *Pseudostichopus villosus* samples were spread around the left hand side of the plot, having a high percentage contribution of β-carotene, with a small percentage contribution from different carotenoids in each sample, leading to the spread of samples (Fig 5.5b). *Oneirophanta mutabilis* samples to the bottom left of the plot had a high β-carotene percentage contribution; the sample to the top right had low β-carotene and high echinenone percentage contribution (Fig. 5.5b).

5.2.5 Interspecies comparison of gut wall and ovarian carotenoid biochemistry – M6

Abyssocucumis abyssorum contained the greatest ovarian carotenoid concentrations (µg gDW^{-1}) of the species sampled at M6, with high percentage

contributions from canthaxanthin, echinenone and β-carotene (Fig. 5.6 and Table 5.7). *Peniagone* spp. had the second highest concentration of carotenoids in its gut wall and ovary (µg gDW^{-1}), followed by *Psychropotes longicauda* (Fig.5.6). *Molpadia blakei* and *Benthodytes* sp. had the lowest concentrations (µg gDW^{-1}) of carotenoids in their gut wall and ovary (Fig. 5.6). *Molpadia blakei* had a greater number of carotenoids in the ovarian samples than the gut wall. Carotenoids that occurred in both the gut wall and ovary of *M. blakei* and were found in greater concentration in the ovary (Fig. 5.6). Diadinoxanthin (9%), alloxanthin (25%), diatoxanthin (18%) and zeaxanthin (15%) had relatively high percentage contributions in *M. blakei* ovaries in comparison to the other species sampled at M6 (Fig. 5.6 and Table 5.8). *Psychropotes longicauda* had a high percentage contribution of diatoxanthin (Table 5.8).
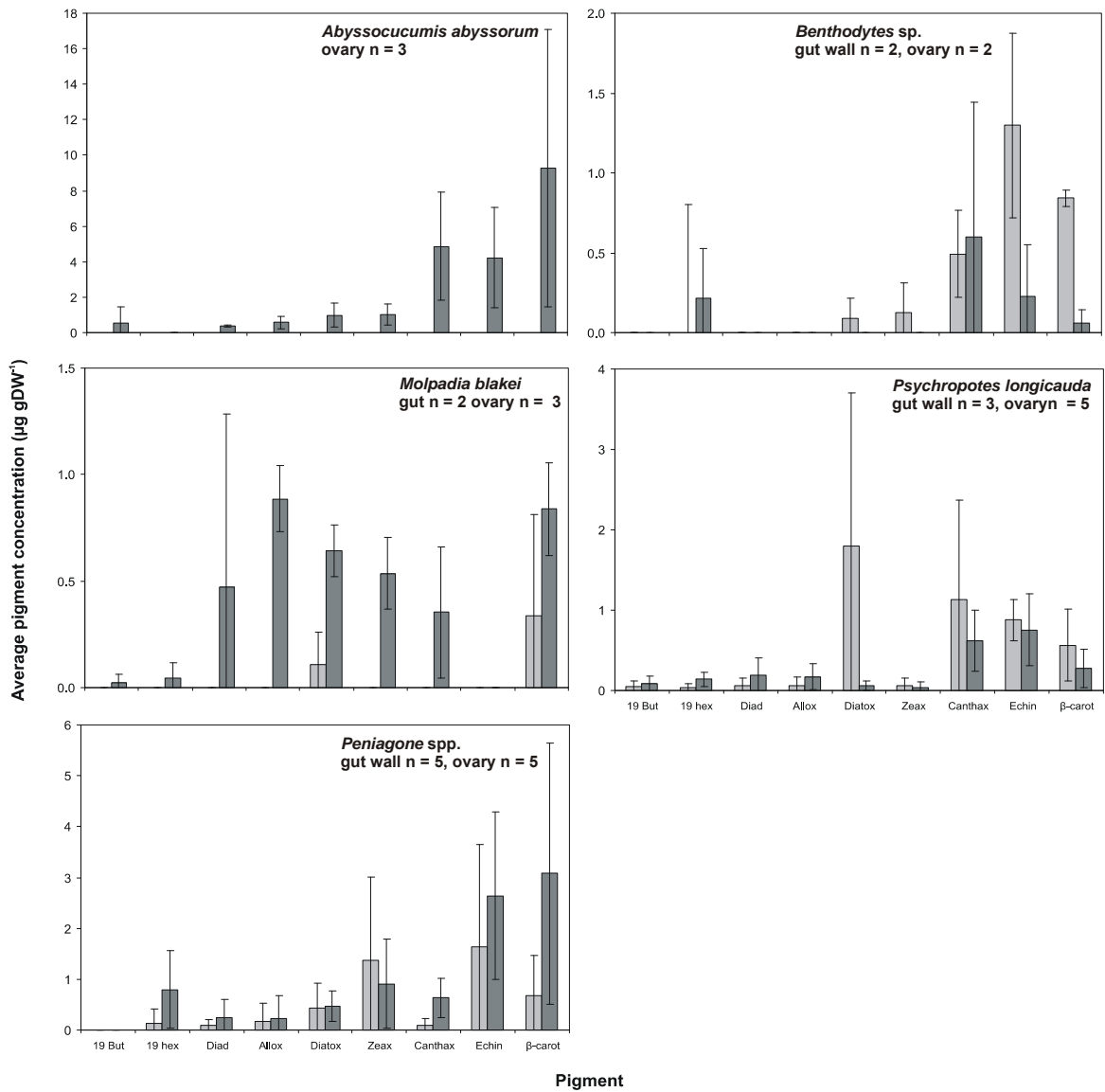
Figure 5.6. Pigment concentrations (mean μg gDW-1 ± SD) in the gut wall (light grey) and ovary (dark grey) of holothurians sampled at M6 during cruise 2 (Dec 2005-Jan 2006). 19'-but = 19'-butanoyloxyfucoxanthin; 19'-hex = 19'-hexanoyloxyfucoxanthin; Diad = diadinoxanthin; Allox = alloxanthin; Diatox = diatoxanthin; Zeax = zeaxanthin; Canthax = canthaxanthin; Echin = echinenone; β-carot = β-carotene. (note different scales on y-axis)

	M. blakei (n=2)	M. blakei % of total	Benthodytes sp. (n=2)	Benthodytes sp. % of total	Peniagone spp. (n = 5)	Peniagone spp. % of total	P. longicauda (n=3)	P. longicauda % of total
19'-but	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.04	0.5%
							0.08	*0.9%*
19'-hex	0.00	0.0%	0.00	0.0%	0.13	1.3%	0.03	0.3%
					0.29	*2.9%*	*0.05*	*0.6%*
Diad	0.00	0.0%	0.00	0.0%	0.09	0.8%	0.06	0.7%
					0.12	*1.2%*	*0.10*	*1.2%*
Allox	0.00	0.0%	0.00	0.0%	0.16	1.4%	0.06	0.7%
					0.36	*3.2%*	*0.10*	*1.2%*
Diatox	0.11	12.1%	0.09	2.4%	0.43	12.5%	1.80	34.0%
	0.15	*17.1%*	*0.13*	*2.8%*	*0.50*	*13.7%*	*1.90*	*10.4%*
Zeax	0.00	0.0%	0.13	3.5%	1.38	30.4%	0.06	0.7%
			0.18	*4.0%*	*1.63*	*22.7%*	*0.10*	*1.2%*
Canthax	0.00	0.0%	0.49	16.8%	0.10	0.9%	1.13	21.3%
			0.27	*8.8%*	*0.14*	*1.3%*	*1.24*	*7.5%*
Echine	0.00	0.0%	1.30	45.3%	1.65	22.1%	0.88	25.0%
			0.58	*26.1%*	*2.01*	*20.2%*	*0.26*	*11.5%*
ß-carot	0.34	37.9%	0.84	32.1%	0.68	10.5%	0.56	16.9%
	0.48	*53.6%*	*0.05*	*18.1%*	*0.78*	*10.5%*	*0.45*	*13.5%*

Table 5.7 Pigment concentrations (µg gDW^{-1}) and percentage contribution of pigments to the total pigment load in the gut wall of holothurians sampled at M6. 19'-but = 19'-butanoyloxyfucoxanthin; 19'-hex = 19'-hexanoyloxyfucoxanthin; Diad = diadinoxanthin; Allox = alloxanthin; Diatox = diatoxanthin; Zeax = zeaxanthin; Canthax = canthaxanthin; Echin = echinenone; β-carot = β-carotene. (Standard deviation in italics)

	A. abyssorum (n=3)	A. abyssorum % of total	M. blakei (n=2)	M. blakei % of total	Benthodytes sp. (n=2)	Benthodytes sp. % of total	Peniagone spp. (n = 5)	Peniagone spp. % of total	P. longicauda (n=3)	P. longicauda % of total
19'-but	0.52	1.4%	0.02	0.5%	0.00	0.0%	0.00	0.0%	0.08	3.2%
	0.91	*2.5%*	*0.04*	*0.8%*					*0.09*	*3.6%*
19'-hex	0.00	0.0%	0.04	0.8%	0.22	9.9%	0.80	8.6%	0.14	4.9%
			0.07	*1.5%*	*0.31*	*14.0%*	*0.76*	*9.5%*	*0.09*	*3.0%*
Diad	0.38	2.8%	0.47	9.3%	0.00	0.0%	0.24	1.8%	0.20	6.0%
	0.06	*2.7%*	*0.82*	*16.2%*			*0.35*	*2.6%*	*0.21*	*5.8%*
Allox	0.59	3.2%	0.89	25.2%	0.00	0.0%	0.23	3.4%	0.17	5.5%
	0.35	*1.6%*	*0.16*	*9.3%*			*0.44*	*6.9%*	*0.16*	*4.5%*
Diatox	0.99	4.4%	0.64	18.3%	0.00	0.0%	0.47	4.2%	0.06	1.8%
	0.68	*0.2%*	*0.12*	*8.1%*			*0.30*	*2.5%*	*0.06*	*1.7%*
Zeax	1.01	4.8%	0.53	14.5%	0.00	0.0%	0.91	7.1%	0.04	1.1%
	0.59	*0.5%*	*0.17*	*4.9%*			*0.88*	*6.7%*	*0.07*	*2.0%*
Canthax	4.87	23.4%	0.35	8.6%	0.60	27.1%	0.63	5.9%	0.62	22.3%
	3.06	*10.5%*	*0.31*	*7.9%*	*0.85*	*38.3%*	*0.38*	*3.6%*	*0.38*	*14.5%*
Echine	4.22	19.3%	0.00	0.0%	0.23	10.3%	2.64	23.9%	0.75	26.3%
	2.83	*0.4%*			*0.32*	*14.6%*	*1.64*	*13.5%*	*0.45*	*15.5%*
ß-carot	9.28	40.8%	0.84	22.7%	0.06	2.7%	3.08	25.2%	0.28	8.8%
	7.83	*9.9%*	*0.22*	*5.9%*	*0.08*	*3.8%*	*2.56*	*18.3%*	*0.24*	*6.5%*

Table 5.8 Pigment concentrations (µg gDW⁻¹) and percentage contribution of pigments to the total pigment load in the ovaries of holothurians sampled at M6. 19'-but = 19'-butanoyloxyfucoxanthin; 19'-hex = 19'-hexanoyloxyfucoxanthin; Diad = diadinoxanthin; Allox = alloxanthin; Diatox = diatoxanthin; Zeax = zeaxanthin; Canthax = canthaxanthin; Echin = echinenone; β-carot = β-carotene. (Standard deviation in italics)
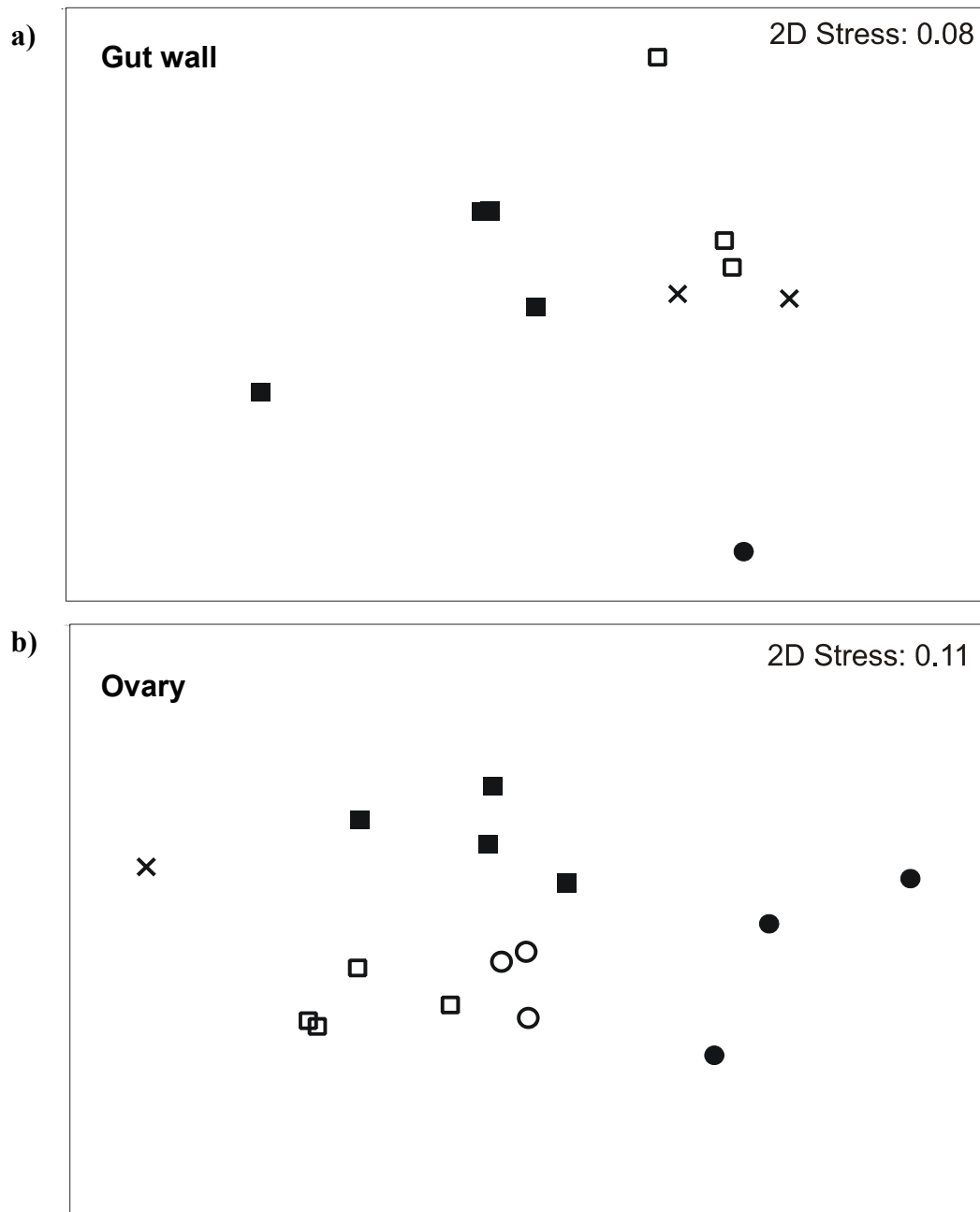
Figure 5.7 MDS ordination of individual holothurian gut wall (a) and ovary (b) samples from M6 cruise 2 (Dec 2005-Jan 2006), based on √-transformed pigment percentage contributions and Bray-Curtis similarities. Key: filled squares = *Peniagone* **spp.; open squares =** *Psychropotes longicauda***; open circles =** *Abyssocucumis abyssorum***; crosses =** *Benthodytes typica***; filled circles =** *Molpadia* **sp.**

The MDS ordination plot of square root transformed pigment percentage contributions to the M6 holothurian gut wall samples showed loose species

specific clustering (Fig. 5.7a). Samples that contained no pigments were removed from the plot because their inclusion increased similarity between the other samples to form one large cluster on the MDS ordination plot. One *Peniagone* sp. and one *Molpadia blakei* gut wall sample contained no carotenoids and are not shown on the plot. The outlying *Peniagone* sp. gut wall sample, lower left of the plot contained only diatoxanthin and zeaxanthin; the outlying sample in the middle of the plot did not contain diadinoxanthin or canthaxanthin (Fig. 5.7a). The outlying *Psychropotes longicauda* sample to the top of the plot has small percentage contributions spread across all carotenoids identified, whereas the other two samples contained only diatoxanthin, echinenone, canthaxanthin and β-carotene (Fig. 5.7a).

All species were clustered separately on the MDS ordination plot of M6 holothurian ovarian samples (Fig. 5.7b). One specimen each of *Benthodytes* sp., *Peniagone* spp. and *Psychropotes longicauda* contained no pigments and were excluded from the plot. *Abyssocucumis abyssorum* showed the tightest species specific clustering. The spread of the *Molpadia blakei* samples (from right to left) can be attributed to the increasing number of carotenoids contributing a small percentage of the total in each sample. The spread of *Peniagone* spp. samples is attributed to the varying percentage contribution of 19'-hexanoyloxyfucoxanthin. The outlying *Psychropotes longicauda* sample to the right had a relatively large percentage contribution of alloxanthin and diadinoxanthin and a low contribution of canthaxanthin (Fig. 5.7b).

5.2.6 Between-site intraspecies comparison

Benthodytes sp., *Peniagone* spp., *Psychropotes longicauda* and *A. abyssorum* (ovary only) were sampled at M5 and M6. Between-site comparison of the average concentration of carotenoids (μg gDW^{-1}) in the gut wall showed the majority of carotenoids were found in greater concentrations in specimens sampled from M5 (Fig. 5.8). 19'-butanoyloxyfucoxanthin, 19'-hexanoyloxyfucoxanthin, diadinoxanthin and alloxanthin were found in one

specimen of *Benthodytes* sp. at M5, but not in those collected at M6. Too few individuals of *Benthodytes* sp. from each site were sampled (n = 2) to perform statistical analysis of the data. Appendix 3 lists the statistical results of between site intra-species gut wall carotenoid concentration comparisons. All carotenoids in the gut wall of *Peniagone* spp., with the exception of 19'-hexanoyloxyfucoxanthin and β-carotene, were found to be in significantly greater concentrations at M5 than at M6 (Mann-Whitney; t-test, P<0.05; Appendix 3). One M6 gut wall sample of *Peniagone* spp. did not contain any pigment. Only 19'-butanoyloxyfucoxanthin was found in significantly greater concentration in the M5 gut wall samples of *Psychropotes longicauda* (Mann-Whitney, P<0.05, Appendix 3) than at M6. Greater concentrations of echinenone and β-carotene occurred in *P. longicauda* but they were very variable and so no significant differences between M5 and M6 could be detected. Diatoxanthin occurred in greater concentrations in specimens of *P. longicauda* at M6 (Fig. 8), although this was not significantly different from M5 (Mann-Whitney, P>0.05, Appendix 3).

In the ovaries of species collected at M5 and M6, the average concentrations of carotenoids were often higher in the M5 specimens (Fig. 5.9). There were too few ovarian samples of *Benthodytes* sp. to perform statistical analysis (n = 2). However one sample from M6 contained no pigment and the M5 samples contained a greater number of carotenoids. 19'-butanoyloxyfucoxanthin, 19'-hexanoyloxyfucoxanthin and diadinoxanthin were found in significantly greater concentrations in M5 samples of *A. abyssorum* than in M6 samples (t-test; Mann-Whitney, P<0.05, Appendix 3). All other carotenoids in *A. abyssorum* were found in similar concentrations between sites. Only 19'-butanoyloxyfucoxanthin was the found in significantly greater concentrations (Mann-Whitney, P<0.05, Appendix 3) in the M5 ovaries of *Peniagone* spp.. Differences in average concentrations of the pigments zeaxanthin, echinenone and β-carotene were also apparent between sites (Fig 5.9), but they were not statistically significant because of the high variability and small sample size affected the statistical outcome. One M6 *Peniagone* spp. ovary sample contained no pigment. Canthaxanthin, echinenone and β-carotene had higher average concentrations in the ovaries of *Psychropotes longicauda* from

M5 than at M6 (one *P. longicauda* specimen collected at M6 contained no pigment). High variability and low sample size affected the statistical outcome so that concentrations from the two sample sites were not significantly different (t-test, P>0.05, Appendix 3).
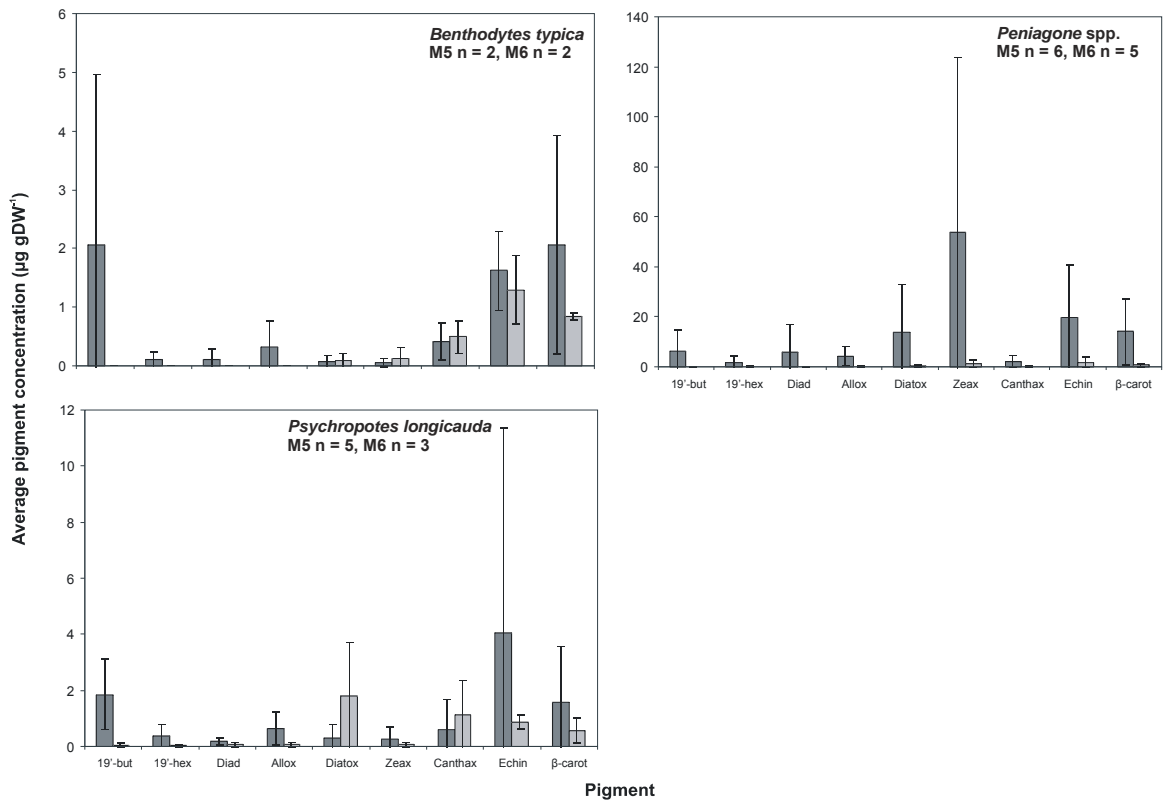
Figure 5.8 Pigment concentrations (mean μg gDW-1 ± SD) in the gut wall of holothurians sampled at M5 (dark grey) and M6 (light grey) during cruise 2 (Dec 2005-Jan 2006). 19'-but = 19'-butanoyloxyfucoxanthin; 19'-hex = 19'-hexanoyloxyfucoxanthin; Diadinox = diadinoxanthin; Allox = alloxanthin; Diatox = diatoxanthin; Zeax = zeaxanthin; Canthax = canthaxanthin; Echin = echinenone; β-carot = β-carotene. (note different scales on y-axis)

Figure 5.9 Pigment concentrations (mean μg gDW-1 ± SD) in the ovaries of holothurians sampled at M5 (dark grey) and M6 (light grey) during cruise 2 (Dec 2005-Jan 2006). 19'-but = 19'-butanoyloxyfucoxanthin; 19'-hex = 19'-hexanoyloxyfucoxanthin; Diad = diadinoxanthin; Allox = alloxanthin; Diatox = diatoxanthin; Zeax = zeaxanthin; Canthax = canthaxanthin; Echin = echinenone; β-carot = β-carotene. (note different scales on y-axis)
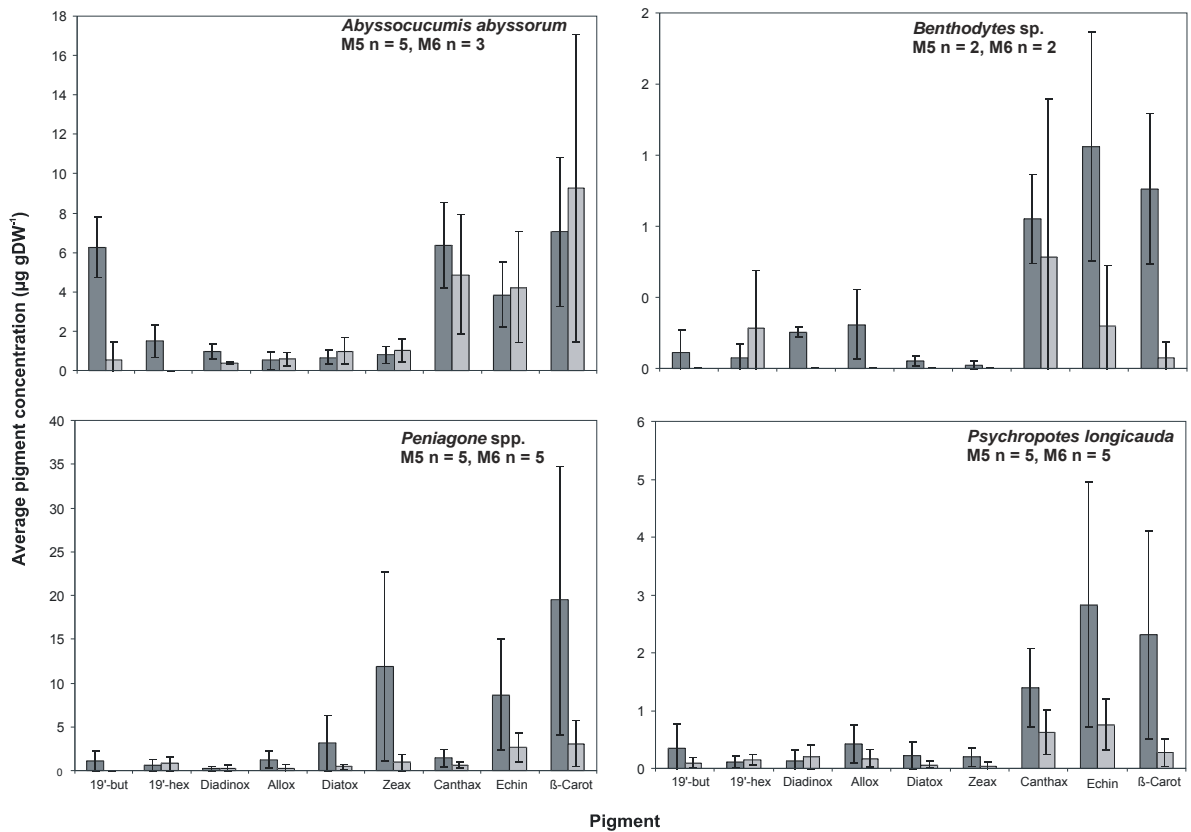
MDS ordination plot of gut wall pigment percentage contribution showed loose species clustering with some separation between M5 and M6 samples (Fig. 5.10a). M5 and M6 *Peniagone* spp. and *Psychropotes longicauda* sample clusters were separated by the occurrence of alloxanthin and 19'-butanoyloxyfucoxanthin in the M5 samples (one M6 *P. longicauda* sample contained these carotenoids and was found near to the M5 samples). ANOSIM analysis showed *Psychropotes longicauda* gut wall samples were significantly different in percentage composition between sites (ANOSIM R = 1, P<0.05; Table 5.9). *Benthodytes* sp. gut wall samples were not found to be significantly different between sites (ANOSIM R = 0, P>0.05; Table 5.9) because of the small number of samples.

Peniagone spp. gut wall samples were found to be significantly similar in pigment composition between sites despite the separation on the MDS plot (ANOSIM R = 0.251, P<0.05). Outlying samples from both sites and the closeness of the two groups led to a small R-statistic, which suggests samples from one site are as similar to the other site as they are to each other.

The occurrence of 19'-hexanoyloxyfucoxanthin and increased percentage contribution of 19'-butanoyloxyfucoxanthin in *A. abyssorum* M5 ovarian samples separated them from M6 samples and made them significantly different (ANOSIM R = 0.959, P<0.05) (Fig. 5.10b; Table 5.9). *Peniagone* spp. ovarian samples from M5 and M6 were similar, but some between-site separation on the ovarian MDS ordination plot suggests why the R-statistic was not significant (ANOSIM R = 0.269, P>0.05; Table 5.9). 19'-butanoyloxyfucoxanthin was absent and zeaxanthin contributed a relatively low proportion in the M6 *Peniagone* spp. samples. ANOSIM analysis suggests *Psychropotes longicauda* ovarian samples were similar between sites, however the higher percentage contribution of β-carotene in the samples from M5 and the variable composition of all samples led to the R-statistic being not significant (ANOSIM R = 0.325, P>0.05; Table 5.9).

Figure 5.11a shows the MDS ordination plot of the square root transformed raw pigment concentration data (differences on the plot can be attributed to concentrations of carotenoids as well as the pigment composition of samples) of gut wall samples. There is a clear divide between M5 and M6 samples attributed to the higher concentrations and between site differences in pigment composition of the samples. The gut wall samples of *Psychropotes longicauda* were significantly different between sites (ANOSIM R = 0.6, P<0.05; Table 5.10). *Benthodytes* sp. gut wall samples were very variable, this coupled with low sample size meant the samples were not different in composition between sites as indicated by the low, non-significant R-statistic (ANOSIM R = -0.250, P>0.05; Table 5.10). Although separation can be seen on the MDS plot between M5 and M6 *Peniagone* spp. gut wall samples (Fig 5.11a), ANOSIM analysis suggests they are similar because of the high variability of the samples (ANOSIM R = 0.445,

P<0.05; Table 5.10). The MDS ordination plot of ovarian square root transformed raw pigment concentration data also gives a clearer intraspecies separation between sites (Fig. 5.11b), although only because *A. abyssorum* ovarian samples are significantly different between sites (ANOSIM R = 0.621, P<0.05; Table 5.10). Low sample size (*Benthodytes* sp.), high variability within each site (*Psychropotes longicauda* and *Peniagone* spp.), and an outlying sample (*Peniagone* spp. – M5 sample to the left middle of the plot), gave an ANOSIM result that suggests the samples are as similar between sites as they are within (Table 5.10). This was only significant for *Peniagone* spp. samples (ANOSIM R = 0.272, P<0.05).

Figure 5.10 MDS ordination of individual holothurian gut wall (a) and ovary (b) samples from species sampled at M6 and M5 during cruise 2 (Dec 2005-Jan 2006), based on √-transformed *pigment percentage contributions* **and Bray-Curtis similarities. Key: filled squares =** *Peniagone* **spp. M5; open squares =** *Peniagone* **spp. M6; crosses =** *Benthodytes typica* **M5; plus signs =** *Benthodytes typica* **M6; filled triangles =** *Psychropotes longicauda* **M5; open triangles =** *Psychropotes longicauda* **M6; filled circles =** *Abyssocucumis abyssorum* **M5; open circles =** *Abyssocucumis abyssorum* **M6.**
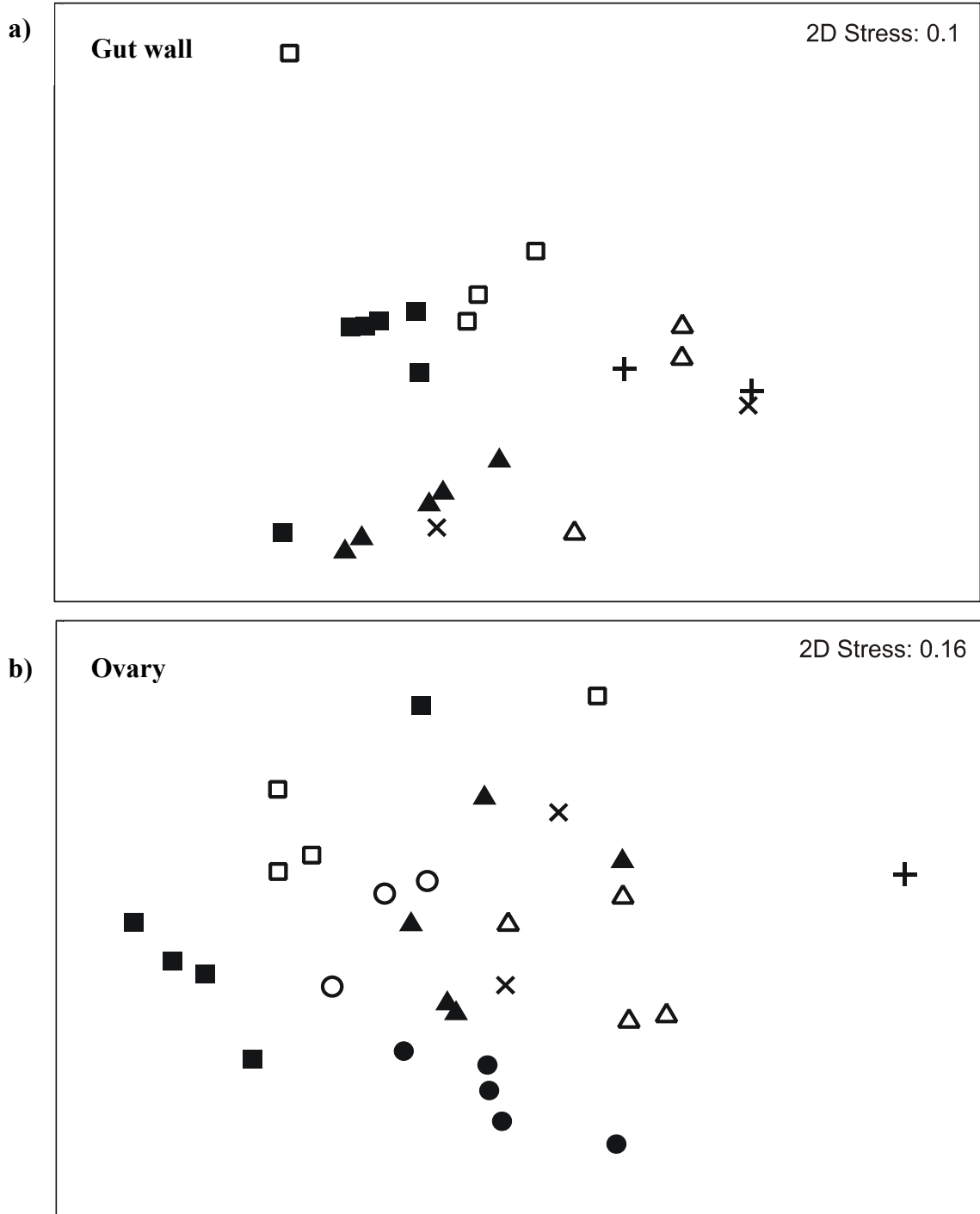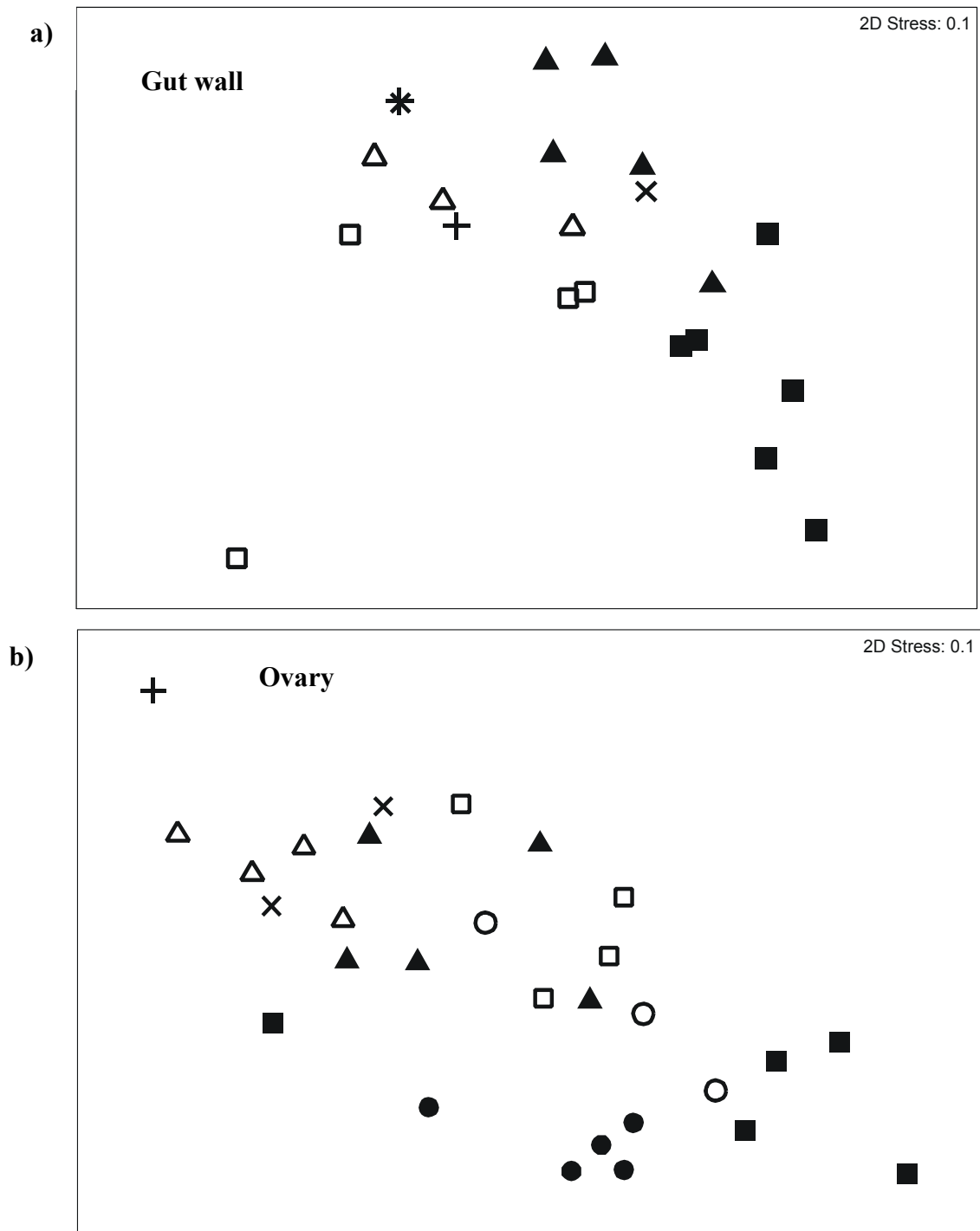
Figure 5.11 MDS ordination of individual holothurian gut wall (a) and ovary (b) samples from species sampled at M6 and M5 during cruise 2 (Dec 2005-Jan 2006), based on √-transformed *pigment concentrations* and Bray-Curtis similarities. Key: filled squares = *Peniagone* sp. M5; open squares = *Peniagone* sp. M6; crosses = *Benthodytes typica* M5; plus signs = *Benthodytes typica* M6; filled triangles = *Psychropotes longicauda* M5; open triangles = *Psychropotes longicauda* M6; filled circles = *Abyssocucumis abyssorum* M5; open circles = *Abyssocucumis abyssorum* M6. (note overlapping plus sign and cross on gut wall plot)

Group (% contribution data)	R-statistic	Significance level
Benthodytes sp. M5 v *Benthodytes* sp. M6 gut wall	0	P = 0.667
Benthodytes sp. M5 v *Benthodytes* sp. M6 ovary	0.25	P = 0.333
P. longicauda M5 v *P. longicauda* M6 gut wall	1	P = 0.018*
P. longicauda M5 v *P. longicauda* M6 ovary	0.325	P = 0.087
Peniagone spp. M5 v *Peniagone* spp. M6 gut wall	0.251	P = 0.011*
Peniagone spp. M5 v *Peniagone* spp. M6 ovary	0.269	P = 0.071
A. abyssorum M5 v *A. abyssorum* M6 ovary	0.959	P = 0.018*

Table 5.9 Results of similarity test (ANOSIM) comparing holothurian gut wall and ovarian pigment percentage contribution to the total load between M5 and M6. R-statistic = 1 only if all replicates within a sample are more similar to each other than any other replicates from different samples. * = significant (analysis includes samples with no pigments)

Group (raw data)	R-statistic	Significance level
Benthodytes sp. M5 v *Benthodytes* sp. M6 gut wall	-0.250	P = 1
Benthodytes sp. M5 v *Benthodytes* sp. M6 ovary	0.250	P = 0.333
P. longicauda M5 v *P. longicauda* M6 gut wall	0.600	P = 0.018*
P. longicauda M5 v *P. longicauda* M6 ovary	0.200	P = 0.063
Peniagone spp. M5 v *Peniagone* spp. M6 gut wall	0.445	P = 0.002*
Peniagone spp. M5 v *Peniagone* spp. M6 ovary	0.272	P = 0.040*
A. abyssorum M5 v *A. abyssorum* M6 ovary	0.621	P = 0.018*

Table 5.10 Results of similarity test (ANOSIM) comparing holothurian gut wall and ovarian pigment square root transformed data between M5 and M6. R-statistic = 1 only if all replicates within a sample are more similar to each other than any other replicates from different samples. * = significant (analysis includes samples with no pigments)

5.3 Discussion

5.2.1 Supply of material to the sea floor at M5 and M6

Similar chlorophyll *a* levels in the particulate organic matter (POM) sampled below the chlorophyll *a* maximum may reflect the composition of material exporting from the upper ocean at stations M5 and M6 at the time of sampling (December 2005 to January 2006). The phytoplankton bloom to the north of the Crozet Isles was declining at the time of sampling (December to January). The bloom in this region starts mid-late September, peaks late October and declines throughout November to early January (Pollard et al., 2002; Venables et al., 2007). The waters to the north of the Islands have a eastward flow and so pass over benthic station M5 to the east (Pollard and Read, 2001). A small chlorophyll *a* peak was observed in the surface waters at M6, located in the HNLC region, in December 2004 (Pollard et al., 2002; Venables et al., 2007), suggesting there is some productivity in the upper water column at that station.

Chlorophyll *a* to phaeophorbide ratios, indicating freshness (Thiel et al., 1989), suggest the POM below the chlorophyll *a* maximum was fresher at M5 (15.4 ± 3.2) than at M6 (7.3 ±2.0). Low variability in the percentage contribution of the pigments found in the POM below the chlorophyll *a* maximum suggests POM composition was consistent within sites (Fig. 5.1). Differences in the phytopigment composition of this POM between M5 and M6 are attributed to differences in 19'-hexanoylofucoxanthin and fucoxanthin concentrations; the latter was found in a higher concentration in the POM sampled at M6 below the chlorophyll *a* maximum, while the former was found in higher, but variable concentrations at M5. Fucoxanthin is a marker for diatoms while 19'-hexanoylofucoxanthin is typical of *Phaeocystis* spp. (Jeffrey et al., 1997). There are significant correlations between diatom biomass and fucoxanthin, and between *Phaeocystis* biomass and 19'-hexanoylofucoxanthin, in the Crozet region (Poulton et al., 2007). The upper water POM biochemical compositional data from the present study concur with data on the phytoplankton community in the region.

Hence, north of the Crozet Plateau, the phytoplankton community is dominated by *Phaeocystis antarctica*, while to the south, although there was a mixed community, the large diatoms *Corethron pinnatum* and *Fragilariopsis kerguelensis* dominate biomass (Poulton et al., 2007). Pigment analysis of the upper water column in December 2004 suggested the cyanobacterium genus *Prochlorococcus* was present at M6, based on the incidence of its signature pigment divinyl chlorophyll *a* (Seeyave et al., 2007); this was also supported by flow cytometry data (Zubkov et al., unpublished data). Pigment markers of cyanobacteria (zeaxanthin and divinyl chlorophyll *a*; Jeffrey et al., 1997) were not observed in the POM sampled beneath the chlorophyll maximum at M6 in December 2005. This may be because they were either not present, or were not being exported from the upper ocean. Violaxanthin was found in low concentrations at M6 but not at M5. Violaxanthin is a marker of chlorophytes, prasinophytes and eustigmatophytes (small pico-nano phytoplankton) (Jeffrey et al., 1997).

The lipid composition of the POM collected by the SAPS in the present study was also analysed (Fisher E.H., pers. comm.). Below the chlorophyll *a* maximum, the lipid composition was similar between sites, although there were two main differences.

1) Higher concentrations of alkenones at M6 in comparison to M5 suggest there were more haptophytes (Volkman et al., 1998) at this station than at M5. The carotenoids 19'-hexanoylofucoxanthin and 19'-butanoylofucoxanthin are found in two Classes of haptophytes – Prymnesiophytes and Coccolithophores. An exception in the Prymnesiophytes are the species in the Family Isochrysidaceae; they also contain fucoxanthin, but not 19'-hexanoylofucoxanthin and 19'-butanoylofucoxanthin (Jeffrey and Wright, 1994). The lower concentrations of 19'-hexanoylofucoxanthin and 19'-butanoylofucoxanthin at M6 than at M5 may suggest the enhanced concentrations of alkenones found at M6 were derived from Isochrysidaecean haptophytes and therefore they may also have contributed to the higher fucoxanthin concentrations found at M6.

2) A higher concentration of the sterol $C_{28}\Delta^{5,22}$ was found at M5. This sterol is a marker of haptophytes and diatoms (Volkman et al., 1998) and is consistent with the enhanced 19'-hexanoylofucoxanthin (biomarker of prymnesiophytes and coccolithophores) concentrations found at M5.

A prominent difference in pigment concentration and composition of the POM sampled in the deep waters above the seabed at each site is consistent with the lack of observable phytodetritus at M6 (Wolff, 2006) (Fig. 5.12). The absence of chlorophyll a in the deep SAPS sample from M6 suggests there was no fresh material in suspended POM above the sea floor. Only two carotenoids previously shown to be important to holothurian reproduction (present study, Chapter 2), 19'-hexanoylofucoxanthin and alloxanthin, were found in the POM above the seafloor at benthic station M6. The lipid composition of this POM also shows a large contrast between sites. Higher concentrations of all fatty acids and sterols were found at M5 than at M6 (Fisher E.H., pers. comm.). β-hydroxy acids are derived mainly from bacterial sources (Volkman et al., 1998) and were found at M5, but were absent at M6.

The phytopigments arriving at the seafloor at M5 are derived from the flux of material from a wide area to the north and northeast of the Crozet Islands; the eastward flow of water from the north of the islands deposits the export flux of the bloom to the east. This is supported by the higher amount of material found in the deep (3000m) sediment trap in comparison to the relatively shallower sediment trap (2000m) at M5 (Wolff, 2006; Salter, 2007). The benthic station M6 to the south in the HNLC region is thought to receive little flux, with differing phytoplankton community composition (Wolff, 2006). The sediment trap data showed there was a sustained large flux of material from January 2005 through to May 2005 at M5 and a very short (two week), but large flux at M6 in January 2005 (Wolff, 2006). The flux period at M6 may have been longer – the sediment trap record does not precede January 2005. The short period of high mass flux at M6 was also observed at a shallower station M2, also located in the HNLC region to the South of the Islands (Wolff, 2006). That M6 and M2 received a short, high

flux of material suggests the HNLC region to the south of the Crozet Islands is not permanently oligotrophic. It is not known if this short, large flux event is typical of the region on an annual or regular basis.

An anti-cyclonic flow around the Crozet plateau advects a small filament of primary production (determined though satellite chlorophyll a data) southwards around the western side of the plateau (Pollard et al., 2007b; Venables et al., 2007). It has been suggested that this filament of high chlorophyll observed in the surface waters between October and end of November was received in the sediment trap at M6 late December early January (assuming phytodetritus settles at 100-200m d^{-1} (Diercks and Asper, 1997)) (Venebles, H., pers. comm.). This hypothesis is not supported by the phytoplankton community found in the deep sediment trap at M5 and M6. If the short high flux of material at M6 had originated from a filament of chlorophyll a enhanced water from the North, both sites would have similar dominant phytoplankton species in their respective sediment traps. This was not the case. The OM flux at M6 was dominated by the diatom *Fragilariopsis kerguelensis*, and at M5 by another diatom *Eucampia antarctica*, although towards the end of the flux profile at M5, *E. antarctica* became less important and *F. kerguelensis* began to increase in abundance (sediment trap composition; Salter, 2007). It is important to note that although *Phaeocystis* spp. may have contributed to the flux of organic matter, this genus is difficult to enumerate and quantify in the OM collected by sediment traps, as there is no mineralised component to the cell. It is probable the short high mass flux event at M6 derived from the small chlorophyll a peak observed in the surface waters in the HNLC region in December (Pollard et al., 2002; Venables et al., 2007)

The similarity of sediment pigment biochemistry at the two benthic sites around the Crozet Islands, and the significant differences in samples taken in the austral summers of 2004/05 and 2005/06, suggests there were temporal, but not spatial differences in the sediment pigment composition. Zeaxanthin and alloxanthin were the most abundant carotenoids in the top 5mm sediment during cruise 1

(December 2004 to January 2005). These are biomarkers of cyanobacteria and cryptophytes (nanoplanktonic flagellates that may be found in endosymbiotic association with organisms such as the red ciliate *Mesodinium rubrum*; (Jeffrey et al., 1997, respectively. In contrast, cruise 2 (December 2005-January 2006) had low concentrations alloxanthin and very low levels of zeaxanthin. In addition, four extra pigments were observed in the austral summer of 2005/04, which were absent in the summer of 2005/06. These pigments and their associated phytoplankton were 19'-hexanoylofucoxanthin (*Phaeocystis* sp. and other Prymnesiophytes), violaxanthin (chlorophytes, prasinophytes and eustigmatophytes), diatoxanthin (diatoms and dinoflagellates) and β-carotene (universal phytoplankton indicator). Although these sediment samples were taken at a comparable time of year, differences in sediment pigment biochemistry between sites may have been more apparent if the sediments were sampled at other times. For example, following the short high mass flux event at M6 in January 2005, or during the extended large flux of OM to M5 between January and May 2005 (3000m sediment trap data, Wolff, 2006).

Chlorophyll *a* concentrations in the sediment were similar between cruises, but 3 times greater at M5 than at M6. This corresponds with the findings of the ANTARES 1 programme, which recorded higher chlorophyll *a* concentrations at Station 10 (directly comparable to M5) than at Station 8 (northeast of M6 in the HNLC region) (Riaux-Gobin et al., 1997). The chlorophyll *a* concentrations determined in the present study were notably higher than those reported from the ANTARES samples, which were taken in April-May 1993, well after the phytoplankton bloom period (Riaux-Gobin et al., 1997). Total organic carbon (TOC) in the surficial sediments (top 1cm) of M5 were marginally higher than at M6, while values for total nitrogen were similar between sites (Hughes et al., 2007). TOC and TN values did not reflect the higher fluxes of organic material at M5 than at M6, which is surprising since chlorophyll *a* concentrations were three times greater at M5. Similar invariant TOC values have been recorded at the PAP over the period 1996-1998, despite significant differences in the concentrations of labile organic compounds (Ginger et al., 2001).

Pigment concentrations in the sediment at M5 during cruise 1 were higher than at M6 (with the exception of diadinoxanthin and phaeophytin, which were similar), suggesting that M5 received a higher flux of fresh OM than M6. During cruise 2, a clear between-site difference in individual pigment concentrations was only apparent for chlorophyll *a* (greater at M5) and phaeophytin (greater at M6). Concentrations of other pigments (excluding chlorophyll *a*) were similar at M5 and M6, which at first glance may suggest the supply of these pigments at each site was similar. However, this is not supported by the pigment composition and concentration of the POM sampled above the seafloor and the sediment chlorophyll *a* concentrations. Lipids found in the sediment during cruise 2 showed similar trends to the phytopigments; these had similar or even greater concentrations at M6 compared to M5 (Fisher E.H., pers. comm.).

The similarity in the concentrations of pigments (excluding chlorophyll *a*, which was three times greater at M5 than at M6) and lipids found in the sediment may be attributed to the fauna associated with the sediment. Holothurian abundance, biomass and dominant species differed considerably between M5 and M6, according to trawl data and photographic evidence (Wolff, 2006). Holothurian biomass alone was more than three times greater at M5 than at M6 – an average value of 14068 g(WW) per hectare of holothurians was found at M5, compared to 3687 g(WW) per hectare at M6 (Wolff, 2006). Dominant holothurian species at M5 were *Pseudostichopus villosus*, *Abyssocucumis abyssorum*, *Peniagone* spp. and *Psychropotes longicauda*; at M6 Peniagone spp., *Benthodytes* spp. and *Psychropotes longicauda* were dominant (*Peniagone* species were different at each site – Ian Cross pers. comm.). The rate of sediment re-working has been shown to be affected by the benthic community (Bett et al., 2001). It is probable the enhanced biomass and abundance of the benthic community at M5 controlled pigment and lipid concentrations in the sediment by assimilation and re-working of the sediment, potentially creating transformation products not identified by the methods used in the present study. For example, metabolites of fucoxanthin include Fucoxanthinol, Isofucoxanthinol and loliolide (Repeta and Gagosian,

1982; Repeta and Gagosian, 1984; Sinninghe Damsté and Koopmans, 1997). Spectral information and standards for such derivatives are not readily available to enable their quantification using the method of the present study. The determination of such compounds would require the use of an HPLC-MS (High Performance Liquid Chromatography – Mass Spectrometer).

A decrease of phytopigment concentration with sediment depth was not observed at either M5 or M6 during cruise 2, although average concentrations for the 0.5 to 1cm section of sediment were more variable. Chlorophyll *a* specifically was highly variable in the lower section of sediment at both stations. This is in agreement with some studies of phytopigment concentrations through sediment depth (Witbaard et al., 2000) but contrasts with previous studies (present study - chapter 4, Riaux-Gobin et al., 1997). A study of depth-related changes of protein and lipid concentrations through the sediment show high variability between cores and may relate to the distribution and activity of the benthic community (Santos et al., 1994). The high variability of the pigments in the 5-10mm section of sediment at M5 and M6 may reflect the spatial variability in concentrations at depth. This in turn may be related to low rates of vertical mixing as observed at the PAP in 2000 using ^{13}C as a tracer (Witte et al., 2003).

5.3.2 Chlorophyll a *in the gut sediments of holothurians*

Abyssocucumis abyssorum gut sediment chlorophyll *a* concentration was an order of magnitude greater than in any other species sampled at M5. *Abyssocucumis abyssorum* leaves distinct traces on the seafloor and exhibits a run and mill (foraging) behaviour (Kaufmann and Smith, 1997). This species may also be a suspension feeder in addition to deposit feeding (pers obs., Lauermann et al., 1997). During a high flux period at a site in the NE Pacific, the concentration of excess ^{210}Pb in the gut sediment of *A. abyssorum* indicated that approximately 91% derived their nutrition from material similar to that in the overlying sediment traps (Lauermann et al., 1997). At M5, flocculent green aggregates were observed

on the sediment surface on the images taken with the Robust BIOdiversity lander (ROBIO) and the Wide-Angle Seabed Photography system (WASP, Wolff, 2006, Fig. 5.12). During periods of increased tidal flow (tidal-driven currents of up to 31 cm s^{-1}) the phytodetritus was re-suspended. During one WASP deployment at M5, no phytodetrital patches were observed on the seabed but there were large amounts of suspended particles above the sea floor (Wolff, 2006). Presumably, this flocculate green material was the same as that sampled with the deep SAPS at M5. This material had high chlorophyll *a* concentration (11.6 ng L^{-1}). Suspension feeding by *A. abyssorum* allowed the species to feed on chlorophyll *a*-rich POM as shown by the elevated chlorophyll *a* concentration in its gut. Detailed examination of the WASP footage from M5 (Fig. 5.13) shows a specimen of *A. abyssorum* (identification confirmed by Henry Ruhl, pers. comm.) exhibiting suspension feeding behaviour (photo from Owen, 2007).

The large, but variable chlorophyll *a* concentrations in the gut sediments of *Amperima robusta* and *Peniagone* spp. at M5 suggest these species feed on fresh material when they can find it. The chlorophyll *a* concentrations were variable as one (*Peniagone* spp.) or two (*A. robusta*) samples out of a total of four and three respectively contained very high chlorophyll *a* values. *Peniagone diaphana*, *P. affinis* and *Amperima rosea* have been shown to be selective feeders through $\delta^{15}N$ analyses (Iken et al., 2001) and their gut sediment chlorophyll *a* concentrations (*P. diaphana* and *A. rosea*, present study, Chapter 4; Wigham et al., 2003). *Peniagone vitrea* can swim, which may facilitate the location of food. This 'searching behaviour' decreases when food availability is high (Kaufmann and Smith, 1997). *Amperima rosea* exhibits a high tracking rate of 110 cm^2 d/m^2, which was 20 times greater than other holothurians during the same period at the Porcupine Abyssal Plain (Bett et al., 2001). Both *Peniagone* and *Amperima* spp. have a velum that is often positioned into the current, which may enable them to be transported by currents to search for fresh material (Gebruk, 1995; Bluhm and Gebruk, 1999).
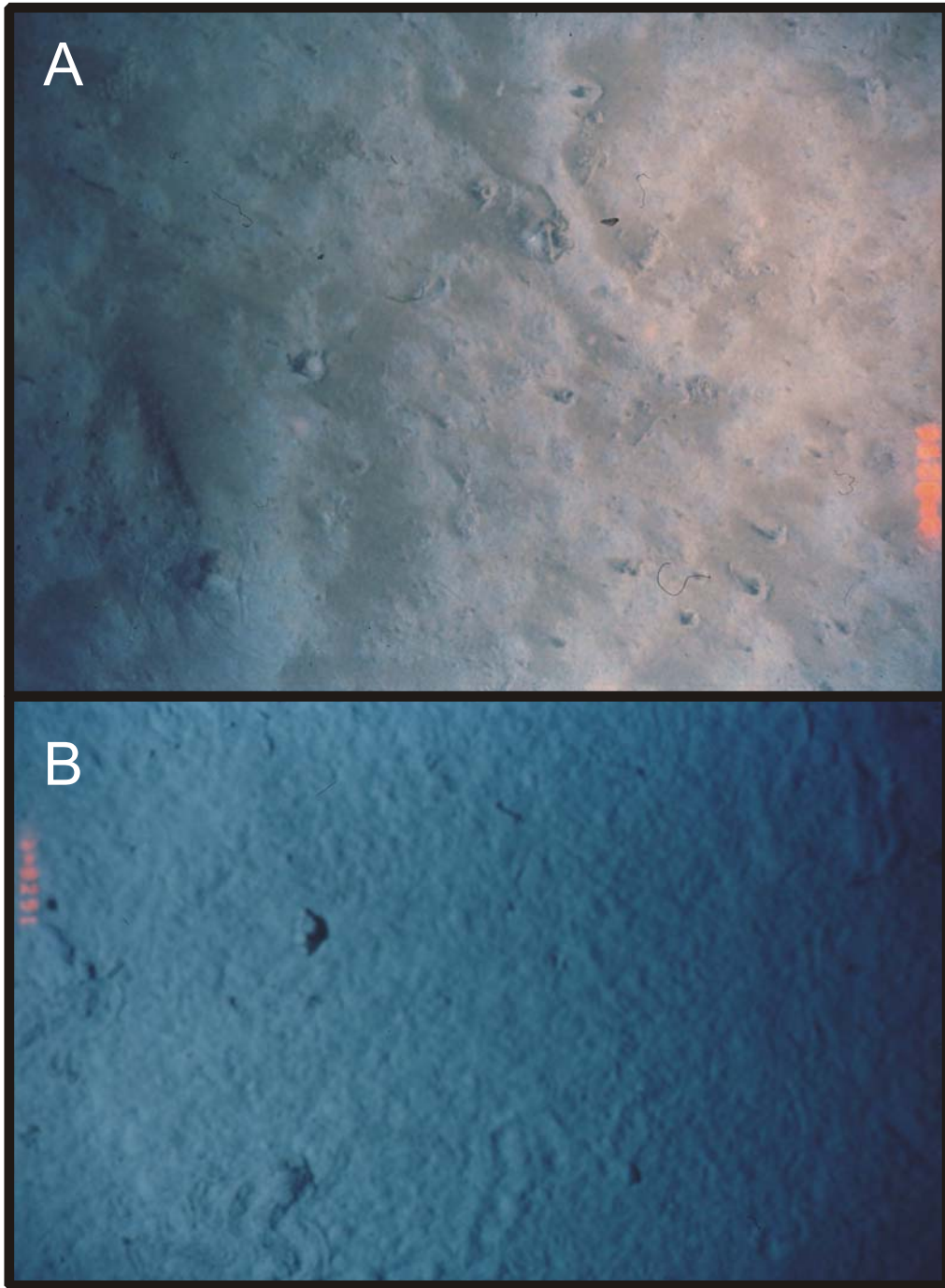
Figure 5.12 WASP photographic footage of the seafloor at M5 (A) clearly showing phytodetrital patches (area shown = 5.4 m^2) and M6 (B) with no phytodetrital material (area shown = 5.22 m^2) during Crozet cruise 2 (January 2005 to December 2006). (Photo from Owen, 2007)

Figure 5.12 WASP photographic footage of *Abyssocucumis abyssorum* exhibiting filter feeding behaviour at benthic station M5 during Crozet cruise 2 (January 2005 to December 2006). (Photo from Owen, 2007)

Oneirophanta mutabilis and *Scotoplanes globosa* have low chlorophyll *a* gut sediment concentrations in comparison to other species sampled at M5 (excluding *Benthodytes* sp., which had similarly low chlorophyll *a* gut sediment concentrations). *Oneirophanta mutabilis* and *S. globosa* can feed selectively on fresh material (Lauermann et al., 1997; Miller and Smith, 2000; Iken et al., 2001). Phytodetritus occurs at benthic station M5 and so species should have enhanced chlorophyll *a* concentrations in their gut contents. *Scotoplanes globosa* exhibits run and mill (foraging) behaviour and aggregates around patches of phytodetritus (Smith and Hamilton, 1983; Gutt and Piepenburg, 1991; Kaufmann and Smith, 1997). *Oneirophanta mutabilis* has papillae that allow it to 'walk' on the sediment in search of food. It can take advantage of increased food supply over *Pseudostichopus villosus* and *Psychropotes longicauda* (present study, Chapter 4; Neto et al., 2006). It is difficult to explain why these two species, which have been shown to be selective feeders, have similar or lower gut sediment chlorophyll *a* concentrations to species that feed on poorer quality food (i.e. *Psychropotes longicauda* (less selective) and *Pseudostichopus villosus* (sub surface feeder) (present study, Chapter 4; Iken et al., 2001)). A higher gut sediment chlorophyll *a* concentration has previously been recorded for *O.*

mutabilis (2.60 µg gDW^{-1}, present study), suggesting this species sampled at M5 was not at capacity (i.e. the species can only forage and handle a certain amount of fresh material at one time). However, it is more likely that the observed flocculent 'fresh' material at M5 (Wolff, 2006), which is easily fed upon by suspension feeding *Abyssocucumis abyssorum* and more easily located by *Peniagone* spp. and *Amperima robusta*, may not have settled completely on the sea floor in the M5 area (as demonstrated by the lack of visible phytodetritus on the cores). The flocculent material may be transported by the currents, so that a portion settles, but not in large enough patches, or for long enough for *O. mutabilis* and *S. globosa* to locate and have time to process. The observation of re-suspended phytodetritus (Wolff, 2006) supports this idea.

All species sampled at both sites (*Abyssocucumis abyssorum*, *Peniagone* spp., *Benthodytes* sp. and *Psychropotes longicauda*) had greater chlorophyll *a* gut sediment concentrations at M5 than at M6. This was particularly obvious at M5 for *A. abyssorum* and may be explained by the lower chlorophyll *a* sediment concentration at M6, the lack of chlorophyll *a* in the deep M6 SAPS sample and by the absence of visible phytodetritus (Wolff, 2006). *Molpadia blakei* had no chlorophyll *a* in its gut sediment, as observed at the PAP (present study, Chapter 4; Wigham et al., 2003). This species is a subsurface indiscriminate feeder (Miller and Smith, 2000; Iken et al., 2001).

5.3.3 Carotenoids in holothurian gut wall and ovaries – M5

Carotenoids were found in the gut wall and ovaries of all species sampled, with the exception of *Abyssocucumis abyssorum*. This species has a very thin gut wall, which was difficult to sample. Echinenone and β-carotene were often found in the greatest concentrations in the gut wall and ovaries of all the holothurians sampled at M5. Echinenone is found in many echinoderm species, especially in their ovaries. It can be metabolised from β-carotene (Tsushima and Matsuno, 1990b; Tsushima et al., 1993b; Matsuno and Tsushima, 1995) and occurs in the gut

epithelium via its precursor β-isocryptoxanthin (Tsushima et al., 1993b; Plank et al., 2002). Echinenone can then be converted to canthaxanthin (Matsuno and Tsushima, 1995). The metabolism of these carotenoids is supported by their occurrence in the gut wall of *A. abyssorum*, but not the sediment or POM. Converting β-carotene through to echinenone and canthaxanthin increases the anti-oxidant activity of the carotenoid, by increasing the number of conjugated double bonds (Chapter 1, Figure 1.1) (Di Mascio et al., 1991). This is beneficial for the developing larvae, since it quenches oxygen free radicals released by rapid metabolism that may otherwise cause mutagenesis (Bendich and Olson, 1989; Di Mascio et al., 1991; Olson, 1996; Linan-Cabello et al., 2003). It should be noted that the concentrations of echinenone, canthaxanthin and β-carotene were most variable in the gut walls and ovaries of *A. abyssorum*. Variability in carotenoid concentrations has been related to the stage of sexual maturity (Funk and Hobson, 1991). Therefore, the variability of these carotenoids in holothurians may be attributed to the asynchronous reproduction. Continuous reproduction is the dominant pattern in deep-sea echinoderms (Gage and Tyler, 1991) and has been reported for the species analysed in the present study (Tyler et al., 1984; Tyler et al., 1985; Tyler and Billett, 1987; Tyler et al., 1987), with the exception of *Scotoplanes globosa*, *Benthodytes typica*, *Pseudostichopus villosus* and *Abyssocucumis abyssorum*, whose reproductive biology is unknown.

The gut wall MDS ordination plot shows some species clustering together, reflecting the high percentage contributions of either zeaxanthin (*Peniagone* spp.), or alloxanthin and β-carotene (*Oneirophanta mutabilis*). The absence of echinenone in *Scotoplanes globosa* gut wall samples led to the clustering of these samples. It is possible that the conversion of β-carotene does not occur in the gut wall of *S. globosa*, but in its ovaries, since echinenone is present in the ovaries. *Psychropotes longicauda* also showed species specific clustering because of the enhanced contribution of 19'-butanoylofucoxanthin. This suggests *P. longicauda* does not discriminate between carotenoids when assimilating organic compounds, because the concentration of 19'-butanoylofucoxanthin in the sediment and the POM above the seabed was high.

Enhanced concentrations of 19'-butanoylofucoxanthin in the ovaries of *A. abyssorum* may also indicate a lack of discrimination in its assimilation of the carotenoids found in the diet. 19'-butanoylofucoxanthin concentration was high in the POM above the seabed at M5 and this species may feed directly on this material (see 5.3.2). *Peniagone* spp. ovarian samples were tightly clustered because of the high percentage contribution of zeaxanthin in the samples. Zeaxanthin was found in the sediment in 2004, but in a much lower concentration in 2005 (this may be related to the supply or to the assimilation by the fauna). Zeaxanthin does not contribute to such a high percentage (>20% in *Peniagone* spp.) of the total in ovaries of the other species sampled, which suggests that *Peniagone* spp. selectively assimilates this carotenoid. *Oneirophanta mutabilis* may have shown selective assimilation as indicated by the high (>60%) percentage contribution of β-carotene and absence of 19'-butanoylofucoxanthin and 19'-hexanoylofucoxanthin in its ovaries.

5.3.4 Carotenoids in holothurian gut wall and ovaries –M6

Diatoxanthin concentration was high, but variable in the gut walls of *M. blakei* and *Psychropotes longicauda*. This carotenoid was found in low concentrations in the sediment and was absent in the POM above the seabed, suggesting these species selected diatoxanthin, although the high variability of this carotenoid in the gut wall samples indicates otherwise. Canthaxanthin, echinenone and β-carotene, were found in the gut wall and ovaries of *Peniagone* spp., *Benthodytes* sp., *Abyssocucumis abyssorum* (ovaries only) and *Psychropotes longicauda*. As discussed previously, these carotenoids occur in the ovaries of shallow water echinoderms and it is thought canthaxanthin and echinenone are converted from β-carotene to increase their anti-oxidant capabilities (Bendich and Olson, 1989; Tsushima and Matsuno, 1990b; Di Mascio et al., 1991; Tsushima et al., 1993b; Matsuno and Tsushima, 1995; Olson, 1996; Linan-Cabello et al., 2003). *Molpadia blakei* contains canthaxanthin and β-carotene in its ovaries, with other carotenoids

also contributing similar concentrations to the total. This may be attributed to *M. blakei* feeding on poorer food and not discriminating in its assimilation of the carotenoids in the sub-surface sediment. Sediment pigment concentrations were similar down to 1cm sediment depth in the present study. Another study in the region has shown a decrease in chlorophyll *a*, *b*, and *c* with sediment depth (down to 8cm), suggesting deeper sediment is pigment depleted (Riaux-Gobin et al., 1997).

5.3.5 Food supply affecting reproductive fitness?

As discussed in section 5.2.1, the supply of OM available to the benthic fauna was more limited at M6 than at M5 and this may explain the between site differences in the pigment biochemistry of individual holothurian species. Of the tissue samples of holothurians from M6, one gut wall sample from *Molpadia blakei* and *Peniagone* spp., and one ovarian sample of *Peniagone* spp., *Benthodytes* sp. and *Psychropotes longicauda* contained no carotenoids. All tissue samples from M5 contained carotenoids. Significant between-site differences in pigment concentrations of the gut wall and ovaries in holothurian conspecifics between sites were apparent. Most prominently, carotenoids important to echinoderm reproduction (canthaxanthin, echinenone and β-carotene; (Tsushima and Matsuno, 1990b; Matsuno and Tsushima, 1995) were found in greater concentrations in the ovaries of conspecifics from M5 than M6. This difference was most pronounced in *Peniagone* spp. and may by attributed to the collection of different species at each site. *Peniagone affinis* and *P. willemeösi* were dominant species at M6; *P. challengeri* and *Peniagone* sp. nov. were dominant at M5 (Ian Cross, pers comm.). Different food regimes appear to favour different species of the genus *Peniagone*. At an abyssal site in the northeast Pacific, changes in the dominant species of *Peniagone* have been correlated to climate fluctuations and OM supply to the seabed (Ruhl and Smith, 2004). These changes in *Peniagone* sp. may not have occurred directly through food supply. Other megafaunal species also changed in dominance, which may have forced a change in *Peniagone* spp. by competition

for resources. *Abyssocucumis abyssorum* ovarian pigment biochemistry reflected the increased supply of 19'-butanoylofucoxanthin, 19'-hexanoylofucoxanthin and diadinoxanthin at M5 (as seen in the POM above the seabed), by having a significantly greater concentration of these carotenoids in ovarian samples taken from M5. This suggests the ovarian carotenoid biochemistry of *A. abyssorum* can be influenced by changes in the composition of the OM supply to the seafloor.

Shallow-water studies have shown that the quantity as well as the quality of the carotenoids available to echinoderms can affect reproductive output. Increased supply of carotenoids enhances the colour of the roe in shallow water echinoderms, suggesting carotenoid concentrations in the ovaries are increased (George and Young, 1998; George et al., 2001; Mclaughlin and Kelly, 2001; George and Lawrence, 2002; Robinson et al., 2002). An increase in ovarian carotenoid concentration increases fecundity, larval maturation and survival in echinoderms (George and Young, 1998; George et al., 2001; Mclaughlin and Kelly, 2001; George and Lawrence, 2002). Greater carotenoid concentrations in the ovaries of species at M5 are a result of enhanced supply of the carotenoids at this station. If the same reproductive advantages are conferred on abyssal holothurians, those species found at M5 will have a higher reproductive output than those of their conspecifics at M6.

The carotenoid composition of the diet can also affect reproductive output in echinoderms. The oxygen free-radical quenching ability of carotenoids can vary greatly, depending on the structures of the carotenoids (Hirayama et al., 1994). Larvae of the sea urchin *Lytechinus variegatus* from parents fed on xanthophylls (oxygen containing carotenoids) were larger throughout development, developed faster, had higher survival rates and attained metamorphic competence faster than those fed just β-carotene. The numbers of juveniles originating from parents fed xanthophylls were also significantly higher (George et al., 2001). Changes in the carotenoid composition of the OM may therefore also have an affect on the reproductive output of the species at both sites. The selectivity or requirement for specific carotenoids by some holothurians (i.e. zeaxanthin by *Peniagone* spp. and

β-carotene by *Oneirophanta mutabilis*) may give them a reproductive advantage or disadvantage, depending on the specific carotenoids supplied in the OM. Species that can metabolise specific carotenoids to carotenoids with greater anti-oxidant properties may also gain a a reproductive advantage. However, apart from the conversion of β-carotene to echinenone and canthaxanthin (Tsushima and Matsuno, 1990b; Tsushima et al., 1993b; Matsuno and Tsushima, 1995), the metabolic pathways of carotenoid in echinoderms are unknown. Changes in the carotenoid composition in the supply of OM may also affect the reproductive output of holothurians that do not discriminate in their assimilation of carotenoids. Reproductive output in these species will depend on the oxygen free radial quenching ability of the carotenoids in the supply of OM to the seabed.

5.4 Conclusion

The quantity of organic matter reaching the seafloor differs between stations M5 and M6 (to the east and south of the Crozet Isles respectively), mirroring differences in the productivity of the overlying upper water column at each site. M5 receives a greater flux of material, as indicated by the sediment trap record and suspended phytodetritus found above the seabed, in comparison to M6 (Wolff, 2006). The POM above the seabed was fresher and contained more phytopigments at M5 than at M6 and this was reflected in the diet of some abyssal holothurian species, depending on their ability to take advantage of the often resuspended phytodetrital material. *Abyssocucumis abyssorum* fed directly on this phytodetrital material. *Peniagone* spp. and *Amperima robusta* were able to exploit this material when they could find it because of their feeding and foraging modes. *Oneirophanta mutabilis* and *Scotoplanes globosa* fed on the same material as other species that have previously been shown to be less selective in their feeding (present study - chapter 4, Neto et al., 2006). The resuspension of the phytodetritus at M5 (Wolff, 2006) did not allow *Oneirophanta mutabilis* and *Scotoplanes globosa* to find fresh phytodetrital patches. That M6 receives a lower flux of fresh material is highlighted by the diet of holothurians sampled at M6 in comparison to conspecifics specimens at M5. Similar between-site sediment pigment concentrations in December 2005 to January 2006 are attributed to the higher abundance and biomass of megafauna at M5. Phytopigment composition between the sites was also similar, although temporal differences were observed.

The relatively high concentrations of canthaxanthin, β-carotene and echinenone in the ovaries of the abyssal holothurians studied suggest that they may be important in reproduction. These carotenoids also showed the highest variability in concentrations, possibly related to the reproductive state of the specimens. The ovarian biochemistry of the holothurians is determined by the selectivity of the species and the supply of carotenoids to the sea floor. Greater carotenoid supply at M5 than at M6 is mirrored in the concentrations of carotenoids assimilated into the ovaries of species sampled at both stations (*Abyssocucumis abyssorum*,

Peniagone spp. and *Psychropotes longicauda*). Enhanced carotenoid concentration in the ovaries of echinoderms increases reproductive output and survival (George and Young, 1998; George et al., 2001; Mclaughlin and Kelly, 2001; George and Lawrence, 2002). Some holothurian species showed selectivity for specific compounds – *Peniagone* spp. for zeaxanthin and *Oneirophanta mutabilis* (and possibly *Pseudostichopus villosus*) for β-carotene. Other species, *Abyssocucumis abyssorum*, *Psychropotes longicauda* and *Molpadia blakei*, do not discriminate so highly between carotenoids and assimilate carotenoids available to them in their diet. Changes in the composition of the organic material supplied to the abyssal sea floor may affect holothurian reproductive output by 1) supplying specific carotenoids essential to specific holothurian species thus giving them a reproductive advantage, or 2) enhancing or decreasing reproductive output of the non-selective species by supplying carotenoids with contrasting oxygen free radical quenching abilities.

The timing and make-up of the phytoplankton bloom, planktonic interactions, and recycling and repackaging of organic matter can be affected by climate warming and increasing atmospheric CO_2 (Turner, 2002; Richardson and Schoeman, 2004; Orr et al., 2005). The present study suggests that the material arriving at the sea floor is dependent on the biogeochemistry of the overlying surface waters. The quantity and composition of this material could potentially exert a control on holothurian reproductive output. If this is true then global changes in upper ocean ecosystems will ultimately affect abyssal sediment community structure and diversity, initiating large community shifts as observed at the Porcupine Abyssal Plain in the northeast Atlantic (Billett et al., 2001) and at Station M, an abyssal time-series station in the northeast Pacific (Ruhl and Smith, 2004).

Chapter 6 – The link between diet and abyssal holothurian ovarian biochemistry – a synthesis of PAP and Crozet data

6.1 Introduction

The deep-sea benthos is a key component of the carbon cycle, affecting long-term bioturbation, remineralisation and sequestration rates of carbon over large areas of the Earth's surface (Ruhl, 2007). Deep-sea benthic megafauna affect the sequestration of carbon by re-distributing and reworking organic material (OM), as well as oxidising sediments through bioturbation. The rates of these processes can be affected by the community composition (Bett et al., 2001), which in turn reflect the supply of food (Smith et al., 1993; Kaufmann and Smith, 1997). Variability in the supply of food is also a major controlling factor in the population dynamics of benthic animals (Carney, 1989). Therefore, it is important to examine feeding adaptations and variations in the utilisation of OM, the quantity and composition of organic matter supplied to the seafloor, and the affect changing supply has on the biochemistry of the fauna dependent on it, in order to fully understand deep-sea ecosystem functioning.

The present study has so far examined the link between diet and ovarian biochemistry in abyssal holothurians at three sites; the Porcupine Abyssal Plain (PAP) and two benthic sites around the Crozet Islands (M5 and M6). A temporal comparison at the PAP, NE Atlantic, has shown 1) the supply of organic material can affect the diet of holothurians, depending on their feeding adaptations and 2) holothurian ovarian biochemistry can be affected by compositional differences in the OM reaching the seafloor, although the extent of this influence appears to differ between species. The two abyssal sites around the Crozet Islands, Southern Ocean, were investigated to compare contrasting OM supply on the diet and ovarian biochemistry of holothurians. The sites are only 460km apart, with no topographic boundary to separate them; however, they are subject to differing overlying primary productivity regimes. Therefore, differences can be ascribed to the composition and amount of organic matter reaching the sea-floor at each site.

The results showed that 1) the quantity of OM reaching the seafloor at each site differed, mirroring the overlying primary productivity regimes. This was reflected in the diet of some holothurian species, depending on their ability to take advantage of the fresh material. 2) The ovarian biochemistry of the holothurians sampled at both sites showed quantitative differences, mirroring the supply of OM to each benthic site.

The present chapter synthesises the data from all three sites, comparing the supply and composition of the OM and relating this to the diet and ovarian biochemistry of the holothurians. Holothurians that are common to the different sites are examined for their feeding selectivity (based on gut sediment chlorophyll *a* concentrations) and their ovarian biochemistry, in terms of quantitative comparisons and biochemical consistency between and within sites. Intra- and interspecies differences are discussed in relation to feeding modes, reproductive requirement and OM supply. Details on the methods of sample collection and analysis are given in Chapter 2.

6.2 Results

6.2.1 Comparison of phytopigments in the sediment

Only the sediment sampled during the second Crozet cruise (Dec. 2005 to Jan. 2006) will be examined here for comparison with the holothurian biochemistry – holothurians were not sampled during the first Crozet cruise. Concentrations of chlorophyll *a* in the top 5mm sediment for each sample site/period differed, with M5 containing the greatest concentration and the PAP in July 2005 the least (Fig. 6.1). The top 5mm sediment at M6 contained greater but variable chlorophyll *a* concentration than the same sediment fraction during either year at the PAP. Phaeophytin and diadinoxanthin were also found in greater concentrations at M5 and M6 than during either year at the PAP (Fig. 6.1).

All identified pigments were found at all four sample sites/periods with the exception of β-carotene, violaxanthin, 19'-butanoloxyfucoxanthin and 19'-hexanoloxyfucoxanthin, which were absent in the top 5mm sediment sampled at the PAP June 2004 (Fig. 6.1). With the exception of β-carotene, these carotenoids were found in greater concentrations at M5 and M6 than in July 2005 at the PAP. Zeaxanthin is the only pigment that was found in greater concentration in the top 5mm sediment at the PAP (in June 2004) than either M5 or M6 (Fig. 6.1).

Average concentrations of pigments in the 5 to 10mm section sediment at the PAP in 2005 were always lower than that recorded for the same section of sediment at M5 and M6 (Fig. 6.2). Alloxanthin, diatoxanthin, chlorophyll *a*, β-carotene, zeaxanthin and violaxanthin were observed in the 5 to 10mm sediment sampled at M5 and M6, but were absent from this section of sediment at the PAP in 2005 (Fig. 6.2).
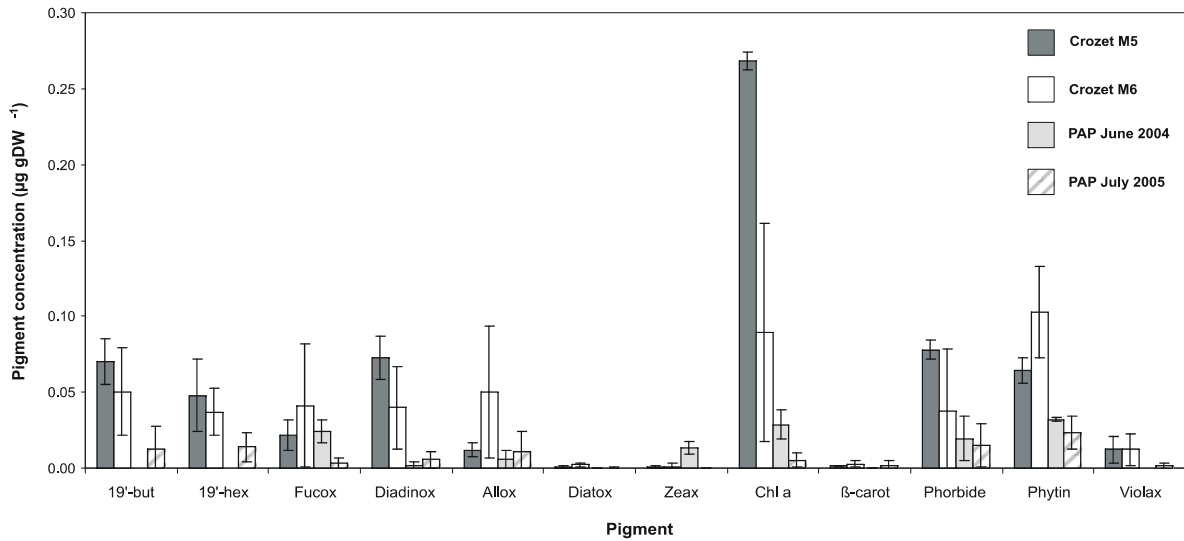
Figure 6.1 Average concentration (µg gDW^{-1} ± SD) of phytopigments found in the top 5mm sediment at M5 (dark grey), M6 (white) (both sampled in Dec 2005 to Jan 2006), PAP June 2004 (light grey) and PAP July 2005 (stripe). 19'-but = 19'-butanoyloxyfucoxanthin; 19'-hex = 19'-hexanoyloxyfucoxanthin; Fucox = fucoxanthin; Diad = diadinoxanthin; Allox = alloxanthin; Diatox = diatoxanthin; Zeax = zeaxanthin; Chl a = chlorophyll *a*; β-carot = β-carotene; Phorb = phaeophorbide; Phytin = phaeophytin; violax = violaxanthin.

Figure 6.2 Average concentration (µg gDW^{-1} ± SD) of phytopigments found in the 5 to 10mm sediment at M5 (dark grey), M6 (white) (both sampled in Dec 2005 to Jan 2006) and PAP July 2005 (stripe). 19'-but = 19'-butanoyloxyfucoxanthin; 19'-hex = 19'-hexanoyloxyfucoxanthin; Fucox = fucoxanthin; Diad = diadinoxanthin; Allox = alloxanthin; Diatox = diatoxanthin; Zeax = zeaxanthin; Chl a = chlorophyll *a*; β-carot = β-carotene; Phorb = phaeophorbide; Phytin = phaeophytin; violax = violaxanthin.
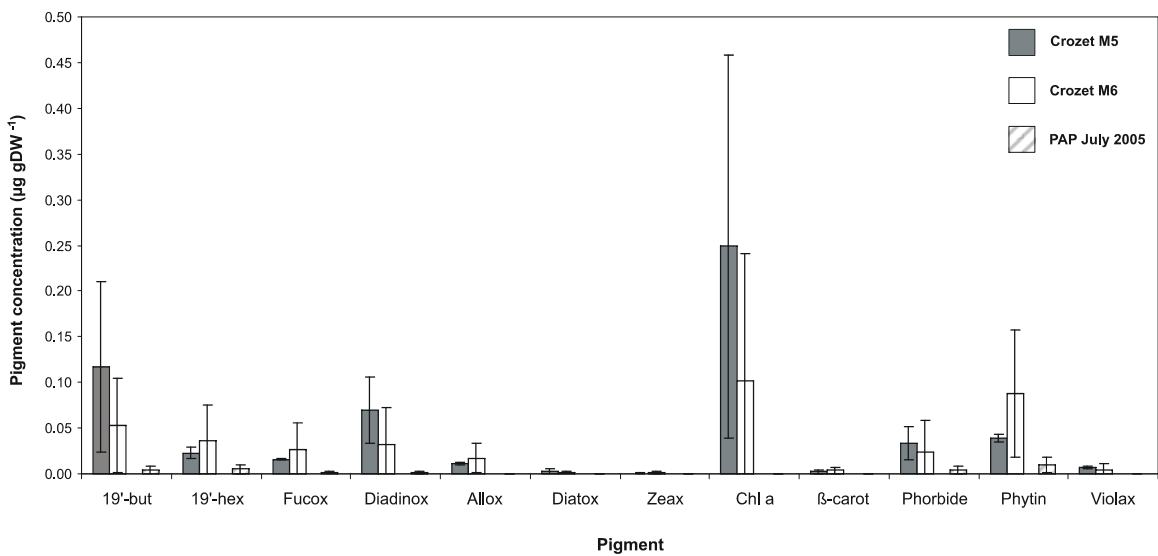
6.2.2 Comparison of holothurian feeding selectivity

No chlorophyll *a* was found in the gut sediment of *Molpadia blakei* sampled at the PAP in 2005, or at stations M5 and M6 around the Crozet Islands (Figure 6.3). Average gut sediment chlorophyll *a* concentrations were higher in species sampled at M5 in comparison to the gut sediment of the same species sampled at M6 and the PAP in both years (Figure 6.3). *Oneirophanta mutabilis* was an exception to this; M5 average gut sediment chlorophyll *a* concentration was greater than the average recorded for the PAP 2005 samples (t(8) = 2.58, P<0.05), but similar to the average concentration recorded in the PAP 2004 samples (t(12) = 1.18 P>0.05). A significant difference was seen between M5 and PAP 2005 gut sediment chlorophyll *a* concentration in *Pseudostichopus villosus* (W(9,5) = 50.5, P<0.05). The high variability of *P. villosus* chlorophyll *a* concentration at M5 is attributed to a single sample containing a relatively high concentration (18.88 µg gDW^{-1}). Without this sample the average gut sediment chlorophyll *a* concentration would be 1.47 (±1.28) µg gDW^{-1}. Gut sediment samples of *Amperima* spp. and *Peniagone* spp., at M5 had higher average chlorophyll *a* concentrations than those recorded in the PAP 2004/2005 samples, but they were not significantly different because of high variability (t(4) = 1.83, P>0.05 and W(4,5) = 15, P>0.05 respectively). *Psychropotes longicauda* gut sediment chlorophyll *a* concentration at the PAP in 2004 and 2005, M5 and M6 were significantly different (F(3,16) = 8.66, P<0.05) – with M5 having the highest concentration.

Peniagone spp. and *Psychropotes longicauda* gut sediment chlorophyll *a* concentrations at M6 were not significantly different from those recorded in samples from the PAP collected in 2004 (W(4,5) = 16.5, P>0.05; t(7) 2.09, P>0.05, respectively). Three out of four M6 *Peniagone* spp. gut sediment samples contained no chlorophyll *a*, the last sample contained high chlorophyll *a* concentration leading to the high variability seen in Figure 6.3. *Psychropotes longicauda* gut sediment concentration recorded at M6 was not significantly different to that at the PAP in 2005 (W(4,8) = 21, P>0.05).
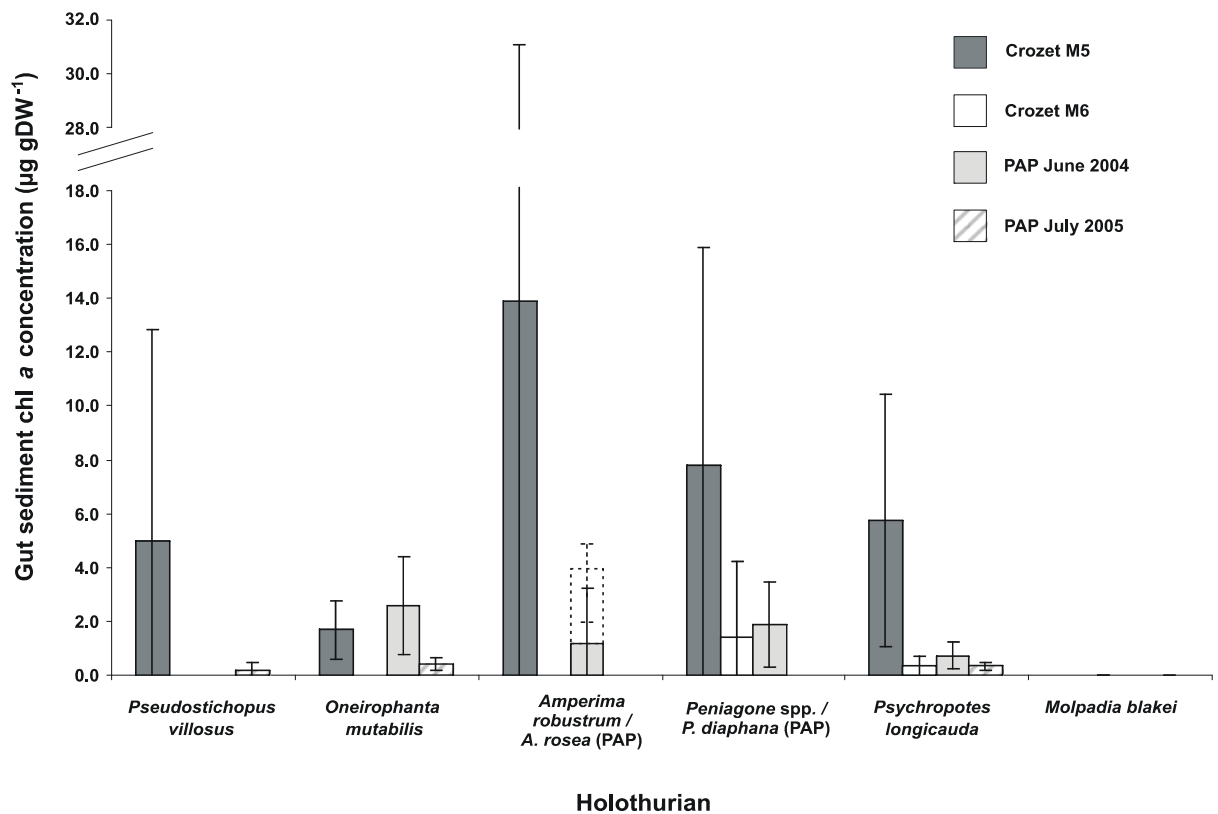
Figure 6.3 Chlorophyll *a* concentration (mean μg gDW^{-1} ± SD) in the gut sediment of holothurians sampled at M5 (dark grey), M6 (white), PAP June 2004 (light grey) and PAP July 2005 (stripe). Average *Amperima rosea* gut sediment chlorophyll *a* values excluding samples with no chlorophyll *a* shown with dotted line. (note break in y-axis to accommodate *Amperima robusta* data)

6.2.3 Comparison of holothurian pigment biochemistry

6.2.3.1 Quantitative intraspecies between-site differences

Concentrations of individual carotenoids found in the gut wall and ovaries of holothurian genera/species from the PAP and the two Crozet sites (M5 and M6) are plotted to show between-site comparisons (Figs. 6.4, 6.5, 6.6, 6.7, 6.8 and 6.9). Average concentrations of diadinoxanthin, alloxanthin, zeaxanthin and diatoxanthin were statistically greater (W(14,4) = 161, P<0.05) in the gut wall of *Amperima rosea* sampled at the PAP in 2004 than in *Amperima robusta* sampled at M5 (Fig. 6.4); concentrations of canthaxanthin, echinenone and β-carotene

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were not significantly different between sites (t(16) = 0.91, 0.37, 0.71 (respectively), P>0.05). Diadinoxanthin (W(16,5) = 216, P<0.05), alloxanthin (W(16,5) = 216, P<0.05), diatoxanthin (t(19) = 6.97, P<0.05), zeaxanthin (t(19) = 6.16, P<0.05) canthaxanthin (W(16,5) = 216, P<0.05) and echinenone (W(16,5) = 214, P<0.05) were all found in significantly greater concentrations in the ovaries of *Amperima rosea* in comparison to *Amperima robusta* (t-test, P>0.05). The concentration of β-carotene was not significantly different in the ovaries of the two *Amperima* species (t(19) = 0.3, P>0.05).

Diatoxanthin, zeaxanthin, echinenone and β-carotene were found in higher concentrations in the gut wall of *Peniagone* spp. at M5 than those at M6 or in *Peniagone diaphana* at the PAP in 2004 (Fig. 6.5). Only diatoxanthin was found in significantly different concentrations (W(4,6) = 12, P<0.05). In the ovaries, zeaxanthin, echinenone and β-carotene were found in higher concentrations in the M5 *Peniagone* spp. (Fig. 6.5), but this was not statistically significant (t(7) = 1.96, P>0.05; W(5,4) = 29, P>0.05; W(5,4) = 31, P>0.05 respectively) because of the high variability of the samples.

The carotenoid biochemistry of the gut wall of *Molpadia blakei* sampled at Crozet site M6 and the PAP 2005 show large differences in pigment composition (Fig. 6.6). Four carotenoids, 19'-butanoloxyfucoxanthin, alloxanthin, zeaxanthin and canthaxanthin were found in the gut wall of the species sampled at the PAP in 2005, but not in the samples from M6. Conversely, diatoxanthin was found in the M6 gut wall samples, but not the PAP 2005 specimens. β-carotene was found in the gut wall of specimens from both sites; the average concentration was higher in the M6 samples, but not significantly so (W(2,6) = 10.5, P>0.05). An increased number of carotenoids was observed in the ovaries of specimens from both sites in comparison to the gut wall. 19'-hexanoloxyfucoxanthin was observed in the ovaries of specimens from M6, but not the PAP in 2005 (Fig. 6.6). Alloxanthin, diatoxanthin and β-carotene were found in significantly greater concentrations in the M6 ovaries of *M. blakei* (t(6) = 4.67; t(6) = 8.30; t(6) = 4.30 (respectively), P<0.05); zeaxanthin and 19-butanoloxyfucoxanthin were found in significantly

greater concentration in the ovaries of *M. blakei* sampled at the PAP in 2005 (t(6) = 2.92; t(6) = 3.28, P>0.05).

Alloxanthin, diatoxanthin, zeaxanthin, echinenone and β-carotene were all found in greater average concentrations in the gut wall of *Oneirophanta mutabilis* of specimens from M5 than in those taken at the PAP in either June 2004 or July 2005 (Fig. 6.7). Of these however, only β-carotene was found in significantly greater concentration (W(8,4) 37, P>0.05). In the ovaries of *O. mutabilis*, β-carotene was found in concentrations that were not significantly different in specimens from M6, or the PAP in June 2004 and in July 2005 (F(2,13) = 0.29, P>0.05). The average concentrations of the carotenoids diadinoxanthin, alloxanthin, diatoxanthin, zeaxanthin, canthaxanthin and echinenone were all higher in the *O. mutabilis* ovarian samples taken at the PAP in June 2004. Of these, diadinoxanthin, alloxanthin and canthaxanthin were found in significantly greater concentrations (t(11) = 3.12; t(11) = 3.78; W(9,4) = 80 (respectively), P<0.05).

Echinenone and β-carotene were found in significantly greater concentrations in the gut walls of *Pseudostichopus villosus* sampled at M5, compared to the average concentrations in specimens from the PAP in 2005 (t(10) = 2.52; (10) = 3.80 (respectively, P<0.05). β-carotene occurred in greater concentration in the ovaries of this species sampled at M5, but was not significantly so (t(10) = 1.83, P>0.05). Despite the higher concentrations of 19'-hexanaloxyfucoxanthin, alloxanthin and canthaxanthin in PAP 2005 gut wall samples in comparison to those from M5 (Fig. 6.8), they were not significantly different (W(6,6) = 46; W(6,6) = 40; W(6,6) 50 (respectively), P>0.05).

Psychropotes longicauda is the only holothurian that was sampled at M5, M6 and the PAP in June 2004 and July 2005. Most carotenoids are found in similar concentrations in its gut walls at the different sites (Fig. 6.9). The exception to this is 19'-hexanoloxyfucoxanthin, which was found in significantly greater concentration (W(6,5) = 23, P>0.05) at M5 in comparison to the PAP in 2005

(19'-hexanoloxyfucoxanthin was absent from June 2004 samples). Echinenone was found in greater (but not significantly; $W(5,5) = 25$, $P>0.05$) concentrations from samples taken at M5 in comparison to the next greatest concentration of this carotenoid in PAP 2004 samples. Canthaxanthin, echinenone and β-carotene were found in the greatest concentrations in *P. longicauda* ovarian samples taken from M5, ($F(3,17) = 4.65$; $F(3,17) = 3.85$; $F(3,17) = 6.18$ (respectively, $P<0.05$). Ovarian samples of *P. longicauda* taken at the PAP in 2005 contained the lowest average concentrations of canthaxanthin, echinenone and β-carotene (Fig. 6.9).
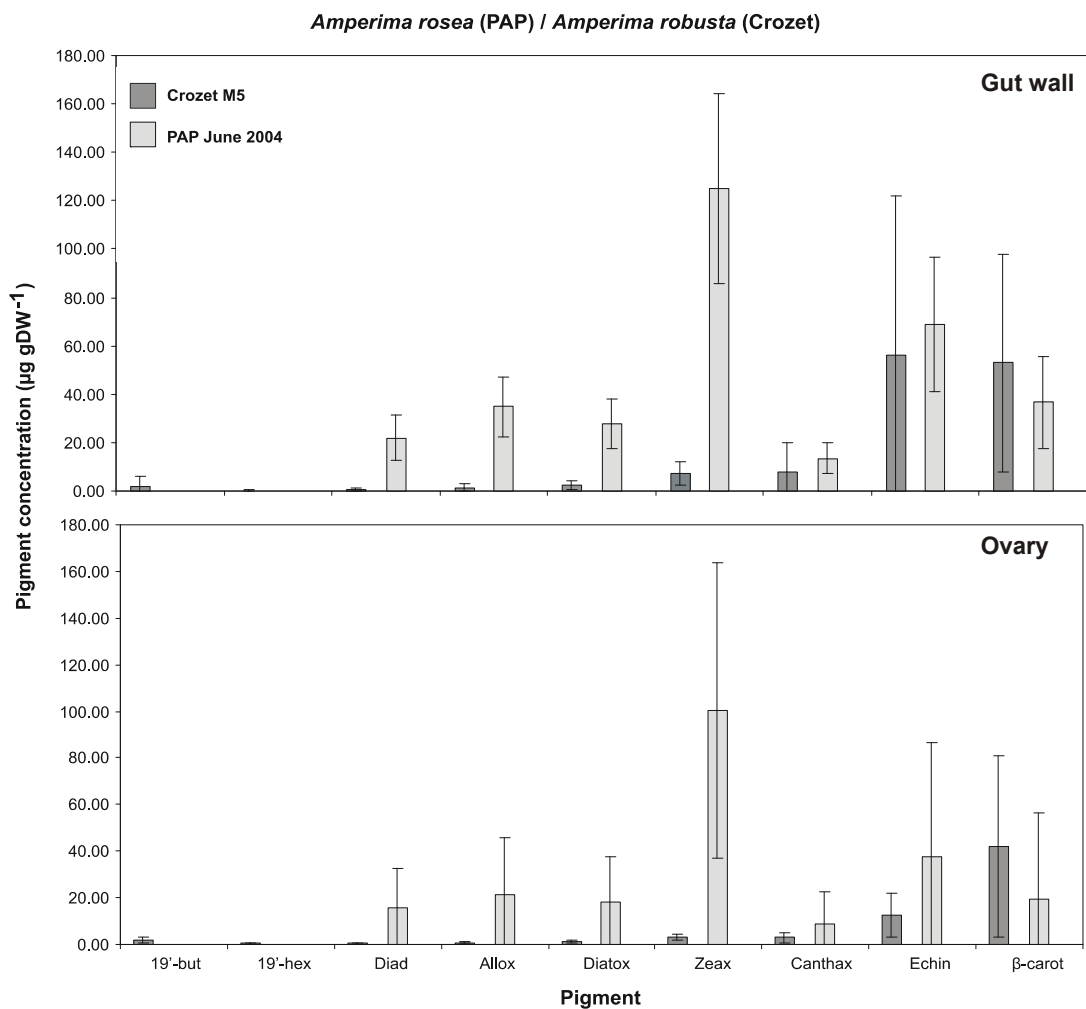
Figure 6.4 Carotenoid concentrations (mean μg gDW^{-1} ± SD) in the gut wall and ovary of *Amperima rosea* (PAP) and *Amperima robustrum* (Crozet) sampled at M5 (dark grey) and the PAP June 2004 (light grey). (note different scales on y-axis)
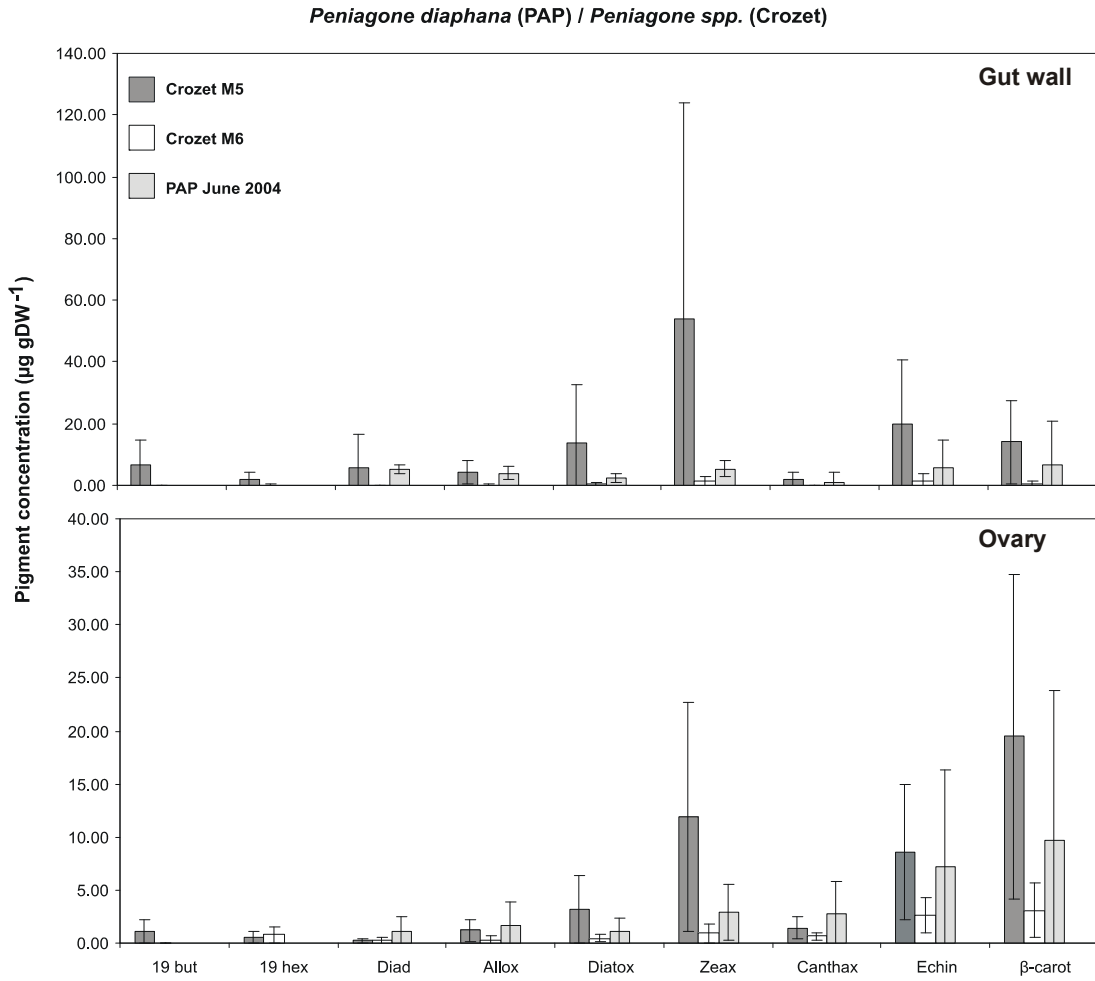
Figure 6.5 Carotenoid concentrations (mean μg gDW^{-1} ± SD) in the gut wall and ovary of *Peniagone diaphana* (PAP) and *Peniagone* spp. (Crozet) sampled at M5 (dark grey), M6 (white) and PAP June 2004 (light grey).

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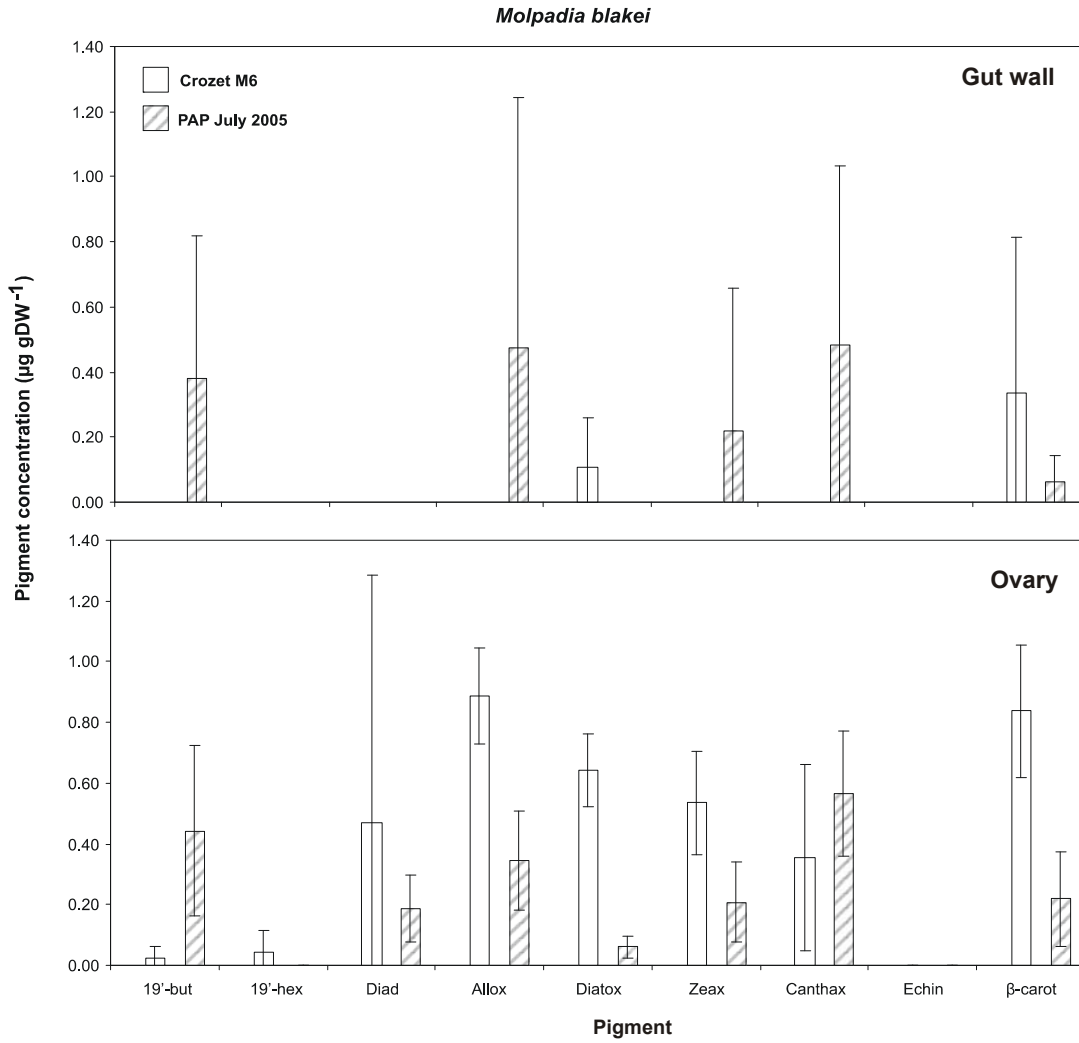
Figure 6.6 Carotenoid concentrations (mean µg gDW^{-1} ± SD) in the gut wall and ovary of *Molpadia blakei* sampled at M6 (white) and PAP July 2005 (stripes).

Oneirophanta mutabilis
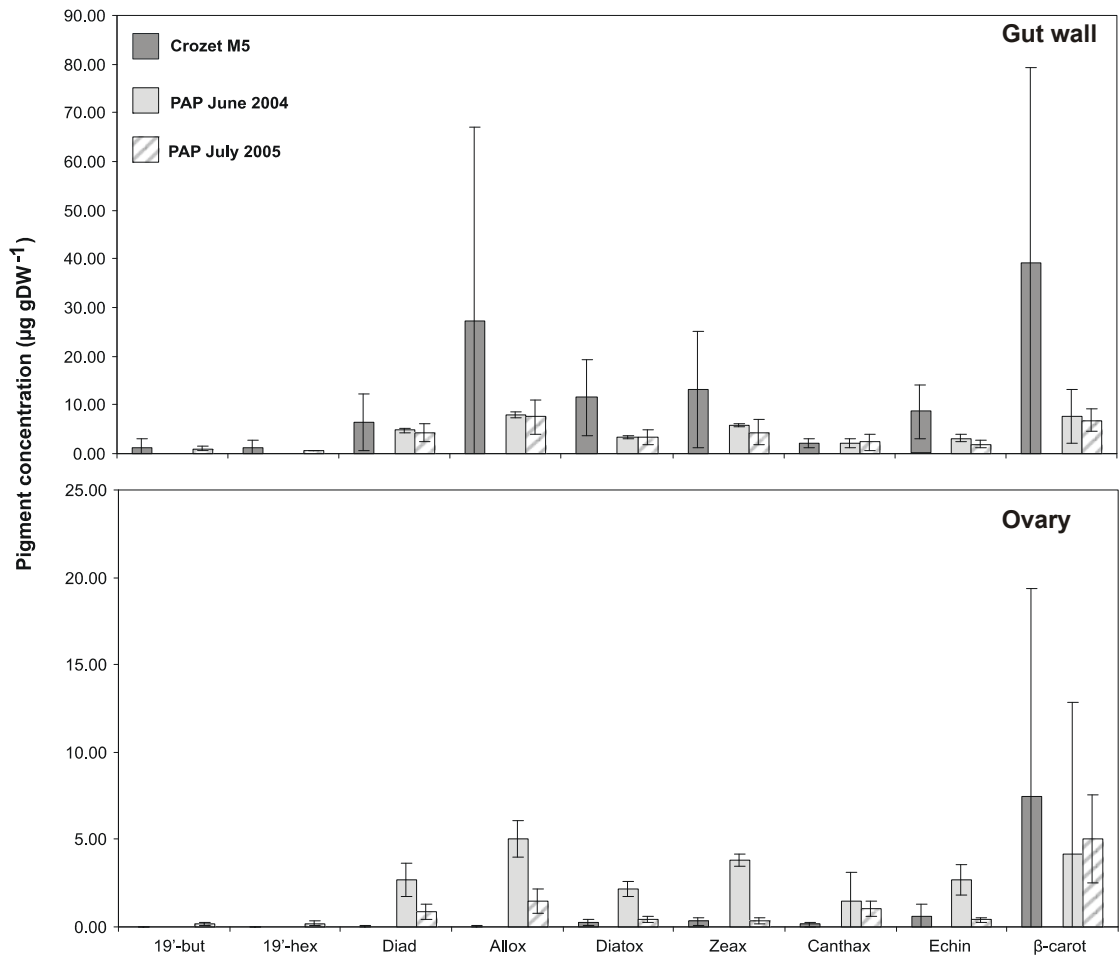
Figure 6.7 Carotenoid concentrations (mean μg gDW^{-1} ± SD) in the gut wall and ovary of *Oneirophanta mutabilis* sampled at M5 (dark grey), PAP June 2004 (light grey) and PAP July 2005 (stripes). (note different scales on y-axis)

Pseudostichopus villosus
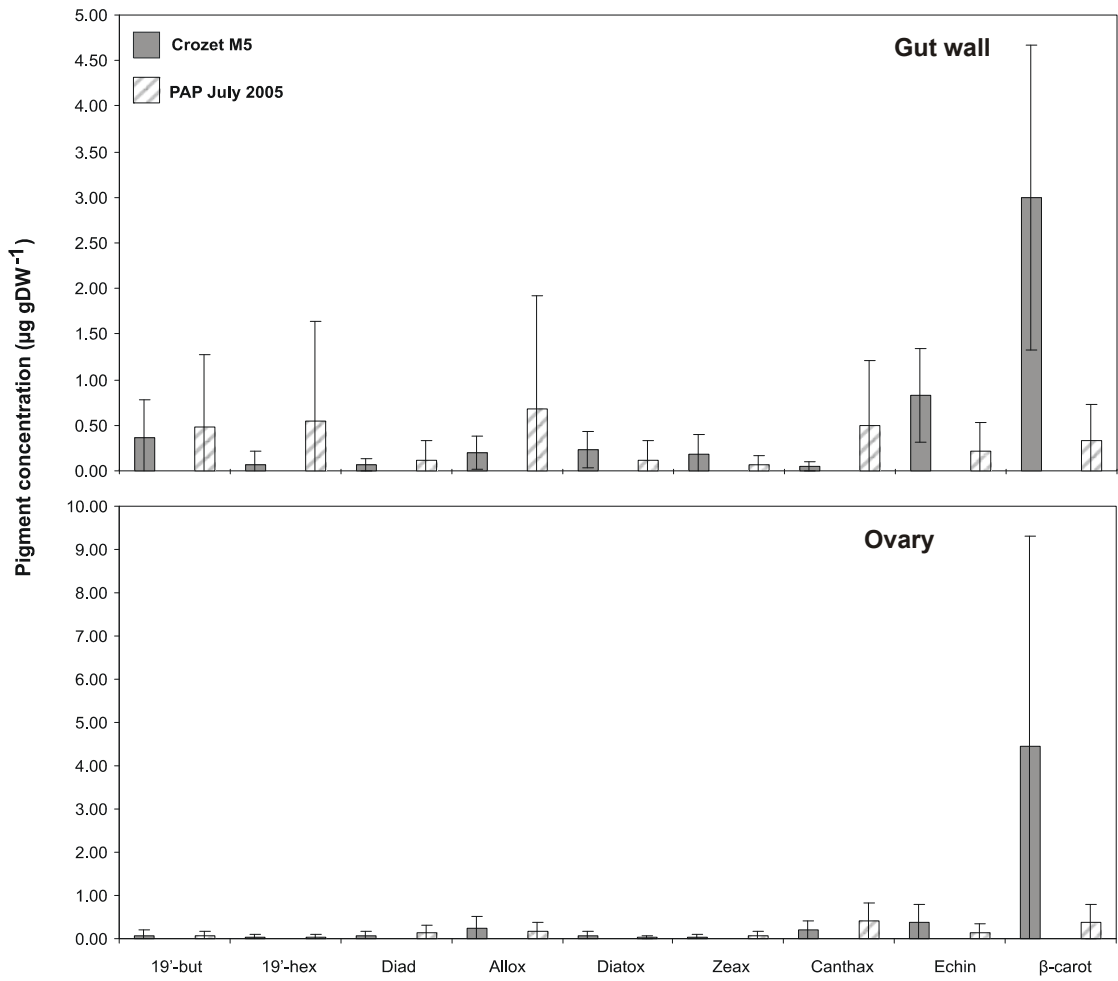
Figure 6.8 Carotenoid concentrations (mean µg gDW^{-1} ± SD) in the gut wall and ovary of *Pseudostichopus villosus* sampled at M5 (dark grey) and PAP July 2005 (stripes). (note different scales on y-axis)
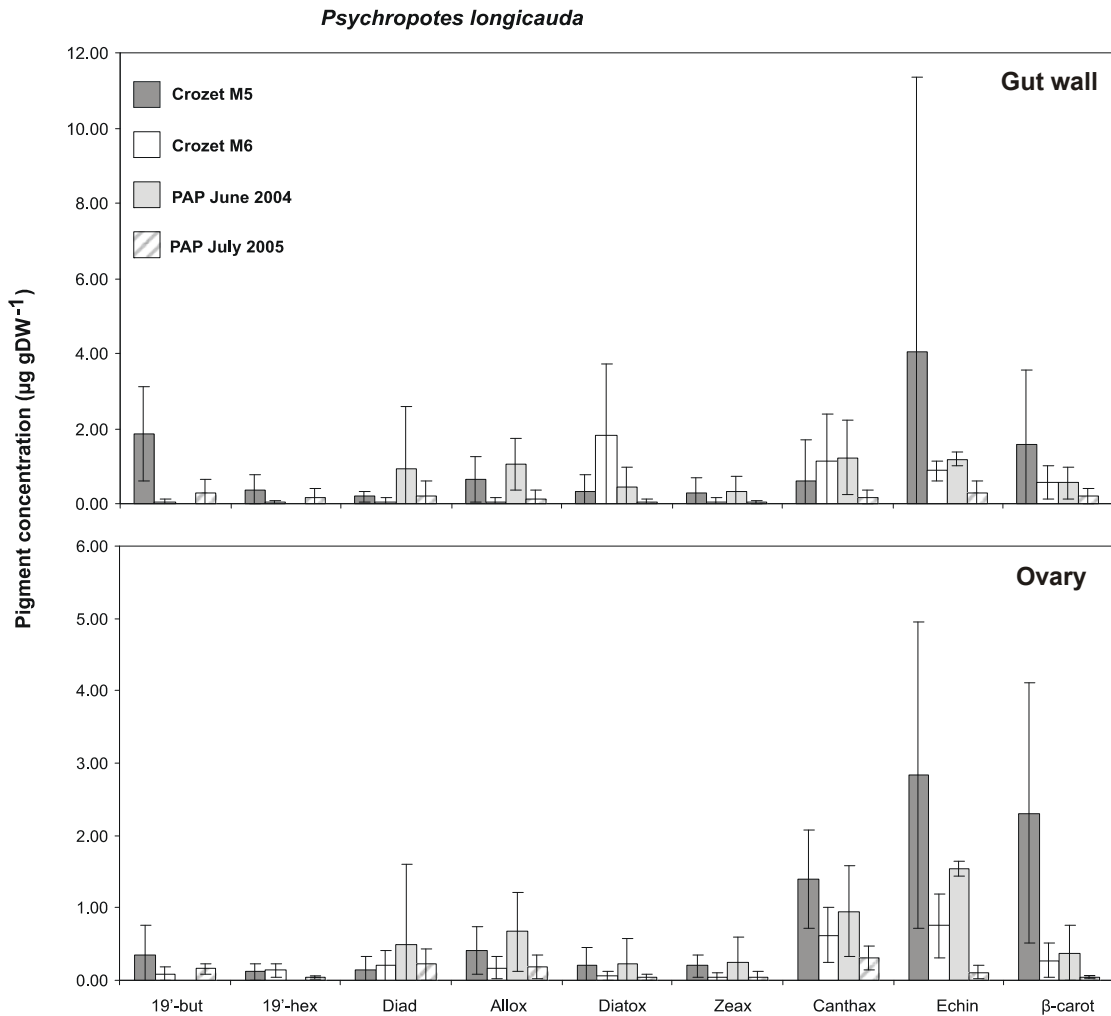
Figure 6.9 Carotenoid concentrations (mean µg gDW^{-1} ± SD) in the gut wall and ovary of *Psychropotes longicauda* sampled at M5 (dark grey) M6 (white), PAP June 2004 (light grey) and PAP July 2005 (stripes). (note different scales on y-axis)

6.2.3.2 Compositional differences and intra-specific consistency between years

Pigment percentage contributions in the gut wall and ovaries of each specimen of species common to the different sites were plotted on an MDS ordination plot to show differences between sites in pigment composition (Figs. 6.10 and 6.11). The gut wall MDS ordination plot shows *Molpadia blakei* samples had the least between-site clustering – samples from M6 were located on the opposite side to the plot to the one sample taken at the PAP in 2005 (the other sample from the

PAP in 2005 contained no pigment and was excluded from the plot). The pigment composition of *M. blakei* samples was different between sites. This is highlighted by ANOSIM analysis (ANOSIM R = 1, P>0.05; Table 6.1), which suggested the samples taken at M6 are more similar to each other than any other samples from the PAP in July 2005. The R-statistic was not significant, however, because of the small sample number.

Oneirophanta mutabilis gut wall samples showed the tightest intra-specific clustering between and within each site on the MDS ordination plot (Fig. 6.10). ANOSIM analysis indicated samples taken at M5 and the PAP in 2005 were similar to each other (ANOSIM R = 0.250, P<0.05; Table 6.1) but those of M5 and the PAP in 2004 were more similar within-site than they were between sites (ANOSIM R = 0.684, P<0.05; Table 6.1).

Pseudostichopus villosus gut wall samples showed between-site separation on the MDS ordination plot (Fig. 6.10) and ANOSIM analysis indicated the samples within M5 and the PAP in 2005 were more similar to each other than to samples from the other site (ANOSIM R = 0.8, P<0.05; Table 6.1). Samples of *P. villosus* from the PAP in 2005 had higher percentage contributions from canthaxanthin, alloxanthin and 19'-hexanoloxyfucoxanthin.

Psychropotes longicauda gut wall samples from the PAP in 2004 were grouped separately from M5 and M6 samples on the MDS plot (Fig. 6.10). Samples of *P. longicauda* from the PAP in 2004 had no 19'hexanoloxyfucoxanthin or 19'-butanoloxyfucoxanthin, and had higher percentage contribution from diadinoxanthin and alloxanthin. ANOSIM analysis indicated the samples from the PAP in 2004 are more similar to each other than they are to either M5 or M6 samples (ANOSIM R = 0.723 (M6), R = 0.88 (M5), P<0.05; Table 6.1). *Psychropotes longicauda* gut wall percentage pigment composition samples from the PAP in 2005 were similar to the samples from M5 and M6 because of the high variability of the PAP 2005 samples, as seen on the MDS ordination plot. The negative R-statistic generated for the PAP 2005 vs. M6 samples (ANOSIM R = -

0.080, P>0.05; Table 6.1) indicated greater dissimilarity among samples from one site than occurred between samples from both sites (Chapman and Underwood, 1999). This dissimilarity was highlighted on the MDS plot where samples of *P. longicauda* from the PAP 2005 had been sampled from differnt 'states' i.e. two samples together above the M6 samples on the plot and the three samples located at the bottom of the plot and one outlying sample to the left of the plot (6.10). The two *P. longicauda* samples above the M6 samples contained a greater number of carotenoids than the three samples to the bottom of the plot.
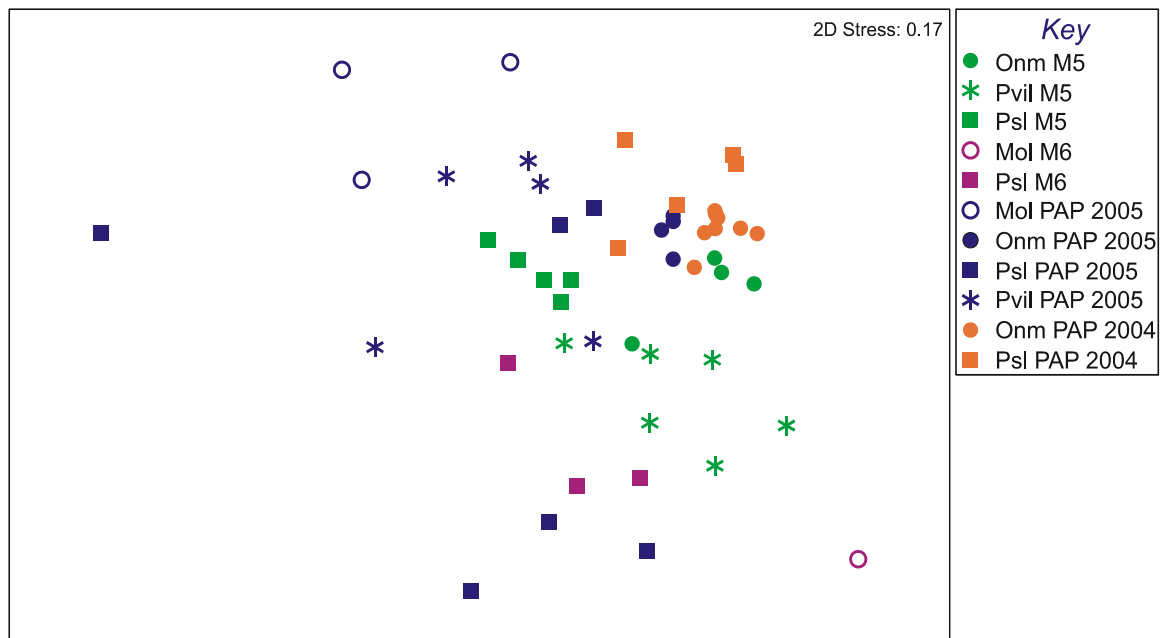

Figure 6.10 MDS ordination of 50 individual holothurian gut wall samples from M5, M6, the PAP in June 2004 and the PAP July 2005, based on √-transformed pigment percentage contributions and Bray-Curtis similarities. Key: Onm = *Oneirophanta mutabilis*; Mol = *Molpadia blakei*; Psl = *Psychropotes longicauda*; Pvil = *Pseudostichopus villosus*.

Group	R-statistic	Significance level
O. mutabilis M5 vs *O. mutabilis* PAP 2005	0.250	P = 0.029*
O. mutabilis M5 vs *O. mutabilis* PAP 2004	0.684	P = 0.020*
P. villosus M5 vs *P. villosus* PAP 2005	0.800	P = 0.020*
P. longicauda M6 vs *P. longicauda* PAP 2005	-0.080	P = 0.548
P. longicauda M6 vs *P. longicauda* PAP 2004	0.723	P = 0.018*
P. longicauda M5 vs *P. longicauda* PAP 2005	0.192	P = 0.067
P. longicauda M5 vs *P. longicauda* PAP 2004	0.880	P = 0.080
M. blakei M6 vs *M. blakei* PAP 2005	1	P = 0.250

Table 6.1 Results of similarity test (ANOSIM) comparing holothurian gut wall sample pigment percentage contribution to the total load between M5 and the PAP in June 2004, M5 and the PAP in July 2005, M6 and the PAP in June 2004, and M6 and the PAP in July 2005 (where samples were taken from the sites). R-statistic = 1 only if all replicates within a sample are more similar to each other than any other replicates from different samples. * = significant

Oneirophanta mutabilis ovarian samples from the PAP in 2005 and 2004 showed some degree of within-site and between-site clustering on the MDS ordination plot based on pigment percentage contributions (Fig. 6.11). All *O. mutabilis* samples had a high percentage contribution from β-carotene. *Oneirophanta mutabilis* M5 samples show the least within-site specific sampling; the outlying sample to the bottom of the MDS plot contains a relatively high percentage contribution from echinenone and a relatively lower percentage contribution (27%) of β-carotene in comparison to other *O. mutabilis* samples (>50%). ANOSIM analysis indicates the samples are similar between sites M5 and the PAP in 2004 (ANOSIM R = 0.266, P>0.05; Table 6.2) and M5 and the PAP in 2005 (ANOSIM R = 0.352, P>0.05; Table 6.2), although this is not significant; the high variability of the M5 samples producing a low R-statistic, but also reducing the probability it is true.

Molpadia blakei ovarian samples taken at the PAP in 2005 show a high degree of clustering on the MDS ordination plot, with the exception of one sample that contained no 19'-butanoloxyfucoxanthin in comparison to the other samples (Fig. 6.11). ANOSIM analysis gives a significant R-value of 0.487 (P<0.05; Table 6.2) indicating that samples between each site are overlapping, but are more similar

within-site than to those from the other site. Samples of *Pseudostichopus villosus* from M5 show variability in their pigment composition, as seen on the MDS ordination plot, despite β-carotene dominating the pigment load in terms of percentage composition (>60%). The spread of these samples arise from the presence or absence of some carotenoids contributing a small percentage to the total. Samples from the PAP in 2005 are split across the plot (Fig. 6.10). *Pseudostichopus villosus* PAP 2005 samples to the left of the plot have high percentage contribution from β-carotene, the outlying sample at the top contains only canthaxanthin and the group to the middle right are very similar in their pigment composition, containing ~25% each of canthaxanthin and β-carotene. The R-statistic given by ANOSIM analysis (Table 6.2) indicates there is no difference in pigment composition between sites, although this is not significant (ANOSIM R = 0.148, P>0.05; Table 6.2) because of the high variability of the PAP 2005 samples.

The pigment percentage contribution of ovarian samples of *Psychropotes longicauda* show distinct clustering within-sites when plotted on an MDS ordination plot (Fig. 6.11). ANOSIM analysis indicates that samples taken at the PAP in 2004 are more similar in their pigment percentage contribution to each other than they are to M5 or M6 samples (ANOSIM, R = 1, P<0.05; Table 6.2). PAP 2004 samples of *P. longicauda* are clustered away from M5 and M6 samples on the MDS plot (Fig. 6.11). Samples of *P. longicauda* from the PAP in 2004 do not contain 19'hexanoloxyfucoxanthin or 19'-butanoloxyfucoxanthin, and have relatively high pigment percentage contributions from diadinoxanthin and zeaxanthin. M5 and M6 *P. longicauda* samples differ through the higher percentage contribution of β-carotene in ovaries taken from M5. *Psychropotes longicauda* PAP 2005 samples are also clustered away from M5 and M6 on the ordination plot (Fig. 6.11), having a comparitively lower percentage contribution from β-carotene and echinenone. ANOSIM analysis shows PAP 2005 to be significantly different from M5 (ANOSIM R = 0.873, P<0.05; Table 6.2) and different from M6, although this is not significant (ANOSIM R = 0.75, P>0.05; Table 6.2).
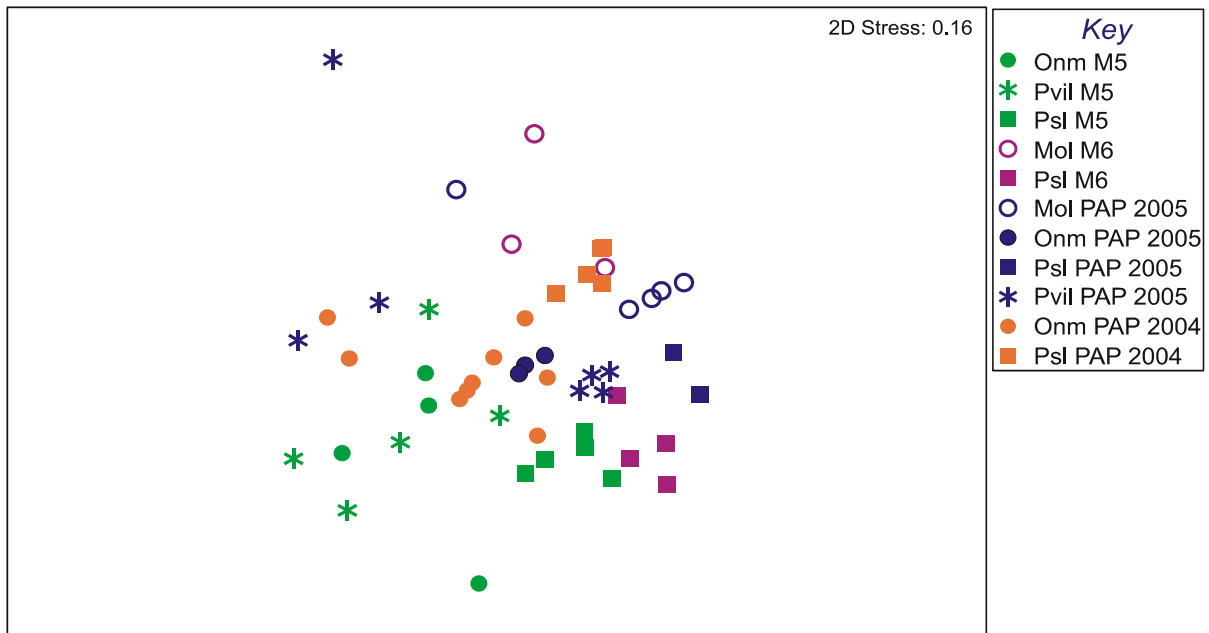
Figure 6.11 MDS ordination of 52 individual holothurian ovary samples from M5, M6, the PAP in June 2004 and the PAP July 2005, based on √-transformed pigment percentage contributions and Bray-Curtis similarities. Key: Onm = *Oneirophanta mutabilis***; Mol =** *Molpadia blakei***; Psl =** *Psychropotes longicauda***; Pvil =** *Pseudostichopus villosus***.**

Group	R-statistic	Significance level
O. mutabilis M5 vs *O. mutabilis* PAP 2005	0.352	P = 0.086
O. mutabilis M5 vs *O. mutabilis* PAP 2004	0.266	P = 0.078
P. villosus M5 vs *P. villosus* PAP 2005	0.148	P = 0.114
P. longicauda M6 vs *P. longicauda* PAP 2005	0.75	P = 0.067
P. longicauda M6 vs *P. longicauda* PAP 2004	1	P = 0.008*
P. longicauda M5 vs *P. longicauda* PAP 2005	0.873	P = 0.048*
P. longicauda M5, vs *P. longicauda* PAP 2004	1	P = 0.008*
M. blakei M6 vs *M. blakei* PAP 2005	0.487	P = 0.036*

Table 6.2 Results of similarity test (ANOSIM) comparing holothurian ovary sample pigment percentage contribution to the total load between M5 and the PAP in June 2004, M5 and the PAP in July 2005, M6 and the PAP in June 2004, and M6 and the PAP in July 2005 (where samples were taken from the sites). R-statistic = 1 only if all replicates within a sample are more similar to each other than any other replicates from different samples. * = significant

6.3 Discussion

6.3.1 Comparison of organic matter supply at each site

Determining the differences in the supply, freshness and composition of organic matter at the three sites, and during the two sampling periods at the PAP, will facilitate the understanding of how diet affected the ovarian biochemistry of the holothurians. Phytodetritus was observed on the sediment from recovered cores at the PAP in 2004 and 2004, and in the photos of the seafloor at M5 in December 2005/January 2006 (Chapters 4; Wolff, 2006). The phytodetritus at M5 was often resuspended during periods of increased tidal flow (Wolff, 2006), which may have contributed to the lack of observable phytodetritus on the surface of the sediment cores retrieved at this station. No phytodetritus was observed at M6 in December 2005/January 2006 (Wolff, 2006), which is consistent with the absence of chlorophyll *a* in the POM above the seabed at this site (Chapter 5, present study). The phytodetritus (or Particulate Organic Matter (POM)) was sampled using different methods at each site; gently removed by pipette from the top of the cores sampled at the PAP in both years, and by SAPS at M5 and M6. Therefore, concentrations of pigments in the POM/phytodetritus cannot be compared because of the methodological artefacts associated with the two methods (Turnewitsch et al., 2007) and differing unit measurements.

The ratio of chlorophyll *a* to phaeophorbide in the phytodetritus (PAP) and POM (M5) may be used to indicate the degree of freshness of the phytodetrital material analysed (Thiel et al., 1989). Chlorophyll *a* to phaeophorbide ratio of the POM above the seabed at M5 was 0.81 and the phytodetritus on the sediment at PAP 0.67 ±0.15 (June 2004) and 0.29 ±0.49 (July 2005) (Chapters 4 and 5, present study). This suggests the POM at M5 was fresher than the phytodetritus sampled from the sediment surface at the PAP.

Differences in the concentrations of pigments in the top 5mm sediment at each site/sampling period is a function of the supply of OM as well as the effect of the

benthic fauna on the reworking and assimilation of organic compounds. A relatively high concentration of chlorophyll a at M5, in comparison to M6 and PAP (June 2004 and July 2005), suggests fresher material was available to the benthos at M5 at the time of sampling. Despite the presence of fresh phytodetrital material at the PAP in both years, M6 had higher concentrations of most pigments (with the exception of diatoxanthin, β-carotene and zeaxanthin) than did the PAP surface sediments in either June 2004 or July 2005. M6 received a short, high flux of organic material in January 2005, but little else for the rest of the year (Wolff, 2006; Salter, 2007). Organic compounds may have persisted in the sediments at M6 after this flux event, (degradation rates of pigments are unknown in the deep sea) because of the comparatively low faunal biomass at M6. At the PAP and M5 on the other hand, the relatively higher biomass of benthic fauna (Wolff, 2006) may have assimilated essential compounds and created transformation products through the reworking of OM and oxygenation through bioturbation. This is supported by the phaeophorbide and phaeophytin concentrations at M6. The concentration of phaeophytin, a general breakdown product of chlorophyll a, is greatest at M6, suggesting that chlorophyll a has been broken down by non-grazing processes at this site.

Mass flux, determined by sediment trap data may give extra clues about the quantitative differences between site/sample periods. At M6 a single large flux event occurred in January 2005, generating particle fluxes of 500mg $m^{-2}d^{-1}$ (3000m sediment trap; Salter, 2007). This flux occurred in the initial two weeks after the sediment trap was deployed. It is not known if the single, large flux event in January is typical (on an annual or regular basis) in the HNLC region to the South of the Crozet Islands. At M5, an initial large two week flux of 450 mg $m^{-2}d^{-1}$ (January 2005), followed by a lower magnitude flux (~150 mg $m^{-2}d^{-1}$) sustained for a longer time period of 173 days was observed (3000m sediment trap; Salter, 2007). At the end of this period, late June 2005, no flux of material was recorded until a one week flux of 100 mg $m^{-2}d^{-1}$ was recorded in December 2005. This was just before the sediment and benthic fauna were sampled at both sites. Mass flux of OM to the seafloor at the PAP in June 2004 was between 150-200 mg $m^{-2}d^{-1}$,

over double that of 20-50mg m^{-2}d^{-1} in June/July 2005. The mass flux of material in the month leading up to the time of sampling was also twice as large in 2004 than in 2005 (3000m sediment trap; Lampitt, 2008). The organic carbon flux (3000m sediment trap) just before the time of sampling was also greatest at the PAP in June 2004 (11mg C m^{-2} d^{-1}), followed by M5 (6mg C m^{-2} d^{-1}) and M6 (no flux). No organic carbon flux data is available for the PAP in July 2005 (Salter, 2007; Lampitt, 2008). These flux data suggest that in terms of quantity during the month before the sampling of sediment and holothurians, the PAP in June 2004 received the greatest flux of material, followed by M5, the PAP in July 2005 and M6.

The chlorophyll a to phaeophorbide ratio of the POM/phytodetritus and the chlorophyll a concentration in the top 5mm sediment suggests M5 received fresher material in comparison to the PAP in June 2004. Differences in the biogeochemical processes in the water column (phytoplankton and zooplankton community structure, remineralisation) may have led to the larger flux of material reaching the seafloor at the PAP in June 2004 being less fresh than that at M5. This highlights the importance of understanding the influence of the biogeochemistry of the water column on the supply and reworking on OM reaching the sea-floor. It is difficult to ascertain the influence the supply of OM has on the benthos, if the knowledge of the quality as well as quantity of OM supply is patchy or uncertain. Necessary

The data show that in terms of composition, the supply at the PAP in June 2004 was the most different to the other sampling period/sites. β-carotene, violaxanthin, 19'-butanoloxyfucoxanthin and 19'-hexanoloxyfucoxanthin were not found in the top 5mm sediment in June 2004, although β-carotene was present in the phytodetritus on the sediment at this site. These four pigments were present in the top 5mm sediment at M5, M6 and the PAP in 2005. The presence of 19'-butanoloxyfucoxanthin and 19'-hexanoloxyfucoxanthin at the PAP in July 2005, M5 and M6 suggests some of the detrital input was derived from prymnesiophytes (Coccolithophores, *Phaeocystis* sp.; Jeffery et al., 1997). Zeaxanthin was found in

greater concentration in the sediment at the PAP in June 2004 in comparison to the PAP in 2005, M5 and M6, suggesting cyanobacteria (Jeffrey et al., 1997) contributed to the flux of OM.

Chlorophyll *a* shows the highest contrast in concentration between M5/M6 and the PAP in 2005, suggesting fresher material was found in the 5 to 10mm sediment section at M5 and M6. It is surprising the chlorophyll *a* concentrations at M6 are higher than that in the 2005 PAP 5 to 10mm sediment, because of the relatively low (or non-existent!) flux of fresh OM at M6. The phytopigments may have been introduced to the deeper sediments at the Crozet sites by bioturbation and reworking of the sediment by the benthic fauna; resh phytodetrital particles have been shown to be rapidly subducted into the burrows of animals (Bett and Rice, 1993). However, bioturbation also introduces oxygen into the sediment, which in turn enhances pigment degradation (Leavitt, 1988; Abele-Oeschger, 1991). Subduction of chlorophyll *a* rich particles by bioturbation is unlikely to have occurred at M6 as no POM was observed at the site and no flux of material was recorded in the deep sediment trap eleven months before sampling. Also, if the abundance and biomass of the infaunal macrofauna mirrors that of the megafauna at the two sites, M6 would not have been subjected to as much bioturbation. It is possible the pigments found in the deeper sediment at M6 had persisted from the high flux eleven months before sampling and that the low benthic biomass aided the preservation of the compounds. Unfortunately there is no quantitative information on the infaunal macrofauna at the Crozet sites.

The results from the study of the three sites indicate that it is important to know the quality and quantity of the OM flux, as well as the consistency/longevity of supply. Sediment chlorophyll *a* concentration alone cannot be used to quantify the OM reaching the seafloor. Although mass flux to the PAP in 2004 was greatest out of the three sites/sample periods before the sampling of the holothurians and sediment, the data suggest the OM available to the benthos was freshest at M5. M6 received a relatively high, but short flux of OM eleven months before the sampling period; the low benthic biomass at this site is thought to have

contributed to the preservation of chlorophyll *a* at this site. Pigment degradation rates in the deep sea are currently unknown. Further studies are required to determine degradation rates and to ascertain how long labile compounds like chlorophyll *a* can persist.

6.3.2 Between-site comparison of holothurian feeding selectivity

The present study gives an opportunity to compare holothurian diet under differing OM supply regimes. *Molpadia blakei* consistently showed no evidence of feeding on fresh material, as demonstrated by the lack of chlorophyll *a* in the samples of gut sediment taken at both the PAP (present study; Wigham et al., 2003) and at the M6 site. Molpadiid holothurians burrow head-down into the sediment, feeding on refractory material (Khripounoff and Sibuet, 1980; Roberts et al., 2000). This is supported by isotopic analysis of the species. Its body wall is enriched in the heavy isotope of ^{15}N; the naturally occurring stable isotope of nitrogen shows a stepwise enrichment between prey and consumer during assimilation processes (Deniro and Epstein, 1978; Iken et al., 2001). The gut sediment of *M. blakei* sampled previously at Station M in the NE Pacific also had relatively low levels of ^{234}Th in comparison to that of superficial sediment feeders (Lauermann et al., 1997).

The average gut sediment chlorophyll *a* concentration of *Pseudostichopus villosus*, *Psychropotes longicauda*, *Amperima* spp. and *Peniagone* spp. suggests specimens sampled at M5 were feeding on fresher material in comparison to their congeners at the PAP in 2004 or 2005. This supports the inference that the organic material available to the fauna was fresher at station M5 than at the PAP in 2004 at the time of sampling. *Amperima spp.* and *Peniagone spp.* are surficial sediment feeders, feeding on the freshest material when they can find it (Billett, 1991; Iken et al., 2001; Wigham et al., 2003a). This may account for the high, but variable gut sediment chlorophyll *a* concentrations of these species at M5. The feeding adaptations of *Amperima* and *Peniagone* allow them to take advantage of the

chlorophyll-rich POM at M5, despite it often being resuspended at times of increased tidal flow (Wolff, 2006). Higher chlorophyll *a* concentrations than that recorded for *Amperima robusta* at M5 13.88 µg gDW^{-1} (± S.D. 15.94) have previously been measured in *A. rosea* at the PAP 45.94 µg gDW^{-1} (± 40.41) and 30.85 µg gDW^{-1} (± 3.63) µg gDW^{-1} (October 2000 and March 2002 respectively; Wigham et al., 2003). This suggests that members of this genus can process and ingest fresher material when it is available or when they locate it.

Psychropotes longicauda feeds on the sediment surface and has a peltate tentacle structure (sweeping sediment into the mouth), suggesting it is less selective than holothurians with a digitate tentacle structure (that are able to select particles) (Roberts et al., 2000). This may explain why that although the chlorophyll *a* concentration in the top 5mm sediment at M6 was higher than that at the PAP in 2004 or 2005, it was not reflected in the gut sediment chlorophyll *a* concentration of *P. longicauda*. The high variability of the chlorophyll *a* in the top 5mm sediment at M6 may reflect high spatial variability. The low gut sediment chlorophyll *a* concentration in *P. longicauda* at M6 may also be as a consequence of the high spatial variability of fresh OM at this site.

Pseudostichopus villosus is a sub-surface deposit feeder, ingesting sediments from 0 to 2cm depth (Billett, 1991; Moore and Roberts, 1994). The feeding modes of *P. villosus* and *Psychropotes longicauda* suggest they would have lower chlorophyll *a* concentrations in their gut sediment in comparison to *Oneirophanta mutabilis*, which is a surficial feeder, using digitate tentacles to select particles (Roberts et al., 2000). That *O. mutabilis* feeds on fresher material to *P. longicauda* and *Pseudostichopus villosus* is supported by isotopic analysis (Iken et al., 2001). In samples taken at the PAP in June 2004, *O. mutabilis* did have higher gut sediment chlorophyll *a* concentrations than *Psychropotes longicauda* and *Pseudostichopus villosus*. Gut sediment samples of these three species taken at the PAP in July 2005 have similar chlorophyll *a* concentrations, which is attributed to the low availability of fresh OM leading up to and during the time of sampling (Chapter 4). However, *Psychropotes longicauda* and *Pseudostichopus villosus* gut sediment

samples taken at M5, had higher chlorophyll *a* concentrations than *O. mutabilis*. This has been attributed to *O. mutabilis* not being able to take advantage of the often resuspended phytodetritus (Chapter 5; Wolff, 2006; Owen, 2007), and also to the number of samples taken. For example, one specimen of *Pseudostichopus villosus* at M5 had a relatively high chlorophyll *a* concentration, suggesting it had ingested a 'fresh' patch, increasing the average chlorophyll *a* concentration to above that of *O. mutabilis*. This highlights the need for collecting as many replicates as possible and should be considered when planning future deep-sea specimen collection for analysis. Gut sediment samples of *Amperima rosea* taken at the PAP in June 2004 (chlorophyll *a* was absent in ten out of fourteen samples).

Results from the present study suggest that chlorophyll *a* is a good indicator of feeding selectivity, but only when used in temporal comparisons and when the quality and quantity of OM available to the fauna is known. For example, if only the PAP 2005 gut sediment chlorophyll *a* data had been used to ascertain the feeding selectivity of abyssal holothurians, then an erroneous conclusion that *O. mutabilis*, *Psychropotes longicauda* and *Pseudostichopus villosus* have similar feeding selectivity might have been made. Therefore, sound conclusions on feeding selectivity using chlorophyll *a* gut sediment data taken at a single period cannot be made with confidence. The concentration of chlorophyll *a* in the gut sediment of abyssal holothurians is a complex function of their feeding modes and supply of organic material – in terms of quantity, quality and the way it is supplied (i.e. if the phytodetritus settles on the sediment surface or is often resuspended). Gut sediment chlorophyll *a* concentration can be used to establish inter-specific trophic relationships under specific food regimes. For example, *O. mutabilis* is a selective feeder, but when fresh OM is scarce the species feeds on the same sediment as other large species that show little or no temporal variation in their diet (Neto et al., 2006; Chapter 4, present study). Elucidation of such trophic relationships is facilitated if the supply of OM to the seafloor is quantified prior to sample collection. It yields vital information on the food regime prior to the sampling event and may also be used to provide information on the influence of OM supply on the biochemistry of the animal.

6.3.3 Between-site comparison of holothurian pigment biochemistry

6.3.3.1 Quantitative intraspecies among-site comparisons

<u>*Amperima* and *Peniagone* spp.</u>

In comparison to *A. robusta* (Crozet, M5), *A. rosea* (PAP, 2004) had significantly greater concentrations of the carotenoids diadinoxanthin, alloxanthin, zeaxanthin and diatoxanthin in the gut wall and diadinoxanthin, alloxanthin, zeaxanthin, diatoxanthin canthaxanthin and echinenone in the ovaries. This may be assigned to differences in supply, but is more likely to be a result of differing pigment biochemistry between the species. Zeaxanthin appears to be an important carotenoid in the ovaries of *A. rosea*, as seen in the present and previous studies (Chapter 4; Wigham et al., 2003; Hudson, 2004), but zeaxanthin does not feature as a dominant carotenoid in the gut wall or ovaries of *A. robusta*. Comparative biochemical studies of shallow-water echinoderms have shown that species from the same genera can have different carotenoid biochemistry in terms of concentration (Tsushima et al., 1995; Borisovets et al., 2002). Therefore, comparing species from the same genera to ascertain the influence of supply on their pigment biochemistry is not valid. The between-site comparison has shown, however, that both species of *Amperima* contained high concentrations of carotenoids in their ovaries (μg gDW^{-1}) in comparison to other species studied (Chapter 4, present study), suggesting this genus may assimilate a high carotenoid load into its ovaries to gain a reproductive advantage.

A similar result was observed for the *Peniagone* spp. sampled at all sites. Although between-species comparisons cannot be made to ascertain the affect of supply on the carotenoid biochemistry of the gut wall and ovaries, the *Peniagone* species studied had relatively large carotenoid concentrations (μg gDW^{-1}), when compared to other species sampled, with the exception of *A. rosea*. Both *Amperima* and *Peniagone* are selective feeders, feeding on the freshest material (that has the greatest pigment concentrations) when they can find it. This facilitates the assimilation of carotenoids to yield enhanced ovarian pigment loads,

which in turn increases reproductive output and larval survival (George et al., 2001). Studies have shown that *Amperima* and *Peniagone* are sexually mature at a small size (Tyler et al., 1985; Wigham et al., 2003b), this would suggest that these genera respond to fresh inputs of OM, by reproducing quickly and giving their eggs and larvae a greater chance of survival.

Molpadia blakei

Average concentrations of carotenoids in the gut wall of *M. blakei* are subject to high variability because of the total absence of pigments from the gut wall of some specimens. The carotenoids that were present in the gut walls of specimens taken at the PAP in 2005 that were absent in specimens from M6, were not found in greater concentrations in the sediment at the time of sampling at the PAP 2005. It is possible the carotenoids found in tissue samples of *M. blakei* at one site but not the other were provided in the supply of OM prior to the time of sampling. The biochemistry of *M. blakei* may reflect historic compositional supply of essential compounds because of the feeding mode of the species (head burrowed in deeper sediment). The low number of replicates may also explain between-site differences; only two samples of *M. blakei* gut wall were obtained from M6, one of which was depleted in carotenoids. Again, this highlights the need for a high number of replicates to be able to give conjectures about the feeding and reproductive ecology of the species with confidence. The ovarian pigment biochemistry of *M. blakei* from both sites indicates specimens sampled from M6 have higher and more consistent concentrations of the carotenoids alloxanthin, diadinoxanthin, zeaxanthin and β-carotene. Ovarian samples taken at the PAP in 2005 have a higher average concentration of 19'-butanoloxyfucoxanthin. The concentrations of pigments in the deeper section of sediment were greater at M6/M5 than at the PAP in 2005. Some pigments found at M5/M6 were absent in the lower section of sediment at the PAP 2005. These differences may have given *M. blakei* access to enhanced carotenoid concentrations at M6 in comparison to its congener at the PAP in 2005 and may have led to the higher concentrations of alloxanthin, diatoxanthin, zeaxanthin and β-carotene in the samples taken at M6. However, a higher concentration of 19'-hexanoloxyfucoxanthin was found in the

5 to 10mm sediment at the PAP in 2005 than at M6, but was not mirrored in the ovarian biochemistry. Further studies to include a higher number of replicates of *M. blakei* and the surrounding sediment it feeds upon are needed to confirm that enhanced pigment concentrations in deeper sediment are conferred into the biochemistry of the animal.

Oneirophanta mutabilis

Between-site differences in pigment concentrations in *O. mutabilis* gut wall samples may be related to the fresher material arriving at M5 at the time of sampling. However, gut sediment chlorophyll *a* concentration suggests the species was not able to take advantage of the often resuspended fresh POM. It is possible the number of specimens sampled at M5 for their gut sediment chlorophyll *a* concentration was not enough to sample a specimen that had found a 'fresh' food patch. *Oneirophanta mutabilis* may have ingested fresh POM infrequently at this site, assimilating the pigments into its gut wall. The absence of OM, followed by a large flux just before sampling at M5, means that *O. mutabilis* may not have had time to transfer the relatively higher carotenoid concentration (in relation to its congener at the PAP) from the gut wall into the ovaries. Assimilation rates in abyssal holothurians are unknown. The lack of evidence that *O. mutabilis* was able to process the fresh POM at M5 means that this explanation for the enhanced gut wall pigment concentration at M5 remains speculative.

Differences in the ovarian pigment concentration of *O. mutabilis* ovarian samples between sites may be related to the supply of fresh material in the period before each sample collection. This would suggest the species builds up the carotenoid load in its ovaries over a long time-scale. Average concentrations of the carotenoids diadinoxanthin, alloxanthin, diatoxanthin, zeaxanthin, canthaxanthin and echinenone were all higher in the *O. mutabilis* ovarian samples taken at the PAP in June 2004, with diadinoxanthin, alloxanthin and canthaxanthin being significantly greater. Sample collection at M5 in December 2005/January 2006 coincided with the start of the seasonal flux of material to the seafloor. The sediment trap record shows that the mass flux of material in December 2005

measuring 100 mg m^{-2}d^{-1}, before this, no flux had been recorded since late June 2005 (3000m sediment trap, Salter, 2007). Conversely, megafauna at the PAP sampled in 2004 and 2005 had been subject to a prolonged enhanced flux since March (June 2004 samples) and early May (July 2005 samples) (Lampitt, 2008), enabling specimens to assimilate higher concentrations of carotenoids into their ovaries in comparison to their congeners at M5. No periods of zero flux were recorded at the PAP between July 2003 to July; the lowest mass flux observed was that of 10.98 m^{-2}d^{-1}, averaged over a January 2005 and 10.49 m^{-2}d^{-1} averaged over a two week period in June 2005 (Lampitt, 2008). This suggests the concentrations of carotenoids in the ovaries of *O. mutabilis* are controlled by the supply of OM to the sea-floor. If supply is seasonal and shows high contrast in flux between periods (high flux to zero flux), the data from this study suggests the concentration of carotenoids in the ovaries of *O. mutabilis* would show a seasonal pattern. The reproductive biology of *O. mutabilis* sampled at the PAP shows no seasonality, although oozyte-size frequency and fecundity can be affected by changes in the benthic community limiting available resources (Tyler and Billett, 1987; Ramirez-Llodra et al., 2005). It would have been interesting to investigate if this competition for resources affected the concentrations of carotenoids in the ovaries of the species; competition for resources may have the same affect as the variation in the flux of material does on ovarian biochemistry. If the pattern of the flux of material observed during the present study at M5 is typical of the region on an annual and regular basis, it is likely that the seasonal absence following a presence of high flux at this site would force seasonality upon the fauna that feed selectively on the fresh material. Whether this seasonality is reflected only in the biochemistry of the animals, or whether it is transferred to periodic (seasonal) increases in reproductive output, remains to be investigated.

Psychropotes longicauda and *Pseudostichopus villosus*

The gut wall and ovarian pigment concentrations of *Psychropotes longicauda* and *Pseudostichopus villosus* show between-site intraspecific similarities, with the exception of the carotenoids that appear to be most abundant; β-carotene in *P. villosus* and echinenone and β-carotene in *Psychropotes longicauda*. These

dominant carotenoids are found in higher concentration in the specimens taken at M5 than at any other site/sampling period at the PAP. Both species contain low carotenoid concentrations (μg gDW^{-1}) in their ovaries, suggesting they do not assimilate enhanced carotenoid loads as a means to increase reproductive output. *Psychropotes longicauda* is an epibenthic feeder, but is less selective than genera like *Peniagone* and *Amperima* (Chapter 4, present study; Iken et al., 2001). *Pseudostichopus villosus* has a feeding mode that 'ploughs' slowly through the sediment, probably ingesting sediments from 0 to 2cm depth (Billett, 1991; Moore and Roberts, 1994). Therefore, the greater average concentration of the carotenoids dominating their gut wall and ovarian biochemistry may be attributed to the following factors.

1) The existence of fresher material in the sediment at M5, as seen in the enhanced levels of chlorophyll *a* in the top 5mm and 5 to 10mm sediment at the time of sampling. If this is the case, the ovarian biochemistry of these species does not reflect the historic enhanced, consistent supply at the PAP. This may be because *Psychropotes longicauda* and *Pseudostichopus villosus* do not accumulate large concentrations of carotenoids into their ovaries or gut walls (as does, for example, *O. mutabilis*).

2) Reduced competition for resources at M5. The significance of this is presently difficult to ascertain. Isotopic analysis of the species would have elucidated their trophic positions, in terms of the freshness of OM they were ingesting in comparison with other species at the site. This data could have then been compared to isotopic information of the same species at the PAP (Iken et al., 2001) to see how trophic positioning may vary at different sites. Future studies could include isotopic studies of the sediment fauna to make comparisons on the trophic positioning of species between abyssal sites. Temporal comparison of such data may also elucidate if changes in the supply of OM affects the trophic position of a species at a site.

6.2.3.2 Compositional differences and intra-specific consistency between years

Comparisons of the consistency of the pigment biochemical profiles of species sampled at the PAP, M5 and M6 are made only with holothurians identified to species level; *Oneirophanta mutabilis*, *Psychropotes longicauda*, *Pseudostichopus villosus* and *Molpadia blakei*. Comparative biochemical studies of shallow-water echinoderms have shown that species from the same genera can have different carotenoid biochemistry in terms composition (Matsuno and Tsushima, 2001; Lawrence et al., 2004). Therefore, combining species from the same genera to ascertain the influence of supply on their pigment biochemistry may not yield valid conclusions. Comparison of the pigments found in the two *Amperima* species sampled at the PAP in 2004 and at M5 show that that they have very different profiles, with *Amperima rosea* (PAP) assimilating a high concentration (contributing to a high pigment percentage of the total) of zeaxanthin in comparison to its congener *Amperima robusta* (M5).

Molpadia blakei

The MDS ordination plot based on the pigment percentage contributions highlights the inconsistent biochemical profile of *Molpadia blakei* gut wall samples taken at M6 and the PAP in 2005. The dissimilarity of the samples between sites can be attributed to different pigments contributing to the biochemistry of the gut wall at each site, suggesting the species passively assimilates carotenoids that are available in its diet. However, the ovarian pigment biochemistry of the specimens from that PAP in 2005 shows some degree of clustering on the MDS ordination plot. The refractory diet of *Molpadia blakei* (present study; (Khripounoff and Sibuet, 1980; Roberts et al., 2000; Iken et al., 2001) would presumably dictate a non-selective adaptation in the species; selectivity would seem futile if the supply of essential organic compounds are in short supply in the deeper sediment. It is possible that the four biochemically similar specimens from the PAP in 2005 had been subjected to a similar (in terms of pigment composition) diet. In future, specimens of *M. blakei* should be

analysed to see if its ovarian biochemical profile of the species at the PAP is truly consistent.

Oneirophanta mutabilis

The gut wall and ovarian pigment biochemistry of *O. mutabilis* is dominated by the carotenoid β-carotene, which is found in percentage contributions >30% up to ~70%. This dominance of β-carotene drives the similarity in biochemical profiles of samples of this species between each site. However, the biochemical profile of the species can be influenced by differences in the composition of the OM supply, as demonstrated by the within-site clustering of the gut wall and ovarian samples on the MDS ordination plot (Fig 6.10 and 6.11). This is driven by the presence of 19'-hexanoloxyfucoxanthin and 19'-butanoloxyfucoxanthin in the gut wall and ovaries of *O. mutabilis* sampled at the PAP in 2005 and M5, but not at the PAP in 2004. These carotenoids were present in the phytodetritus/POM and the sediment at the PAP in 2005 and M5, but not at the PAP in 2004. *Oneirophanta mutabilis* samples taken at M5 show the least species specific clustering. This may be related to the supply of POM at this site being difficult for the species to process and/or because of timing of sampling just after high flux event following a period of zero flux (Salter, 2007).

Psychropotes longicauda

P. longicauda shows the most consistent within-site gut wall and ovarian biochemical pigment profile on the MDS ordination plot of the pigment percentage contributions. This is seen most clearly in the ovarian samples. That the POM/phytodetritus and sediment sampled at the PAP in 2004 showed the greatest difference in composition to that sampled at the PAP in 2005, M5 and M6, is mirrored in the ovarian biochemistry of *P. longicauda*. This suggests this species assimilates carotenoids present in its diet. 19'-hexanoloxyfucoxanthin and 19'-butanoloxyfucoxanthin are not found in the ovaries of *P. longicauda* sampled at the PAP in 2004, but is found in specimens from the PAP in 2005, M5 and M6. Zeaxanthin contributes a higher percentage to the pigment load in the ovaries of *P. longicauda* at the PAP in 2004 in comparison to the other sites/sampling period.

This may be related to the higher concentration of zeaxanthin in the top 5mm sediment at the PAP in 2004 in comparison to the PAP in 2005, M5 and M6.

Pseudostichopus villosus

The gut wall biochemical profile of *P. villosus* appears to be very variable within each site, although samples from the two sites are more similar to each other than they are to samples from the other site. This may be related to the feeding mode of the species – moving slowly and feeding on deeper sediments of 0 to 2cm depth (Billett, 1991; Moore and Roberts, 1994). Therefore, *P. villosus* will only be able to exploit the deeper carotenoid-depleted (PAP 2005) or variable (in terms of carotenoid concentration) sediment (M5). These differences in pigment composition and concentrations in the 5 to 10mm sediment between the two sites may account for the between-site differences in the gut wall pigment biochemistry of the species. The within site variability may be a result of the variable pigment concentration in the 5 to 10mm sediment section at M5 and the low concentrations of pigments in the 5 to 10mm sediment section at the PAP in 2005.

The ovarian biochemical profile of four specimens of *P. villosus* taken at the PAP in 2005 show some consistency, while three others are completely different and contain a limited number of carotenoids. The biochemical profile of the ovaries of *P. villosus* from M5 are inconsistent, which can be observed on the MDS ordination plot, although β-carotene dominates the pigment load in terms of percentage composition (>60%). The spread of these samples are slightly misleading as it is driven by the presence or absence of carotenoids contributing a small percentage (<5%) to the total load. The contribution of β-carotene to the ovarian pigment load is very variable from samples taken at the PAP in 2005. *Pseudostichopus villosus* may therefore be selective for β-carotene when carotenoid concentrations in the sediment are enhanced (5 to 10mm sediment M5), and less selective when sediment concentrations are depleted. This may be supported by the dominance of β-carotene in the ovaries of *P. villosus* sampled at the PAP in October 2003 (Hudson, 2004). Although only seven carotenoids were identified, β-carotene was found in the highest concentration (Hudson, 2004). The

concentrations of pigments measured in the present study are not comparable with values given for sediment analysed in October 2003 (top 5mm and 5 to 10 sediment, present study; top 1mm Hudson, 2004). However, mass flux prior to and at the time of sampling was similar to that recorded in June 2004 i.e. greater than at the PAP in July 2005 (3000m sediment trap; Lampitt, 2008).

6.4 Conclusion - How do changes in the supply of organic material affect holothurian ovarian biochemistry?

The three abyssal sites studied have shown differences in the supply of OM to the seafloor in terms of quantity, quality, composition and duration of the flux events. This has enabled a comparison of the effects these variables have on the diet and ovarian biochemistry of abyssal holothurians that are common to the different sites.

The influence of OM supply on the diet of the holothurians was dependant on the quantity and quality of the OM and the feeding adaptations of each species. Some selective species (*Amperima robusta* and *Peniagone* spp.) can take advantage of the fresher OM arriving at the seafloor at M5. However, the often resuspended POM at this site was not exploited by the normally selective *Oneirophanta mutabilis* (Hudson, 2004; Wigham et al., 2004; Chapter 4, present study). *Psychropotes longicauda* and *Pseudostichopus villosus* benefited from enhanced chlorophyll *a* concentrations in the top 5mm and deeper 5 to 10mm sediment at M5 by having enhanced chlorophyll *a* concentrations in their gut sediment. *Molpadia blakei* consistently showed no evidence of feeding on fresh material.

The ovarian biochemistry of the abyssal holothurians is a complex function of the composition, magnitude and duration of the flux of organic material, as well as the feeding adaptations and selectivity of the holothurians. The effect each of these variables had on the ovarian pigment biochemistry appears to differ between species. Deep-sea holothurians can also exhibit contrasting rates of feeding and digestion (Hudson et al., 2005); more variables that could have affected the ovarian biochemistry of the animals by enhancing food processing rates. None of the holothurians analysed for the present study exhibited the same feeding adaptation, reproductive biology and ovarian biochemistry, suggesting ecological niche separation. This allows species to partition resources so that one species does not out-compete the other. Changes in the magnitude, composition and duration of the OM flux to the seafloor affected the ovarian biochemistry of each

holothurian species in different ways. This may give species a reproductive advantage or disadvantage, leading to community change.

Quantitative differences in supply affected the carotenoid concentrations in the ovaries of all the holothurians species studied. This was observed in species that were common between the sites M5 and M6 (*A. abyssorum* and *Psychropotes longicauda*) where greater carotenoid supply at M5 than at M6 was mirrored in the concentrations of pigments in the ovaries. This was also observed in species that were common to the M5/M6 sites and the PAP, although differences in the way the flux arrived at the seafloor, in conjunction with the duration of the POM flux, appears to add another dimension to how far the ovarian biochemistry of the species were affected. For example, the ovarian carotenoid loads of *Psychropotes longicauda* and *Pseudostichopus villosus* reflected the fresher material found at M5 than at the PAP in 2004 or 2005, whereas the relatively enhanced carotenoid load of *O. mutabilis* ovarian samples at the PAP in 2004 reflected the prolonged, steady flux of OM prior to the sampling period at the PAP. The subsurface indiscriminate feeder *Molpadia blakei* also showed a positive response in its ovaries to increased carotenoid concentration in the surrounding deeper section sediment at M6 in comparison to that at the PAP in 2005. This suggests that if pigments persist in deeper sediments, or are subducted through bioturbation, *Molpadia blakei* may gain a reproductive advantage. Experiments on shallow water echinoderms have shown that the larvae of adults fed on a carotenoid-rich diet were larger throughout development, developed faster and had higher survival rates (Tsushima et al., 1997; George et al., 2001; George and Lawrence, 2002). This suggests that enhanced carotenoid concentrations in the ovaries of the abyssal holothurians would confer to a higher reproductive output.

In terms of the consistency of ovarian biochemical profiles, *O. mutabilis* showed the greatest consistency between sites and years. *Amperima rosea* showed the tightest within-sample period consistency (Chapter 4), but this consistency was not tested temporally. However, previous studies have shown that zeaxanthin is assimilated in large concentrations in the ovaries of the species (Wigham et al.,

2003a; Hudson, 2004), which corresponds with the findings of the present study (Chapter 4). If a species is selective in the carotenoids it requires for its reproduction, its reproductive output may be enhanced or inhibited by changes in the composition of the OM reaching the deep-sea floor. It has been suggested the increased supply of zeaxanthin (associated with cyanobacteria) can give *Amperima rosea* a competitive advantage by increasing its reproductive output (Wigham et al., 2003a). This may have driven the increase in abundance of the holothurian by more than two orders of magnitude between 1996 and 1999 (Billett et al., 2001; Wigham et al., 2003a). The present study still supports this, but further samples of *Amperima rosea* are needed to confirm the very consistent nature of its ovarian pigment biochemistry, which is dominated by zeaxanthin. Following this hypothesis, an increase in the availability of the carotenoid β-carotene to *O. mutabilis* may be beneficial to this holothurian species.

For those holothurians that show little consistency in their pigment biochemistry, the composition of the OM may also affect their reproductive output by supplying carotenoids of varying biochemical value. Depending on the structures of carotenoids, the compounds can show differences in their free radical quenching ability (Hirayama et al., 1994). An increase in the number of conjugated double bonds (carbon atoms covalently bonded with alternating single and double bonds) increases the quenching ability of the carotenoid (Foote et al., 1970; Lee and Min, 1990). Larvae of the sea urchin *Lytechinus variegatus* from parents fed on xanthophylls (oxygen containing carotenoids) were larger throughout development, developed faster, had higher survival rates and attained metamorphic competence faster than those fed just β-carotene. The numbers of juveniles originating from parents fed xanthophylls were also significantly greater (George et al., 2001). The compositional ovarian biochemistry of *Psychropotes longicauda* differed between sites. *Pseudostichopus villosus* showed some selectivity for β-carotene, but this only occurred when enhanced carotenoid concentrations were found in the sediment. *Molpadia blakei* showed no consistency in its biochemical pigment profile.

Species that can metabolise specific carotenoids for their needs may give themselves a reproductive advantage, by increasing the quenching ability of the carotenoids found in their diet. However, apart from the conversion of β-carotene to echinenone and canthaxanthin (Tsushima and Matsuno, 1990b; Tsushima et al., 1993b; Matsuno and Tsushima, 1995), the metabolic pathways of carotenoids in echinoderms are unknown.

Holothurians that have been shown to feed selectively on fresh material (*Amperima* spp. *Peniagone* spp. and *Oneirophanta mutabilis*) assimilate high concentrations of carotenoids into their ovaries, presumably as an adaptation to increase reproductive output. Carotenoids reduce the harmful effects of reactive oxygen species given off during the rapid metabolism of lipids in the egg, increasing larval survival (Blount et al., 2000; 2004; Lotocka et al., 2004). Survival of post-larvae is an important factor in response to the seasonal flux of phytodetritus, contributing to population structure and density (Wigham et al., 2003b). However, if a holothurian species is a selective feeder, it does not predispose the species to assimilate high concentrations of carotenoids into its ovaries. The species *Abyssocucumis abyssorum* (sampled at M5 and M6) is an example of this. This species feeds on the freshest material at M5 and M6 in comparison to the other species studied, but had carotenoid concentrations in its gut wall and ovaries of the same order of magnitude as those of less selective feeders such as *Pseudostichopus villosus* and *Psychropotes longicauda* (Chapter 5, present study).

The timing and make-up of the phytoplankton bloom, planktonic interactions, and recycling and repackaging of organic matter can be affected by climate warming and increasing atmospheric CO_2 (Turner, 2002; Richardson and Schoeman, 2004; Orr et al., 2005). Such changes will affect the biogeochemistry of the upper ocean and ultimately influence the magnitude and composition of the organic material arriving at the deep-sea floor. The present study clearly shows that a change in the quantity and composition of OM flux exerts a control on holothurian ovarian biochemistry. The extent and impact this influence has is dependant on the species

and how the OM supply has changed. Global changes in upper ocean ecosystems will ultimately affect abyssal sediment community structure and diversity and has the potential to change deep-sea community structure as observed at the Porcupine Abyssal Plain in the northeast Atlantic and at Station M, an abyssal time-series station in the northeast Pacific (Billett et al., 2001; Ruhl and Smith, 2004). Such community structure and biodiversity changes will affect ecosystem functioning; a change in the dominant species can affect sediment re-working rates (Bett et al., 2001) and a loss of biodiversity reduces bioturbation (Solan et al., 2004). In turn, this will dramatically impact the functioning of our biosphere if such changes occur on global scales.

6.5 Summary of the major findings of this study

1. There was a contrast in the quantity, quality (in terms of freshness) and composition of the OM reaching the seafloor at each of the abyssal sites studied.

2. The quantity and quality of the OM supply affected the diet holothurians. However, the extent of this influence differed between species.

3. The ovarian biochemistry of the holothurians studied was a complex function of the feeding adaptations and selectivity of each species for specific carotenoids, as well as the composition and magnitude of the flux of OM.

    a) Some holothurian species assimilate relatively high concentrations of carotenoids into their ovaries, presumably to increase reproductive output and larval survival.
    b) Enhanced carotenoid supply in the flux of OM was mirrored in the concentration of carotenoids in the holothurian ovaries and in turn may increase reproductive output. However, differences in the way the OM flux arrived at the seafloor, in conjunction with the duration of the flux, influenced to what extent enhanced carotenoid supply was translated to enhanced ovarian carotenoid concentrations in each species because of their feeding adaptations.
    c) Some holothurians showed very consistent carotenoid profiles, which suggests these species require specific carotenoids. Enhanced supply of these carotenoids may give such species a reproductive advantage.
    d) Some holothurian species showed less consistency in their ovarian biochemistry, suggesting less selectivity for specific compounds. These species assimilated carotenoids supplied in the flux of OM. The reproductive output of these species may be affected by the varying biochemical value of carotenoids in the flux of OM.

4. No consistent relationship can be made between the feeding adaptation of the species, the ovarian carotenoid concentration and the intraspecific requirement of for specific carotenoids.

5. This study proposes that changes in phytoplankton composition, the timing and extent of the bloom, as well as the recycling and repackaging of OM in the upper water column will influence the ovarian biochemistry of abyssal holothurians. This in turn may affect the reproductive output of the holothurians and lead to community change.

6.6 Limitations of the study and ideas for future work

One of the main problems encountered during the present study was the high variability of pigment concentrations in the samples. This highlights the need for more than 5 replicates of each sample type and this should be taken into consideration when designing future sampling programmes. Variability in gut samples chlorophyll *a* concentrations may be attributed to the patchy nature of the phytodetritus and sediment (through supply and bioturbation) and the encounter rate of the species. Carotenoid concentrations in the ovaries can differ between stages of gonadal maturity – which may explain variance between samples in a species (Borisovets et al., 2002; Lawrence et al., 2004). Future work should assess the reproductive biology and biochemistry of a species at the time of sampling to see if the two are linked. Detailed inspection of the eggs in the ovary of *O. mutabilis* shows that some eggs are more orange (indicating higher carotenoid loads; Mclaughlin and Kelly, 2001) than others (Ramirez-Llodra et al., 2005; pers. obs.). This may be related to the developmental stage of the egg. Time constraints and the small egg size of some species did not allow for the analysis of individual eggs. Further work could quantify the variation of carotenoid concentration (and possibly composition) in the eggs of the ovaries of one specimen. A few eggs of similar colour from the same specimen would need to be pooled into one sample so that the carotenoid concentration is above the detection limit of the analytical procedure.

The analytical method used for the present study enabled the correct identification and quantification of more pigments than previous studies (Hudson et al., 2003; Wigham et al., 2003a). This was especially the case for carotenoids that eluted in the diadinoxanthin to zeaxanthin region (see Chapter 2 for details). The methodology used to analyse the 2004 samples was not responsible for the non-detection of 19'-butanoloxyfucoxanthin and 19'-hexanoloxyfucoxanthin in samples from these years as the method has successfully been used to detect these pigments (Barlow et al., 1993). Hudson (2004) experienced difficulty in distinguishing between chlorophyll *a* and echinenone because they elute closely

together. Correct identification was made during the present study because the spectral property of each carotenoid is quite different, which facilitated identification. Some carotenoids in the gut wall and ovarian samples were not identified. These pigments are probably transformation products. The biotransformation pathways of some carotenoids in echinoderms are known. β-carotene was shown to be metabolised to echinenone (the major carotenoid constituent in the gonad accounting for up to 82% of the total) and sequestered in the gonad of the shallow water echinoid *Lytechinus variegates* (Plank et al., 2002). This has also been shown in other echinoderms by Tsushima and Matsuno (1990b); Tsushima et al (1993b) and Matsuno and Tsushima (1995). The metabolism of β-carotene to echinenone occurs in the gut wall of the echinoid *Pseudocentrotus depressus* via its precursor β-isocryptoxanthin (Tsushima et al., 1993b). In sea cucumbers, Matsuno and Tsushima (1995) have elucidated the metabolic pathways that convert β-carotene to astaxanthin and canthaxanthin to cucumariaxanthin (a novel marine carotenoid). It is possible that astaxanthin, isozeaxanthin, isocryptoxanthin and cucumariaxanthin account for some of the unknown pigment peaks. LC-MS of the holothurian samples, in comparison with known standards of astaxanthin, isocryptoxanthin, isozeaxanthin and cucumariaxanthin would confirm this.

Carotenoid 'metabolic pathways' in animals are largely speculative, with little direct evidence. The rates of conversion are generally slow and various alternative sequences are possible (George Britton – pers comm.). Pathways may differ between orders and at species level – especially in nutritionally poor environments like the deep sea where resource partitioning is observed (Hudson et al., 2003). The metabolic pathway of carotenoids in echinoderms other than that of β-carotene to canthaxanthin and echinenone through precursors of isocryptoxanthin and isozeaxanthin (respectively) (Goodwin, 1980; Tsushima et al., 1993b; Matsuno and Tsushima, 2001) are unknown. It is assumed in the present and previous studies (Hudson et al., 2003; Wigham et al., 2003a) that the zeaxanthin in the gut wall and ovaries of *Amperima rosea* has been assimilated though its diet. However, there is the possibility that this species may be able to

convert other carotenoids into zeaxanthin. This would need to be assessed using an experimental approach. The biochemical pathways detailed by Tsushima and Matsuno (1990b); Tsushima et al (1993b), Matsuno and Tsushima (1995) and Plank et al. (2002) were determined by providing specimens with an experimental diet over a long time scale. Such feeding experiments would be unrealistic in the deep sea because of sampling and time constraints.

Recent deep-sea *in situ* experiments have used ^{13}C to help elucidate the response and affect a pulse of phytodetritus has on deep-sea deposit feeders (Witte et al., 2003; Nomaki et al., 2005; Nomaki et al., 2006). The application of ^{13}C labelled phytoplankton makes it possible to follow directly the pathway and transfer of carbon and individual compounds into animals. This is in contrast to previous studies involving controlled diets, where only the changes in compositions were taken as evidence for these processes (Graeve et al., 2005). Such isotopic studies could be used to study the uptake of carbon by deep-sea megafauna such as holothurians. Labelled carbon could be used to assess the assimilation rates of carbon into different body parts (gut walls/ovaries) and compared between species. For example, the present study suggests that *Psychropotes longicauda* and *Pseudostichopus villosus* respond quickly to an enhanced pulse of phytodetritus at M5, as shown by the enhanced ovarian carotenoid load. However, the ovarian biochemistry of *Oneirophanta mutabilis* reflected the historic supply of enhanced prolonged flux at the PAP. This contrast may reflect different assimilation rates and responses to the flux of OM. Carbon uptake rates of foraminifera have been shown to vary depending on the trophic niche of the species (Nomaki et al., 2005). An extension to isotopic holothurian feeding studies could be applied by introducing different labelled algal/bacteria (representative of phytodetritus/OM in the sediments) to examine selectivity further in holothurians. This approach may determine if holothurians 1) ingest fresh phytodetritus selectively, 2) ingest fresh phytodetritus selectively, but can take advantage of sedimentary OM when phytodetritus is absent, or 3) ingest sedimentary organic matter at random. Such a study may be better than using gut sediment chlorophyll *a* concentrations as an indicator of selectivity because of the erroneous conclusions that may be derived

if chlorophyll *a* concentrations are not compared temporally under different OM supply regimes.

The isotopic labelling method can also be used to trace different fatty acids produced by different algal groups into the body walls and ovaries of the holothurians. This would help to determine the selective assimilation and differences between holothurians in their response to a pulse of phytodetritus. Temporal comparisons of the lipid biochemistry of deep-sea holothurians and sediment have previously been used as evidence of these processes (Ginger et al., 2000; Hudson et al., 2004; Neto et al., 2006). Although individual lipids can be traced and analysed using GC/IRMS (Gas Chromatography - Isotope Ratio Mass Spectroscopy), the labelling, and subsequent analysis of individual pigments and their biotransformation products is more difficult. Either the algae would need to be uniformly labelled to produce meaningful results on the LC-MS (George Wolff – pers comm.) or liquid chromatography isotope ratio mass spectrometry (LC/IRMS) would need to be used, but few of these machines are in existence. Labelling algae also presents problems in tracing pigment pathways as phytoplankton often have more than one type of associated pigment, therefore it would not be clear which carotenoid was involved in the biosynthesis of new carotenoids. Producing a feed containing one pure ^{13}C labelled pigment would be required for this kind of study.

The present study also highlighted differences in deeper sediment sections that could not be easily explained by OM supply. Further work could examine and compare bioturbation rates at deep-sea sites with contrasting environmental regimes. Shallow water studies have shown that bioturbation profiles differ between communities with different dominant species (Biles et al., 2002). Such studies may elucidate if the enhanced pigment concentration (or other labile compounds) at sediment depth is attributed to the subduction of labile material by infauna, or through the persistence of the compounds into the deeper sediment.

Experimental techniques are the future where the understanding of ecosystem functioning in the deep sea is concerned. Environmental conditions can be controlled and manipulated to measure individual or community response to different variables. Traditional sampling methods only give a snap-shot view of what is occurring at the time of sampling. Resources, in terms of experimental equipment and the means to deploy it (Remotely Operated Vehicles) need to be developed and/or made available for such experimental work to progress.

7. References

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8. Appendices

8.1 Appendix 1

A. rosea gut sediment sample	Chl c3	Chl c2	Fucox	Diadinox	Allox	Diatox	Zeax	Canthax	Chl a	Echine	ß-carot	Phorb	Phytin
1	0.00	0.00	0.00	10.24	13.44	10.90	64.67	8.96	0.00	17.21	9.41	0.00	0.25
2	0.41	0.48	2.69	3.19	3.82	2.94	11.00	1.55	2.76	5.66	3.45	4.98	1.25
3	0.56	0.58	12.45	9.23	11.35	8.28	32.29	3.64	4.13	13.09	7.79	13.24	2.90
4	1.06	0.53	12.71	2.37	1.70	1.41	1.28	0.30	5.33	0.23	1.90	11.92	3.54
5	0.79	0.25	8.84	6.36	7.24	5.33	16.41	1.81	4.42	6.62	5.25	15.61	1.79
6	0.00	0.75	6.24	7.24	9.86	6.39	31.10	3.88	0.00	13.93	5.25	8.63	2.04
7	0.50	0.92	5.09	16.86	21.32	18.73	71.12	13.02	0.00	56.68	19.46	6.45	1.34
8	1.40	0.54	3.79	6.81	10.65	8.50	30.43	4.11	0.00	15.30	6.86	5.60	1.31
9	0.42	0.16	4.72	5.93	9.59	6.92	31.10	2.24	0.00	14.40	6.84	7.41	2.11
10	0.00	0.00	3.25	9.44	15.17	14.34	41.02	6.23	0.00	34.49	12.78	4.92	1.39
11	2.06	0.89	5.30	4.66	6.70	4.46	18.28	2.12	0.00	7.43	7.25	6.75	1.40
12	3.14	0.84	6.13	6.89	7.53	4.31	18.79	2.04	0.00	1.86	3.03	8.64	2.16
13	0.00	0.00	0.00	3.79	5.29	2.59	4.63	1.41	0.00	4.14	2.11	3.97	0.63
14	0.00	0.00	7.86	5.98	6.53	4.26	13.00	0.61	0.00	6.86	6.78	11.20	2.93

Appendix 1. Phytopigment concentration (µg/gDW) of individual *Amperima rosea* gut sediment samples.

8.2 Appendix 2

a) *Oneirophanta mutabilis* gut wall

	Year of highest concentration (if sig. different)	Statistical outcome
Diad	-	t(10)=0.64, P>0.05
Allox	-	t(10)=0.53, P>0.05
Diatox	-	t(10)=0.44, P>0.05
Zeax	-	t(10)=1.02, P>0.05
Canthax	-	t(10)=0.01, P>0.05
Echine	-	t(10)=1.38, P>0.05
B-carot	-	t(10)=0.61, P>0.05

b) *Oneirophanta mutabilis* ovary

	Year of highest concentration (if sig. different)	Statistical outcome
Diad	-	t(10)=0.68, P>0.05
Allox	-	t(10)=1.23, P>0.05
Diatox	-	t(10)=0.51, P>0.05
Zeax	-	t(10)=1.09, P>0.05
Canthax	-	t(10)=1.49, P>0.05
Echine	-	t(10)=1.80, P>0.05
B-carot	-	t(10)=1.56, P>0.05

c) *Psychropotes longicauda* gut wall

	Year of highest concentration (if sig. different)	Statistical outcome
Diad	2004	t(9)=2.75, P<0.05
Allox	2004	t(9)=2.91, P<0.05
Diatox	2004	t(9)=3.63, P<0.05
Zeax	2004	t(9)=2.81, P<0.05
Canthax	2004	t(9)=2.42, P<0.05
Echine	-	t(9)=1.32, P>0.05
B-carot	-	t(9)=1.87, P>0.05

d) *Psychropotes longicauda* ovary

	Year of highest concentration (if sig. different)	Statistical outcome
Diad	-	t(5)=1.21, P>0.05
Allox	-	t(5)=1.09, P>0.05
Diatox	-	t(5)=1.27, P>0.05
Zeax	-	t(5)=1.69, P>0.05
Canthax	-	t(5)=0.72, P>0.05
Echine	-	t(5)=0.14, P>0.05
B-carot	-	t(5)=1.76, P>0.05

e) *Paroriza prouhoi* gut wall

	Year of highest concentration (if sig. different)	Statistical outcome
Diad	-	t(7)=1.44, P>0.05
Allox	-	t(7)=0.51, P>0.05
Diatox	-	t(7)=0.87, P>0.05
Zeax	-	t(7)=1.73, P>0.05
Canthax	-	t(7)=1.03, P>0.05
Echine	-	No echinenone in 2004 samples
B-carot	-	t(7)=1.11, P>0.05

f) *Paroriza prouhoi* ovary

	Year of highest concentration (if sig. different)	Statistical outcome
Diad	-	t(6)=1.05, P>0.05
Allox	-	t(6)=1.75, P>0.05
Diatox	-	t(6)=1.28, P>0.05
Zeax	-	t(6)=1.13, P>0.05
Canthax	-	t(6)=1.04, P>0.05
Echine	-	t(6)=1.62, P>0.05
B-carot	-	t(6)=2.44, P>0.05

Appendix 2. Between-year statistical analysis of the average concentrations of carotenoids found in the gut wall and ovaries of holothurians sampled at the PAP in June 2004 and July 2005 (statistical package used: minitab)

8.3 Appendix 3

a) *Peniagone spp.* gut wall

	Year/site of highest concentration (if sig. different)	Statistical outcome
19 but	M5	W(6,5)=51, P<0.05
19 hex	-	W(6,5)=44, P>0.05
Diad	M5	t(9)=2.6, P<0.05
Allox	M5	W(6,5)=50, P<0.05
Diatox	M5	W(6,5)=50, P<0.05
Zeax	M5	U(6,5)=49, P<0.05
Canthax	M5	W(6,5)=47.5, P<0.05
Echine	M5	W(6,5)=49, P<0.05
B-carot	-	t(9)=2.45, P>0.05

b) *Peniagone spp.* ovary

	Year/site of highest concentration (if sig. different)	Statistical outcome
19 but	M5	W(5,5)=37.5, P<0.05
19 hex	-	t(8)=0.42, P>0.05
Diad	-	t(8)=0.16, P>0.05
Allox	-	t(8)=2, P>0.05
Diatox	-	t(8)=1.94, P>0.05
Zeax	-	t(8)=2.28, P>0.05
Canthax	-	t(8)=1.65, P>0.05
Echine	-	t(8)=2.04, P>0.05
B-carot	-	t(8)=2.36, P>0.05

c) *Psychropotes longicauada* gut wall

	Year/site of highest concentration (if sig. different)	Statistical outcome
19 but	-	W(5,3)=30, P>0.05
19 hex	-	W(5,3)=29, P>0.05
Diad	-	W(5,3)=27, P>0.05
Allox	-	W(5,3)=29, P>0.05
Diatox	-	W(5,3)=17, P>0.05
Zeax	-	W(5,3)=26, P>0.05
Canthax	-	W(5,3)=18, P>0.05
Echine	-	W(5,3)=24, P>0.05
B-carot	-	W(5,3)=24, P>0.05

d) *Psychropotes longicauada* ovary

	Year/site of highest concentration (if sig. different)	Statistical outcome
19 but	-	t(8)=1.46, P>0.05
19 hex	-	t(8)=0.38, P>0.05
Diad	-	t(8)=0.48, P>0.05
Allox	-	t(8)=1.49, P>0.05
Diatox	-	t(8)=1.41, P>0.05
Zeax	-	t(8)=2, P>0.05
Canthax	-	t(8)=2.2, P>0.05
Echine	-	t(8)=2.15, P>0.05
B-carot	-	t(8)=2.51, P>0.05

e) *Abyssocucumis abyssorum ovary*

	Year/site of highest concentration (if sig. different)	Statistical outcome
19 but	M5	t(6)=6.61, P<0.05
19 hex	M5	W(5,3)=30, P<0.05
Diad	M5	t(6)=3.24, P<0.05
Allox	-	t(6)=0.26, P>0.05
Diatox	-	t(6)=0.75, P>0.05
Zeax	-	t(6)=0.53, P>0.05
Canthax	-	t(6)=0.73, P>0.05
Echine	-	t(6)=0.21, P>0.05
B-carot	-	t(6)=0.46, P>0.05

Appendix 2. Between-site statistical analysis of the average concentrations of carotenoids found in the gut wall and ovaries of holothurians sampled at M5 and M6 (statistical package used: minitab)

8.4 Appendix 4

Published paper:

Hughes JA, **Smith T**, Chaillan F, Bett BJ, Billett DSM, Boorman B, Frenz M, Wolff GA (2007). Two abyssal sites in the Southern Ocean influenced by different organic matter inputs: environmental characterization and preliminary observations on the benthic foraminifera. *Deep-Sea Research II* 54: 2275-2290