THE UNIVERSITY OF LIVERPOOL

THE BIOCHEMICAL RESPONSE OF DEEP-SEA HOLOTHURIANS TO TEMPORAL VARIATION IN FOOD SUPPLY AT THE DEEP-SEA FLOOR

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by

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Abstract

This thesis investigates biochemical changes in holothurians as a response to fluctuations of food supply. The approach involved analysis of lipids in their body tissues and in the sediments on which they feed. Samples were collected on five cruises in the Porcupine Abyssal Plain (PAP; ~ 5000 m water depth), in the northeast Atlantic, over a period of more than two years (March-1997 to May-1999). These provide a view of temporal variability at that site. Regional differences between holothurian populations were addressed by comparison of the species collected at the PAP (*Oneirophanta mutabilis, Pseudostichopus villosus, Psychropotes longicauda, Deima validum, Paroriza prouhoi, Amperima rosea* and *Molpadia blakei*) with those collected at a contrasting site on the West Antarctic Peninsula Shelf (WAP; ~600 m water depth; *Molpadia musculus, Peniagone* sp.*, Scotoplanes globosa, and Bathyplotes* sp.) in February 2001.

The lipid contents of species of both locations were highly complex in which may reflect their opportunistic feeding mode and their ability to assimilate dietary organic matter. Lipids may also have a role in the negative buoyancy of holothurians. The lack of or low concentration of triacylglycerols and other forms of chemical energy storage in holothurians indicates that they do not accumulate energy reserves. However, steryl sulphates may act as a store of sterols in low food periods. Molecular parameters distinguished species collected at the PAP and at the WAP, suggesting that the differences in available food (quality and quantity), ambient pressure, and feeding mode influence the biochemistry of these animals.

Feeding mode and food availability also influence the temporal variations of their biochemistry. Hence, the lipid contents of *Psychropotes longicauda* showed a striking correlation to those of sediments. Lipid contents of other species were also variable, but showed no relationship with the sedimentary organic matter pool, which was attributed to differences of feeding mode. Species feeding on deeper sediments were those which responded least to food supply to the surficial sediments. Finally, the results indicate that holothurians may have potential as indicators of temporal variation of food composition and supply to the deep-sea.

I certify that the work described in this thesis is my own except where otherwise stated and has not previously been submitted for a degree at this, or any other university.

Renato Rodrigues Neto.

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% Bact FA – Percentage of the concentration of bacterially-derived compounds relatively to total concentration of fatty acids;

$^{234}Th_{xs}$ – Excess ^{234}Th;

ACN – Acetonitrile;

A_{is} – Area of the internal standard;

AmR – *Amperima rosea*;

APCI – Atmospheric pressure chemical ionization;

A_{st} – Area of the standard being quantified;

A_{tc} – Area of the peak of the target compound;

Bathy – *Bathyplotes* sp.;

BBL – Benthic boundary layer;

BENGAL – High-resolution and spatial study of the Benthic biology and Geochemistry of a north-eastern Atlantic abyssal locality;

BIO-C-FLUX - Biological Carbon Flux in the Benthic Boundary Layer of the Deep Sea;

BIOTRANS – Biological carbon flux in the Benthic boundary layer of the deep sea;

BR – Batch reactors;

BSTFA – N,O-*bis*-(trimethylsilyl)trifluoroacetamide;

C – Carbon;

CID – Collision induced dissociation;

C_{is} – Concentration of the internal standard;

C_{st} – Concentration of the standard being quantified;

CSTR – Continuous-flow stirred-tank reactors;

C_{tc} – Concentration of the target compounds;

CV – Coefficient of variation;

DCM – Dichloromethane;

DmV – *Deima validum*;

ESI – Electrospray ionization;

FA – Fatty acids;

FAB – Fast atom bombardment;

FAMEs – Fatty acid methyl esters;

FOODBANCS – Food for benthos on the Antarctic Continental Shelf;

FSI – Flux Stability Index;

GC – Gas chromatography;

GC-EI-MS – Gas chromatography-eletronic ionization-mass spectrometry;

GC-IR-MS – Gas chromatography isotope ratio mass spectrometer;

GC-MS – Gas chromatography-mass spectrometry;

HPLC – High performance liquid chromatography;

LC-MS – Liquid chromatography-mass spectrometry;

LC-UV-Vis – Liquid chromatography-Ultraviolet/Visible detector;

LF – Low flux period;

MBl – *Molpadia blakei*;

MeOH – Methanol;

Mmus – *Molpadia musculus*;

N – Nitrogen;

NABE – North Atlantic Bloom Experiment;

OM – Organic matter;

Omt – *Oneirophanta mutabilis;*

PAP – Porcupine Abyssal Plain;

Pen – *Peniagoni* sp.;

PFRs – Continuous plug flow reactors ;

Ph – Phthalate;

PHF – Post-high flux period;

Plg – *Psychropotes longicauda*;

POC – Particulate organic carbon;

POM – Particulate organic matter;

PPr – *Paroriza prouhoi;*

PsV – *Pseudostichopus villosus*;

PUFAs – Polyunsaturated fatty acids;

Q_{sample} – Weight of the aliquot of the extracted sample (g);

RF – Response factor;

SCB – Santa Catalina Basin;

SCOC – Sediment community oxygen consumption;

SD – Standard variation;

SGl – *Scotoplanes globosa*;

SIM – Single ion monitoring;

SOC – Southampton Oceanography Centre;

TIC – Total inorganic carbon;

TLC – Thin layer chromatography;

TN – Total nitrogen;

TOC – Total organic carbon;

WAP – West Antarctica Peninsula Shelf;

W_{is} – Weight of the Internal Standard added to the sample (ng);

Chapter 1

THE BENTHIC RESPONSE TO FLUCTUATIONS IN FLUX OF PARTICULATE ORGANIC MATTER TO THE DEEP SEA AND CHEMICAL METHODS TO ASSESS IT

1.1 GENERAL INTRODUCTION

This thesis is focused mainly on the response of the benthos to the arrival of particulate organic matter (POM) at the sea floor. This introductory chapter discusses the response of benthic organisms to a variable supply of POM to the deep sea and the methods used to assess it. The materials and methods employed in the present study are described in Chapter 2. In Chapter 3, a new method of analysis of intact steryl sulphates in invertebrates by liquid chromatography-mass spectrometry is presented. Chapters 4-6 describe and discuss the lipid contents of 11 species of large invertebrates (holothurians), a comparative study between the lipids of holothurians from two different regions, the Porcupine Abyssal Plain (PAP), in the northeast Atlantic, and the West Antarctica Peninsula (WAP) and their relationship to food availability, and the temporal variability of the concentration of these compounds in their body tissues, respectively. Finally, the summary of conclusions and future work are presented in Chapter 7.

1.2 SEASONALITY AND INTER-ANNUAL VARIATION IN THE DEEP SEA

The detailed investigation of organic carbon cycling in estuarine and marine environments has stimulated the development of multidisciplinary concepts, research and sampling strategies, as well as analytical tools since early 1980s (Saliot *et al.*, 1991). In spite of all efforts, there are many chemical and physical processes in the oceans, which are either unknown or poorly understood. This is particularly the case in the deep sea because its depth and associated pressures, which make sampling and *in situ* observations extremely difficult.

The influence of external forcing factors such as the variability of supply of POM to the deep-sea ecosystem (Billett *et al*., 1983) and its impact on life at the benthic boundary layer (BBL; Tyler *et al.*, 1982; Fleeger *et al.*, 1989; Gooday and Turley, 1990; Altenbach, 1992; Pfannkuche, 1993; Goedkoop and Johnson, 1996; Drazen *et al.*, 1998; Gooday and Rathburn, 1999; Pfannkuche *et al.*, 1999; Danovaro *et al.*, 2000b; Billett and Rice, 2001; Witbaard *et al.*, 2001) as well as the complex interplay of processes such as bioturbation and remineralization of organic carbon (*e.g.* Pope *et al.*, 1996) are still poorly constrained.

The deep-sea environment had traditionally been considered as one of the least variable on the surface of the Earth (Deuser *et al.*, 1981, Dickson *et al.*, 1982). However, in the 1970s, the development of new photographic and video equipment, which could cope with elevated pressures, led to important physicochemical and biological observations (see *section 1.3.2*; Paul *et al.,* 1978; Deuser *et al*., 1981; Tyler *et al.*, 1982; Billett *et al.*, 1983;

Lampitt and Burnham, 1983; Rice *et al.*, 1986; Thurston *et al.*, 1994; Smith *et al.*, 1996; Beaulieu and Smith, 1998; Gutt *et al.*, 1998; Bett *et al.*, 2001; Billett *et al.*, 2001; Brown *et al.*, 2001), which have completely changed the scientific view of the deep sea. For instance, Billett *et al.* (1983) showed photographic evidence of phytodetritus covering the seabed at the Porcupine Seabight (1370-4100 m water depth), as a thin green layer of apparently fresh POM, in the spring-early summer 1982. More recently, it has become apparent that there is inter-annual variability in the timing of the arrival of phytodetritus and its coverage of the sea floor (Bett *et al.*, 2001).

Gooday and Turley (1990) classified the particles (POM) that reach the sea-floor from the euphotic zone into the four main groups:

(1) Large animal remains (mainly vertebrate carcasses).

(2) Large plant remains (macroalgae, seagrasses, terrestrial material).

(3) Large particles (faecal pellets, zooplankton carcasses, crustacean moults, skeletal remains of zooplankton and phytoplankton).

(4) Macroaggregates (marine snow).

POM of types 3 and 4 are the dominant food for holothurians (*e.g.* Billett, 1991; Roberts *et al.*, 2000). As food for the deep-sea benthos is scarce and is mainly derived from the remains of biological material, which arrives from overlying waters, organisms inhabiting the BBL are likely to be greatly affected by inter-annual changes in food supply (*i.e.* change in the absolute flux of POM; Rowe and Staresinic, 1979; Gooday and Turley, 1990).There are considerable practical difficulties in assessing the response of the benthos to changes of POM flux since the approaches adopted for their measurement are incompatible. Sediments traps are used to study the

chemical and biological composition, and flux of POM (*e.g.* Fowler and Knauer, 1986; Wakeham *et al.*, 1997; Kiriakoulakis *et al.*, 2001). On the other hand, biological and sediment samples are collected by trawls, sediment multi-corers and box corers and are analysed on board research vessels or in the laboratory (*e.g.* Bett *et al*., 2001; Billett *et al.*, 2001). Observations and measurements have also been made *in situ* by using cameras, landers and other equipment (*e.g.* Smith *et al.*, 1983; Gage and Tyler, 1991; Billett *et al*., 2001; Witbaard *et al*., 2001).

The mass sedimentation of material produced in the photic zone to the ocean floor following a surface bloom was first described in detail at the Porcupine Sea Bight (51°N, 13°W; Billett *et al.*, 1983; Lampitt, 1985; Rice *et al.*, 1986). Similar seasonal deposition has since been observed in many deep-sea regions including: the Rockall Trough, the Porcupine Abyssal Plain, and the Bay of Biscay, in the northeast Atlantic; Gay Head Bermuda, in the northwest Atlantic; the Scotia Sea, in the South Atlantic, the Sub-Artic Pacific, the California Upwelling, in the northeast Pacific, and in the Panama Basin (Tyler, 1988 and references therein), the central equatorial Pacific (Smith *et al.*, 1996; Brown *et al.*, 2001), the Gulf of Lions and the Catalan Sea in the western Mediterranean (Danovaro *et al.*, 1999) in the Cretan Sea off north of Crete (Danovaro *et al.*, 2000a&b), in the Arabian sea (Honjo *et al*., 1999; Rixen *et al*., 2000) and on the Antarctic shelf (Gutt *et al.*, 1998).

Rice *et al.* (1986) suggested that the seasonal deposition of POM as phytodetritus is a regular phenomenon and that the material undergoes relatively little degradation during its passage through the water column. Considering that more than 40% of the annual particulate organic carbon

(POC) flux may reach mid to high latitude northeast Atlantic sediments following the spring bloom (Honjo and Manganini, 1993), such events may impart significant temporal variability to nutrient fluxes and carbon burial at the seafloor (Smith *et al.*, 1992; Pfannkuche, 1993). Biochemical analyses, microbial/meiofaunal standing stocks and growth rates, and selective ingestion suggest that phytodetritus constitute a high-quality food source for the deep-sea benthos (Smith *et al.*, 1996 and references therein). Thus, the abundance and biomass of benthic organisms is related directly to the amount of food reaching the sediment surface (Gooday and Turley, 1990, and references therein), and the presence and persistence of life itself on the ocean floor can be viewed as a response to organic inputs (Gooday and Turley, 1990).

There are indirect and direct means of characterizing benthic response to variations of flux of POM to the deep sea. The former includes measurement of processes or chemical signals in samples other than in the organisms themselves (*e.g.* chemical analyses of sediments and POM, sediment community oxygen consumption; SCOC). Indirect observations carried out in the water column and in sediments can be influenced by physical, chemical and biological processes such as dilution, currents, bioturbation, decompression, etc. Direct measurements, on the other hand, are made directly on the organisms (*e.g.* body size, size of oocytes, chemical composition of body tissues). For instance, by measuring oocyte sizes, Tyler *et al.* (1982) and Harrison (1988) developed the hypothesis that there is a direct link between the deposition of phytodetritus and the

seasonal patterns of reproduction observed in some deep-sea macrofaunal and megafaunal populations.

In this Chapter, the impact of a pulse of POM to the deep-sea floor and the chemical tools (direct and indirect) used to address these processes are reviewed and discussed. Recent biological reviews of deep-sea seasonality and related biological response can be found elsewhere (Tyler, 1988; Gooday and Turley, 1990; Billett, 1991; Gooday and Rathburn, 1999; Gooday, 2002). Thus, in the following sections there is more emphasis on a chemical perspective of determining processes and community response to POM enrichment.

1.2.1 VARIATION IN COMPOSITION OF POM FLUX

There are intra (depth and temporal) and inter-regional differences in the POM flux to the deep sea. The Flux Stability Index (FSI) measures the seasonal variations in flux, which is the minimum number of days required for half of the annual flux to be collected in any one year (Lampitt and Antia, 1997). This index had little variation over a period of 10 y (1989-1999) at the PAP (Lampitt *et al.*, 2001). On the other hand, temporal variations in the composition of the particulate flux were observed for particulate inorganic carbon, particulate organic carbon, particulate nitrogen, and opaline silica, although the relative abundance of these biogenic components did not vary with depth (Lampitt *et al*., 2001). In contrast, the lipid composition varied both with depth and temporally (Kiriakoulakis *et al*., 2001) over a period of two years.

Individual chemical compound classes have different reaction and degradation rates during transport through the water column. Labile POM, namely relative fluxes of phytopigments, proteins and carbohydrates varied temporally between September 1996 and October 1998 at the PAP (Danovaro *et al.*, 2001). Although this is too short period to evaluate inter-annual fluctuations, the available fractions of these biochemical groups (definite as enzymatic hydrolysable, which account for < 10% of the total pool), showed strikingly temporal variability in the sediments (Danovaro *et al.*, 2001). It was concluded that the temporal changes and possibly inter-annual variability of the organic content of deep-sea sediments is evident only when the biochemical composition of the sediment is investigated (Danovaro *et al.*, 2001). Thus, the changes of faunal populations described by Billett *et al.* (2001) are more likely to be driven by the bioavailable fraction of POM, rather than by the total flux of C and N deposited at the sea floor.

Inter-regional differences in the chemical composition of POM are striking. For example, protein fluxes were 6 times higher at the PAP than in the Cretan Sea, while carbohydrate fluxes were two times higher in the Cretan Sea (Danovaro *et al.*, 2000a; Fabiano *et al.*, 2001). This difference may reflect the productivity of the regions (Cretan Sea-oligotrophic; PAP-mesotrophic) rather than differences of depth or latitude (Danovaro *et al.*, 1999c in Fabiano *et al*., 2001). However, Kirialoulakis *et al.* (2001) suggested that intact proteins in POM at the PAP are only “semi-labile”; furthermore, proteins are not totally hydrolizable by enzymes (Dell’Anno *et al.*, 2000). Thus, the relatively high flux of proteins arriving at the PAP sea

floor could be driven by its “recalcitrant” characteristics (Fabiano *et al.*, 2001).

The distinction between recalcitrant and labile POM is important in assessing the impact of its flux on the BBL (section 1.3.2.2) and several methods have been used to quantify the “freshness” of the depositing material (*e.g.* Pfankuche, 1993).

Chlorophyll *a* degrades rapidly in oxic waters producing many different products, including phaeopigments. The ratio of chlorophyll *a* to phaeopigments has been employed as a “freshness” indicator (Pfankuche, 1993; Goedkoop and Johnson, 1996; Pace and Carman, 1996; Drazen *et al.*, 1998; Gerino *et al.*, 1998; Witbaard *et al.*, 2000; Fabiano *et al.*, 2001; Witbaard *et al.*, 2001). Sedimentary chlorophyll *a* and phaeopigment concentrations vary inter-annually in both the North Pacific (Drazen *et al.*, 1998) and North Atlantic (Witbaard *et al.*, 2000; Fabiano *et al*., 2001). These variations may reflect:

1) Inter-annual variability of primary production, which controls POM export to the deep sea (Lampitt and Antia, 1997)
2) Variation in the activity of the deep-sea benthos (Billett *et al*., 2001; Smallwood *et al.*, 1999), which could be responsible for accelerated rates of remineralisation of incoming POM (Ginger *et al.*, 2001; equally a change of quality of the organic matter could drive the variability in benthic activity; Billett *et al*., 2001).

Chlorophyll *a* has also been used as an indicator of phytoplankton sedimentation. As an example, Pfannkuche (1993) observed that the flux of chlorophyll *a* varied seasonally by one order of magnitude at the PAP,

hence sedimentation of phytodetritus begins around April-early May with a maximum sedimentation rate in June-July. Chlorophyll *a* is non-specific, however, the composition of microorganisms contributing to POM can be assessed by using lipid biological markers (biomarkers; Volkman *et al*., 1986; Rieley *et al*., 1991; Saliot *et al*., 1991; Meyers and Ishiwatari, 1993; Madureira *et al*., 1995; Canuel and Martens, 1996; Bourbonniere and Meyers, 1996; Colombo *et al*., 1996a&b). For instance, Skerratt *et al.* (1995) observed seasonal and inter-annual changes in planktonic biomass and community structure in eastern Antarctica by using specific lipids. During the period of diatom blooms (predominantly *Nitzchia* spp.) the dominant sterol and fatty acid were cholesta-5,22-dien-3*β*-ol (**3**)[1] and 20:5(n-3)[2], respectively, which were accompanied by a high ratio of 16:1(n-7)/16:0, and the presence of relatively high amounts of polyunsaturated fatty acids (PUFAs), particularly 20:5(n-6) and 22:6(n-3). However, blooms of the prymnesiophyte *Phaeocystis* sp., were characterized by high amounts of 14:0 fatty acids and (22*E*)-24-methylcholesta-5,22-dien-3*β*-ol (brassicasterol, **8**) and low levels of PUFAs and a low ratio of 16:1(n-7)/16:0.

In the Pacific, meridional trends for (**8**) indicate that there are elevated inputs of phytoplankton lipids around the equator, while trends of hexadecanol and cholest-5-en-3*β*-ol (**5**) suggest reduced inputs of zooplankton lipids (Wakeham *et al.*, 1997). These authors also showed that PUFAs are the most labile compounds among the investigated lipids, being

[1] The structure, short hand notation and name of sterols and other lipids are listed in Appendix A.
[2] Short hand notation for fatty acids X:Y(n-Z), where X=the chain length(number of carbons), Y=number of double bonds, and Z=position of the double bond numbering the chain from the opposite end of the acid group.

rapidly lost from particles during sedimentation. The mass sedimentation of phytodetritus as observed by Billett *et al.* (1983) may, nevertheless, increase the flux of these labile compounds to the BBL.

1.2.2 BENTHIC RESPONSE TO VARIATIONS OF POM

The inter-species difference of response of organisms inhabiting the deep sea to POM enrichment (Tyler, 1988) may reflect their different morphologies (Billett *et al.*, 1988; Jumars *et al.*, 1990; Billett, 1991; Roberts *et al.*, 1991; Roberts *et al.*, 1996; Roberts *et al.*, 2000). However, the study of animal morphology does not necessarily allow feeding mechanisms to be established. For example, both Sokolova (1958) and Hansen (1975) noted the diversity of tentacle structure in deep-sea holothurians, but were unable to detect any relationship with the gut contents of the holothurians. As predicted by Billett *et al.* (1988), chemical analyses have been useful in the study of feeding guilds and the benthic response to POM enrichment (food availability). The approaches that have been employed include analyses of sediments, gut contents and tissues for: stable isotopic composition (^{13}C, ^{15}N), radionuclides tracers (^{234}Th, ^{210}Pb), biological makers (including chlorophyll *a* and phaeopigments), sediment community oxygen consumption (SCOC), and the bulk biochemical composition (proteins, carbohydrates, lipids, pigments). Nevertheless, these chemical parameters are frequently interpreted to reinforce biological observations, which are discussed in the next section (1.2.2.1).

1.2.2.1 Biological observations

There are a number of reviews on the biological response to food supply in the deep sea (Tyler, 1988; Gooday and Turley, 1990; Gooday and Rathburn, 1999; Gooday, 2002), hence this subject will be only briefly reviewed here.

Seasonality of reproduction in deep-sea benthic invertebrates was first suggested by Schoener (1968) for two species of ophiuroids, *Ophiura ljungmani* and *Ophiomusium lymani*. Later, Tyler *et al*. (1982) provided evidence for annual periodicity and inter-species synchrony in the reproductive cycles of three deep-sea echinoderms, *Ophiura ljungmani*, *Plutonaster bifrons*, and *Echinus affinis.* Tyler *et al*. (1990) and Sumida *et al.* (2000) observed the same phenomena for *Dytaster grandis* and *Ophiocten gracilis*, respectively. These species produce relatively small-sized eggs (<150µm diameter; Tyler *et al.*, 1982), however other echinoderms that produce larger eggs (up to 4 mm) did not show any indication of seasonality in gametogenesis, eggs presumably being produced continuously throughout the year (Tyler *et al.*, 1982). Species that produce larger eggs do not have a larval stage, while those that produce small eggs develop free-swimming larvae that feed in the plankton (Tyler *et al.*, 1982 and references therein). Campos-Creasey *et al.* (1994) have provided further evidence that there is a coupling between the vertical flux of phytodetritus and the diet and seasonal life history of *Echinus affinis*. However, in other cases no relationship between reproduction and food availability has been noted and

direct links have yet to be found (Ambrose and Renaud, 1997, and references therein).

Bacteria do respond to food supply. A very rapid community response to the deposition of phytoplankton blooms occurs both in shallow water (Graf *et al.,* 1983) and in deep-sea environments (Gooday and Turley, 1990). Indeed, it has been suggested that bacteria and other organisms could respond in a matter of hours or days to undecomposed detrital material (Aller and Aller, 1986). There are no significant differences between total counts and biomass estimates for bacteria in phytodetritus, and sediments not overlain by phytodetritus. However, the microbial activity is greater within and immediately beneath the detrital aggregates, than in normal sediments (Rice *et al.* 1986).

Danovaro *et al.* (2000b & c) showed that there was a significant correlation between bacterial and meiofaunal abundance and biomass and indicators of food quality and quantity (chlorophyll *a*, phaeopigments, protein and soluble carbohydrate concentrations) in sediments in the Mediterranean Sea, reinforcing the views of Gooday and Turley (1990). The latter authors hypothesized that when phytodetritus arrives on the sediment surface in the late spring and early summer, it is colonized by benthic foraminifera that feed on the detritus and associated organisms. They subsequently reproduce rapidly and build up large populations. In well-oxygenated environments, the distributions of some foraminifera are controlled by the quantity and quality of available POM, rather than by bottom water masses as been commonly supposed (Gooday and Turley, 1990; Gooday and Rathburn, 1999).

There is evidence to suggest that other small organisms also respond rapidly to POM enrichment at the sea floor. Populations of meiofauna including an opheliid polychaete, which lives in the surface or sub-surface of sediments at the PAP, feeds on phytodetritus, increased rapidly in abundance after a pulse of POM in summer 1996 (Vanreusel *et al.*, 2001). Macrofaunal polychaetes, on the other hand, showed a lagged response to the same event, and their abundance increased more slowly (Vanreusel *et al.*, 2001). It might be expected therefore, that larger invertebrates, such as holothurians, would respond more slowly still.

The megabenthic community at the PAP has been monitored for more than ten years and temporal variability has been observed in terms of taxon/species composition, body size distributions, abundance and biomass (Billett *et al.*, 2001). One dramatic change had been the increase in abundance and biomass of the holothurian *Amperima rosea*. Using time-lapse photography (Bathysnap), Bett *et al.* (2001) found that the abundance of this species increased from 4 to 6457 specimens per hectare between 1994 and 1997. The bloom probably occurred in a relatively short time between the summers of 1996 and 1997 and may have resulted from changes in the quality of POM arriving the sea floor (Billett *et al.,* 2001).

Although some studies have failed to substantiate a link between POM supply and benthic activity (*e.g.* Gooday *et al.*, 1996), there is enough biological evidence to conclude that seasonal variability in POM flux to the deep sea can influence (directly or indirectly) the benthos (micro-, meio-, macro- and megafauna).

1.2.2.2 SCOC

The response time of SCOC to the arrival of phytodetritus has been estimated as less than 8 d at bathyal depths in the Norwegian Sea (Graf, 1989). The measurements of oxygen consumption and nutrient exchange rates of deep-sea sediments in aerobic environments provide some indication of the rate at which the POM that reaches the seafloor is remineralized (Smith *et al.*, 1983) as a function of benthic respiration.

The abyssal northeast Pacific is the only deep-sea region where SCOC has been examined for more than 20 years (Smith *et al.*, 1983; Drazen *et al.*, 1998; Smith *et al.*, 1998). However, other deep-sea sites have also been monitored for relatively long periods, albeit < 10 years (Gooday, 2002). These include the northeast Atlantic (Pfannkuche, 1993; Duineveld *et al.*, 1997; Witbaard *et al.*, 2000) and the Mediterranean (Tselepides and Polychronaki, 2000).

SCOC measurements at the PAP, in northeast Atlantic range from 400.8 to 542.4 μmol m^{-2} d^{-1} (Witbaard *et al*., 2000). These are of the same order of magnitude to those recorded in the Pacific (510 to 700 μmol m^{-2} d^{-1}; Smith *et al.*, 1998). Apparently, SCOC seasonality in the Pacific is much more pronounced than in the Atlantic (Gooday, 2002 and references therein). This may be due to primary productivity differences between these two oceans (Hinga, 1985). Smith *et al.* (1992) noted that at the slope in the eastern North Pacific there is very effective transfer of coastal production to the deep sea floor thus enhancing SCOC. On the other hand, the high eddy kinetic energy at abyssal depths in the western North Atlantic increases

bottom resuspension, resulting in a long residence time of the organic matter in the water column, decreasing remineralization at the seabed (Smith *et al*., 1992).

Temporal and spatial variability in SCOC have been observed (Smith *et al.*, 1983; Smith and Baldwin 1984) being attributed to variations of depth, primary productivity and macrofaunal abundance (Smith *et al.*, 1983; Smith, 1987). SCOC tends to decrease with increasing water depth (Smith *et al.*, 1983; Duineveld *et al.*, 1997) as a response to decreasing benthic density (see Duineveld *et al.*, 1997), and because of the decreasing primary productivity with distance from coastal upwelling areas towards central oligotrophic waters (Smith *et al*., 1983).

Several studies in both the North Atlantic and North Pacific indicate that primary productivity (flux of POM) and SCOC are decoupled (Smith, 1978; Smith and Baldwin, 1984; Smith, 1987; Lampitt *et al*., 1995; Duineveld *et al*., 1997 Witbaard *et al*., 2000). This may reflect the degradation (remineralization and repackaging) of POM during transport through the water column (Smith *et al.*, 1983, Duineveld *et al*., 1997) or large-scale horizontal advection (Smith, 1987). Thus, best estimates of SCOC are obtained if the flux of POM arriving at the BBL is monitored. The lack of response of SCOC to the peak deposition of particulate organic carbon (POC) on the northeast Atlantic Slope suggests that the POC was either refractory or biologically unavailable (Duineveld *et al*., 1987). SCOC may therefore be influenced only by labile material and bioavailable organic compounds, *i.e*. enzymatically hydrolysable proteins, carbohydrates and lipids (Danovaro *et al.*, 2001) and not by the gross POC flux.

Experimental errors in the determination of SCOC are also significant and may explain the absence of its relationship with the flux of POM (Smith, 1987). There is certainly an important difference between *in situ* and on-board measurements (Wittbard *et al.*, 2000 and references therein). For instance, Smith *et al.* (1983) noted that laboratory-measured SCOC varied by only 4% over a range of water depth (150 to 7000m), whilst *in situ* measurements spanned three orders of magnitude. There are several methods of making *in situ* measurements. The studies carried out in the Pacific were usually performed with a free vehicle grab respirometer, where sediment grabs (~ 400 cm^2) are fitted with polarographic oxygen sensors. Each grab is incubated until a significant change is noticed (1-3 days; Smith *et al.*, 1983). Witbaard *et al.* (2000) measured SCOC with chambers fitted with oxygen electrodes attached to landers at the PAP. They noted that the total diffusive flux of oxygen across the sediment-water interface had a positive relationship with the concentration of chlorophyll *a* in the sediment and suggested that this compound is representative of reactive organic matter. However, there was no variation of *in situ* SCOC between the sampling times. Thus, either the *in situ* SCOC measurements were underestimated, because of the 'blows' caused by the arrival of the lander, or the diffusive fluxes estimated from the pore-water oxygen profiles, which were measured on board with recovered multi-cores were too high (Witbaard *et al*., 2000).

Although different instruments can have different responses, the main problem of *in situ* measurements is generally attributed to the small number of replicate samples taken and the timing of collection (Smith, 1987;

Witbaard *et al*., 2000). Indeed, when Smith *et al.* (1992) carried out a long-term study, with a high degree of replication, they found a close match between SCOC and supply of POC (within 85%).

Problems with on-board measurements could arise from the fact that microbial metabolism is stimulated by the warming (up to 10 °C) upon retrieval of the sediment core and decompression, which causes lysis of pressure and temperature sensitive organisms (Glud *et al*., 1994). The release of organic matter could lead to an increase in the oxygen uptake in decompressed cores (Witbaard *et al*., 2000).

SCOC, nevertheless, is commonly employed to measure the response of benthos to variation of supply of POM. The major drawback of this method (other than experimental error) is that it does not differentiate between various benthic groups (Drazen *et al.*, 1998) and excludes megafauna. When monitoring the response of SCOC to POM deposition, it is also important to monitor the quality of POM, since it has been found that the latter is not necessarily coupled with its biochemical constituents (Kiriakoulakis *et al.*, 2001 *cf.* Lampitt *et al.*, 2001).

1.3. RATES AND MODES OF FEEDING OF MEGAFAUNA

Deep-sea benthic invertebrates feed mainly on sedimentary organic matter (*e.g.* Billett, 1991), hence their response to enrichment by POM may depend on rates and modes of feeding of individual groups. Studies of the bulk composition of POM and sediments have been employed to access their nutritional value. Total carbohydrates, proteins, lipids, total organic

carbon and nitrogen (TOC and TN, respectively) are the most commonly measured chemical groups. Proteins are the main source of N, while carbohydrate and lipids supply C to the animals. Because N has been considered a limiting element in the deep sea (*e.g.* Jumars *et al.*, 1990), organic matter with low C/N ratio (~<6) has been considered to be of high nutritional value to the deep-sea benthic community (Linke *et al.*, 1995). On the other hand, lipids can yield up to twice as much metabolic energy per unit weight as proteins and carbohydrates (Hadley, 1985).

Bulk analyses have also been used to measure the rate of absorption of organic matter in the guts of holothurians and the nutritional value of the food that these animals feed on. For instance, the gut contents of two species of holothurians at the Demerara Abyssal Plain, *Pseudosthicopus villosus* and *Deima validum*, had concentrations of proteins soluble in NaOH (1 M) approximately 10-fold greater than the total lipids, and about 4 to 5-fold more concentrated than carbohydrates soluble in NaOH (1M; Sibuet *et al.*, 1982). Furthermore, proteins were also the class of compound most readily absorbed by the guts relative to total absorbed organic constituents (18% for *Deima validum* and 16% for *Pseudostichopus villosus vs.* just 1.9 and 1.54% of lipids, respectively).

Recently, excess ^{234}Th ($^{234}Th_{xs}$) has been used to study the selectivity and rates of megafaunal deposit feeding (Lauerman *et al.*, 1997; Miller *et al.*, 2000). $^{234}Th_{xs}$ has a radioactive half-life of 24.1 d and is commonly used as a geochemical tracer for the fate of recently deposited (<100-d old) particles in the quiescent deep sea (Miller *et al.*, 2000). Lauerman *et al.* (1997) found high $^{234}Th_{xs}$ activities in the holothurians *Abyssocucumis*

abyssorum and *Oneirophanta mutabilis* (~4100-m depth off central California), indicating that they were feeding on particles 12-13 d old. Miller *et al*. (2000) applied $^{234}Th_{xs}$ to access feeding selectivity of six species of surface deposit feeders, four from the Santa Catalina Basin (SCB), and two from the Hawaiian slope. The guts of the species from SCB had $^{234}Th_{xs}$ activities 14-17 fold greater than those of the top 5 mm of sediment. The highly mobile elasipodid holothurians, *Pannychia moseleyi* and *Scotoplanes globosa*, were most enriched in gut $^{234}Th_{xs}$ activity, suggesting that these two species fed on very recently deposited particles (~20 d). However, the gut contents of the two surface deposit feeders, the synallactid holothurians *Mesothuria carnosa* and *Paleopatides retifer*, from the Hawaiian slope, were not enriched in $^{234}Th_{xs}$. In contrast, chlorophyll *a* analyses showed that the guts of the latter were 2.7-12 fold enriched in comparison to surficial sediments suggesting that these animals are selective feeders.

The difference, between the enrichment of $^{234}Th_{xs}$ and chlorophyll *a*, can be attributed to lateral advection (downslope transport), since the Hawaiian slope site is located at the foot of a ~22° slope of more than 1000 m height. The horizontal transport of relatively old particles would mask the $^{234}Th_{xs,}$ decoupling this tracer from fresh organic material. Thus, this tracer is only reliable at the quiescent deep-sea sites, which do not receive refractory material from horizontal advection (Miller *et al.*, 2000). Indeed, Gerino *et al.* (1998) showed that $^{234}Th_{xs}$ distributions predict generally lower overall organic matter transport than do chlorophyll *a* and luminophores.

$^{234}Th_{xs}$, chlorophyll *a*, and lipids have also been used to determine the rate at which the benthos process food. For instance, Miller *et al.* (2000),

using a mass-flux model based on $^{234}Th_{xs}$, determined that the populations of *Pannychia moseleyi*, *Chiridota sp*. (a burrowing, surface feeder, chiridotid holothurian), and *Bathybembix bairdii* (a surface deposit-feeding trochid gastropod) were responsible for reworking 39-52% of the daily flux of POC to the SCB floor. Witbaard *et al.* (2001) using chlorophyll *a* as a marker for ingested OM, with degradation rate constant k=55 y^{-1}, gut residence times of > 5 d, and a gut volume (not specified), calculated that *Oneirophanta mutabilis* grazes at a rate of ~ 0.22 m^2 d^{-1}, and travels 747 cm d^{-1} at an average speed of 31 cm h^{-1}. However, Mackenzie (2000) calculated higher values of grazing rate based on lipid concentrations in gut contents, namely that an *Oneirophanta mutabilis* individual could graze 1.67 m^2 d^{-1} and would metabolise 76% of the total sterols passing through its guts, although selective feeding might have led to these values being over-estimates. For comparison, the estimates of locomotion rate based on a free-vehicle camera for this species were about 84.4 cm h^{-1} (~1946 cm d^{-1}, Smith *et al*., 1997), in which, assuming that *Oneirophanta mutabilis* is ~2.5 cm wide, it transforms ~0.5 m^2 d^{-1}. Hence, holothurians have a significant impact on their sedimentary environment (Roberts *et al.*, 2000).

The distribution of lipids has also been used for determining feeding modes. For example, the free sterol distributions of abyssal holothurians are complex, with mixtures of C_{26} – C_{30} sterols being present in all animals (Ginger *et al.,* 2000). However, 24-ethyl-5α(H)-cholest-7-en-3β-ol (**25**) is the main free sterol of *Oneirophanta mutabilis, Pseudostichopus villosus,* and *Deima validum,* whereas 24-ethyl-5α(H)-cholestan-3β-ol (**20**) is predominant in *Psychropotes longicauda,* and *Molpadia blakei. Amperima rosea* has a

different distribution, 5α(H)-cholest-7-en-3β-ol (**7**) being the main free sterol (Ginger *et al.*, 2000). The sterol distributions reveal significant inter-species differences that might be ascribed to differences in feeding strategy. *A. rosea* is the only holothurian that clearly assimilates 4-α-methylsterols. This may reflect its ability to exploit more fully the phytodetritus at sea-floor, which is its main food source (Ginger *et al*., 2000; Ginger *et al*., 2001).

Feeding modes of megafauna have also been assessed by isotopic analyses of C and N. Suchanek *et al.* (1985) provided evidence from the stable isotopic composition of carbon ($\delta^{13}C$) that three species of holothurians: *Mesothuria verrilli, Psychropotes semperiana,* and *Benthodytes lingua*, and two species of sea urchins, *Hygrosoma petersi* and *Salenocidaris profundi*); feed on shallow-water seagrass detritus in the Caribbean deep-sea (2455-3950 m water depth). Apart from gut analyses, they compared $\delta^{13}C$ of abyssal seagrass detritus (from –5.8 to –9.0‰) and other potential carbon sources (POM; -20.1 to -28.2‰) with those for epidermis (-9.1 to -12.6‰), longitudinal muscles (-12.9 to -13.9‰), respiratory tissue (-13.4 to –16.0‰), guts wall (-13.1‰) and gonads (-14.6 to -17.7‰) of the study animals.

In summary, the rates and feeding guilds of benthic fauna can be assessed by a number of different techniques. However, all methods show that fauna are capable of processing a high amount of sediment, and that they absorb a high percentage of ingested OM.

1.4. TRACERS FOR FOOD WEB ELUCIDATION

The trophic structure in pelagic ecosystems is commonly assessed by determining the stable isotopic composition of carbon and nitrogen (*e.g.* Fry, 1988). Selective feeding or metabolic fractionation leads to the preferential loss of lighter isotopes during respiration (^{12}C vs. ^{13}C) and excretion (^{14}N vs. ^{15}N; DeNiro and Epstein, 1978). The consumer tissues thus tend to become enriched relative to that of their prey; hence relative trophic positions can be addressed (*e.g.* Fry 1988). $\delta^{13}C$ values may be biased by lipid contents, because rates of $\delta^{13}C$ enrichment depend on different chemical groups (proteins, carbohydrates, etc.). Nevertheless, $\delta^{13}C$ can be a good trophic maker if the samples are defatted. An alternative is to use $\delta^{15}N$, since lipids contain negligible quantities of N (Iken *et al*., 2001).

Such an approach has recently been employed to address the trophic structure of a benthic deep-sea ecosystem, by analysis of macro- and magafaunal organisms, including deposit feeders, suspension feeders and predators/scavengers (Iken *et al.*, 2001). $\delta^{15}N$ values overlapped between classes and covered a large range within feeding types, indicating a strong overlap in food sources and a high degree of competition for food (Table 1.1, Iken *et al.*, 2001). However, these values were dependent on the feeding mode of species of the same class, hence species feeding on fresher organic material had lighter $\delta^{15}N$ values (Iken *et al.*, 2001). For instance, the holothurian *Amperima rosea*, which feeds preferentially on fresh material, had a $\delta^{15}N$ value of +10.75 ‰, while *Molpadia blakei,* which feeds with its head down in the sediment had a mean value of +15.87 ‰.

Table 1.1. Ranges of mean $\delta^{13}C$ and $\delta^{15}N$ values (‰) of taxonomic groups at the PAP (after Iken *et al*., 2001).

Benthic groups at PAP	Range of $\delta^{13}C$	Range of $\delta^{15}N$
Pisces	-16.27 to -18.62	+10.28 to +15.52
Tunicata	-16.79 to -17.71	+14.80 to +15.11
Holothuroidea	-13.91 to -20.35	+10.75 to +16.18
Ophiuroidea	-17.85 to -19.08	+10.70 to +12.97
Asteroidea	-12.89 to -18.31	+12.59 to +17.13
Crinoidea	-17.09	+14.18
Decapoda	-16.20 to -20.36	+12.12 to +15.61
Cirripedia	-16.92 to -19.27	+14.93 to +16.91
Isopoda	-17.36	+13.43
Echiurida	-13.80 to -16.20	+12.80 to +13.61
Polychaeta	-16.18 to -18.39	+10.84 to +17.66
Cephalopoda	-18.94	+13.56
Sipunculida	-15.05	+14.03
Cnidaria	-13.64 to -17.57	+12.31 to +16.23
POM	-21.90	+8.15

Food web studies have occasionally employed lipid markers (Nichols *et al.* 1982, 1986; Kharlamenko *et al*. 1995; Graeve *et al*., 2001; Pond *et al*., 2002). The advantage of using the lipids in feeding studies is that trophic lipid markers integrate longer time periods than conventional methods (Graeve *et al*., 2001). The approach is based on the specific composition of microorganisms and algae and on the inability of animals to synthesize these compounds. Animals obtain a considerable portion of their fatty acids *via* diet (Sargent and Whittle, 1981), therefore, comparison of the fatty acid

composition of the animals and of their potential food sources may be used as a tool in food web studies. For example, brown algae contain high concentrations of PUFAs, 18:4(n-3) being dominant in *Alaria fistulosa* and *Arthrothamnus bifidus* and 20:4(n-6) in *Alaria angusta* and *Fucus evanescens.* The diatom *Thalassiosira anguste-lineata* is rich in 16:4(n-3), 18:4(n-3) and 20:5(n-3) (48.2% of the total fatty acids). The red alga, *Turnerella mertensiana*, is also enriched in 20:5(n-3) (39.2%; Kharlamenko *et al.*, 1995). Thus, the presence of these compounds in animal bodies in significant quantities suggests that they feed on these algae.

In tropical holothurians the branched chain fatty acids (bacterial biomarkers) constitute only about 1% of total fatty acids. At the same time, holothurians from temperate waters contained higher amounts of branched fatty acids (~15%; Svetashev *et al.*, 1991). Although Svetashev *et al.* (1991) did not carry out any analyses of food sources; they argued that this difference is most probably caused by the different composition in their food. However, it may be that these differences of bacterial biomarkers are caused by different gut structure and associated bacteria, which is also linked to feeding mode (this thesis, chapters 5 and 6). Animals feeding on symbiotic bacteria would be expected to have high concentrations of bacterial fatty acids and low concentrations of PUFAs (Convey and McDowell Capuzzo, 1991; Zhukova *et al.*, 1992; Fullarton *et al.*, 1995a&b; Kharlamenko *et al.* 1995). However, both biomarkers are found in holothurians. Bacterial fatty acids (branched acids and 18:1(n-7)) have been detected in high concentrations in shallow water and deep-sea holothurians (Kharlamenko *et al.*, 1995; Ginger *et al.*, 2000). High concentrations of

20:5(n-3) have also been found in these animals. In addition, the concentration of 16:1(n-7) much exceeds that of 16:0. Such a combination of markers may be observed in consumers of diatoms and large quantities of bacteria or microbial mats as food sources (Kharlamenko *et al.*, 1995). However a more likely scenario is that diatoms were used as the main food source and bacteria were involved in food transformation (Kharlamenko *et al.*, 1995). These authors also found a remarkable quantity of 22:6(n-3) acid in *Chone sp*., which implies that this species also feeds on animals.

Conclusions based on distribution of PUFAs can be misleading, however, since even infaunal deep-sea holothurians such as *Molpadia blakei* contain high quantity of PUFAs (Ginger *et al*., 2000; this thesis), although they are absent or present in very low quantities in subsurficial deep-sea sediments (Santos *et al.,* 1994; Makcenzie, 2000); this implies that *M. blakei* biosynthesises PUFAs.

1.5 SUMMARY

Research in the deep sea over the last 25 years has showed that this is a highly variable environment. Mass sedimentation of POM occurs periodically (*e.g.* Billett *et al.*, 1983) and benthic organisms can respond to this pulse of phytodetritus in a matter of hours. As a consequence, the POM becomes patchily distributed and this probably plays an important role in structuring ocean-floor communities, for example, by helping maintain high species diversity and increasing population heterogeneity (Gooday and Turley, 1990).

Although a number of studies have been carried out, the influence of a varying food supply on the reproduction (*e.g.* Tyler *et al.*, 1982), recruitment (Ambrose and Renaud, 1997), respiration (Duineveld *et al.*, 1997), population (Billett *et al.*, 2001) and biochemistry (this Thesis) of animals inhabiting the deep sea are still not fully understood. The influence of food supply on organisms probably depends on their grazing rates, feeding modes, and trophic niche; organisms, which selectively feed on fresh material are more likely to be influenced by variation of its supply.

1.6. OBJECTIVES

The main objective of this study is to evaluate the biochemical response of the holothurians to food supply. To tackle this issue, four points were assessed:

- Does the biochemistry of the animals reflect the ambient level of nutrition (quantity + quality of food supply)?
- Does the biochemistry of the animals reflect biological processes? *e.g.* growth, reproduction, etc.
- Does biochemical response depend on feeding (guild) mode, gut structure, metabolism and life mode?
- Do bacteria play an important role in the nutrition of holothurians?

These questions are addressed using the lipid approach discussed above.

Chapter 2

MATERIALS AND METHODS

2.1. CHEMICALS AND MATERIALS

All chemicals, suppliers, and grade are listed in Table 2.1.

TABLE 2.1. List of chemicals used in the present study.

Chemicals	**Suppliers**	**Grade**
Methanol (MeOH)	BDH (Poole, U.K.)	HiPerSolv
Dichloromethane (DCM)	BDH (Poole, U.K.)	HiPerSolv
Cyclohexane	BDH (Poole, U.K.)	AnalaR
Diethylether	BDH (Poole, U.K.)	AnalaR
Acetonitrile (ACN)	BDH (Poole, U.K.)	HiPerSolv
Water	BDH (Poole, U.K.)	HiPerSolv
Petroleum ether	Aldrich (Milwaukee, USA)	Spectrophotometric
Ethyl acetate	Prolabo (Fontenay, France)	HPLC
Acetyl chloride	Sigma (Steinheim, Germany)	-[1]
BSTFA	Sigma (Steinheim, Germany)	-
Silica 60	Merck (Darmstadt, Germany)	-
Sodium Sulphate	BDH (Poole, U.K.)	-

Table 2.1. Continued.

Chemicals	Suppliers	Grade
Cholesterol 3-sulphate[2]	Sigma (Steinheim, Germany)	-
Cholecalciferol 3-sulphate[2]	Sigma (Steinheim, Germany)	-
Cholest-5-en-3β-ol ($C_{27}\Delta^5$; **5**)	Sigma (Steinheim, Germany)	99.8%
4,4-Dimethyl-5α(H)-cholesta-8(9),24-dien-3β-ol (4-4-dimethyl-$C_{29}\Delta^{8(9),24(25)}$; **27**)	Sigma (St Louis, USA)	97.4%
5α(H)-Cholest-7-en-3β-ol ($C_{27}\Delta^7$; **7**)	Sigma (St Louis, USA)	99%
5α(H)-Cholestan-3β-ol ($C_{27}\Delta^0$; **6**)	Sigma (St Louis, USA)	-
(22*E*)-24-Ethylcholesta-5,22-dien-3β-ol ($C_{29}\Delta^{5,22}$; **17**)	Sigma (St Louis, USA)	95%
24-Ethyl-5α(H)-cholestan-3β-ol ($C_{29}\Delta^0$;**20**)	Sigma (St Louis, USA)	96.7%
Mixture of fatty acids[3]	Sigma (Steinheim, Germany)	-
Heptatriaconta-15E,22E-dien-2-one **(28)**	Prof. J.R.Maxwell, Univ. of Bristol	-
Carotenoid standards[4]	Dr. G. Britton, Univ. of Liverpool	-

[1]Grade not specified.
[2]For structures see figure 3.1.
[3]Fatty acids are those listed in table 2.2 (except cholanic acid)
[4]Fucoxanthin; zeaxanthin; canthaxanthin; 3S,3'S-astaxanthin; lutein; α-carotene, and lycopene.

2.2. GENERAL PROCEDURES

All glassware was soaked in a solution of Decon-90 (2%, 6 h), and then rinsed with tap water and Milli-Q® water (18.2 MΩ cm^{-1}). Non-volumetric flasks were wrapped in clean aluminum foil and dried in an oven (60-80° C, 48h) and

finally muffled at 400°C (12 h). Volumetric flasks were air dried and rinsed with distilled DCM. All glassware was stored in aluminum foil.

Methanol, cyclohexane, *n*-hexane and dichloromethane used for organic trace analyses were distilled in an all glass still, using a fractional distillation method. Acetyl chloride was distilled prior use, using a small distillation column.

Sodium sulphate, anti-bumping granules, glass and cotton wool, and granular silica were soxhlet-extracted (24 h; dichloromethane: methanol; 9:1 v/v) in cellulose thimbles. The material was then allowed to dry in a fume cupboard (48 h), transferred to glass jars and combusted overnight (400°C, except glass and cotton wool that were stored in combusted jars). Glass vials and Pasteur pipettes were extracted using the same system, wrapped in aluminum foil and combusted (400°C, 12h). Sodium sulphate and silica were stored in an oven (60-80°C). Fully activated silica was used in this study.

2.3. AREAS OF STUDY

Samples were collected in two areas, the Porcupine Abyssal Plain (PAP; 48°N 20°W; ~4850 m water depth, about 270 km southwest of Ireland) in the northeast Atlantic Ocean (Fig 2.1) and the West Antarctic Peninsula Shelf (WAP, 64°5 'S 65°W; ~500 m water depth) in the Antarctic Ocean (Fig 2.2).

The PAP area was chosen as a study site because large seasonal fluctuations in the deposition of particulate material were detected in the 1980s and 1990s (Billett and Rice, 2001) and there is little influence from down-slope

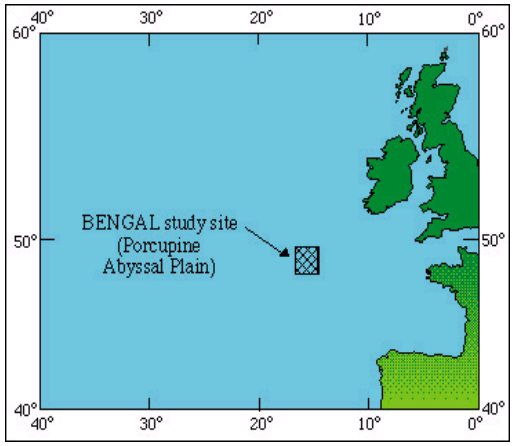
processes (Rice *et al.*, 1994). The samples were collected during the BENGAL project (High-resolution and spatial study of the BENthic biology and Geochemistry of a north-eastern Atlantic abyssal Locality), which was a long-term multidisciplinary study of the response of abyssal benthic boundary layer (BBL) to the pulses of phytodetritus, and of the transformation of the deposited material by the benthos (Billett and Rice, 2001).

The samples from the WAP were collected through the FOODBANCS project (FOOD for Benthos on the ANtarctic Continental Shelf), which attempted to evaluate the seasonal food availability to the benthos at the BBL (Smith, 2002).

2.4. SAMPLING

Animals from the PAP were collected by beam-trawl and Otter-trawl (OTSB14; Rice *et al*., 1990) during cruises of *RRS Discovery* (29 August to 24 September 1996, cruise D222; Rice, 1996; 02-31 July 1997, D229; Bett, 1998; 28 February to 30 March-1998; Rice, 1998; D231; and 25 September to 08 October 1998, D237; Sibuet, 1999) and *RRS Challenger* (15 to 30 October 1997, C135; Billett, 1998; and 19 April to 19 May 1999, C142, Billett, 2000). Hereafter the cruises will be referred as September-96, July-97, October-97, March-98, October-98 and April-99. Specimens from WAP were sampled (February 2001) by otter-trawl during a cruise on the *RVS Laurence M. Gould*

(a)



(b)

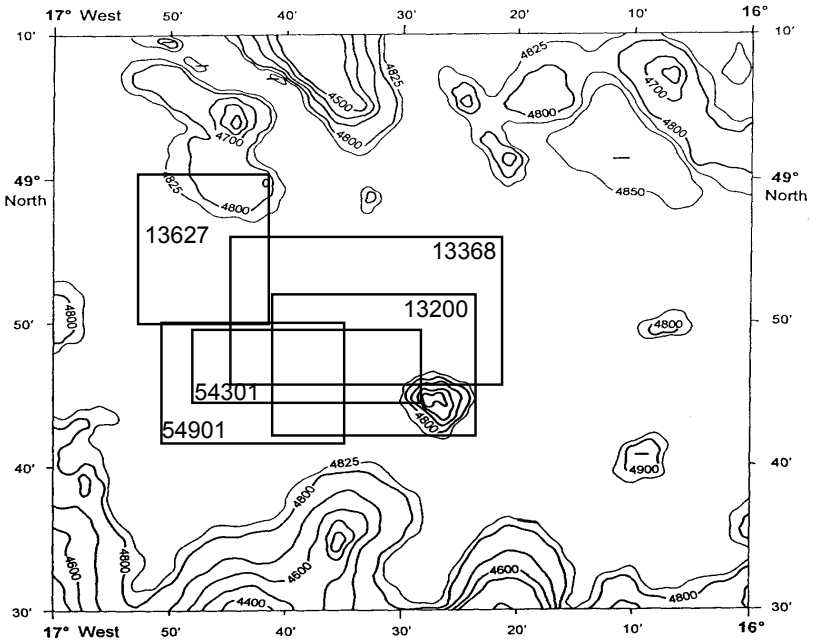
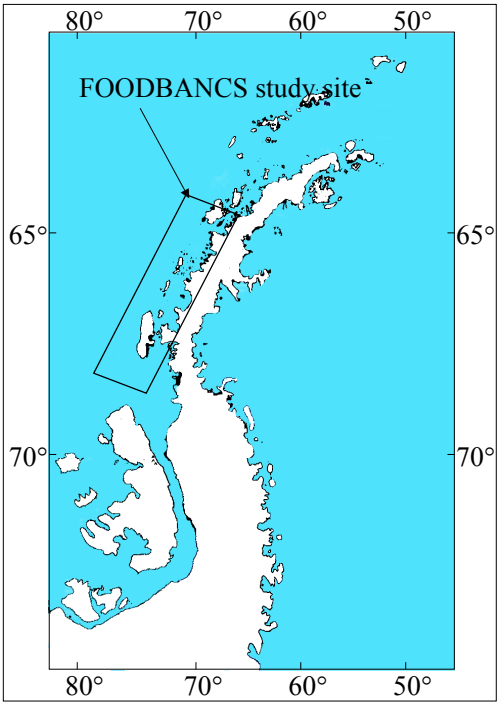


Figure 2.1. Porcupine Abyssal Plain, Northeast Atlantic (a) General locality and (b) Detailed bathymetric map (Courtesy of Dr. David Billett, SOC). See Table B.2, Appendix B, for stations).

(a)



(b)

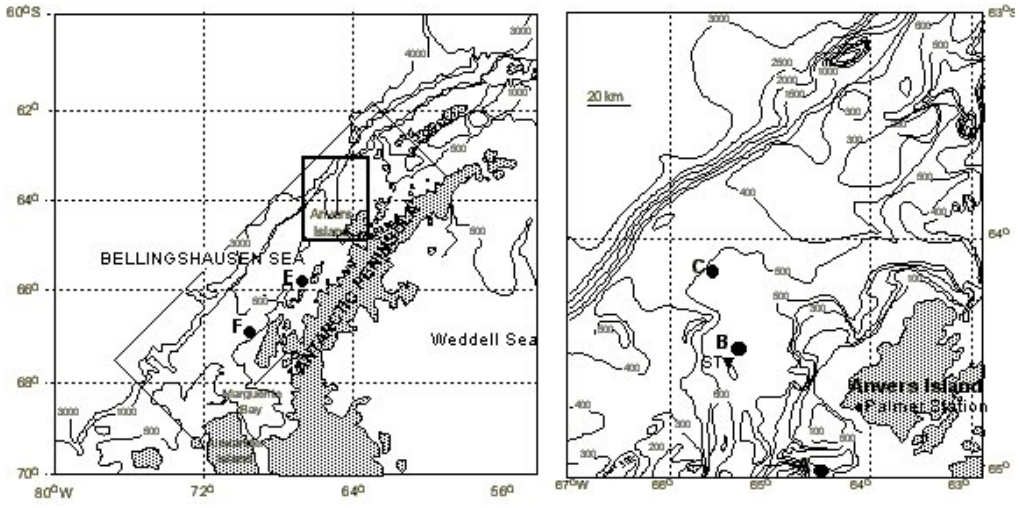


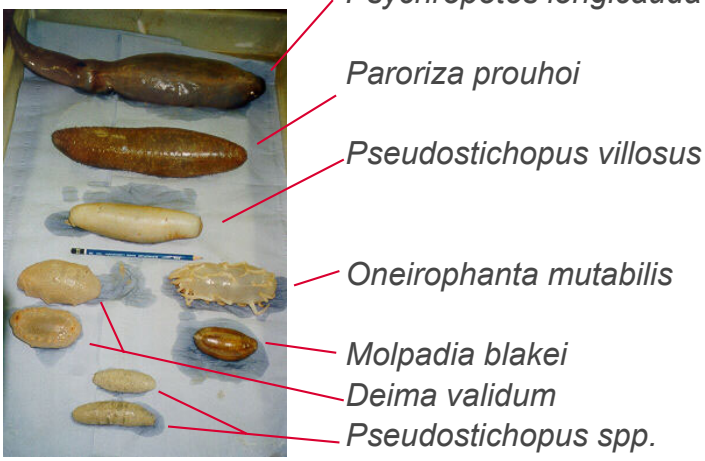
Figure 2.2. (a) General locality and (b) detailed bathymetric map of the sampling stations (A-F) at the West Antarctic Peninsula (WAP). See Table B.3, Appendix B, for stations.

(cruise n° LMG01-02). Specimens collected from the PAP included *Oneirophanta mutabilis, Pseudostichopus villosus, Psychropotes longicauda, Deima validum, Paroriza prouhoi, Amperima rosea* and *Molpadia blakei* (Fig. 2.3). Animals sampled from WAP included *Molpadia musculus, Peniagone* sp.*, Scotoplanes globosa, and Bathyplotes* sp. (Fig. 2.4). On recovery of the trawls, animals in good condition were selected and stored in pre-chilled seawater (4°C). The animals were dissected within 2 h in a cold room (6°C) at the PAP and in an aquarium room (<6°C) at the WAP.

The PAP sediment samples analyzed in this study were collected in April 1999 on *RRS Challenger* (Cruise C142); additionally, for comparison with the biochemistry of the holothurians, sediment data for the period 1996-1998 collected by Mackenzie (2000) are also used. Sediment cores were sampled using a muti-corer (Barnett *et al.*, 1984) and were collected independently (different deployments). Sediment cores from the WAP were collected using a mega-core sampler (Barnett *et al.*, 1984). Cores were sliced in 7 sections (0-5, 5-10, 10-20, 20-30, 30-40, 40-50 and 50-60 mm), however, only the surficial sediments were analyzed in this study.

Appendix B describes all sampled species, number of specimens, locations, times, treatment, and analyses employed.

a)



b)

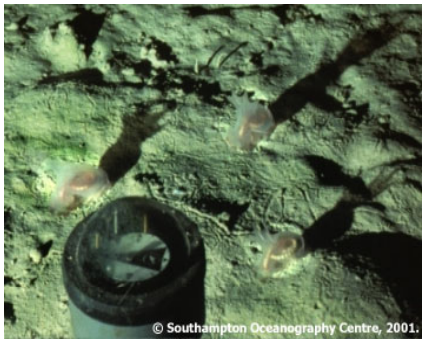
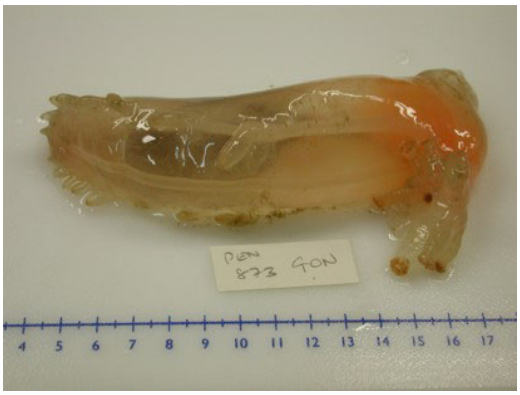
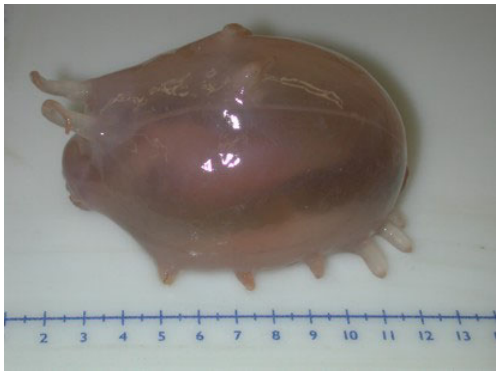


Figure 2.3. Holothurians sampled from the Porcupine Abyssal Plain (a) Holothurians recovered from trawls (b) *Amperima rosea, in situ* photographed by Bathysnap camera (Courtesy of Dr. David Billett, SOC).

(a)



(b)



(c)

Figure 2.4. Holothurians sampled in the West Antarctica Peninsula (WAP): (a) *Peniagone* sp. (b) *Scotoplanes globosa* and (c) *Bathyplotes* sp (All three photos are courtesy of Professor Paul Tyler, SOC).

2.5. DISSECTION OF ANIMALS

Most of the dissections were carried out on board of the ship, as described by Mackenzie (2000) using the method of Khripounoff and Sibuet (1980). Briefly, the oesophagus was identified as the dark section posterior to the mouth. The anterior gut was taken to be the part between the first and second constriction. Following was the posterior gut until the third and strongest constriction that characterizes the beginning of the rectum, which ends at the cloaca. The gut parts were not analyzed here, but were kept for future work.

After the dissection, the body wall were stored in clean, aluminum foil-wrapped, pre-weighed petri dishes, or were just wrapped in aluminum foil when they were bigger than the dishes. On board, all samples were stored in freezers (-80 to -60°C). Samples were packed with dry ice to be transported from the ships to the laboratory. In the lab, samples were stored (-20°C) and freeze-dried. Once dry, samples were frozen in liquid nitrogen and ground to a coarse powder with a pestle and mortar, and finally stored (-20°C) prior to analysis.

2.6 ANALYTICAL METHODOLOGY

2.6.1. LIPID ANALYSIS

2.6.1.1. Extraction

Aliquots (50-80 mg) of dried tissue were soxhlet-extracted (24 h; dichloromethane (DCM):methanol; 9:1 v/v). Internal standards (5-α(H)-cholestane, **29,** and 5-β(H)-cholanic acid; **30**) were added to the aliquots prior to extraction. Lipid extracts were concentrated *in vacuo* (to ~ 1 mL) after extraction, transferred to a small pre-weighed vial and then the solvents were removed under a stream of N_2.

The extract was then re-dissolved in DCM and was dried by passing through a column of anhydrous sodium sulphate. The solution was divided into two aliquots: one for lipid analyses by gas chromatography-mass spectrometry (GC-MS) and the other for steryl sulphate analysis by liquid chromatography-mass spectrometry (LC-MS; Chapter 3). Prior to analysis by GC-MS, the aliquot was methylated and silylated using the methods of Christie (1982), and Chambaz and Horning (1969), as described in the sections 2.6.1.2 and 2.6.1.3, respectively.

Blank extractions were carried out with every batch of extractions, where the internal standards were added to the extraction thimbles containing clean glass wool. The resulting extract was then treated as a normal sample.

2.6.1.2. Methylation

The lipid extracts were transferred to Reacti-Vials (5 mL; Pierce Chemicals Ltd.), and the solvents were completely evaporated under a stream of N_2. The methylating reagent was prepared in an ice bath (0°C), where acetyl chloride (2

mL) was added drop-wise to cold methanol (20 mL). The resulting solution (2 mL) was then added to the extracts and the vials were maintained (40°C, 12h) under a N_2 atmosphere. Finally, the solvents were removed under a N_2 stream, and the methylated samples transferred to clean vials with DCM. Trans-esterification or esterification by this procedure occurs according to the general scheme:

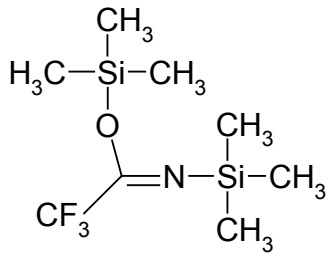
$$R'COOR + CH_3OH \xrightarrow{H_3O^+} R'COOCH_3 + ROH \qquad \text{(reaction 2.1)}$$

Where:

R = H (esterification) and R= C_nH_{2n+1} (trans-esterification)

2.6.1.3. Silylation

After methylation, N,O-*bis*-(trimethylsilyl)trifluoroacetamide (BSTFA; 30 μL) was added to dry extracts and left to react (40 °C; 1h) under N_2 atmosphere (reaction 2.2). Then, BSTFA was evaporated under a N_2 stream. Silylated hydroxyl groups have better chromatographic properties than their free counterparts and are easier to identify because their mass spectra show characteristic (and intense) fragmentation involving the loss of a methyl group ($[M-15]^{+\cdot}$).



BSTFA

+ ROH ⟶ $ROSi(CH_3)_3$ (reaction 2.2)

2.6.1.4. Gas Chromatography-Mass Spectrometry (GC-MS)

Lipids 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). The oven was initially held 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 (total time=87.33 min). 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 MS 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.

2.6.1.5. Identification and Quantification

Identification of individual lipids was based on mass spectral interpretation, comparison of mass spectra and retention times with those of authentic standards, where available and by comparison with literature mass spectra (*E.g.* Hydroxyacids: Eglinton and Hunneman, 1968; Eglinton *et al*., 1968a&b; fatty acids: Waller, 1972;Murphy, 1993; sterols: Goad and Toshihiro, 1997).

Quantification of individual lipids was carried out relatively to the internal standards, after correction for relative response factors, which were calculated based on analyses of a mixture of fatty acids (FA), sterols, a ketone (*E*-37:2, **28**), and the internal standards, 5α(H)-cholestane (IS1, **29**) and 5α(H)-cholanic acid (IS2, **30**; Fig. 2.5). This mixture was prepared as follows:

a) A mixture of 32 FA's from Sigma (100 mg) was diluted to 5 mL (cyclohexane);
b) Cholest-5-en-3β-ol ($C_{27}\Delta^5$; **5**), 5α(H)-cholest-7-en-3β-ol ($C_{27}\Delta^7$; **7**), 5α(H)-cholestan-3β-ol ($C_{27}\Delta^0$; **6**), 24-ethylcholesta-5,22-dien-3β-ol ($C_{29}\Delta^{5,22}$; **17**, 24-ethyl-5α(H)-cholestan-3β-ol ($C_{29}\Delta^0$; **20**), (E)37:2 (**28**), and the internal standards were weighed (0.3–0.6 mg) in separate vials and dissolved in 5 mL of solvent (DCM:Cyclohexane, 1:1, v/v)
c) An aliquot of the mixed FA solution (15 μL) was mixed with aliquots (40 to 100 μL) of sterol, *E*-37:2 ketone and the internal standard solutions.

This resultant mixture was then submitted to the same treatment as samples (methylation and silylation) and analysed by GC-MS (Fig 2.5). The

positions of the double bounds of some of the unsaturated fatty acids were identified by comparison of retention time with authentic standards.

Response factors used in the quantification of fatty acids and sterols are listed in Table 2.2 and were calculated according to Equation 2.1.

$$RF = (C_{st} * A_{is}) / (C_{is} * A_{st}) \quad \text{(Eq. 2.1.)}$$

Where:

RF = Response Factor;

A_{is} = Area of the internal standard;

A_{st} = Area of the standard being quantified;

C_{is} = Concentration of the internal standard;

C_{st} = Concentration of the standard being quantified.

The concentrations of the target compounds (C_{tc}) of the sample extracts were then calculated according to equation 2.2:

$$C_{tc}\ (ng/g) = [(W_{is} * A_{tc}) / (A_{is} * Q_{sample})] * RF \quad \text{(Eq. 2.2)}$$

Where:

W_{is} = Weight of the Internal Standard added to the sample (ng)

A_{tc} = Area of the peak of the target compound.

A_{is} = Area of the peak of the Internal standard.

Q_{sample} = Weight of the aliquot of the extracted sample (g)

The RF of identified chemicals not in the standard mixture were assumed to be identical to similar compounds. As an example, the RF of *trans*-18:1(n-9) was applied to the quantification of 19:1.

Table 2.2. Response factors for all compounds of the mixture of standards calculated in relation to cholanic acid.

Compound	RF	Concentration(ng/μL)[1]
C14:0	1.78	64.0
C15:1cis10	1.66	32.0
C15:0	1.43	32.0
C16:1cis9	1.48	32.0
C16:0	1.21	96.0
C17:1cis10	1.56	32.0
c17:0	1.35	32.0
C18:3cis6,9,12	2.41	32.0
C18:2cis9,12	1.6	32.0
C18:1trans9+C18:2trans9,12+C18:3cis9,12,15	-[2]	-
C18:2trans9,12	1.71	32.0
C18:1trans9	2.07	64.0
C18:0	1.12	64.0
C20:4cis5,8,11,14	2.64	32.0
C20:5cis5,8,11,14,17	2.52	32.0
C20:3cis8,11,14	1.9	32.0
C20:2cis11,14	1.6	32.0
C20:1cis11+C20:3cis11,14,17	-[2]	-
C20:0	1.09	64.0

Table 2.2. Continued.

C21:0	1.27	32.0
C22:6cis4,7,10,13,16,19	3.58	32.0
C22:2cis13,16	1.85	32.0
C22:1cis13	1.14	32.0
C22:0	1.03	64.0
C23:0	1.33	32.0
C24:1cis15	1.55	32.0
C24:0	1.13	64.0
$C_{27}\Delta^5$ (**5**)	1.95	26.4
$C_{27}\Delta^0$ (**6**)	1.21	27.2
$C_{27}\Delta^7$ (**7**)	1.42	23.8
$C_{29}\Delta^{5,22}$ (**17**)	1.47	22.6
$C_{29}\Delta^0$ (**20**)	1.37	24.8

[1]Final volume of the solution prior the injection = 200 μL

[2] As these compounds co-eluted under the analytical conditions employed (section 2.6.1.4), their RF's were not calculated.

2.6.1.6. Reproducibility

5 Aliquots of a *Psychropotes longicauda* specimen were extracted and treated as described in the sections 2.6.1.1, 2.6.1.2 and 2.6.1.3, and injected as in section 2.6.1.4 in order to determine the reproducibility of the technique. The most concentrated compound showed the highest variability, but, not more than ±15% (Table 2.3).

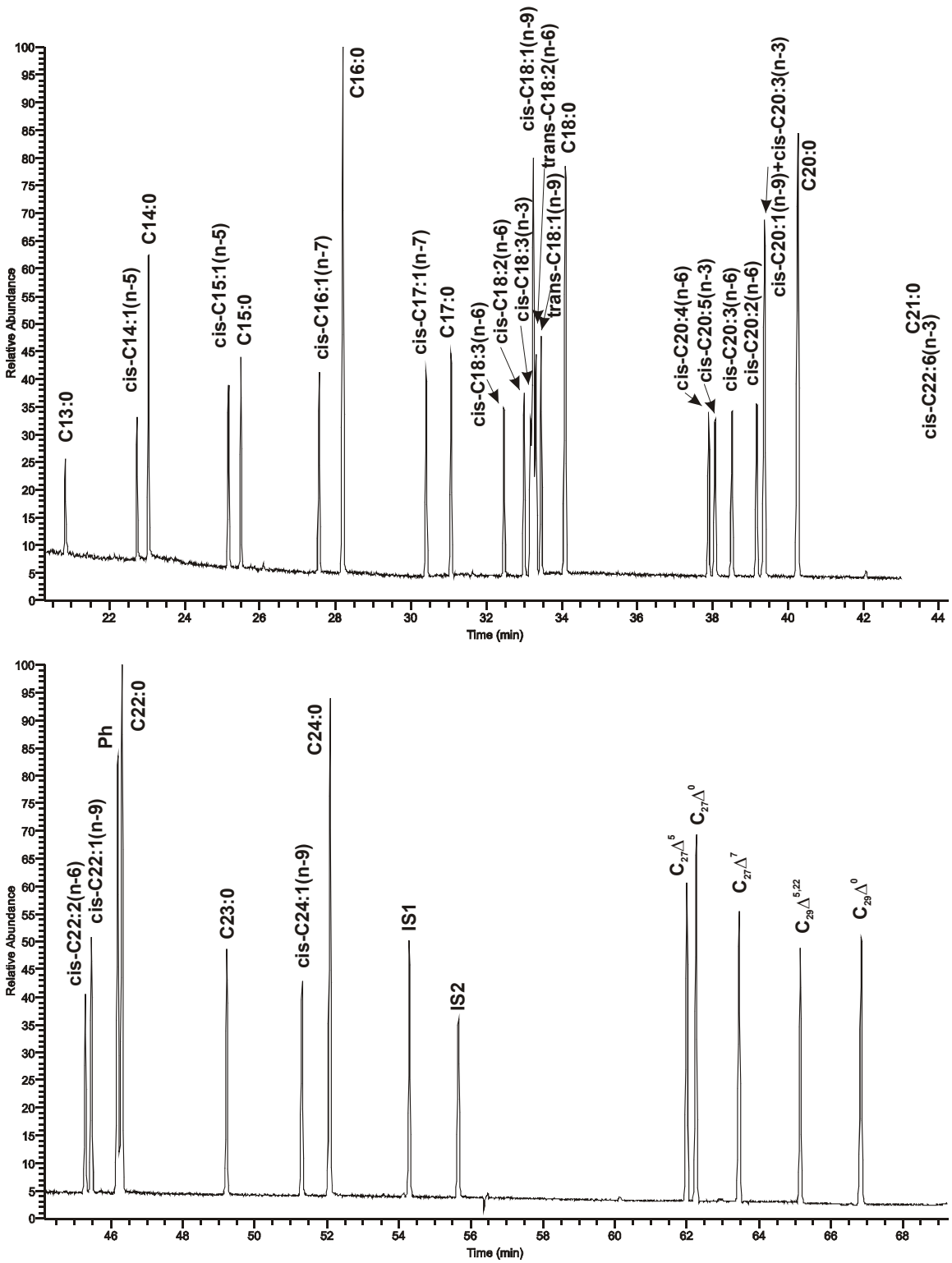


Figure 2.5. Total ion chromatogram (TIC) of a solution of lipid standards (E-$C_{37:2}$ standard not included in this mixture) obtained by GC-MS (top. 20-44 min, bottom 44-70 min; Ph= phthalate).

Table 2.3. Mean, standard variation (SD) and coefficient of variation (CV) of the method of analysis of lipids.

Compound	Mean±SD	CV(%)
16:0	34.06±1.84	5.41
18:1	107.42±7.84	7.30
19:0	19.99±1.89	9.43
20:4	778.06±70.02	9.00
20:5	565.32±56.82	10.05
23:1	610.47±51.14	8.38
HAc24:1	772.40±56.12	7.27
$C_{27}\Delta^{5}$ (**5**)	469.75±54.94	11.70
$C_{28}\Delta^{5,22}$ (**9**)	105.74±15.16	14.33
$C_{28}\Delta^{22}$ (**10**)	566.57±60.54	10.68
$C_{29}\Delta^{5}$ (**19**)	1310.80±192.75	14.71
$C_{29}\Delta^{0}$ (**20**)	2758.73±373.06	13.52

2.6.2. STERYL SULPHATES

The method for steryl sulphate analyses using LC-MS was developed as part of the present study, and is discussed and presented in Chapter 3.

2.6.3. CAROTENOIDS

2.6.3.1. Extraction

All procedures for carotenoid analyses were carried out in dim light. Aliquots (1-5 g) of dried body tissue were weighed in glass centrifuge tubes, acetone (20 mL) was added and the samples were then sonicated (15 min). The tubes were centrifuged and the supernatant separated from the solids. The procedure was then repeated (x2). The supernatants were combined and concentrated *in vacuo* (~1 mL), transferred to a small vial and dried under a stream of N_2.

Prior to analysis, the extracts were cleaned up by passing through a Pasteur pipette filled with silica (2g) in DCM (3 mL). The pigments were eluted with MeOH (5mL) to fresh vials, dried under a stream of N_2 and kept in a freezer until analysis.

2.6.3.2. Chromatography

Total extracts of *Amperima rosea* were separated using thin layer chromatography (TLC) plates (silica gel 60, from Merck, Darmstadt, Germany), which were developed using diethyl ether:petrol (1:1 v/v). Each visible band was immediately scraped from the plates and filtered with DCM through extracted glass wool in Pasteur pipettes. Carotenoid fractions were dried under a stream of nitrogen and stored under nitrogen at –20°C until analysis (no more than 5 days).

2.6.3.3. Liquid Chromatography-Mass Spectrometry (LC-MS)

LC-MS analyses of carotenoids were carried out using a Spectra System P4000 auto-sampler coupled with an AS1000 Spectra System High Performance Liquid Chromatography (HPLC). Samples were separated on an Hypersil BDS C18 column (250 X 3mm, i.d., 5 μm particle size) using the solvent system described in Table 2.4. The Atmospheric Pressure Chemical Ionisation (APCI) mode has widely been used for carotenoid analyses (Lacker *et al*., 1999), and so this mode was therefore employed for the present study. Typical operating conditions for the APCI source were as follows: vaporizer temperature 500°C, heated capillary 200°C, with the corona discharge set at 5μA. All other parameters and settings are as in Chapter 3.

Table 2.4. Solvent program for carotenoid analyses.

Time (min)	Flow rate (mL min^{-1})	% MeOH:Water(80:20)	% MeOH:DCM (80:20)
0	0.25	100	0
30	0.25	20	80
45	0.25	20	80
50	0.25	100	0
65	0.25	100	0

Carotenoid identification was based on mass spectral interpretation and comparison of mass spectra (tandem) and retention times with authentic standards.

2.6.3.4. Liquid Chromatography-Ultra violet/Visible (LC-UV/Vis)

LC-UV-Vis analyses of carotenoids were carried out in a SP8800 Ternary High Performance Liquid Chromatography (HPLC, Spectra-Physics), coupled with an UV/Vis detector SP8450 (Spectra-Physics), a Rheodyne injector (Cotati, USA), and with an Hypersil BDS C18 column (250 X 3mm, i.d., 5 μm particle size). The solvent system is the same as in Table 2.3. A variable wavelength UV-Vis detector was used, with the absorption wavelength set at 480 nm.

2.6.4. CARBON (C) AND NITROGEN (N).

Total and organic elemental C (TC and TOC), and N (TN) were analysed using a Carlo Erba 1106 CHN elemental analyser. TC and TN were determined as in Smallwood (1998). Sediments were accurately weighed into a tin capsule with a Cahn 21 Automatic Electrobalance, placed into a vertical quartz and heated (1050°C) in a continual stream of helium. The stream was momentarily enriched in pure oxygen once the sample had been introduced, the gases were

then passed over Cr_2O_3 to ensure quantitative combustion of the sample. Excess oxygen was then removed by passing the combustion gases over a copper packed reactor (650°C). The gases where then immediately introduced to the chromatographic column (packed with Porapak QS, 80°C) where the products were separated as N_2 and CO_2.

TOC was determined after de-carbonation of samples, which was carried out as follows: Samples were weighed in to silver capsules (6x4 mm, Elemental Microanalyses Limited), which were placed in PTFA holders, wetted with dionized water and left overnight in a dessicator in a HCl saturated atmosphere. Then, the samples were dried in an oven (60°C, 2h), the capsules sealed and analysed as above. TOC was then determined with the same procedure describe for TC and TN above. TOC values were corrected as follows:

$$\%TOC = \%C \times (W_f/Wo)$$

Where:

W_0 = weight of dry sample before de-carbonation

W_f = weight of dry sample after de-carbonation

%C = Percentage of carbon measured by the instrument.

2.6.4.1. Reproducibility

Aliquots of a homogenised specimen of *Paroriza prouhoi* were analysed for TOC, TC and TN (x8). Table 2.5 lists their mean, standard of deviation and coefficient of variation.

Table 2.5. Mean, standard deviation (SD), and coefficient of variation (CV) of TC, TOC, and TN of a *P. prouhoi* sample (body tissue, n=8).

	Mean	**SD**	**CV**
TC	26.09	±1.58	6.05
TOC	21.38	±0.90	4.20
TN	6.07	±0.57	9.31

2.7. STATISTICS

Data were tested with Shapiro-Wilk and Levene's tests in order to evaluate their distribution and variance, respectively (Dytham, 1999), respectively. When a variable was found not to have a normal distribution ($P < 0.05$), the Dixon's Q test (Miller and Miller, 1993) was applied in order to assess if any outlier could be deleted from the data set. If one outlier was deleted, Shapiro Wilk's test was applied again successively until no outliers were found.

If the distribution was still not normal and/or the variance was not homogenous, the Kruskall-Wallis test was applied to find any statistical

variation between the sampling periods. If variables had a normal distribution and a homogeneous variance, one-way ANOVA was applied to find any variation in the groups. Similarly, when values of two samples were compared, if the data set was not normally distributed and/or if its variance was not homogeneous, the Mann-Whitney *U*-test was applied instead of the independent samples' *t*-test (Dytham, 1999). Data were analysed using Excel (2000) and SPSS (Ver. 11.0.0) software.

Table 2.6 lists the number of replicates used in this study. Correlation analyses were carried out using the Spearman correlation-raking test (Dytham, 1999).

Table 2.6. Number of replicates of samples analysed from the PAP and from the WAP.

	Jul-97 (PAP)	Oct-97 (PAP)	Mar-98 (PAP)	Oct-98 (PAP)	Apr-99 (PAP)	Feb-2001 (WAP)
Sediments	6	6	6	4	3	4
Oneirophanta mutabilis	7	2	6	7	5	-[1]
Psychropotes longicauda	3	2	5	3	4	-
Pseudostichopus villosus	3	4	5	4	5	-
Paroriza prouhoi	-	-	3	2	5	-
Deima validum	-	-	1	2	2	-
Amperima rosea	-	-	20 (pooled)	-	20 (pooled)	-
Molpadia blakei	-	-	2	-	1	-
Bathyplotes sp.	-	-	-	-	-	2
Molpadia musculus	-	-	-	-	-	3
Scotoplanes globosa	-	-	-	-	-	1
Peniagone sp.	-	-	-	-	-	3

[1]Not sampled

Goad, L. J. and Toshihiro, A. (1997) *Analysis of sterols*. Chichester, Blackie Academic & Professional.

Chapter 3

DETERMINATION OF STERYL SULPHATES IN INVERTEBRATE TISSUE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

3.1 INTRODUCTION

Steryl sulphates or sulphated sterols are one of the many naturally-occurring organic sulphate esters. They are present in a wide range of animal tissues including invertebrates (Makarieva *et al.*, 1993; Ginger *et al.*, 2000), mice (Metzer *et al.*, 1995), and humans (Jetten *et al.*, 1989; Griffiths *et al.*, 1999). Their biological function in invertebrates remains unknown although it has been suggested that steryl sulphates act as a reserve of sterols, and that the losses of sulphate and the Δ^5-double bond could be pre-requisites to incorporation of sterols into cell membranes (Ginger *et al.*, 2000).

Steryl sulphates are involatile and thermally labile compounds, consequently, their direct analysis by gas chromatography (GC) is precluded, although steryl sulphates can be analysed by GC after solvolysis, which transforms steryl sulphates to free sterols, by reflux in dioxan-acetic acid (99:1, 4h; Burstein and Liebermann, 1958). However, sterols are prone to oxidation in the presence of light, oxygen or high storage temperature (Bosinger *et al.*, 1993; Paniangvait *et al.*, 1995) and the solvolysis may lead

to some losses of labile compounds (Ginger and Wolff, pers. comm.). Thus, a method for the direct determination and structure identification of steryl sulphates is desirable. Furthermore, as steryl sulphates are present in complex mixtures (including isomers) in invertebrates (up to 34 compounds in *Eupentacta fraudatix*; Makarieva *et al.*, 1993), their chromatographic separation prior to mass-spectrometry is essential for their reliable identification and quantification. In this report, I describe a method for determination of intact steryl sulphates in a biological matrix (body tissues of holothurians), with only one clean-up step. The method involves LC separation and mass spectrometry, coupled *via* electrospray (ESI) and atmospheric pressure chemical ionisation (APCI) interfaces.

3.2. MATERIALS AND METHODS

3.2.1. CHEMICALS AND MATERIALS

All solvents were filtered through a cellulose nitrate filter (Whatman 0.45 μm) before use. Other chemicals and materials were as described in Chapter 2.

Figure 3.1 shows the structures, names, abbreviations and the measured masses of the singly charged pseudomolecular ions of all standards used in this work. Cholesterol 3-sulfate and cholecalciferol 3-sulfate were purchased from Sigma-Aldrich Gmbh (Steinheim, Germany). 5α-Cholest-7-enyl-3-sulphate (SO_4-$C_{27}\Delta^7$), 5α-cholestanyl-3-sulphate (SO_4-$C_{27}\Delta^0$), 8,24-lanostadienyl 3-sulphate (SO_4-4,4-dimethyl-$C_{29}\Delta^{8(9),24(25)}$), 24-ethyl-5,22-

cholestadienyl 3-sulphate (SO_4-$C_{29}\Delta^{5,22}$) and 24α-ethyl-5α(H)-cholestanyl-3-sulphate (SO_4-$C_{29}\Delta^{0}$) were synthesized from their free sterol forms, which were purchased from Sigma Chemical co., St . Louis, USA ($C_{27}\Delta^{7}$, $C_{27}\Delta^{0}$, and 4,4-dimethyl-$C_{29}\Delta^{8(9),24(25)}$) and from Sigma-Aldrich Gmbh, Steinheim, Germany ($C_{29}\Delta^{5,22}$ and $C_{29}\Delta^{0}$), as follows:

Concentrated sulphuric acid (~0,2 mL) was added drop-wise to the solutions of each sterol (~50 mg) in dioxane (2 mL). The solution was left at room temperature under N_2 (4 h) and was then saturated with sodium sulphate to avoid the reverse reaction, after which cold NaOH (10%, 10mL) was added and a white precipitate was formed. The mixture was filtered through a Whatman 0.45 μm cellulose nitrate filter in a Buchner funnel and washed with water. The white gel held in the filter was dissolved in DCM and evaporated to dryness *in vacuo*. To eliminate completely traces of water, the crude steryl sulphates were salted out with a NaCl solution (10% w/v) and ether. The organic phase was isolated in a separatory funnel and its volume reduced *in vacuo* (~ 2 mL). The solution was then transferred to a small vial and dried under a stream of N_2. The steryl sulphates standards were stored in a freezer (-20°C).

3.2.2. LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY

ESI and APCI sources were operated with an APCI/ESI controller (Finnigan MAT, San Jose, CA) using a ThermoFinnigan TSQ 7000 triple-stage quadrupole tandem mass spectrometer which was automatically tuned and calibrated by infusing a mixture of 1-methionyl-arginyl-phenylalanyl-

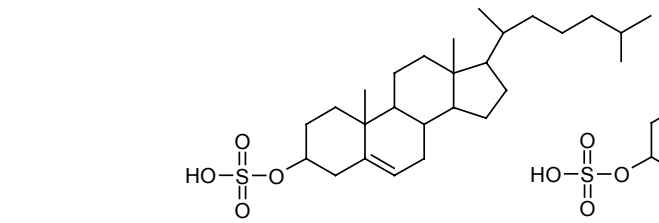
alanine acetate (MRFA; 20nM/mL) and apomyoglobin (5 nM/mL) in methanol/water (50:50,v/v, 1% acetic acid). Typical operating conditions for the ESI source were as follows: spray voltage 4.5kV, sheath gas (N_2), 80psi; auxiliary gas (N_2), 40 units; and capillary temperature set at 250 ºC.

The voltage of the tube lens in APCI was optimised with loop injection of a solution of 10 pg/μL cholesterol 3-sulphate in MeOH after ESI interface parameters were optimised. The APCI source was operated as follows: Vaporizer temperature 500°C, heated capillary 200 °C, with the corona discharge set at 5 μA, sheath gas (N_2), 80 psi, auxiliary gas (N_2) 40 units[1].

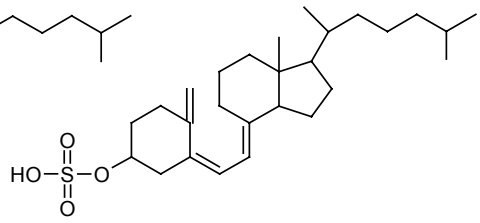
A regular scan mode was achieved using the first quadrupole to monitor from m/z 50 to 500 every 1s. In selected ion monitoring (SIM) mode, twenty ions diagnostic for sterol sulphate moieties (26-30 carbons) with 0-3 double bonds each were monitored. Thus, in positive mode, ion current diagnostic of $[M+H-H_2SO_4]^+$ (see Results and Discussion) were acquired at m/z 351, 353, 355, 357, 365, 367, 369, 371, 379, 381, 383, 385, 393,395, 397, 399, 407, 409, 411 and 413. In negative mode, ions characteristic of $[M-H]^-$ were monitored at m/z 447, 449, 451, 453, 461, 463, 465, 467, 475, 477, 479, 481, 489, 491, 493, 495, 503, 505, 507, 509. Collision induced dissociation (CID) of selected precursor ions in the rf-only quadrupole collision cell was achieved using argon as the collision gas and offset voltage as collision energy (2.8 mT, collision energy 30eV, see section 3.3). The first quadrupole monitored selected ions (maximum of 10 ions in each of 10 holothurian species tested; results shown for *Amperima rosea,* only) whereas the third quadrupole scanned the fragment ions from m/z 50 to 500. The dwell time

[1] Arbitrary units, *i.e*. not specified by the maker of the instrument.

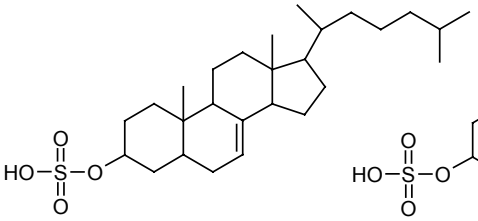
for each selected ion was set to 0.05s. The electron multiplier voltage was set to 1400 V for SIM and regular scan mode and 1750 V for CID.



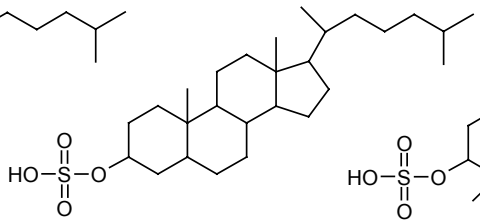
Cholesterol 3-sulphate
(SO_4-$C_{27}\Delta^5$)
$[M-H]^-$ = 465.5
$[M+H-H_2SO_4]^+$ = 369.5



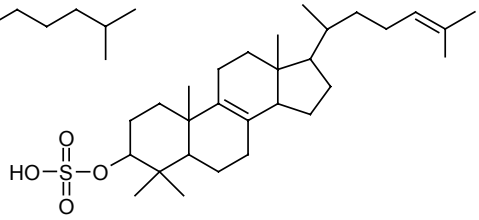
Cholecalciferol 3-sulphate
No abbreviation.
$[M-H]^-$ = 463.5
$[M+H-H_2SO_4]^+$ = 367.5



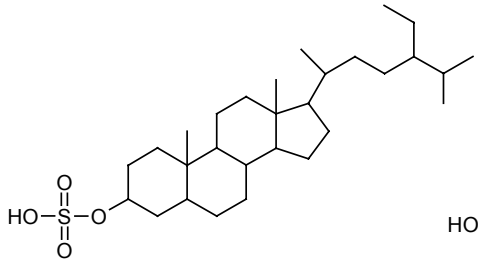
5α(H)-Cholest-7-enyl-3-sulphate
(SO_4-$C_{27}\Delta^7$)
$[M-H]^-$ = 465.5
$[M+H-H_2SO_4]^+$ = 369.5



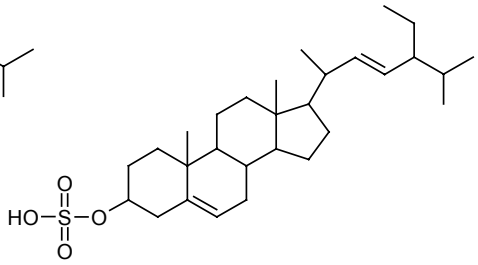
5α(H)-Cholestanyl-3-sulphate
(SO_4-$C_{27}\Delta^0$)
$[M-H]^-$ = 467.5
$[M+H-H_2SO_4]^+$ = 371.5



8,24-Lanostadienyl-3-sulphate
(4,4-dimethyl SO_4-$C_{29}\Delta^{8(9),24(25)}$)
$[M-H]^-$ = 491.5
$[M+H-H_2SO_4]^+$ = 395.5



24α(H)-Ethyl-5α-cholestanyl-3-sulphate
(SO_4-$C_{29}\Delta^0$)
$[M-H]^-$ = 495.5
$[M+H-H_2SO_4]^+$ = 399.5



24-Ethyl-5,22-cholestadienyl-3-sulphate
(SO_4-$C_{29}\Delta^{5,22}$)
$[M-H]^-$ = 491.5
$[M+H-H_2SO_4]^+$ = 395.5

Figure 3.1. Structures, names, abbreviations and m/z values of the pseudomolecular ions of steryl sulphate standards.

Standard solutions of cholesterol-3-sulphate and cholecalciferol were infused (20 µL/min) directly into the ESI source of the mass spectrometer *via* a Harvard pump (Pump Model 11, Harvard Apparatus, Holliston, MA).

In the APCI mode, the standards were introduced into the mass spectrometer by loop injection (5 µL loop fitted post-column) with a solvent flow provided by a P4000 quaternary gradient pump (Thermo Separation Products, Manchester, UK) fitted with a reverse phase HPLC column (Hypersil BDS C18, 250 x 3mm, i.d., 5 µm particle size) and a pre-column (50 x 3 mm i.d. 5µm reverse phase, Hypersil). For on-line HPLC (AS1000 Spectra System, Thermo Separation Products, Manchester, UK), the standards and samples were injected in APCI mode (see section 3.3) using a Spectra System P4000 auto-sampler (Thermo Separation Products, Manchester, UK) fitted with a 20µL loop and a mobile phase flow rate of 0.6 mL/min. The solvent program which gave rise to the best chromatographic separation was a binary system with a starting composition of water:ACN (60:40; 1 min solvent delay), with a linear change to water:ACN (10:90) after 23 min. Then, the solvent system was held isocratically for 5 min, and returned to the initial conditions linearly after a total time of 32 min. No ionisation reagent was used in the solvents because they can damage the mass spectrometer. Data were processed using X-calibur software v1.0.

3.2.3. SAMPLE PREPARATION AND EXTRACTION.

Sample collection and preparation have been described by Ginger et al., (2000) and in Chapter 2. Prior to analysis, lipid extracts were cleaned up

over a small silica gel-60 column (~ 4g in a Pasteur pipette). Less-polar material was eluted with DCM (5 mL) and discarded, followed by slightly polar compounds (including steryl sulphates) eluted with DCM:MeOH (7:3 v/v; 5 mL).

3.3. RESULTS AND DISCUSSION

3.3.1. ESI *versus* APCI

ESI is best suited for polar and large compounds whereas APCI is more appropriate for non-polar and small molecules. As steryl sulphate compounds have a polar group (sulfate) and a relatively non-polar one (cyclic and side chain), both ESI and APCI had to be tested.

The mass spectrum of cholesterol sulphate in negative ESI mode (Fig. 3.2a) shows a pseudomolecular ion $[M-H]^-$ and the characteristic fragment of a protonated sulphate group at m/z 97. Analogous ions have been found for cholesterol sulphate using fast-atom bombardment (FAB) mass spectrometry (Veares *et al.*, 1990) and ESI (Metzer *et al.*, 1995) and for other organic sulphate esters using FAB (Shackleton and Straub, 1982), nano-ESI (Griffiths *et al.*, 1999), and secondary ion mass spectrometry (Shackleton and Straub, 1982). Organic esters of sulphates are readily ionised by negative ESI. Indeed, Chatman *et al*. (1999) employed sulphation in the analysis of steroids, arguing that it increases sensitivity and allows precursor ion scanning because of the sulphate (HSO_4^-) fragment ion. On the other hand, no easily identifiable fragmentations of cholesterol sulphate

were apparent in positive mode (Fig. 3.2b), although some fragmentation was evident. Although the intensity of the base peak in negative polarity is lower (~30%) than that obtained in positive mode, the former is potentially more useful, providing some information about the molecular structure, *i.e.* the presence of the sulphate group and the molecular mass.

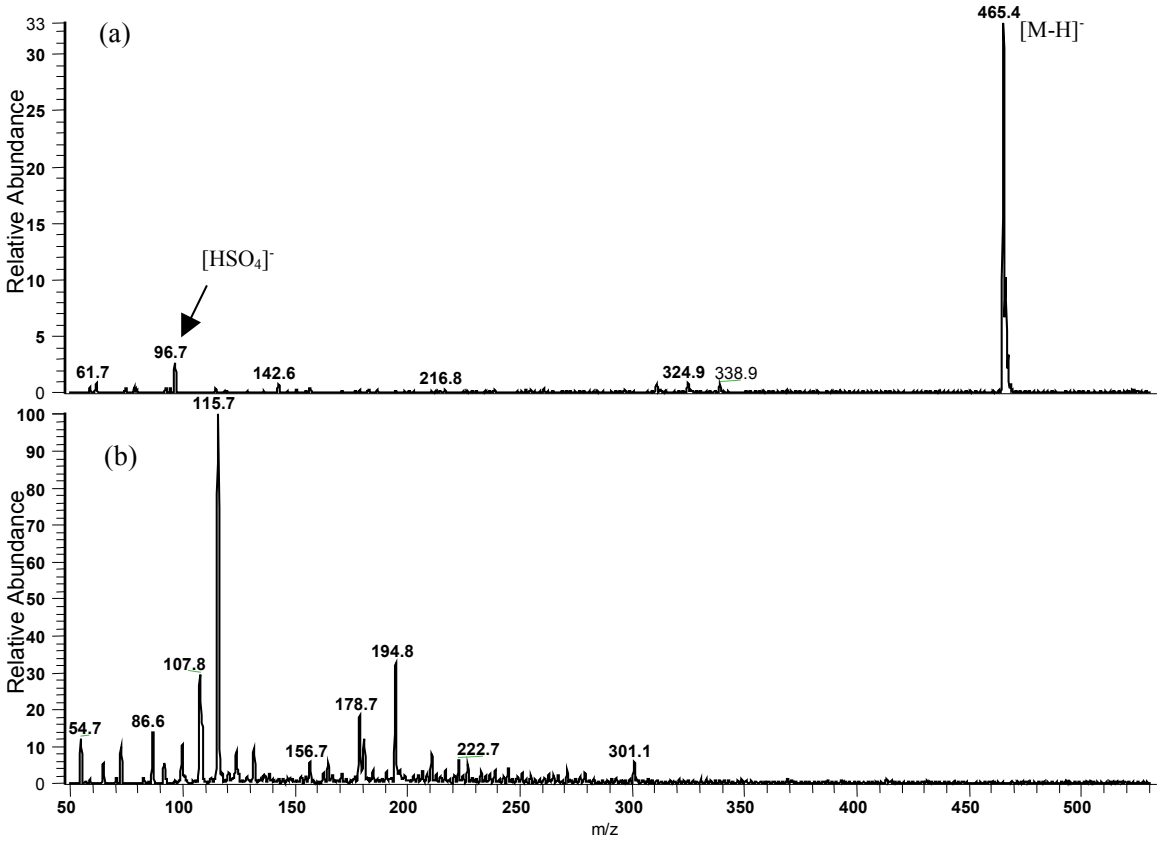


Figure 3.2. ESI mass spectra of cholesterol 3-sulphate in (a) negative and (b) positive polarities (direct injection).

The APCI spectra of cholecalciferol sulphate (the chosen internal standard) are illustrated in Figure 3.3. The APCI source usually generates more fragmentation than ESI (Chatman *et al.*, 1999), but in negative mode, the spectrum was similar to that obtained in ESI, being dominated by the

pseudomolecular ion, $[M-H]^-$. In contrast to the spectrum generated by ESI, the spectrum acquired in positive APCI mode showed some diagnostic fragmentation of steryl sulphates. The intense base peak at m/z 367.5 corresponds to $[M+H-H_2SO_4]^+$ whilst other peaks corresponding to adducts $[M+H-H_2SO_4+H_2O]^+$, $[M+H-H_2SO_4+CH_3OH]^+$ and $[M+H-H_2SO_4+2Na]^+$ are also observed at m/z 385.5, 399.5 and 413.5 respectively. The intensity of these adducts has been showed to be correlated to structure and could potentially be used for identification (Honing *et al.*, 2000). However, one should note that the intensities of these adducts could change when LC/MS rather than direct (loop) injections is employed. Similar spectra have been obtained when free sterols (Toh *et al.*, 2001), and cholesterol oxides (Razzazi-Fazeli *et al.*, 2000) are analysed by liquid inlet-MS. Thus, other steroids may also give rise to similar spectra and where a mixture of free sterols and conjugates are present, it is important that LC is employed to separate these.

3.3.2. COLLISION ENERGY FOR CID

In order to optimise the fragmentation of steryl sulphates in CID, a range of collision energies from 10 to 50 eV was applied; cholecalciferol 3-sulphate was infused directly and the first quadrupole set to select $[M-H]^-$ at m/z 463.5 in negative ESI and APCI mode, and $[M+H-H_2SO_4]^+$ at m/z 367.5 in positive APCI. For negative ESI and APCI, no further fragmentation occurred; the ion characteristic of HSO_4^- at m/z 97 became more intense, whilst the ion $[M-H]^-$ decreased in abundance, when the collision energy was increased (spectra

not shown). Nano-ESI in negative polarity has been used for complete structural identification of steryl sulphates using high-energy CID (Griffiths *et al.*, 1999), and of sulphated bile acids using low-energy CID (Lemonde *et al.*, 1999). In contrast, no characteristic side-chain and ring fragmentations were

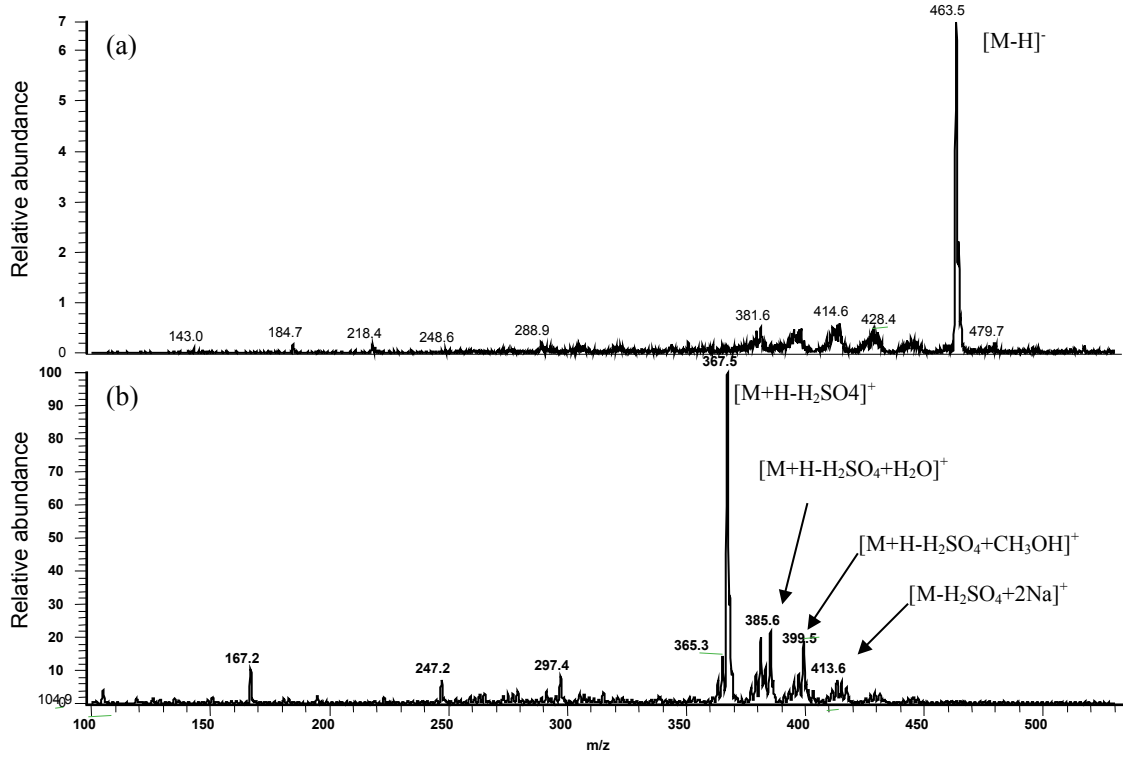


Figure 3.3. APCI mass spectra of cholecalciferol 3-sulphate in (a) negative and (b) positive polarities (direct injections).

observed in our spectra, perhaps because of the relatively higher quantity of sample injected and the complexity of the matrix. On the other hand, in positive APCI mode, more fragmentation was observed when a collision energy of –30 eV was applied (Fig. 3.4a-b). The fragmentation became more intense at higher energies, but resulting spectra gave no additional structural

information, hence 30 eV was chosen as optimal for the identification of steryl sulphates.

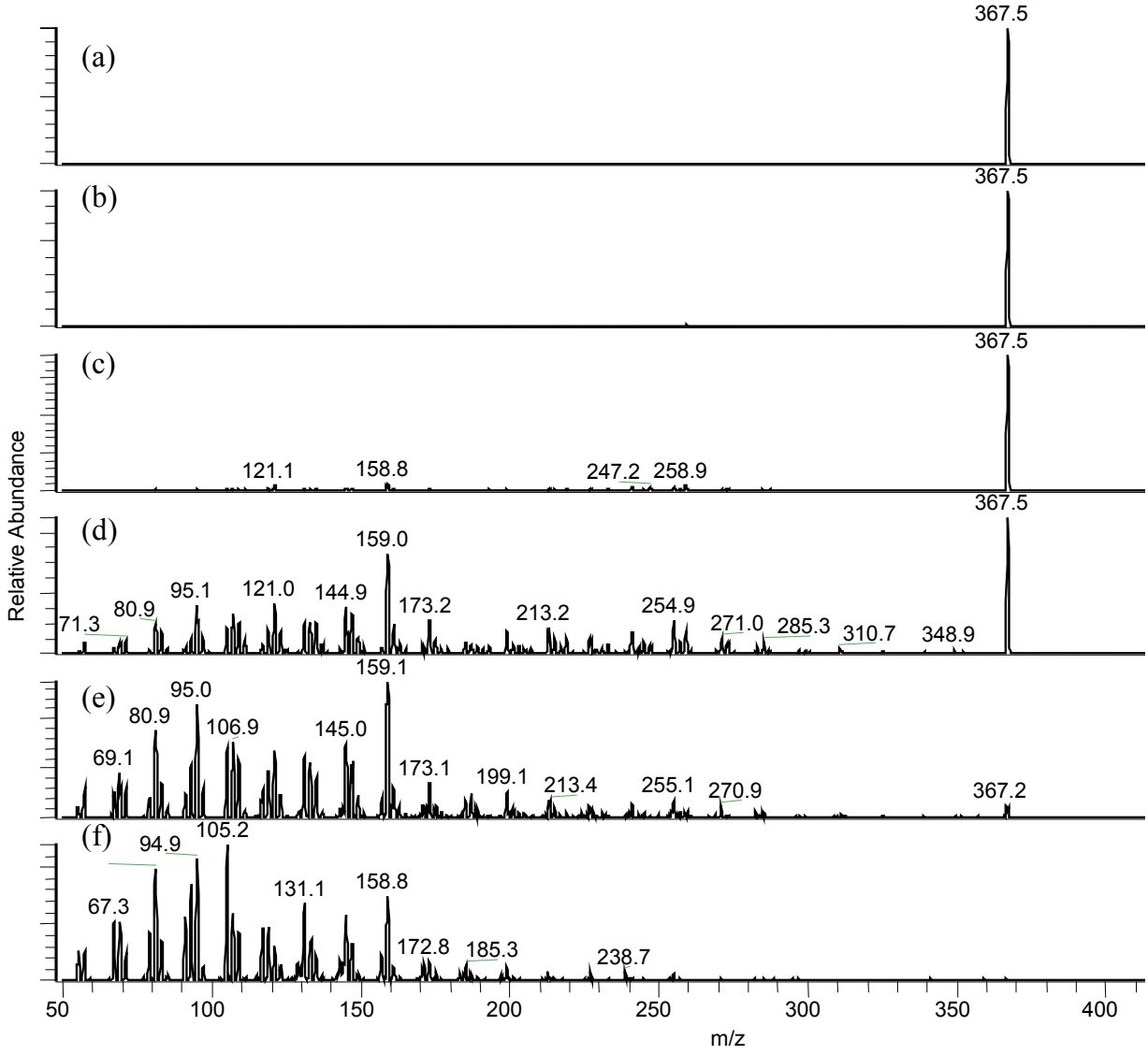


Figure 3.4. CID mass spectra of cholecalciferol 3-sulphate generated at collision energies of (a) 10, (b) 15, (c) 20, (d) 30, (e) 40, (f) 50 eV in positive mode.

3.3.3 STRUCTURE ELUCIDATION

CID mass spectra of 4 standard steryl sulphates (SO_4-$C_{29}\Delta^0$, SO_4-$C_{29}\Delta^{5,22}$, SO_4-$C_{27}\Delta^7$, and SO_4-$C_{27}\Delta^5$) acquired in positive APCI are illustrated in Figure 3.5. The spectra show more fragmentation than those reported for free sterols acquired by GC-EIMS (Goad and Toshihiro, 1997). However, the fragmentation appears to be similar to that for free sterols observed in positive APCI mode (Toh *et al.*, 2001).

The dominant ions result from the loss of C_nH_{2n}, C_nH_{2n+2}, and C_nH_{2n+4} units, and the formation of $[C_nH_{2n+1}]^+$, $[C_nH_{2n-1}]^+$, and $[C_nH_{2n-3}]^+$ (Fig. 3.5). This is diagnostic of charge-remote fragmentation (when fragmentation occurs without the involvement of the ionic charged site; Wysocki and Ross, 1991). It is also possible that steryl sulphates undergo charge-directed fragmentation and that certain fragment ions in the CID spectra may arise *via* pathways similar to those which yield key ions in the EIMS spectra of free sterols. For instance, in EIMS, ions at m/z 213 and 255 are diagnostic for free sterols that have one double bond in the A or B ring (Goad and Toshihiro, 1997). The former is analogous to the ion at m/z 215 in the CID spectra of SO_4-$C_{29}\Delta^{5,22}$, SO_4-$C_{27}\Delta^7$ and SO_4-$C_{27}\Delta^5$ (Fig. 3.5) and to that at m/z 243.4 in the CID spectrum of 4,4-dimethyl SO_4-$C_{29}\Delta^{8(9),24}$ (not shown). In the case of SO_4-$C_{29}\Delta^0$, this fragment shifts to m/z 217 reflecting the lack of the double bond in the A or B ring. The difference of 2 Thomsons presumably reflects the difference in parent ions, namely $[M\text{-}H_2O]^+$ in EIMS *vs*. $[M+H\text{-}H_2SO_4]^+$ in APCI-CID and fragmentation with abstraction of an additional H atom in the latter case. The same is true for the ion that arises

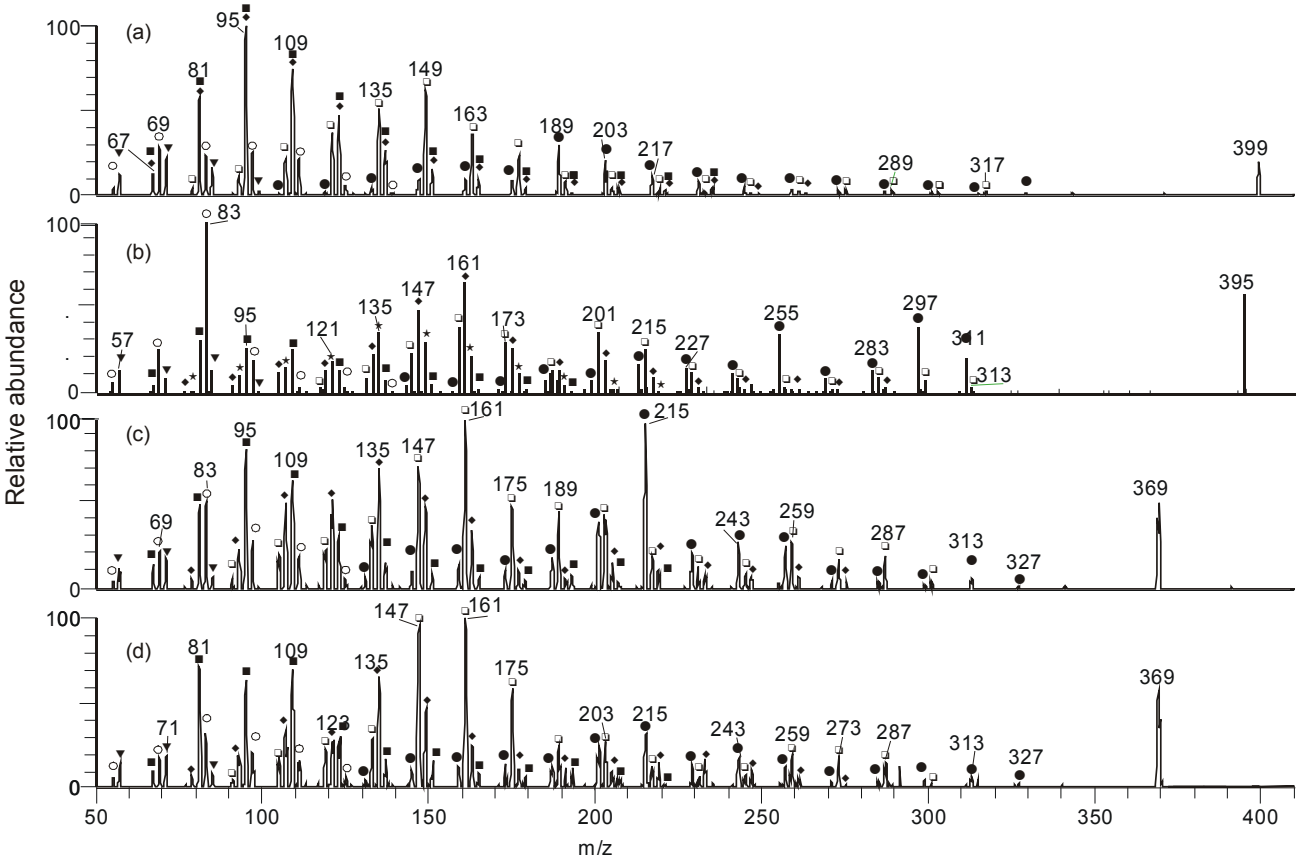


Figure 3.5. CID mass spectra obtained in positive mode (collision energy, -30eV) of (a) SO_4-$C_{29}\Delta^0$, (b) SO_4-$C_{29}\Delta^{5,22}$, (c) SO_4-$C_{27}\Delta^7$, and (d) SO_4-$C_{27}\Delta^5$. (a), (c) and (d) were loop-injected. (b) was injected on-line using the autosampler (▼=C_nH_{2n+1};❍ =C_nH_{2n-1}; ■ =C_nH_{2n-3}; ● =M-C_nH_{2n}; ❑ =M-C_nH_{2n-2}; ◆ =M-C_nH_{2n-4}; ★=M-C_nH_{2n-6}).

from loss of the side chain, at m/z 255, in the CID spectra of SO_4-$C_{29}\Delta^{5,22}$ and 4,4-dimethyl SO_4-$C_{29}\Delta^{8(9),24}$; other steryl sulphates showed a similar fragment ion at m/z 257 (Fig. 3.6a). On the other hand, the peak at m/z 255 could arise *via* cleavage of the A-ring (Fig. 3.6b). The ions at m/z 159 for cholecalciferol 3-sulfate, m/z 161 for SO_4-$C_{29}\Delta^{5,22}$, SO_4-$C_{27}\Delta^7$ and SO_4-

$C_{27}\ \Delta^5$, and m/z 163 for SO_4-$C_{29}\Delta^0$ probably result from cleavage in the B ring, as shown for SO_4-$C_{29}\Delta^{5,22}$ (Fig. 3.6b) and could also be diagnostic for the determination of the number of double bonds in the rings A or B. Note that this fragmentation would be expected to give rise to an ion of m/z 189 in the spectrum of 4,4-dimethyl SO_4-$C_{29}\Delta^{8(9),24(28)}$. This is absent; instead the B ring cleavage gives rise to m/z 191, which implies that the $\Delta^{8(9)}$ bond rearranges during CID, possibly to the $\Delta^{8(14)}$ position.

Certain fragment ions are present in all spectra, albeit with different intensities; this provides additional information that is helpful in the identification of the different compounds, including isomers. For example, the ion at m/z 83 is present in all spectra, but is the base peak only in the spectrum of SO_4-$C_{29}\Delta^{5,22}$. This characteristic ion presumably derives from the cleavage of the bond between C23 and C24 in the side chain (Fig. 3.6b). It is also possible to distinguish between the spectra of the isomers SO_4-$C_{27}\Delta^7$ and SO_4-$C_{27}\Delta^5$. In the former case, the base peak is at m/z 215 and its ratio to the ion at m/z 161 is ~ 1. In contrast, the base peak for SO_4-$C_{27}\Delta^5$ is at m/z 161, and ratio m/z 215:161 is much lower (~ 0.2). Thus, the CID spectra of standard steryl sulphates in positive APCI mode are compound specific, providing enough information for structure elucidation, including the position of the double bonds and the identification of certain isomers. It should be noted that to elucidate completely the mechanisms of the steryl sulphate fragmentation in CID, more research with labelled compounds is required.

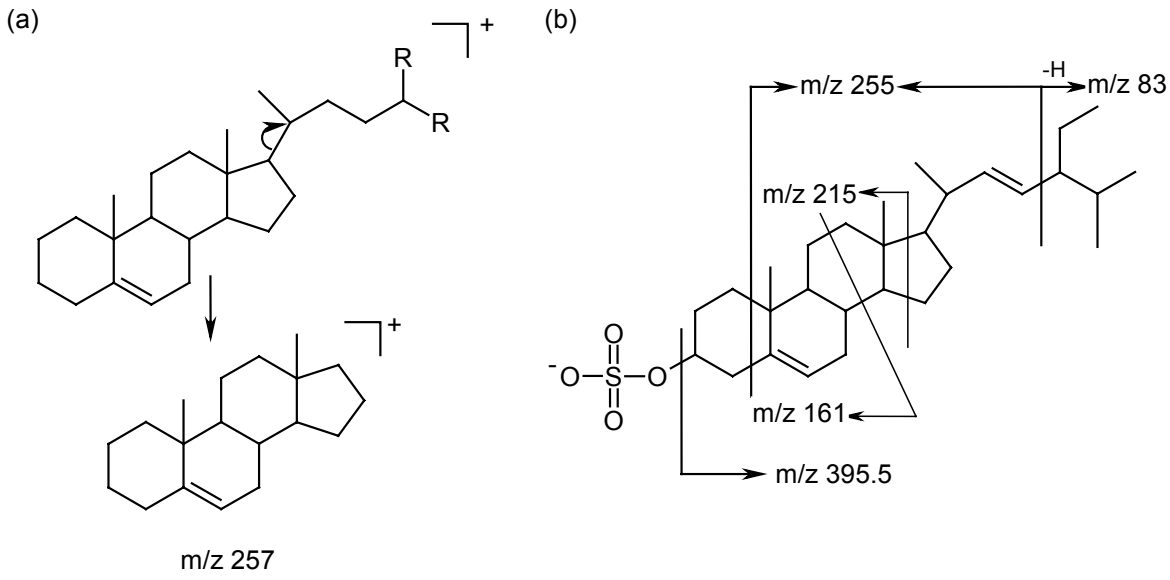


Figure 3.6. (a) Suggested general fragmentation leading to an ion at m/z 257. (b) Suggested fragmentation of SO_4-$C_{29}\Delta^{5,22}$.

3.3.4. LC ANALYSES

LC analyses were carried out both in positive and negative APCI mode, in order to assess detection limits. Initially, an aliquot of the organic extract of *Amperima rosea* was injected in SIM mode (Fig. 3.7), and then CID was performed on individual chromatographic peaks based on the results of SIM analyses (see Material and Methods). Thus, compounds present in the tissue of *Amperima rosea* were identified based on their CID spectra and retention times relative to the standards. The elution order is consistent with that which might be expected; hence, increasing carbon number and decreasing numbers of double bonds lead to increased retention times (de Souza and Nes, 1969). No steryl sulphates with 27

carbons and three double bonds were identified in the body tissue of *Amperima rosea* (chromatograms not shown).

In the analyses of tissue samples, the chromatograms generated in negative APCI are much cleaner than those acquired in positive APCI. Although they elute later in the chromatogram, free sterols (Toh *et al.*, 2001), sterol oxides (Razzazi-Fazeli *et al.*, 2000), and probably other sterol conjugates also contribute to the monitored ion current in positive mode. Since these compounds give similar spectra to the steryl sulphates, there is the potential for interference. Free sterols and related compounds, however, are not detected in negative ion APCI. Furthermore, although the ion current acquired in positive mode is ~ 1 order of magnitude more intense than that acquired in negative mode, the signal/noise ratio (S/N) is lower, which reflects the generation of positive ions by the sample matrix. Finally, the stability of the positive ions of steryl sulphates probably depends on the presence of double bonds in the steroid nucleus, which in turn influences their response factors significantly (Ma and Kim, 1997). This may explain why SO_4-$C_{27}\Delta^0$ was not detectable in the tissue of *Amperima rosea* by positive APCI, but appears in the negative APCI chromatogram (Fig. 3.7) of the same sample. Based on these observations, it can be concluded that quantitative analyses of steryl sulphate mixtures should be carried out in negative APCI mode after their identification in positive APCI mode.

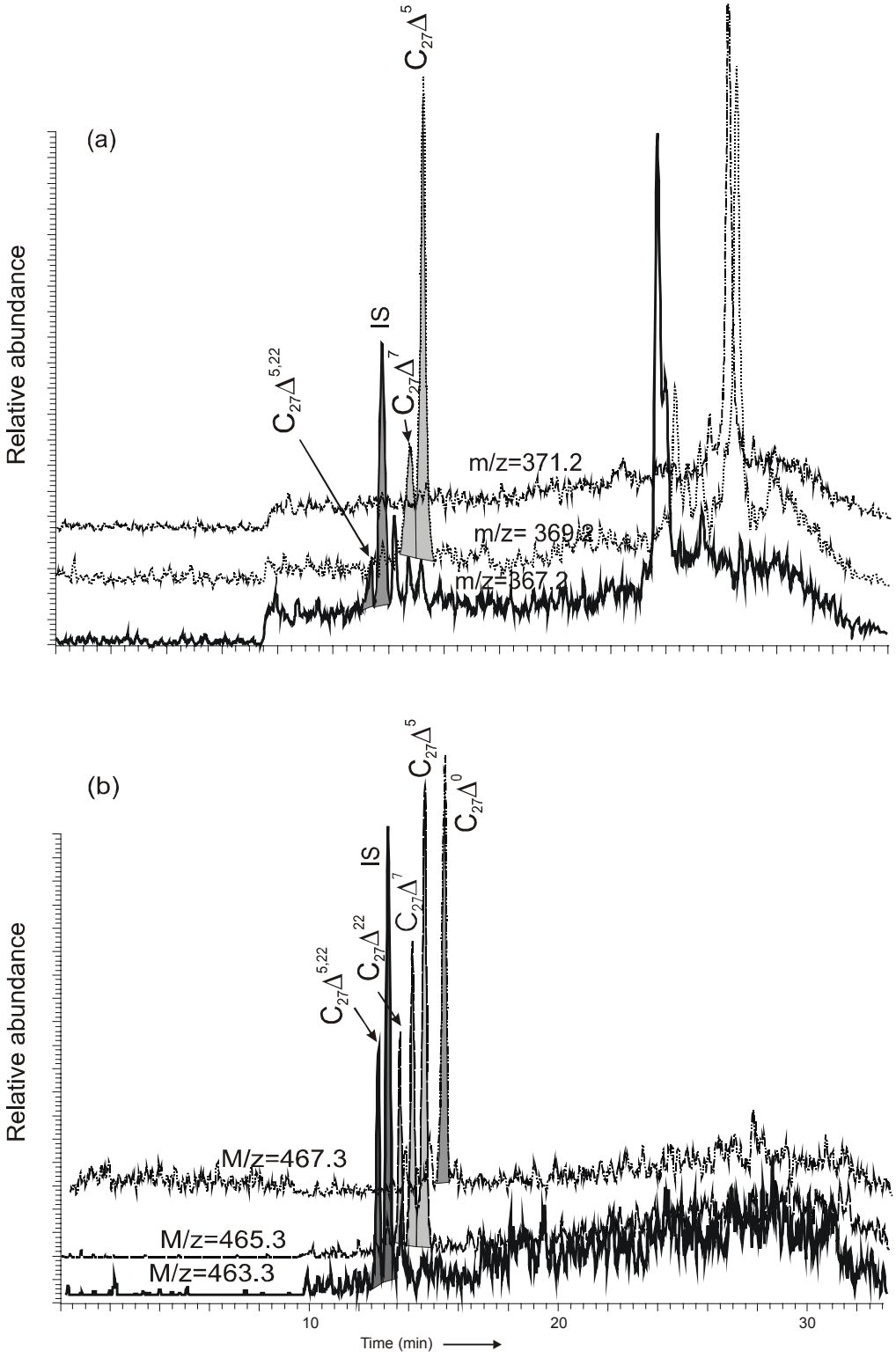


Figure 3.7. SIM (a) positive and (b) negative APCI chromatograms of steryl sulphates with 27 carbons, and 0-2 double bonds present in the extracts of *Amperima rosea*.

3.3.5. LINEARITY AND DETECTION LIMITS

The detection limits (S/N=3) based on cholesterol 3-sulphate in positive and negative APCI mode are 3.66 and 0.73 pmol μL^{-1}, respectively, the latter having the better detection limit, because of its greater selectivity (see above). Calibration plots and response factors for cholesterol 3-sulphate relative to the internal standard**,** cholecalciferol 3-sulphate, in both positive and negative polarities, were linear in the concentration range 1.22 to 16.4 pmol/μL with good coefficients of determination (R^2> 0.98; Fig. 3.8). The tendency to form adducts or multimers often gives rise to irreproducible and non-linear calibration curves (Lagerwerf *et al.*, 2000); nevertheless, in the present study variations in the intensities of these ions did not affect the linearity of the calibration.

The method shows sufficient sensitivity, precision, specificity and robustness for the analyses of steryl sulphates in our samples, and the technique meets the recommendations for method validation proposed by Green (1996).

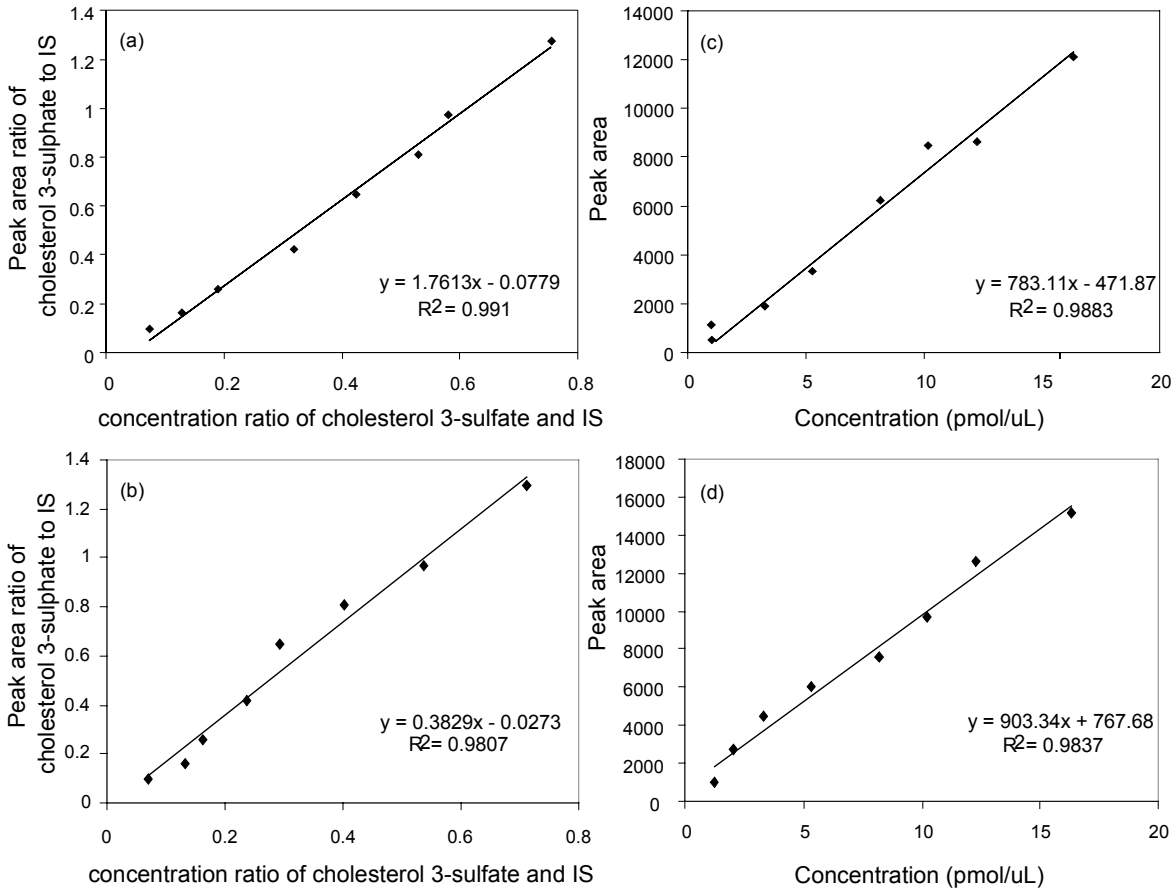


Figure 3.8. Response factors curves of cholesterol 3-sulfate in relation to the internal standard (cholecalciferol 3-sulfate) in both (a) positive and (b) negative polarities. Analytical curves of cholesterol 3-sulfate in both (c) positive and (d) negative polarities.

3.4 CONCLUSIONS

The analysis of steryl sulphates using positive and negative ionisation APCI and negative ESI modes generated spectra with strong pseudomolecular ions ($[M+H-H_2SO4]^+$ or $[M-H]^-$). The negative polarities in both ionisation modes also showed an ion at m/z 97 for HSO_4^- whereas

positive APCI generated adducts $[M+H- H_2SO_4+H_2O]^+$, $[M+H- H_2SO_4+CH_3OH]^+$ and $[M+H-H_2SO_4+2Na]^+$. Thus, any of the three methods could be used for characterization of sulphated sterols. The drawback with the negative polarities is that under CID conditions, the steryl sulphates did not fragment further and thus yielded no structural information on the steroid moiety. On the other hand, CID in positive APCI (collision energy, -30eV) generated several fragments that gave significant information for elucidation of steryl sulphate structures, and allowed isomers to be distinguished. Thus, it is suggested that identification of these compounds must be carried out in positive APCI mode. In contrast, quantification using positive APCI mode must be carried out cautiously, since it seems that the intensity of ionisation depends on the position and presence of double bonds in the molecules that can significantly influence their response factors relative to the internal standard. Therefore, it is recommended that the quantification should be carried out in negative APCI mode, which also shows a better S/N ratio. This method is fast, with only one clean-up step, and has sufficient sensitivity, precision, specificity and robustness for the routine analyses of steryl sulphates in biological matrices.

Chapter 4

THE LIPID CONTENTS OF DEEP-SEA HOLOTHURIANS

4.1 INTRODUCTION

Deposit-feeding holothurians (Phylum Echinodermata) are amongst the most abundant invertebrates that populate the deep-sea floor and represent a large component of the biomass at several locations (Khripounoff and Sibuet *et al*., 1980; Sibuet *et al*., 1982, Billett, 1991). Their dominance at hadal depths (>6000 m) is so great that this habitat is considered to be the kingdom of the holothurians (Belyaev, 1970). Some species use tentacles around the mouth to feed on particles of food suspended in the water. Most of them, however, roam over the seabed, feeding on the top layer of the sediment (Billett, 1986) reworking, redistributing and remineralizing the deposited POM (Chapters 1 and 6).

The morphology and biology of many deep-sea elasipodid holothurians have been described (Hansen, 1975), however, there are very few accounts of their biochemistry (Walker *et al.,* 1987a&b; Billett, 1991; Ginger *et al*., 2000). Recently, Ginger *et al*. (2000) presented one of the first detailed descriptions of the lipids of holothurians collected from an abyssal location, in the northeast Atlantic Ocean. By definition, lipids are compounds that are soluble in organic (fatty) solvents. Nevertheless, they are widely distributed in the waters of the oceans as part of the POM pool (*e.g.* Wakeham *et al.*,

1997; Kiriakoulakis *et al.*, 2001) and are present in all marine organisms (*e.g.* Lawrence and Guille, 1982; Harvey *et al.*, 1987; Harvey *et al.*, 1988; Svetashev *et al.*, 1991; Volkman *et al.*, 1998; Montgomery *et al.*, 1999; Ginger *et al.*, 2000; Carballeira *et al.*, 2002). They have several functions, being involved in energy storage (Hagen and Schnack-Schiel, 1996), cell membrane structure (Ayanoglu *et al.*, 1990; Dai *et al.*, 1991; Ohvo-Rekila *et al.*, 2002), and buoyancy (Phleger, 1998).

Most biochemical analyses of holothurians have concentrated on their bulk composition (Prim *et al*., 1976; Sibuet and Lawrence, 1981; Lawrence and Guille, 1982; Walker *et al.,* 1987a&b; Jaya Sree *et al.*, 1994; David and MacDonald, 2002). However, some studies of lipids at molecular level have also been published for both shallow water and deep-sea species (Voogt and Over, 1972; Goad *et al.*, 1986; Makarieva *et al.*, 1993; Ginger *et al.*, 2000).

The dominant constituent of the body tissue of holothurians is refractory organic matter, principally insoluble proteins (Walker *et al.,* 1987a&b). These are important constituents of the connective tissue, which is common in the body, gut and ovary walls of a number of species (Walker *et al.*, 1987a and references therein). Of the soluble constituents analysed in 10 holothurian species by Walker *et al.* (1987a&b), lipids were the dominant biochemical component in all tissues being most abundant in the ovaries. They are an important constituent of the yolk that develops directly and acts as a high-energy food-reserve (Walker *et al.,* 1987a&b). However, concentrations of lipids can vary significantly depending on the species,

tissue (Walker *et al.,* 1987a&b), gender, season, and feeding mode (David and MacDonald, 2002; Chapters 5 and 6).

Seven of the deep-sea species (*Oneirophanta mutabilis, Pseudostichopus villosus, Psychropotes longicauda, Deima validum, Paroriza prouhoi, Amperima rosea* and *Molpadia blakei*), which were collected at the PAP as part of the present study, have been investigated previously by Ginger *et al.* (2000), although, their data were qualitative. Here, quantitative data for all lipids (namely fatty acids, sterols and hydroxyacids) are presented. Furthermore, the results presented here differ from those of Ginger *et al.* (2000) because a different analytical method was employed in the preparation of the fatty acid methyl esters (FAMEs). Hence, transesterification (see Chapter 2) is a straightforward process that methylates all compounds that have an ester bond or a free acid group, with a low risk of artefact formation. It also leads to the hydrolysis of any steryl sulphates (see Chapter 3), to the free sterols. The method provides a reliable means of determining total lipids (including glycosides, etc.) in a single analysis. For this reason, the general composition of lipids (specifically sterols) differs slightly from those presented by Ginger *et al*. (2000).

In this Chapter, the detailed composition of lipids (more than 170 compounds; Fig. 4.1) of 11 deep-sea species of holothurians (7 described above from the PAP, and 4 from the WAP; *Peniagone* sp., *Scotoplanes globosa, Bathyplotes* sp., *Molpadia musculus*) will be described and briefly discussed. However, regional and seasonal differences and their

relationship to food availability are discussed in Chapters 5 and 6, respectively.

4.2 RESULTS AND DISCUSSION

All compounds found in the investigated species and their concentrations are listed in the Tables 4.1-4.10. The concentration of most compounds show high standard deviations, which may reflect the different genders and sizes of the replicate specimens, as well as food availability at the time of sampling and the ability of different specimens to exploit it.

4.2.1. FATTY ACIDS

More than 90 different fatty acids (ranging from 14 to 25 carbons) were identified among the different species investigated here (Fig. 4.1; Tables 4.1 and 4.2). The highly complex distributions of fatty acids are typical of organisms that do not have any specialisation in lipid and fatty acid composition (biosynthesis and/or dietery uptake), and demonstrate strongly opportunistic feeding behaviour (Graeve *et al.*, 2001). However, there are common trends in all species, except for *Molpadia musculus, a* subsurficial feeder. For instance, the high concentrations of the monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) are striking; 18:1(*n*-7), 20:4(*n*-6), 20:5(*n*-3), 21:4, 22:6, 23:1 and 24:1 being the most abundant

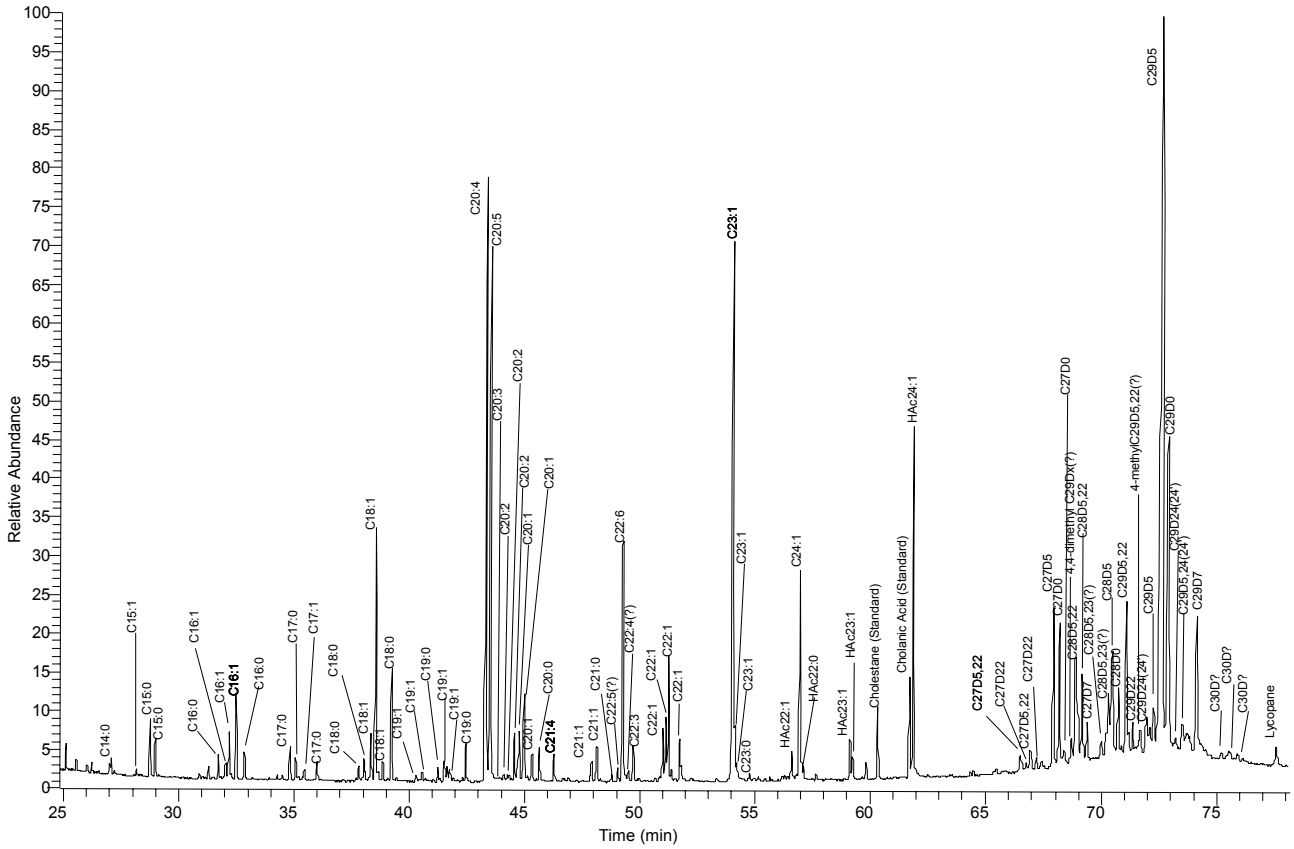


Figure 4.1. Total ion chromatogram of the lipids of the body tissue of *Oneirophanta mutabilis.*

fatty acids (Tables 4.1 and 4.2). High concentrations of PUFAs are also observed in shallow-water species (Svetashev *et al.*, 1991), but relatively higher amounts of unsaturated compounds in the deep-sea animals are ascribed to an adaptation of the latter to maintain membrane fluidity at high pressure and low temperature (Ginger *et al.*, 2000; Chapter 5).

It has been suggested that the high concentration of 20:4(n-6) could arise from dietary uptake of Rhodophyceae, since these algae are rich in this compound and have been identified by microscope in gelatinous detrital material at the PAP sediments (Bühring *et al.*, 2002). However, the high concentrations of the other PUFAs were not discussed by Bühring *et al.* (2002). Furthermore, PUFAs are absent or present in very low concentrations in deep-sea sediments because they are very labile, being degraded rapidly in a matter of days to weeks (Santos *et al.*, 1994; Wakeham *et al.*, 1997; Mackenzie, 2000). It is possible that "hot spots" of labile material may be present for short periods even at the abyssal sea floor. These may not be effectively sampled because of the small number of replicates collected, or through loss of flocculent surface material on recovery. Nevertheless, holothurians have the key starting materials for the biosynthesis of the PUFAs (14:0, 16:0 and 18:0; Carballeira *et al*., 2002), and it is certainly possible that holothurians can biosynthesise these compounds from these fatty acids, as do other marine organisms when PUFAs are not available in their diet (*e.g.* Bell *et al.*, 2001; Pond *et al.*, 2002)

The dominance of the PUFAs 20:4(*n*-6), 20:5(*n*-3), 21:4 and 22:6,

probably as phospholipids, reflects their role as constituents of cell membranes (*e.g.* Graeve *et al*., 2001; Bühring *et al.*, 2002) and suggests that other lipid pools, such as triacylglycerides and wax esters are subordinate. Ginger and Wolff (pers. commun.) did not find any wax esters in *Psychropotes longicauda* and Walker *et al.* (1987a&b) also indicated that deep-sea holothurians do not have large deposits of energy-rich lipids in their body walls. Additionally, glycogen, which is the main storage carbohydrate in many marine animals, has been found only in low concentrations in holothurians (Jaya Sree *et al*., 1994; David and MacDonald, 2002). Moreover, it has been postulated that instead of storing food, holothurians transform ingested food into reserve tissues, which, because of the weight-specific respiration of larger animals, last longer than in proportionally smaller animals (Peters, 1983; Gage and Tyler, 1991). Separate analyses of triacylglycerols, wax esters and phospholipids would have given a better insight into the way in which deep-sea holothurians deal with high and low periods of food supply, however, such an approach was not adopted in the present study because of time constraints. Additionally, the correlation between temporal variation of lipid content and food supply for some species of deep-sea holothurians (Chapter 6) is striking and indicates that these animals do not store triacylglycerols as reserve of energy in long terms.

The lipid composition may also have a role in the buoyancy of the animals (Phleger, 1998). The major lipids that have a direct role in buoyancy of marine fish are wax esters, squalene, and alkyldiacylglycerols (Phleger,

1998). Most deep benthopelagic fishes that live at depths in excess of 800 m in the ocean have fat-containing swimbladders that are still fully functional (Bowne, 1982). This fat deposit is rich in cholesterol and phospholipids (1:1 ratio) formed by highly unsaturated fatty acids (72-89%; Phleger, 1991). Thus, the absence (or low quantity) of wax esters and tryacylglycerols and the high level of PUFAs could be linked to the negative buoyancy of the investigated holothurians.

Bathyplotes sp. and *Oneirophanta mutabilis* had highest concentrations of bacterially-derived fatty acids (Tables 4.9 and 4.10), which could be reflect of the high bacterial activity in their guts or in their tissue. The gut of *Bathyplotes* sp. may act as a bacterial fermentation chamber since it is relatively bigger than those of the other species (pers. obs.). The gut of *Oneirophanta mutabilis*, on the other hand, does not include fermentation chambers (Roberts *et al.*, 1996). Thus, the high concentration of bacterial compounds in their body tissue could arise from bacteria present in their tentacles (Chapter 5). However, no bacterial counts were undertaken in the body tissues or in the gut contents of the specimens analysed here, precluding any definite conclusions.

It is important to note that *Molpadia musculus* has a very different distribution of fatty acids from all other species (low quantity of PUFAs). This difference is difficult to explain, but it may be related to feeding mode and food availability, which are discussed in Chapter 5.

4.2.2. HYDROXYACIDS

The carbon chain length of the hydroxy acids identified in the studied species ranged from 17 to 25 carbons, the compounds being monounsaturated or fully saturated (Tables 4.3 and 4.4). They are *α*-substituted, dominated by *α*-HAc24:1[1], which is present in concentrations of one order of magnitude higher than any other hydroxy acid (Tables 4.3 and 4.4). Aliphatic *α*- and *β*-monohydroxy fatty acids occur in a wide range of organisms (Downing, 1961; Cardoso and Eglinton, 1983) and are typically produced as intermediates of *α*- and *β*-oxidation of monocarboxylic fatty acids. *β*-oxidation is more common than *α*-, although the latter does operate in plants, animals and bacteria (Volkman *et al.*, 1998). Thus, the *α*-oxidation of fatty acids could be a source of *α*-hydroxy fatty acids found in all species. Indeed, *Psychropotes longicauda*, which may absorb more enteric bacteria because of its gut morphology (Chapter 5), had the highest concentrations of these acids (Tables 4.3 and 4.4). On the other hand, *Amperima rosea*, which feeds on fresh organic material (Ginger *et al.*, 2000; Ginger *et al.*, 2001; Iken *et al.*, 2001) and probably has little bacterial activity in its gut, had the second lowest value. Only *Molpadia musculus*, which is a subsurficial feeder, had lower values, which again are difficult to explain, since this species presumably feeds on refractory material, and thus might

[1] Short hand notation for hydroxy acids: (position of hydroxyl group)-HAc(length of carbon chain):(number of unsaturations).

be expected to extract food by fermentation in its guts, having a high concentration of bacterially-derived compounds.

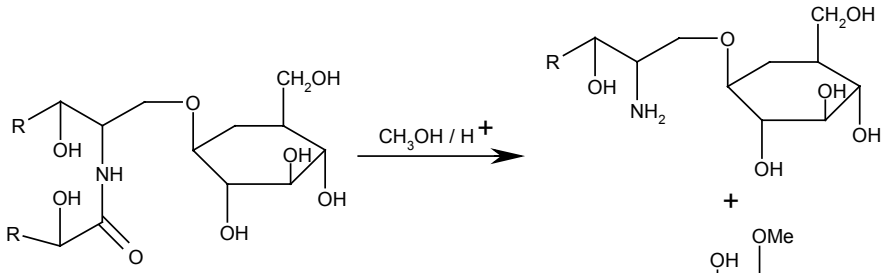
Saturated and monounsaturated *α*-hydroxy fatty acids have been found as major lipid components of the cell walls of several marine chlorophytes (Gelin *et al*., 1997) and could also be a source of these compounds. However, although *β*-hydroxyacids have been found in deep-sea sediments (Cardoso & Eglinton, 1983), *α*-hydroxyacids have not been reported (Cardoso & Eglinton, 1983; Madureira *et al*., 1995). Hence it is likely that holothurians can biosynthesise these compounds *de novo*.

The function of hydroxyacids is very likely to be related to membrane structure. Indeed, *α*-hydroxy acids, mainly *α*-HAc24:1, have been identified in sphingolipids, namely glycosphingolipids and sialosphingolipids (Kochetkov *et al.*, 1976), which are present in the cell membranes of all eukariots and have many different functions from regulation of cellular growth to modulation of cell-cell communication (Merrill *et al*., 1997). Although very little work has been carried out in holothurians, sphingolipids have been identified in many other marine organisms. Indeed other classes of the phylum Echinodermata have high contents of glycosphingolipids (Kochetkov and Smirnova, 1986).

The analytical method employed in the present study probably transmethylates the amide bonds of sphingolipids releasing the hydroxyacids (Fig. 4.2) and may explain why the latter compounds have not been identified previously in holothurians (Ginger *et al.*, 2000).

4.2.3. STEROLS

Sterols in marine organisms are functionally involved in a number of processes including, growth and cell membrane structure (Dai *et al.*, 1991). These compounds are found in representative animals belonging to all marine invertebrate phyla; they have been extensively examined and many novel sterols have been discovered (Goad *et al.*, 1986; Kanazawa, 2001).



Glycosphingolipid Hydroxyacid

Figure 4.2. Transmethylation of a glycosphingolipid yielding a hydroxyacid (R= alkyl chain usually between 14 and 26 carbons).

Echinoderms have been proved to be particularly interesting from the viewpoint of their sterol content and their metabolites (Goad *et al.*, 1986) with respect to chemotaxonomy and animal nutrition (Kanazawa, 2001). Many marine invertebrates depend on dietary sterols, because they cannot biosynthesise sterols *de novo* (Kanazawa, 2001). However, echinoderms including holothurians may be capable of synthesizing 5α(H)-cholest-7-enol (**7**) from acetate or mevalonate probably via squalene and lanosterol

(Kanazawa, 2001). Nevertheless, they cannot alkylate the sterols at the C-24 position or introduce a double bond at the C-22 position of (**7**) (Kanazawa, 2001). Thus, the C_{27} compounds may be biosynthesised *de novo*, while C_{26}, C_{28}, and C_{29} compounds have exclusively exogenous sources (Kanazawa, 2001).

Both shallow-water and deep-sea holothurians contain a large quantity of sterols ranging from 26 to 30 carbons and are dominated by Δ^7 sterols (Ginger *et al.*, 2000; Kanazawa, 2001). The results shown here differ, because of the transmethylation employed in the sample treatment. This process converts conjugated sterols (*e.g.* steryl esters, steryl sulphates) to free sterols. Thus, the main sterols (as of the total pool) in the deep-sea holothurians are Δ^0's and Δ^5's (Fig. 4.1; Tables 4.5 and 4.6), reflecting in part the distribution of steryl sulphates (Tables 4.7 and 4.8; Ginger *et al.*, 2000). 24-ethylcholet-5-en-3*β*-ol (**19**) was the main sterol found in all species, except in *Psychropotes longicauda*, *Molpadia blakei* and *Molpadia musculus* (Tables 4.5 and 4.6) in which 24-ethyl-5α(H)-cholestan-3*β*-ol (**20**) was dominant (Tables 4.5 and 4.6). C_{29} compounds dominated the sterols of all holothurians (Tables 4.9 and 4.10), except for *Molpadia musculus*, in which C_{28} compounds were the most abundant. Interestingly, the main sterols in the PAP and WAP sediments are the C_{29} compounds, being 24-ethylcholet-5-en-3*β*-ol (**19**) the dominant sterol (Chapter 5).

The difference between the concentrations of Δ^5 and Δ^0 sterols may reflect the feeding modes of the holothurians (Chapter 5). Both bacteria (*e.g.* Wakeham *et al*., 1997) and holothurians (Chapter 5) can reduce the Δ^5

double bond. Thus, the higher the level of Δ5 sterols, the fresher is the pool of sterols (Chapter 5) in their bodies. *Amperima rosea* and *Oneirophanta mutabilis*, species that feed on relatively fresher material (Ginger *et al.*, 2000; Iken *et al.*, 2001; Chapter 5), have high Δ^5/Δ^0 ratios (Table 4.9; Chapter 6). On the other hand, both *Molpadia musculus* and *Molpadia blakei*, species that feed with their head down in the sediments ingesting more refractory material (Iken *et al*., 2001), have low Δ^5/Δ^0 values (Table 4.9). *Psychropotes longicauda* also has a low Δ^5/Δ^0 ratio, but in this case, it is more likely that this ratio reflects the high bacterial activity in its gut, which has a rectum that function as a fermentation chamber (Roberts *et al.*, 2000).

Thus, as previously shown by Ginger *et al.* (2000), sterols in deep-sea holothurians reflect their diet and their feeding mode.

4.2.4. STERYL SULPHATES.

Although the role of steryl sulphates in holothurians is still unclear (Makarieva *et al.*, 1993; Ginger *et al.*, 2000), it has been postulated that they act as a reserve of sterols and that the loss of the Δ^5-bond could be a pre-requesite prior to incorporation of sterol into cell membranes (Ginger *et al.*, 2000).

Again, our results differ from those presented by Ginger *et al.* (2000) probably because of the different analytical method employed. *Amperima rosea* showed a complex mixture of steryl sulphates, although their

concentration was rather low in contrast to the results of Ginger *et al.* (2000) (Table 4.7). *Psychropotes longicauda*, *Oneirophanta mutabilis*, *Pseudostichopus villosus,* and *Molpadia blakei* contained the highest amounts (Table 4.7); the species from PAP had higher average steryl sulphate contents than those from the WAP (Table 4.8). This difference may be explained by the more limited supply of sterols to the PAP, thus there is a requirement for their storage (Chapters 5 and 6), while this is not the case at the WAP (Chapter 5). Thus, the concentrations of steryl sulphates may be related to food availability, and will be discussed in more detail in Chapter 5 and 6.

4.2.5. BULK PARAMETERS

Values of total organic carbon (TOC), total inorganic carbon (TIC) and total nitrogen (TN) varied from 5.92±3.38% to 25.99±3.28%, 2.58±0.29% to 11.33±6.46%, and 2.02±0.49 to 8.21±0.88% (all dry weight), respectively (Tables 4.11 and 4.12).

Deima validum and *Molpadia musculus* were the only species with higher contents of TIC than of TOC (Tables 4.11 and 4.12). Inorganic contents (ash) of eleven deep-sea species are higher in the body walls (35-69.5%) than in the gonads (10-35%; Walker *et al.*, 1987a&b). Species such as *Deima validum*, which have a high percentage of ash (Walker *et al.*, 1987b), also have an abundance of spicules in the body wall (Hansen, 1975), suggesting that much of the ash is calcium carbonate (Walker *et al*.,

1987b). This variation of ash level in the body walls of holothurians may be responsible for the variation in organic contents between species and tissue types (Prim *et al.*, 1976; Walker *et al.,* 1987a+b). Indeed, *Deima validum* had the highest TIC values and the lowest concentration of total lipids among the PAP species. However, no correlation between concentrations of total lipids and %TIC (both dry weight) was apparent (Spearman's rank correlation test, r_s= 0.06, *P*=0.59, N=98; Fig. 4.3).

On the other hand, a good (59%) and highly significant correlation was observed between the concentration of total lipids and the %TOC (Spearman's rank correlation test, r_s=0.59, *P*<0.000001, N=98; Fig. 4.4). Considering the means of the total concentrations of lipids (10.8±6.4 mg/g) and TOC (158.8±54.80 mg/g), the pool of lipids accounts only for 5.40% of the TOC, assuming lipids are formed by 20:5 fatty acids. This indicates that although there is a good correlation between lipids and TOC, other components may be more important in controlling the variation of the latter. As carbohydrates contents are even lower than lipids (1.4-1.8 % against 1.8-3.0% of lipids; Sibuet and Lawrence, 1981), TOC must be driven by other uncharacterised material.

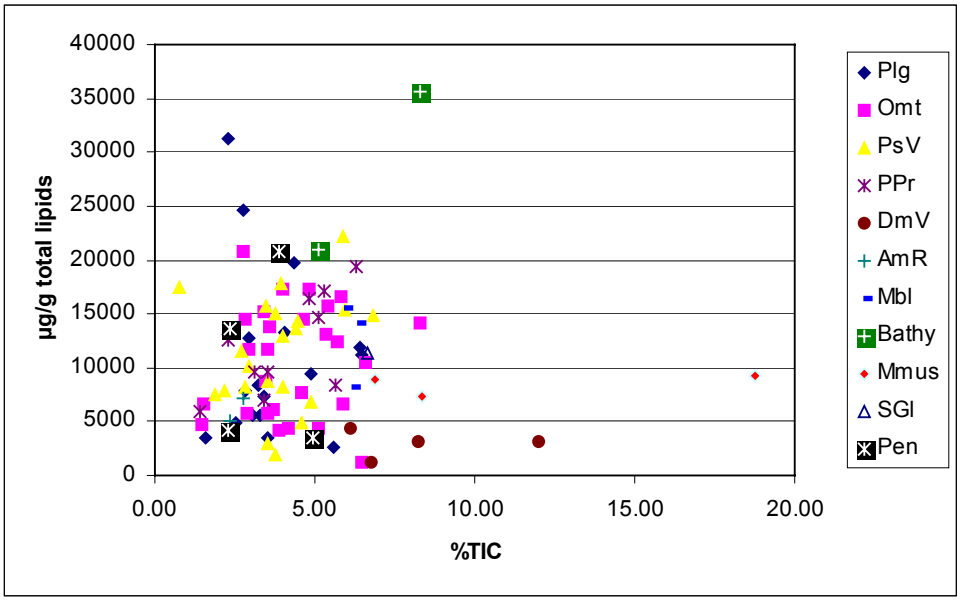


Figure 4.3. Relationship between concentrations of total lipids and %TIC (both dry weight) in holothurians (n=98, 11 different species from the PAP and the WAP, for legend see Tables 4.1 and Table 4.2).

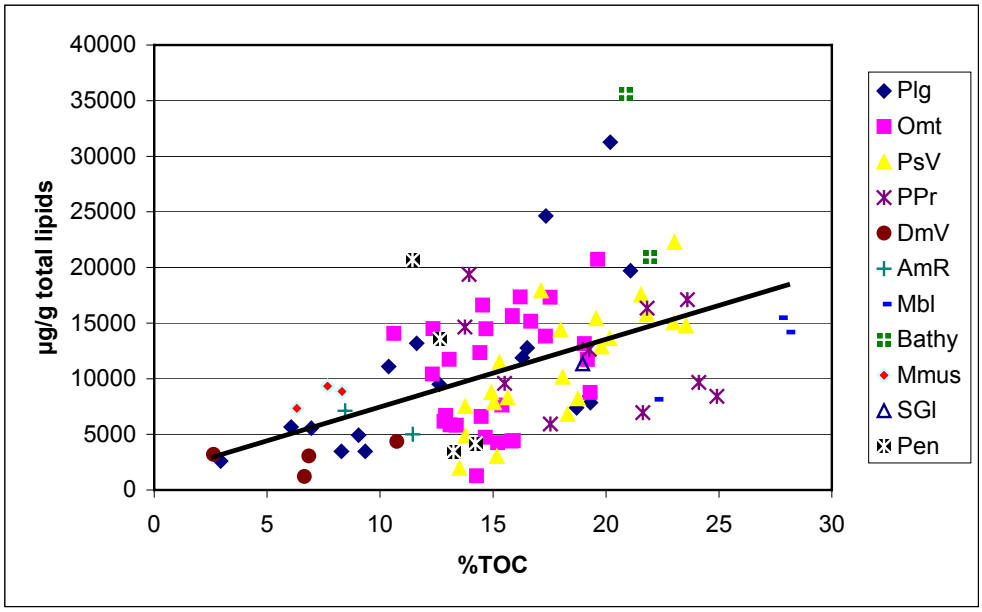


Figure 4.4. Correlation between concentrations of total lipids and %TOC (both dry weight) in holothurians (n=98, 11 different species from the PAP and the WAP; for legend see Tables 4.1 and Table 4.2).

4.3. CONCLUSIONS

- Holothurians have a very complex mixture of lipids (>170 compounds), which reflect in part the opportunistic feeding mode of these animals and their ability to assimilate lipid.
- Fatty acids, which are constituents of phospholipids, showed a common trend for all species, namely high concentrations of 18:1(*n*-7), 20:4(*n*-6), 20:5(*n*-3), 21:4, 22:6, 23:1 and 24:1, in all but *Molpadia musculus*.
- Although some authors suggest that PUFAs derive from dietary uptake, the absence (or low concentration) of these compounds in POM and sediments at the PAP suggests that holothurians biosynthesise them *de novo*.
- The lack or low concentration of triacylglycerols and other forms of chemical energy storage in holothurians, indicates that they do not accumulate energy reserves. However, steryl sulphates may act as a store of sterols in low food periods. Lipids may also have a role in the negative buoyancy of holothurians.
- Hydroxyacids are reported for the first time in deep-sea holothurians. It is likely that these compounds are released from glycosphingolipids during work up.
- The pool of total sterols is dominated by C_{29} compounds (**19** and **20**), and reflects the sterol distribution in the sediments. (**19**) is also dominant in the sulphated form.

- The Δ^5/Δ^0 ratio may be dependent on feeding mode.
- The distribution of sterols in deep-sea holothurians reflects their diet and their feeding mode, as previously shown by Ginger *et al.* (2000).
- *Deima validum* and *Molpadia musculus* have high values of IC, which may reflect the high concentration of calcium carbonate in spicules present in its body tissues (at least for *D. validum*).
- TOC and the concentration of total lipids were significantly correlated (59%), however, the latter account for only 5.4% of TOC, indicating that other compounds are also responsible for the variation of TOC.

Table 4.1. Fatty acid composition (µg/g dry weight) of holothurians collected at the Porcupine Abyssal Plain.

	PLg[1](n=18)	OMt (n=28)	PsV (n=22)	PPr (n=11)	DmV (n=5)	AmR (n=2x20pooled)	Mbl (n=3)
14:0[2]	0.67 ±0.20	10.18 ±11.25	5.22 ±6.44	8.31 ±12.39	0.91 ±1.27	2.05 ±2.90	0.75 ±1.31
14:0	5.33 ±5.37	9.96 ±8.11	6.73 ±8.30	7.31 ±11.09	3.90 ±3.03	12.11 ±7.19	2.92 ±5.06
15:1	0.91 ±1.90	3.50 ±6.82	0.52 ±1.56	0.15 ±0.49	*n. d.*[3]	2.70 ±3.82	*n. d.*
i-15:0[4]	13.46 ±12.56	89.52 ±68.97	43.84 ±28.59	59.67 ±87.15	7.55 ±7.87	12.73 ±3.88	12.95 ±5.40
a-15:0	17.70 ±12.53	72.91 ±61.27	64.69 ±44.09	86.51 ±123.37	8.87 ±7.20	21.75 ±1.13	21.76 ±3.00
15:0	2.29 ±4.06	1.85 ±3.41	1.32 ±2.12	2.07 ±2.61	2.12 ±1.71	5.24 ±0.32	5.03 ±0.82
16.0	0.32 ±0.91	39.07 ±31.79	11.24 ±10.89	12.75 ±16.08	1.52 ±3.41	11.43 ±6.21	9.05 ±15.68
16.0	12.22 ±7.52	5.58 ±22.76	*n. d.*	*n. d.*	3.47 ±6.58	*n. d.*	10.42 ±3.10
16:1	5.89 ±4.49	30.30 ±33.39	9.33 ±11.95	7.65 ±11.75	11.00 ±8.17	11.04 ±0.11	14.55 ±12.92
16:1(n-7)	23.98 ±19.05	58.33 ±44.94	56.05 ±109.90	29.71 ±16.17	12.00 ±4.23	42.05 ±14.82	87.54 ±24.89
16:1	3.16 ±4.65	10.17 ±9.15	10.16 ±15.12	4.80 ±10.13	2.80 ±2.20	7.94 ±2.07	13.06 ±18.53
16:1	10.90 ±6.88	133.88 ±117.87	79.66 ±62.06	85.65 ±109.35	8.08 ±8.62	22.94 ±10.45	54.58 ±12.20
16:0	32.09 ±15.99	38.08 ±26.22	28.24 ±20.85	33.81 ±25.37	29.75 ±15.70	74.11 ±15.02	78.04 ±41.42
17:0	n. *d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	13.02 ±5.27
17:1	0.19 ±0.59	5.56 ±9.02	2.23 ±5.54	3.11 ±7.12	0.42 ±0.60	3.02 ±2.02	*n. d.*
17:1	*n. d.*	4.31 ±6.04	2.32 ±3.95	2.99 ±5.39	0.08 ±0.19	*n. d.*	*n. d.*
i-17:0	13.34 ±9.45	56.74 ±46.96	9.22 ±5.50	7.79 ±4.55	4.59 ±2.44	11.61 ±3.69	24.42 ±5.79
a-17:0	14.20 ±8.56	44.56 ±39.43	22.17 ±13.14	18.59 ±16.07	6.25 ±3.70	10.27 ±1.78	20.60 ±3.60
17:1	0.36 ±1.08	6.60 ±10.16	13.96 ±16.80	7.10 ±9.54	0.83 ±1.24	8.71 ±0.08	9.75 ±2.76
17:1	2.67 ±3.36	18.40 ±27.58	10.18 ±11.72	15.50 ±25.63	0.49 ±1.10	43.21 ±61.10	23.33 ±6.83
17:0	14.34 ±7.52	28.61 ±20.68	25.02 ±15.92	19.80 ±12.26	6.48 ±3.62	19.42 ±6.41	10.73 ±1.36
18:2	*n. d.*	0.22 ±1.14	1.76 ±3.53	16.44 ±19.32	*n. d.*	1.42 ±2.01	6.69 ±1.23
18:0	9.40 ±8.53	16.63 ±11.43	7.32 ±4.85	17.75 ±22.64	1.83 ±0.94	5.61 ±0.33	2.21 ±2.03
18:2	2.75 ±7.52	1.43 ±3.58	8.63 ±8.21	20.69 ±19.30	*n. d.*	2.47 ±3.50	15.97 ±11.38
18:0	3.99 ±4.60	32.60 ±17.84	3.47 ±3.90	17.89 ±12.20	4.07 ±1.83	*n. d.*	11.10 ±6.52
18:2	1.24 ±2.32	*n. d.*	1.79 ±2.61	17.65 ±36.25	*n. d.*	*n. d.*	*n. d.*
18:1	39.14 ±28.71	125.92 ±75.35	65.16 ±80.53	79.30 ±57.42	81.79 ±90.69	121.46 ±95.16	88.49 ±30.54
18:1	227.25 ±116.03	564.12 ±333.72	403.07 ±188.34	981.92 ±805.47	99.96 ±39.05	220.65 ±14.75	313.70 ±110.80
18:1	12.96 ±6.32	24.39 ±29.47	24.19 ±18.38	33.07 ±37.60	2.79 ±2.26	19.38 ±3.02	8.15 ±9.32

[1]Shorthand notation for species; Plg = *Psychropotes longicauda*; Omt=*Oneirophanta mutabilis*; PsV= *Pseudostichopus villosus*; PPr=*Paroriza prouhoi*; DmV=*Deima validum*; AmR=*Amperima rosea*; MBl= *Molpadia blakei*; [2] Short hand notation is used for the fatty acids; *e.g.* 18 (carbon number):3 (number of unsaturations)(n-(the position of the double bound counting from the aliphatic end of the chain)); [3]n.d.=non-detected; [4] *i*- = iso and *a*- = anteiso

Table 4.1. Continued…

	PLg[1](n=18)	OMt (n=28)	PsV (n=22)	PPr (n=11)	DmV (n=5)	AmR (n=2x20pooled)	Mbl (n=3)
18:1	11.80 ±6.46	35.44 ±31.01	123.52 ±115.51	54.59 ±33.21	18.96 ±8.68	11.95 ±0.21	8.42 ±3.32
18:1	1.81 ±2.36	*n. d.*	14.18 ±19.58	*n. d.*	*n. d.*	5.66 ±0.05	3.59 ±6.22
18:0	103.60 ±52.94	128.34 ±73.93	102.38 ±41.28	74.86 ±38.83	31.59 ±12.39	109.12 ±24.56	62.91 ±39.46
19:1	2.77 ±6.24	7.52 ±6.46	0.89 ±2.63	6.48 ±11.17	*n. d.*	*n. d.*	1*n. ±8.84*
19:0	4.04 ±3.76	16.26 ±12.03	*n. d.*	2.50 ±5.23	0.11 ±0.24	*n. d.*	11.14 ±9.89
19:1	1.80 ±4.08	11.71 ±10.22	*n. d.*	*n. d.*	0.55 ±1.23	*n. d.*	6.51 ±11.27
19:0	1.56 ±1.81	32.89 ±19.41	1.68 ±2.52	2.16 ±3.65	4.82 ±2.74	3.68 ±2.81	*n. d.*
19:0	10.06 ±8.08	8.06 ±21.28	4.91 ±3.80	8.41 ±9.99	4.14 ±2.81	*n. d.*	22.55 ±3.06
19:1	5.50 ±9.15	27.46 ±18.80	26.60 ±22.90	21.52 ±13.42	2.61 ±1.30	6.95 ±1.24	27.56 ±12.41
19:1	3.34 ±5.38	22.98 ±15.52	12.26 ±7.52	20.20 ±16.34	1.65 ±1.34	5.59 ±7.90	12.40 ±2.68
19:1	0.34 ±0.98	5.40 ±8.44	21.70 ±16.26	15.97 ±15.51	1.42 ±1.25	2.73 ±3.87	7.02 ±4.43
19:0	10.44 ±5.82	47.02 ±27.39	14.81 ±8.64	10.12 ±6.37	9.91 ±3.19	12.42 ±1.50	9.33 ±3.79
20:4(n-6)	1490.38 ±1302.12	978.83 ±814.09	2079.59 ±1317.55	2722.36 ±1734.48	338.65 ±361.05	754.58 ±284.78	2940.73 ±931.61
20:1	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*
20:5(n-3)	1059.42 ±882.34	681.99 ±579.25	1790.18 ±1030.08	1254.94 ±474.62	264.38 ±322.53	1127.46 ±460.74	755.32 ±445.57
20:3	13.61 ±14.51	4.14 ±6.88	17.05 ±31.78	42.78 ±26.84	0.48 ±1.07	12.24 ±17.32	56.50 ±60.31
20:1	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*
20:1	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*
20:2	5.52 ±6.39	4.72 ±7.11	12.90 ±10.98	63.67 ±36.20	1.28 ±1.96	3.14 ±4.44	55.79 ±34.76
20:0	1.53 ±2.43	2.30 ±3.13	*n. d.*	*n. d.*	0.58 ±1.31	*n. d.*	*n. d.*
20:2	5.02 ±5.66	10.25 ±20.02	8.66 ±7.41	31.58 ±17.80	2.19 ±2.48	6.77 ±0.84	74.00 ±57.54
20:0	0.73 ±1.63	4.14 ±6.03	0.41 ±1.49	2.59 ±4.50	*n. d.*	*n. d.*	*n. d.*
20:2	67.19 ±46.30	46.29 ±39.57	107.88 ±58.21	85.47 ±32.85	5.15 ±11.51	46.91 ±2.81	70.80 ±30.41
20:1	13.39 ±9.72	71.50 ±37.08	40.66 ±18.87	56.22 ±30.93	39.97 ±13.46	24.13 ±12.98	119.94 ±123.10
20:1	27.16 ±14.47	99.20 ±53.84	24.51 ±18.50	55.25 ±30.27	11.86 ±3.33	23.13 ±5.80	17.73 ±30.71
20:1	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*
20:1	3.71 ±9.49	8.22 ±13.36	8.33 ±13.21	28.06 ±21.63	8.30 ±6.78	13.95 ±19.73	43.07 ±40.35
20:1	1.08 ±4.47	3.56 ±8.18	0.08 ±0.39	*n. d.*	2.93 ±4.92	*n. d.*	*n. d.*
20:0	17.31 ±15.26	36.59 ±21.54	26.31 ±17.94	8.05 ±5.65	9.69 ±3.62	17.38 ±3.40	14.99 ±4.15
21:4	183.45 ±141.88	41.27 ±34.67	191.68 ±115.90	310.99 ±90.50	43.21 ±42.10	110.69 ±34.18	208.82 ±152.83
21:1	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*
21:1	*n. d.*	*n. d.*	12.55 ±42.54	1.60 ±3.43	*n. d.*	*n. d.*	*n. d.*

Table 4.1. Continued…

	PLg[1](n=18)	OMt (n=28)	PsV (n=22)	PPr (n=11)	DmV (n=5)	AmR (n=2x20pooled)	Mbl (n=3)
21:1	7.50 ±5.11	17.21 ±11.87	*n. d.*	2.48 ±4.80	5.44 ±2.18	2.63 ±1.47	*n. d.*
21:0	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*
21:2	*n. d.*	*n. d.*	*n. d.*	12.80 ±8.62	*n. d.*	*n. d.*	*n. d.*
21:1	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*
21:1	31.63 ±18.88	37.99 ±21.82	31.34 ±17.68	11.60 ±18.33	5.88 ±1.94	7.12 ±0.12	45.66 ±11.21
21:1	4.38 ±4.39	11.69 ±17.24	12.27 ±16.09	63.68 ±27.33	0.33 ±0.48	3.52 ±0.16	*n. d.*
21:1	*n. d.*	*n. d.*	*n. d.*	4.55 ±4.27	*n. d.*	*n. d.*	*n. d.*
21:0	4.60 ±3.25	8.24 ±4.51	16.98 ±12.89	5.85 ±2.93	1.80 ±0.73	2.05 ±0.82	9.12 ±1.04
20:5?	17.15 ±14.91	37.28 ±89.60	27.14 ±16.75	48.67 ±17.21	2.82 ±3.27	3.08 ±0.97	40.84 ±7.69
22:6	296.88 ±253.79	408.82 ±448.00	444.93 ±324.81	425.23 ±201.53	65.20 ±80.15	425.62 ±181.78	388.79 ±236.85
22:4?	0.18 ±0.76	9.44 ±9.70	4.09 ±6.85	1.52 ±3.29	0.12 ±0.27	*n. d.*	*n. d.*
22:4?	12.46 ±14.31	19.10 ±18.13	27.04 ±39.10	97.13 ±177.87	2.02 ±3.10	9.52 ±1.95	32.10 ±6.10
22:3	23.55 ±21.90	31.84 ±35.79	29.93 ±27.50	73.42 ±27.97	3.22 ±5.12	28.63 ±11.18	49.59 ±18.72
22:2	*n. d.*	*n. d.*	*n. d.*	34.20 ±49.38	*n. d.*	*n. d.*	*n. d.*
22:2	*n. d.*	*n. d.*	*n. d.*	52.62 ±53.36	*n. d.*	*n. d.*	*n. d.*
22:1	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*
22:1	11.23 ±5.82	48.67 ±24.11	10.84 ±8.56	5.68 ±5.74	6.54 ±6.37	5.58 ±0.73	9.88 ±2.43
22:1	24.03 ±11.74	48.48 ±26.90	32.30 ±21.94	11.70 ±8.17	9.89 ±5.43	12.23 ±5.76	18.92 ±5.66
22:1	129.63 ±69.74	130.15 ±68.53	201.88 ±144.25	513.45 ±365.92	9.14 ±6.33	90.81 ±106.74	159.13 ±151.58
22:1	7.30 ±3.28	15.43 ±15.67	15.37 ±19.68	11.35 ±9.91	7.24 ±6.65	83.72 ±108.91	57.37 ±68.58
22:1	6.52 ±5.76	*n. d.*	35.50 ±91.14	164.90 ±376.82	0.61 ±0.83	*n. d.*	*n. d.*
22:0	7.83 ±5.24	13.65 ±11.65	24.53 ±50.58	8.65 ±9.61	3.10 ±2.69	3.98 ±4.26	21.49 ±7.57
23:2	*n. d.*	*n. d.*	0.43 ±1.37	8.74 ±7.24	*n. d.*	*n. d.*	*n. d.*
23:2	0.62 ±1.03	2.32 ±5.88	3.11 ±6.93	9.10 ±6.95	0.09 ±0.20	5.16 ±1.38	5.21 ±0.73
23:1	442.24 ±316.77	534.19 ±272.74	373.58 ±172.24	278.53 ±89.05	120.12 ±56.81	153.01 ±6.93	233.30 ±98.24
23:1	9.53 ±9.73	54.89 ±29.49	60.31 ±35.17	61.30 ±27.37	2.35 ±2.11	38.55 ±12.88	14.02 ±13.82
23:1	10.59 ±9.22	17.49 ±11.66	25.64 ±15.20	56.90 ±22.30	1.73 ±0.83	3.67 ±5.19	24.95 ±14.66
23.0	1.99 ±2.19	8.33 ±4.69	6.57 ±7.56	5.22 ±3.26	1.08 ±0.81	2.56 ±0.01	8.39 ±8.87
24:2	*n. d.*	*n. d.*	0.78 ±2.23	9.08 ±9.31	*n. d.*	*n. d.*	*n. d.*
24:1	87.48 ±52.26	324.21 ±173.79	289.21 ±148.36	298.49 ±103.42	29.77 ±11.27	231.15 ±11.60	80.90 ±3.27
24:0	1.90 ±2.42	5.24 ±5.66	1.18 ±2.75	1.16 ±2.08	0.57 ±0.66	3.20 ±0.13	2.04 ±3.53
25:0	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*

Table 4.2. Fatty acid composition (µg/g dry weight) of holothurians collected at the West Antarctica Peninsula.

	Bathy[1] (n=2)	Mmus (n=3)	SGI (n=1)	Pen (n=3)
14:0	174.49 ±127.22	13.96 ±1.85	7.46	3.89 ±5.49
14:0	200.23 ±180.94	27.57 ±6.13	46.31	190.94 ±77.20
15:1	128.81 ±177.80	n. d.	n.d	n. d.
i-15:0	1381.54 ±654.01	63.24 ±12.56	46.90	144.34 ±67.37
a-15:0	684.02 ±336.66	67.11 ±5.92	41.43	44.26 ±28.54
15:0	25.25 ±22.27	8.85 ±1.45	12.46	29.12 ±14.26
16.0	225.49 ±92.90	29.80 ±3.85	56.61	74.24 ±38.82
16.0	n. d.	n. d.	n.d	n. d.
16:1	340.31 ±116.95	33.92 ±10.79	n.d	94.42 ±44.19
16:1(n-7)	694.81 ±235.99	263.39 ±69.68	202.31	1036.24 ±552.84
16:1	183.64 ±85.61	31.31 ±21.90	25.60	18.10 ±25.59
16:1	659.46 ±336.43	52.11 ±43.43	71.30	218.81 ±113.70
16:0	411.46 ±235.79	108.09 ±19.40	220.67	536.13 ±256.48
17:0	n. d.	2.70 ±2.34	n.d.	n. d.
17:1	62.63 ±53.09	n. d.	n.d.	n. d.
17:1	65.97 ±4.79	n. d.	n.d.	n. d.
i-17:0	400.94 ±129.84	57.20 ±14.87	59.81	107.50 ±44.47
a-17:0	134.80 ±41.39	46.18 ±6.05	25.83	56.61 ±27.04
17:1	n. d.	21.75 ±3.09	n.d.	n. d.
17:1	139.37 ±60.14	19.50 ±2.57	n.d.	29.50 ±17.79
17:0	204.46 ±102.07	23.46 ±6.75	53.31	76.43 ±26.96
18:2	n. d.	33.27 ±21.29	n.d.	n. d.
18:3(?)	31.68 ±13.86	n. d.	20.75	68.52 ±18.90
18:0	37.18 ±21.73	2.22 ±3.84	10.26	27.98 ±12.18
18:2	42.54 ±25.92	13.53 ±3.47	n.d.	n. d.
18:0	117.38 ±73.30	40.41 ±10.06	19.15	n. d.
18:2	n. d.	n. d.	19.64	39.21 ±6.36
18:1	308.33 ±33.33	237.46 ±31.87	192.54	530.93 ±250.78
18:1	2485.53 ±796.45	310.03 ±73.04	428.36	734.44 ±275.31
18:1	155.55 ±60.31	14.03 ±6.92	25.43	57.42 ±25.41
18:1	318.81 ±68.69	9.28 ±2.99	31.94	83.34 ±26.80
18:1	28.30 ±9.48	n. d.	6.89	32.02 ±9.12
18:0	551.41 ±219.19	49.35 ±19.80	226.28	310.33 ±61.44
19:1	51.05 ±72.20	175.79 ±24.20	30.98	n. d.
19:0	n. d.	75.75 ±12.51	13.82	n. d.
19:1	85.30 ±47.11	2.81 ±4.87	n.d.	n. d.
19:0	36.58 ±49.51	3.15 ±5.45	n.d.	n. d.
19:0	61.51 ±17.58	45.52 ±13.50	n.d.	n. d.
19:1	114.56 ±36.06	7.93 ±2.08	36.86	25.76 ±10.33
19:1	69.66 ±39.91	5.42 ±5.98	10.81	26.09 ±12.15
19:1	41.38 ±21.66	8.22 ±2.01	20.29	33.39 ±9.59
19:0	185.65 ±38.45	18.03 ±10.90	36.81	44.97 ±8.99
20:4(n-6)	2648.54 ±944.22	n. d.	2162.68	1518.14 ±505.90
20:1	n. d.	54.20 ±29.19	n.d.	n. d.
20:5(n-3)	5067.30 ±1027.86	n. d.	2247.20	3459.88 ±837.70
20:3	31.14 ±8.08	n. d.	15.89	24.01 ±5.70
20:1	n. d.	29.32 ±19.34	n.d.	n. d.
20:1	n. d.	25.02 ±8.05	n.d.	n. d.
20:2	n. d.	19.69 ±6.86	14.00	29.81 ±10.85
20:0	21.00 ±29.71	n. d.	n.d.	3.42 ±4.83

[1]Short hand notation for holothurians collected in the WAP; Bathy= *Bathyplotes* sp.; Mmus=*Molpadia musculus*; SGI=*Scotoplanes globosa*; Pen=*Peniagone* sp.

Table 4.2. Continued…

	Bathy (n=2)	Mmus (n=3)	SGI (n=1)	Pen (n=3)
20:2	74.70 ±62.69	34.03 ±4.96	*n.d.*	7.81 ±11.04
20:0	21.15 ±29.91	*n. d.*	*n.d.*	*n. d.*
20:2	226.00 ±83.87	7.38 ±6.46	67.88	78.36 ±21.70
20:1	630.51 ±131.31	53.75 ±11.86	13.37	59.40 ±33.16
20:1	364.41 ±62.79	48.55 ±5.49	26.00	44.65 ±18.60
20:1	11.86 ±4.10	40.61 ±12.99	*n.d.*	*n. d.*
20:1	275.45 ±145.00	13.53 ±4.35	46.40	136.02 ±48.88
20:1	*n. d.*	*n. d.*	*n.d.*	*n. d.*
20:0	115.08 ±17.03	34.93 ±23.63	49.00	44.51 ±14.09
21:4	88.55 ±25.01	*n. d.*	93.18	86.64 ±31.12
21:1	21.50 ±30.41	*n. d.*	10.46	19.99 ±12.23
21:1	44.16 ±62.45	23.56 ±16.61	*n.d.*	*n. d.*
21:1	*n. d.*	*n. d.*	*n.d.*	*n. d.*
21:0	*n. d.*	*n. d.*	*n.d.*	*n. d.*
21:2	5.88 ±8.31	4.72 ±4.09	*n.d.*	*n. d.*
21:1	*n. d.*	*n. d.*	*n.d.*	15.66 ±5.99
21:1	61.83 ±39.28	13.21 ±22.87	5.59	*n. d.*
21:1	75.33 ±52.66	21.74 ±19.02	18.88	56.02 ±15.87
21:1	12.63 ±2.54	4.34 ±7.53	11.65	17.65 ±4.14
21:0	74.27 ±60.91	33.62 ±22.70	22.25	42.03 ±16.84
20:5?	214.76 ±23.37	2.20 ±3.82	22.04	97.88 ±43.37
22:6	4192.04 ±1230.78	2.48 ±4.30	1069.95	2776.70 ±932.05
22:4?	*n. d.*	*n. d.*	2.85	*n. d.*
22:4?	75.45 ±22.88	*n. d.*	6.48	31.21 ±11.26
22:3	368.18 ±104.39	*n. d.*	42.89	111.37 ±50.58
22:2	*n. d.*	*n. d.*	*n.d.*	*n. d.*
22:2	*n. d.*	*n. d.*	*n.d.*	*n. d.*
22:1	24.50 ±3.26	*n. d.*	*n.d.*	*n. d.*
22:1	128.73 ±22.71	33.94 ±13.10	14.28	44.25 ±20.01
22:1	18.93 ±2.31	39.79 ±8.45	13.19	17.19 ±3.49
22:1	515.09 ±139.77	294.04 ±29.31	396.17	373.59 ±53.71
22:1	17.20 ±10.84	5.89 ±5.30	7.63	7.44 ±2.19
22:1	*n. d.*	7.24 ±3.88	*n.d.*	*n. d.*
22:0	42.48 ±3.40	22.54 ±8.71	30.33	17.21 ±4.95
23:2	*n. d.*	*n. d.*	*n.d.*	*n. d.*
23:2	17.57 ±11.08	*n. d.*	5.05	6.08 ±2.07
23:1	499.69 ±210.98	121.16 ±206.53	231.53	145.20 ±51.86
23:1	30.84 ±3.27	102.55 ±155.05	39.08	21.98 ±5.80
23:1	18.71 ±7.67	8.91 ±7.90	15.45	10.03 ±5.49
23.0	22.07 ±14.71	9.42 ±1.79	53.92	16.36 ±23.14
24:2	28.01 ±25.90	*n. d.*	*n.d.*	7.02 ±9.92
24:1	337.14 ±128.80	52.29 ±36.86	752.13	419.85 ±121.39
24:0	7.15 ±1.64	4.56 ±1.71	14.14	3.30 ±1.45
25:0	*n. d.*	*n. d.*	*n.d.*	*n. d.*

Table 4.3. Hydroxyacid composition (µg/g dry weight) of holothurians collected at the Porcupine Abyssal Plain

	PLg(n=18)	OMt (n=28)	PsV (n=22)	PPr (n=11)	DmV (n=5)	AmR (n=2x20pooled)	Mbl (n=3)
HAc17:0[1]	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	1.98 ±*3.42*
HAc17:0	*n. d.*	1.09 ±*3.14*	1.49 ±*3.05*	*n. d.*	0.45 ±*0.53*	*n. d.*	5.80 ±*3.49*
HAc18:0	18.67 ±*9.01*	27.52 ±*17.00*	19.46 ±*12.63*	*n. d.*	4.58 ±*5.41*	14.38 ±*20.34*	32.87 ±*24.37*
HAc19:0	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	43.49 ±*70.63*
HAc22:1	2.64 ±*5.04*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*
HAc22:1	15.36 ±*11.22*	31.59 ±*22.34*	36.17 ±*36.88*	118.81 ±*71.66*	5.74 ±*5.92*	3.15 ±*4.45*	35.37 ±*18.50*
HAc22:0	2.30 ±*2.23*	5.78 ±*6.44*	10.47 ±*10.52*	30.20 ±*22.52*	0.10 ±*0.22*	5.24 ±*7.41*	9.75 ±*9.71*
HAc23:1	27.33 ±*20.85*	34.71 ±*25.73*	49.03 ±*47.30*	67.79 ±*43.65*	12.51 ±*10.53*	9.39 ±*13.27*	16.84 ±*13.75*
HAc23:1	15.64 ±*11.19*	20.06 ±*13.83*	37.21 ±*32.90*	39.12 ±*26.95*	3.89 ±*3.72*	3.51 ±*4.96*	12.07 ±*10.53*
HAc23:0	*n. d.*	*n. d.*	1.99 ±*7.23*	*n. d.*	*n. d.*	*n. d.*	9.96 ±*14.44*
HAc24:1	565.04 ±*349.43*	350.37 ±*244.39*	329.04 ±*265.96*	300.82 ±*167.06*	110.52 ±*88.79*	77.23 ±*62.35*	114.80 ±*84.75*
HAc24:1	*n. d.*	*n. d.*	1.15 ±*3.54*	8.77 ±*9.43*	*n. d.*	*n. d.*	8.36 ±*12.62*
HAc24:0	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	5.07 ±*5.84*

[1]Short hand notation for hydroxy-acids: HAc(number of carbons):(number of unsaturations).

Table 4.4. Hydroxyacid composition (µg/g dry weight) of holothurians collected at the West Antarctica Peninsula

	Bathy (n=2)	Mmus (n=3)	SGI (n=1)	Pen (n=3)
HAc17:0	*n. d.*	*n. d.*	*n.d.*	*n. d.*
HAc17:0	13.10 ±*18.53*	7.76 ±*2.61*	*n.d.*	8.27 ±*11.69*
HAc18:0	17.49 ±*24.73*	13.60 ±*13.81*	*n.d.*	10.12 ±*14.31*
HAc19:0	*n. d.*	*n. d.*	*n.d.*	*n. d.*
HAc22:1	29.14 ±*41.21*	*n. d.*	*n.d.*	*n. d.*
HAc22:1	*n. d.*	8.26 ±*2.60*	19.20	34.47 ±*3.66*
HAc22:0	8.44 ±*7.93*	11.41 ±*2.87*	22.26	5.11 ±*1.53*
HAc23:1	31.01 ±*37.93*	6.08 ±*2.77*	37.44	49.22 ±*7.18*
HAc23:1	4.35 ±*6.15*	3.37 ±*1.27*	7.98	15.98 ±*1.69*
HAc23:0	*n. d.*	*n. d.*	*n.d.*	*n. d.*
HAc24:1	166.68 ±*201.11*	60.89 ±*22.96*	222.58	500.47 ±*53.17*
HAc24:1	*n. d.*	1.01 ±*1.75*	*n.d.*	*n. d.*
HAc24:0	*n. d.*	11.05 ±*5.09*	*n.d.*	*n. d.*
Hac25:1	*n. d.*	*n. d.*	*n.d.*	*n. d.*

Table 4.5. Sterol composition (µg/g dry weight) of holothurians collected at the Porcupine Abyssal Plain

	PLg(n=18)	OMt (n=28)	PsV (n=22)	PPr (n=11)	DmV (n=5)	AmR (n=2x20pooled)	Mbl (n=3)
$C_{26}\Delta^{0}$ (**1**)[1]	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	22.58 ±*23.50*
$C_{28}\Delta^{X,Y}$	*n. d.*	0.07 ±*0.38*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*
$C_{26}\Delta^{22}$	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*
$C_{27}\Delta^{5,22}$ (**2** or **3**)	16.13 ±*13.62*	19.04 ±*12.07*	29.64 ±*21.38*	21.48 ±*11.37*	2.73 ±*2.42*	12.14 ±*2.71*	4.44 ±*1.72*
$C_{27}\Delta^{22}$ (**4**)	24.32 ±*22.20*	4.93 ±*3.95*	11.82 ±*10.55*	11.55 ±*4.45*	2.64 ±*2.21*	8.47 ±*2.33*	101.08 ±*40.34*
$C_{27}\Delta^{5,22}$ (**2** or **3**)	58.44 ±*53.08*	30.20 ±*20.90*	33.60 ±*25.27*	17.71 ±*9.35*	4.68 ±*4.38*	33.82 ±*11.49*	16.60 ±*12.06*
$C_{27}\Delta^{22}$ (**4**)	68.76 ±*60.58*	9.90 ±*10.73*	13.23 ±*9.22*	13.84 ±*7.29*	8.03 ±*7.15*	11.95 ±*1.14*	159.26 ±*95.42*
$C_{27}\Delta^{x}$?	6.75 ±*9.13*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	2.22 ±*3.14*	*n. d.*
$C_{27}\Delta^{5}$ (**5**)	268.57 ±*268.89*	290.62 ±*185.92*	122.54 ±*87.76*	121.03 ±*65.17*	75.80 ±*87.21*	241.80 ±*9.16*	65.71 ±*23.43*
$C_{27}\Delta^{7,22}$?	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	16.03 ±*22.67*	*n. d.*
$C_{27}\Delta^{0}$ (**6**)	456.71 ±*413.16*	150.89 ±*80.84*	73.47 ±*43.16*	102.06 ±*49.82*	85.46 ±*56.68*	82.76 ±*8.19*	363.13 ±*201.43*
$C_{27}\Delta^{0}$ or $C_{27}\Delta^{7,22}$	20.09 ±*14.65*	16.01 ±*14.10*	8.38 ±*9.96*	10.68 ±*5.66*	3.19 ±*3.96*	30.87 ±*18.75*	35.48 ±*16.87*
4,4dimet-$C_{29}\Delta^{X}$	8.71 ±*17.18*	27.13 ±*32.49*	9.63 ±*12.55*	10.96 ±*11.93*	0.81 ±*1.81*	17.72 ±*3.71*	3.82 ±*6.62*
$C_{28}\Delta^{5,22}$ (**8** or **9**)	60.69 ±*45.42*	166.72 ±*100.06*	229.30 ±*190.53*	80.15 ±*33.09*	13.57 ±*7.85*	129.79 ±*64.39*	88.42 ±*55.05*
$C_{28}\Delta^{22}$ (**10**)	158.46 ±*201.87*	68.73 ±*30.42*	69.78 ±*46.96*	58.57 ±*28.47*	18.63 ±*15.78*	35.23 ±*11.51*	312.77 ±*129.82*
$C_{27}\Delta^{7}$ (**7**)	18.58 ±*27.69*	68.44 ±*93.99*	14.60 ±*14.78*	9.98 ±*9.27*	6.17 ±*9.36*	46.17 ±*50.31*	11.23 ±*11.33*
$C_{28}\Delta^{5,23?}$	23.66 ±*32.53*	7.39 ±*11.93*	21.84 ±*32.11*	15.38 ±*13.69*	0.46 ±*1.03*	2.98 ±*4.22*	*n. d.*
$C_{28}\Delta^{5,23?}$	123.65 ±*98.26*	119.92 ±*103.37*	128.29 ±*102.16*	59.89 ±*48.62*	16.00 ±*13.40*	68.03 ±*56.99*	100.28 ±*29.80*
$C_{28}\Delta^{0}$ (**14**)	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	46.23 ±*15.37*	*n. d.*
$C_{28}\Delta^{5}$ (**13**)	235.56 ±*201.57*	185.08 ±*106.50*	195.41 ±*155.84*	139.21 ±*75.47*	19.18 ±*18.27*	42.57 ±*60.20*	153.21 ±*47.30*
$C_{28}\Delta^{0}$ (**14**)	172.15 ±*144.47*	48.45 ±*24.59*	69.54 ±*60.66*	50.31 ±*32.92*	17.16 ±*15.64*	5.68 ±*8.03*	134.43 ±*54.88*
$C_{29}\Delta^{5,22}$ (**17**)	71.26 ±*69.53*	145.73 ±*72.63*	144.68 ±*131.28*	73.97 ±*37.42*	14.92 ±*15.22*	83.08 ±*37.45*	114.86 ±*60.14*
$C_{29}\Delta^{22}$ (**18**)	33.95 ±*43.10*	14.97 ±*8.67*	19.79 ±*21.87*	17.64 ±*11.39*	4.13 ±*4.18*	27.49 ±*20.12*	110.97 ±*74.09*
$C_{28}\Delta^{5,7}$ (**16**)	*n. d.*	10.29 ±*15.17*	12.23 ±*19.89*	*n. d.*	*n. d.*	8.96 ±*1.57*	*n. d.*
$C_{28}\Delta^{7}$ (**15**)	*n. d.*	19.47 ±*33.87*	18.26 ±*50.30*	*n. d.*	*n. d.*	*n. d.*	*n. d.*
4met-$C_{29}\Delta^{5,22}$	72.01 ±*83.75*	12.52 ±*24.69*	24.38 ±*38.58*	14.76 ±*19.67*	4.43 ±*6.09*	7.88 ±*11.14*	44.66 ±*77.36*
$C_{29}\Delta^{24(24')}$ (**21**)	37.79 ±*52.49*	20.18 ±*25.87*	21.26 ±*27.67*	38.90 ±*49.21*	0.69 ±*1.54*	*n. d.*	105.70 ±*17.05*
$C_{29}\Delta^{5}$?	29.95 ±*36.34*	70.90 ±*40.93*	121.23 ±*142.61*	84.37 ±*74.14*	3.74 ±*5.24*	31.36 ±*16.74*	82.92 ±*75.41*
$C_{29}\Delta^{5}$ (**19**)	1262.34 ±*1105.46*	2076.75 ±*1302.13*	1458.09 ±*1145.94*	1057.04 ±*552.83*	281.65 ±*216.49*	670.83 ±*260.12*	919.08 ±*236.30*
$C_{29}\Delta^{0}$ (**20**)	1893.27 ±*1815.45*	429.53 ±*170.53*	571.11 ±*482.23*	583.07 ±*354.57*	183.39 ±*111.98*	97.79 ±*35.51*	2269.04 ±*1110.84*
$C_{29}\Delta^{5,24(25)?}$ (**23**)	*n. d.*	9.21 ±*18.53*	*n. d.*	*n. d.*	5.15 ±*7.38*	26.66 ±*16.37*	*n. d.*

[1]Short hand notation for sterols: $C_{(\text{number of carbons})}\ \Delta^{(\text{Positions of unsaturations})}$; x,y and z refers to non-identified position of the double bonds.

Table 4.5. Continued…

	PLg(n=18)	OMt (n=28)	PsV (n=22)	PPr (n=11)	DmV (n=5)	AmR (n=2x20pooled)	Mbl (n=3)
$C_{29}\Delta^{24(24')}$ (**24**)	20.27 ±19.34	24.70 ±32.33	27.13 ±48.72	11.86 ±9.50	*n. d.*	*n. d.*	113.38 ±72.49
$C_{29}\Delta^{5,24}$ (**24**)	14.09 ±17.84	25.24 ±22.61	49.77 ±40.54	30.63 ±17.63	3.10 ±3.91	9.55 ±0.95	9.87 ±8.63
$C_{30}\Delta^{5,22}$	*n. d.*	*n. d.*	11.04 ±20.44	20.72 ±23.76	*n. d.*	11.05 ±1.23	*n. d.*
$C_{29}\Delta^{24(24')}$ (**22**)	26.24 ±39.21	*n. d.*	2.81 ±10.57	7.27 ±11.70	*n. d.*	*n. d.*	128.66 ±14.88
$C_{30}\Delta^{x,y,z}$	3.36 ±8.62	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*
$C_{29}\Delta^{7}$ (**25**)	35.76 ±41.97	260.78 ±297.65	93.16 ±104.80	53.35 ±46.45	63.47 ±86.83	28.42 ±22.68	135.14 ±41.46
$C_{30}\Delta$?	5.17 ±8.27	2.70 ±6.17	*n. d.*	12.92 ±13.15	0.81 ±1.11	4.15 ±1.22	19.94 ±8.49
$C_{30}\Delta$?	4.04 ±9.23	0.96 ±3.10	0.10 ±0.46	7.09 ±9.67	*n. d.*	*n. d.*	11.55 ±8.18
$C_{30}\Delta$?	4.14 ±11.25	*n. d.*	0.20 ±0.93	*n. d.*	*n. d.*	*n. d.*	9.08 ±13.41

Table 4.6. Sterol composition (µg/g dry weight) of holothurians collected at the West Antarctica Peninsula

	Bathy (n=2)	Mmus (n=3)	SGl (n=1)	Pen (n=3)
$C_{26}\Delta^{0}$ (**1**)	*n. d.*	*n. d.*	*n.d*	*n. d.*
$C_{28}\Delta^{X,Y}$	*n. d.*	*n. d.*	*n.d*	*n. d.*
$C_{26}\Delta^{22}$	*n. d.*	84.80 ±9.80	*n.d*	*n. d.*
$C_{27}\Delta^{5,22}$ (**2** or **3**)	5.02 ±7.10	21.56 ±2.53	7.49	11.62 ±0.88
$C_{27}\Delta^{22}$ (**4**)	2.41 ±3.41	75.72 ±17.85	4.83	5.41 ±0.30
$C_{27}\Delta^{5,22}$ (**2** or **3**)	9.52 ±13.47	36.21 ±10.88	41.43	39.77 ±0.65
$C_{27}\Delta^{22}$ (**4**)	5.02 ±7.11	223.37 ±65.75	18.06	7.26 ±0.73
$C_{27}\Delta^{x}$?	*n. d.*	*n. d.*	*n.d*	1.19 ±1.68
$C_{27}\Delta^{5}$ (**5**)	52.73 ±74.58	139.38 ±46.61	247.54	407.55 ±28.28
$C_{27}\Delta^{7,22}$?	*n. d.*	*n. d.*	*n.d*	*n. d.*
$C_{27}\Delta^{0}$ (**6**)	32.14 ±45.45	467.16 ±71.90	54.08	109.64 ±9.95
$C_{27}\Delta^{0}$ and $C_{27}\Delta^{7,22}$	3.11 ±4.41	37.73 ±18.76	17.25	37.00 ±2.25
4,4dimet-$C_{29}\Delta^{X}$	*n. d.*	*n. d.*	*n.d*	*n. d.*
$C_{28}\Delta^{5,22}$ (**8** or **9**)	120.90 ±170.97	323.87 ±107.61	158.75	96.86 ±9.31
$C_{28}\Delta^{22}$ (**10**)	55.05 ±77.86	920.25 ±175.71	55.28	306.71 ±46.16
$C_{27}\Delta^{7}$ (**7**)	49.75 ±70.36	47.74 ±26.49	29.31	53.13 ±3.78
$C_{28}\Delta^{5,23?}$	*n. d.*	*n. d.*	*n.d*	*n. d.*
$C_{28}\Delta^{5,23?}$	4.47 ±6.33	316.15 ±131.44	55.05	30.99 ±0.42

Table 4.6. Continued…

	Bathy (n=2)	Mmus (n=3)	SGI (n=1)	Pen (n=3)
$C_{28}\Delta^{0}$ (**14**)	*n. d.*	*n. d.*	*n.d*	*n. d.*
$C_{28}\Delta^{5}$ (**13**)	13.25 ±*18.74*	361.90 ±*64.98*	23.32	13.94 ±*19.72*
$C_{28}\Delta^{0}$ (**14**)	9.40 ±*13.29*	257.79 ±*100.06*	*n.d*	10.92 ±*15.44*
$C_{29}\Delta^{5,22}$ (**17**)	10.16 ±*14.37*	47.44 ±*15.88*	49.20	24.64 ±*3.60*
$C_{29}\Delta^{22}$ (**18**)	9.81 ±*13.87*	95.13 ±*12.26*	55.49	56.38 ±*3.48*
$C_{28}\Delta^{5,7}$ (**16**)	3.23 ±*4.57*	*n. d.*	22.67	8.08 ±*11.42*
$C_{28}\Delta^{7}$ (**15**)	*n. d.*	118.03 ±*60.95*	*n.d.*	*n. d.*
4met-$C_{29}\Delta^{5,22}$	11.84 ±*16.74*	*n. d.*	22.27	*n. d.*
$C_{29}\Delta^{24(24')}$ (**21**)	*n. d.*	91.59 ±*16.39*	*n.d.*	11.02 ±*15.58*
$C_{29}\Delta^{5}$?	23.92 ±*33.83*	*n. d.*	31.57	48.09 ±*0.52*
$C_{29}\Delta^{5}$ (**19**)	142.99 ±*202.22*	480.98 ±*126.56*	412.38	324.79 ±*7.91*
	Bathy (n=2)	Mmus (n=3)	SGI (n=1)	Pen (n=3)
$C_{29}\Delta^{0}$ (**20**)	79.52 ±*112.46*	711.81 ±*179.36*	98.40	261.02 ±*6.94*
$C_{29}\Delta^{5,24(25)?}$ (**23**)	*n. d.*	*n. d.*	9.20	13.34 ±*0.27*
$C_{29}\Delta^{24(24')}$ (**24**)	6.01 ±*8.51*	44.15 ±*19.39*	*n.d.*	*n. d.*
$C_{29}\Delta^{5,24}$ (**24**)	11.37 ±*16.08*	23.28 ±*26.13*	26.82	31.91 ±*4.88*
$C_{30}\Delta^{5,22}$	14.13 ±*19.98*	*n. d.*	*n.d.*	49.86 ±*1.35*
$C_{29}\Delta^{24(24')}$ (**22**)	*n. d.*	125.15 ±*70.29*	*n.d.*	*n. d.*
$C_{30}\Delta^{x,y,z}$	*n. d.*	*n. d.*	31.57	*n. d.*
$C_{29}\Delta^{7}$ (**25**)	26.07 ±*36.88*	126.34 ±*61.99*	34.87	46.70 ±*8.49*
$C_{30}\Delta$?	3.31 ±*4.68*	0.99 ±*1.71*	*n.d.*	85.28 ±*19.06*
$C_{30}\Delta$?	8.12 ±*11.48*	4.94 ±*5.19*	*n.d.*	*n. d.*
$C_{30}\Delta$?	1.72 ±*2.44*	9.91 ±*8.94*	*n.d.*	*n. d.*

Table 4.7. Steryl sulphate composition (µg/g dry weight) of holothurians collected at the Porcupine Abyssal Plain.

	PLg(n=18)	OMt (n=28)	PsV (n=22)	PPr (n=11)	DmV (n=5)	AmR (n=2x20pooled)	Mbl (n=3)
$C_{27}\Delta^{x,y,z}$	*n. d.*	*n. d.*	16.33 *±53.15*	2.25 *±6.72*	*n. d.*	9.72 *±6.55*	*n. d.*
$C_{27}\Delta^{7}$ [1]	2.72 *±10.89*	1.35 *±5.17*	4.56 *±19.87*	7.15 *±13.02*	*n. d.*	3.79 *±1.35*	*n.*
$C_{27}\Delta^{5}$	18.24 *±26.38*	57.97 *±114.48*	61.25 *±80.95*	25.55 *±13.86*	10.09 *±22.55*	26.16 *±14.35*	*n. d.*
$C_{28}\Delta^{x,y,z}$	*n. d.*	2.52 *±8.29*	*n. d.*	3.03 *±9.09*	*n. d.*	*n. d.*	*n. d.*
$C_{28}\Delta^{x,y,z}$	*n. d.*	2.38 *±7.87*	*n. d.*	*n. d.*	*n. d.*	7.10 *±10.04*	*n. d.*
$C_{28}\Delta^{5,22}$?	8.41 *±17.06*	34.70 *±38.69*	70.73 *±46.92*	38.72 *±31.18*	*n. d.*	8.58 *±12.14*	*n. d.*
$C_{28}\Delta^{5,22}$??	*n. d.*	5.28 *±18.59*	*n. d.*	*n. d.*	1.44 *±3.23*	20.11 *±7.47*	*n. d.*
$C_{28}\Delta^{X}$	9.59 *±15.90*	12.15 *±14.64*	12.81 *±21.85*	22.55 *±16.08*	*n. d.*	11.91 *±10.63*	*n. d.*
$C_{28}\Delta^{y}$	*n. d.*	1.27 *±5.04*	33.68 *±106.65*	4.71 *±5.92*	*n. d.*	*n. d.*	*n. d.*
$C_{28}\Delta^{z}$	28.29 *±41.81*	51.87 *±62.42*	80.74 *±66.45*	50.29 *±24.45*	8.24 *±18.41*	13.57 *±9.87*	10.95 *±9.63*
$C_{29}\Delta^{x,y,z}$	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	29.13 *±29.14*	*n. d.*
$C_{29}\Delta^{x,y,z}$	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	10.16 *±1.00*	*n. d.*
$C_{29}\Delta^{5,22}$?	*n. d.*	*n. d.*	*n. d.*	*n. d.*	*n. d.*	19.52 *±6.43*	31.09 *±26.96*
$C_{29}\Delta^{5,22}$?	24.26 *±52.94*	30.77 *±61.80*	137.35 *±84.15*	79.01 *±36.88*	*n. d.*	44.42 *±26.45*	16.35 *±14.18*
$C_{29}\Delta^{7}$	7.24 *±12.38*	73.04 *±71.56*	88.13 *±52.01*	62.25 *±46.32*	5.00 *±6.52*	16.23 *±9.75*	18.28 *±2.57*
$C_{29}\Delta^{5}$	973.06 *±1100.45*	718.52 *±855.86*	498.60 *±372.53*	413.47 *±196.31*	26.14 *±24.22*	133.31 *±56.73*	416.11 *±46.84*
$C_{30}\Delta x,y$	*n. d.*	*n. d.*	8.35 *±32.50*	*n. d.*	*n. d.*	*n. d.*	*n. d.*
$C_{30}\Delta x,y$?	*n. d.*	*n. d.*	11.22 *±48.92*	*n. d.*	*n. d.*	*n. d.*	*n. d.*

[1]For structure numbers, see analogue free sterols in table 4.5.

Table 4.8. Steryl sulphate composition (µg/g dry weight) of holothurians collected at the West Antarctica Peninsula

	Bathy (n=2)	Mmus (n=3)	SGI (n=1)	Pen (n=3)
$C_{27}\Delta^{x,y,z}$ [1]	*n. d.*	*n. d.*	*n.d.*	*n. d.*
$C_{27}\Delta^{7}$	4.81 *±6.80*	1.76 *±3.05*	12.62	8.09 *±3.58*
$C_{27}\Delta^{5}$	26.58 *±9.43*	11.06 *±10.94*	55.20	15.91 *±5.60*
$C_{28}\Delta^{x,y,z}$	*n. d.*	*n. d.*	*n.d.*	*n. d.*
$C_{28}\Delta^{x,y,z}$	*n. d.*	*n. d.*	61.20	*n. d.*
$C_{28}\Delta^{5,22}$?	*n. d.*	*n. d.*	*n.d.*	*n. d.*
$C_{28}\Delta^{5,22}$??	9.25 *±13.08*	26.54 *±11.45*	*n.d.*	10.09 *±14.27*
$C_{28}\Delta^{X}$	*n. d.*	*n. d.*	*n.d.*	*n. d.*

Table 4.8. Continued…

	Bathy (n=2)	Mmus (n=3)	SGl (n=1)	Pen (n=3)
$C_{28}\Delta^{y}$	*n. d.*	*n. d.*	*n.d.*	*n. d.*
$C_{28}\Delta^{z}$	15.86 ±*7.23*	17.32 ±*5.50*	17.14	*n. d.*
$C_{29}\Delta^{x,y,z}$	*n. d.*	*n. d.*	*n.d.*	*n. d.*
$C_{29}\Delta^{x,y,z}$	*n. d.*	*n. d.*	*n.d.*	*n. d.*
$C_{29}\Delta^{5,22}$?	20.02 ±*28.32*	*n. d.*	78.95	18.21 ±*25.75*
$C_{29}\Delta^{5,22}$?	8.43 ±*11.92*	8.17 ±*14.15*	37.52	39.30 ±*31.44*
$C_{29}\Delta^{7}$	15.14 ±*8.34*	10.61 ±*4.18*	19.47	17.98 ±*6.43*
$C_{29}\Delta^{5}$	88.58 ±*34.07*	77.91 ±*39.91*	199.47	64.03 ±*4.13*
$C_{30}\Delta$x,y	*n. d.*	*n. d.*	*n.d.*	*n. d.*
$C_{30}\Delta$x,y?	*n. d.*	*n. d.*	*n.d.*	*n. d.*

[1]For structure numbers, see analogue free sterols in Table 4.6.

Table 4.9. Total lipid composition (µg/g dry weight) of holothurians collected at the Porcupine Abyssal Plain.

	PLg(n=18)	Omt (n=28)	PsV (n=22)	PPr (n=11)	DmV (n=5)	AmR (n=2x20pooled)	Mbl (n=3)
Total lipids	10788.29 ±*7871.13*	10581.81 ±*5142.14*	11344.47 ±*5256.86*	12062.18 ±*4628.55*	2553.01 ±*1451.79*	6062.14 ±*1481.03*	12599.01 ±*3908.67*
Total FA	4656.50 ±*3251.43*	5554.22 ±*3165.67*	7236.17 ±*3423.56*	8756.34 ±*3862.28*	1385.02 ±*959.12*	4107.58 ±*923.46*	6599.64 ±*2127.89*
total Hydroxy-FA	646.98 ±*384.14*	471.11 ±*298.62*	486.01 ±*388.74*	565.51 ±*319.89*	137.77 ±*112.86*	112.90 ±*112.79*	296.36 ±*197.53*
Total Sterols	5234.88 ±*4675.93*	4337.44 ±*2192.62*	3606.29 ±*2630.06*	2736.40 ±*1397.43*	839.98 ±*557.69*	1841.67 ±*444.78*	5647.30 ±*1879.98*
total SaturatedFA	304.92 ±*147.43*	757.35 ±*491.63*	428.26 ±*173.82*	421.82 ±*340.32*	148.71 ±*57.37*	340.73 ±*19.48*	384.96 ±*101.29*
Total Unsat-FA	4351.58 ±*3136.31*	4796.87 ±*2888.63*	6807.91 ±*3283.57*	8334.52 ±*3608.07*	1236.32 ±*934.87*	3766.85 ±*942.94*	6214.69 ±*2028.01*
Total monounsFA	1172.15 ±*619.06*	2518.89 ±*1324.98*	2050.34 ±*867.32*	2995.45 ±*1601.79*	507.53 ±*158.73*	1229.16 ±*51.16*	1513.53 ±*470.34*
total diuns-FA	82.34 ±*56.19*	65.24 ±*47.86*	145.95 ±*70.99*	362.03 ±*194.24*	8.70 ±*12.83*	65.87 ±*1.20*	228.46 ±*106.72*
Total PUFA	3097.09 ±*2525.33*	2212.74 ±*1908.04*	4611.62 ±*2498.87*	4977.04 ±*2296.91*	720.08 ±*809.85*	2471.82 ±*992.90*	4472.70 ±*1458.49*
Bacterial compound	58.70 ±*40.53*	263.74 ±*212.33*	139.92 ±*80.40*	172.56 ±*226.87*	27.26 ±*20.50*	56.36 ±*10.49*	79.73 ±*5.94*
Sterols total-C_{27}	938.35 ±*831.71*	590.03 ±*342.63*	307.28 ±*183.05*	308.34 ±*148.87*	188.70 ±*148.42*	470.20 ±*58.20*	756.93 ±*385.35*
Sterols total-C_{28}	774.17 ±*697.66*	606.58 ±*310.79*	726.38 ±*541.76*	403.50 ±*212.82*	85.00 ±*64.16*	293.24 ±*92.92*	789.11 ±*284.49*
Sterols total-C_{29}	3400.43 ±*3051.15*	2947.73 ±*1535.21*	2378.56 ±*1812.53*	1892.22 ±*1011.55*	541.27 ±*357.06*	863.56 ±*298.08*	3812.28 ±*1372.82*
Sterols total-Δ^{5}	1796.42 ±*1565.13*	2623.34 ±*1577.89*	1897.27 ±*1459.03*	1401.65 ±*712.62*	380.37 ±*280.08*	986.56 ±*346.23*	1220.92 ±*267.14*
Sterols total-Δ^{0}	2542.23 ±*2369.49*	644.88 ±*256.29*	722.49 ±*570.22*	746.12 ±*421.82*	289.20 ±*175.53*	217.10 ±*16.60*	2802.07 ±*1202.74*
Δ^{5}/Δ^{0}	0.70 ±*0.26*	3.87 ±*1.30*	2.96 ±*2.26*	1.93 ±*0.29*	1.22 ±*0.71*	4.50 ±*1.25*	0.46 ±*0.10*
total Steryl Sulfates	1008.76 ±*1179.70*	777.68 ±*1023.51*	926.25 ±*746.57*	638.08 ±*375.58*	50.90 ±*38.57*	353.72 ±*143.26*	492.77 ±*95.54*
total free sterols	4226.12 ±*3743.09*	3559.76 ±*2065.33*	2680.04 ±*2467.18*	2098.32 ±*1202.96*	789.08 ±*544.69*	1487.95 ±*301.52*	5154.54 ±*1973.46*

Table 4.10. Total lipid composition (µg/g dry weight) of holothurians collected at the West Antarctica Peninsula.

	Bathy (n=2)	Mmus (n=3)	SGI (n=1)	Pen (n=3)
Total lipids	28256.42 ±10382.29	8506.18 ±1049.61	11338.65	17142.32 ±5053.91
Total FA	27269.97 ±9068.83	3091.55 ±242.03	9522.33	14425.61 ±4937.66
total Hydroxy-FA	270.20 ±300.54	123.42 ±46.61	309.46	623.63 ±41.22
Total Sterols	715.00 ±1011.17	5193.34 ±748.19	1506.86	2093.07 ±75.03
total SaturatedFA	5135.62 ±2440.76	787.66 ±138.99	1046.75	1773.58 ±713.88
Total Unsat-FA	22134.34 ±6628.07	2303.89 ±104.93	8475.58	12652.03 ±4223.78
Total monounsFA	9000.50 ±3056.90	2186.58 ±116.82	2674.63	4289.41 ±1713.01
total diuns-FA	394.69 ±201.15	112.63 ±19.12	106.57	168.29 ±61.94
Total PUFA	12717.65 ±3400.44	4.69 ±8.12	5683.91	8174.34 ±2436.60
Bacterial compound	2601.31 ±1161.90	233.73 ±36.62	173.97	352.71 ±167.41
Sterols total-C_{27}	159.72 ±225.88	1048.87 ±166.72	420.00	672.56 ±17.22
Sterols total-C_{28}	206.31 ±291.77	2179.95 ±427.68	315.07	467.50 ±83.01
Sterols total-C_{29}	301.73 ±426.72	1603.29 ±330.96	626.32	723.52 ±27.35
Sterols total-Δ^5	232.90 ±329.37	982.25 ±175.22	714.82	794.37 ±0.13
Sterols total-Δ^0	124.17 ±175.61	1474.48 ±248.07	169.73	418.58 ±30.08
Δ^5/Δ^0	1.87 ±0.45	0.67 ±0.14	4.21	1.90 ±0.14
total Steryl Sulfates	188.69 ±78.57	153.38 ±59.46	481.55	173.60 ±4.02
total free sterols	526.32 ±932.60	5039.96 ±727.36	1025.31	1919.47 ±71.01

Table 4.11. Percentage values of TOC, TN, and TIC, and C/N ratios of holothurians collected at the Porcupine Abyssal Plain.

	PLg(n=18)	Omt (n=28)	PsV (n=22)	PPr (n=11)	DmV (n=5)	AmR (n=2x20pooled)	Mbl (n=3)
TOC	13.11 ±5.56	15.21 ±2.32	17.87 ±3.28	21.93 ±4.15	5.92 ±3.38	9.95 ±2.12	25.99 ±3.28
TN	3.84 ±1.66	4.24 ±0.84	5.27 ±1.21	7.59 ±4.14	2.02 ±0.49	2.72 ±1.01	8.21 ±0.88
TIC	3.58 ±1.50	4.34 ±1.55	3.74 ±1.43	3.99 ±1.52	8.89 ±2.67	2.58 ±0.29	6.15 ±0.19
C/N	3.50 ±0.47	3.65 ±0.45	3.43 ±0.35	3.29 ±0.83	2.93 ±1.47	3.77 ±0.62	3.16 ±0.18

Table 4.12. Percentage values of TOC, TN, and TIC, as well as C/N ratio of holothurians collected at the West Antarctic Peninsula.

	Bathy (n=2)	Mmus (n=3)	SGI (n=1)	Pen (n=3)
TOC	21.40±*0.77*	12.78±*6.28*	11.77	10.75±*0.96*
TN	7.93±*0.29*	5.97±*1.54*	5.12	3.70±*0.47*
TIC	6.71±*2.25*	13.89±*10.87*	6.63	3.11±*1.09*
C/N	2.70±*0.20*	4.22±*1.92*	2.30	3.74±*0.08*

Chapter 5

THE LIPID POOL IN HOLOTHURIANS FROM THE ANTARCTIC SHELF AND THE PORCUPINE ABYSSAL PLAIN, NORTHEAST ATLANTIC, AND ITS RELATIONSHIP TO FOOD AVAILABILITY

5.1 INTRODUCTION

Fluctuating environmental factors, such as food supply, can evoke changes in the biochemistry of organisms, which could influence biological processes (*e.g.* reproduction, Bradshaw *et al.*, 1990; Wigham *et al.*, *in prep*.; Chapter 6). However, holothurians have not received much attention to date despite being an important megafaunal class, particularly in the abyssal and hadal oceans (Billett *et al*., 1991, Ginger *et al*., 2000). Deep-sea holothurians are benthic dwellers, which depend on organic matter originating from the photic zone for their metabolic needs (Wiebe *et al*., 1976). As the quantity and quality of food arriving at sea floor depends, among many other factors, on the depth of the water column, the BBL at the PAP (~5000) and WAP (~600) certainly receive different inputs of POM.

In this Chapter, the differences in the quantity and quality of organic matter (available food; lipids) between the surficial sediments (0-5mm including the phytodetritus layer; hereafter, surficial sediments are termed sediments) from the WAP and the PAP are identified and compared with the lipids of the holothurians from the two regions in order to assess the

relationship between their diet and biochemistry of the different populations. The approach involves the identification of regional differences of specific lipid indices in sediments, and comparison with those found in the holothurians. Here, the data for the PAP are based on specimens and sediments collected in May 1999 only in order to avoid temporal differences, because the sediment was relatively richer in organic matter at that time (Mackenzie, 2000; Chapter 6), and because more specimens were available. Samples from the WAP were collected in February 2001 (Chapter 2).

5.2 RESULTS

Although the sediments from the WAP had higher contents of TOC (1.59±0.43%) relative to those from the PAP (0.43±0.04%), the TOC of the animals of both locations were within the same range (4.74-27.71% at the PAP, and 10.75-21.40% at the WAP; Fig. 5.1). *Deima validum* had the lowest values of all species (4.74±2.99 %).

TN had the same pattern as TOC (0.03±0.00% in the sediments, and 1.96-8.24% in the holothurians at the PAP, and 0.22±0.03% in the sediments, and 3.70-7.93% in the animals at the WAP; Fig. 5.2), *Deima validum* again being the species with the lowest value (1.96±0.01%). As both TOC and TN had the same distribution among species from both sites, C/N ratio values were practically the same (2.43-3.78; Fig. 5.3). On the other hand, sediments from PAP had higher C/N values (12.55±0.51)

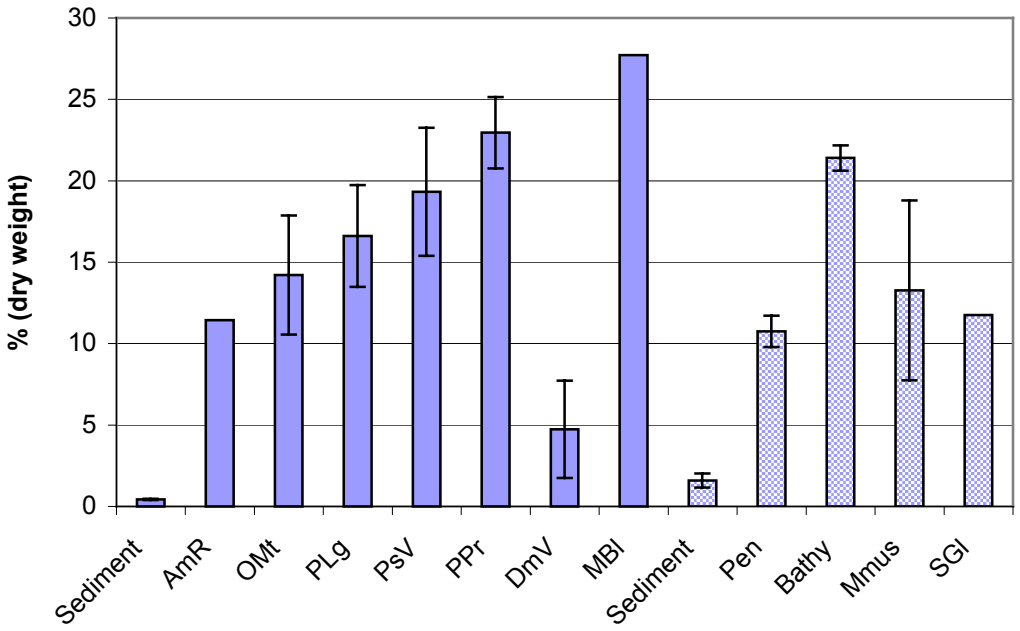


Figure 5.1. %TOC (dry weight) of samples collected at the WAP (checked bars) and PAP. Error bars represent standard deviation from mean.

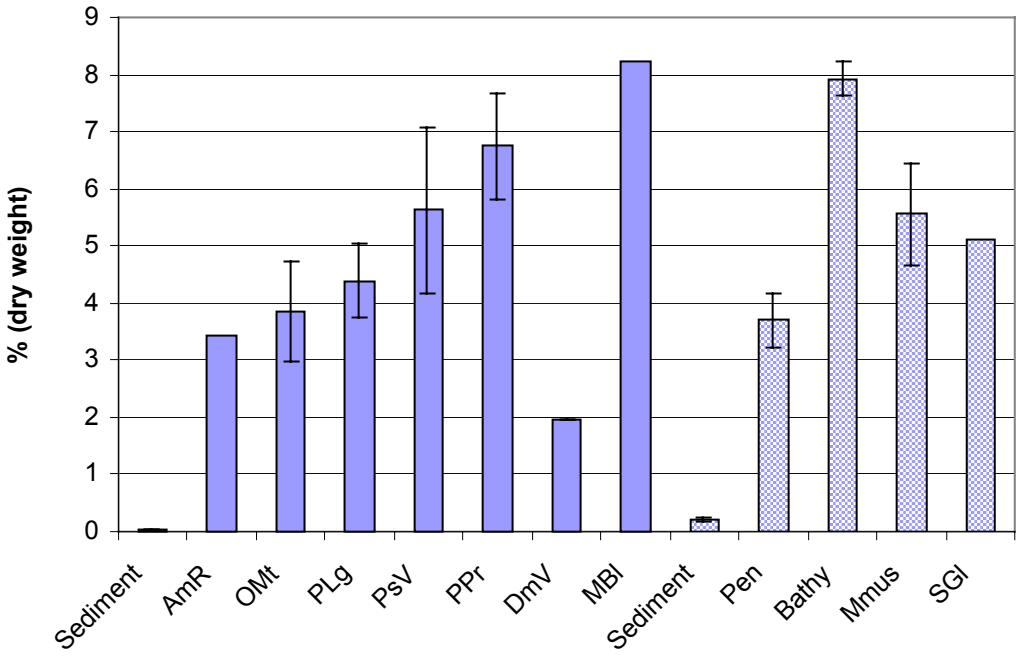


Figure 5.2. %TN (dry weight) of samples collected at the WAP (checked bars) and PAP. Error bars represent standard deviation from mean.

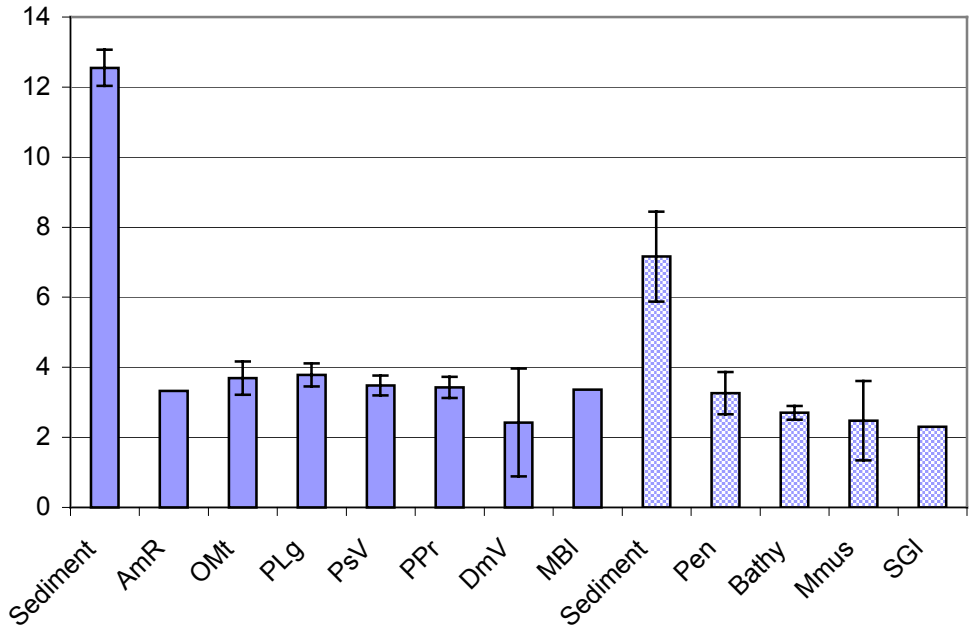


Figure 5.3. C/N (dry weight) of samples collected at the WAP (checked bars) and PAP. Error bars represent standard deviation from mean.

relative to those from the WAP (7.16±1.28; Fig. 5.3). TIC contents were similar in animals (2.38-10.13% at the PAP, and 3.11-11.33% at the WAP; Fig. 5.4), but sedimentary contents were much higher at the PAP (7.56±0.09%) than at the WAP (0.15±0.04; Fig. 5.4).

Differences in the distributions of lipids in sediments between the PAP and WAP were apparent (Fig. 5.5), but are best assessed *via* lipid indices [total lipids, total fatty acids, total hydroxyacids, total sterols, total unsaturated and saturated fatty acids, unsaturated/saturated ratio, stenols(Δ^5)/stanols(Δ^0) ratio, C_{-27}/C_{-29} sterols ratio (C_{27}/C_{29}), % of 20:5+22:6 relative to total PUFA's, (% 20:5+22:6 to PUFAs), 16:1(n-7)/16:0 ratio, total

18:x[1]/ total 16:x ratio (T18/T16), concentration of bacterial compounds, and distribution of the main sterols in sediments]. As these lipid indices were used in this study only to indicate differences in the quality of organic matter, the values of the sediments were compared to those found in the body tissues of the holothurians (Figs. 5.6-5.20).

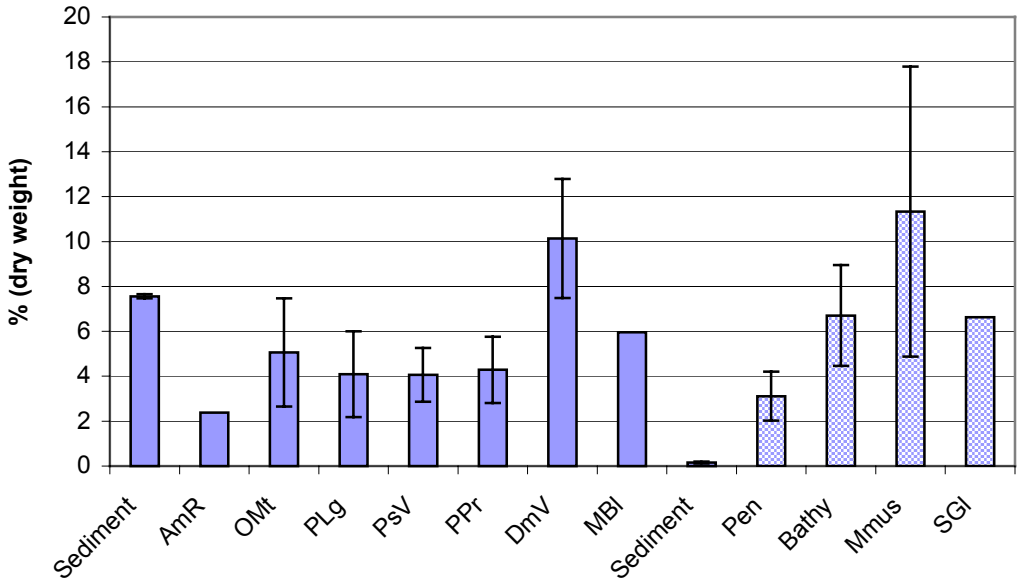


Figure 5.4. %TIC (dry weight) of samples collected at the WAP (checked bars) and PAP. Error bars represent standard deviation from mean.

Although the concentration of total lipids in the sediments from the WAP were at least an order of magnitude higher than those at the PAP (Table 5.1), the values in the animals were more similar; again, *Deima validum* had the lowest value (Fig. 5.6). The total concentrations of fatty acids, hydroxyacids and sterols (Figs. 5.7-5.9, respectively) had a similar pattern

[1] X= 1, 2, 3…, i.e. all unsaturated compounds with 18 carbons.

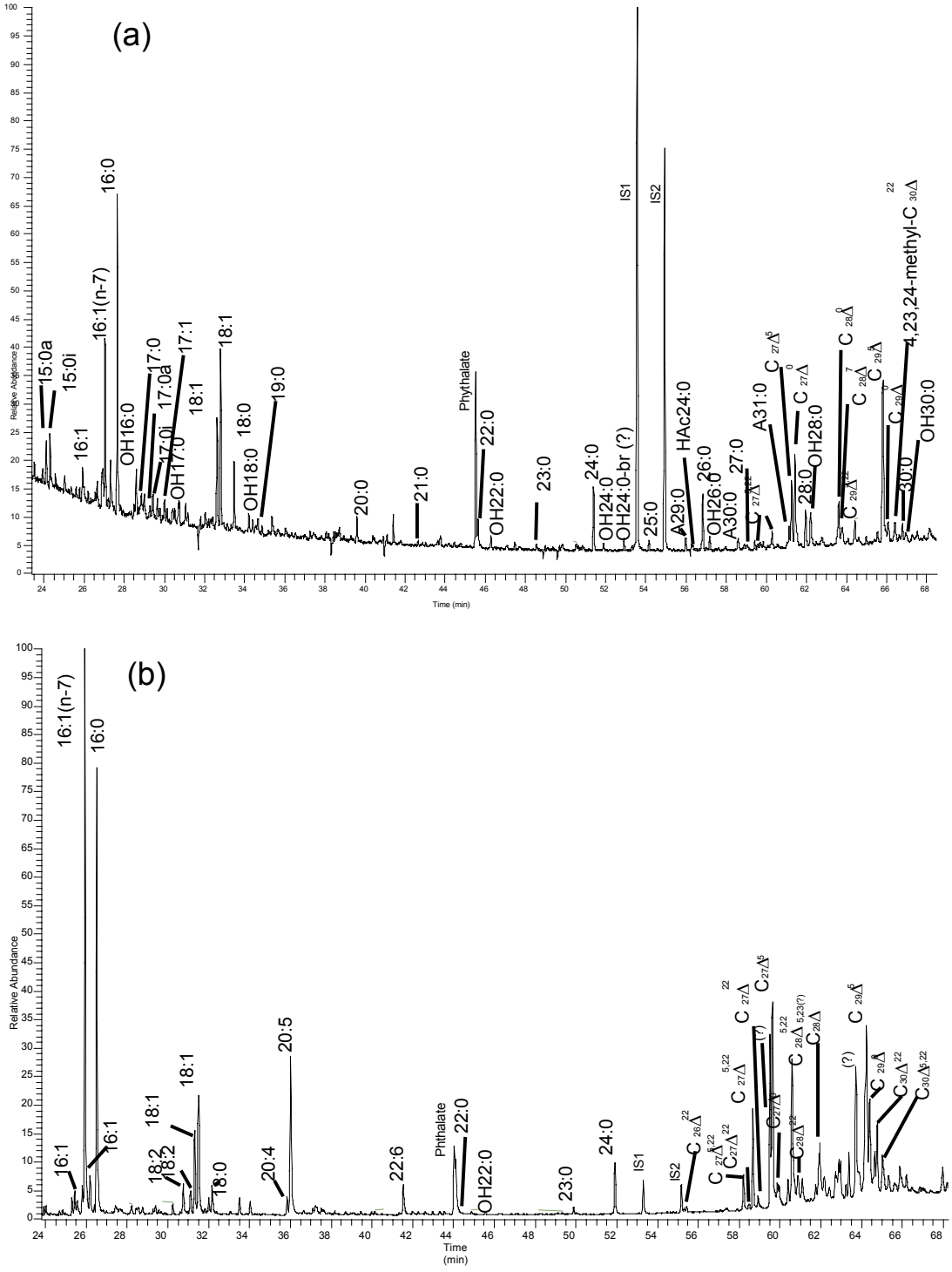


Figure 5.5. Total ion chromatogram of sediments (0-5 mm) collected at the (a) PAP and at the (b) WAP.

to the total lipids, although *Bathyplotes* sp. (WAP) had a much higher concentration of fatty acids relative to sterols than any other species.

The distributions of the lipids in the two populations did show some differences. For example, the concentrations of saturated fatty acids of the two groups of holothurians differed significantly (Mann-Whitney *U* test, $p<0.00001$, Fig. 5.10) as did unsaturated/saturated ratio (Mann-Whitney *U* test, $p<0.0002$); the animals from the PAP were richer in unsaturated acids, although the sediments from the WAP showed higher values than the PAP sediments (Fig. 5.12).

% 20:5+22:6 to PUFAs of both populations of holothurians were statistically different (Mann-Whitney *U* test, $p< 0.00005$; Fig. 5.13; *Molpadia musculus* was not included because PUFAs were present in very low concentration in its tissue; Chapter 4), as were 16:1(n-7)/16:0 ratio, T18/T16 and total bacterial fatty acids (Mann-Whitney *U* test, $p< 0.0003$, $p<0.0000003$, and $p<0.0005$; Figs. 5.15-5.17, respectively); a similar trend was apparent in the sediments. The concentration of bacterial compounds in *Bathyplotes* sp. was one order of magnitude higher than in any other species.

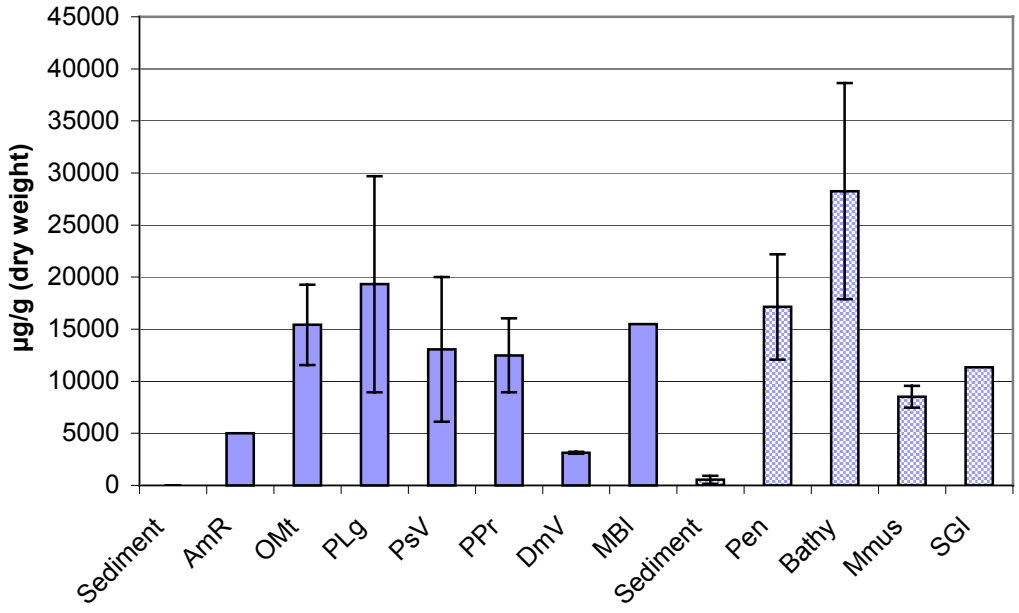


Figure 5.6. Concentration of total lipids of samples collected at the WAP (checked bars) and PAP. Error bars represent standard deviation from mean.

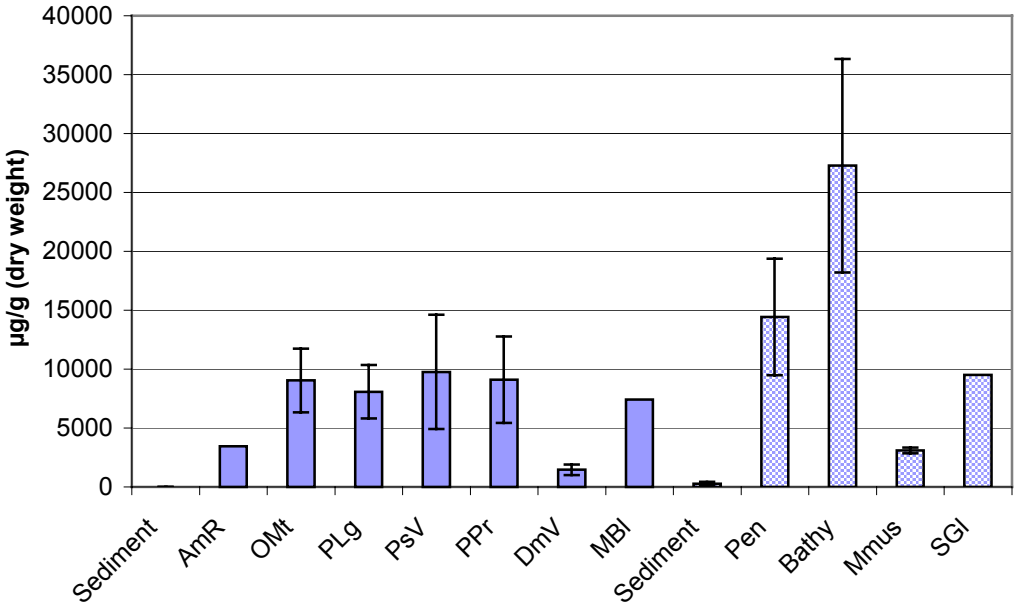


Figure 5.7. Concentration of total fatty acids of samples collected at the WAP (checked bars) and PAP. Error bars represent standard deviation from mean.

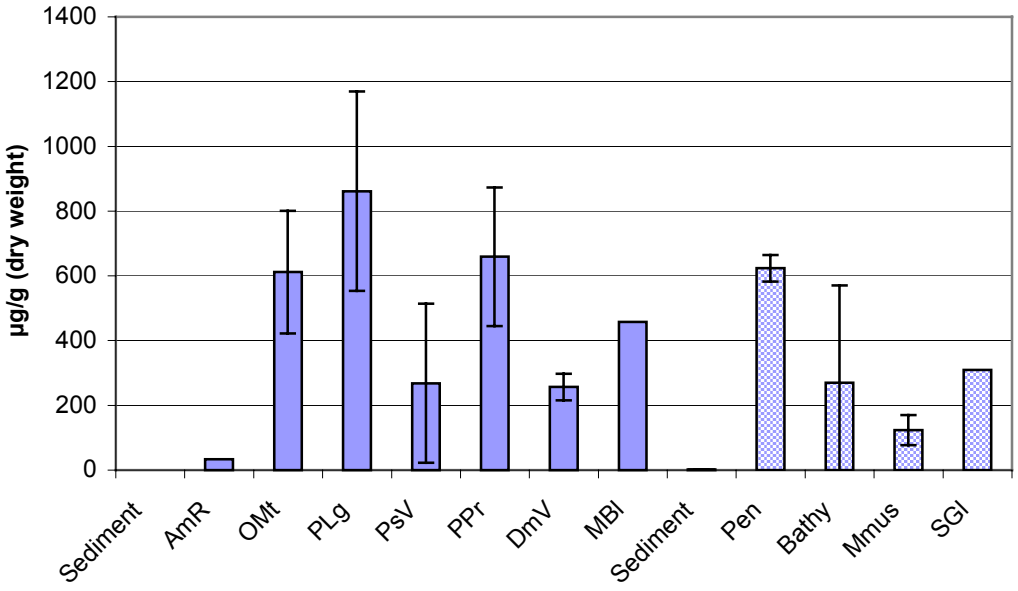


Figure 5.8. Concentration of hydroxyacids of samples collected at the WAP (checked bars) and PAP. Error bars represent standard deviation from mean.

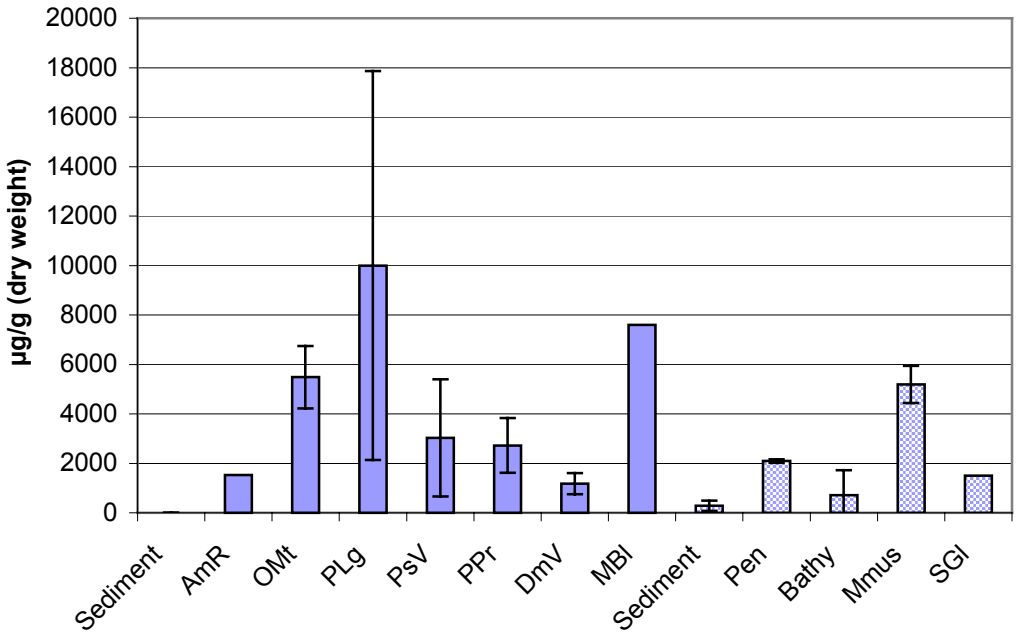


Figure 5.9. Concentration of sterols of samples collected at the WAP (checked bars) and PAP. Error bars represent standard deviation from mean.

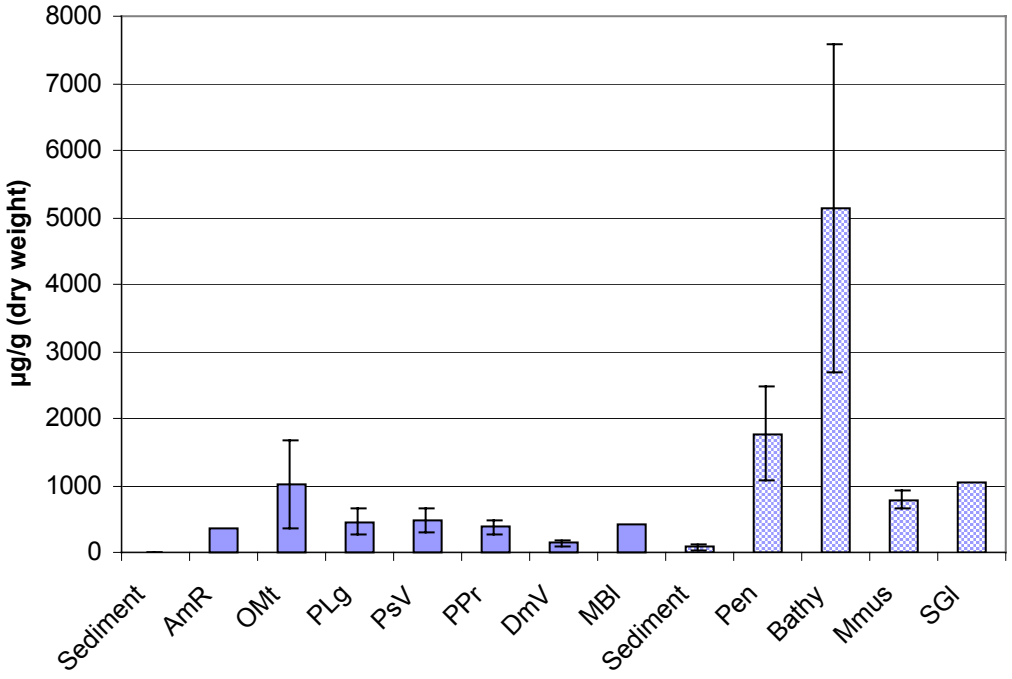


Figure 5.10. Concentration of saturated fatty acids of samples collected at the WAP (checked bars) and PAP. Error bars represent standard deviation from mean.

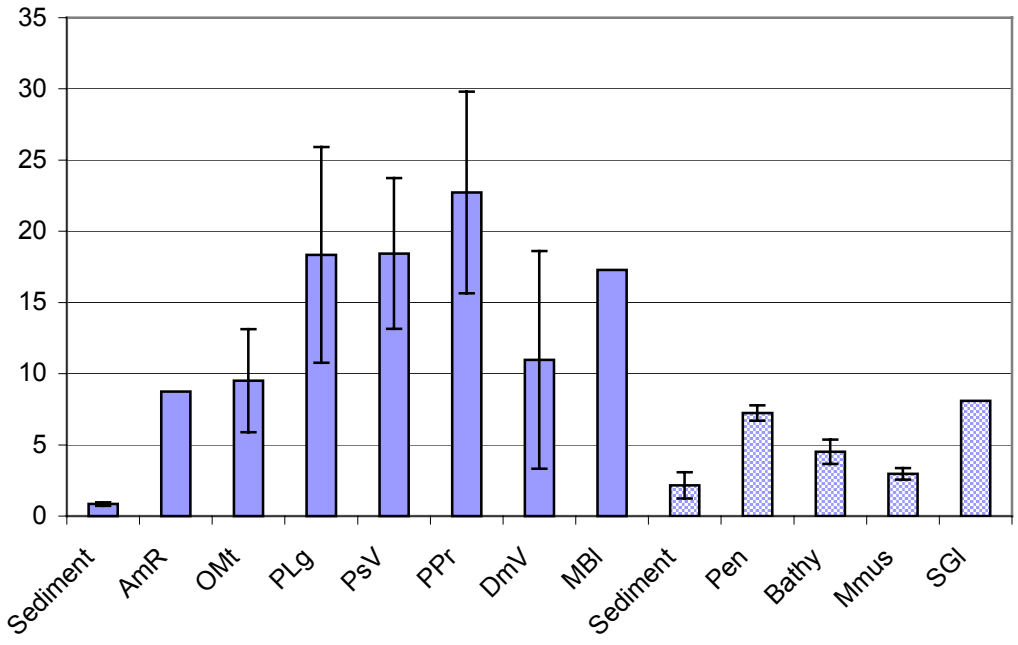


Figure 5.11. Unsaturated/saturated ratio of samples collected at the WAP (checked bars) and PAP. Error bars represent standard deviation from mean.

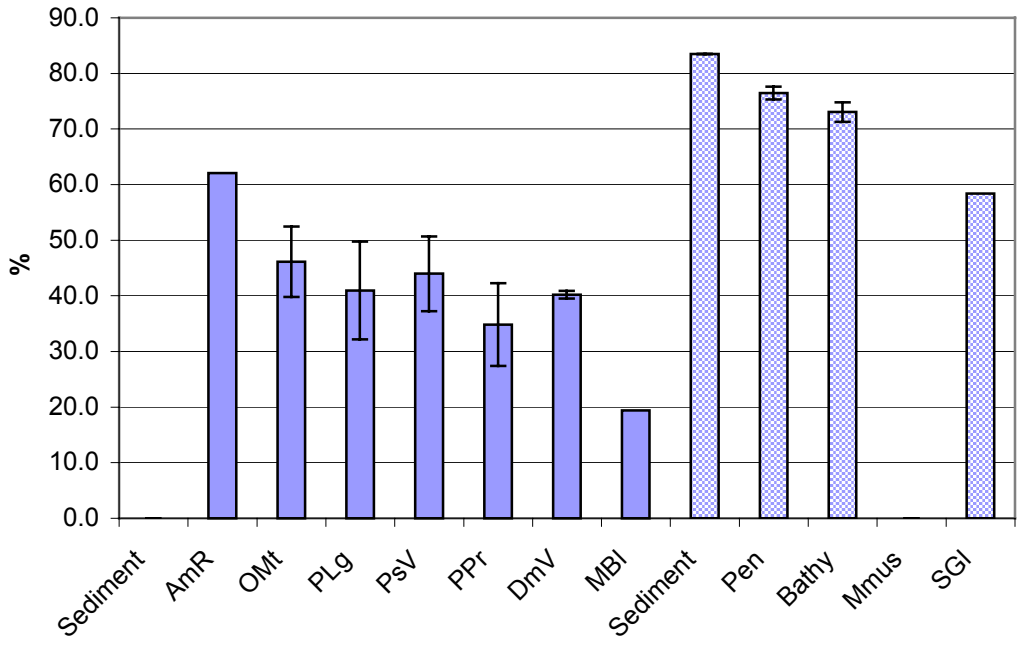


Figure 5.12. % of 20:5+22:6 in relation to total PUFAs (% 20:5+22:6 to PUFAs) of samples collected at the WAP (checked bars) and PAP. Error bars represent standard deviation from mean.

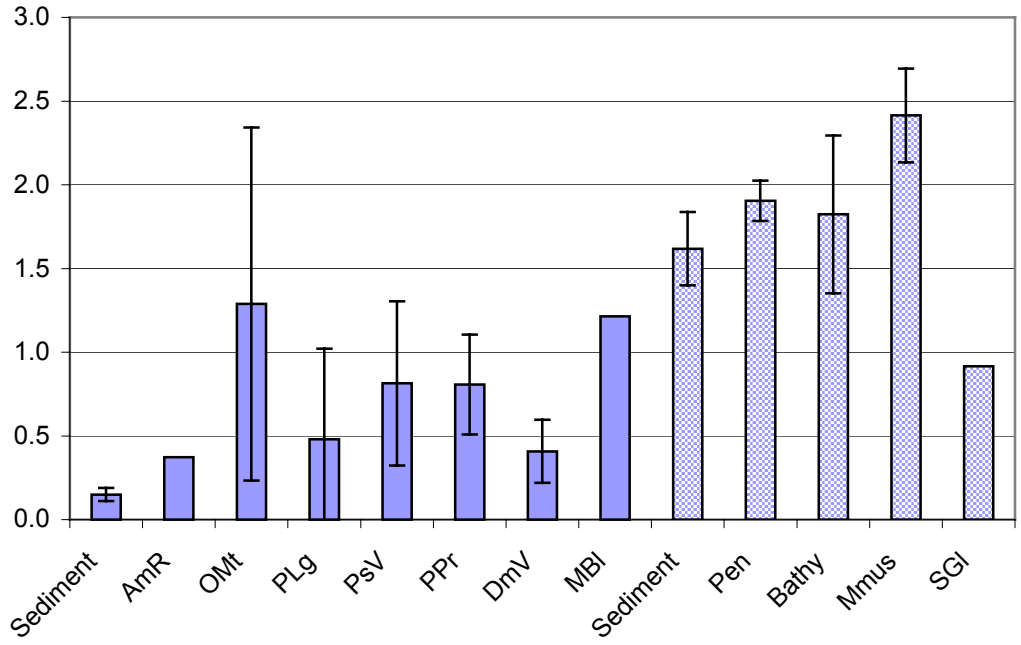


Figure 5.13. 16:1(n-7)/16:0 ratio of samples collected at the WAP (checked bars) and PAP. Error bars represent standard deviation from mean.

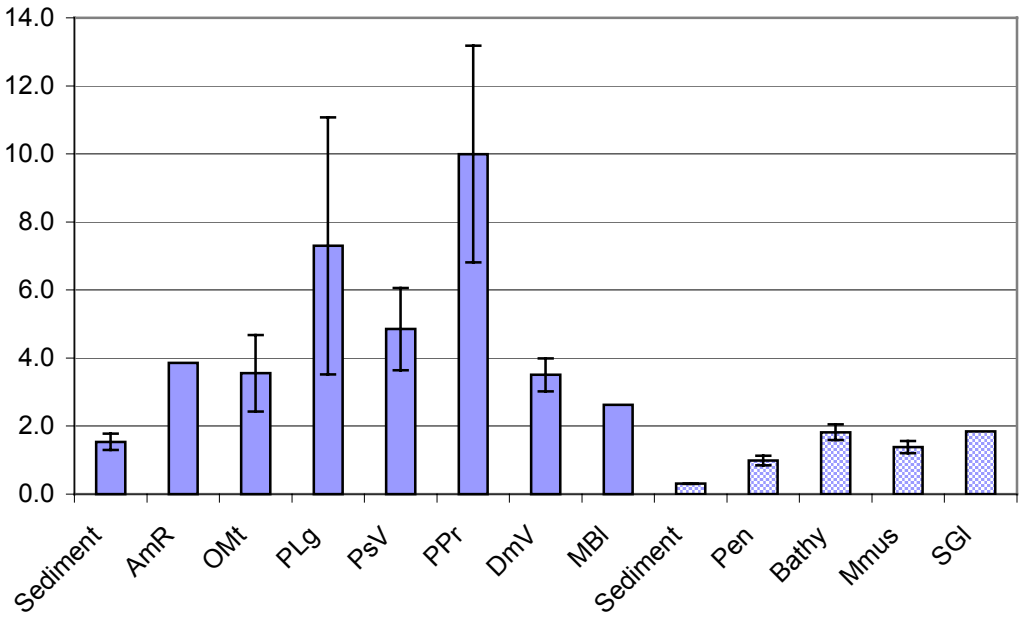


Figure 5.14. T18/T16 in samples collected at the WAP (checked bars) and PAP. Error bars represent standard deviation from mean.

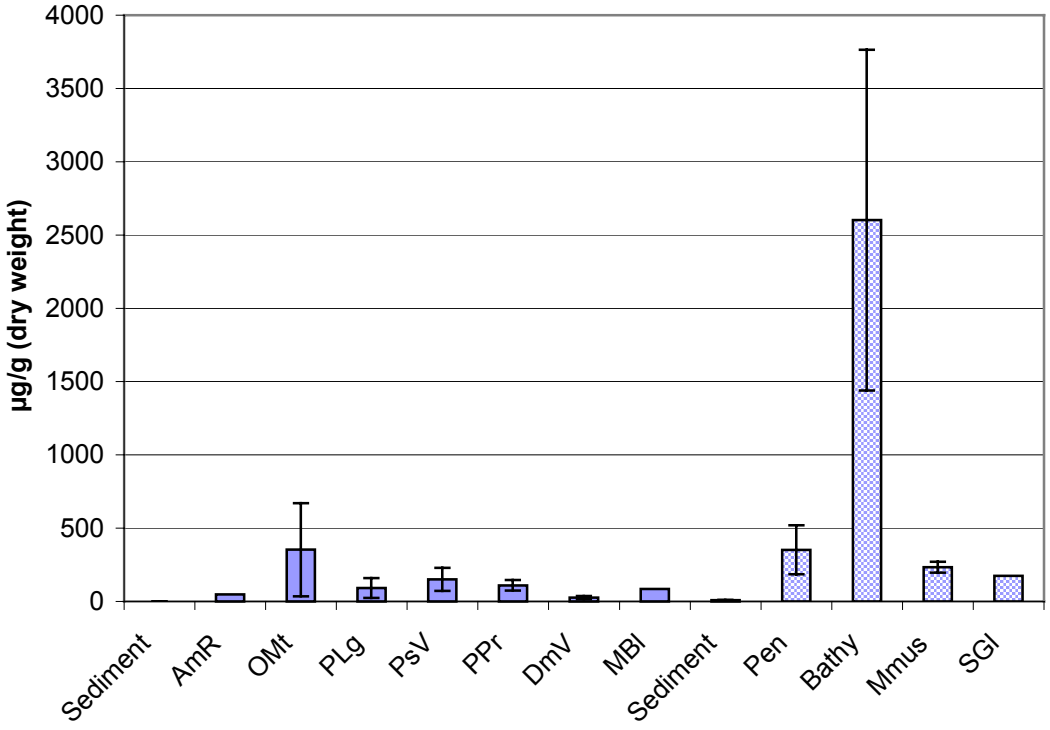


Figure 5.15. Total concentration of bacterially-derived compounds of samples collected at WAP (checked bars) and PAP. Error bars represent standard deviation from mean.

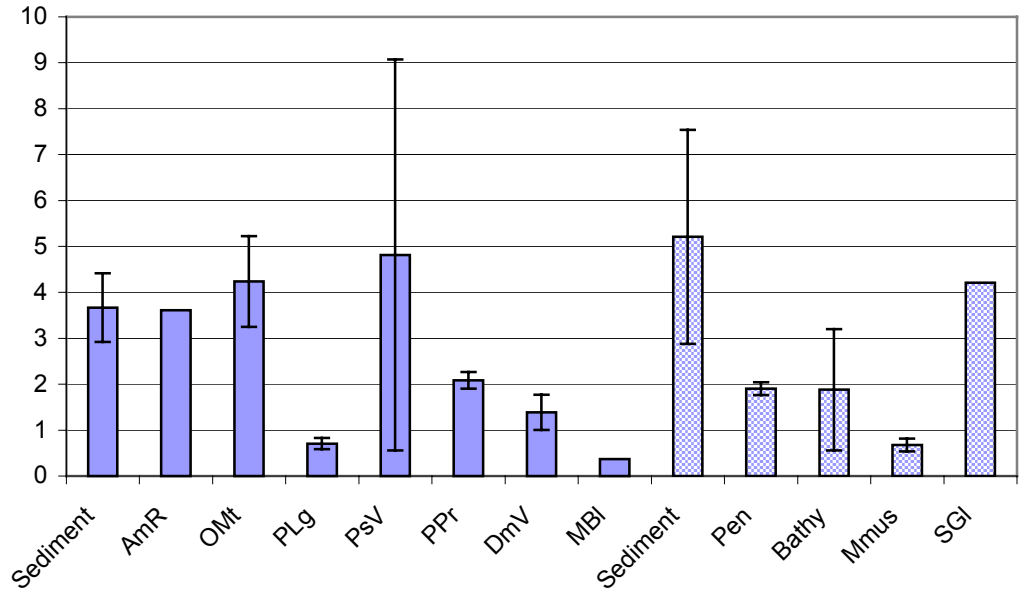


Figure 5.16. Stenols(Δ^5)/stanols(Δ^0) ratios of samples collected at WAP (checked bars) and PAP. Error bars represent standard deviation from mean.

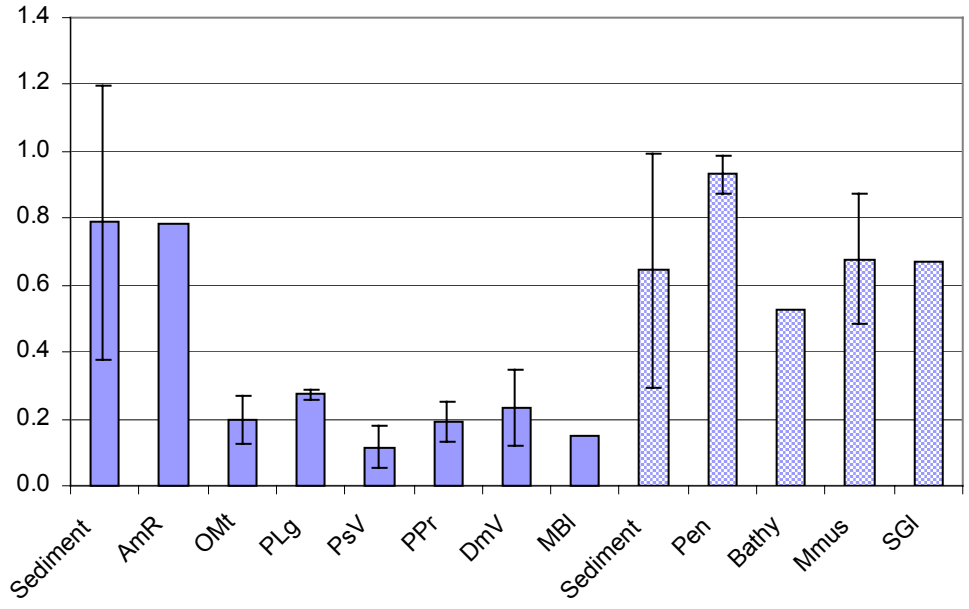


Figure 5.17. C_{27}/C_{29} ratios of samples collected at WAP (checked bars) and PAP. Error bars represent standard deviation from mean.

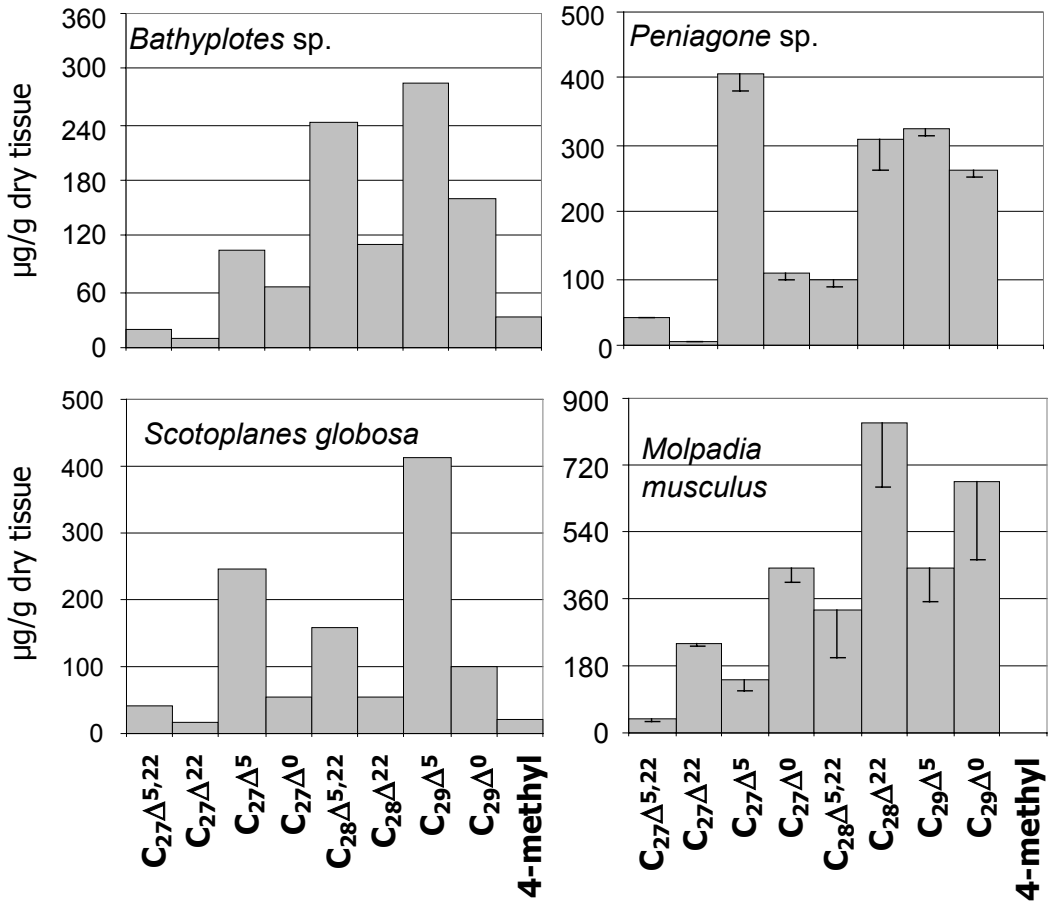


Figure 5.18. Distribution of the main sterols in the species from the WAP. Error bars represent standard deviation from mean.

The stenols(Δ^5)/stanols(Δ^0) ratios of animals and sediments collected from the two sites were not distinct (Fig. 5.17). In contrast, the C_{27}/C_{29} of holothurians from the WAP, were significantly higher than animals from the PAP (Mann-Whitney *U* test, $p<0.00002$, Fig. 5.18), although *Amperima rosea* was exceptional at the PAP. C_{29} compounds dominate sediments at the two locations (Fig. 5.20).

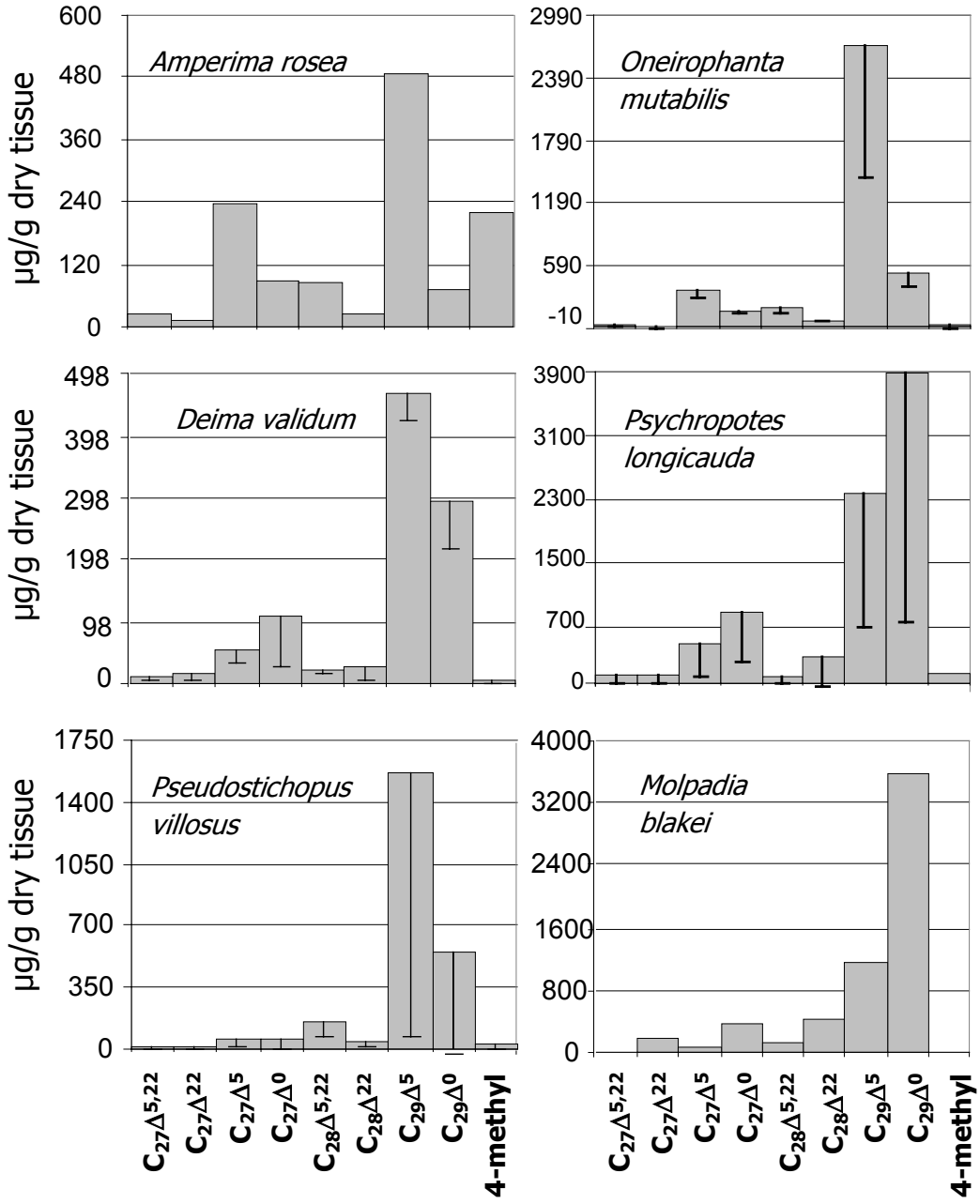


Figure 5.19. Distribution of the main sterols in the holothurians species from the PAP. Error bars represent standard deviation from mean.

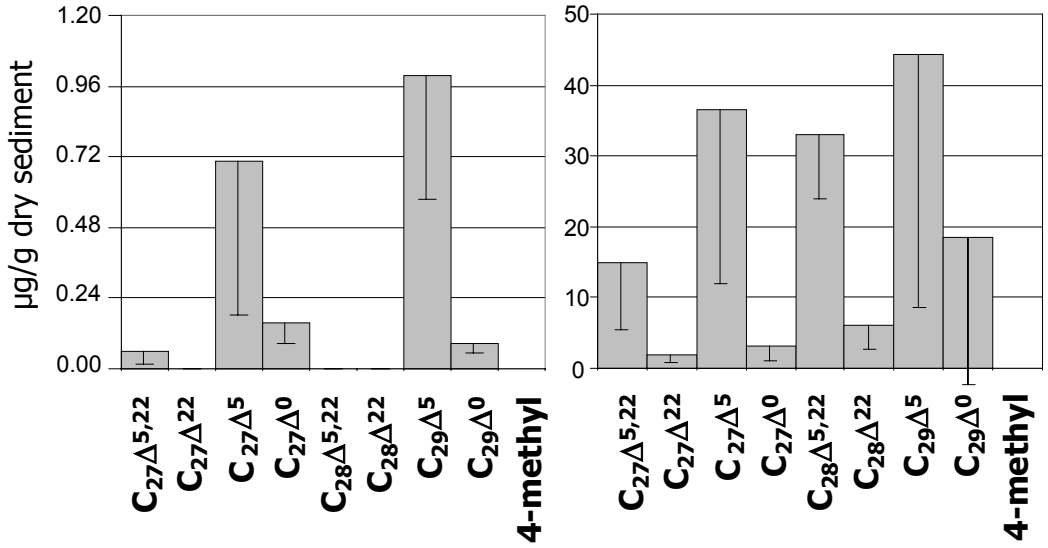


Figure 5.20. Distribution of the main sterols in sediments from the (a) PAP and the (b) WAP. Error bars represent standard deviation from mean.

5.3 DISCUSSION

5.3.1 SEDIMENTARY ENVIRONMENT

The main differences and similarities between the PAP and WAP regions are listed in Table 5.1 and can be visualised by comparing distributions of lipids in Figure 5.5. Both locations are characterised by a pulsed deposition of phytodetritus during late spring-early summer (Billett *et al.*, 1983; Smith *et al*., 2002). However, the phytodetritus layer on the seabed at the PAP formed by the spring bloom of the primary producers appears to degrade or become incorporated into seafloor sediments on timescales of 4-8 weeks (Lampitt, 1985, Rice *et al.*, 1986, Lochte and Turley, 1988). It is possible that the megafauna are responsible in part for

the rapid disappearance of the detrital layer (Rice *et al.*, 1986 Mackenzie, 2000; Bett *et al*., 2001; Ginger *et al*., 2001). On the other hand, the organic matter deposited in the WAP is believed to last longer, serving as a food bank for the benthic community through the year (Smith *et al*., 2002). The reasons for this difference are unclear, but are probably related to the apparent higher quantity of material deposited in the WAP (Fig. 5.21,Table 5.1).

Table 5.1. Some characteristics of surficial sediments of the WAP and PAP.

	PAP	**WAP**
Depth:	~5000 m	~600m
Pulse of phytodetritus?	YES	YES
TOC	0.43±0.04	1.59±0.43
TN	0.03±0.00	0.22±0.03
C/N	12.55±0.51	7.16±1.28
Flux of organic matter[1]	0.13 mMoles C m^{-2} y^{-1}	5.5 mMoles C m^{-2} y^{-1}
Total lipids in superficial sediments (0-5mm)[2]	10.12 ±7.72 µg/g (n=3)	542.41±389.51 µg/g (n=4)
Temperature	~2.5 ºC	<1.0 ºC
Sampling period	April-May/1999	February-March/2001

[1] Measured at 150 mab (WAP; Thomas *et al*., 2002) and at 100 mab (PAP; Lampitt *et al*., 2001).

[2] The WAP sediments had a thick layer of phytodetritus covering its surface (Fig. 5.21).

Figure 5.21. A sediment core from the WAP, where a thick layer of phytodetritus covering the sediment was observed over the period of sample collections in February-2001.

There is a clear difference in the levels of TOC and TN at the PAP and WAP (Figs. 5.1 and 5.2, respectively; Table 5.1), which reflects the presence of a thick layer of phytodetritus on the sediments from the WAP. The lower C/N values of the WAP sediment (Fig. 5.3) is typical of freshly deposited material (phytodetritus~6; *e.g.* Santos *et al*., 1994). The TOC and lipid-enriched WAP sediments reflect the high production of surface water and the relatively shallow water depth. Indeed, the measured flux of organic carbon in a near-bottom sediment trap (150 metres above the bottom; mab) was 5.5 mMoles C m^{-2} y^{-1} between October 2000 and February 2001

(Thomas *et al.*, 2002). On the other hand, the flux of organic carbon in the PAP was 40 times lower (0.13 mMoles C m^{-2} y^{-1} at 100 mab) between 25 April 1999 and 24 May 1999 (Lampitt *et al.*, 2001).

%TIC was higher in the sediments from the PAP, which probably reflects the important input of carbonate secreting organisms to the sediments (particularly foraminifera) *vs.* the dominant input of diatoms at the WAP.

5.3.2. DO LIPIDS IN HOLOTHURIANS REFLECT THE FOOD AVAILABILITY?

5.3.2.1 Bulk parameters and lipid indices

TOC and TN contents of animals at the two sites are similar as might be expected. The particularly low values of TOC in *Deima validum* results from the high quantity of spicules in its body (Prim *et al.*, 1976; Walker *et al.,* 1987a&b, Chapter 4). As TOC and TN had the same pattern, C/N values of the different populations of holothurians were within the same range (Fig. 5.4).

The concentration of total lipids, total fatty acids, total hydroxyacids and total sterols had the same trend as for TOC (Chapter 4), being more abundant in the sediments of WAP, and showing no difference between species sampled in the two locations (Figs. 5.6-5.9). On the other hand, the concentrations of saturated fatty acids were lower in the animals from the PAP, reflecting the lower concentrations of these compounds in the sediments (Fig. 5.10). In contrast, the ratio of unsaturated/saturated fatty

acids was lower in the holothurians from the WAP, the reverse of the trend in the sediments (Fig. 5.11). This observation is consistent with the conclusions of Ginger *et al.* (2000) who attributed relatively higher concentrations of unsaturated compounds in deep-sea relative to shallow water animals to an adaption of the former to maintain membrane fluidity at high pressure. Thus, the data shown here reinforce this hypothesis, since the proportion of saturated/unsaturated fatty acids in the holothurians does not reflect the available food source.

% 20:5+22:6 to PUFAs of WAP and PAP holothurians, however, were statistically different and reflected in part the values of the sediments (Fig. 5.12). The relatively high abundance of 20:5 and 22:6, which have been often used as diatom biomarkers (*e.g.* Skerratt *et al*., 1995), in the sediments of the WAP (Fig. 5.5) can be attributed to the high amount of the diatom *Corethron* sp. found in the phytodetritus overlying the sediments (Smith and DeMaster, Pers. Comm.). Thus, high values of %20:5+22:6 in WAP animals (except *Molpadia musculus*) might be attributed to dietary uptake and assimilation (Chapter 4). On the other hand, PUFAs are absent in sediments from PAP, but abundant in the PAP holothurians, albeit less so them in WAP animals (Fig. 5.12). These observations suggest that holothurians can biosynthesise PUFAs, but that part of the total pool comes from their diet. The absence of PUFAs in the *Molpadia musculus* body tissues is intriguing since it contrasts markedly with its counterpart from the same order, *Molpadia blakei*, which has relatively high concentration of PUFAs (Chapter 4). It is possible that *Molpadia musculus* is incapable of biosynthesising these compounds *de novo* or unable to absorb them.

Other indices commonly used to assess the contribution of diatoms to sediments, 16:1(n-7)/16:0 and T16/T18 ratios, and the amount of bacterially-derived compounds, were clearly different in the sediments (Fig. 5.5) and showed a similar trend to % 20:5+22:6 to PUFAs, where holothurians of both locations differ significantly, and had values close to the ambient sediments (Figs. 5.13-5.15). These indices, thus, indicate that the biochemistry of the holothurians reflects the quality of available food. The high amount of bacterially-derived compounds in *Bathyplotes* sp., however, is likely to arise from its gut morphology because it had one of the largest gut volumes (pers. obs.), which may help bacterial fermentation.

Stenols(Δ^5)/stanols(Δ^0) ratios between species varied greatly, showing no distinction between the animals collected in different sites (Fig. 5.16). Thus, this ratio is more likely to be influenced by the way that each species selects food and the rate that animals reduce Δ^5 to Δ^0 (Chapter 6) rather than by food availability. The other index based on the sterol distribution, the C_{27}/C_{29}, was significantly different between species from the WAP and PAP, where the animals sampled in each region had values close to those in the ambient sediments (Fig. 5.17). This ratio clearly shows the influence of food source on the biochemistry of the animals. However, *Amperima rosea* (PAP) was an exception, having values close to those animals from the WAP. This is likely to be caused by its feeding mode. *Amperima rosea* feeds selectively on fresh phytodetritus (Iken *et al*., 2001; Wigham, 2002), and for this reason has a sterol distribution similar to POM arriving at the PAP (Kiriakoulakis *et al*., 2001). It is important to note that the sterols in *Peniagone* sp., which feeds on the water column (Tyler, pers. comm.), were

dominated by cholest-5-en-3β-ol, $C_{27}\Delta^5$ (**5**; Fig 5.18), a characteristic sterol of zooplankton (*e.g.* Wakeham, 1997). The gut contents of this species show high ^{234}Th activity (~160 dpm g^{-1}), implying that the gut organic matter is fresher; other species [*Bathyplotes* sp. and *Scotoplanes globosa* (~90-120 dpm g^{-1}) and *Molpadia musculus* (<30 dpm g^{-1}); DeMaster *et al.*, 2002)] have much lower values. Thus, taken together, these data suggest that *Peniagone* sp. feeds on fresh POM in the water column and may select it.

The low C_{27}/C_{29} of the PAP species (except *Amperima rosea*) reflects the high amount of 24-ethyl-cholet-5-en-3*β*-ol, $C_{29}\Delta^5$ (**19**), and 24-ethyl-5α(H)-cholestan-3*β*-ol, $C_{29}\Delta^0$ (**20**) (Fig. 5.19) present in their bodies. Interestingly, these compounds are dominant in the sediments of both locations (Fig. 5.20). Thus, the high concentration of **19** and **20** in species from PAP may be caused by the ability/necessity of the holothurians from the PAP to store these sterols, since they may be limiting nutrient compounds at the PAP region (Ginger *et al.*, 2001). *Amperima rosea*, which is a selective feeder (Iken *et al*., 2001; Wigham, 2002) and has a high locomotion rate (Bett *et al.*, 2001), may not need to store these compounds because of its ability to exploit food. On the other hand, it has been hypothesised that the bloom of algae in the late spring/earlier summer in the WAP provide enough food to the holothurians to feed all year round (Mincks *et al.*, 2002; Smith *et al.*, 2002), thus it is likely that WAP species do not need to store sterols.

5.3.2.2. Cluster analysis of lipid contents

Fig. 5.22 is a dendrogram of a hierarchical cluster analyses based on the percentages of the concentration of all compounds relative to the totals of their classes (fatty acids, hydroxyacids and sterols). The species of holothurians were sorted by their biochemical contents, except for *Oneirophanta mutabilis* and *Deima validum,* which were spread into two groups (Fig. 5.22). Interestingly, when Wigham (2002) performed the same test based on pigment distribution in the guts of the PAP animals, specimens of *Oneirophanta mutabilis* were also split into two groups. This implies that this species has a high variability on its biochemical content, which in turn may indicate that it is a diverse feeder ingesting different types of food.

Specimens of *Molpadia musculus* were classified as a different group from all other holothurians, probably because of its different distribution of fatty acids (Chapter 4). The other species, then, were divided into two sub-groups: 1) the unique sample of *Molpadia blakei* and 2) the rest of the animals (Fig. 5.22). Consequently, the latter was divided into 5 sets. Interestingly, the WAP species were clustered (except for *Molpadia musculus*) in one of these sets (at the top of the dendrogram, Fig. 5.22), indicating that the lipid contents of the WAP population are different, probably because of the available food. *Paroriza prouhoi*, *Pseudostichopus villosus* and *Psychropotes longicauda* specimens were grouped separately. Thus, this mathematical analysis shows that species of holothurians differ at the molecular level and can be sorted by its lipid contents.

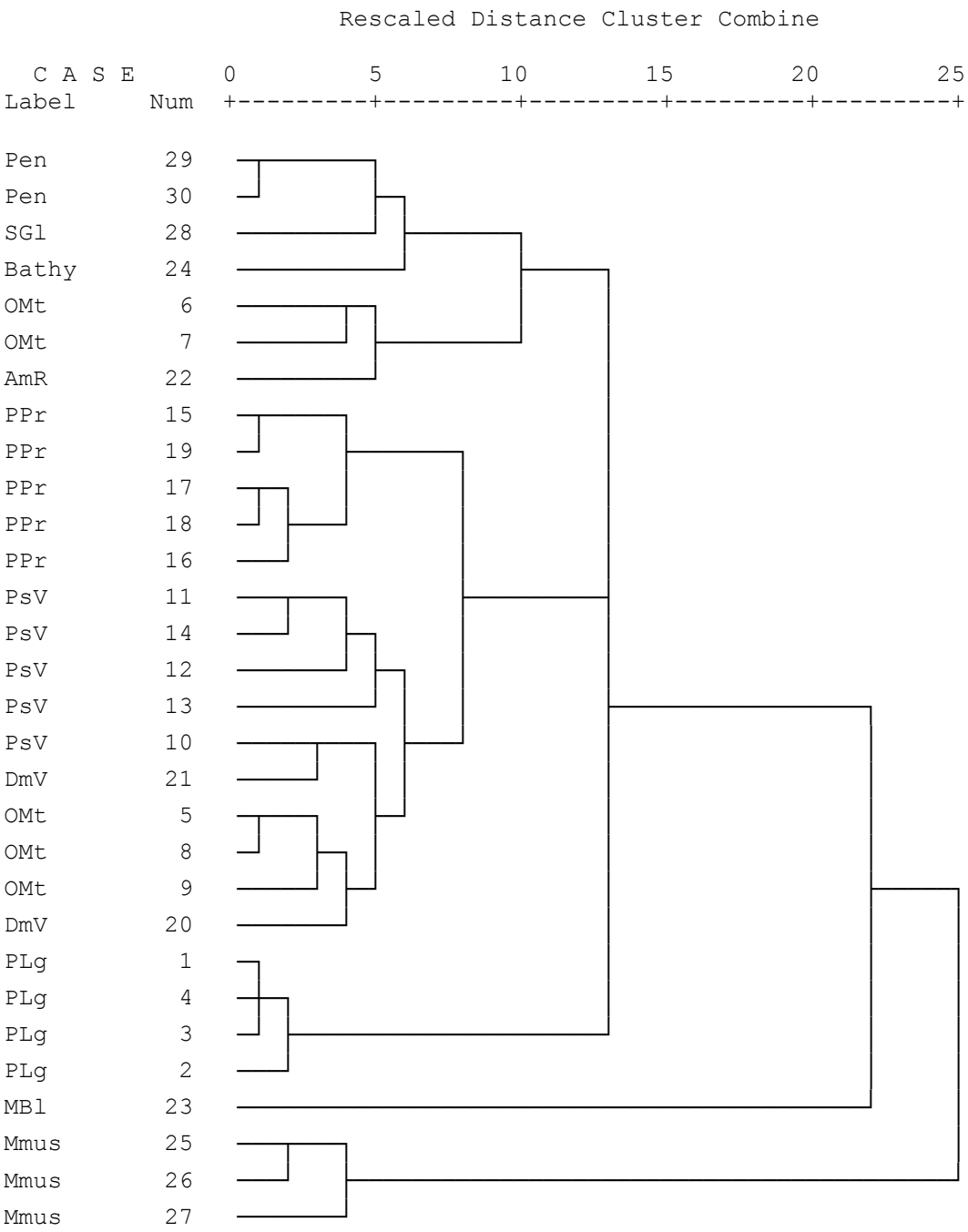


Figure 5.22. Dendrogram (hierarchical cluster analyses) of the percentages of all compounds in relation to the total concentration of their class (fatty acids, hydroxyacids and sterols) using Average Linkage (Between Groups; SPSS software; see Table 4.2 for species codes).

5.4 CONCLUSIONS

The concentrations of total lipids and TOC were one order of magnitude and three times higher, respectively, in the sediments from the WAP than in those from the PAP, but not in the holothurians. The organic carbon and biomass of the two populations is probably very different and not dependent of the POM flux to the sea floor. Nevertheless, indices of the quality of the organic matter showed that the biochemistry of the animals is influenced by the quality of available nourishment. Furthermore, the ratios of unsaturated/saturated fatty acids at the two sites reflect the ambient pressure regimes rather than food availability.

Finally, different species of holothurians have distinct lipid compositions, which can be separated by hierarchical cluster analysis. The data shown here indicate that the inter-species differences are caused mainly by differences of 1) food sources, 2) feeding mode, and 3) pressure.

Chapter 6

THE INFLUENCE OF THE PULSED DEPOSITION OF PHYTODETRITUS ON THE BIOCHEMISTRY OF DEEP-SEA HOLOTHURIANS AT THE PORCUPINE ABYSSAL PLAIN

6.1 INTRODUCTION

6.1.1 HOLOTHURIANS AND BIOCHEMICAL VARIATIONS AT THE PAP

Holothurians (Phylum Echinodermata) are ubiquitous marine particulate-feeding invertebrates ranging in size from 7 mm to > 1 m. They dominate large parts of the deep sea, both in terms of biomass and abundance (Sibuet *et al*., 1982; Billett, 1991; Roberts, 2000). At the Porcupine Abyssal Plain (PAP; NE Atlantic Ocean) the holothurians account for ~ 76 and 93%, of the megabenthos abundance and biomass, respectively (Billett *et al.*, 2001).

It is known that holothurians have high grazing rates and can have a significant impact in the redistribution of sediments (Chapter 1). It has been estimated 100g of sediment can pass through the gut of a single specimen per day (Deming and Colwell 1982). Ginger *et al.* (2001) suggested that a bloom of the species, *Amperima rosea* and *Ellipinion molle* over a period of three months may have led to the complete removal of phytosterols deposited at the PAP. Although there are considerable differences in the estimates of holothurian impact on OM reworking at the deep-sea floor, they

certainly appear to have an important role at the sediment/water interface and in the biogeochemistry of the deep sea.

POM are the dominant food for holothurians (*e.g.* Billett, 1991; Roberts *et al.*, 2000) and are the source of dietary proteins, carbohydrates, pigments, sterols, fatty acids, and organic nutrients (Kiriakoulakis *et al.*, 2001; Witbaard *et al.*, 2001; Wigham, 2002). The flux of POM to the deep sea and its quality, however, depends on a number of factors (sedimentation rates, the activity of pelagic marine heterothrophs, intensity of the blooms of phytoplankton occurring in the photic zone, depth of the water column, currents, degradation, atmospheric dust input, etc.; McCave, 1975; Deuser *et al*., 1981; Lampitt, 1985; Fowler and Knauer, 1986; see Chapter 1). The supply of organic nutrients to the deep-sea is not constant, for instance, it has been estimated that more than 40% of the annual particulate organic carbon (POC) flux reaches the northeast Atlantic sediments as a pulse of phytodetritus after the spring bloom (Honjo and Manganini, 1993); providing an important food source for the deep-sea benthos (Billett *et al.,* 1983).

The seasonality of the POM supply at PAP, the location of the present study, has been studied in numerous projects (*e.g.* NABE, BIO-C-FLUX; Pfannkuche, 1993; DEEPSEAS, Rice *et al.*, 1994; BIOTRANS, Pfannkuche *et al.,* 1999; BENGAL, Billett and Rice, 2001) in the 1980s and 1990s. Although there is little inter-annual variation in the downward flux of POM at the PAP (Lampitt *et al.*, 2001), there is, nonetheless, a high degree of biochemical variability in the water column (Kiriakoulakis *et al.*, 2001), at the sediment/water interface, and in both surficial (0-5cm) and deeper layers

(up to 15cm) of sediment (Mackenzie 2000; Danovaro *et al.*, 2001; Fabiano *et al.*, 2001). Hence, Danovaro *et al.* (2001) showed that there was temporal variation in the concentrations of total proteins, total carbohydrates, total lipids, hydrolysable proteins and carbohydrates, and biopolymeric and bioavailable organic carbon in PAP sediments. Mackenzie (2000) found that there was also temporal variability at the molecular (lipid) level. Photographs of the seafloor show that phytodetritus arriving at the seabed becomes very patchy after some hours or days following its deposition (Lampitt, 1985). This patchiness is also apparent in the biochemical character of the surficial sediments (Santos *et al.,* 1994).

There has been no apparent trend either in the measured or integrated annual fluxes of POM at the PAP since 1989 (Lampitt *et al.,* 2001). Thus, any variation in the abundance or biomass of megafauna caused by fluctuations in the benthic diet can only be attributed only to variation of the quality of the POM arriving at the sea-floor (Kiriakoulakis *et al.,* 2001; Fabiano *et al.,* 2001), or in the sediments (Mackenzie, 2000; Fabiano *et al.,* 2001; Danovaro *et al.,* 2001).

The response of holothurians to the OM quality has been the subject of several studies. Billett *et al.* (2001) showed that holothurians varied in size intra- and inter-annually and suggested that their abundance (major taxonomic variation) at the PAP is related to environmental parameters, such as food supply. Most striking was the bloom of *Amperima rosea*, which increased in abundance from less than 10 individuals per hectare in April 1994 to more than 6400 individuals per hectare in 1997 (estimated from Bathysnap observations; Bett *et al.*, 2001). Other short-term and/or

seasonal changes in deep-sea populations (micro-, meio- and macro-fauna) have also been attributed to the deposition of organic matter (Billett *et al*. 1983; Rice *et al*., 1994; Smith *et al*., 1994; Smith *et al*., 1996; Gutt *et al*., 1998; Danovaro *et al.*, 2000b+c)

The hypothesis to be tested here is, therefore that the biochemistry of holothurians responds to periodic POM enrichment at the deep sea floor, and that this may influence the population dynamics at the PAP (see Billett *et al*., 2001)

6.1.2 FOOD EXPLOITATION AND ABSORPTION BY HOLOTHURIANS.

Different species of holothurians probably respond differently to pulses of food supply and/or food availability, since some organisms respond physiologically (*e.g.* reproduction) to food enrichment, whilst others do not (Tyler, 1988). As the distribution of freshly-arrived POM at the deep-sea floor tends to be very patchy and its arrival is rare for most of the year, the way and the effectiveness with which each species exploits and assimilates the food may drive its response to the variation of nutritional supply. Pelagic and bentho-pelagic holothurians use tentacles around the mouth to feed on food particles suspended in the water (Billett, 1991). Benthic animals, however, roam over the seabed, feeding on the top layer of the sediment (Billett, 1986), while others feed on deeper sediments with their mouth buried (Billett 1991). Most of the species living at the PAP are benthic, but still have very different interspecific morphologies and living habits, which may make them respond unequally to food supply.

Variables that can influence the response of different species to food availability include feeding mode (Roberts *et al.,* 1996; Roberts *et al.,* 2001), rate of locomotion (Roberts *et al*., 2001), gut anatomy (Penry and Jumars 1986; Roberts *et al.,* 1996; Roberts *et al.,* 2001), tentacle structure (Roberts *et al.,* 1996; Roberts *et al.,* 2001), efficiency of organic matter absorption (Sibuet *et al.,* 1982; Pfannkuche *et al.,* 1999; Miller *et al.,* 2000), and requirement and turnover of specific organic substrates in their bodies (Ginger *et al.,* 2001).

Feeding strategies (Roberts *et al.,* 1996) and efficiency of absorption are reflected in gut anatomy. Penry and Jumars (1986, 1987) modelled digestive processes in terms of chemical reactors based on theoretical models of chemical engineering. These included: (a) batch reactors (BRs); (b) continuous plug flow reactors (PFRs); and (c) the continuous-flow stirred-tank reactors (CSTRs). In BRs, all reactants are loaded, mixed and allowed to react in discrete batches. PFRs are characterised by a continuous, orderly flow of material through the reaction vessel with no axial mixing along the tube. On the other hand, reagents undergo continuous mixing in CSTR type guts. In this respect, the more the food is allowed to mix and react in the guts with enzymes and bacteria, the more efficient the extraction of nutrients will be. Thus, efficiency of absorption of food is also dependent on gut residence times and bacterial density in the holothurians gut (Jumars *et al.,* 1990). Bacteria in their guts (Deming and Colwell 1982; Roberts *et al.,* 1996; Roberts *et al.,* 2001) and in their tentacles (Roberts *et al.,* 1991) may play an important role in exploitation of food, helping holothurians to extract more from their poor diet by breaking down refractory

material, and/or by providing limiting nutrients such as nitrogenous compounds (Roberts *et al.,* 1991).

The holothurians, being large and mobile, seem to exploit better the phytodetritus supplied by pulsed depositions than more sedentary organisms (Wigham, 2002). Within holothurians, however, there are interspecies differences in the locomotion rates (Billett, 1991) and thus in foraging ability. Billett (1991) affirmed that the degree of sediment bioturbation caused by holothurians depends on the method of locomotion. Holothurians with poor developed tubefeet rely on the alternate extension and contraction of the whole body to move over the seabed (Billett 1991). Other holothurians, however, have well-developed tubefeet (especially the Elasipodida), which do not disturb the sediment, apart from a series of pin-point marks (Billett 1991, and references therein). Faster organisms tend to better exploit fresh phytodetritus when it is patchily distributed in the seafloor (Roberts *et al.,* 2001).

Some deep-sea epibenthic holothurians (those living in the superficial sediment) are able to locate food heterogeneously distributed at the sea-floor (Billett *et al.,* 1988). Some species can also select a wide range of items including, detritus, foraminifera, spores, pollen, faecal pellets and the giant rhizopod protozoans Xenophyophorea and Komokiacea (Billett 1991; Roberts *et al.*, 2000). This degree of selectivity may be associated with tentacle structure (Hansen 1975), which has been divided into five classes by Massin (1982): dendritic, peltate, pinnate, digitate and peltodendritic. Suspension-feeders are thought to have mainly dendritic or peltodendritic tentacles with which they capture suspended particles (Hyman, 1955 and

Massin, 1982). Digitate tentacles are used to shovel sediments and peltate ones are used to sweep sediments into the mouth (Roberts *et al.,* 1991).

Consequently, these differences in food exploitation and absorption could lead the diverse species of holothurians to respond differently to the variation of food availability at the seafloor. The main question posed by Tyler (1988) was if some deep-sea organisms respond to seasonality of food supply, why do the majority that do not show seasonality in their physiological processes not do so? This question, and the others set in the Introduction (*Section 1.6*), will be addressed in the present Chapter.

6.2. THE APPROACH

The temporal variations (Jul-97 to Apr-99) of the biochemical contents, specifically the lipids of four of the main species of holothurian (*Oneirophanta mutabilis, Pseudostichopus villosus, Psychropotes longicauda,* and *Paroriza prouhoi*; Billett *et al.* 2001), which live in the PAP, are reported here (see Table 2.6). Lipids were chosen as the primary target biochemicals for this temporal study, as they are functionally involved in both energy storage (fatty acids as triacylglycerides) and in hormonal regulation (*e.g.* steroids), as well as in cell membranes (phospholipids, steroids; Ginger *et al*., 2000). Their relationship with the distributions and concentrations of the lipids in sediments, the flux of organic matter to the deep-sea, and other variables presented in literature are discussed.

6.3. RESULTS

6.3.1. TOC, TIC, TN, and C/N

Concentrations of total organic carbon (TOC), total inorganic carbon (TIC) and total nitrogen (TN) in *Oneirophanta mutabilis* ranged between 14.4 - 17.5, 1.45 - 8.32, and 3.67 - 4.86% of dry tissue respectively. There was no temporal variation in TOC, TIC and TN contents (Figs. 6.1a, 6.2a and 6.3a; Table 6.1). However, values for TN in July and October 1997 are significantly different (Mann-Whitney *U* test, $p<0.05$).

Psychropotes longicauda showed significant variation in both TOC and TN during the study period (one-way ANOVA $p<0.001$and $p<0.005$, respectively; Figs. 6.1b and 6.3b, Table 6.1). TIC was invariant for this species (Fig. 6.2b; Table 6.1). Mean values of TOC, TIC and TN ranged from 6.9 - 18.2, 1.57 - 6.48, and 2.15 - 5.44% of dry tissue respectively.

TOC (14.3 - 20.4 % of dry tissue) for *Pseudostichopus villosus* varied significantly during the sampling period (one-way ANOVA, $p<0.05$; Fig. 6.1). On the other hand, TIC and TN values (0.75 - 5.95 and 4.32 - 6.70% of dry tissue, respectively) were invariant (Figs. 6.2c and 6.3c; Table 6.1).

TOC and TN (18.3 - 23.0 and 5.09 - 7.67 % of dry tissue, respectively) contents in *Paroriza prouhoi* were the highest of all four species and were statistically similar through the sampling periods (Figs 6.1d and 6.3d, Table 6.1). TIC values in *Paroriza prouhoi* (1.42 - 6.27% dry weight) were similar to all other species, and were also temporally invariant (Fig.6.2d). There were no specimens available in 1997.

TOC/TN (C/N) mass ratios of all four species were typical of marine samples (C/N < 10), and were similar for all four species (Fig. 6.4) and were virtually constant through the study period.

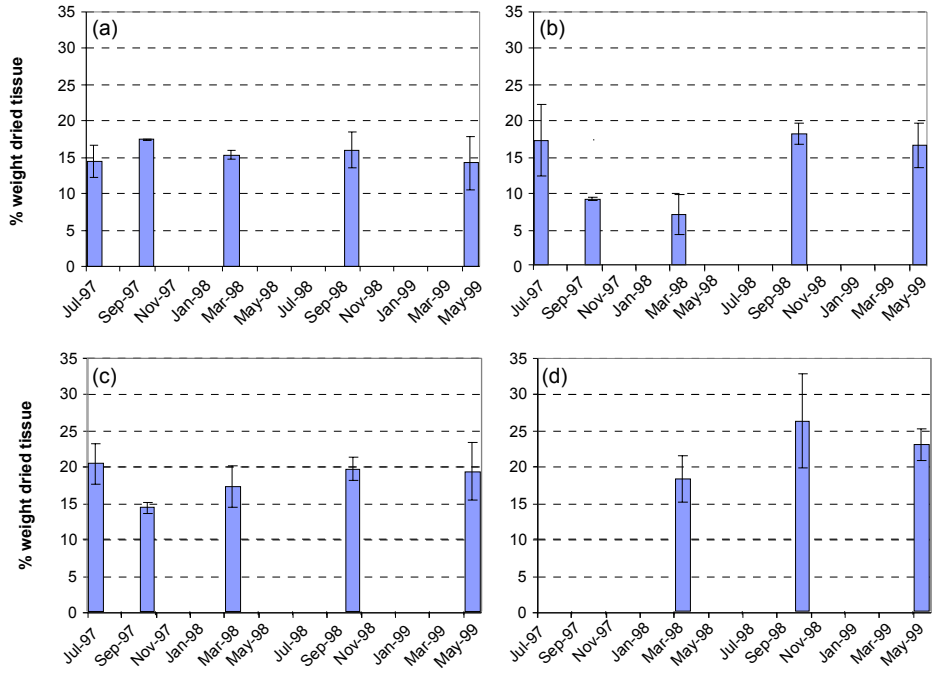


Figure 6.1. Temporal variation of TOC content of body tissues of (a) *Oneirophanta mutabilis*, (b) *Psychropotes longicauda*, (c) *Pseudostichopus villosus,* and (d) *Paroriza prouhoi*. Error bars represent standard deviation from mean.

Table 6.1. Mann-Whitney *U* test (underlined) and one-way ANOVA results comparing different sampling periods (Jul-97 to Apr-99) for *Oneirophanta mutabilis*, *Psychropotes longicauda*, *Pseudostichopus villosus*, and *Paroriza phouhoi*. Values in brackets are normalized to TOC. Significant values are in bold print.

	Oneirophanta mutabilis	***Psychropotes longicauda***	***Pseudostichopus villosus***	***Paroriza prouhoi***
Lipids	**0.0255 (0.0007)**	**0.0372** (0.1157)	0.1981 (0.2124)	0.8370 (0.8553)
Fatty acids (FA)	**0.0451 (0.0021)**	**0.0071** (0.2154)	0.0719 (0.1022)	0.7191 (0.9637)
Hydroxy-fatty acids	0.1159 (0.1221)	**0.0226** (0.3075)	**0.0451 (0.0247)**	0.2318 (0.3186)
Saturated FA	0.3401 (0.0919)	0.1020 (0.1920)	0.0843 (0.2248)	0.3245 (0.7275)
Unsaturated FA	**0.0318 (0.0015)**	**0.0068** (0.1994)	0.0714 (0.0964)	0.7573 (0.9386)
Monounsaturated FA	0.1230 (0.0507)	**0.0224** (0.1198)	0.0671 (0.0639)	0.7275 (0.9315)
Diunsaturated FA	0.1376 (0.0910)	**0.0089** (0.4786)	0.2173 (0.4128)	0.8475 (0.7937)
PUFA	**0.0055 (0.0026)**	**0.0459** (0.1946)	0.0686 (0.0790)	0.7918 (0.7418)
Sterols	**0.0057 (0.0028)**	0.1664 (0.5097)	0.2172 (0.2094)	0.8331 (0.4112)
Steryl sulphates	0.1359 **(0.0267)**	**0.0108 (0.0402)**	0.0784 (0.3103)	0.9465 (0.1877)
Sterols - C_{27}	**0.0366 (0.0019)**	0.1381 (0.2858)	0.3640 (0.4341)	0.7834 (0.6487)
Sterols - C_{28}	**0.0321 (0.0012)**	0.3347 (0.7174)	0.2648 (0.2931)	0.6021 (0.6881)
Sterols - C_{29}	**0.0010 (0.0005)**	0.1427 (0.3816)	0.0931 (0.1358)	0.7484 (0.0942)
Sterols Δ^5	**0.0004 (0.0003)**	0.0985 (0.2724)	0.2271 (0.2161)	0.8095 (0.4222)
Sterols Δ^0	0.0548 **(0.0003)**	0.2441 (0.2361)	0.1684 (0.1900)	0.7038 (0.0941)
Δ^5/Δ^0 ratio	**0.0222** (0.0651)	0.1089 (0.3331)	0.3348 (0.9392)	0.2231 (0.5967)
Bacterial FA	0.5621 (0.2856)	0.4526 (0.4727)	0.2778 (0.3020)	0.8475 (0.8584)
%Bacterial FA[1]	**0.0280 (0.0134)**	**0.0462 (0.0094)**	0.0569 **(0.0126)**	0.5630 (0.7530)
%PUFA[1]	0.0811 (0.0619)	0.1794 (0.9551)	0.0589 (0.0913)	0.6964 (0.1482)
Lycopane	0.6141 **(0.0331)**	-[2]	**0.0387 (0.0259)**	0.1295 (0.1295)
TOC	0.3880	**0.0007**	**0.0440**	0.0905
TIC	0.7622	0.8466	0.4174	0.5613
TN	0.1300	**0.0019**	0.0930	0.3175

[1]Percentages relative to total fatty acids.
[2]Lycopane was not detected in *Psychropotes longicauda.*

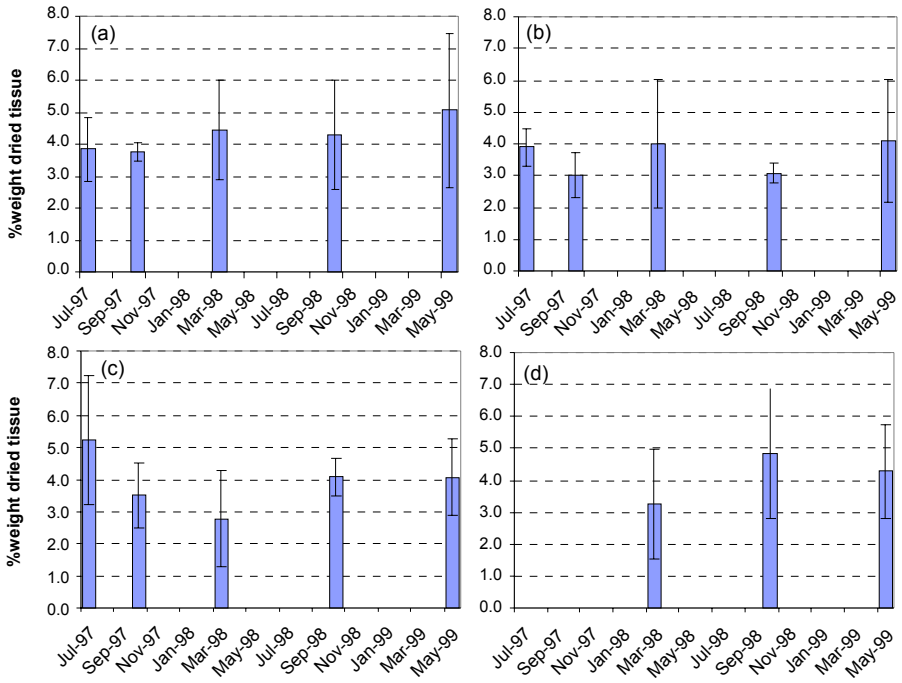


Figure 6.2. Temporal variation of TIC in body tissues of (a) *Oneirophanta mutabilis*, (b) *Psychropotes longicauda*, (c) *Pseudostichopus villosus,* and (d) *Paroriza prouhoi*. Error bars represent standard deviation from mean.

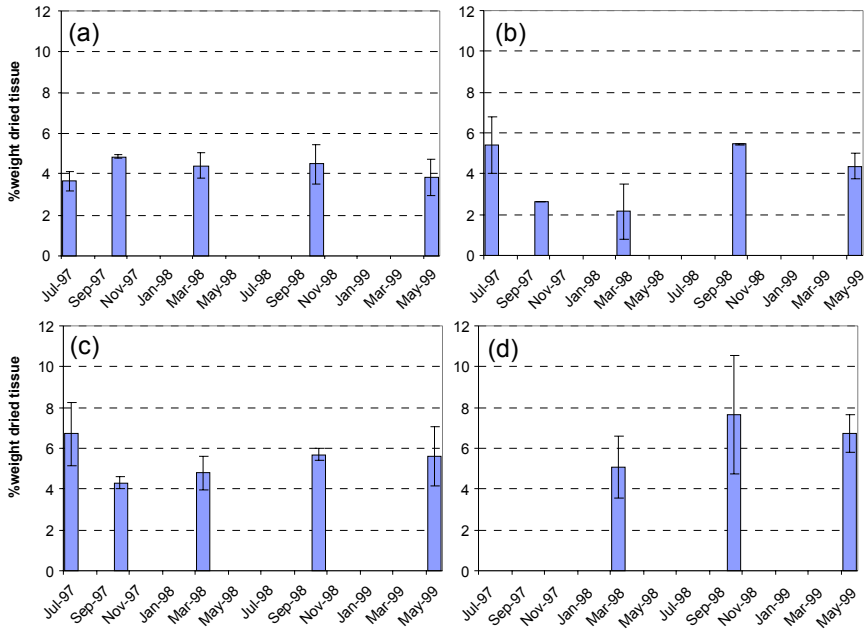


Figure 6.3. Temporal variation of TN in body tissues of (a) *Oneirophanta mutabilis*, (b) *Psychropotes longicauda*, (c) *Pseudostichopus villosus,* and (d) *Paroriza prouhoi*. Error bars represent standard deviation from mean.

6.3.2 LIPIDS

6.3.2.1. Compound classes

Concentrations (mg g^{-1} dry tissue) of total sterols, total fatty acids and total hydroxyacids were highly variable for all species (Table 6.2). Total concentrations of lipids and fatty acids in *Oneirophanta mutabilis* and *Psychropotes longicauda* varied temporally (one way ANOVA, $p < 0.05$), whereas they did not show any statistically significant variation in *Paroriza prouhoi* and *Pseudostichopus villosus* (Figs. 6.5 and 6.6). However, the

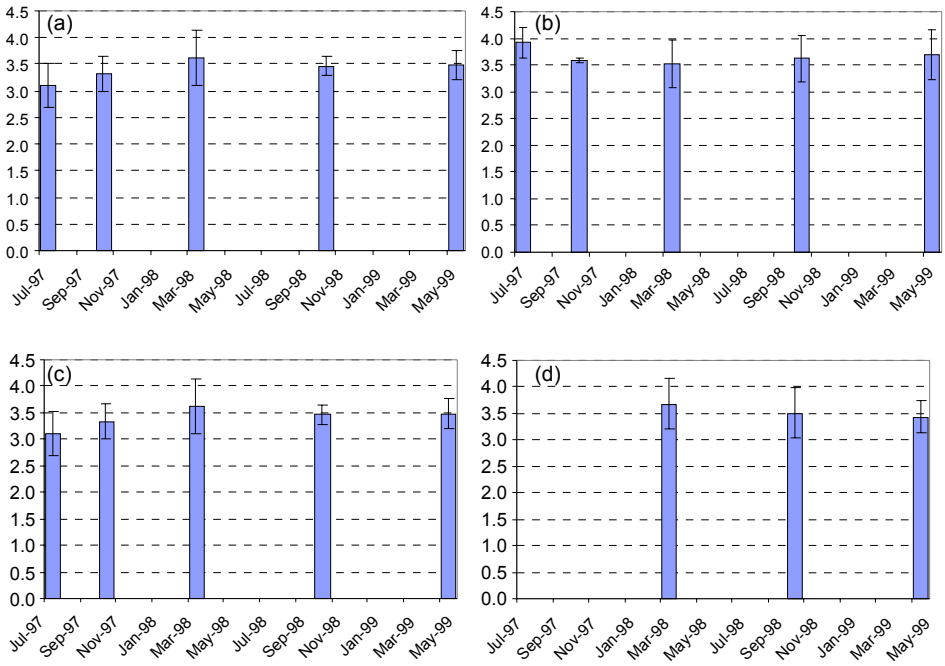


Figure 6.4. Temporal variation of C/N ratio in body tissues of (a) *Oneirophanta mutabilis*, (b) *Psychropotes longicauda*, (c) *Pseudostichopus villosus,* and (d) *Paroriza prouhoi*. Error bars represent standard deviation from mean.

Table 6.2. Range of concentration (mg/g of dried tissue) of total sterols, total fatty acids, total hydroxy-acids, and total lipids of *Oneirophanta mutabilis, Psychropotes longicauda, Paroriza prouhoi* and *Pseudostichopus villosus.*

	Species			
Total concentrations (mg g^{-1} of dried tissue)	*Oneirophanta. mutabilis*	*Psychropotes longicauda*	*Paroriza prouhoi*	*Pseudos-thicopus. villosus*
Sterols	0.58-9.75	0.54-19.7	1.46-5.76	0.26-11.2
Fatty acids	0.51-12.4	0.48-10.1	2.62-15.3	1.54-15.2
Hydroxy-acids	0.05-0.95	0.05-1.22	0.11-1.09	0.09-1.40
Lipids	1.23-20.7	2.59-31.2	4.53-19.3	1.95-22.3

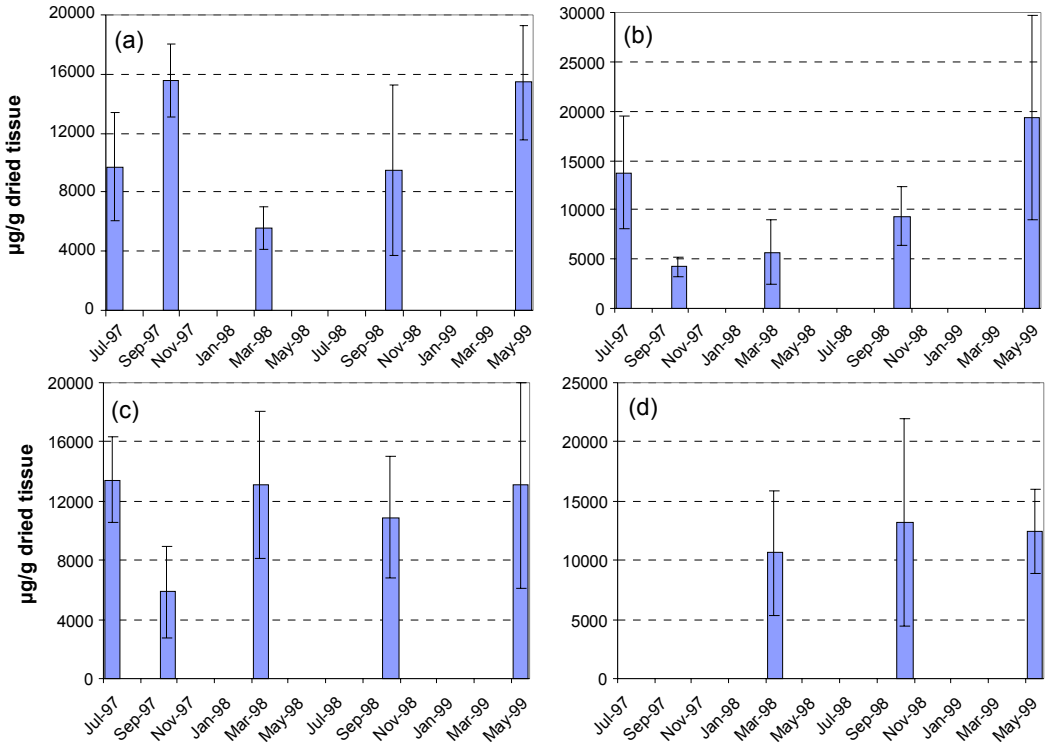


Figure 6.5. Temporal variation of total lipids in body tissues of (a) *Oneirophanta mutabilis*, (b) *Psychropotes longicauda*, (c) *Pseudostichopus villosus,* and (d) *Paroriza prouhoi*. Error bars represent standard deviation from mean.

latter exhibited a significant difference in these parameters between July and October 1997 (independent *t*-test, $p<0.05$). *Oneirophanta mutabilis* and *Psychropotes longicauda* also showed a significant difference in total lipid concentrations over this period. Thus, total lipid concentrations of *Pseudostichopus villosus* and *Psychropotes longicauda* decreased in the same way as those of surficial sediments (Fig. 6.7a; Mackenzie, 2000), whereas the values for *Oneirophanta mutabilis* increased, mainly reflecting the increase in relative concentration of the total sterols (Fig. 6.8a; the values for total fatty acids were similar, Fig. 6.6a).

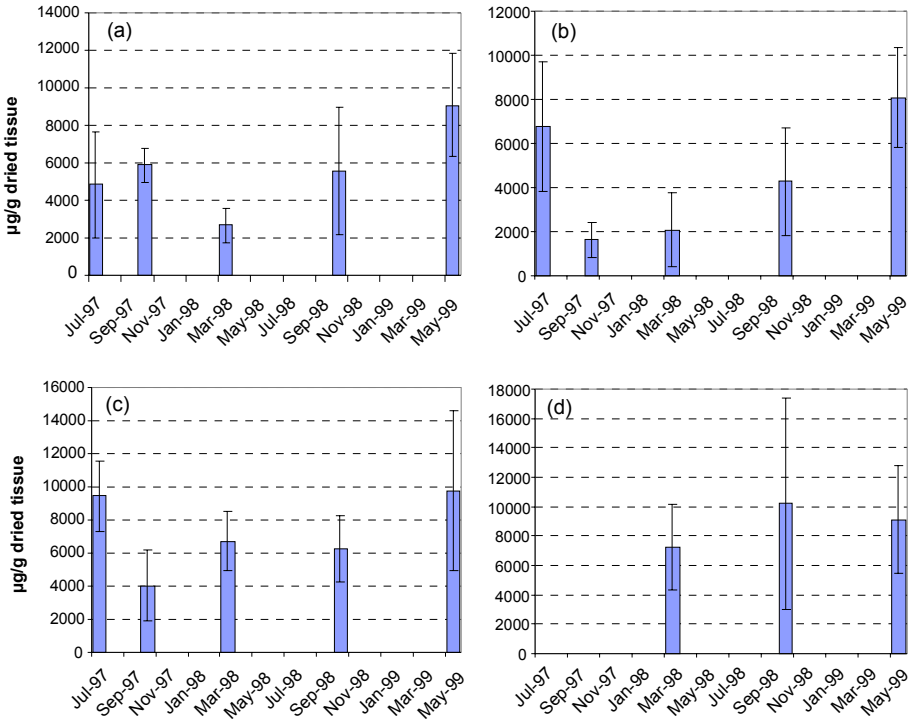


Figure 6.6. Temporal variation of total fatty acids in body tissues of (a) *Oneirophanta mutabilis*, (b) *Psychropotes longicauda*, (c) *Pseudostichopus villosus,* and (d) *Paroriza prouhoi*. Error bars represent standard deviation from mean.

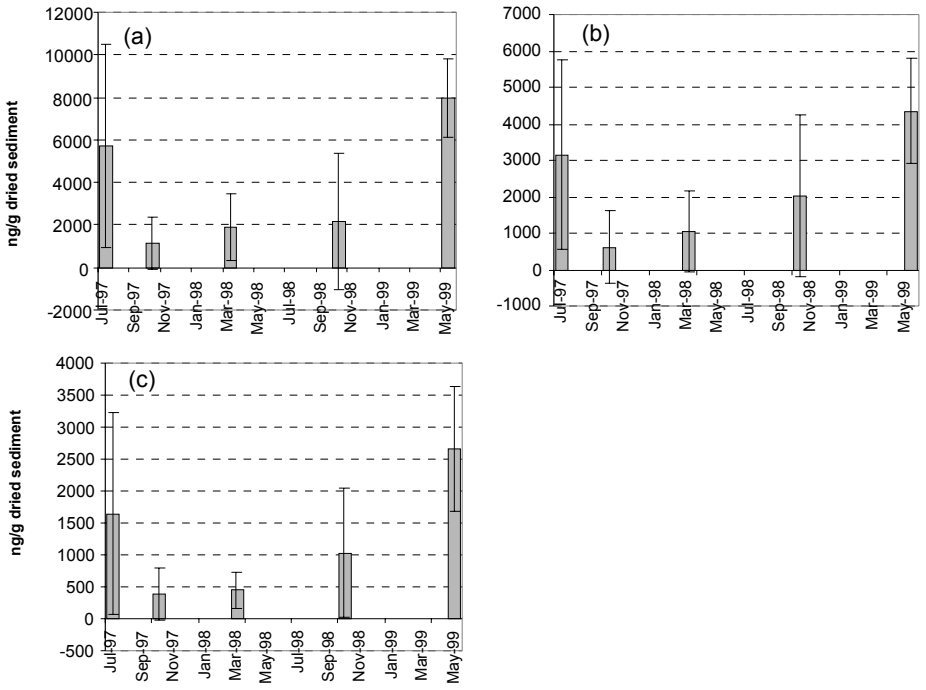


Figure 6.7. Temporal variation in the concentrations of (a) total lipids (b) fatty acids and (c) sterols in surficial sediments (0-5mm; data for July-97, October-97, March-98, and October-98 are from Mackenzie, 2000). Error bars represent standard deviation from mean.

Oneirophanta mutabilis was the only species to show variation in sterol content over the study period (one way ANOVA, $p < 0.01$, Fig. 6.8). *Psychropotes longicauda* did not show any statistically significant variation in its sterol, mainly because of the high standard deviation for May-99. However, differences between the sampled periods in 1997 were evident (independent *t*-test, $p < 0.005$, Fig. 6.8).

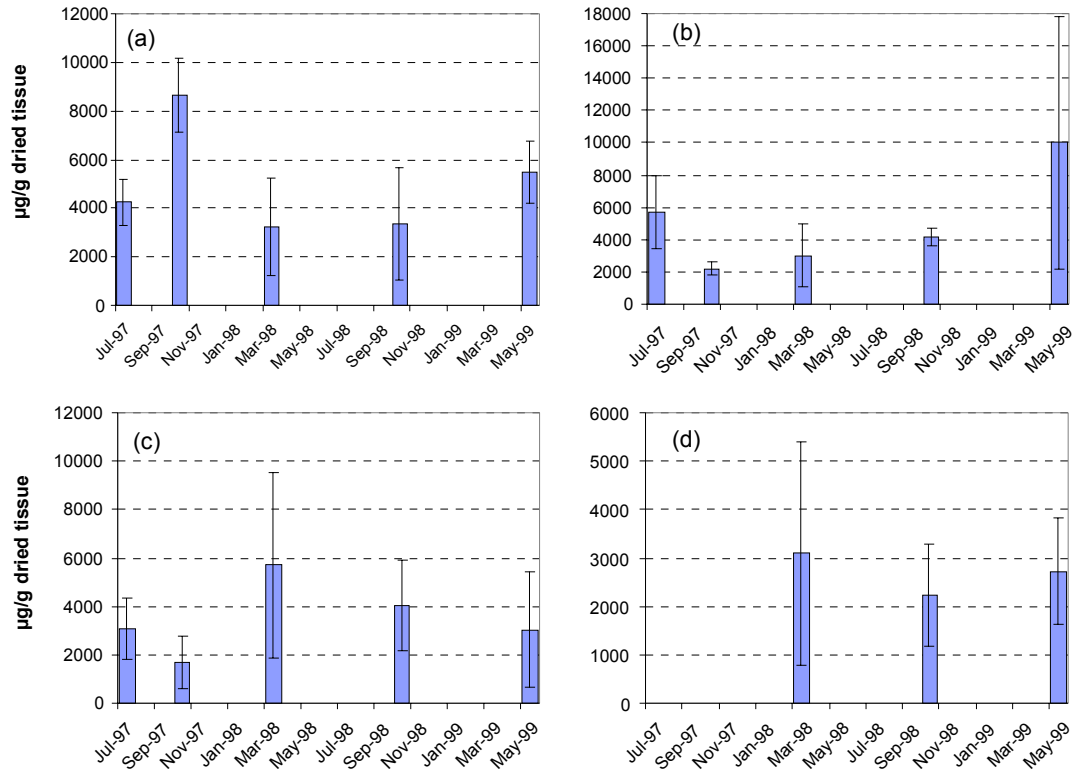


Figure 6.8. Temporal variation of total sterols in body tissues of (a) *Oneirophanta mutabilis*, (b) *Psychropotes longicauda*, (c) *Pseudostichopus villosus,* and (d) *Paroriza prouhoi*. Error bars represent standard deviation from mean.

Sterol contents of *Pseudostichopus villosus* and *Paroriza prouhoi* did not show any significant variation. In the case of *Pseudostichopus villosus* this was probably driven by the high standard deviation.

Hydroxy-acid concentrations in the body tissues of all species (except *Paroriza prohoi*) showed some variability. *Pseudostichopus villosus* and *Psychropotes longicauda* varied periodically (one way ANOVA, $p < 0.05$, Fig. 6.9). *Oneirophanta mutabilis* had significant differences in the hydroxyacid

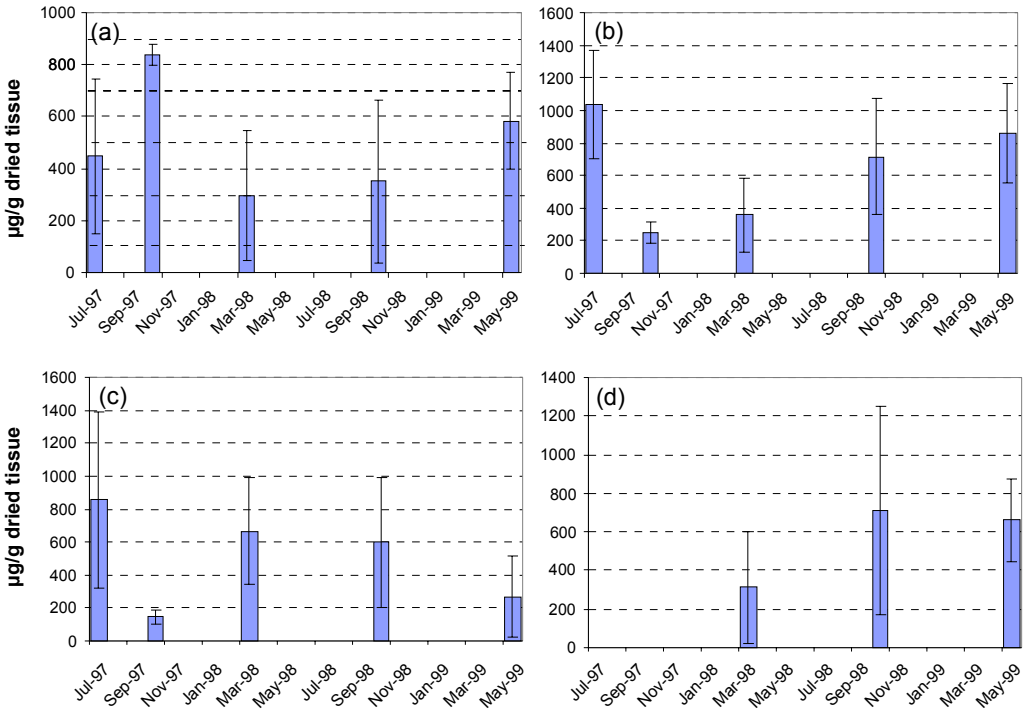


Figure 6.9. Temporal variation of total hydroxy-acids in body tissues of (a) *Oneirophanta mutabilis*, (b) *Psychropotes longicauda*, (c) *Pseudostichopus villosus,* and (d) *Paroriza prouhoi*. Error bars represent standard deviation from mean.

contents between July and October 1997 (independent *t*-test, $p<0.05$, equal variances not assumed). Note that the variation of total hydroxyacid contents of *Oneirophanta mutabilis* and *Psychropotes longicauda* correlated strongly with the variation in total sterols (Spearman rank correlation, R_s = 1.0, $P<0.001$; and R_s = 0.9, $P<0.05$; respectively).

6.3.2.2. Δ^5-Stenols/ 5α(H)-stanols (Δ^0) ratio

Oneirophanta mutabilis was the only species to show variability in the Δ^5/Δ^0 ratio over the study period (one way ANOVA, $p<0.05$, Fig. 6.10). The

variation in this ratio for *Psychropotes longicauda* was also significantly different between July and October 1997 (independent *t*-test, $p<0.005$, Fig. 6.10).

6.3.2.3. Steryl sulphates

24-Ethylcholest-5-en-3β-ol ($C_{29}\Delta^5$; **19**) was the main component of the pool of steryl sulphates for all species (55-100%; see Chapter 4). Thus, fluctuations in the concentration of this compound drove any variation in the total steryl sulphate pool.

Psychropotes longicauda was the only species to show a significant variability in steryl sulphate concentration over the study period (one way ANOVA, $p <0.05$, Fig. 6.11). The temporal variation in the concentrations of these conjugate sterols in *Psychropotes longicauda* had the same trend as the free 24-ethylcolest-5-en-3β-ol (**19**) in the sediments (Fig. 6.12; Mackenzie, 2000).

Although, the total steryl sulphate pools were not significantly different for *Oneirophanta mutabilis* over the study period, they did show a statistically significant fluctuation when normalized to TOC (Kruskall-Wallis test, $p<0.05$, figure not shown).

6.3.2.4.Lycopane

Lycopane (**29**) was identified as a major component in the tissues of *Oneirophanta mutabilis* (26.2±18.3 µg/g dried tissue, n=27). Small quantities were also present in *Pseudostichopus villosus* (1.20±1.82 µg/g dried tissue,

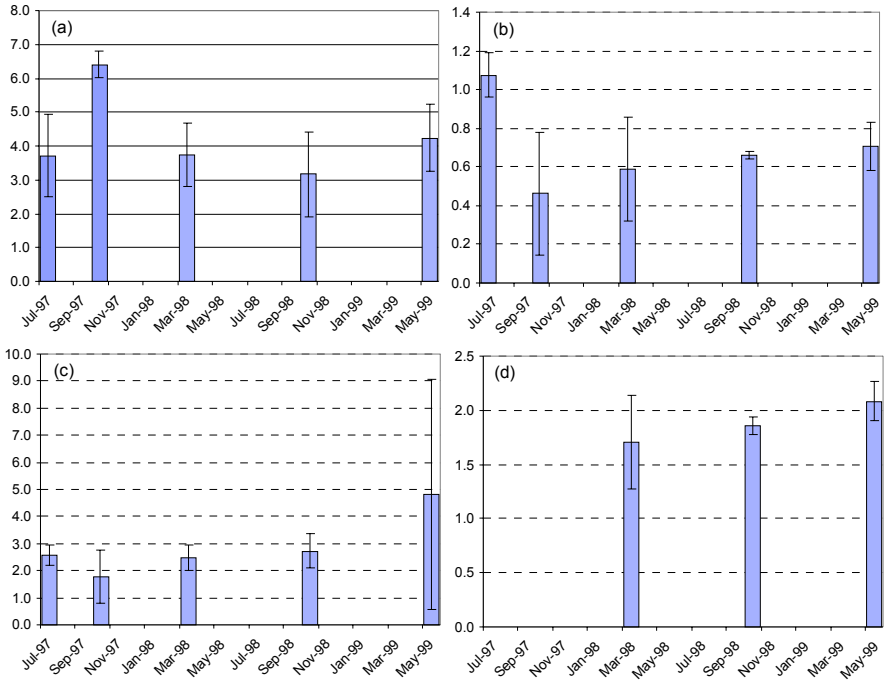


Figure 6.10. Temporal variation of Δ^5/Δ^0 ratio in body tissues of (a) *Oneirophanta mutabilis*, (b) *Psychropotes longicauda*, (c) *Pseudostichopus villosus,* and (d) *Paroriza prouhoi*. Error bars represent standard deviation from mean.

n=21) and in *Paroriza prouhoi* (3.93±4.08 µg/g dried tissue, n=10), but lycopane was absent in *Psychropotes longicauda.* As the compound was absent or close to detection limits in the latter three species, its temporal variation is discussed only for *Oneirophanta mutabilis*. Lycopane

concentrations (µg/g dried tissue) did not show any correlation with food availability (total lipids in sediments). Nevertheless, the variability of lycopane concentration normalized to TOC (Fig. 6.13) in *Oneirophanta mutabilis* showed a strong correlation with the total lipid concentrations in sediments (Mackenzie, 2000). Again, this could reflect the decrease of the standard deviations of the values, when the concentrations were normalized to TOC, simply because TOC values had lower variability in general. Error bars represent standard deviation from mean.

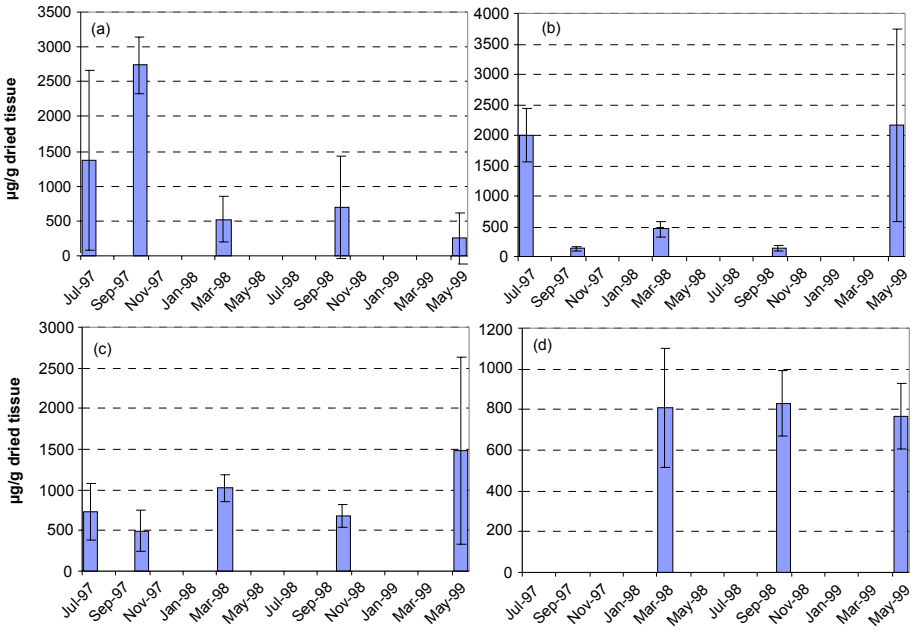


Figure 6.11. Temporal variation of total steryl sulphate in body tissues of (a) *Oneirophanta mutabilis*, (b) *Psychropotes longicauda*, (c) *Pseudostichopus villosus,* and (d) *Paroriza prouhoi*. Error bars represent standard deviation from mean.

6.3.2.5 PUFAs

For the purpose of the present study, the polyunsaturated fatty acids (PUFA's) were considered to be those with three or more degrees of unsaturation. For both *Oneirophanta mutabilis* and *Psychropotes longicauda*

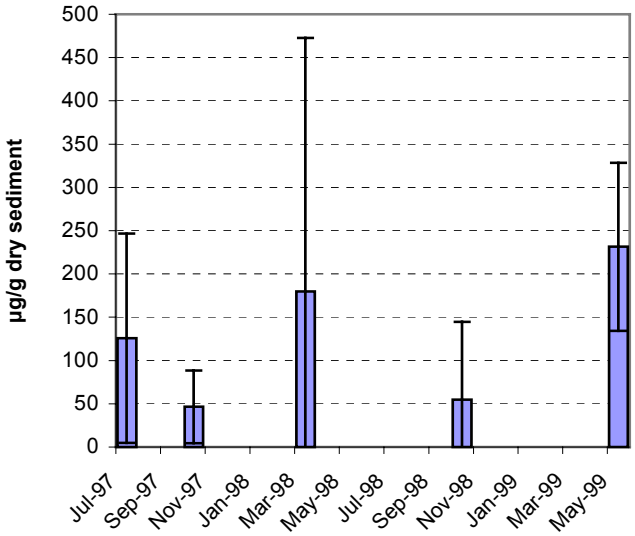


Figure 6.12. Temporal variation of 24-ethylcolest-5-en-3β-ol ($C_{29}\Delta^5$; **19**) present in sediments of the PAP (Data for July, October-1997, and March, October-1998 are from Mackenzie, 2000). Error bars represent standard deviation from mean.

their concentrations varied significantly (Figs 6.14a and 6.14b, respectively; Table 6.1). Only the latter showed any correlation with fatty acid concentrations in the sediments (Spearman rank correlation, R_s = 1.0, P<0.001; Mackenzie, 2000). PUFAs in both *Pseudostichopus villosus* and *Paroriza prouhoi* did not show a temporal variation over the study period (Figs 6.14c and 6.14d, respectively; Table 6.1).

6.3.2.6. Bacterial lipids

The total concentrations of the branched saturated fatty acids, *iso*-C15:0 (**30**), *anteiso*-C15:0 (**31**), *iso*-C17:0 (**32**) and *anteiso*-C17:0 (**33**) (Fig. 6.15), characteristic biomarkers of bacteria (*e.g.* Volkman *et al.,* 1980), were

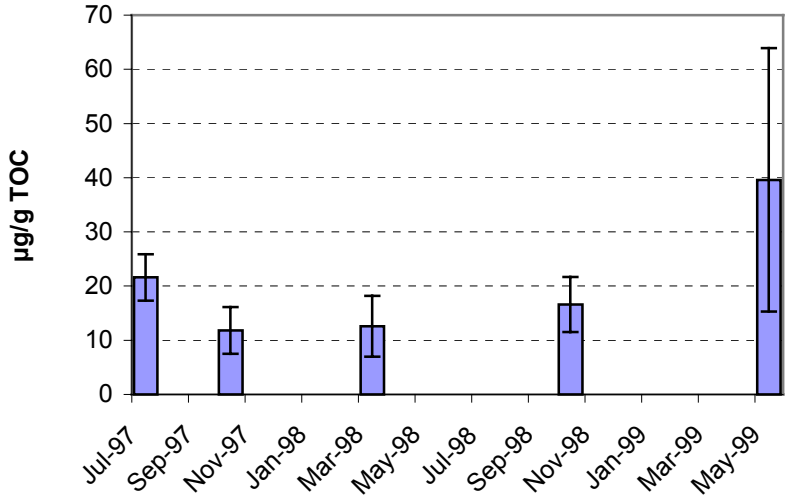


Figure 6.13. Temporal variation of lycopane (µg/g TOC) in *Oneirophanta mutabilis* body tissue. Error bars represent standard deviation from mean.

employed as potential markers of temporal variability in enteric bacterial activity. The bacterial biomarkers did not vary significantly in any of the species investigated (µg/g dried tissue, Fig. 6.16). However, their abundance relative to total fatty acids (% Bact FA) did vary significantly in *Oneirophanta mutabilis* and *Psychropotes longicauda* over the study period (Kruskall-Wallis test, $p<0.05$, Fig. 6.17). Error bars represent standard deviation from mean.

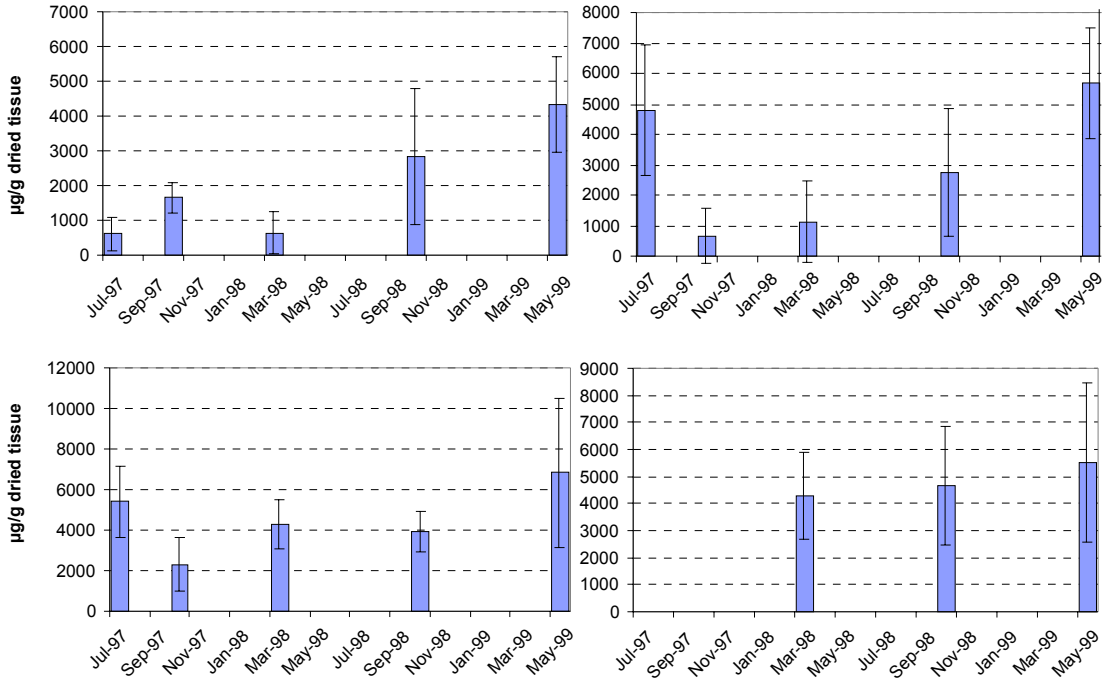


Figure 6.14. Temporal variation of total PUFAs in body tissues of (a) *Oneirophanta mutabilis*, (b) *Psychropotes longicauda*, (c) *Pseudostichopus villosus,* and (d) *Paroriza prouhoi*. Error bars represent standard deviation from mean.

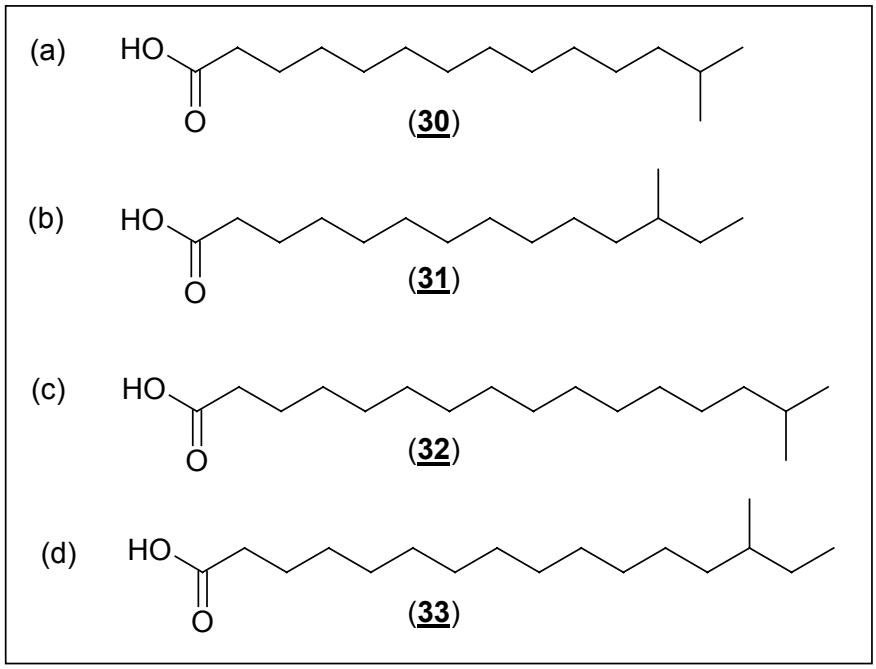


Figure 6.15. Bacterial biomarkers (a) *iso*-C15:0, (b) *anteiso*-C15:0, (c) *iso*-C17:0, and (d) *anteiso*-C17:0.

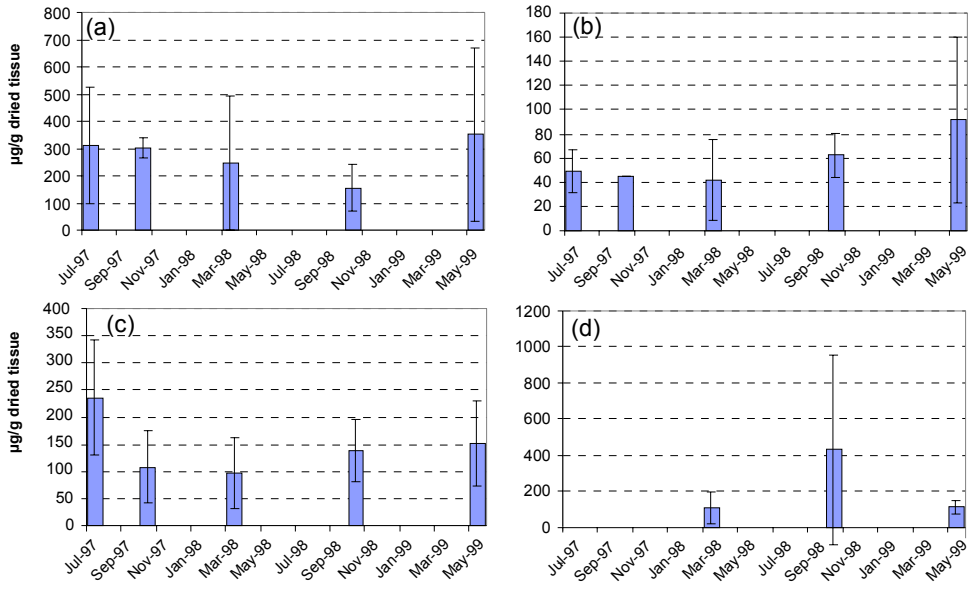


Figure 6.16. Temporal variation in concentrations of total bacterial biomarkers in body tissues of (a) *Oneirophanta mutabilis*, (b) *Psychropotes longicauda*, (c) *Pseudostichopus villosus,* and (d) *Paroriza prouhoi*. Error bars represent standard deviation from mean.

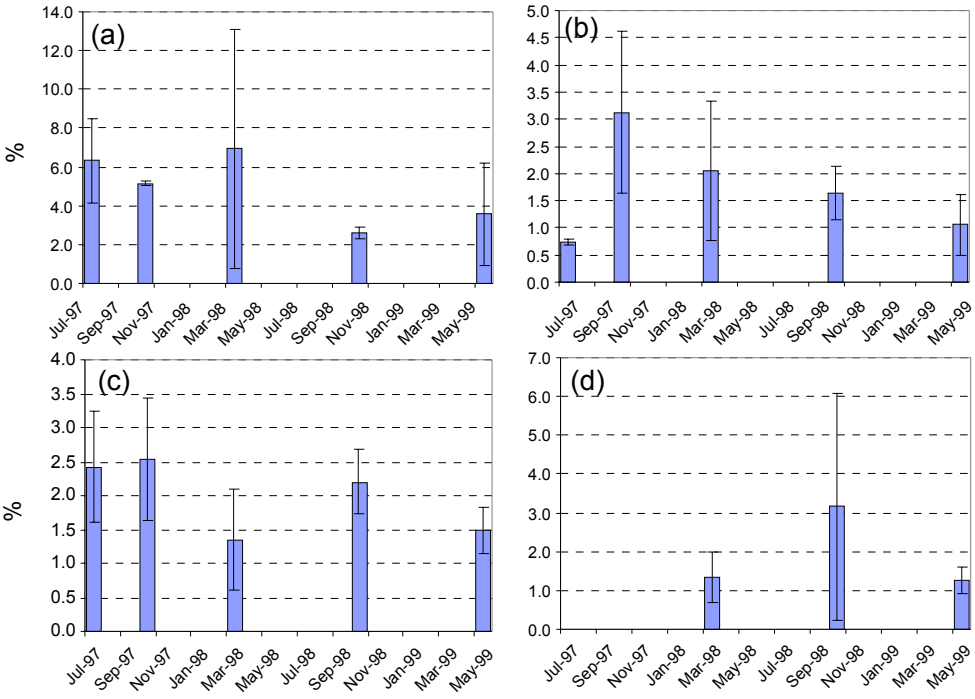


Figure 6.17. Variation in the percentage of total bacterial biomarkers relative to total fatty acids in (a) *Oneirophanta mutabilis*, (b) *Psychropotes longicauda*, (c) *Pseudostichopus villosus,* and (d) *Paroriza prouhoi*. Error bars represent standard deviation from mean.

6.4 DISCUSSION

6.4.1 TOC, TIC, TN, and C/N

TIC did not show any temporal variation in any of the studied species. Since this represents mainly calcium carbonate, this observation is not surprising. *Psychropotes longicauda* was the only species, which showed significant variability in TOC and TN contents, whereas *Oneirophanta mutabilis* only showed statistically significantly different concentrations of TN between July-1997 and October-1997. On the other hand, TOC values for *Pseudostichopus villosus* varied significantly, whereas TN values for this species were invariant over the study period.

The temporal variation of TOC and TN for *Psychropodes longicauda* is highly correlated and the C/N ratio is constant over the sampled period suggesting that the proportion of lipids+carbohydrates/proteins does not change with food supply (the same observation was made for all of the species), considering that the main forms of organic carbon and nitrogen in the holothurians' tissue are carbohydrates/lipids and proteins, respectively.

Organic compounds containing nitrogen have been suggested to be the limiting nutrients at deep-sea (Jumars *et al.,* 1990; Kiriakoulakis *et al.,* 2001). Invariant C/N ratios therefore imply that uptake of carbohydrates and lipids may be dependent on TN supply. The differences in temporal variability in TN and TOC contents among species may reflect the difference of feeding mode and diet of each species (see 6.4.2).

6.4.2. TOTAL CONCENTRATIONS OF LIPIDS

The total lipid contents of *Psychropotes longicauda* showed a strong positive relationship with the concentrations of lipids in surficial sediments at the PAP (0-5mm), as did components of the total lipids, namely the sterols and total fatty acids. This provides direct evidence that the metabolism of holothurians responds to food availability. Although the lipid content of *Oneirophanta mutabilis* did not correlate significantly with the lipids in surficial sediments (0-5 mm), they were nonetheless variable.

The main difference between these two species is that, for the former, the total lipid content decreased from July to October 1997 (Fig. 6.5b) in the same way as in the sediments (Fig. 6.7a), whereas those of *Oneirophanta mutabilis* increased over the same period. This increase was principally driven by total sterols (Fig. 6.8a), because total fatty acid concentrations (Fig. 6.6a) were invariant between July and October 1997. The inter-species differences are likely to reflect different responses to the variation of food supply, but the underlying factors need to be established (see *Section 6.1.2*).

Psychropotes longicauda and *Oneirophanta mutabilis* are both elasipodids, but they have completely different feeding strategies. Hence, their gut anatomies (Roberts *et al.,* 1996), morphologies (Hansen, 1975), rates of locomotion (Roberts et al, 2000) and tentacle structures (Roberts, 2000) differ. An explanation for the different biochemical response of these two species to food availability may be that their different feeding modes leads them to exploit different parts of the sedimentary OM. *Psychropotes*

longicauda is considered to use its peltate tentacles to sweep sediments into the mouth (Roberts, 2000). This feeding mode would tend to exploit the organic matter in the top ~5 mm of the sediments (Roberts, 2000), *i.e.* at the same level as the analytical resolution used for sediment lipid analyses in this study (also Mackenzie, 2000). Hence, the high correlation between lipid contents of *Psychropotes longicauda* body tissue and those found of the sampled sediments may reflect its feeding guild.

By way of contrast, *Oneirophanta mutabilis* is a picker, using its digitate tentacles to transfer sediment into its mouth, and thus tends to be more selective and to feed on the surface (~1 mm depth?) of the sediment (Roberts *et al.*, 2000). Witbaard *et al.* (2001) showed that temporal patterns of chlorophyll *a* content in the guts of *Oneirophanta mutabilis* was highly correlated to those found in the upper millimetre of the sediment, confirming that this species feeds on the top layer of sediment.

Kiriakoulakis *et al.* (2001) analysed the lipids of POM deposited in sediment traps at the PAP and observed that their composition varied temporally and with depth in the water column. During periods of high flux in 1997, fatty acids and alcohols predominated. Sterol concentrations were highest during low flux period 1 (LF1; 11 May 1997 to 01 June 1997) and post-high flux period 2 (PHF2; 13 July 1997 to 07 September 1997) at 1000m depth and 2 mab, respectively. Assuming that the POM trapped at 1000m in the LF1 period would take slightly more than 30 days to arrive at the seafloor (using a rate of deposition of 100 to 150 m/day; Tyler, 1988), then the sediment trap POM collected in both LF1 at 1000 m and PHF2 at 2 mab, represents material produced at the same time in the photic zone, and

was enriched in sterol. Therefore the increase in their concentration in the body tissues of *Oneirophanta mutabilis* observed between July and October 1997 (Fig. 6.8a) presumably reflects its ability to exploit this resource. Although there was no apparent enrichment in sedimentary sterols at this time (Mackenzie, 2000), this may simply reflect their removal by selecting feeding or the resolution of sediment sampling (to 5 mm), which may have masked (diluted) any temporal trend. Indeed, sterols (concentration in µg/g dried sediments) are the class of lipid compounds, which suffer most degradation with sediment depth (>60% within the first 5 mm; Madureira *et al.,* 1995). It is also possible that the distribution of sterol-rich material at the sea floor was patchy (Santos *et al.*, 1994), and the rather limited sampling missed enriched patches. *Oneirophanta mutabilis* would nonetheless have been able to search for and exploit such patches, because of its relatively rapid locomotion and its feeding guild (Billett, 1991; Witbaard *et al*, 2001).

In support of the hypothesis of selective feeding on sterol-rich material by *Oneirophanta mutabilis*, its Δ^5/Δ^0 sterol ratio was also much higher in October 1997 than at any other time (*cf. Psychropotes longicauda*; Fig. 6.10a&b). The ratio of 5α(H)-stanols/Δ^5-stenols has been used elsewhere (*e.g.* Wakeham *et al*., 1997) as a measure of bacterial degradation of OM, because bacteria can anaerobically reduce the double bound in the carbon at the 5-position (*e.g.* Fig. 6.18). However, the inverse ratio (Δ^5-stenols/5α(H)-stanols; Δ^5/Δ^0) is used here as a parameter of the "freshness" of *assimilated* sterols present in the body tissue of the four species. Deep-sea holothurians seem not to biosynthesise sterols *de novo*, relying on dietary sterols (Ginger *et al.*, 2000, 2001), which at the PAP (and

elsewhere) are dominated by Δ^5 compounds, both in POM and in the sediments (*e.g.* Santos *et al.,* 1994; Wakeham *et al*., 1997; Kiriakoulakis *et al.,* 2001). Δ^0 sterols are considered to be intermediates in the conversion of the dietary Δ^5 to Δ^7 sterols (Fig. 6.18), the latter being the main free sterol in most echinoderms (Kanazawa, 2001). In this study, free sterols were quantified together with the sulphated sterol pool, which was dominated by Δ^5 compounds and which probably acts as a sterol store. Hence, the higher the Δ^5/Δ^0 ratio, the "fresher" the pool of *assimilated* sterols is. It can be argued that *Oneirophanta mutabilis* (Fig. 6.10) was feeding on the sterol-rich material identified in sediment traps over the period July-October-1997 (Kiriakoulakis *et al*., 2001).

The depletion of sterols in surficial sediments at the PAP between July and October 1997 in comparison to 3000 m trap was considerably greater than that for fatty acids (Kiriakoulakis *et al*., 2001). This was attributed to the bloom of *Amperima rosea* in July 1997 (Billett *et al*., 2001; Ginger *et al*., 2001). The present study suggests that *Oneirophanta mutabilis* may also have contributed to the depletion of sterol in the PAP sediments.

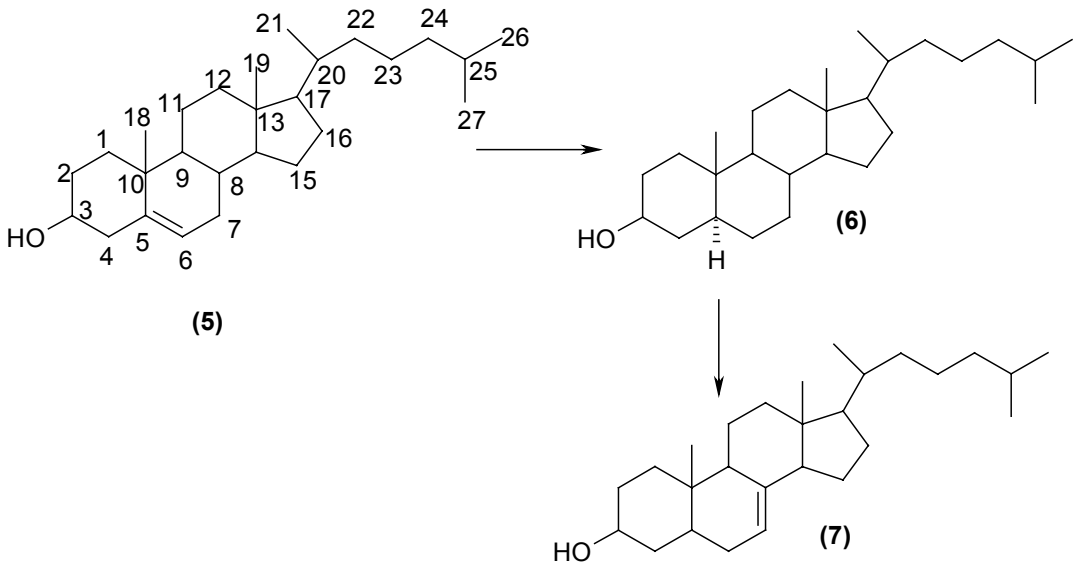


Figure 6.18. The conversion of cholest-5-en-3β-ol (**5**) to cholest-7-en-3β-ol (**7**) as an example of conversion of Δ^5 sterols to Δ^7 intermediated by Δ^0 in holothurians.

It is interesting to note that after October 1997 sterol contents of both *Oneirophanta mutabilis* and *Psychropotes longicauda* followed the same trend. This suggests that the former selects OM-enriched particles when possible, but that when these "hot spots" are absent, the two species compete for the same material. The gut contents of *Oneirophanta mutabilis* and *Psychropotes longicauda* contained similar amounts of this material (30% fresh phytodetritus, 70% sediment) in only one of the three periods studied by Iken *et al.* (2001), but the average $\delta^{15}N$ composition of *Oneirophanta mutabilis* tissue indicates that it primarily exploits fresh phytodetrituts as a food source. *Psychropotes longicauda* on the other hand appears to feed on more degraded material (Iken *et al.*, 2001).

Surprisingly, Wigham (2002) did not observe any difference in the pigments present in the guts of these two species, suggesting that they were feeding on the same sort of material. Furthermore, Witbaard *et al.* (2001), determined relatively low selection coefficients in *Oneirophanta mutabilis*, although these were influenced by food-poor periods at the PAP. Selection coefficients measure the concentrations of components of organic matter present in the guts relative to the sediments. The coefficient should be treated with caution, since if food is scarce, the quantity of organic matter present in the guts of the animals would tend to be close to that in sediments. This reinforces the idea that coefficient of selection could be seasonal (Billett *et al*., 1988) and "hot spot"-dependent.

According to Wigham (2002), the three major pigments in the guts of *Pseudostichopus villosus*, *Oneirophanta mutabilis* and *Psychropotes longicauda* were peridinin, fucoxanthin and 19' hexanoyloxyfucoxanthin (19'hex), which are major taxonomic pigments of dinoflagellates (Jeffrey *et al*., 1997). Kiriakoulakis *et al*. (2001) reported that the main sterol present in all trap sediments was cholest-5-en-3β-ol ($C_{27}\Delta^5$, **5**), apart from the 2 mab trap, which was dominated by 5α(H)-4α-methylcholestan-3β-ol (4-methyl-$C_{28}\Delta^0$; **34**). Cholest-5-en-3β-ol (**5**) is a major sterol of zooplankton (*e.g.* Wakeham *et al*., 1997). However, no zooplankton remains were identified in holothurians guts (Iken *et al*., 2001).

On the other hand, cholest-5-en-3β-ol (**5**) is also found as a main sterol in some prymnesiophyceae and dinoflagellates, which are abundant in surface waters overlying the PAP (Weeks *et al*., 1993). Moreover, 4-methylsterols are biomarkers of dinoflagellates, albeit they are also found in

some prymnesiophytes. Thus, based on the sterols distributions in the body tissues (this thesis), pigments and qualitative analysis of material in the guts (Wigham, 2002 and Iken *et al.*, 2001, respectively), it is possible to conclude that *Oneirophanta mutabilis* feeds preferentially on these planktonic species. In addition, Wigham (2002) suggested that the 'longer' and 'finer' tentacles of *Amperima rosea* and *Oneirophanta mutabilis* may allow them to 'select' and manipulate larger aggregates of phytodetritus, which the tentacles of the other surface deposit feeders would find hard to pick up.

Pseudostichopus villosus and *Paroriza prouhoi* did not show any statistically significant variation in total fatty acids, total sterols or total lipid contents. TOC and TC were variable in *Pseudostichopus villosus* but there was no correlation with sediment lipids. Roberts *et al.* (2001) found that harpaticoids were present in a greater number in *Pseudostichopus villosus* than in any other species. This species also had a low concentration of chlorophyll *a* in its gut contents, indicating ingestion of dominantly degraded material (Wigham, 2002). This is consistent with its relatively enriched $\delta^{15}N$ isotopic composition (16.18‰), and similar values for *Paroriza prouhoi* (15.83‰) indicates that both species feed on more refractory material than other holothurians (Iken *et al*., 2001). Only *Molpadia blakei*, which feeds in deeper layers of sediment (50-70mm) with it head buried in the sediments (Billett, 1991), had a similar isotopic composition (15.87‰). In addition, Tyler *et al.* (1992) did not found any seasonal variation on the reproduction and gut contents of two species of the genus *Paroriza*.

Thus, there is evidence that both *Pseudostichopus villosus* and *Paroriza prouhoi* feed sub-surficially, which probably explains why there is no

temporal variation in their lipid content (sedimentary lipid contents below 5 mm did not vary significantly over the study period, Mackenzie, 2000).

Based on these observations, it appears that some megafauna can respond directly to availability of food, but the impact of the pulsed deposition of phytodetritus depends on the feeding guild of each species. More exactly, it depends on which layer of sediments they are feeding on. Species feeding on fresh phytodetritus will be more influenced by variation in food supply than those which feed on deeper layers of sediments, where the food availability is less related to the pulse of phytodetritus.

These conclusions are important since they could explain why some organisms respond physiologically to pulses of phytodetritus whereas others do not (Tyler, 1988). For instance, the abundance of certain megafauna species at the PAP has changed, whereas others have not (Billett *et al.,* 2001). Based on the idea that the response to food availability depends on the ability to exploit food, the "*Amperima* event" (Billett *et al.*, 2001) could be explained in part by changes in food quality at the PAP. It is known that *Amperima rosea* feeds on "fresh" organic matter (Iken *et al.,* 2001). Thus, its bloom in 1997 (Billett *et al.,* 2001) could have been "fuelled" by the deposition of high quality POM in September 1996 (Witbaard *et al.*, 2001). Interestingly, the variation of the abundance of *Psychropotes longicauda* (although possibly not significant, Billett *et al.,* 2001) over the study period has the same pattern of variation as the lipids in sediments, and in its body tissue. Hence, its abundance decreased from July to October 1997, then increased from March to October 1998 (Billett *et al.,* 2001), and increased further to April 1999 (Wigham, 2002). This implies that the abundance of the

Psychropotes longicauda may be affected by food availability. The same argument could be used to explain the bloom of *Amperima rosea* (Billett *et al.,* 2001*)*, although there is no direct evidence to date than can confirm this.

6.4.3. TEMPORAL VARIATION OF STERYL SULPHATES.

The role of the steryl sulphates is still unclear (Chapter 3 and 4). However, the relationship between the variation of total steryl sulphate of *Psychropotes longicauda* and the temporal variation of the free 24-ethylcolest-5-en-3β-ol, the main sterol present in the sediments (Mackenzie, 2000) is striking and has a number of implications:

1. Part of the ingested free sterol pool is converted to steryl sulphates.
2. The variation of the concentration of these compounds in the free form in the diet is reflected by the concentration of the sulphated conjugate in the body tissues of this species. This confirms that the biochemistry and metabolism of holothurians is directly influenced by changes in food availability.
3. The pool of steryl sulphate is used as a store of sterols, and may be used when the supply of dietry sterols is low.
4. Sterols may be limiting micronutrients in the deep sea (Ginger *et al.*, 2001).

6.4.4. LYCOPANE

Oneirophanta mutabilis is the only species to contain high concentrations of lycopane (**29**), which was absent in the body tissues of *Psychropotes longicauda*, and present at very low concentrations in *Pseudostichopus villosus* and in *Paroriza prouhoi*. The interesting question is why is this unusual compound present in high concentrations only in *Oneirophanta mutabilis*?

Lycopane may have several origins, from:

1) Algaenan, a highly aliphatic, non-hydrolysable, insoluble macromolecular constituent of cell walls from phytoplankton (Freeman *et al*., 1994), most specifically from micro-algae (Behar *et al*., 1995).
2) Faecal pellets; there is a significant positive relationship between lycopane contents in POM and the relative contribution of faecal pellets, suggesting that this compound may derive from zooplankton grazing (Raoux *et al*., 1999). The presence of compounds in faecal pellets was not linked to the zooplankton themselves, but rather to external sources. Zooplankton pack dissolved and particulate matter into fast-sinking faecal pellets (Fowler *et al*., 1987).
3) Surface waters of the ocean, while additional sources from anaerobic microbial action on algal precursors may also be important (Wakeham *et al*., 1993). However, the well-oxygenated character of sediments at the PAP (250-290 μmol L^{-1} at

sediment/water interface; Rabouille *et al.*, 2001) makes this source less likely.

4) Kerogens, which could form from algaenans (*Salmon et al.*, 1997) and are very refractory. Hence, it is possible that lycopane could be extracted by the bacterial activity present in *Oneirophanta mutabilis* (based on microbial biomarkers, see Section 6.4.2.5).

5) Bacteria, since this compound has been found in thermophilic Archea (Lattuati *et al.*, 1998). Thermophilic bacteria have not been reported in the PAP sediments, but other Archea have been identified in the guts of *Oneirophanta mutabilis* (McInerney *et al*., 1995).

Lycopane concentrations normalized to TOC in *Oneirophanta mutabilis* showed a strong correlation with total fatty acids, total sterols and total lipid concentrations in the sediments (Spearman rank correlation, R_s =1.0, P<0.001). On the other hand, the concentrations of this compound did not significantly correlate with bacterially-derived compounds in its tissue. This suggests that lycopane derives in some way from food present in sediments (which may be extracted by gut bacteria). Alternatively, others sources that may respond to food supply, such as Archea could influence the biosynthesis of this compound.

The relationship between faecal pellets and lycopane is interesting. Some holothurians are able to select these particles (Billett, 1988), which have been observed in the guts of *Oneirophanta mutabilis* (Roberts *et al.*, 2000). The high concentration of lycopane in its body tissues suggests that this species may selectively feed on faecal pellets that are enriched in OM.

6.4.5. HOW DOES THE VARIATION OF FOOD SUPPLY AFFECT HOLOTHURIANS?

One of the impacts that sinking POM has on the benthos is that it may provide a food source for developing larvae in the water column and a labile food source at the sea bed for gamete development in adult echinoderms undergoing seasonal reproduction (Tyler and Gage, 1984). Indeed, the biomass of megabenthos changes most vigorously in response to a reduction of the carbon supply when compared to bacteria and macrofauna (Sibuet *et al.*, 1984, 1993). The question that arises is how are the holothurians affected by food supply? This discussion tackles this issue from a chemical perspective.

The temporal variation of α-hydroxyacids contents is strongly correlated with those of sterols in the body tissues of *Psychropotes longicauda* and *Oneirophanta Mutabilis*. This suggests that the function/storage of these two chemicals may depend on one another. Sterols are an essential component in the plasma membranes of animals, with multiple effects on the physical properties of membranes including membrane order (fluidity), phase behaviour, thickness, and permeability (Crockett, 1998). They play an important role in the health of animals and the variation in the sterol contents of the diet of holothurians at the PAP (Mackenzie *et al*., 2000; Kiriakoulakis *et al*., 2001) could affect their metabolism in general. Furthermore, marine sterols (Ayanoglu *et al.,* 1986; Ayanoglu *et al.,* 1988a&B; Li *et al.,* 1988; Ayanoglu *et al.,* 1990; Dai *et al.,* 1991) and hydroxyacids of the same chain length as those identified in this study (C_{24}),

have been observed to interact with and to be part of phospholipids, which are major components in cell membranes and are responsible for many of their properties (Thain and Hickman, 2001). Thus the lipid contents of PAP holothurians probably reflect their physiological condition. It should be noted that excess dietery lipids stored as triacylglycerols act as nutrient reserves (Hadley, 1985) and can be used during starvation (Lemcke and Lembert, 1975 in Meier *et al.*, 2000) and reproduction (Cargil *et al.*, 1985) in many animals. However, it is unlikely that deep-sea holothurians store tryacylglycerols as reserve of energy (Chapter 4), thus changes in food supply may have a large impact.

The other impact that pulsed phytodetritus deposition may have on deep-sea holothurians is the supply of key compounds necessary for reproduction. For example, eicosanoids, prostaglandins and their derivatives (Fig. 6.19) regulate reproduction in many marine invertebrates, including echinoderms (Howard and Stanley, 1999 and references therein). Eicosanoids play a role in egg hatching, spawning, egg release, vitellogenesis, induction of ovulation, egg production, egg mass deposition, oocyte maturation, intracellular calcium release from eggs, and prevention of polyspermic fertilization (Howard and Stanley, 1999 and references therein). There are no reports of eicosanoids in the abyssal environments to the knowledge of the author. However, these compounds are biosynthesised by enzymatic oxygenation of three C_{20} PUFAs: 20:3(*n*-6), 20:4(*n*-6), or 20:5(*n*-3) (Stanley, 1998), which are the major compounds (apart from 20:3(*n*-6), which is present in low quantities) in most of the holothurians analysed in this study (Chapter 4).

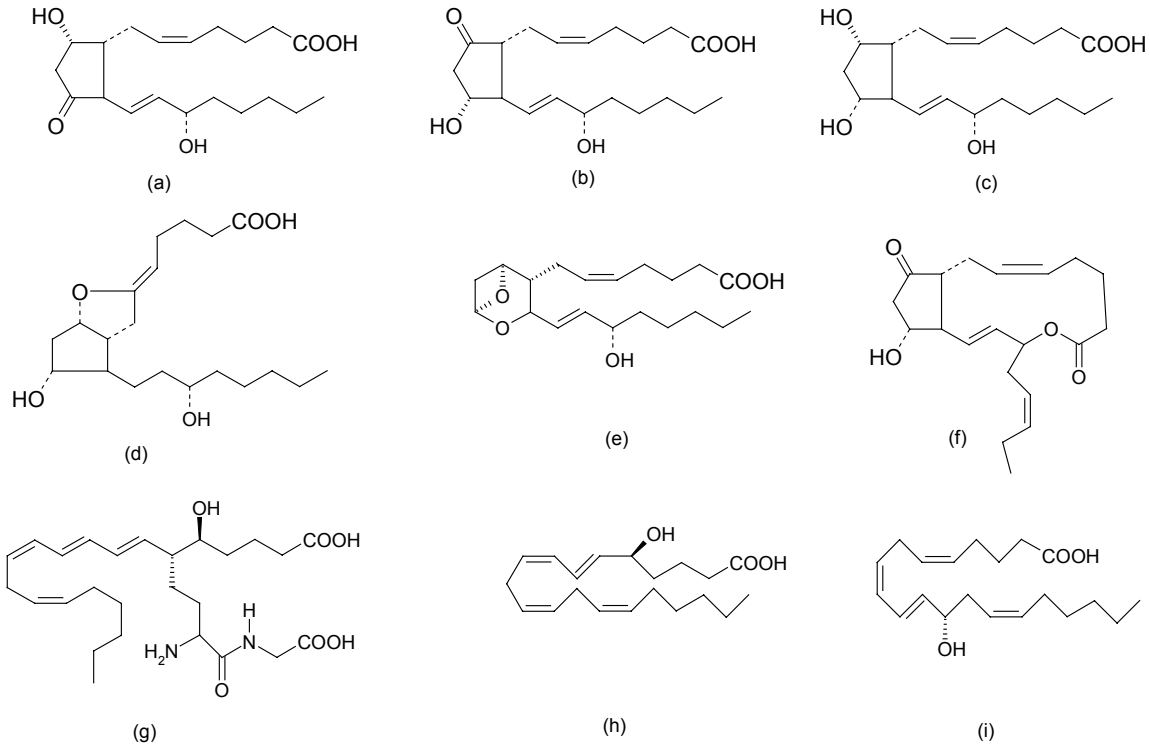


Figure 6.19. Eicosanoids reported from invertebrate organisms. (a) PGD_2, (b) PGE_2, (c) $PGF_{2\alpha}$, (d) PGI_2, (e) Thromboxane A_2, (f) PGE_3-1,15-lactone, (g) Leukotriene D_4, (h) 5-(S)-hydroxyeicosatetraenoic acid, (i) 12-(S)-hydroxy-eicosatetraenoic acid. Eicosanoids includes prostaglandins and their derivatives (a-f), leukotrienes (g), and hydroxyeicosatetraenoic acids (h,g; from Howard and Stanley, 1999).

The origin of these PUFAs in deep-sea holothurians is not yet clear, but it is believed that they are biosynthesized from other fatty acids (Ginger *et al*., 2000; Chapter 4) since PUFAs are not found in most of deep-sea sediments (Mackenzie, 2000; Wakeham *et al.,* 1997). The sea urchin *Psammechinus miliaris* can biosynthesize PUFAs when they are not present in their diet, (Bell *et al*., 2001); they are probably biosynthesized from C_{18}

fatty acids (Erwin and Bloch, 1964; Bell *et al.*, 2001), which are usually dominant lipids in deep-sea sediments (Mackenzie 2000; Wakeham *et al.,* 1997), and are also present in high quantities in the body tissues of holothurians (Chapter 4; Ginger *et al.,* 2000).

Thus, any extra supply of eicosanoids precursors or eicosanoids to the holothurians diet could modify reproduction process. As there is a significant variation of PUFA concentrations in the body tissue of *Psychropotes longicauda* relative to the fatty acid concentrations in surficial sediments, it might be expected that the levels of eicosenoids would fluctuate too, and as a consequence, influence reproductive processes.

In short, the pulse of phytodetritus to the deep-sea may supply key chemicals, such as eicosenoid precursors that play important roles in the reproduction. The variability of supply of these chemicals, thus, may influence seasonal and inter-annual changes in the reproduction processes of deep-sea fauna.

6.4.6. TEMPORAL VARIATION OF BACTERIAL BIOMARKERS.

Bacteria inhabit both the intestinal tracts (Deming and Colwell 1982; Roberts *et al.,* 1996) and the tentacles (Roberts *et al.,* 1991) of deep-sea holothurians. The presumed role of the gut bacteria is to help break down refractory organic matter into chemicals that can be easily assimilated by holothurians, for example by providing N-containing compounds which may be limiting (Deming and Colwell 1982; Jumars *et al.,* 1990; Roberts *et al.,* 1991; Roberts *et al.,* 1996; Roberts *et al.,* 2001).

It is known that bacteria in sediments can respond to the pulse of phytodetritus in a matter of hours (Gooday and Turley 1990). Enteric bacteria also seem to respond to food uptake by holothurians (Gage and Tyler, 1991). Temporal variation of bacterial contributions was investigated by examining the concentrations of lipid biomarkers, specific to bacteria in the holothurian body tissues.

The total concentrations of the bacterial biomarkers did not show any significant variation over the study period for any of the species (Fig. 6.16). On the other hand, their percentages relative to total fatty acids (% bact. FAs) varied for *Oneirophanta mutabilis* and *Psychropotes longicauda* (Fig. 6.17). The % bact. FAs for *Psychropotes longicauda* showed an inverse relationship with lipids present in both sediments and in its body tissues. This suggests two phenomena.

1. Bacteria are an important source of fatty acids and other nutrients to this species in periods of poor nourishment availability.

2. Bacterial abundance is higher during periods of low food availability, allowing them to break down refractory organic compounds and transform them to more readily absorbed compounds; and relatively lower when more organic matter is present in the sediments.

Although, these two roles may operate simultaneously, the latter is not supported by data presented by Roberts *et al.* (2001), where no temporal variation (2 cruises; August-September 1996 and March-April 1997) in bacterial abundance and activity in the guts of *Pseudostichopus villosus*, *Oneirophanta mutabilis* and *Psychropotes longicauda* was evident. On the

other hand, their data for *Psychropotes longicauda* were incomplete and the study missed the critical period between July and October 1997.

The observation of apparent variation in bacterial activities in *Psychropodes longicauda* may reflect its digestive strategy. Roberts *et al.* (2000 and 2001) suggested that the expanded rectum of *Psychropotes longicauda* functions as a fermentation chamber. Hence, the rectum contents of freshly dissected specimens have high bacterial abundances and a characteristic odour. Although Roberts *et al.* (1996) showed that the oesophagus and the rectum of *Psychropotes longicauda* have approximately the same volume, Deming and Colwell (1982) observed that an important component of the ingested bacterial population appears to survive the initial digestive processes of the foregut and to flourish in the hindgut.

Animals that employ hindgut fermentation tend to exploit more refractory material, whereas those with foregut fermentation exploit fresher material (Roberts *et al.*, 1996). Thus, it seems that *Psychropotes longicauda* uses this fermentation chamber as a plug-flow reactor (see above), to extract organic matter from sediments when the food is present in low quantity.

The other species have different gut structures, not having an expanded hindgut (Roberts *et al*., 1996) and the role of bacteria is not clear. Bacterial biomarkers increased in the gut contents from the oesophagus to rectum in *Oneirophanta mutabilis* (19.1-29.0%; Mackenzie, 2000). This reinforces the view that the gut of *Oneirophanta mutabilis* works as a continuous plug flow reactor (Roberts *et al*., 1996) and the high quantity of bacterial biomarkers present in the body tissues of this species (Chapters 4 and 5) may be

reflected by bacteria present in the tissue and tentacles. Roberts *et al.* (2000) reported on the presence of bacteria in the tissues of *Pseudostichopus villosus, Paroriza prouhoi, Oneirophanta mutabilis* and others. Indeed, the large tentacles of *Oneirophanta mutabilis* and their associated bacteria may play an important role in extracting food from its surrounded environment, perhaps more than the other species.

Based on the comments above, it seems that enteric bacteria help extract food from sediments and thus are important in the nourishment for deep-sea holothurians mainly when food is scarce; at least in the case of *Psychropotes longicauda*. On the other hand, *Oneirophanta mutabilis* does not have the expanded rectum, but its high content of bacterial biomarkers suggests that they may have a elevated bacterial activity associated with the tentacles, which can help this species to extract organic nutrients from its environment as suggested by Roberts *et al.* (1991).

6.5 CONCLUSIONS

- Direct biochemical evidence indicates that holothurians can respond to food supply. Until now, only smaller organisms were thought to be influenced by the deposition of phytodetritus mainly because they have a shorter generation time. Nevertheless, this long temporal study shows a clear response is some larger organisms.
- The correlation between lipids in sediments and in body tissues of *Psychropotes longicauda* is striking, and contradicts the idea that deep-sea biological processes proceed at slower rates than those in

shallow waters (Roberts *et al.*, 2000). Indeed biochemical compounds could have a turnover time of weeks or possibly days.

- The response of each species to a pulse of phytodetritus depends on its feeding mode, tentacle structure and gut morphology. The relationship between the biochemistry of the holothurians and the presence or absence of phytodetritus depends on their feeding guilds.
- Roberts *et al.* (2000) pointed out that there is not enough evidence that bacteria contribute directly to diet of holothurians up to date, however, from the data presented here, it seems that bacteria help to extract food from sediments and may also be an important source of nourishment for *Psychropotes longicauda*, particularly when food is scarce. This holothurian has an expanded hindgut that is used as a fermentation chamber (PFR), where bacteria probably transform refractory material to easily assimilated compounds.
- The sterols of *Oneirophanta mutabilis* and their variability together with data presented in the literature (Iken *et al*., 2001; Wigham, 2002) suggest that this species feeds preferentially on particles derived from dinoflagellates and prymnesiophyceae.
- Although *Oneirophanta mutabilis* is more selective than *Psychropotes longicauda*, it may feed on the same material at some period of times when particles rich in organic matter are scarce. Shifts in feeding modes are known for some species of holothurian (Roberts *et al.*, 2000).
- *Oneirophanta mutabilis* fed preferentially on particles rich in sterols over the period between July and October 1997. Thus, it is suggested

that this species also contributes to the rapid removal of sterols in the sediments observed by Mackenzie (2000) over that period, which was previously attributed to *Amperima rosea and Ellipinion molle* (Ginger *et al.*, 2001).

- The supply of key nutrient chemicals could accelerate biological processes, including reproduction (the “eicosaniods” hypothesis), and thus, could alter recruitment of these animals in the deep-sea.
- Lycopane present in *Oneirophanta mutabilis* is most likely to derive from algaenans and faecal pellets or Archea. However, more research is needed to clarify its origin.

Chapter 7

GENERAL CONCLUSIONS AND FUTURE WORK

7.1 SUMMARY

In order to discuss the conclusions of this thesis, the key questions regarding the nutrition of holothurians in the deep sea and their biochemical composition (outlined in Chapter 1) are addressed here:

1) ***Does the biochemistry of the holothurians reflect the ambient level of nutrition (quantity + quality of food supply)?***

   The observations described in Chapters 5 and 6 show that the biochemistry of these animals reflects their diet. Bulk parameters such as sedimentary TOC, TN and TIC do not influence their biochemistry. On the other hand, the concentration of total lipids and the sub-groups (fatty acids, sterols, steryl sulphates and total hydroxyacids), as well as their molecular distributions seem to reflect the quality of available nourishment. This is most noticeable for sterols, probably as most of these compounds are not biosynthesised *de novo*, deriving solely from dietary assimilation. The dominant steryl sulphate in all holothurians is 24-ethylcholest-5-en-3*β*-ol (**19**), which is also one of the main free sterols in the sediments. Thus, it is suggested that steryl sulphates act as a reserve of sterols. Certain fatty acids, such as the PUFAs, on the other hand, may be

biosynthesised by holothurians, although other compounds, such as the bacterially-derived fatty acids are assimilated directly.

2) Does the biochemistry of the animals reflect biological processes? e.g. growth, reproduction, etc.

This question is still open and is addressed in section 7.2.

3) Does the biochemical response to enrichment of the sea floor by an influx of POM depend on feeding (guild) mode, gut structure, metabolism and life mode?

A) The correlation between the lipid contents in *Psychropotes longicauda* and those in sediments (top 0-5mm) is striking and probably reflects the feeding mode of this species. On the other hand, species that are thought to be more selective feeders, such as *Oneirophanta mutabilis* and those animals that feed on deeper sediments, namely *Paroriza prouhoi* and *Pseudosthicopus villosus* showed some temporal variability, but no correlation with surficial sediment lipids was apparent. There was, however, a significant relationship between sterol contents of *Oneirophanta mutabilis* and the sterol supply to the sea floor. Thus, this author suggests that these animals can respond rapidly to food availability. It can be concluded that all animals respond to fluctuations in their nourishment, understandably, animals feeding on deeper sediments will be less affected by the pulse of phytodetritus.

b) *Psychropotes longicauda* has a rectum that may acts as a fermentation chamber. Hence, the inverse relationship between % bact. FAs in *Psychropotes longicauda* and lipids present in both sediments and in its body tissues may reflect the reliance of this animal on bacterial fermentation to break down refractory OM during periods of food scarcity.

c) Sterol distribution in species from the WAP and the PAP were similar to those found in its surrounding surficial sediments, apart from *Amperima rosea* (at the PAP). The distinct sterol biochemistry of this species may arise from their life mode. *Amperima rosea,* which has a sterol distribution similar to that of fresh phytodetritus, is known for its rapid rate of locomotion and its selective feeding mode.

d) Similarly, *Peniagone* sp. seems to feed in the water column, and may feed selectively on zooplankton; thus its sterol composition is dominated by a zooplankton biomarker, cholesta-5-en-3β-ol (**5**).

Thus, the present study provides enough evidence to show that the biochemistry of holothurians depends on their feeding and life modes, as well as their morphology (*e.g.* gut structure) and that the animals respond to variations of food supply. The differences between species with different feeding guilds may also shed light on the dilemma that some animals respond to varying food supply while others do not (Tyler, 1988).

4) Do bacteria play an important role in the nutrition of holothurians?

Two species had particularly high concentrations of bacterially-derivated fatty acids, *Oneirophanta mutabilis* and *Bathyplotes* sp. The gut of the former does not function as a fermentation chamber (Roberts *et al*., 2000). On the other hand, this species has long tentacles, and subcutaneous bacteria may be a source of these compounds (Roberts *et al*., 1991). The gut volume of the latter is much bigger and may allow bacterial fermentation, however, more research is needed to be definitive. As discussed above, bacteria may help *Psychropotes longicauda* to extract food in periods of low food availability. Thus, high concentrations of bacterial biomarkers in some species show that bacteria are important in the nutrition of these animals.

The objectives of the present study have been met, at least in part (except question 2, this Chapter). Additionally, other important conclusions were made:

- The concentration of unsaturated fatty acids in animals from the PAP is significantly higher than those in holothurians from the WAP. This difference probably reflects the difference in water depth (5000 vs. 500 m, respectively) and thus the pressure regimes at the two sites (see Ginger *et al*., 2000).
- The apparent lack of, or low concentrations of triacylglycerols and other forms of chemical energy storage in holothurians, would suggest that they do not possess energy reserves. However, steryl sulphates may act as a

store of sterols in low food periods. Lipids may also have a role in the negative buoyancy of holothurians.

- Although there are some common trends in the distribution of lipids between species, there were inter-species differences, suggested by cluster analyses.
- Photographic evidence suggested that there was an absence of POM arriving at the PAP sediment surface in 1997 (Bett *et al.*, 1997). However, the striking relationship between the biochemical contents of holothurians and their available food shows that these animals can function as a food marker. Apparently, they do not store chemical at a long term, and their response to food availability may be quicker than previously though. As a consequence, the biochemical contents of these animals may be good indicators of temporal variability in the deep-sea. Both sediment cores and sediment traps have associated problems and difficulties in their deployment and recovery. Sediment cores often arrive on-board disturbed, and sediment traps may suffer the action of “swimmers” or other problems (Lampitt *et al.*, 2001). Furthermore, the limited sampling opportunities of these techniques make representative studies of temporal variability in the deep sea difficult. On the other hand, although still difficult, holothurians, collected frequently by other-trawl, do not suffer any disturbance (apart from the wall guts, which may suffer cell lysis; Ginger *et al.*, 2000). These

animals living on the seabed are certainly better "biological-samplers" than conventional methods of determining temporal variation of flux of POM. Different species may well respond to change at different levels; *Oneirophanta mutabilis* could be a good indicator of food availability in surficial sediments, while *Psychropotes longicauda* would probably respond to temporal variation in the top 5 mm.

7.2 FUTURE WORK

The discussion and conclusions of the present study gave rise to several ideas for future work:

1) Starting from the unanswered question (2) in Section 7.1, this author speculates that these biochemical temporal variation driven by food availability, may have significant impact on the biology of holothurians. In Chapter 5, the importance of eicosanoids in the reproduction of some marine organisms is discussed. However, further work has to be carried out to fully tackle this point. A temporal study of the concentration of eicosanoids in the bodies and gonads of holothurians would, shed light on this question.
2) To study coupling between SCOC and labile organic matter markers such as unsaturated fatty acids. It seems that the lack of correlation between SCOC and flux of organic matter to the deep-sea is characterized mainly by the poor quality of organic matter (Duineveld *et al*., 1997). Thus, it

would be expected that the SCOC would respond better to more bioavailable chemical compounds. This would improve the use of lipids as biomarkers of benthic response to food supply.

3) Th_{xs} has been proved to be a powerful indicator of labile or refractory OM in both sediments and animals (Pope *et al.*, 1996; Miller *et al*., 2000). It would be fruitful, to perform a series of analyses of lipids and Th_{xs} in body tissue samples to find out if the signal of the latter is linked to some individual or group of compounds. This would underpin the hypothesis that the rate of assimilation of labile OM can be measured by a lipid "freshness" index (*e.g.* Δ^5/Δ^0).

4) The analysis of $\delta^{13}C$ can be biased by the lipid content of organisms being investigated (Iken *et al*., 2001 and references therein). However, the determination of the isotopic composition of $\delta^{13}C$ of individual lipids by gas chromatography isotope ratio mass spectrometer (GC-IR-MS) may be fruitful in the assessment of trophic levels. Furthermore, it would provide a means of identifying assimilated *vs.* biosynthesised biochemicals.

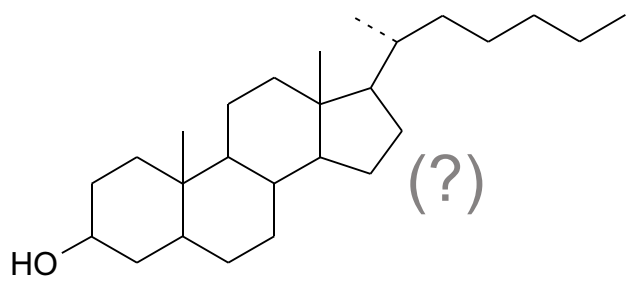
5) The lipid indexes discussed in Chapter 5 could be further developed for holothurians that feed in different layers of the sediment, and in the sediments itself. This would lead to more evidence that animals feeding at different layers of sediment respond differently to phytodetritus deposition.

Finally, although, the present study has advanced in the understanding of holothurians' responses to food supply, there are still plenty of unknown

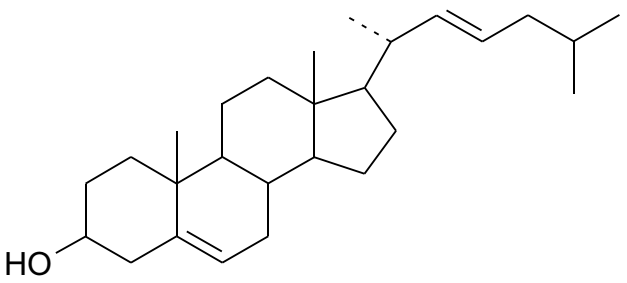
processes at the BBL (at a regional and temporal basis), which involves phytodetritus deposition and benthic activity. Future work involving long term studies, perhaps over longer time periods than this work, may lead to further clues of how primary productivity, phytodetritus deposition and benthic activity are coupled.

APPENDIX A

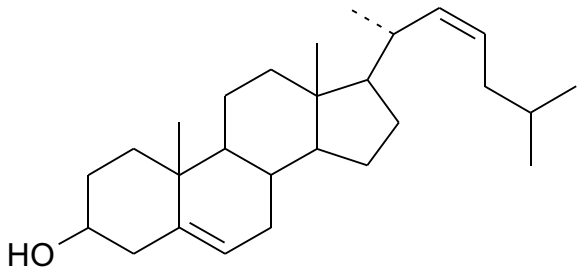
List of names, short hand notations, structures and reference numbers of sterols and other relevant lipids



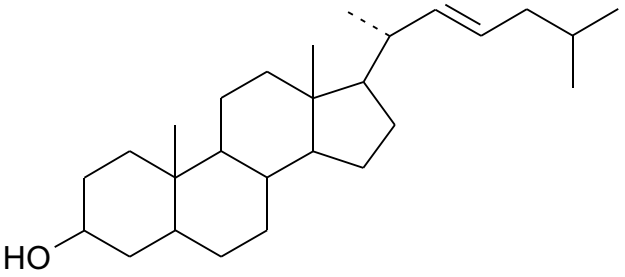
$C_{26}^{\ 0}$ **(1)**



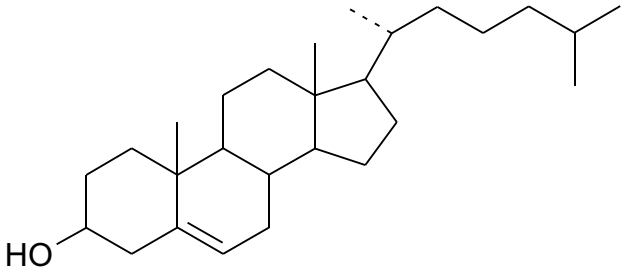
Z-$C_{27}^{\ 5,22}$ **(2)**
Z-cholesta-5,22-dien-3 -ol



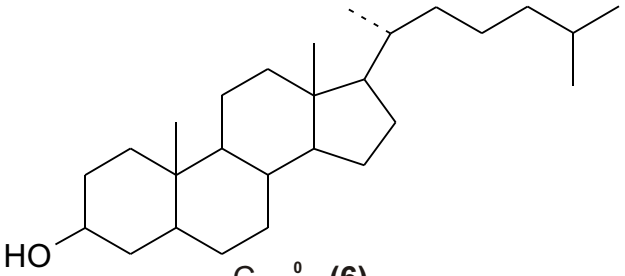
E-$C_{27}^{\ 5,22}$ **(3)**
E-cholesta-5,22-dien-3 -ol



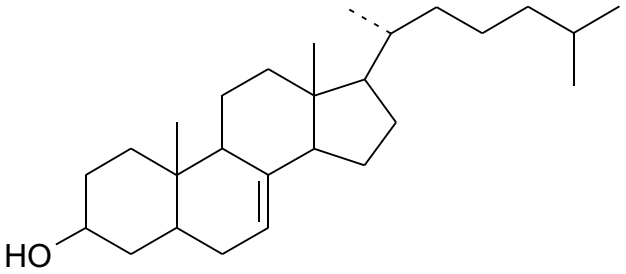
$C_{27}^{\ 22}$ **(4)**
Cholest-22-en-3 -ol



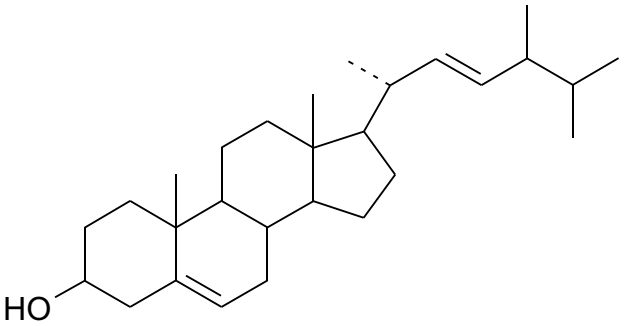
$C_{27}^{\ 5}$ **(5)**
Cholest-5-en-3 -ol
(cholesterol)



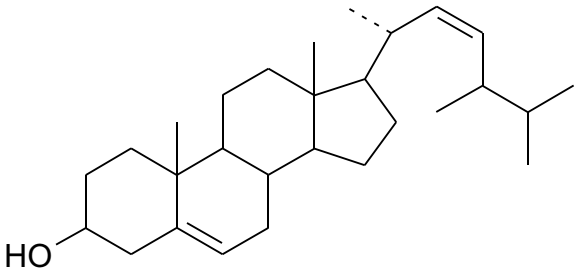
$C_{27}^{\ 0}$ **(6)**
5 (H)-Cholestan-3 -ol
(dihydrocholesterol)



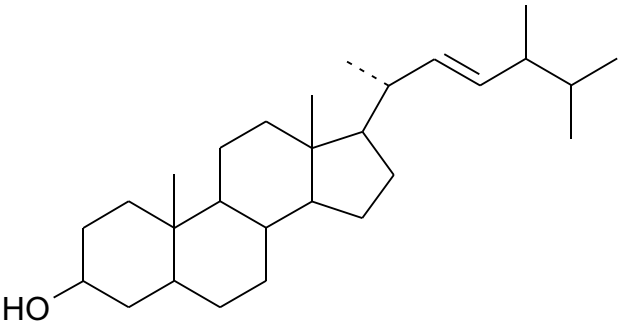
$C_{27}^{\ 7}$ **(7)**
Cholest-7-en-3 -ol
(*lathosterol)*



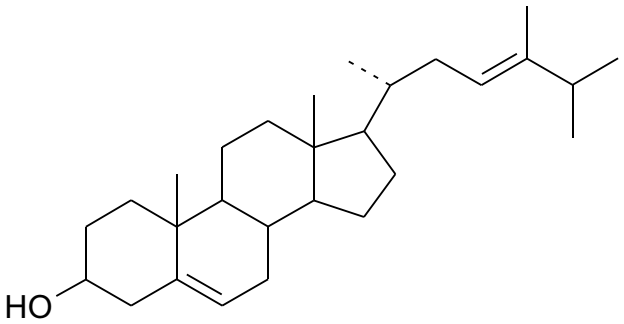
Z-$C_{28}^{\ 5,22}$ **(8)**
Z-24-methylcholesta-5,22-dien-3 -ol
(brassicasterol)



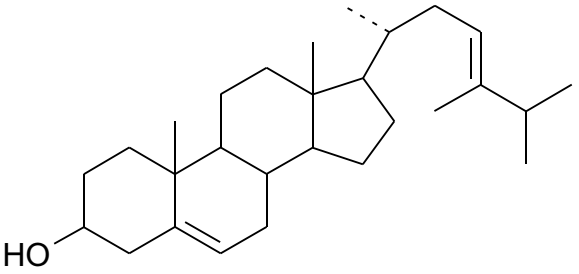
E-C_{28} ^{5,22} **(9)**
E-24-methylcholesta-5,22-dien-3 -ol



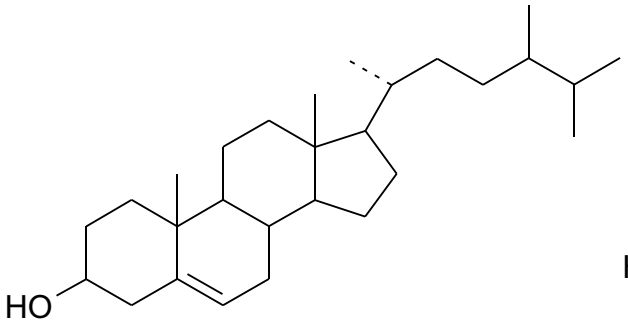
C_{28} ^{22} **(10)**
24-methylcholest-22-en-3 -ol



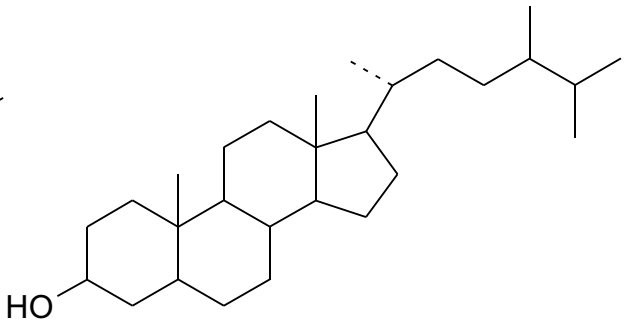
Z-C_{28} ^{5,24} **(11)**
Z-24-methylcholesta-5,24-dien-3 -ol



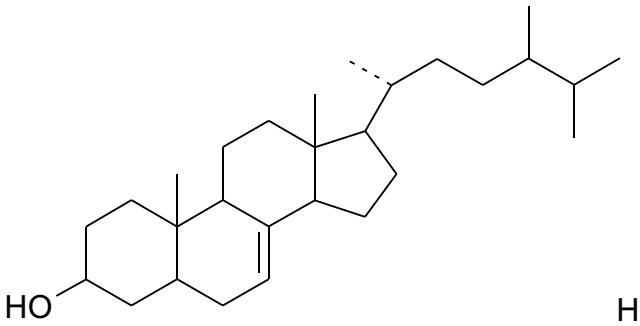
E-C_{28} ^{5,24} **(12)**
E-24-methylcholesta-5,24-dien-3 -ol



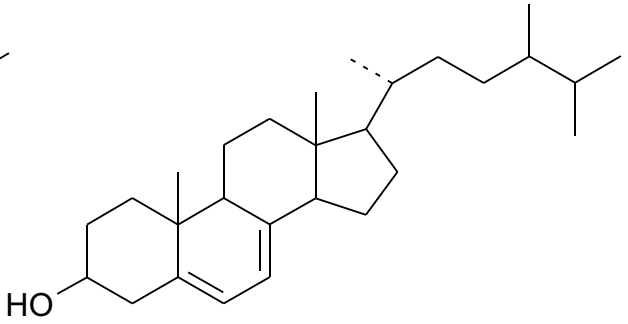
C_{28} ^{5} **(13)**
24-methylcholest-5-en-3 -ol
(campesterol)



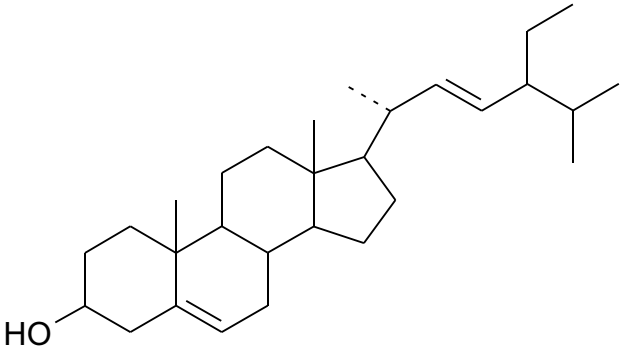
C_{28} ^{0} **(14)**
24-methylcholestan-3 -ol



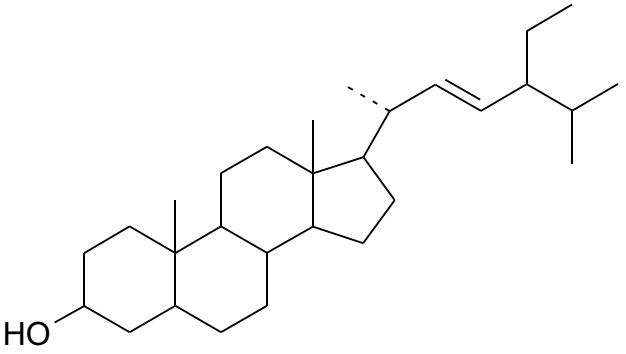
$C_{28}{}^{7}$ **(15)**
24-methylcholest-7-en-3 -ol



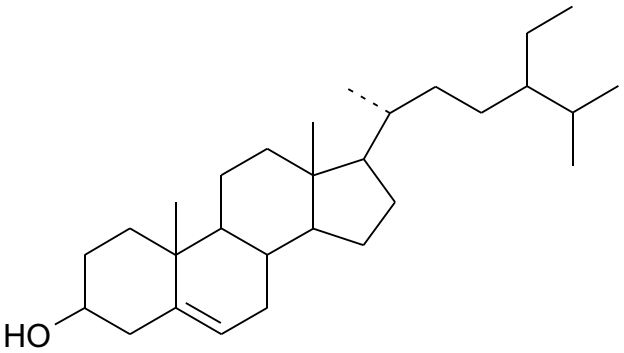
$C_{28}{}^{5,7}$ **(16)**
24-methylcholesta-5,7-dien-3 -ol



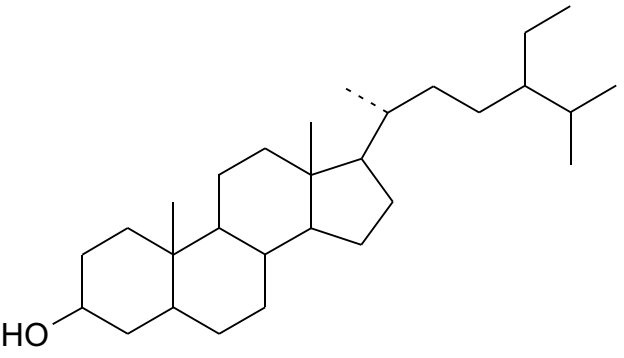
$C_{29}{}^{5,22}$ **(17)**
24-ethylcholesta-5,22-dien-3 -ol
(stigmasterol)



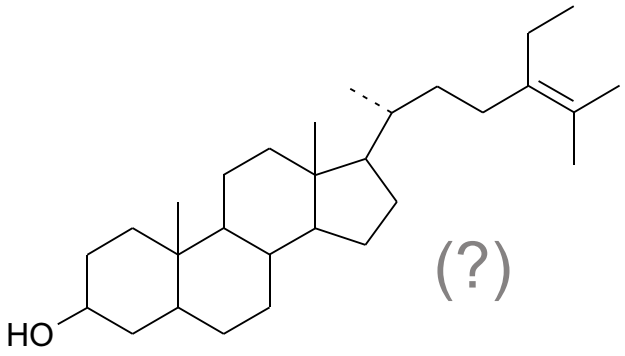
$C_{29}{}^{22}$ **(18)**
24-ethylcholest-22-en-3 -ol



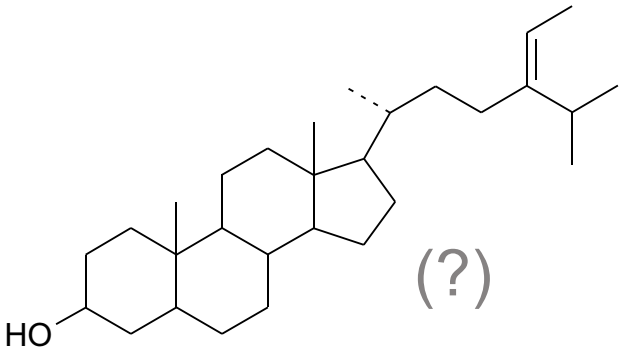
$C_{29}{}^{5}$ **(19)**
24-ethylcholest-5-en-3 -ol
(-sitosterol)



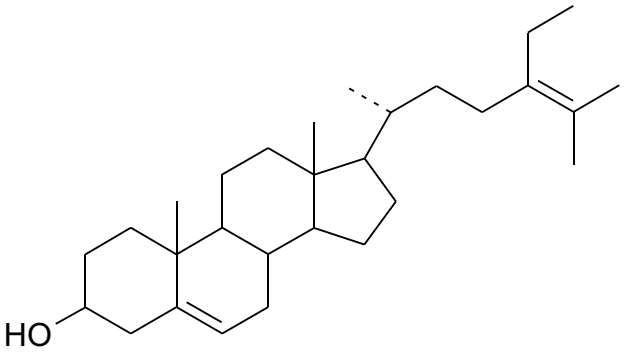
$C_{29}{}^{0}$ **(20)**
5 (H)-24-ethylcholestan-3 -ol
(Stigmastanol)



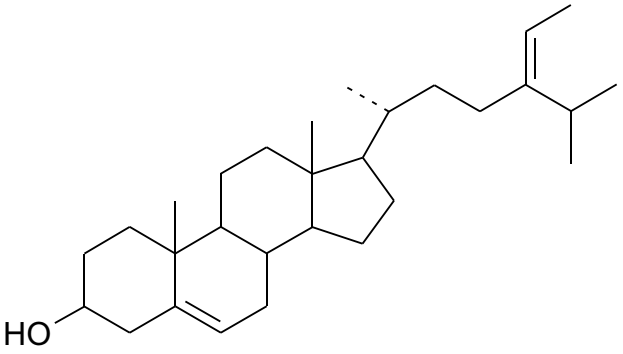
C_{29} $^{24(25)}$ **(21)**
24-ethylcholest-24(25)-en-3 -ol



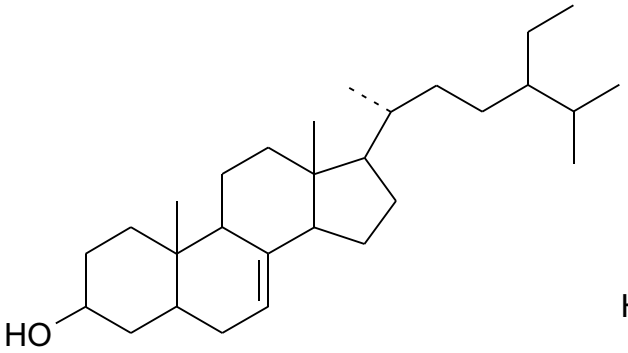
C_{29} $^{24(24')}$ **(22)**
24-ethylcholest-24(24')-en-3 -ol



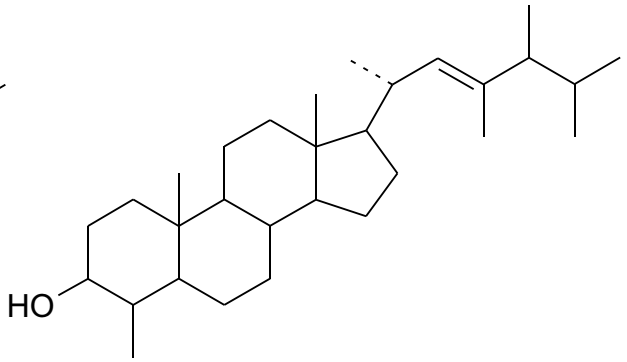
C_{29} $^{5,24(25)}$ **(23)**
24-ethylcholesta-5,24(25)-dien-3 -ol



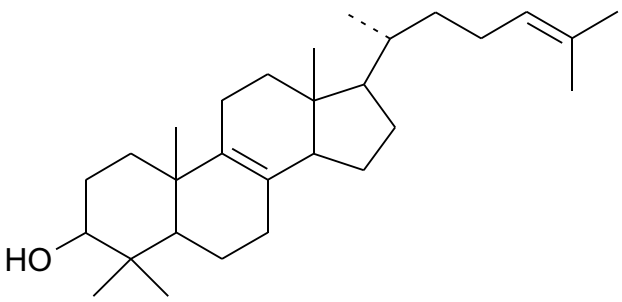
C_{29} $^{5,24(24')}$ **(24)**
24-ethylcholesta-5,24(24')-dien-3 -ol



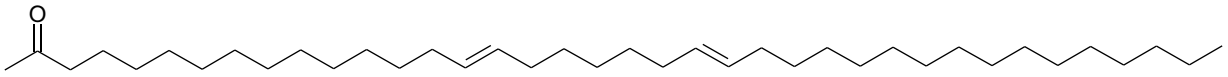
C_{29} ^{7} **(25)**
24-ethylcholest-7-en-3 -ol



4,23,24-C_{30} ^{22} **(26)**
4 ,23,24-trimethyl-5 -cholest-22-en-3 -ol
(dinosterol)

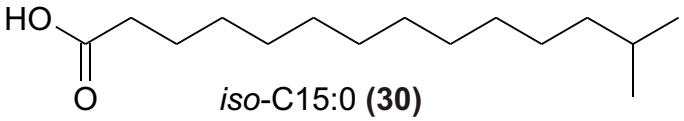


4,4-dimethyl-C_{29} $^{8(9),24(25)}$ **(27)**
4,4-dimethyl-5 -cholesta-8(9),24(25)-dien-3 -ol
(Lanosterol)

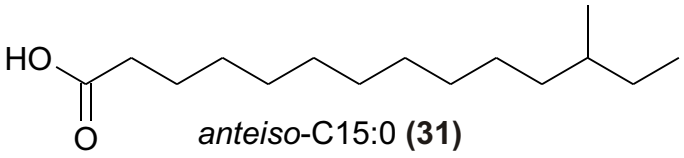


(E)37:2 **(28)**
Heptatriaconta-15E,22E-dien-2-one

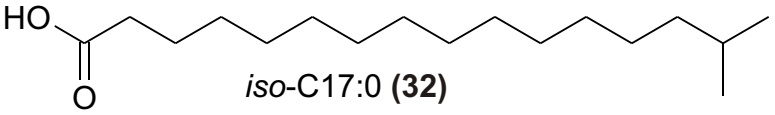
Lycopane **(29)**



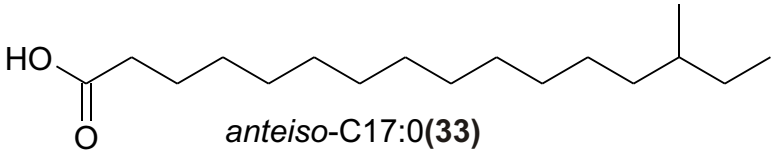
iso-C15:0 **(30)**



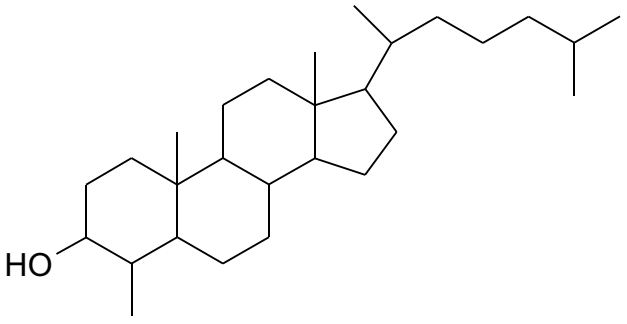
anteiso-C15:0 **(31)**



iso-C17:0 **(32)**



anteiso-C17:0**(33)**



4-methylcholestan-3-ol **(34)**

APPENDIX B

List of samples and locations

Table B.1 List of samples from the PAP

	D229	*n.*[1]	C135	*n.*	D231	*n.*	D237	n.	C142	n.
	Jul-97		Oct-97		Mar-98		Sep-98		Apr-99	
Oneirophanta mutabilis	13200#9	*2*	54301#8	*1*	13368#24	*1*	13627#23	4	54901#5	*1*
	13200#35	*3*	54301#?	*1*	13368#51	*1*	13627#10	3	54901#5	*3*
	?	*2*			13368#?	*1*			54901#7	*1*
					13368#23	*3*				
Pseudostichopus villosus	13200#9	*2*	54301#8	*2*	13368#52	*1*	13627#10	4	54901#5	*1*
	13200#60	*1*	54301#6	*2*	13368#51	*1*			54901#2	*2*
					13368#23	*2*			54901#7	*2*
					13368#24	*1*				
Psychropotes longicauda	13200#60	*1*	54301#8	*2*	13368#52	*5*	13627#10	3	54901#2	*3*
	13200#27	*1*							54901#7	*1*
	13200#9	*1*								
Paroriza prouhoi					13368#24	*3*	13627#24	2	54901#2,1	*3*
									54901#9,1	*1*
									54901#7,1	*1*
Deima validum					13368#24	*1*	13627#10	2	54901#9	*2*
Molpadia blakei					13368#23	*2*			54901#9	*1*
Amperima rosea					13368#23	*20*[2]			54905#1	*20*[2]

[1]Number of specimens analysed.
[2]Pooled samples

Table B.2. List of the locations of the stations at the PAP.

Cruise	Station#deployment	Latitude	Longitude
D229 (Jul-97)	13200#9	48°51.9'-48°46.2'N	16°24.6'-16°31.6'W
	13200#27	48°52.5'-48°42.4'N	16°29.1'-16°29.0'W
	13200#35	48°44.0'-48°55.8'N	16°32.8'-16°40.2'W
	13200#60	48°52.1'-48°48.3'N	16°26.7'-16°42.0'W
C135 (Oct-97)	54301#6	48°46.9'-48°48.6'N	16°49.7'-16°40.5'W
	54301#8	48°49.1'-48°50.5'N	16°38.4'-16°27.0'W
D231 (Mar-98)	13368#23	48°50.7'-48°44.8'N	16°28.3'-16°40.4'W
	13368#24	48°50.3'-48°57.0'N	16°37.3'-16°46.0'W
	13368#51	48°49.7'-48°48.9'N	16°28.4'-16°20.6'W
	13368#52	48°48.3'-48°46.6'N	16°26.0'-16°23.0'W
D237 (Sep-98)	13627#10	48°53.6'-49°02.0'N	16°42.6'-16°53.3'W
	13627#24	48°50.5'-48°52.5'N	16°44.4'-16°42.5'W
C142 (Apr-99)	54901#2	48°42.2'-48°48.0'N	16°51.6'-16°50.4'W
	54901#5	48°44.9'-48°48.2'N	16°40.5'-16°36.2'W
	54901#7	48°47.4'-48°50 8'N	16°48.9'-16°46.0'W
	54901#9	48°46.9'-48°50 6'N	16°41.6'-16°36.4'W
	54905#1	50°32.7'-50°28 7'N	16°57.8'-16°59.5'W

Table B.3. Details of samples from the WAP retained for lipid analyses.

Description	Dep. number	Location	Station
4 *Molpadia musculus*	CRS734	65°08.25'S 64°43.62'W to 65°10.53'S 64°47.27'W	Station A
1 *Bathyplotes sp.*	CRS734	65°08.25'S 64°43.62'W to 65°10.53'S 64°47.27'W	Station A
2 Cores	CRS736	65°10.41'S 64°47.24'W	Station A
1 Core	CRS749	64°48.017'S 65°21.270'W	Station B
6 *Scotoplanes globosa*	CRS754?	64°52.19'S 65°24.76'W to 64°49.47'S 65°19.62'W	Station B
1 *Peniagone sp.*	CRS754?	64°52.19'S 65°24.76'W to 64°49.47'S 65°19.62'W	Station B
2 *Peniagonesp.* (1 male and 1 female)	CRS775	64°12.12'S 65°27.93'W to 64°10.44'S 65°24.49'W	Station C
1 *Scotoplanes globosa*	CRS775	64°12.12'S 65°27.93'W to 64°10.44'S 65°24.49'W	Station C
1 *Bathyplotes sp.*	CRS776	64°11.49'S 65°22.84'W to 64°10.39'S 65°22.18'W	Station C
1 Core	CRS788	66°54.80'S 69°05.68'W	Station F?

APPENDIX C

Preliminary results of carotenoid analyses in deep-sea holothurians

C.1 CAROTENOIDS

The identification of key carotenoids in the body and gut of *Amperima rosea* is likely to be very important, since these compounds are potentially excellent biomarkers for food sources in individual species. The present preliminary study of carotenoids has concentrated on the body tissues of *Amperima rosea*, the key species in the megafaunal bloom at the Procupine Abyssal Plain in the northeastern Atlantic Ocean (Billett *et al*., 2001; Ginger *et al*., 2001). This appendix shows the preliminary results of these analyses.

Figure C.1 shows a chromatogram of carotenoids extracted from an *Amperima rosea* body tissue. All the main peaks are carotenoid esters. Only one of these has been tentatively identified as 6-hydroxysiphonixanthin-trans-Δ2-dodecenoate on the basis of its mass spectrum and collision induced fragmentation spectrum (Fig. C.2). This compound has been found only in Prasinophytes to date (Egeland *et al*., 1997), which may can be a source of carotenoids for this species. However, 6-hydroxysiphonaxanthin-trans-Δ2-dodecenoate could be alternatively derived from other carotenoids.

Other minor carotenoids that were identified on basis of SIM chromatogram, their mass spectra, and co-injection with authentic standards, were zeaxanthin, canthaxanthin, echinenone, and astaxanthin (ratio 0.25:1.0:0.78:0.47, respectively; Fig. C.3-C4). The origin of these carotenoids are as follows:

- Astaxanthin: is present in some green algae and is also a transformation product in marine animals (Jeffrey *et al*., 1997);

- Canthaxanthin: is a minor or trace pigment in some green algae and cyanobacteria, diatoms, prymnesiophytes, and eustigmatophytes (Jeffrey *et al*., 1997);
- Zeaxanthin: is present in prochlorophytes, cyanobacteria (coccoid), green algae, most chrysophytes, and raphidophytes (Jeffrey *et al*., 1997);
- Echinonenone: Major carotenoid of freshwater and estuarine cyanobacteria (blue-green algae); minor or trace carotenoid in some green algae and euglenophytes (Jeffrey *et al*., 1997);

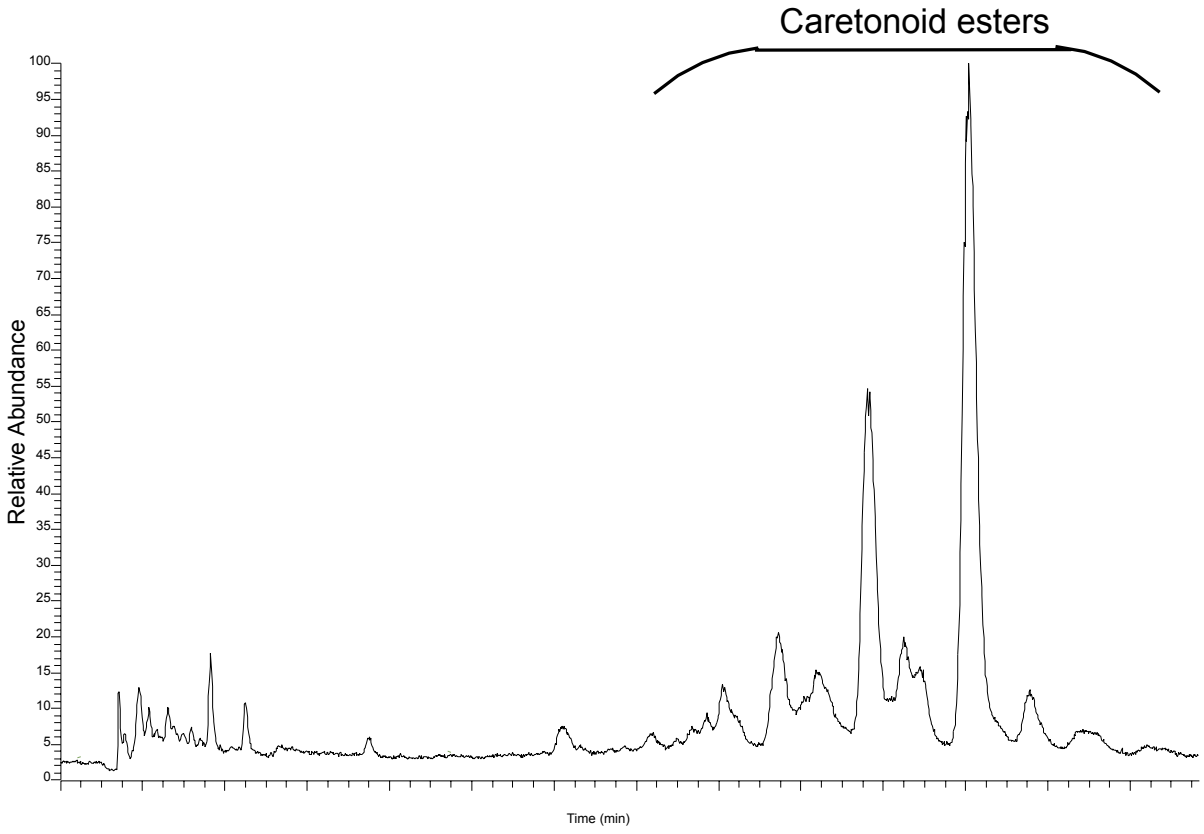


Figure C.1. Total ion chromatogram of carotenoids extracted from the body tissues of *Amperima rosea*.

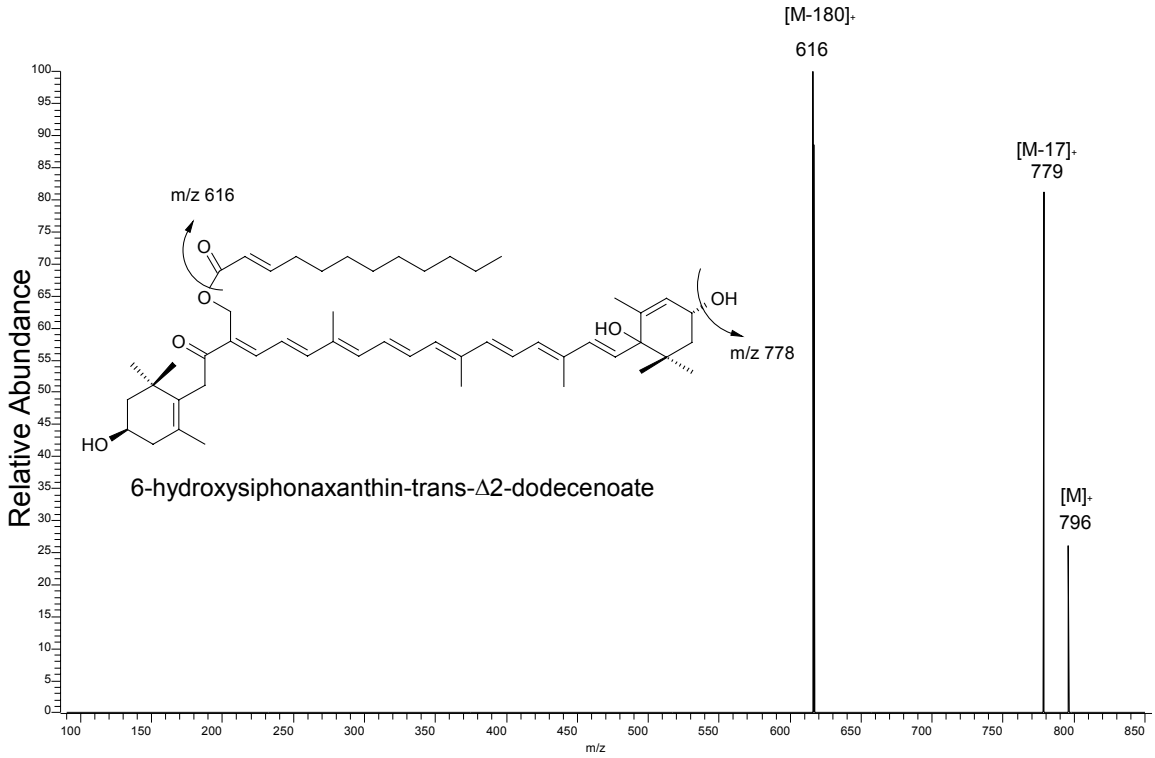


Figure C.2. MS-MS chromatogram (@ 796 Thomsons) and structure of 6-hydroxysiphonaxanthin-trans-Δ2-dodecenoate.

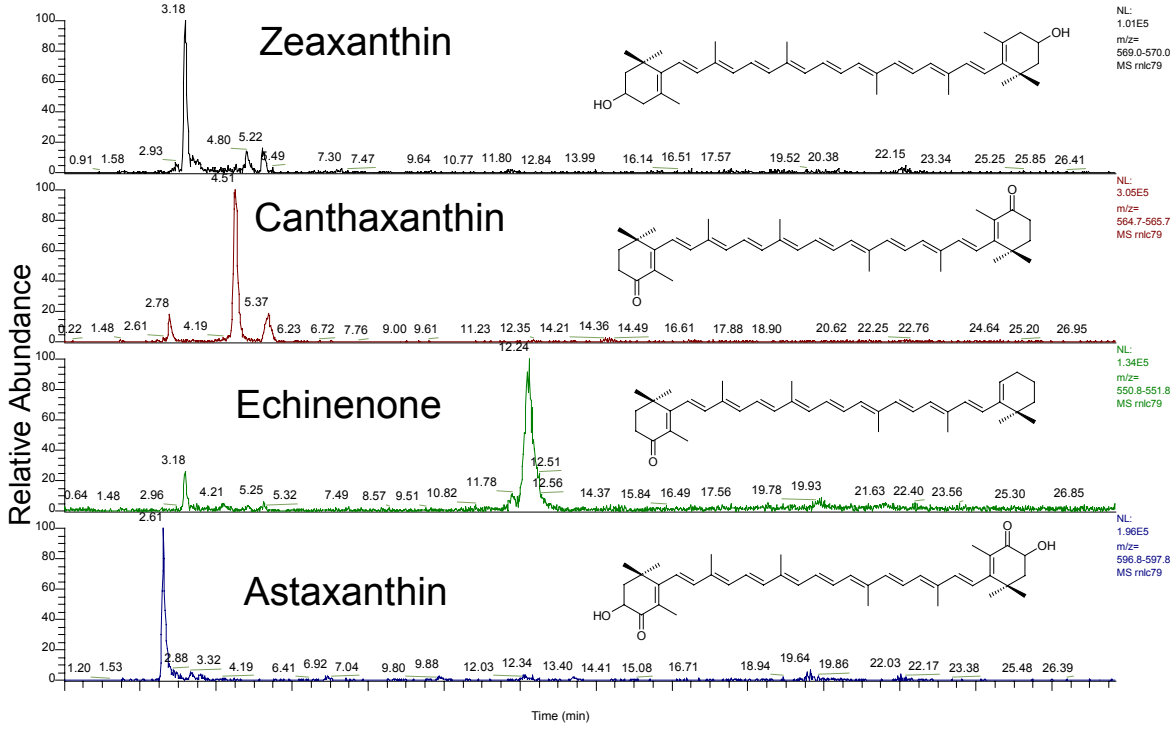


Figure C.3. Positive SIM chromatograms and structures of zeaxanthin, canthaxanthin, echinenone, and astaxanthin of an *Amperima rosea* extract.

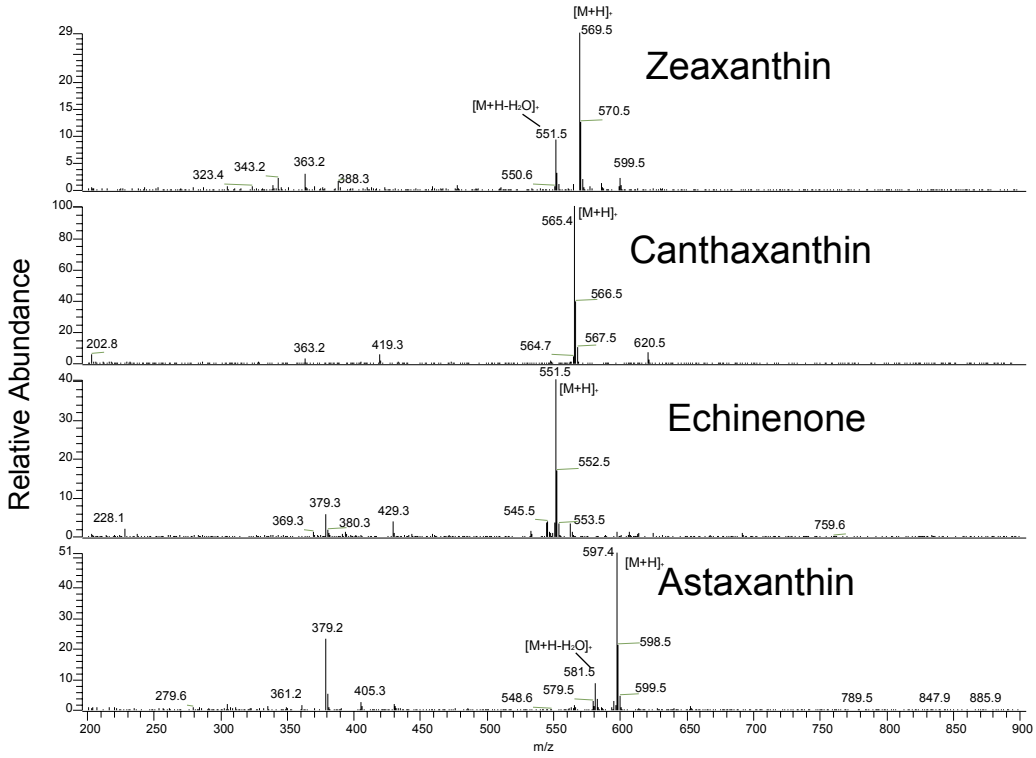


Figure C.4. Mass spectra of zeaxanthin, canthaxanthin, echinenone, and astaxanthin from an *Amperima rosea* extract.

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