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Lipid, sterols and fatty acid composition of abyssal holothurians and ophiuroids from the North-East Pacific Ocean: Food web implications

Jeffrey C. Drazen [a,*], Charles F. Phleger [b,c], Michaela A. Guest [d], Peter D. Nichols [b,c]

[a] University of Hawaii, Department of Oceanography, 1000 Pope Road, Honolulu, HI 96822, USA
[b] CSIRO, Marine and Atmospheric Research, Food Futures Flagship, Hobart, Tasmania 7000, Australia
[c] Antarctic and Climate Ecosystems CRC, Hobart, Tasmania 7001, Australia
[d] Tasmanian Aquaculture and Fisheries Institute, Taroona, Nubeena Crescent, Tasmania 7053, Australia

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ABSTRACT

The lipid, fatty acid (FA), and sterol composition of two ophiuroids and four holothurians from the abyssal eastern North Pacific were analysed to assess their feeding habits and to ascertain their composition for use in a larger study to examine food web dynamics and trophic ecology. Holothurians were rich in phytosterols and algal derived FA such as docosahexaenoic acid and eicosapentaenoic suggesting tight trophic coupling to phytodetritus. Large proportions of stanols were found, probably a result of enteric bacteria but they may come from sterol metabolism in the holothurians themselves. *Oneirophanta mutabilis* was distinct with much higher levels of stanols and bacterially derived FA suggesting specific selection of bacteria rich detrital particles or the activity of enteric and integumental bacteria. The ophiuroids sterol and FA compositions differed greatly from the holothurians and reflected consumption of animal material in addition to phytodetritus. Large proportions of energy storage lipids suggested a sporadic food supply. Several unusual fatty acids were found in these abyssal echinoderms. Tetracosahexaenoic acid, 24:6ω3, in ophiuroids and 23:1 in holothurians may be good biomarkers for food web studies. We report the first occurrence of αOH 24:1 in holothurians with none detected in ophiuroids. Its function is presently unknown.

1. Introduction

Echinoderms dominate the megafauna of the abyssal seafloor. In visual and trawl sampling of the abyssal plain holothurians and ophiuroids are usually the most abundant taxa observed (Billett, 1991; Ruhl, 2007). Most are considered deposit feeders and isotope tracer studies show that at least some species (*Abyssocucumis abyssorum* and *Oneirophanta mutabilis*) consume freshly deposited detrital material from the seafloor (Lauerman et al., 1997). Therefore, these animals can play a pivotal role in abyssal food webs by consuming and transferring the allochthonous supply of phytodetrital material, generated in surface waters, to higher trophic levels. However little is known of specific abyssal echinoderm feeding habits and strategies.

Lipid biomarkers are useful tools to study trophic ecology and determine food web connections. Organisms can have unique fatty acid and sterol compositions, or at least profiles that can be traceable, and many of these compounds are transferred from predator to prey without modification (Nichols et al., 1986a; Phleger et al., 1998; Dalsgaard et al., 2003). For instance most animals cannot synthesize longer chain polyunsaturated fatty acids such as eicosapentaenoic acid (EPA; 20:5ω3), arachidonic acid (AA; 20:4ω6) and docosahexaenoic acid (DHA; 22:6ω3). Instead these are formed by phytoplankton and some bacteria and are transferred through the food web (Volkman et al., 1989; Brown et al., 1993) such that high levels of these fatty acids are suggestive of herbivory. Similarly phytosterols are synthesized only by algae and plants (Volkman, 1986, 2003) and are incorporated into herbivores with minimal modification. These types of markers are not exclusively from primary producers. Calanoid copepods have been reported as the major synthesizers for long chain monounsaturated fatty acids, including the 20:1 and 22:1 isomers, in marine food webs (Sargent et al., 1981).

Relatively few studies have employed these techniques to study feeding ecology and food webs in the deep-sea. Studies of hydrothermal vent ecosystems have recently been completed (Phleger et al., 2005a,b). Signature lipid studies of abyssal echinoderms in the Atlantic have helped to identify specific feeding niches and have confirmed the general deposit feeding foraging mode (Ginger et al., 2000; Howell et al., 2003; Hudson et al., 2004; Neto et al., 2006). Temporal investigations showed that for some species the relative

Abbreviations: AA, Arachidonic acid; BSTFA, *N,O*-bis-(trimethylsilyl)-trifluoroacetamide; DAGE, Diacylglycerol ether; DHA, Docosahexaenoic acid; FAME, Fatty acid methyl esters; FFA, Free fatty acids; GC–MS, Gas chromatography–mass spectrometry; PL, Phospholipid; ST, Sterols (ST); TAG, Triacylglycerol; TSE, Total solvent extract; WE, Wax ester.

* Corresponding author. Tel.: +1 808 956 6567; fax: +1 808 956 9516.
E-mail address: jdrazen@hawaii.edu (J.C. Drazen).

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amounts of fatty acids and sterols varied with the lipid composition and quantity of the particle flux (Hudson et al., 2004; Neto et al., 2006). In addition lipid profiles and temporal trends were modulated by selectivity of feeding and whether the species fed at the sediment surface or subsurface (Neto et al., 2006). The lipid profiles observed result from differences in particle selection (Howell et al., 2003), but may also arise from differences in assimilation or the enteric microflora (Ginger et al., 2000). Thus a great deal has been learned about echinoderm food habits through the use of lipid signatures. No studies have yet used lipid biomarker techniques to study abyssal echinoderms in the Pacific.

In this study the fatty acid and sterol composition of two ophiuroids and four holothurians from a site in the abyssal eastern North Pacific were analysed. Holothurians and ophiuroids are the most abundant echinoderms at this site. Recent investigations indicate that they exhibit interannual fluctuations in abundance, size distributions, and community composition related to changes in the productivity of overlying surface waters which controls resource availability (Ruhl et al., 2004; Ruhl, 2007, 2008). The goal of the present effort was to use lipid biomarkers to assess their feeding habits and also to ascertain their composition for use in a larger study to examine food web dynamics and trophic ecology of top predators in the ecosystem (Drazen et al., in press).

2. Materials and methods

2.1. Sampling

All samples were collected at station M (34° 50′ N; 123° 00′W, 4100 m depth) in the north-east Pacific, 220 km west of Point Conception, California during August, 2006. For a complete description of the physical and biological attributes of this station see Smith and Druffel (1998). In brief, this site is located 220 km west of Pt. Conception, California on the Monterey Abyssal Fan and lies underneath the California Current. The bottom is flat with sediments composed of fine silt and clay. Particulate matter fluxes to the seafloor show a distinct seasonal cycle following the cycle in surface water productivity. August of 2006 represented peak seasonal flux for the year at 10.26 mg C m^{-2} d^{-1}.

Peniagone vitrea, *Abyssocucumis abyssorum*, *Protankyra brychia* and *Ophiacantha* sp. were collected using the submersible ALVIN. All *Ophiura bathybia* and *O. mutabilis*, and additional specimens of *Ophiacantha* sp., *A. abyssorum*, and *P. brychia* were collected using a 12.3 m otter trawl. Only specimens in very good condition showing little or no signs of damage were rinsed free of any adhering sediment and sampled. *A. abyssorum*, *O. mutabilis* and *P. vitrea* were large (6–12 cm in length) and were split in half longitudinally with one half analysed for lipids. For all speciments, contents from the gastrointestinal tract were removed. Echinoderms were frozen in cryovials under liquid nitrogen on board ship and stored at −80 °C in the laboratory. Samples were freeze-dried, ground and then shipped to CSIRO Marine and Atmospheric Research, Hobart, Tasmania, Australia, for lipid analyses. Freeze dried sample masses, as extracted for lipid, were between 0.02 and 0.39 g.

2.2. Lipid extraction

Samples were quantitatively extracted overnight using a modified Bligh and Dyer (1959) one-phase methanol–chloroform–water extraction (2:1:0.8 v/v/v). The phases were separated by the addition of chloroform–water (final solvent ratio, 1:1:0.9 v/v/v methanol–chloroform–water. The total solvent extract (TSE) was concentrated using rotary evaporation at 40 °C.

2.3. Lipid classes

An aliquot of the TSE was analysed using an Iatroscan MK V TH10 thin-layer chromatography-flame ionization detector (TLC-FID) analyser (Tokyo, Japan) to quantify individual lipid classes (Fraser et al., 1985; Volkman et al., 1991). Samples were applied in duplicate to silica gel SIII chromarods (5 μm particle size) using 1 μL micropipettes. Chromorods were developed in a glass tank lined with pre-extracted filter paper. The primary solvent system used for the lipid separation was hexane-diethyl ether-acetic acid (60:17:0.1), a mobile phase resolving non-polar compounds such as wax ester (WE), triacylglycerol (TAG), free fatty acids (FFA) and sterols (ST) from phospholipid (PL). A second non-polar solvent system of hexane-diethyl ether (96:4) was used to resolve hydrocarbons, WE, TAG, and diacylglycerol ether (DAGE). After development, the chromorods were oven dried and analysed immediately to minimize absorption of atmospheric contaminants. The FID was calibrated for lipid class (phosphatidylcholine, cholesterol, cholesteryl oleate, oleic acid, squalene, TAG (derived from fish oil), WE (derived from orange roughy, *Hoplostethus atlanticus*, oil) and DAGE (derived from shark liver oil); 0.1–10 μg range). Peaks were quantified on an IBM compatible computer using DAPA Scientific software (Kalamunda, Western Australia, Australia). TLC-FID results are generally reproducible to ±10% of individual lipid class abundances (Volkman et al., 1991).

2.4. Fatty acids

An aliquot of the TSE was *trans*-methylated to produce fatty acid methyl esters (FAME) using methanol–chloroform–conc. hydrochloric acid (10:1:1, 80 °C, 2 h (Christie, 1982). FAME were extracted into hexane–chloroform (4:1, 3×1.5 mL). FAME fractions were treated with *N*,*O*-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA 50 μL, 60 °C, overnight) to convert ST, alcohols, and hydroxyl fatty acids to their corresponding TMSi ethers.

Gas chromatographic (GC) analyses were performed with an Agilent Technologies 6890N GC (Palo Alto, CA, USA) equipped with an HP-5 cross-linked methyl silicone fused silica capillary column (50 m×0.32 mm i.d.), an FID, a split/splitless injector and an Agilent

Table 1
Lipid class composition of abyssal echinoderms

	n	Percentage composition												Lipid content (% dry wt)	
		WE		DAGE		TAG		FFA		ST		PL			
		Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd
Holothuroidea															
Abyssocucumis abyssorum	3	0		0		9	4.3	3.6	2.8	0.7	0.4	86.7	3.6	9	5.8
Oneirophanta mutabilis	3	2.6	1.8	0		0		4	2.2	0.4	0.2	93	3.6	2.6	1
Peniagone vitrea	1	0		1		0.5		24.4		5.3		68.8		3.2	
Protankyra brychia	3	0.9	0.3	0		0		2.3	0.9	1.7	0.2	95.2	0.8	3.8	0.6
Ophiuroidea															
Ophiacantha sp.	3	32.2	28	2.5	1.8	3	2.3	2	1.2	2.4	1.7	58.1	21	5.4	1.8
Ophiura bathybia	2	3.1	0.1	0		19.9	2.8	1.7	0.5	4.1	0.3	71.4	2.2	3.8	0.8

WE, wax ester; DAGE, diacylglyceryl ether; TAG, triacylglycerol; ST, sterol; PL, phospholipid.

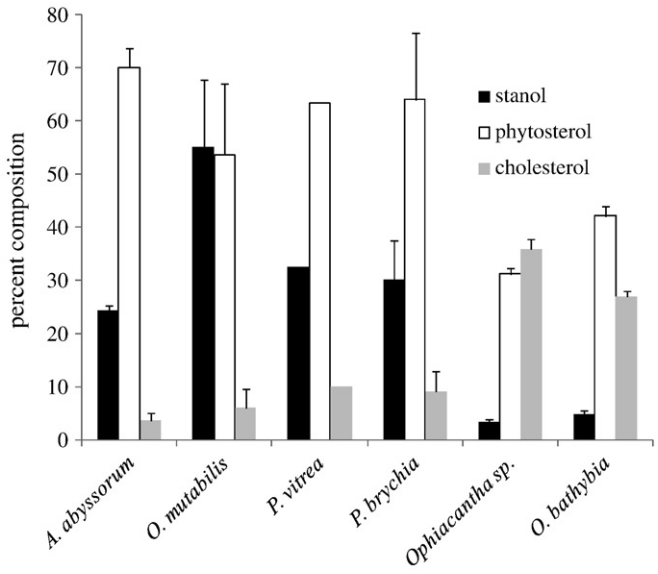

Fig. 1. Percent sterol composition (cholesterol, phytosterols, stanols) by group for abyssal echinoderms. Phytosterols include all C_{28} and C_{29} sterols (# 9–10 and 12–19). Values are means and standard deviations.

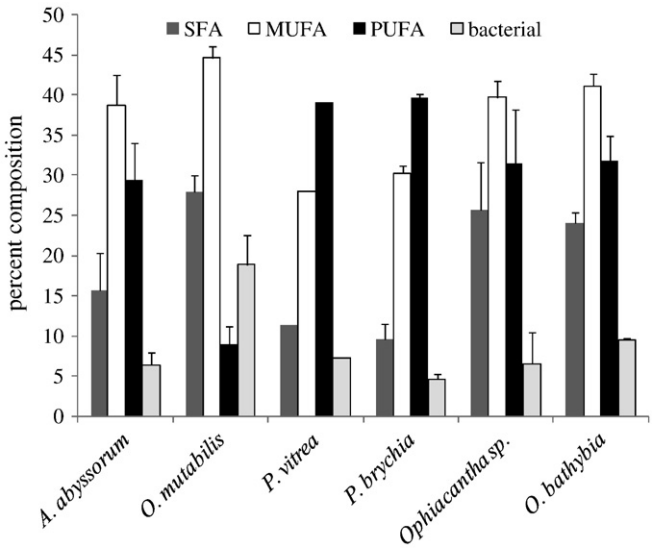

Fig. 2. Percent fatty acid composition by group for abyssal echinoderms. Bacterial FA include i15:0, a15:0, i17:0, a17:0, 17:0, 18:1ω7. Values are means and standard deviations.

Technologies 7683 Series auto sampler and injector. Helium was the carrier gas. Following addition of methyl nonodecanoate internal injection standard, samples were injected in splitless mode at an oven temperature of 50 °C. After 1 min, the oven temperature was raised to 150 °C at 30 °C min^{-1}, then to 250 °C at 2 °C min^{-1} and finally to 300 °C at 5 °C min^{-1}. Peaks were quantified with Agilent Technologies GC ChemStation software (Palo Alto, CA, USA). Individual components were identified using mass spectral data and by comparing retention time data with those obtained for authentic and laboratory standards. GC results are subject to an error of ±5% of individual component area as determined by replicate analysis. GC–mass spectrometric (GC–MS) analyses were performed on a Finnigan Thermoquest GCQ GC–mass spectrometer fitted with an on-column injector using Thermoquest Xcalibur software (Austin, TX, USA). The GC was fitted with a capillary column similar to that described above.

2.5. Statistical analysis

Fatty acid profiles were compared among taxa using Principal Component Analysis (PCA). PCA reduces the number of dimensions produced by the large number of variables and uses linear correlations (components) to identify those fatty acids that contribute most to the separation between observed groups (Best et al., 2003). Fatty acids that contributed a mean of less than 1.0% (of total fatty acids) to the profile were omitted from statistical analyses. All analyses were performed on % composition data and results were confirmed by analysis of mg/g fatty acid data (data not shown, also see Phillips et al.

Table 2
Sterol composition of abyssal echinoderms

	Sterol	Percent composition										
		Holothuroidea							Ophiuroidea			
		A. abyssorum		*O. mutabilis*		*P. vitrea*	*P. brychia*		*Ophiacantha* sp.		*O. bathybia*	
		Avg	Sd	Avg	Sd		Avg	Sd	Avg	Sd	Avg	Sd
1	24-norcholesta-5,22E-dien-3β-ol	1.7	0.6	0.0	0.0	0.6	0.0	0.0	1.7	0.2	1.0	0.0
2	24-nor-5α-cholest-22E-en-3β-ol	0.1	0.1	0.0	0.0	0.2	0.0	0.0	0.4	0.2	0.0	0.0
3	cholesta-5,22Z-dien-3β-ol	3.3	0.6	1.8	0.4	1.4	2.5	1.0	3.8	0.6	4.4	0.1
4	5α-cholest-22Z-en-3β-ol	0.7	0.4	0.3	0.5	0.4	0.0	0.0	0.0	0.0	0.0	0.0
5	cholesta-5,22E-dien-3β-ol	2.9	1.1	10.7	14.3	3.1	4.2	1.8	18.2	1.5	18.9	0.8
6	5α-cholest-22E-en-3β-ol	1.6	0.6	2.2	0.5	1.4	2.3	1.1	1.5	0.0	1.3	0.2
7	cholest-5-en-3β-ol	3.7	1.3	5.9	3.6	9.9	9.1	3.8	35.7	2.0	27.0	0.8
8	5α-cholestan-3β-ol	3.3	0.4	20.4	3.9	9.8	7.6	2.9	1.1	0.1	2.3	0.4
9	24-methylcholesta-5,22E-dien-3β-ol	16.3	1.3	4.9	4.5	7.7	11.0	0.8	14.1	1.0	16.0	0.0
10	24-methyl-5α-cholest-22E-en-3β-ol	6.6	0.6	8.5	2.0	4.2	5.4	1.2	0.3	0.2	0.8	0.2
11	24-methylcholesta-5,24(28)-dien-3β-ol	0.0	0.0	0.0	0.0	0.8	0.4	0.7	0.9	0.1	0.0	0.0
12	24-methylcholest-5en-3β-ol	5.1	0.6	3.0	2.6	3.9	5.3	1.3	2.6	0.1	3.8	0.3
13	24-methyl-5α-cholestan-3β-ol	2.3	0.3	4.4	0.5	2.0	2.4	0.7	0.0	0.0	0.0	0.0
14	23,24-dimethylcholesta-5,22E-dien-3β-ol	1.0	0.9	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0
15	24-ethylcholesta-5,22E-dien-3β-ol	8.2	1.3	4.3	3.8	4.6	5.8	0.4	2.6	0.1	3.2	0.5
16	24-ethyl-5α-cholest-22E-en-3β-0l	1.7	0.5	2.1	0.8	1.8	0.8	0.1	0.0	0.0	0.0	0.0
17	23,24-dimethylcholest-5-en-3β-ol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.1	0.3
18	24-ethylcholest-5-en-3β-ol	20.8	1.6	9.3	14.0	26.4	21.6	18.2	10.8	1.7	15.8	1.2
19	24-ethyl-5α-cholestan-3β-ol	8.0	1.7	17.0	5.9	12.6	11.5	1.6	0.0	0.0	0.4	0.0
	Other	12.6	0.2	5.0	2.4	9.1	9.9	3.0	5.6	0.5	3.0	2.3
	Total	100		100		100	100		100		100	

(2003)). Multivariate statistical analyses were performed using PRIMER 6 software (PRIMER-E, Plymouth, UK).

3. Results

3.1. Lipid content and composition

Holothurian lipid composition was dominated by PL (69–95% of total lipid) with low storage lipid (TAG and WE; Table 1). *A. abyssorum* had 9% TAG. PL was less in ophiuroids (58–71%). ST were <6% in all the species (Table 1). Ophiuroids had more storage lipid (20% TAG in *O. bathybia* and 32% WE in *Ophiacantha* sp.). One specimen of *Ophiacantha* sp. had no WE while the other two specimens contained 46–51% WE, resulting in the high standard deviation for this species (Table 1). FFA were <4% except *P. vitrea* with 24%. Low FFA indicates little PL and TAG breakdown, low lipolytic and enzyme activity, and confirms the stability and low temperature of samples during transport and storage. Ginger et al. (2000) considered FFA in holothurian gut contents and assigned this to lipolysis during recovery. Diacylglyceryl ethers were not detected in most samples except for 3% in *Ophiacantha* sp. and 1% in *P. vitrea*. Hydrocarbons were not detected in any of the samples.

3.2. Sterols

Both holothurians and ophiuroids were characterized by high phytosterols (C_{28} and C_{29}-sterol numbers 9–10 and 12–19, 31–70% of total ST) including significant stanol levels in the holothurians (Fig. 1). However there were large differences between the two groups in overall sterol composition (Table 2) notably a much higher contribution of cholesterol for the ophiuroids (Fig. 1).

The specific sterols that were most abundant varied between the species (Table 2). The two most abundant ST in the holothurian *A. abyssorum* were 24-ethylcholest-5-en-3β-ol and 24-methylcholesta-5,22E-dien-3β-ol. The ST profile in *O. mutabilis* was dominated by the stanols 5α-cholestan-3β-ol and 24-ethyl-5α-cholestan-3β-ol. Cholesta-5,22E-dien-3β-ol was more abundant in this species than in the other holothurians. The most abundant ST in *P. vitrea* were 24-ethylcholest-5-en-3β-ol and 24-ethyl-5α-cholestan-3β-ol. *P. brychia* ST were similar to those of *P. vitrea*. As stated above, the ophiuroids had

Table 3
Fatty acid composition of abyssal echinoderms

	Holothuroidea							Ophiuroidea			
	A. abyssorum		*O. mutabilis*		*P. vitrea*	*P. brychia*		*Ophiacantha* sp.		*O. bathybia*	
	Avg	Sd	Avg	Sd		Avg	Sd	Avg	Sd	Avg	Sd
4,8,12TMTD	0.6	0.1	3.2	2.0	0.3	0.0	0.0	0.0	0.0	0.1	0.0
14:0	0.8	0.3	0.3	0.1	0.5	0.3	0.1	1.8	0.9	6.8	1.7
i15:0	0.9	0.3	3.5	1.4	0.6	0.2	0.1	0.5	0.2	1.3	0.1
a15:0	0.5	0.1	2.4	0.9	0.5	0.4	0.2	0.3	0.1	0.7	0.1
16:0	5.0	3.9	1.5	0.7	3.1	2.5	1.5	3.6	1.3	5.9	1.1
16:1ω5c	0.3	0.1	3.1	1.6	1.3	0.7	0.2	0.2	0.1	0.3	0.0
16:1ω7c	5.2	1.8	1.4	1.1	2.6	1.1	0.4	1.8	0.3	1.1	0.5
17:0	0.4	0.1	1.5	0.5	0.5	0.6	0.0	0.8	0.4	1.0	0.1
i17:0	0.9	0.2	2.0	0.2	0.6	0.3	0.0	0.4	0.3	0.3	0.1
a17:0	0.4	0.1	1.6	0.2	0.5	0.2	0.0	0.3	0.2	0.4	0.1
17:1ω8c	0.5	0.1	0.3	0.3	0.5	0.4	0.0	0.3	0.1	0.2	0.1
18:0	2.2	0.6	4.1	1.4	1.6	2.3	0.4	4.1	1.6	3.4	0.7
18:1ω5c	0.5	0.1	0.8	0.2	0.3	0.7	0.0	0.4	0.3	0.4	0.1
18:1ω7c	2.8	0.4	6.7	2.0	3.5	2.6	0.3	3.5	2.4	4.0	0.3
18:1ω9c[a]	6.2	5.2	2.4	0.2	1.3	2.0	1.2	5.1	0.6	8.8	2.4
18:2ω6	0.7	0.4	0.2	0.1	0.3	0.2	0.0	0.6	0.1	0.6	0.1
18:4ω3/i18:0	0.9	0.2	1.6	0.4	1.0	0.3	0.1	0.6	0.2	0.5	0.1
20:0	1.4	0.3	1.5	1.1	1.0	1.6	0.1	11.8	10.0	0.2	0.0
20:1ω7c	1.3	0.1	1.9	0.1	0.7	2.1	0.0	1.0	0.7	0.6	0.1
20:1ω9c	1.3	0.3	2.0	0.3	0.3	0.8	0.2	13.5	11.7	3.2	3.7
20:1ω11c	0.0	0.0	0.0	0.0	0.0	0.6	0.1	4.4	7.6	12.7	8.0
20:2ω6	0.7	0.0	0.4	0.1	1.1	1.1	0.0	0.0	0.1	0.0	0.0
20:2NMIb	0.1	0.1	0.2	0.3	0.1	0.1	0.1	0.4	0.3	0.8	0.1
20:3ω6	0.1	0.1	0.0	0.0	0.2	0.3	0.0	0.4	0.1	1.1	0.5
20:4ω3	0.3	0.1	0.4	0.3	0.3	0.4	0.1	0.5	0.2	1.2	0.3
20:4ω6	6.8	1.7	2.2	0.4	8.5	12.1	0.2	2.8	1.0	2.7	0.4
20:5ω3 EPA	13.5	2.1	2.9	1.1	17.8	16.6	1.0	9.4	2.8	11.3	1.6
21:5ω3	0.1	0.1	0.0	0.0	0.3	0.0	0.0	0.3	0.2	0.2	0.0
21:1	0.5	0.1	0.7	0.1	0.2	0.6	0.1	0.9	0.4	1.5	1.1
22:1ω7c	3.8	1.2	3.3	0.8	4.3	5.8	0.4	0.3	0.1	0.5	0.1
22:1ω9c+16:0 GED	0.9	0.4	1.6	0.1	0.8	0.6	0.0	1.3	0.3	1.3	0.4
22:1ω11+13c	0.0	0.1	0.0	0.1	0.0	0.0	0.0	3.7	1.7	1.0	0.6
22:3ω6+22:2NMI	0.1	0.0	0.2	0.3	0.0	0.1	0.1	1.1	1.9	0.3	0.4
22:4ω3+22:2NMI	0.2	0.0	0.1	0.1	0.2	0.1	0.0	0.9	0.5	0.1	0.1
22:4ω6	0.3	0.0	0.1	0.1	0.1	0.2	0.0	0.2	0.2	0.2	0.1
22:5ω3 DPA(3)	0.6	0.2	0.8	1.1	0.9	0.5	0.1	1.0	1.3	0.5	0.1
22:5ω6 DPA(6)	0.4	0.1	0.1	0.1	0.3	0.5	0.0	0.1	0.0	0.4	0.1
22:6ω3 DHA	5.4	0.9	1.5	0.8	8.3	7.2	0.1	1.0	0.4	2.1	1.3
23:1	8.8	2.7	7.9	2.6	4.2	4.2	0.6	0.6	0.2	0.7	0.1
αOH 23:1	3.1	1.1	1.5	0.4	4.3	3.2	0.6	0.1	0.1	0.2	0.0
24:1	4.3	1.2	8.0	2.2	5.0	6.2	0.6	1.6	0.3	1.8	0.4
αOH 24:1	10.5	2.4	14.1	3.8	12.6	12.0	1.5	0.0	0.0	0.0	0.0
24:6ω3 THA	0.1	0.0	0.0	0.0	0.2	0.1	0.0	2.3	0.2	10.3	1.0
C26PUFA	0.0	0.0	0.0	0.0	0.3	0.3	0.2	10.7	1.9	0.6	0.2
Other	7.0		12.0		8.9	8.3		5.3		8.7	
	100.0		100.0		100.0	100.0		100.0		100.0	

[a] 18:3ω3 is a minor FA co-eluting with 18:1ω9c.

much higher levels of cholesterol than the holothurians (27–36%; Table 2). Other ST comprising more than 10% of the composition in these ophiuroids included cholesta-5,22E-dien-3β-ol, 24-methylcholest-5,22E-dien-3β-ol, and 24-ethylcholest-5-en-3β-ol. A PCA analysis of the sterol profiles clearly separated the ophiuroids from the holothurians based on the differences noted above (data not shown), and was similar in result to a PCA analysis using fatty acids (see below).

3.3. Fatty acids

The fatty acid (FA) profiles are largely reflective of phospholipid fatty acids (PLFA), since the samples contained 58–95% PL (Table 1). Total polyunsaturated fatty acids (PUFA) were high in almost all holothurian and ophiuroid species (29–40% of total FA, Fig. 2). *O. mutabilis* was an exception, with relatively low total PUFA. Highest PUFA values occurred in *P. vitrea* and *P. brychia* with lower levels in *A. abyssorum* and the two ophiuroid species (Fig. 2).

PUFA compositions varied amongst the echinoderms (Table 3). Levels of DHA (docosahexaenoic acid, 22:6ω3) were 1–8% with the highest proportions in the holothurians *A. abyssorum*, *P. vitrea*, and *P. brychia* and the lowest in *O. mutabilis* and in both ophiuroid species. Other C22 PUFA, including DPA (3) (docosapentaenoic acid, 22:5ω3) and DPA (6), were low in all species (0.1–1.1%). EPA (eicosapentaenoic acid, 20:5ω3) levels were higher than DHA in these deep-sea echinoderms. EPA in the holothurians *A. abyssorum*, *P. vitrea* and *P. brychia* was 14–18% of total FA, but considerably lower in *O. mutabilis*. EPA comprised 9–11% of total FA in both ophiuroids (Table 3). AA (arachidonic acid, 20:4ω6) was higher in the holothurians *A. abyssorum*, *P. vitrea* and *P. brychia* than in *O. mutabilis* or both ophiuroids. The unusual very long chain PUFA (≥C24, VLC-PUFA) 24:6ω3 was markedly more abundant in the ophiuroids (10% in *O. bathybia* and 2% in *Ophiacantha* sp.) than in the holothurians (0.1–0.2% of total FA, with none detected in *O. mutabilis*) (Table 3). C26 PUFA comprised 11% in *Ophiacantha* sp. and only 1% in *O. bathybia*. <0.5% C26 PUFA was present in *P. vitrea* and *P. brychia* and none were detected in the other two holothurian species.

Total monounsaturated fatty acids (MUFA) for all echinoderm species were 28–45% of total FA (Fig. 2). Lowest MUFA levels occurred in *P. vitrea* and *P. brychia* and highest in *O. mutabilis*. The MUFA compositions differed between holothurians and ophiuroids. The MUFA 23:1 comprised 4–9% of total FA in holothurians versus 1% in ophiuroids (Table 3). The MUFA 24:1 was also greater in holothurians (4–8%) than in ophiuroids (2%). This difference was most pronounced for 20:1ω11c, 20:1ω9c, and 20:1ω7c which were 1–4% for holothurians and 17–19% for ophiuroids. Relative proportions of other MUFA differed slightly between holothurians and ophiuroids (Table 3). 16:1ω7c and 16:1ω5c comprised 2–6% of total FA in holothurians and were 1–2% in ophiuroids. The MUFA 18:1ω9c, 18:1ω7c, and 18:1ω5c were 5–10% in holothurians and 9–13% in ophiuroids. The MUFA 22:1ω11c, 22:1ω9c, and 22:1ω7c were 5–6% for holothurians and 3–5% for ophiuroids.

Total saturated fatty acids (SFA) were lower than either MUFA or PUFA in all species except *O. mutabilis* (Fig. 1). The principal SFA included 16:0, 18:0, 14:0 (<1% holothurians, 2–7% ophiuroids) and 20:0. *Ophiacantha* sp. had 12% of 20:0 whereas *O. bathybia* had 0.2%. There were minor amounts (<1.3%) of odd-carbon SFA i15:0, a15:0, i17:0, a17:0, and 17:0 in all species except the holothurian *O. mutabilis* with 11% total odd-carbon SFA (Table 3).

Several other fatty acids were present in the echinoderm profiles. The holothurians had 11–14% of the hydroxy acid αOH 24:1 which was not detected in the ophiuroids (Table 3). Holothurians also had 2–4% of αOH 23:1 versus 0.1–0.2% in the ophiuroids. The glyceryl ether diol-1-hexa-decylglyceryl ether (16:0), which co-eluted with 22:1ω9c, was detected in minor amounts in all species of holothurians and ophiuroids. Nonmethylene interrupted diunsaturated FA (20:2 NMI and 22:2 NMI) occurred at low levels in all species (Table 3).

Differences in the fatty acid profiles between holothurians and ophiuroids were clearly separated by the principal components analysis (PCA, Fig. 3). Fatty acids that contributed most to the separation of groups along PC1 were 20:1ω9c, 20:0, C26PUFA, αOH24:1, 23:1 and 22:6ω3 which collectively explained 45.0% of the total variance. The

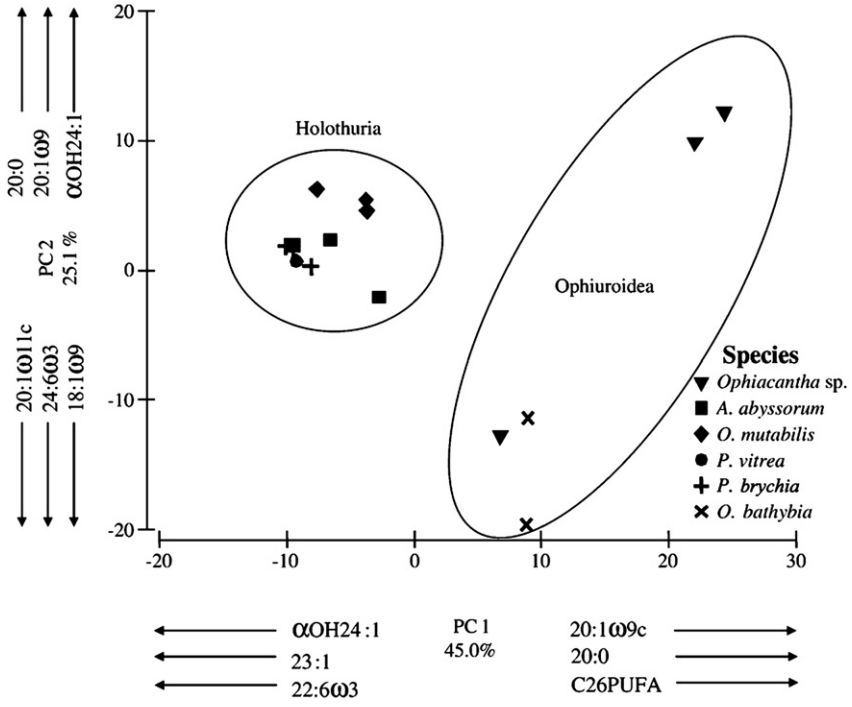

Fig. 3. Biplot of the first and second PC derived from the fatty acid composition of individual holothurian and ophiuroid specimens. PC1 explained 45.0% of the variability between species. PC2 explained 25.1% of the variability. Arrows indicate fatty acids contributing most to the distribution of species along each component. Holothurians are: *Abyssocucumis abyssorum*, *Oneirophanta mutabilis*, *Peniagone vitrea* and *Protankyra brychia*. Ophiuroids are: *Ophiacantha mutabilis* and *Ophiura bathybia*.

fatty acid profiles of holothurians and ophiuroids were most clearly separated along PC1, but holothurians also formed a discrete group along PC2. The ophiuroids were more scattered along PC2 which explained 25.1% of the total variance. The major fatty acids contributing to PC2 were 20:0, 20:1ω9c, αOH24:1, 20:1ω11c, 24:6ω3 and 18:1ω9c. The specimen of *Ophiacantha* sp. which had much lower WE than the other two individuals clustered closely with the *O. bathybia*. It had much higher 20:1ω11c and much lower 20:0 and 20:1ω9c compared to its two conspecifics.

4. Discussion

4.1. Lipid content and composition

Low levels of lipids (3–9%, Table 1) and low storage lipid (TAG/WE) in holothurians are in agreement with past studies. Previous studies of deep-sea (Artem'ev, 1975; Neto et al., 2006) and shallow water (Svetashev et al., 1991) holothurians indicate lipid levels of ~1–5% of dry weight. It was calculated that utilization of lipid and carbohydrate during starvation of *Holothuria atra* could only meet very short-term energy needs (Lawrence, 1972). The absence of lipid stores is interesting because the flux of their phytodetrital food varies 10 fold seasonally with large variation in the timing of peak flux and phytodetrital flux varies interannually ~2 fold (Baldwin et al., 1998; Lampitt et al., 2001). Perhaps these animals adjust to varying food supply through changes in activity (Smith et al., 1993) or reproductive activity (Ruhl, 2008). The dominance of PL in deep-sea holothuria (Table 1) agrees with data of Thompson et al. (2001) for shallow water holothuria. Glyceryl ethers (5–12% of total lipid) were reported in gut walls and contents of *O. mutabilis* from the Porcupine Abyssal Plain in the NE Atlantic (Santos et al., 2002). *O. mutabilis* in our study from the NE Pacific had none, but another holothurian, *P. vitrea*, had 1% GE (Table 1). In fishes, DAGE is used for buoyancy (Phleger, 1998), but this lipid's function in holothurians and other benthic invertebrates remains obscure (Santos et al., 2002).

Ophiuroids also had low lipid content although they contained more storage lipid than holothurians (Table 1). *O. bathybia* contained 20% TAG and *Ophiacantha* sp. 32% WE. Since TAG is a short short-term energy reserve lipid and WE a long term energy reserve lipid (Lee and Patton, 1989), differences in lipid composition are likely indicative of two different feeding strategies. *O. bathybia*, is the most abundant ophiuroid at Sta. M (Lauerman et al., 1996) and resides in the sediments whereas *Ophiacantha* sp., is found living on the tubes of polychaetes, hexactinellid sponges, or other biogenic structures protruding from the seafloor (Lauerman et al., 1996). Ophiuroids most likely consume detritus and also are known to prey on small infaunal organisms (Pearson et al., 1984), but interspecific differences in food habits are likely. The different life styles of the two species in this study likely result in important diet differences. Both of these species had slightly higher $\delta^{15}N$ and $\delta^{13}C$ isotopic values compared to the holothurians suggesting a diet with less detritus and more animal material (Drazen et al., in press). Feeding on more animal material could result in a more sporadic intake of energy due to less reliability of animal food resources and thus energy reserves are needed. Lipids of *O. bathybia* have shown seasonal fluctuations significantly correlated with particle flux at Station M (Lauerman, 1998). *Ophiura sarsi* from Wakasa Bay, Japan, had 30% TAG (Kawasaki et al., 2000) but in several Arctic species storage lipids are <10% of total lipids (Graeve et al., 1997). Temporal studies of lipid dynamics and samples large enough to separate temporal from intraspecific variation are necessary to truly elucidate the nature of storage lipids in these echinoderms.

4.2. Sterols

The sterol compositions of the echinoderms are indicative of their feeding on phytodetrital material. Phytosterols are synthesized only by algae and plants where their main function is to regulate membrane fluidity (Rozner et al., 2006). They are structurally similar to cholesterol but have an extra hydrophobic carbon chain at the C–24 position. 24-Ethylcholest-5-en-3β-ol is the most abundant phytosterol in land plants (Sasaki et al., 1990; Trautwein et al., 2003) and in the marine environment it is abundant in phytoplankton (Volkman, 1986, 2003). It is one of the most abundant phytosterols in the holothurians and ophiuroids in our study (9–26% of total ST, Table 2) indicating a diet rich in phytoplanktonic material. 24-Methylcholesta-5,22E-dien-3β-ol, reported to be a major ST in some prymnesiophytes, such as *Phaeocystis* (Nichols et al., 1991; Tsitsa-Tzardis et al., 1995), diatoms and other species, was an abundant ST in both holothurians and ophiuroids (5–16% of total ST, Table 2). The abundance of this sterol in the filter feeder *Pyrosoma atlanticum*, has been shown to reflect dinoflagellates and prymnesiophytes as major diet items (Perissinotto et al., 2007). Thus the high phytosterol composition in these abyssal echinoderms is reflective of a diet rich in phytodetritus. A greater proportion of phytodetritus in the diet of the holothurians is evident from their higher proportion of phytosterols compared to the ophiuroids (Fig. 1).

Ophiuroids are known to be opportunistic feeders consuming both phytodetritus and animal material (Warner, 1982; Pearson et al., 1984) and this is reflected in their sterol composition. Cholesterol and cholesta-5,22E-dien-3β-ol were the major sterols in both ophiuroids (27–36% and 18–19%, respectively; Table 2). In holothurians cholesterol and cholesta-5,22E-dien-3β-ol were considerably less (4–10% and 3–11%, respectively). Cholesterol reflects a carnivorous diet (Nelson et al., 2001). Many ophiuroids are facultative carnivores, capturing food with their arms and transferring it to the mouth (Warner, 1982). The two ophiuroids are the only two species studied at this abyssal station with appreciable proportions of cholesta-5,22E-dien-3β-ol. Polychaetes, crustaceans, and fishes all had less than 8% (Drazen, Phleger and Nichols, unpub data). Cholesta-5,22E-dien-3β-ol is an intermediate in cholesterol synthesis. Thus it is likely that the ophiuroids have large amounts due to differences in sterol metabolism rather than diet. Cholesta-5,22E-dien-3β-ol was the major ST in the Antarctic ice diatom *Nitzschia cylindricus* (Nichols et al., 1986b) and in a variety of Antarctic cnidarians, ctenophores (Nelson et al., 2000), and salps (Phleger et al., 1998). Salp faecal pellets are an important means of vertical transfer of particulate matter to the abyssal deep-sea floor. Cyanobacteria and photosynthetic pigments from salp (*S. fusiformis*) faeces are rapidly incorporated into abyssal holothurians including *O. mutabilis* and *Psychropotes longicauda* (Pfannkuche et al., 1993). However, if salp faecal pellets or dead sinking gelatinous organisms were the major source of cholesta-5,22E-dien-3β-ol then the holothurians, which would also eat faecal pellets in detritus, would be expected to have similar proportions compared to the ophiuroids.

The sterol compositions of the holothurians contained many stanols which probably reflect sterol metabolism within the animals and/or bacterial sources from within the gut. The holothurians possessed 8-17% 24-ethyl-5α-cholestan-3β-ol, whereas the ophiuroids had only 0-0.4%. 5α-cholestan-3β-ol levels were also higher in holothurians (3–20%) and lower in ophiuroids (1–2%). Other stanols were detected including 24-methyl-5α-cholestan-3β-ol and 24-ethylcholestan-22E-en-3β-ol and again they were not present in the ophiuroids. These stanols may result from incorporation and biohydrogenation of dietary components or inefficient conversion of Δ5 to Δ7 sterols. In fact this study found no Δ7 sterols although these have been found to be common in shallow and deep living holothurians (Ginger et al., 2000). The presence of stanols in these abyssal holothurians may reflect bacterial metabolism. For instance, 5β-cholestan-3β-ol (coprostanol) is produced by enteric bacterial degradation of cholesterol in mammal digestive tracts (Leeming et al., 1998). Many holothurians have unique bacterial assemblages in their gut to aid in digestion (Roberts et al., 2000). The highest amounts of

stanols were present in *O. mutabilis*. In this species 5α-cholestan-3β-ol and 24-ethyl-5α-cholestan-3β-ol are most abundant (Table 2). The latter stanol results from hydrogenation of 24-ethylcholest-5-en-3β-ol which is much lower in this species than the other holothurians. In addition to bacteria in the gut, *O. mutabilis* possesses bacteria in the integument of the tentacles and body wall (Roberts et al., 2000). It is unknown whether the other species do.

Differences in relative amounts of sterols in the holothurians may reflect feeding strategies. *O. mutabilis*, in particular, exhibits a strong contrast in sterol composition compared to the other species with higher total stanols and lower amounts of phytosterols (Fig. 1). This species is a selective deposit feeder, using its digitate tentacles to transfer food particles to its mouth from the surface of the sediments and a rapid locomotory capability, compared to the other holothurians, to cover large areas of the seafloor in search of rich particles (Kaufmann et al., 1997; Roberts et al., 2000). Sterols of *O. mutabilis* were closely related to particulate organic matter arriving at the sea floor on the Porcupine Abyssal Plain in the northeast Atlantic Ocean, however the high levels of stanols reported here were not evident in the north Atlantic specimens (Neto et al., 2006). In our study the high stanols may result from the selection of bacteria rich particles or from enteric bacteria as indicated above. *A. abyssorum* is a dendrochirotid holothurian which has been shown to selectively ingest fresh phytodetritus from the sediment surface (Lauerman et al., 1997) and it may suspension feed as shallow water members of the order do. It had the highest amounts of phytosterols of all the species (Fig. 1). *P. vitrea* has been observed at detrital aggregates on the seafloor and assumed to be eating them (Lauerman et al., 1998). It has the ability to swim into the water column which may facilitate foraging over large areas or as a mechanism to avoid predators (Billett, 1991). Curiously *P. vitrea*'s sterol profile is very similar to that of *P. byrchia* a burrowing apodan. *P. brychia* is common in trawls at the study site, however, it never appears in camera sled transects (Lauerman et al., 1996; Ruhl, 2007). Little is known of the biology of this species although others in the order are subsurface deposit feeders feeding deep within the sediments or right at the sediment water interface but from below. Therefore, it is somewhat surprising that this species has a sterol composition indicating a diet similar to both *P. vitrea* and *A. abyssorum* which feed on detritus on the top of the sediment. Furthermore, in a study of stable isotopes of benthic fauna at this station *P. brychia* had much greater δ^{15}N and δ^{13}C isotopic values compared to the other holothurians indicating a different diet (Drazen et al., in press). Further research is needed to determine the feeding habits of this enigmatic burrowing holothurian.

4.3. Fatty acids as trophic markers

The differences in diet between the holothurians and ophiuroids were also evident from their fatty acid compositions. The PCA analysis clearly separated these two groups of echinoderms (Fig. 3). The deposit feeding holothurians possessed greater amounts of algal derived FA such as DHA and EPA (Table 3). They also contained large amounts of some unusual FA which are discussed in more detail below. The ophiuroids had greater amounts of 18:1ω9 which is typically derived from animals. There were considerably higher values for the three isomers of 20:1 in ophiuroids (17–19%) as compared to holothurians. Long chain MUFA, including the 20:1 and 22:1 isomers, are evidence for carnivory/omnivory. Calanoid copepods have been reported as the major synthesizers for long chain MUFA in marine food webs (Sargent et al., 1981). Copepod faecal pellets and other remains are very common on the sea floor and could be selectively consumed by the ophiuroids. In addition, *Ophiacantha* sp. are perched on structures elevated above the seafloor where their arms may be capable of the direct capture of small zooplankton from the water column. However at this time, synthesis of these MUFA by the ophiuroids can not be ruled out.

Within the holothurians, *O. mutabilis* has a very different FA composition most likely the result of bacterial influences. There are clear differences in total PUFA and component FA (particularly EPA) between the holothurian *O. mutabilis* (lower values) and all other species of both holothurians and ophiuroids (higher values) (Table 3, Fig. 2). DHA, an essential FA for higher marine organisms, was also low in *O. mutabilis*. A FA profile of *O. mutabilis* from the abyssal NE Atlantic included EPA, AA and DHA as abundant FA and did not differ from the other co-existing holothurians, *P. longicauda* and *Pseudostichopus villosus* (Neto et al., 2006). Only the ST profile differed significantly between these NE Atlantic species. Ginger et al. (2000) reported that EPA and AA were the most abundant FA in *O. mutabilis*, *P. longicauda* and *P. villosus* as well as four other holothurian species also from the abyssal NE Atlantic. Other abyssal holothurians have similar PUFA levels such as *Scotoplanes theeli* from 4400 m depth in the Cedros Trough off Baja California, Mexico, which had high AA and EPA (21 and 18% respectively) and low DHA (5%; Lewis, 1967). *O. mutabilis* in our study certainly is in contrast and the lower total PUFA, as a proportion of total FA, can be explained by this holothurian's much higher proportions of bacterial FA (i15:0, a15:0, i17:0, a17:0, 17:0, 18:1ω7; Fig. 2; Table 3). These bacterial FA markers (Mayzaud et al., 1989; Nichols et al., 1991) indicate a greater microbial input to the diet and assimilation into tissues of this holothurian than to the other species and are supported by the sterol results.

4.4. Unusual fatty acids

The unusual PUFA 24:6ω3 (tetracosahexaenoic acid, THA) may be a good biomarker for ophiuroids. It was present in both ophiuroids but only in trace amounts in three of the holothuroids (Table 3). THA level in *O. bathybia* is similar to that reported for *O. sarsi* (14% of total lipids, Kawasaki et al., 2000). In contrast, a starfish collected from the same area (Wakasa Bay, Japan) with a detrital diet similar to *O. sarsi* had 1.2% THA (Kawasaki et al., 2000). The ophiuroid *Amphiura elandiformis* had high THA (Mansour et al., 2005) like the ophiuroids in the current study, but since THA was absent from the sediment associated with *A. elandiformis*, it was concluded that THA does not originate from the diet (Takagi et al., 1986). THA could also be absent from surrounding sediment due to an expected short lifetime of this polyunsaturated FA in oxygenated abyssal sediments. The FA profile of *A. elandiformis* was consistent with chain elongation of 20:5ω3 to 22:5ω3 and 24:5ω3 followed by desaturation to 24:6ω3 (Buzzi et al., 1997; Sprecher, 2000). THA may accumulate due to low activity for the chain-shortening step. Certainly, 22:6ω3 (DHA) levels were low in *O. bathybia* (Table 3).

Another unusual fatty acid, 23:1 (tricosenoic acid), was highest in the holothurians *A. abyssorum* (9% of total FA) and *O. mutabilis* (8%) with less in *P. vitrea* and *P. brychia* (4%) and <1% in the ophiuroids contributing to the separation of these two groups along PC1 (Table 3, Fig. 3). *O. mutabilis* from the NE Atlantic (Porcupine Abyssal Plain) had 17% 23:1 (Ginger et al., 2000), but this FA was not reported in other holothurians including *O. mutabilis* from the same location although collected a few years latter (Hudson et al., 2004). Earlier studies of FA composition of holothurians also did not report 23:1 (Lewis, 1967; Allen, 1968). Levels of AA in these earlier studies were higher (21%) than in our analyses (Table 3, 2–12%) and in Ginger et al. (2000) (14%). This difference could possibly be due to co-elution of 23:1 with AA in the earlier studies which used short packed columns rather than capillary columns. It was suggested that the elevated levels of 23:1 and other MUFA in the deep-sea holothurians function in the maintenance of membrane fluidity at low temperatures and high pressures (Ginger et al., 2000). In a study of tropical and temperate-water holothurians, 2–7% of 23:1 was reported (Svetashev et al., 1991). The structure of 23:1 in holothurians from Japan shallow coastal waters was determined as *cis*-14-tricosenoic acid (23:1ω9; Kaneniwa et al., 1986). Levels of 23:1 in these holothurians were 1–7% and 23:1 was a minor component of Crinoidea and Ophiuroidea (<1%).

Since *n*−9 MUFA were found in holothurians by Kaneniwa et al., (1986) and our study (Table 3), it was suggested that 23:1 is formed from 24:1 by α-oxidation, but not by desaturation of odd-chain saturated fatty acids. Levels of 24:1 for the holothurians in our study were 4–8% versus 2% in the ophiuroids where 23:1 levels are also low (Table 3). In seal lipids, it has been proposed that 21:5(*n*−3) is formed from 22:5 (*n*−3) by α-oxidation (Mayzaud et al., 1978). It is possible to biosynthesize 24:1 from MUFA *n*−9 precursors including 18:1, 20:1, and 22:1 as has been shown in vegetables. The content of 23:1 was higher in PL in the holothurians (Kaneniwa et al., 1986) and in our study since most FA were from PL (Table 1). Therefore, we also propose that 23:1 must be more important for membrane structure and function than as a component of energy reserve molecules. Sea urchins caught at the same time and location as holothurians lacked 23:1, yet had a similar detrital diet (Kaneniwa et al., 1986). This finding also indicates that the 23:1 does not derive from the holothurian diet and is synthesized *de novo*.

The presence of substantial amounts of the hydroxy acid αOH 24:1 in holothurians with none detected in ophiuroids is very unusual and is the primary separator of these two groups in the PCA (Fig. 3). Similarly, the presence of αOH 23:1 in much greater relative levels in holothurians than in ophiuroids (Table 3) is also unusual and is to our knowledge, the first report of these hydroxy acids in echinoderms. The lack of previous reports of these FA may be in part due to the methods used. The precise role of αOH 24:1 and αOH 23:1 in the holothurians remains to be determined. Numerous reports exist on the occurrence of OH FA in other marine organisms, including microbes where they may be used as structural components of membranes either in phospholipids or lipopolysaccharides (Skerratt et al., 1991, 1992). Our findings indicate the wider than hitherto realized distribution of these components.

5. Conclusions

An analysis of the lipids of abyssal echinoderms in the eastern North Pacific has augmented our information on their feeding habits. Holothurian's were rich in phytosterols and algal derived FA such as DHA suggesting tight trophic coupling to phytodetritus similar to studies in the abyssal Atlantic (Ginger et al., 2000; Neto et al., 2006). The relatively large proportions of stanols are probably the result of enteric bacteria but may come from sterol metabolism in the holothurians themselves. Despite supposedly different feeding modes (subsurface versus surface deposit feeding) three of the four holothurians exhibited only small differences in sterol and FA composition. However, *O. mutabilis* was very different with much higher levels of stanols and bacterially derived FA suggesting specific selection of bacteria rich detrital particles or the activity of enteric and integumental bacteria. The ophiuroids sterol and FA compositions differed greatly from the holothurians and reflected consumption of animal material in addition to phytodetritus. The ophiuroids contained a significant proportion of TAG or WE for energy storage which also may be advantageous to species which feed more sporadically than the deposit feeding holothurians.

Several unusual fatty acids were found in these abyssal echinoderms. 24:6ω3 appears to be a biomarker for ophiuroids and 23:1 appears to be a good biomarker for holothurians. We report αOH 24:1 in the holothurians, the first occurrence of this FA in echinoderms. The precise role of this FA is presently unknown. The characterization of the lipids of these species has aided in understanding their trophic biology and will also assist in larger lipid biomarker studies to elucidate the trophic interactions of the abyssal seafloor community.

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