Lipid, sterols and fatty acids of abyssal polychaetes, crustaceans, and a cnidarian from the northeast Pacific Ocean: food web implications

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ABSTRACT: The lipid, sterol, and fatty acid compositions of the abyssal anemone *Bathyphellia australis*, the 3 polychaetes *Laetmonice* sp., *Paradiopatra* sp. and *Travisia* sp., 3 crustaceans (*Munidopsis* sp. and 2 lysianassid amphipods), and an unidentified caridean shrimp were determined from a site in the northeast Pacific Ocean. Lipid composition was dominated by phospholipids in most species. However, energy storage lipids (triacylglycerols and wax esters) contributed >50% to the total lipids in the lysianassid amphipods and the unidentified caridean shrimp, and lipids made up 19 and 45% of amphipod and 14% of shrimp dry mass, suggesting sporadic feeding and the need for energy storage. The dominant sterol was cholesterol. The presence of phytosterols in the anemone, the polychaetes and *Munidopsis* sp. suggested consumption of phytodetritus, but none of the specimens had levels suggesting that phytodetritus was their main food resource. The levels of essential photosynthetically derived polyunsaturated fatty acids (PUFA) corroborated the sterol results. The ratio of 18:1ω9/18:1ω7 further suggested carnivory as the predominant mode of foraging in the abyssal animals, although to varying degrees. Some distinct differences in the fatty acid (FA) composition of these animals allowed for their separation, confirming that FA profiles will be useful in future biomarker approaches to deep-sea food web studies.

KEY WORDS: Polychaeta · Cnidaria · Crustacea · Deep sea · Trophic biology · Lipid composition · Sterols · Fatty acid biomarkers

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INTRODUCTION

The benthic megafauna are important members of abyssal communities. Echinoderms typically dominate (Billett 1991, Ruhl 2007), while polychaetes, crustaceans, and anemones are also common and often abundant (Lauerman et al. 1996). The trophic relationships of these animals are poorly understood and are often based on functional morphology or assumptions derived from knowledge of shallow water members of the groups. This lack of information has hampered understanding of deep-sea food web function and basic ecological relationships. Studies have identified broad feeding guilds (Fauchald & Jumars 1979) and more detailed niche separation between related species (Cartes et al. 2007). In general, polychaetes and crustaceans can be consumers of surface-derived phytodetrital material, predators on other abyssal fauna, or scavengers on carrion. Anemones are less studied, but the consumption of detritus and benthopelagic plankton are obvious feeding modalities. All these abyssal megafauna are also potential prey for higher trophic levels such as fishes (Martin & Christiansen 1997, Drazen et al. 2008a). Thus, polychaetes, crustaceans, and anemones play an important and diverse role in deep-sea food webs and more information on their trophic relationships would be helpful.

Lipid biomarkers are useful tools for studying trophic ecology and determining food web connections. The relative percentage of lipid classes helps to characterize the trophic status of an organism. Triacylglycerols (TAG) are short-term energy storage lipids, whereas wax esters (WE) are long-term energy storage lipids. During starvation stress, TAG are utilized before WE (Lee & Patton 1989). Phospholipids (PL) and sterols (ST) are primarily membrane structural components, and dominance of these lipid classes in the lipid profile indicates a constant food source. Cholesterol is the main ST in most animals, and the presence of other ST may provide good trophic indicators. For example, phytosterols are synthesized by algae and plants. Their main function is to regulate membrane fluidity (Rozner & Garti 2006). They are structurally similar to cholesterol, although they have an extra hydrophobic carbon chain at the C-24 position. 24-Ethylcholesterol is the major phytosterol in land plants (Trautwein et al. 2003) and may be abundant in select marine phytoplankton (Volkman 1986). Organisms can have unique fatty acid (FA) profiles that can be traceable, and many of these FA are transferred from prey to predator without modification (Nichols et al. 1986, Phleger et al. 1998). FA have been used as dietary tracers in the ocean since the 1960s (Ackman & Eaton 1966, Sargent 1976) to explore dietary relationships in a number of diverse marine organisms (Nichols et al. 2007). The approach is based on the assumption that many FA in the ocean, particularly polyunsaturated FA (PUFA), can only be biosynthesized by certain phytoplankton and macroalgal species and become essential dietary components to higher trophic levels. Phytoplankton and macroalgae are often characterized by very distinct ratios of FA, and these ratios influence the FA profiles of higher organisms, thus making them useful tools for providing information on food webs (Graeve et al. 2002).

Few studies have employed lipid and FA profiles to study feeding ecology and food webs in the deep sea. Studies of hydrothermal vent ecosystems showed a strong bacterial dietary input for all vent invertebrates, while archaeal-derived ether lipids were not detected (Phleger et al. 2005a,b). Signature lipid studies of abyssal zooplankton in the Atlantic have helped to identify specific feeding niches and have suggested ties between bentho-pelagic copepods and phytodetrital food sources even at great depths (Bühring & Christiansen 2001). However, no studies have yet used lipid biomarker techniques to study abyssal megafauna in the Pacific, other than echinoderms (Drazen et al. 2008b).

In this study, the lipid, FA and ST composition of 3 polychaetes, 4 crustaceans, and an anemone from a site in the abyssal northeast Pacific Ocean were analysed. Recent studies at this site indicate inter-annual fluctuations in abundance of the benthic megafauna related to changes in the productivity of overlying surface waters (Ruhl & Smith 2004). The goal of the present study was to assess their feeding habits, using lipid biomarkers, and also to ascertain their lipid profiles for use in a larger study incorporating diet analysis and stable isotope signatures to examine food web dynamics and trophic ecology of top predators in the ecosystem (Drazen et al. 2008a).

MATERIALS AND METHODS

Sampling. All samples were taken during a research cruise on the RV 'Atlantis' to Stn M (34° 50' N, 123° 0' W, 4100 m water depth) in the northeast 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 & Druffel (1998). In brief, this site is 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, with a peak in summer months. August of 2006 showed the peak seasonal flux for the year at 10.26 mg C m^{-2} d^{-1}.

Common cnidarian, polychaete and crustacean species, as determined from previous camera sled and trawl analyses (Lauerman et al. 1996), were sampled. Collections of specimens were made during DSV 'Alvin' dives using scoops and a suction sampler and returned to the surface in plastic sample boxes. Specimens were also collected using a 12.3 m otter trawl deployed from the RV 'Atlantis'. In this case, only specimens in very good condition, showing little or no signs of damage, were used. Specimens were rinsed free of any adhering sediment before tissues were sampled. These included *Paradiopatra* sp., *Laetmonice* sp. and *Munidopsis* sp. Two unidentified species of lysianassid amphipods (given here simply as A and B) were collected using a baited trap. Abdominal muscle tissue of the large galatheid crab *Munidopsis* sp. was sampled. A caridean shrimp was split longitudinally and one-half was used for the signature lipid analysis described below, while the other was used for a different study. The lysianassid amphipods were small (2 to 6 mm), so 7 to 8 individuals were pooled for each sample. The rest of the invertebrates were whole specimens. All samples were frozen and stored in cryovials under liquid nitrogen on board ship and subsequently 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 weights were between 0.02 and 0.39 g.

Lipid extraction. Samples were quantitatively extracted overnight using a modified Bligh & Dyer (1959) one-phase methanol:chloroform:water (2:1:0.8 v:v:v) extraction. 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.

Lipid classes. An aliquot of the TSE was analysed using an Iatroscan MK V TH10 thin-layer chromatography-flame ionization detector (TLC-FID) analyser to quantify individual lipid classes (Volkman & Nichols 1991). Samples were applied in duplicate to silica gel SIII chromarods (5 μm particle size) using 1 μl micropipettes. Chromarods 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 WE, TAG, free fatty acids (FFA) and ST from 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 chromarods 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 to 10 μg range). Peaks were quantified on an IBM-compatible computer using DAPA Scientific software (Kalamunda). TLC-FID results are generally reproducible to ±10% of individual lipid class abundances (Volkman & Nichols 1991).

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

Gas chromatographic (GC) analyses were performed with an Agilent Technologies 6890N GC (Palo Alto) equipped with an HP-5 cross-linked methyl silicone fused silica capillary column (50 m × 0.32 mm inner diameter), an FID, a split/splitless injector and an Agilent Technologies 7683 Series auto sampler and injector. Helium was the carrier gas. Following the 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. Individual components were identified using mass spectral data and by comparing retention time with data obtained for authentic and laboratory standards. GC results are subject to an error of ±5% of the individual component area. GC-mass spectrometric (GC-MS) analyses were performed on a Finnigan Thermoquest GCQ GC-MS fitted with an on-column injector using the software Xcalibur (Thermoquest). The GC was fitted with a capillary column similar to that described above.

Statistical analysis. FA profiles of different taxa were compared 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 FA that contribute most to the separation between observed groups (Best et al. 2003). FA that contributed a mean of less than 1.0% (of total FA) to the profile were omitted from statistical analyses. All analyses were performed on % composition data and results were confirmed by analysis of mg g^{-1} fatty acid data (data not shown). Multivariate statistical analyses were performed using PRIMER 6 software (PRIMER-E).

RESULTS

Lipid content and classes

Total lipid content varied between the animals examined (Table 1). Lipid content was low (ca. 3 to 5%) in the 2 large polychaetes, *Laetmonice* sp. (Aphroditidae) and *Travisia* sp. (Opheliidae), the anemone *Bathyphellia australis*, and the galatheid crab *Munidopsis* sp. The onuphid polychaete *Paradiopatra* sp. and an unidentified caridean shrimp contained more lipid and the 2 lysianassid amphipods contained the most lipid as a proportion of dry weight (18.6 to 44.9%).

Lipid classes were dominated by PL in all organisms, except the shrimp and amphipods (Table 1). Storage lipids (TAG and WE) in polychaetes were generally low, although *Paradiopatra* sp., the species with the highest lipid content of all the polychaetes, had 11% WE. The shrimp and amphipods contained high amounts of storage lipids (39 to 83% TAG), but *Munidopsis* sp. did not. This probably reflects the fact that only *Munidopsis* sp. abdominal muscle was analysed, while for the other species total mass was extracted. In the crustacean *Jasus edwardsii*, the lipid class (and FA) profiles of leg and tail tissues were generally very similar, while those in the hepatopancreas differed

Table 1. Lipid content and composition of abyssal Cnidaria, Polychaeta, and Crustacea. n: number of samples, HC: hydrocarbon, WE: wax ester, DAGE: diacylgylceryl ether, TAG: triacylgylcerol, FFA: free fatty acid, ST: sterol, PL: phospholipid. Means ± SD

	n	HC	WE	DAGE	TAG	FFA	ST	PL	Lipid content (% dry weight)
					% composition				
Cnidaria									
Bathyphellia australis	1	3.3	3.9	0	9.2	3.3	7.4	72.9	4.5
Polychaeta									
Laetmonice sp.	1	0	2.7	0	0	0.9	7.6	88.8	5
Paradiopatra sp.	3	0	10.9 ± 2.8	0.6 ± 0.3	0.6 ± 0.3	3.1 ± 1.5	5.8 ± 0.6	79 ± 1	9.1 ± 0.8
Travisia sp.	2	1.5 ± 0.1	1.2 ± 0.1	0	0	1.1 ± 0.1	12.5 ± 0.8	83.8 ± 1.1	3.0 ± 0.6
Crustacea									
Munidopsis sp.	3	0	0.4 ± 0.3	0	0	1.1 ± 0.8	6.5 ± 0.4	91.9 ± 0.3	5.3 ± 0.3
Caridean shrimp	1	0	9.9	0	39.1	1.6	1.8	47.6	14
Lysianassid amphipod A	1	0	0.7	0	68.9	5.5	1.4	23.4	18.6
Lysianassid amphipod B	1	0	0.7	0	82.6	1.9	0.3	14.5	44.9

(Nelson et al. 2005). It is likely that storage lipids could be found in the hepatopancreas of *Munidopsis* sp. since this is often a lipid-rich tissue in crustaceans (Nelson et al. 2005). The FA of *Munidopsis* sp. were also primarily from PL, while in the other crustaceans, the FA reported were from both storage lipid and PL. ST in the animals generally ranged from 5 to 12%, but was considerably lower in the TAG-rich amphipods and shrimp (Table 1). FFA was generally low in all samples.

Sterols

The ST composition of the different taxa were dominated by cholesterol (73 to 99%, as % total ST), while the smaller amounts of other sterols varied in proportion intraspecifically (Table 2). In the polychaetes,

smaller proportions of *trans*-22-dehydrocholesterol, dihydrobrassicasterol, brassicasterol, sitosterol, and 24-methylenecholesterol were found. ST composition in *Bathyphellia australis* was similar to that of the polychaetes. ST in crustaceans were less diverse than in the other taxa, with only cholesterol found in lysianassid amphipod B (Table 2). While the ST composition of *Munidopsis* sp. was less diverse than that of the other animals, it was much more diverse than either that of the shrimp or of the amphipods, including the presence of several unidentified ST (as 'other' in Table 2). Similar to the shrimp, *Munidopsis* sp. had low levels of *trans*-22-dehydrocholesterol and *trans*-22-dehydrocholestanol with smaller amounts of a few other ST. Total stanols were low in all of the species examined (0 to 5%), but total phytosterols ranged greatly from 0% in the caridean shrimp and lysianassid amphipod B to 16.4% in *Travisia* sp. (Table 2).

Table 2. Sterol composition (of total sterols) of abyssal Cnidaria, Polychaeta, and Crustacea. Means ± SD

Sterol	Cnidaria *Bathyphellia australis*	*Laetmonice* sp.	*Paradiopatra* sp.	*Travisia* sp.	*Munidopsis* sp.	Caridean shrimp	Lysianassid amphipod A	Lysianassid amphipod B
			Polychaeta			Crustacea		
24-nordehydrocholesterol	1.1	0.0	0.2 ± 0.2	1.2 ± 0.1	0 ± 0	0.0	0.0	0.0
Cis-22-dehydrocholesterol/occelasterol	1.5	0.2	1 ± 0.1	0.5 ± 0.1	0 ± 0	0.0	0.0	0.0
Trans-22-dehydrocholesterol	8.6	3.2	6.7 ± 0.2	5.2 ± 0.7	3.8 ± 0.5	3.2	0.0	0.0
Trans-22-dehydrocholestanol	1.5	1.5	1.5 ± 0.2	2.1 ± 0.1	1.8 ± 0.1	0.0	1.8	0.0
Cholesterol	73.2	88.2	78.5 ± 1.7	72.9 ± 0.7	82.2 ± 1.7	96.8	98.2	100
Cholestanol	0.8	0.5	1.4 ± 0.1	0.4 ± 0.2	0.1 ± 0.2	0.0	0.0	0.0
Brassicasterol/crinosterol	3.3	2.1	2.8 ± 0.3	2.6 ± 0.4	0 ± 0	0.0	0.0	0.0
Brassicastanol/crinostanol	0.0	0.0	0 ± 0	2.5 ± 0.2	0 ± 0	0.0	0.0	0.0
24-methylenecholesterol	3.0	0.5	0.5 ± 0.5	0.6 ± 0	0 ± 0	0.0	0.0	0.0
Campesterol/dihydrobrassicasterol	2.1	2.7	2.8 ± 0.4	6 ± 0.4	0.3 ± 0.1	0.0	0.0	0.0
23,24-dimethylcholesta-5,22E-dien-3β-ol	0.0	0.4	0 ± 0	0.6 ± 0.1	0.2 ± 0.1	0.0	0.0	0.0
Stigmasterol/poriferasterol	0.0	0.0	0.3 ± 0.3	0.5 ± 0	0 ± 0	0.0	0.0	0.0
23,24-dimethylcholesta-5-en-3β-ol	0.0	0.0	0 ± 0	0 ± 0	0.3 ± 0.3	0.0	0.0	0.0
Sitosterol/clionosterol	1.4	0.5	3.7 ± 0.3	4.2 ± 0.7	0.3 ± 0.2	0.0	0.0	0.0
Other	3.4	0.2	0.6 ± 0.1	0.7 ± 0.5	11.1 ± 1.3	0.0	0.0	0.0
Total stanols	2.3	2.0	2.9 ± 0	5 ± 0.2	1.9 ± 0.3	0.0	1.8	0.0
Total phytosterols	6.8	5.7	9.6 ± 1	16.4 ± 0.7	1 ± 0.4	0.0	0.0	0.0

Fatty acids

The total proportions of PUFA and its composition varied between the species. Total PUFA were lower in the polychaete *Travisia* sp., compared to *Laetmonice* sp. and *Paradiopatra* sp. (Fig. 1). Total PUFA were highest in the anemone *Bathyphellia australis* and the galatheid crab *Munidopsis* sp. (40%). In contrast, the caridean shrimp and the 2 lysianassid amphipods had the lowest PUFA levels (12 to 20%). Much of the total PUFA difference is reflected in the amounts of eicosa-pentaenoic acid (EPA; 20:5ω3), arachidonic acid (AA; 20:4ω6) and docosahexaenoic acid (DHA; 22:6ω3). EPA levels were highest in *Munidopsis* sp. and lowest in *Travisia* sp. (Table 3). AA levels were mostly much lower than EPA and less variable interspecifically. DHA content was highest in *B. australis* and *Munidopsis* sp., lower in *Laetmonice* sp., *Paradiopatra* sp., the caridean shrimp and the lysiannassid amphipods, and lowest in *Travisia* sp. *Paradiopatra* sp. had much higher 22:2NMI (nonmethylene-interrupted diunsaturated FA) than the other polychaetes, and only small amounts were detected in most other animals. Although *Travisia* sp. had low total amounts of PUFA, it had moderate amounts of C21 PUFA, and 20:2NMI proportions were higher than in other animals.

Monounsaturated fatty acids (MUFA) were the most abundant type of FA and were fairly consistent in total proportions in the anemone, the polychaetes and *Munidopsis* sp. (37 to 47%); however, the caridean shrimp and lysianassid amphipods had higher levels (ca. 61%; Fig 1). MUFA composition differed among these 3 crustaceans and among the other taxa. The lysianassid amphipods contained high proportions of 18:1ω9, whereas the other 2 crustaceans had about half as much
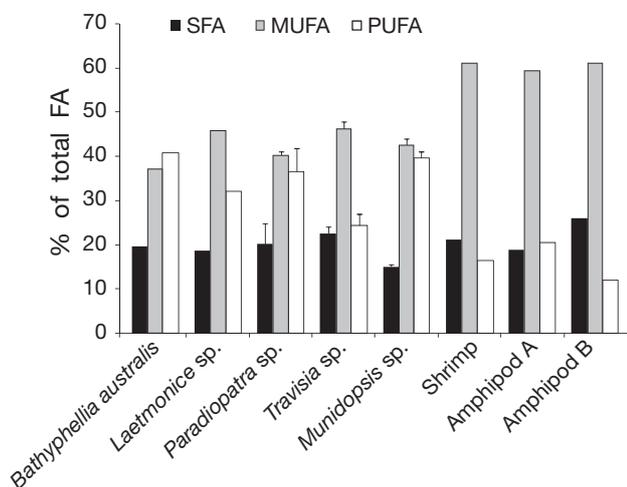

Fig. 1. Fatty acid composition by group for abyssal anemone, polychaetes and crustaceans. FA: fatty acids; SFA: saturated fatty acids; MUFA: monosaturated fatty acids; PUFA: poly-saturated fatty acids. Values are means ± SD

(Table 3) and the other taxa even less. The shrimp, on the other hand, had larger proportions of 16:1ω7c and 20:1ω7c compared to the amphipods or other species. Two other MUFA, 16:1ω5c and 18:1ω7c, occurred in different proportions among taxa (Table 3). C20 MUFA proportions were different both within and between the crustaceans and polychaetes (Table 3).

Total saturated fatty acids (SFA) were similar in most taxa (Fig. 1). Major SFA included 16:0 and 18:0 (Table 2). Total SFA were lower in *Munidopsis* sp. (Fig. 1) than other taxa. In comparison to the other crustaceans, *Munidopsis* sp. had lower levels of 16:0. The amphipods had small but slightly higher proportions of 14:0 compared to the other taxa.

Differences in FA profiles among taxa were clearly separated by the PCA (Fig. 2). FA contributing the most to the separation of groups along PC1 were 18:1ω9c, 16:1ω7, 16:0, 20:5ω3, 22:3ω6, and 18:1ω7, collectively explaining 49% of total variance. The amphipods were most clearly distinguished from other taxa along this axis, and shrimp from polychaetes and *Bathyphellia australis*. Major FA contributing to PC2 were 20:5ω3, 22:6ω3, 18:1ω7c, 20:1ω7c, 20:2NMI, and 22:4ω3, explaining 28% of the total variance. This axis clearly separated *Munidopsis* sp. and *Travisia* sp. from the rest of the taxa.

DISCUSSION

Polychaeta

Lipid content can provide an insight into the tempo of feeding. For instance, polychaetes from hydro-thermal vent environments have large proportions of membrane structural lipids such as PL and ST and low amounts of energy storage lipids such as TAG and WE, which is indicative of their relatively constant food source (Phleger et al. 2005b). Storage lipid content was also low in the 2 large polychaetes *Laetmonice* sp. (Aphroditidae) and *Travisia* sp. (Opheliidae) in our study (Table 1). *Paradiopatra* sp. contained 12% storage lipids, which may indicate a more sporadic diet. *Paradiopatra* sp. is an onuphid polychaete that lives in a mud tube which protrudes from the sediment and then curves back down towards it (Lauerman et al. 1996). Onuphids are opportunistic feeders that consume detritus and prey on other fauna (Fauchald & Jumars 1979). With a sessile lifestyle, *Paradiopatra* sp. relies on food coming to it, which perhaps necessitates greater energy stores. However, with only modest proportions of energy storage lipids, the case is not nearly as clear as for the crustaceans (see below). Reproductive events could also be influencing lipid storage and composition.

Table 3. Fatty acid composition of abyssal Cnidaria, Polychaeta and Crustacea. Ratios are in **bold**. Means ± SD

Fatty acids and ratios	Cnidaria *Bathyphellia australis*	*Laetmonice* sp.	Polychaeta *Paradiopatra* sp.	*Travisia* sp.	*Munidopsis* sp.	Crustacea Caridean shrimp	Lysianassid amphipod A	Lysianassid amphipod B
14:0	0.1	0.6	0.7 ± 0.6	1.2 ± 0	0.5 ± 0.1	0.4	1.3	1.8
a15:0	0.0	0.1	0 ± 0	1.2 ± 0.1	0.1 ± 0	0.2	0	0
15:0	0.4	0.4	0.3 ± 0.1	0.5 ± 0	0.5 ± 0.1	0.3	0.4	0.3
i16:0	0.6	0.2	0.2 ± 0	0.4 ± 0	0.1 ± 0	0.4	0.1	0.1
16:0	9.7	7.8	9.1 ± 4	9.4 ± 1.3	7.6 ± 0.8	12.4	13.2	16.5
16:1+16:2	0.7	0.0	0 ± 0	0 ± 0	0 ± 0	0.0	0	0
16:1ω5c	0.4	0.7	0.2 ± 0	3.8 ± 0.5	0.2 ± 0	0.8	0.2	0.1
16:1ω7c	1.5	3.6	3.3 ± 1.2	2.6 ± 0.6	5.3 ± 1.5	18.1	8.5	15.0
16:1ω9c	0.5	0.6	0.1 ± 0.1	0.4 ± 0.4	0.3 ± 0.1	0.9	0.8	0.4
i17:0	1.4	0.4	1.9 ± 0.3	0.4 ± 0.1	0.5 ± 0.1	0.8	0.2	0.1
a17:0	1.5	0.9	0.4 ± 0	1.6 ± 0.2	0.4 ± 0	1.8	0.2	0.1
17:0	1.8	1.3	1.3 ± 0.2	0.8 ± 0	0.6 ± 0.1	0.7	0.4	0.7
17:1ω6c	0.2	0.2	0.1 ± 0	1.8 ± 0.2	0.2 ± 0	0.2	0.1	0
17:1ω8c	1.0	0.6	0.4 ± 0	0.7 ± 0.1	1 ± 0.2	0.8	0.8	1.1
18:0	3.2	5.4	5.1 ± 0.4	5.2 ± 0	3.2 ± 0.1	2.8	2.3	3.2
18:1ω5c	1.8	1.7	0.2 ± 0	1.7 ± 0.2	1 ± 0.2	1.6	0.3	0.4
18:1ω7c	5.5	5.8	8.6 ± 1.4	7.6 ± 0.4	13.9 ± 0.7	8.6	4.2	3.7
18:1ω9c+18:3ω3	9.3	5.9	6.6 ± 5.2	6.3 ± 0.5	14.2 ± 0.2	14.0	35.9	35.2
18:2ω6	0.4	1.9	0.3 ± 0.1	1.2 ± 0	0.6 ± 0.2	0.7	1.5	1.8
18:4ω3+i18:0	1.1	0.3	0.9 ± 0.2	0 ± 0	1.5 ± 0.5	0.2	0.6	0.6
a19:0+19:1	0.2	0.6	0.2 ± 0	0.9 ± 0	0.4 ± 0	0.6	0.3	0.1
19:1b	1.2	2.6	2.4 ± 0.4	4.5 ± 0.3	1.1 ± 0.2	0.8	0.2	0.1
20:0	0.2	0.5	0.5 ± 0	0.4 ± 0	0.2 ± 0.1	0.2	0	1.9
20:1	0.2	0.4	0.3 ± 0	0.2 ± 0	0.1 ± 0	0.6	0.1	0.1
20:1ω7c	7.7	6.4	4.6 ± 0.7	2.7 ± 0.2	0.3 ± 0.1	8.5	0.8	0.3
20:1ω9c	2.0	0.0	9.4 ± 1.1	0 ± 0	1 ± 0.2	0.0	2.9	1.6
20:1ω11c	0.0	11.8	0.4 ± 0.3	11.2 ± 0.7	0.5 ± 0.2	4.3	1.2	1.3
20:2NMI	0.3	2.5	0.8 ± 0	5.5 ± 0.1	0.2 ± 0	0.8	0	0
20:2ω6	1.2	0.9	0.7 ± 0.2	0 ± 0	1.1 ± 0.1	0.6	0	0
20:3ω6	0.0	0.9	0.3 ± 0	0 ± 0	0.2 ± 0	0.1	0.1	0.1
20:4ω3	0.2	0.0	0 ± 0	0.1 ± 0.2	0.2 ± 0	0.0	0.4	0.6
20:4ω6 AA	3.1	0.2	1 ± 0.2	2.5 ± 0.7	4.1 ± 0.5	1.4	1.9	0.9
20:5ω3 EPA	14.0	8.8	12.3 ± 2.2	2.2 ± 0.4	19.6 ± 1.1	7.2	6.3	2.7
21:1a	0.0	0.6	0.4 ± 0.1	1.1 ± 0.2	0 ± 0	0.2	0	0
C21PUFA	1.1	4.9	2.3 ± 0.2	8.4 ± 0.6	0.5 ± 0.1	0.7	0.1	0
22:1ω7c	0.5	3.2	0.3 ± 0.1	0.1 ± 0.1	1.1 ± 0.1	0.9	0.2	0
22:1ω9c+16:0 GED	2.9	0.8	2.1 ± 1.5	0.8 ± 0.1	0.3 ± 0.2	0.4	1.1	0.3
22:1ω11+13c	0.4	0.2	0 ± 0	0 ± 0	0 ± 0	0.0	0.8	0.7
22:2NMIa[a]	0.0	3.6	9.9 ± 1.2	2.1 ± 0.1	0 ± 0	0.2	0.1	0
22:2NMIb[b]	0.5	1.6	2.5 ± 0.4	5.4 ± 0.6	0.1 ± 0	0.9	0.1	0.1
22:4ω6	2.0	0.8	0.8 ± 0.2	0.6 ± 0.3	0.2 ± 0.2	0.3	0.3	0.2
22:5ω3 DPA(3)	6.0	3.7	2.6 ± 0.3	1.3 ± 0	0.5 ± 0.1	0.8	1.4	1.4
22:5ω6 DPA(6)	0.4	0.0	0.1 ± 0.1	0 ± 0	0.2 ± 0	0.1	0.2	0.2
22:6ω3 DHA	11.5	4.2	3.6 ± 1	0.2 ± 0	12.1 ± 1.5	3.3	7.9	3.7
24:1b	0.3	0.1	0.1 ± 0.1	0 ± 0	0.9 ± 0.4	0.0	0.5	0.1
other	3.2	2.3	2.9 ± 0.5	3.1 ± 0.3	3.2 ± 0.1	1.7	2.0	2.2
18:1ω9/18:1ω7	**1.7**	**1.0**	**0.8**	**0.8**	**1.0**	**1.6**	**8.4**	**9.4**

[a]22:3ω6 co-eluted as minor FA with 22:2NMIa; [b]22:4ω3 co-eluted as minor FA with 22:2NMIb

The ST compositions were dominated by cholesterol, which is the main ST in many animals, but variations that may indicate different feeding strategies were evident. Cholesterol is a major membrane component in worms and crustaceans, regulating membrane fluidity (Goad 1978, Okamura et al. 1985). High levels of cholesterol, such as those seen in this study, are suggestive of a carnivorous diet (Nelson et al. 2001), although polychaetes from hydrothermal vents consuming large amounts of microbial food have also been found to contain cholesterol (Phleger et al. 2005b). *Trans*-22-de-hydrocholesterol, the second-most abundant ST (Table 2), is an intermediate in cholesterol synthesis, but it could also derive from animal food sources. Phytosterols were also present in the polychaetes, indicating a link to detrital food
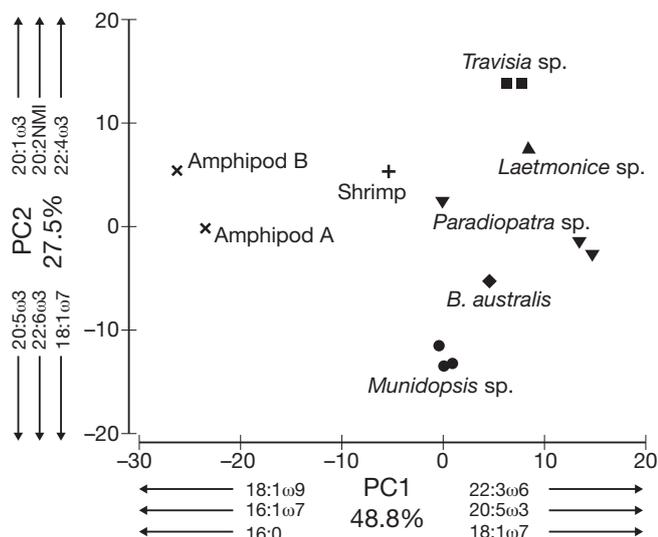
Fig. 2. First and second principal components derived from the fatty acid (FA) composition of Polychaeta (*Laetmonice* sp., *Paradiopatra* sp., and *Travisia* sp.), Cnidaria (*Bathyphellia australis*), and Crustacea (*Munidopsis* sp., caridean shrimp, and lysianassid amphipods A and B). PC1 explained 48.8% of the variability between FA profiles of the species examined. PC2 explained 27.5% of the variability. Arrows indicate FA contributing most to the distribution of species along each axis

sources. Sitosterol is the most abundant phytosterol in land plants (Trautwein et al. 2003); in the marine environment it is abundant in phytoplankton (Volkman 1986), and it is the most abundant phytosterol in some shallow water deposit feeding polychaetes (Judd 2001). The main phytosterols found in polychaetes in this study included sitosterol, brassicasterol and dihydrobrassicasterol. Amongst the polychaetes, *Travisia* sp. contained the highest proportions of phytosterols (Table 2), which reflects the subsurface deposit feeding nature of this burrowing ophelid (Fauchald & Jumars 1979). *Paradiopatra* sp., which probably feeds on phytodetritus as described above, contained ca. 10% phytosterols, while the large predaceous *Laetmonice* sp. contained only 6%. None of the polychaetes or other animals in the study appears to consume as much detritus as the almost exclusively deposit feeding holothurians and ophiuroids at this site, which contained from 31 to 70% phytosterols (Drazen et al. 2008b).

The FA compositions presented in Table 3 provide an initial basis for biomarker studies of abyssal food webs and give some insight into the diet of the examined taxa. Low FFA levels indicate little PL and TAG breakdown, low lipolytic and enzyme activity, and confirm the stability of samples during collection, transport and storage. The PUFA levels of the abyssal polychaetes suggest omnivory and links to the eupho-

tic zone. Most animals cannot synthesize longer-chain PUFA, including EPA, DHA, and AA. Instead, these are formed by phytoplankton, some bacteria and heterotrophic protozoans, and are transferred through the food web. EPA is typically found in higher proportions in diatoms (Volkman et al. 1989), while flagellates contain higher DHA relative to EPA (Brown et al. 1993). Thus, high levels of these FA and the resulting high PUFA levels are suggestive of herbivory. For instance, the commensal deep-sea hydrothermal vent polychaete *Amathys lutzi* contained low amounts of PUFA with no EPA or DHA, indicating low reliance on photosynthetically produced material (Colaço et al. 2007). The pelagic polychaete *Tomopteris carpenteri* had higher total PUFA levels (59% of total FA, with 28% DHA and 21% EPA) (Phleger et al. 1998) than the benthic polychaetes in this study. In contrast, another pelagic polychaete, *Vanadis antarctica*, had similar total PUFA, DHA, and EPA to the benthic polychaetes examined here (Fig. 1). *V. antarctica* may prey upon larval fish, whereas *T. carpenteri* has a more herbivorous diet, relying upon photosynthetically produced material (Phleger et al. 1998). Thus, the relatively low PUFA levels of polychaetes in this study suggest consumption of animal material as well as phytodetritus from surface waters. *Travisia* sp. had the lowest PUFA levels (Fig. 1) and had the greatest relative levels of phytosterols (Table 2), providing contrasting results. Ophelids, such as *Travisia* sp., are generally subsurface deposit feeders (Fauchald & Jumars 1979). Subsurface detritus is usually reworked and more refractory than detritus settling to the seafloor. It might be depleted in PUFA, but not phytosterols. PUFA are rapidly degraded in sinking detritus (Wakeham et al. 1984), but whether their degradation is faster than ST is not known. *Travisia* sp. did have a different PUFA composition compared to the other polychaetes, which might reflect a different source of food but could also be the result of lipid metabolism.

MUFA composition provides further insight into trophodynamics, making it possible to distinguish between carnivory, herbivory and detritivory. The long-chain MUFA, including the 20:1 isomers, were abundant in the polychaetes. Calanoid copepods have been reported as the major synthesizers of long-chain MUFA, including the 20:1 and 22:1 isomers, in marine food webs (Sargent & Whittle 1981). However, 20:1 FA are rich in cold water *Calanus* spp. and are associated with their WE. Not all copepods are WE-rich and contain substantial 20:1 FA. Therefore, it is possible that the levels of 20:1 FA in the predatory polychaete *Laetmonice* sp. indicate the direct consumption of copepods. Copepod remains, including faecal pellets, are common components of the vertical flux to the deep-sea floor, and their consumption could lead to the pres-

ence of 20:1 isomers in *Travisia* sp. and *Paradiopatra* sp. While faecal pellets probably have low FA contents, copepod body parts could contain higher levels of 20:1 isomers.

The ratio of 18:1ω9/18:1ω7 is often used as an indicator of carnivory in marine animals. Many animals can synthesize 18:1ω9 from its saturated precursor 18:0, and 18:1ω7 is of bacterial origin (Mayzaud et al. 1989, Nichols et al. 1991) or synthesized by chain elongation of 16:1ω7, which is algal or bacterial in origin (Graeve et al. 1997). Therefore, proportionally more 18:1ω9 indicates carnivory, whereas more 18:1ω7 indicates herbivory or detritivory (Graeve et al. 1997, Buhring & Christiansen 2001). Although 18:3ω3 co-elutes with 18:1ω9 on the GC column, it is only a very minor component in this study. The polychaetes had ratios of 18:1ω9/18:1ω7 between 0.8 and 1.0 (Table 3), suggesting that they consume more detritus than the other taxa examined in the present study. The benthic polychaete *Onuphis conchylega*, in the same family as *Paradiopatra* sp., had a very low 18:1ω9/18:1ω7 ratio of 0.24 (Graeve et al. 1997), suggesting that *Paradiopatra* sp. relies more on predation than the shallow living *O. conchylega*.

Cnidaria

The lipid biomarkers for the anemone *Bathyphellia australis* suggest a mixed diet of phytodetritus and animal material similar to that of the polychaetes. *B. australis* is an anemone found almost exclusively attached to manganese nodules (Lauerman et al. 1996). Based on isotopic evidence and ST profiles, this animal apparently feeds on some resuspended phytodetritus and also on zooplankton (Drazen et al. 2008a). Its sessile lifestyle is similar to that of the polychaete *Paradiopatra* sp., and indeed it has similar levels of storage lipid, suggesting a somewhat sporadic food supply. In addition, the ST profile of *B. australis* was similar to that of *Paradiopatra* sp., reflecting a mix of phytodetrital and animal material in their diets, despite different modes of feeding. Long-chain MUFA such as 20:1 were abundant in *B. australis*, which, as discussed above, may represent consumption of copepods. The 18:1ω9/18:1ω7 ratio for *B. australis* (1.7, Table 3) indicates a relatively more carnivorous diet than the polychaetes.

Crustacea

Unlike the other animals in this study, the caridean shrimp and the 2 lysianassid amphipods contained >50% storage lipids (TAG + WE), with the latter hav-

ing a high lipid content. It is possible that the high levels of neutral lipid found in these crustaceans are associated with the hepatopancreas. There are numerous reports of TAG-rich amphipods from the continental shelf (Graeve et al. 1997) to the abyss (Buhring & Christiansen 2001). Scavenging lysianassids are well known for their ability to gorge themselves and survive for months between feeding bouts (Hargrave et al. 1994). Clearly, the ability to store energy in the form of lipids is a benefit if feeding is sporadic. The foraging habits of the shrimp are unknown, but based on isotopic evidence it is a high trophic level predator (Drazen et al. 2008a). *Munidopsis* sp. did not have large amounts of lipid or high proportions of storage lipids. It is difficult to compare lipids and FA of *Munidopsis* sp. with the other crustaceans, since only the muscle tissues of this crab were analysed. Whole *M. subsquamosa* from a deep-sea hydrothermal vent system also had low amounts of lipid (10 mg g^{-1} wet weight) and a similar proportion of ST; however, its proportion of TAG (53%) (Phleger et al. 2005a) was much larger than observed in the abyssal taxa examined in this study.

Of the crustaceans, only *Munidopsis* sp. had any phytosterols (1%), suggesting that diets of most taxa almost entirely consisted of animal matter. Since crustaceans can convert phytosterol to cholesterol, it is also possible that phytosterols in the diet were more rapidly converted to cholesterol than in the other invertebrates. Amphipods are well known scavengers, but a diet study employing DNA-based markers suggested that hadal species are facultative scavengers and do consume some detritus (Blankenship & Levin 2007). The absence of phytosterols in amphipods does not suggest that this is the case at Stn M. Bathyal galatheids eat a mixed diet of infauna and detritus (Cartes et al. 2007) and sometimes carrion (Kemp et al. 2006), reflecting an opportunistic foraging strategy. Even a galatheid from a hydrothermal vent area, *Munidopsis subsquamosa*, which is a predator on benthic invertebrates, had small amounts of the phytosterol desmosterol, suggesting phytodetrital inputs to its diet (Phleger et al. 2005a).

The lysianassid amphipods and the caridean shrimp had low proportions of PUFA and high MUFA (Table 1). The only other study of abyssal amphipods yielded almost identical results (Bühring & Christiansen 2001). The FA profiles probably reflect the high TAG content of these animals. However, Nelson et al. (2001) found no correlation between lipid content and MUFA levels in Antarctic amphipod species. As discussed for the polychaetes, the high levels of 20:1 and 22:1 in the shrimp may reflect feeding on copepods. The relatively low amounts of these long-chain

MUFA in the crab and amphipods suggested a different feeding mode such as the consumption of carrion and other large organisms (Hargrave et al. 1994, Kemp et al. 2006).

High levels of the PUFA EPA and DHA in *Munidopsis* sp. contributed to its separation on PC2 of the PCA analysis (Fig. 2). EPA, DHA and total PUFA levels were relatively high compared to *M. subsquamosa* from a hydrothermal vent system (Phleger et al. 2005a), which consumes a variety of benthic animals. The abyssal *Munidopsis* sp. probably consumes a diet rich in PUFA compared to the hydrothermal vent *M. subsquamosa*, indicating a greater reliance on photosynthetically derived material.

The ratio of 18:1ω9/18:1ω7 for *Munidopsis* sp. was 1.0 (Table 1), similar to those for predatory decapods, known to be macrophagous, feeding on small benthic or benthopelagic organisms (Graeve et al. 1997). The ratio for *Munidopsis* sp. is less than the ratio for the hydrothermal vent *M. subsquamosa* (1.4, Phleger et al. 2005a), which with *Munidopsis* sp.'s higher PUFA suggests a greater reliance on detritus in the abyssal species.

The caridean shrimp had a 18:1ω9/18:1ω7 ratio of 1.6 in support of the other data indicating a carnivorous diet. The 2 lysianassid amphipods had very high levels of 18:1ω9 and 18:1ω9/18:1ω7 ratios of 8.4 and 9.4, which clearly distinguished them from the other taxa examined (Fig. 2). Their 18:1ω9/18:1ω7 ratios are very similar to those of other abyssal lysianassids and abyssal benthopelagic copepods from the North Atlantic (Buhring & Christiansen 2001), and such high ratios probably reflect a dominance of carrion in their diets. Arctic benthic amphipods from the continental shelf had lower ratios of 3.3 to 3.6 (Graeve et al. 1997). The variation in this ratio between these animals likely reflects dietary differences, including the relative importance of scavenging on the carcasses of large animals.

Lipid metabolism

Munidopsis sp. had very low levels of NMI (0.3%) compared to *M. subsquamosa* (4%) from hydrothermal vents (Phleger et al. 2005a). The NMI 20:2 is formed by animals that chain elongate and desaturate 18:1ω7 which is derived from bacteria. The lack of NMI in *Munidopsis* sp. indicates that bacteria are not a major part of the diet. Combined with higher DHA levels (12%), it also indicates that chain elongation and desaturation are not occurring to any extent. The higher DHA levels, if arising from other dietary sources along with EPA, likely inhibit the chain elongation and desaturation of C18 FA (Pond et al. 2002).

The 2 lysianassid amphipods also had very low NMI (0.1 to 0.2%) with moderate levels of DHA and EPA (Table 3). Total NMI in the shrimp were 2% and DHA 3%. These levels of NMI and moderate levels of vaccenic acid (18:1ω7, 9%) may indicate that chain elongation and desaturation of C18 FA is occurring in this crustacean. The polychaetes had much more NMI (8 to 13%), relatively low DHA levels, and 6 to 9% 18:1ω7, suggesting that chain elongation and desaturation might be occurring in these animals as well.

SUMMARY

These signature lipid profiles provide insights into the trophodynamics of abyssal communities. The FA profiles are the first for abyssal animals in the Pacific. Lipid stores of scavenging lysianassid amphipods and an unidentified caridean shrimp are very high and suggest sporadic feeding. Phytosterols were present in the anemone *Munidopsis* sp. and the polychaetes, providing evidence for the consumption of phytodetritus albeit as a small component of the diet. These data were corroborated by an analysis of essential PUFA. The ratio of 18:1ω9/18:1ω7 suggested carnivory as the predominant mode of foraging. The high 18:1ω9/18:1ω7 ratios in shrimp and amphipods and the absence of phytosterols suggests diets almost exclusively of animal material. Some distinct differences in the FA composition of the examined taxa allowed for their separation, indicating that FA profiles will be useful in future biomarker approaches to deep-sea food web studies. The abyssal food web is supported by a flux of phytodetritus from the euphotic zone and the abundance of some populations fluctuate inter-annually in concert with changes in this food supply (Ruhl & Smith 2004). Carefully establishing cause and effect requires well-described food webs. The present results assist in achieving that goal. However, in some systems, seasonal and sometimes inter-annual variations in FA composition occur (Nelson et al. 2001, Hudson et al. 2004), which may further influence trophic connections. Future research in the Pacific should investigate to which extent such temporal variations exist.

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