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Feeding ecology of deep-sea seastars (Echinodermata: Asteroidea): a fatty-acid biomarker approach

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ABSTRACT: Fatty-acid biomarkers and stomach content analysis were used to investigate the diets of 9 species of deep-sea seastar. Polyunsaturated fatty acids were the most abundant categories of fatty acid contained in the total lipids of all species. They were dominated by 20:5 (n-3) and 20:4 (n-6), with 22:6 (n-3) present in much lower proportions. Monounsaturated fatty acids were also abundant, particularly 20:1 (n-13) and (n-9). Odd-numbered, branched-chain fatty acids and non-methylene interrupted dienes (NMIDs) were present in relatively high levels in all species. Cluster and multi-dimensional scaling (MDS) analyses of the fatty acid composition separated the seastar species into 3 trophic groups; suspension feeders, predators/scavengers, and mud ingesters. Suspension feeders showed greatest reliance on photosynthetic carbon as indicated by the abundance of fatty-acid biomarkers characteristic of photosynthetic microplankton. By contrast, mud ingesters were found to rely heavily on heterotrophic bacterial carbon, containing high percentages of 18:1 (n-7) and NMIDs. Predator/scavengers occupied a trophic position between the suspension feeders and mud ingesters. *Zoroaster longicauda*, an asteroid of unknown diet, had a similar fatty acid composition to the 3 suspension feeders, *Freyella elegans*, *Brisingella coronata* and *Brisinga endecacnemos.* While the suspension feeders are specialists on benthopelagic copepods, the preferred prey of *Z. longicauda* is unknown, but is likely to be very similar to that of the suspension feeders. Stomach content analysis revealed the diet of *Z. longicauda* also includes benthic echinoderms and crustaceans.

KEY WORDS: Fatty acid biomarkers · Lipids · Diet · Asteroidea · Deep-sea ecology

INTRODUCTION

In comparison to shallow water and terrestrial environments, we know very little about deep-sea ecosystems. Increasing anthropogenic impacts in the deep sea (i.e. oil and gas exploration, deep-sea fishing and waste disposal) have highlighted our lack of understanding. The environment below the permanent thermocline is generally considered to be food-limited (Dayton & Hessler 1972, Thiel 1979). The biomass and, to an extent, abundance of benthic organisms is related directly to the amount of food reaching the sediment surface (Tseylin 1987). In addition, changes in the quality of organic matter deposited on the seafloor may lead to large-scale ecosystem changes (Billett et al. 2001, Ginger et al. 2001). Nutrition plays an important role in shaping the distribution and structure of deep-sea communities. An understanding of the dietary interactions within this ecosystem is therefore essential.

Asteroids are an important megafaunal group and can account for up to 70 % of the megafaunal biomass (Billett 1991). The diets of many species of deep-sea asteroids are largely unknown. Previous studies have been based on analysing the stomach content of key species (Carey 1972, Khripounoff 1979, Jangoux 1982 and references therein, Tyler et al. 1990, 1993). Additional knowledge of asteroid diets and feeding modes have also been gained from submersible and photographic observations (Pawson 1976, Rowe & Staresinic 1979).

Stomach content analysis is subject to a number of problems, particularly in deep-sea species (Hopkins 1985). Deep-sea animals often suffer from deformation

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and loss of their ingesta during capture and retrieval to the surface (Feller et al. 1985). In addition, many asteroid species feed extra-orally and are rarely observed with any material in their stomach (Carey 1972). Other limitations include (1) the identification of partially digested, unidentifiable material (Carey 1972, Feller et al. 1985), (2) feeding by asteroids within the cod end of the sampling net and (3) variable resistance to digestion of different food items (Fukuda & Naganuma 2001). The analysis of stomach contents also tends to bias in favour of the most recently ingested material (Fukuda & Naganuma 2001). Thus, it is difficult to obtain specimens with intact stomach contents suitable for analysis, particularly given the logistic and economic constraints associated with deep-sea sampling. These difficulties suggest the need for alternative and/or complementary methods of examining diet and nutrition.

The analysis of fatty acid biomarkers provides an alternative method of investigating diet. The principle behind this method is relatively simple. Consumers derive their lipid requirements either from their diet or by endogenous lipogenesis from dietary protein and carbohydrate precursors (Gurr & Harwood 1991, Stryer 1995). Dietary lipids are broken down into their constituent fatty acids and are incorporated relatively unchanged into the tissues of the consumer (Lee et al. 1971). Certain fatty acids have specific known sources and these act as 'biomarkers' (Kates & Volcani 1966, Harrington et al. 1970, Sargent et al. 1987, Viso & Marty 1993, Dunstan et al. 1994). Dietary sources may be traced over a number of trophic levels using biomarker fatty acids (Owen et al. 1972, Gatten et al. 1983, Klungsoyr et al. 1989, Linko et al. 1992). Fatty acid analysis has a number of advantages over stomach content analysis in that (1) fewer samples are required, (2) it can be used to examine long-term dietary sources or most recent feeding events depending on the tissue type selected (Sargent & Falk-Petersen 1988, Fukuda & Naganuma 2001) and (3) it is not subject to the biases of stomach content analysis.

There have been few previous investigations that have utilized fatty acid biomarkers to examine trophic interactions within the deep sea (Ginger et al. 2000). The majority of these are studies of hydrothermal vent ecosystems (Ben-Mlih et al. 1992, Pranal et al. 1996, 1997, Allen et al. 2001, MacAvoy et al. 2001). Many vent studies are concerned, in particular, with determining the quantitative significance of photosynthetically derived material for the nutrition of vent communities versus that of bacterial primary production (Pond et al. 1997a,b, 2000a,b, 2002)

This study represents one of the first attempts to examine the diet of non-vent deep-sea animals using fatty acid analysis. The nutritional relationships between selected asteroid species with different modes of feeding were investigated and, in some cases, stomach content analysis was also used. The species were examined in terms of utilization of the 2 available carbon sources in the environment, phytodetrital and benthic heterotrophic bacterial carbon. In addition, fatty-acid biomarkers, together with relationships between fatty acid composition and feeding mode, were used to gain an insight into the diet of an extra-orally feeding seastar species *Zoroaster longicauda*, which rarely contains any identifiable contents in the stomach.

MATERIALS AND METHODS

Sampling. Nine asteroid species were collected from varying depths on the continental slope in the Porcupine Seabight and Porcupine Abyssal Plain in the NE Atlantic (Fig. 1, Table 1). Species were selected based on established or suspected mode of feeding from depths of 900 to 4840 m. Two mud ingesters (*Hyphalaster inermis* and *Styracaster chuni*), 3 predator/scavengers (*Dytaster grandis grandis*, *Bathybiaster vexillifer* and *Hymenaster membranaceus*) and 3 suspension feeders (*Freyella elegans*, *Brisingella coronata* and *Brisinga endecacnemos*) were chosen, as well as one species of unknown feeding type (*Zoroaster longicauda*) (Table 1).

The asteroids were collected in good condition using an otter trawl (Merrett & Marshall 1981). Upon recovery of the trawl, adult specimens of each of the 9 selected species were dissected in a constant temperature laboratory at 4°C. Where available, tube feet were

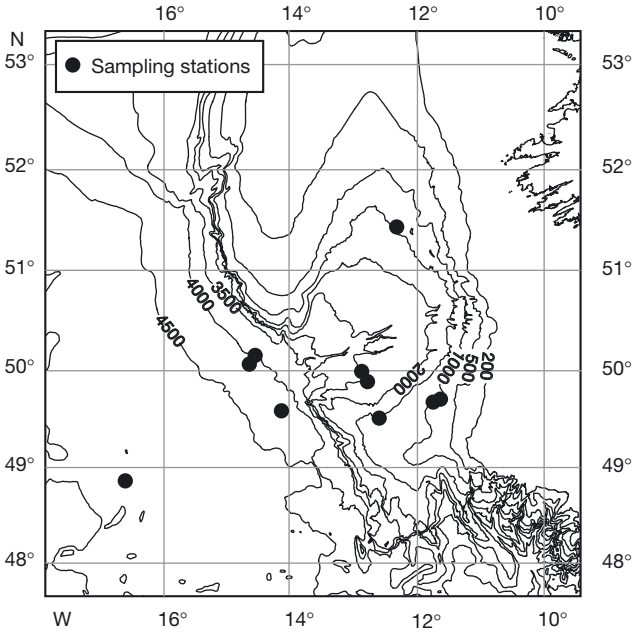

Fig. 1. Bathymetric chart of the Porcupine Seabight and Porcupine Abyssal Plain, NE Atlantic Ocean, with 200 and 500 m depth contours; sampling stations are shown

Table 1. Summary of species sample data, feeding type, and stomach content. Source data for feeding types: Madsen (1961), Khripounoff (1979), Jangoux (1982, and references therein), Gage et al. (1983), Tyler et al. (1990), Tyler et al. (1993), Mortensen (1927), Pawson (1978), Rowe & Staresinic (1979)

Station no.	Sample depth	Sample date (dd/mm/yy)	Species	Feeding type	No. tube feet for analysis	No. stomachs dissected	Total no. with stomach content	Brief description in order of frequency of occurrence
13925	4840	23/09/00	*Hyphalaster inermis*	Mud ingester	5	20	15	Mud
13925	4840	23/09/00	*Styracaster chuni*	Mud ingester	5	20	17	Mud
13906	4001	18/09/00	*Dytaster grandis grandis*	Predator/scavenger	5	10	23	Sediment traces,
13942	4020	19/04/01	"		5	20		echinoderm remains
14141	2504	20/08/01	*Bathybiaster vexillifer*	Predator/scavenger	5	0	n/a	Crustacean moults, mollusc remains, fish scales
13932	2434	16/04/01	*Hymenaster membranaceus*	Predator/scavenger	5	249	75	Pteropod moults, sediment traces, whole crustaceans and fragments, foraminiferans, other invertebrates
14137	4127	19/08/01	*Freyella elegans*	Suspension feeder	5	52	36	Copepods, crustacean remains, foraminiferans
13952	1065	23/04/01	*Brisingella coronata*	Suspension feeder/	3	3	1	Copepods
14163	1455	29/08/01	"	predator				
14164	1053	30/08/01	"					
14165	1462	31/08/01	*Brisinga endecacnemos*	Suspension feeder	3	3	0	
13906	4001	18/09/00	*Zoroaster longicauda*	Unknown	5	20	27	Sediment traces,
13942	4020	19/04/01						echinoderm remains
			"			5	20	crustacean remains, foraminiferans

taken from 5 individuals of each species and stored in chloroform:methanol (2:1 vol/vol) at –70°C until analysis. For *Brisingella coronata* and *Brisinga endecacnemos* only 3 individuals were obtained for analysis. All vials were completely filled with solvent to exclude air and therefore prevent oxidation.

Lipid analysis. In the laboratory all samples were initially homogenised before being filtered through a pre-washed (chloroform:methanol) Whatman No. 1 paper filter. Total lipid was extracted as detailed by Folch et al. (1957), followed by trans-esterification in methanol containing 1.5% sulphuric acid at 50°C for 16 h to generate fatty acid methyl esters (FAME) (Christie 1982). FAME were purified by thin layer chromatography using a hexane:diethyl-ether:acetic acid (90:10:1, vol/vol/vol) solvent system. Purified FAME were dissolved in hexane to a concentration of 2 mg ml^{-1} and analysed by gas chromatography on a Carlo Erba (5190) gas chromatograph (GC) equipped with a 30 m Resteck STABILWAX column using hydrogen as the carrier gas. FAME were identified by reference to standards of known composition. Thirty-nine different fatty acids were identified and are expressed here as a percentage of the total of those identified.

Data analysis. Multivariate analyses of the fatty acid composition of the tube feet were performed for all individuals using the program PRIMER (Plymouth Routines in Multivariate Ecological Research), Version 5 (Clarke & Warwick 1994). Both hierarchical clustering and multi-dimensional scaling (MDS) were performed based on a Bray-Curtis similarity co-efficient applied to the percentage composition data. No transformation was applied to the data set, because those fatty acids that made up only a small percentage of the total composition did not feature heavily in the diet. Giving artificial weight to these minor fatty acids by applying a transformation would therefore be inappropriate. The SIMPER routine in PRIMER was used to investigate the clusters found by both hierarchical cluster analysis and MDS.

The non-parametric Kruskal Wallis ANOVA on ranks test for 3 or more variables (Fowler et al. 1998) was used to compare percentage composition of known biomarker fatty acids between feeding types, at the 95% significance level. Dunn's Method of pairwise multiple comparison (Sokal & Rohlf 1994) was applied post-hoc to investigate statistical differences between specific groups.

Investigation of stomach content. The stomach contents of 8 asteroid species were analysed using varied numbers of individuals of each species. Samples of *Bathybiaster vexillifer* were not investigated because

Table 2. Fatty acid composition (%) of total lipid extracted from 9 species of asteroid from the Porcupine Seabight and Porcupine Abyssal Plain. Standard deviations are given in parentheses. Pred/scav: predator/scavengers; DMA: dimethylacetals

Fatty acid	*Hyphalaster inermis* Mud ingester	*Styracaster chuni* Mud ingester	*Dytaster grandis grandis* Pred/scav	*Bathybiaster vexillifer* Pred/scav	*Hymenaster membranaceus* Pred/scav	*Freyella elegans* Suspension	*Brisingella coronata* Suspension	*Brisinga endecacnemos* Suspension	*Zoroaster longicauda* Unknown
Saturates									
14:0	0.58 (0.65)	1.09 (1.04)	0.12 (0.00)	0.11 (0.00)	0.20 (0.16)	0.11 (0.00)	0.51 (0.36)	0.53 (0.37)	0.11 (0.00)
15:0	0.11 (0.00)	0.12 (0.00)	0.12 (0.00)	0.11 (0.00)	0.12 (0.03)	0.13 (0.06)	0.13 (0.05)	0.29 (0.16)	0.11 (0.00)
Iso 15:0	0.90 (0.53)	0.81 (0.41)	0.86 (0.19)	0.11 (0.00)	0.33 (0.14)	0.11 (0.00)	0.11 (0.00)	0.11 (0.00)	0.20 (0.21)
16:0 DMA	0.11 (0.00)	0.12 (0.00)	0.87 (0.07)	0.77 (0.26)	0.64 (0.35)	0.08 (0.05)	0.97 (1.10)	0.59 (0.48)	2.68 (1.11)
16:0	2.12 (1.31)	3.16 (0.61)	0.76 (0.15)	0.57 (0.14)	1.66 (0.56)	1.58 (0.71)	2.91 (1.19)	3.82 (0.96)	2.11 (0.58)
17:0	0.11 (0.00)	0.12 (0.00)	0.12 (0.00)	0.11 (0.00)	0.31 (0.13)	0.54 (0.25)	0.30 (0.17)	0.31 (0.17)	0.11 (0.00)
18:0 DMA	0.90 (0.65)	1.11 (0.97)	4.81 (0.55)	5.45 (2.44)	6.45 (4.86)	0.79 (1.22)	3.24 (2.75)	1.89 (1.12)	2.55 (1.12)
18:0	4.35 (2.17)	4.45 (0.70)	6.38 (0.56)	8.92 (0.43)	7.30 (1.41)	7.40 (0.76)	6.72 (0.43)	6.26 (0.50)	7.42 (0.83)
20:0	7.11 (4.71)	4.56 (3.20)	3.75 (0.52)	1.21 (0.66)	0.18 (0.07)	0.06 (0.06)	0.11 (0.00)	0.11 (0.00)	0.11 (0.00)
Monoenes									
14:1	1.73 (0.65)	1.73 (0.29)	0.85 (0.11)	0.20 (0.19)	0.47 (0.08)	0.13 (0.05)	0.14 (0.05)	0.11 (0.00)	0.20 (0.22)
16:1 (n-9)	1.50 (0.45)	1.78 (0.28)	0.22 (0.23)	0.11 (0.00)	0.13 (0.05)	0.00	0.07 (0.06)	0.00	0.94 (0.47)
16:1 (n-7)	1.57 (0.74)	2.06 (0.14)	0.23 (0.26)	0.21 (0.23)	0.58 (0.12)	0.61 (0.10)	0.77 (0.39)	0.74 (0.30)	0.34 (0.32)
16:1 (n-5)	0.11 (0.00)	0.09 (0.05)	0.02 (0.05)	0.11 (0.00)	0.14 (0.07)	0.11 (0.00)	0.11 (0.00)	0.07 (0.06)	0.11 (0.00)
18:1 DMA	0.16 (0.12)	0.12 (0.00)	0.12 (0.00)	0.18 (0.16)	0.55 (0.17)	0.06 (0.06)	0.11 (0.00)	0.11 (0.00)	0.11 (0.00)
18:1 (n-9)	1.70 (0.99)	1.44 (0.43)	2.05 (0.43)	0.36 (0.24)	0.79 (0.10)	2.25 (0.42)	1.61 (0.91)	1.11 (0.41)	1.19 (0.11)
18:1 (n-7)	7.89 (1.96)	10.86 (1.42)	3.78 (0.39)	2.87 (0.35)	5.40 (0.77)	2.50 (0.42)	2.61 (0.80)	3.27 (1.13)	2.27 (0.12)
20:1 DMA	2.39 (1.57)	1.64 (1.42)	2.60 (0.23)	4.22 (2.14)	0.22 (0.22)	0.75 (1.20)	0.30 (0.27)	1.06 (0.51)	0.23 (0.33)
20:1 (n-13)/(n-9)	13.20 (0.80)	16.20 (1.39)	16.75 (2.31)	17.16 (1.50)	22.06 (1.44)	28.23 (3.02)	23.42 (0.31)	27.61 (0.70)	30.06 (1.64)
20:1 (n-13)	not resolved	not resolved	15.16 (0.95)	not resolved	12.76 (0.93)	19.14 (2.00)	15.90 (1.01)	16.89 (2.64)	18.30 (0.90)
20:1 (n-9)	not resolved	not resolved	1.59 (0.15)	not resolved	9.30 (0.61)	9.10 (3.19)	7.52 (1.29)	10.72 (2.52)	11.76 (1.71)
20:1 (n-7)	2.75(0.27)	2.67 (0.16)	2.60 (0.27)	2.55 (0.34)	0.85 (0.07)	1.62 (0.24)	0.44 (0.17)	0.75 (0.58)	1.20 (0.14)
22:1 (n-11)	0.00	0.00	0.36 (0.55)	0.50 (0.24)	0.00	0.77 (0.38)	2.10 (0.61)	4.21 (1.82)	0.39 (0.40)
22:1 (n-9)	0.00	0.00	0.12 (0.00)	0.38 (0.26)	0.68 (0.20)	0.69 (0.33)	0.45 (0.17)	0.42 (0.27)	0.36 (0.35)
22:1 (n-7)	0.00	0.00	0.12 (0.00)	0.11 (0.00)	0.39 (0.25)	0.08 (0.05)	0.14 (0.07)	0.11 (0.00)	0.11 (0.00)
24:1 (n-9)	0.11 (0.00)	0.12 (0.00)	0.48 (0.33)	0.96 (0.26)	2.21 (0.33)	0.33 (0.21)	0.93 (0.29)	1.11 (0.36)	0.81 (0.18)
Dienes									
16:2 (n-3)	0.11 (0.00)	0.12 (0.00)	1.10 (0.46)	0.11 (0.00)	0.56 (0.09)	0.18 (0.16)	0.11 (0.00)	0.11 (0.00)	0.21 (0.24)
18:2 (n-6)	0.76 (0.90)	0.67 (0.51)	0.57 (0.27)	0.90 (0.53)	0.17 (0.08)	0.08 (0.05)	0.11 (0.00)	0.00	0.11 (0.00)
20:2 delta 5,11	0.93 (0.52)	0.45 (0.45)	0.64 (0.61)	3.91 (0.82)	2.62 (0.28)	1.99 (0.63)	3.52 (0.38)	2.50 (1.02)	1.70 (0.18)
20:2 delta 5,13	8.51 (0.87)	7.09 (0.92)	2.49 (0.21)	0.71 (0.16)	0.17 (0.06)	0.11 (0.00)	0.41 (0.27)	0.00	0.11 (0.00)
22:2 delta 7,13	0.00	0.00	0.12 (0.00)	0.11 (0.00)	0.18 (0.07)	1.91 (1.12)	0.28 (0.16)	0.90 (0.18)	0.41 (0.42)
22:2 delta 7,15	0.11 (0.00)	0.12 (0.00)	0.12 (0.00)	0.94 (0.21)	0.00	0.68 (1.02)	0.11 (0.00)	0.11 (0.00)	0.11 (0.00)
Polyunsaturates									
16:3 (n-3)/17:1	0.20 (0.21)	0.12 (0.00)	0.12 (0.00)	0.69 (0.53)	0.92 (0.97)	0.49 (0.43)	0.25 (0.25)	0.54 (0.58)	0.04 (0.06)
16:4 (n-1)	0.00	0.00	0.05 (0.06)	0.00	0.12 (0.08)	0.00	0.00	0.00	0.11 (0.00)
18:3 (n-6)	0.11 (0.00)	0.12 (0.00)	0.12 (0.00)	0.11 (0.00)	0.27 (0.10)	0.00	0.22 (0.13)	0.29 (0.17)	0.09 (0.05)
18:3 (n-3)	0.11 (0.00)	0.12 (0.00)	0.07 (0.06)	0.09 (0.05)	0.00	0.08 (0.14)	0.00	0.00	0.02 (0.05)
18:4 (n-3)	0.00	0.21 (0.21)	0.53 (0.70)	0.48 (0.21)	0.07 (0.06)	0.38 (0.72)	0.07 (0.06)	0.00	0.08 (0.05)
20:4 (n-6)	23.21 (1.55)	24.00 (2.08)	23.48 (2.44)	23.65 (2.48)	20.11 (2.82)	14.45 (2.29)	10.53 (0.83)	11.85 (0.83)	14.76 (1.92)
20:4 (n-3)	0.75 (0.39)	0.51 (0.38)	0.12 (0.00)	0.11 (0.00)	0.23 (0.07)	0.11 (0.00)	0.14 (0.06)	0.35 (0.21)	0.11 (0.00)
20:5 (n-3)	15.43 (1.09)	11.35 (0.37)	21.50 (3.38)	18.70 (1.38)	21.57 (1.21)	24.30 (2.05)	26.83 (1.58)	23.02 (4.48)	25.36 (1.87)
22:5 (n-3)	0.11 (0.00)	0.12 (0.00)	0.12 (0.00)	0.60 (0.06)	0.13 (0.06)	0.18 (0.17)	0.62 (0.09)	0.53 (0.08)	0.11 (0.00)
22:6 (n-3)	0.23 (0.28)	0.80 (0.41)	0.81 (0.43)	1.59 (0.38)	1.24 (0.24)	6.13 (0.89)	8.61 (0.65)	5.24 (0.58)	3.01 (1.41)

comprehensive studies of the diet of this species have been conducted previously (Tyler et al. 1993). Although the diet of *Dytaster grandis grandis* has also been studied previously, there is still some confusion over its primary food source. This species is believed to consume a wide range of food types. Owing to the uncertainty of its feeding biology (Tyler et al. 1990), this species was included in the stomach dissection investigations (Table 1).

Photographic observations. Observations of feeding behaviour of the asteroid species investigated in this study were made using photographs (Lampitt & Billett 1988, Tyler et al. 1990, 1993). All photographs, except that of *Hyphalaster inermis*, were taken during previous cruises to the Porcupine Seabight using a camera mounted on an epibenthic sledge (for cruise and station data see Jackson et al. 1991). *H. inermis* was observed using Bathysnap time-lapse photography at the Great Meteor East site in the eastern North Atlantic 31° 17′ N, 25° 24′ W (for cruise and station data see Roe 1985).

RESULTS

Fatty acid composition of tube feet

The fatty acid profiles of tube feet from the 9 species of asteroid were different but showed some

Table 3. Percentage composition of different fatty acid groups and the C18 ratio from 9 species of asteroid from the Porcupine Seabight and Porcupine Abyssal Plain. Standard deviations are given in parentheses. Pred/scav: predator/scavengers; PUFA: polyunsaturated fatty acids; NMID: non-methylene interrupted dienes; DMA: dimethylacetals; odd and branched: odd number and branched chain fatty acids

Fatty acid	*Hyphalaster inermis* Mud ingester	*Styracaster chuni* Mud ingester	*Dytaster grandis grandis* Pred/scav	*Bathybiaster vexillifer* Pred/scav	*Hymenaster membranaceus* Pred/scav	*Freyella elegans* Suspension	*Brisingella coronata* Suspension	*Brisinga endecacnemos* Suspension	*Zoroaster longicauda* Unknown
Saturates	16.30 (3.15)	15.54 (2.27)	17.78 (0.69)	17.36 (2.96)	17.19 (4.09)	10.81 (1.24)	15.00 (2.46)	13.90 (2.16)	15.39 (1.02)
Monoenes	33.12 (3.34)	38.69 (2.14)	30.29 (2.02)	29.93 (1.48)	34.47 (2.04)	38.12 (2.77)	33.19 (3.34)	40.67 (3.74)	38.29 (2.55)
Dienes	10.42 (1.42)	8.44 (0.56)	5.03 (0.54)	6.69 (0.99)	3.69 (0.43)	4.95 (0.74)	4.54 (0.59)	3.61 (1.18)	2.64 (0.68)
Polyunsaturates	39.95 (2.32)	37.22 (2.29)	46.78 (2.25)	45.33 (2.97)	43.73 (3.69)	45.63 (4.17)	47.02 (2.14)	41.29 (4.05)	43.64 (3.09)
C16 PUFA	0.11 (0.21)	0.12 (0.00)	1.15 (0.06)	0.11 (0.53)	0.68 (0.93)	0.18 (0.43)	0.11 (0.25)	0.11 (0.58)	0.32 (0.06)
C18 PUFA	0.98 (0.90)	1.11 (0.63)	1.29 (0.86)	1.58 (0.53)	0.51 (0.14)	0.55 (0.63)	0.40 (0.15)	0.29 (0.17)	0.30 (0.05)
NMID	9.55 (1.11)	7.65 (0.79)	3.36 (0.73)	5.67 (0.99)	2.97 (0.38)	4.69 (0.76)	4.32 (0.58)	3.50 (1.19)	2.32 (0.56)
DMA	3.56 (2.19)	2.98 (2.39)	8.40 (0.63)	10.63 (4.87)	7.86 (5.47)	1.69 (2.48)	4.62 (4.12)	3.64 (2.09)	5.57 (2.42)
Odd and branched	1.13 (0.53)	1.04 (0.41)	1.09 (0.19)	0.34 (0.01)	0.77 (0.25)	0.78 (0.26)	0.54 (0.21)	0.71 (0.33)	0.41 (0.21)
C18 ratio	0.22 (0.11)	0.13 (0.04)	0.54 (0.08)	0.12 (0.08)	0.15 (0.04)	0.90 (0.19)	0.62 (0.16)	0.34 (0.02)	0.52 (0.06)

general similarities (Table 2). Polyunsaturated fatty acids (PUFA) dominated total fatty acids in all species apart from *Styracaster chuni*. PUFA concentrations ranged from 37 (*S. chuni*) to 47% (*Brisingella coronata*) (Table 3). The major polyunsaturated fatty acids for all species were 20:4 (n-6) and 20:5 (n-3), with 22:6 (n-3) present in lower proportions (Table 2). The dominant mono-unsaturated fatty acids (MUFA) in all species were isomers of 20:1 (Table 2). Where the n-13 and n-9 moieties were resolved, n-13 dominated. Isomers of 18:1 were the second most dominant form of MUFA present in most species. Non-methylene interrupted dienes (NMIDs), odd-number and branch-chain fatty acids were present in all species.

A hierarchical cluster analysis separated the 9 asteroid species into 3 distinct groupings at the 80% similarity level (Fig. 2). These groupings corresponded to 3 feeding types. Cluster 1 consisted of *Hyphalaster inermis* and *Styracaster chuni* (mud ingesters), Cluster 2 *Bathybiaster vexillifer*, *Hymenaster membranaceus* and *Dytaster grandis grandis* (predator/scavenegers) and Cluster 3 *Freyella elegans*, *Brisingella coronata*, *Brisinga endecacnemos* and *Zoroaster longicauda* (suspension feeders and unknown). Species in Cluster 2 separated into single species groupings (Fig. 2). However, species in Clusters 1 and 3 did not form perfectly distinct species groupings (Fig. 2), although some separation was still apparent.

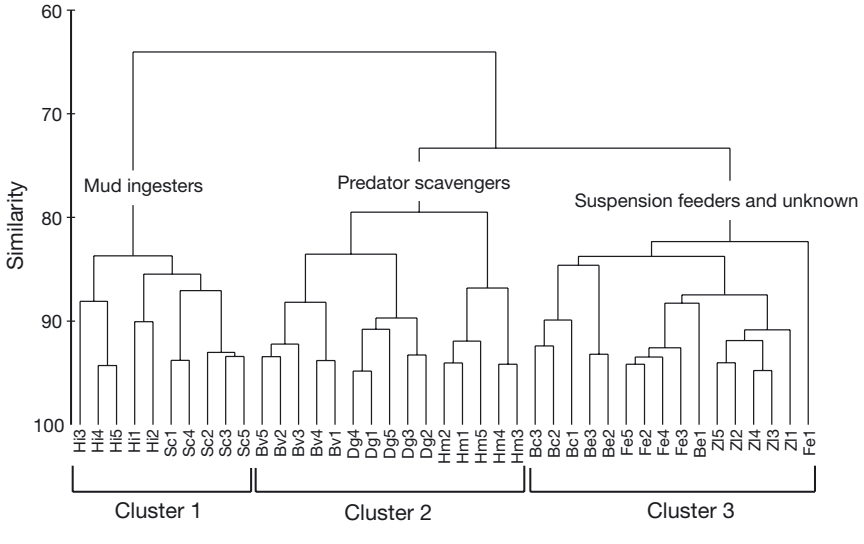

Fig. 2. Hierarchical cluster analysis of fatty acid composition (%) of the total lipid extracted from 9 species of asteroids: Sc—*Styracaster chuni*, Hi—*Hyphalaster inermis*, Dg—*Dytaster grandis grandis*, Bv—*Bathybiaster vexillifer*, Hm—*Hymenaster membranaceus*, Fe—*Freyella elegans*, Bc—*Brisingella coronata*, Be—*Brisinga endecacnemos*, Zl—*Zoroaster longicauda*

From the MDS analysis 3 distinct grouping, were identified and corresponded to the 3 clusters found in the previous analysis (Fig. 3). These groupings had high, within group, similarities (~84% SIMPER analysis). Clusters 1 and 3 (mud ingesters and suspension feeders) were most dissimilar (42%, SIMPER), with Cluster 2 (predator/scavengers) showing similarity to both Clusters 1 (29% dissimilar SIMPER) and 3 (27% dissimilar SIMPER). SIMPER analysis using all fatty acids for each group revealed 20:2 Δ 5, 13 (NMID) and 18:1 (n-7), which are largely of bacterial origin (Ackman & Hooper 1973, Sargent et al. 1987), to distinguish Cluster 1, the mud ingesters, from the other 2 clusters. The 20:1 (n-13) + (n-9) and diatom biomarker 20:5 (n-3) (Sargent et al. 1987) distinguished the cluster with the suspension feeders and *Zoroaster long-*

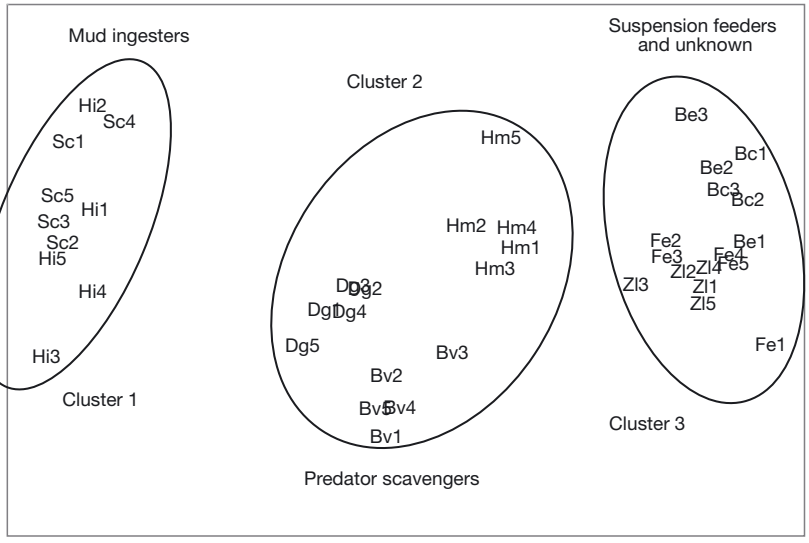

Fig. 3. Multi dimensional scaling plot of fatty acid composition (%) of the total lipid extracted from 9 species of asteroids: Sc—*Styracaster chuni*, Hi—*Hyphalaster inermis*, Dg—*Dytaster grandis grandis*, Bv—*Bathybiaster vexillifer*, Hm—*Hymenaster membranaceus*, Fe—*Freyella elegans*, Bc—*Brisingella coronata*, Be—*Brisinga endecacnemos*, Zl—*Zoroaster longicauda*

icauda (Cluster 3). No fatty acids strongly distinguished Cluster 2, the predator/scavengers.

Analysis of the mean percentage composition of particular biomarker fatty acids for each feeding type revealed clear differences and supported the findings of the SIMPER analysis (Table 4). Mud ingesters contained significantly lower amounts of 20:5 (n-3) than all other feeding types (Kruskal-Wallis, $p < 0.0001$), but significantly higher percentages of 18:1 (n-7) (Kruskal-Wallis, $p < 0.0001$) and NMID (Kruskal-Wallis, $p < 0.0001$). Suspension-feeding asteroids contained significantly lower percentages of 20:4 (n-6) (Kruskal-Wallis, $p < 0.0001$) in their total lipid than the predator/scavengers or mud ingesters, but significantly higher percentages of 22:6 (n-3) (Kruskal-Wallis, $p < 0.0001$) and a higher C18 ratio (Kruskal-Wallis, $p < 0.0001$). Predator/scavengers, in general, contained intermediate or equal percentages of all biomarker fatty acids to those of the mud ingesters and suspension feeders.

Separation of the predator scavengers into clear species groups in cluster and MDS analysis suggested differences in dietary selection. Analysis of specific biomarker fatty acids for these 3 species revealed differences in composition (Table 5). *Hymenaster membranaceus* had a significantly lower mean percentage of 22:1 (n-11) and 20:4 (n-6) than the other predator/scavenger species (Kruskal-Wallis, $p = 0.0068$ and $p = 0.0081$), but a significantly higher mean percentage of 20:1 (n-9) (Kruskal-Wallis, $p = 0.0357$) than *Dytaster grandis grandis* (Table 2). *D. grandis grandis* contained a significantly greater percentage of 18:1 (n-9), than the other predator/scavenger species (Kruskal-Wallis, $p = 0.0019$), but a lower percentage of 22:6 (n-3) (Kruskal-Wallis, $p = 0.0221$). *Bathybiaster vexillifer* contained a lower proportion of both 18:1 (n-9) (Kruskal-Wallis, $p = 0.0019$) and 18:1 (n-7) (Kruskal-Wallis, $p = 0.0025$) than the other 2 species, but contained a significantly greater proportion of the bacterially-derived NMIDs (Kruskal-Wallis, $p = 0.0104$).

Analysis of specific biomarker fatty acids of *Zoroaster longicauda*, a species for which the diet is unknown, revealed this species to contain significantly greater proportions of the photosynthetic biomarkers 20:5 (n-3) and 22:6 (n-3) (Sargent et al. 1987) and a significantly higher ratio of 18:1 (n-9/n-7) than the mud ingesters (Table 4) (Kruskal-Wallis, $p < 0.0001$); but significantly lower percentages of 20:4 (n-6), 18:1 (n-7) and NMIDs than the mud ingesters (Table 4) and significantly lower percentages of odd-numbered and branched-chain fatty acids than both the mud ingesters and predator/scavengers (Kruskal-Wallis, $p < 0.0001$). This resulted in the clustering of this species with the suspension feeders. However, *Z. longicauda* contained a greater percentage of 22:6 (n-3), the monounsaturate 20:1 (n-13/n-9) and the PUFA 20:4 (n-6), clearly distinguishing it from the suspension feeders (SIMPER).

Investigation of stomach contents

The limited investigation of the stomach contents of 8 of the species confirmed some of the findings of previous investigations on these species (Table 1). *Freyella elegans*, suspected to be a suspension feeder from behavioural observations and stomach content analysis of related species, was found to feed predominantly on copepods, presumably present in the benthic boundary layer. *Dytaster grandis grandis*, however, was found to feed predominantly on other echinoderms, in particular echinoids and ophiuroids, with a minor detrital component. A large number of individual *Hymenaster membranaceus* were investigated for stomach content analysis. This species was found to feed on small benthic invertebrates, in particular foraminiferans and crustaceans as well as planktonic fall-out such as pteropod moults and planktonic forams.

Table 4. Summary of the differences in mean fatty acids composition (%) of specific biomarker fatty acids for each feeding type, with standard deviations given in parentheses. PUFA: polyunsaturated fatty acid. Odd no. and br. chain bacterial: odd number and branched chain fatty acids. NMID: non-methylene interrupted dienes; SATS: saturated fatty acids

Fatty acid	Biomarker for	Mud ingesters *Hyphalaster inermis* *Styracaster chuni*	Predator/scavengers *Hymenaster membranaceus* *Dytaster grandis grandis* *Bathybiaster vexillifer*	Suspension feeders *Freyella elegans* *Brisingella coronata* *Brisinga endecacnemos*	Unknown *Zoroaster longicauda*
PUFA	Photosynthetic microplankton	38.59 (2.61)	45.28 (3.09)	44.65 (4.08)	43.64 (3.09)
20:4 (n-6)	? possibly protozoans and microeukaryotes in the sediment[a]	23.61 (1.79)	22.41 (2.93)	12.28 (2.35)	14.76 (1.92)
22:6 (n-3)	Dinoflagellates	0.52 (0.45)	1.21 (0.47)	6.66 (1.53)	3.01 (1.41)
18:1 (n-7)	Bacterial	9.38 (2.25)	4.01 (1.19)	2.79 (0.75)	2.27 (0.12)
Odd no. and br. chain	Bacterial	1.08 (0.45)	0.73 (0.36)	0.68 (0.26)	0.41 (0.21)
18:1 (n-9/n-7)	Measure of bacterial input	0.17 (0.09)	0.27 (0.21)	0.62 (0.29)	0.52 (0.06)
NMID	Bacterially derived	8.60 (1.35)	4.00 (1.42)	4.17 (0.92)	2.32 (0.56)
SATS	Association with sediment	15.92 (2.62)	17.44 (2.74)	13.24 (2.56)	15.39 (1.02)

[a]See 'Discussion' for more on the origin of 20:4 (n-6)

Table 5. Summary of the differences in mean fatty acid composition (%) of specific biomarker fatty acids for each of the predator/scavenger species. Standard deviations are given in parentheses. NMID: non-methylene interrupted dienes

Fatty acid	Biomarker for	*Hymenaster membranaceus*	*Bathybiaster vexillifer*	*Dytaster grandis grandis*
22:6 (n-3)	Dinoflagellates	1.24 (0.24)	1.59 (0.38)	0.81 (0.43)
20:4 (n-6)	? possibly protozoans and microeukaryotes in the sediment[a]	20.11 (2.82)	23.65 (2.48)	23.48 (2.44)
22:1 (n-11)	Copepods	0	0.5 (0.24)	0.36 (0.55)
20:1 (n-9)	Copepods	9.3 (0.61)	No data	1.59
18:1 (n-9)	Deep-sea fish and crustaceans	0.79 (0.10)	0.36 (0.24)	2.05 (0.43)
18:1 (n-7)	Bacterial	5.4 (0.77)	2.87 (0.35)	3.78 (0.39)
NMID	Bacterially derived	2.97 (0.38)	5.67 (0.99)	3.36 (0.73)

[a]See 'Discussion' for more on the origin of 20:4 (n-6)

Where material was present in the stomach of *Zoroaster longicauda* it comprised predominantly of trace amounts of sediment. Only 12 of the 40 *Z. longicauda* examined contained material other than trace amounts of sediment, and these consisted of only trace amounts of animal remains, including echinoderm and crustacean fragments. For all other species where animal remains were present, they were always present at greater than trace amounts.

Photographic observations

Photographs of *Hyphalaster inermis*, *Bathybiaster vexillifer*, *Dytaster grandis grandis*, *Hymenaster membranaceus*, *Freyella elegans* and *Zoroaster fulgens* (a close relative of *Z. longicauda*) revealed important information about the feeding behaviour and diet of these species. These findings will be explored in more detail in the discussion.

DISCUSSION

Although the biology of deep-sea asteroids has been studied for a number of decades, little detailed information is available regarding their nutrition (but see Tyler et al. 1993). This study employed fatty-acid biomarker analysis to examine trophic niches of asteroids in bathyal and abyssal food-limited environments. Previous studies on the fatty acid composition of asteroids have been largely on shallow water specimens. We present here the fatty acid compositions of 9 species of deep-sea asteroids.

Composition

The fatty acid composition of all deep-sea species was consistent with that in shallow-water echinoderms. Echinoderms contain moderate amounts of 18:1, but high proportions of 20:4 (n-6) and 20:5 (n-3),

with lower percentages of 22:6 (n-3) (Lewis 1967, Allen 1968, Bell & Sargent 1985) (Table 2). The ability to accumulate high proportions of 20:4 (n-6) may be characteristic of echinoderms (Takagi et al. 1980). The major monoenoic fatty acids of echinoderms are isomers of 20:1 (Rodegker & Nevenzel 1964, Ferguson 1976, Takagi et al. 1980, Sargent et al. 1983), with the major isomeric moiety varying between n-5, n-7, n-9, and n-13. In this study the n-13 moiety predominated, where separation was achieved, and the n-9 and n-7 moieties varied in second position (Table 2). Bacterially derived NMIDs (Ackman & Hooper 1973), and branched-chain and odd-number fatty acids (Sargent et al. 1983) were also present at levels comparable with shallow water echinoderms (2.3 to 9.6% of the total fatty acids for NMID's and 0.3 to 1.1% for branched-chain and odd-numbered fatty acids (Rodegker & Nevenzel 1964, Allen 1968, Ferguson 1976, Paradis & Ackman 1977, Takagi et al. 1980, Sargent et al. 1983, Kiyashko et al. 1998) (Table 2).

Dietary sources of asteroids

The fatty acid composition of the tube feet suggest the mud ingesters *Hyphalaster inermis* and *Styracaster chuni* rely most heavily on bacterial carbon as a source of nutrition (Table 4). The high percentage composition of fatty acids of bacterial origin, 18:1 (n-7) (Perry et al. 1979), odd-number and branched-chain fatty acids (Sargent et al. 1983), and NMIDs (Ackman & Hooper 1973) within the tissues of the mud ingesters, as compared to the other feeding types, suggests a high bacterial input to the diet of these asteroids.

Relatively high levels of 20:4 (n-6) also suggest an association with sediment dwelling micro-organisms (Table 4). The source of 20:4 (n-6) in the diets of marine invertebrates is not certain and has been attributed to (1) macroalgae (Takagi et al. 1980), (2) certain diatom species (Dunstan et al. 1994), but most frequently to (3) protozoans and microeukaryotes in the sediment (Bell & Sargent 1985, Fullarton et al. 1995). More recently some deep-sea and polar, free-living and endosymbiotic bacteria have been found to possess the metabolic capability to produce 20:4 (n-6) as well as other PUFA (DeLong & Yayanos 1986, Nichols et al. 1993, Jostensen & Landfald 1997, Yano et al. 1997, Russell & Nichols 1999). It is possible that bacterial input to the diet may also be responsible for the high levels of 20:4 (n-6) within *Styracaster chuni* and *Hyphalaster inermis*.

It is likely that bacteria are one of the most important components of sediment organic matter for deep-sea deposit feeders (Sokolova 2000). Previous studies have shown both species to ingest bulk sediment and digest meiofauna, faecal pellets, bacteria and nutrients from organic detritus (Madsen 1961, Shick et al. 1981, Jangoux 1982, Billett 1987). Time-lapse photography of *Hyphalaster inermis* taken by Bathysnap in the Porcupine Abyssal Plain shows this species may remain buried in the sediment for several days, possibly digesting food items from the bulk sediment ingested (Fig. 4A) (Lampitt & Billett 1988). Both species also scavenge on larger animals, as well as take up dissolved organic matter through the cribriform organs (Madsen 1961, Shick et al. 1981, Jangoux 1982, Billett 1987).

Of the 20 individuals of *Styracaster chuni* and *Hyphalaster inermis* dissected within this study, all those that contained material in the stomach were full with sediment (Table 1). The overall similarity in diet of both *S. chuni* and *H. inermis* is reflected in the unclear separation of the 2 species in both cluster and MDS analysis (Figs. 2 & 3). However, some separation is apparent and this may be indicative of a subtle dietary difference between the species, possibly as a result of food-particle selection. Species of the genus *Styracaster* have been reported to select food particles (Sokolova 2000). *S. chuni* and *H. inermis* may, to a degree, select different food particles. Both species, however, do not rely solely on the microbial benthic food web as suggested by the presence of photosynthetic fatty acid biomarkers (Table 2). The source of both 20:5 (n-3) and 22:6 (n-3) in the diet of these 2 species is likely to be photosynthetically derived, seasonally available phytodetritus (Billett et al. 1983, Lampitt 1985). However the possibility of biosynthesis of these PUFA by either free-living or endosymbiotic bacteria cannot be ruled out (DeLong & Yayanos 1986, Temara et al. 1991, Nichols et al. 1993, Jostensen & Landfald 1997, Yano et al. 1997, Russell & Nichols 1999, Ginger et al. 2000, Pond et al. 2002).

The fatty acid data suggests that the suspension feeders *Freyella elegans*, *Brisingella coronata* and *Brisinga endecacnemos* all rely heavily on the pelagic-detrital food web and photosynthetic carbon as a result of the high levels of PUFA within their tissues, particularly 20:5 (n-3) and 22:6 (n-3) (Tables 2 & 4) (Sargent et al. 1987). However, there is also a minor bacterial input to their diet as indicated by the presence of low levels of 18:1 (n-7), odd-number and branched-chain fatty acids, and NMID's within the tissues (Table 4). Brisingids feed at the seabed as suspension feeders (Emson & Young 1994, Sokolova 2000). Heterotrophic bacteria in suspension are a potential food source for benthic suspension feeders (Jorgensen 1966) and are abundant in marine snow (Silver & Alldredge 1981). Khripounoff (1979) suggested *F. elegans* fed on flocculent material in suspension. Ingestion of bacterially-laden marine snow may explain the bacterial element in the diet of these species.

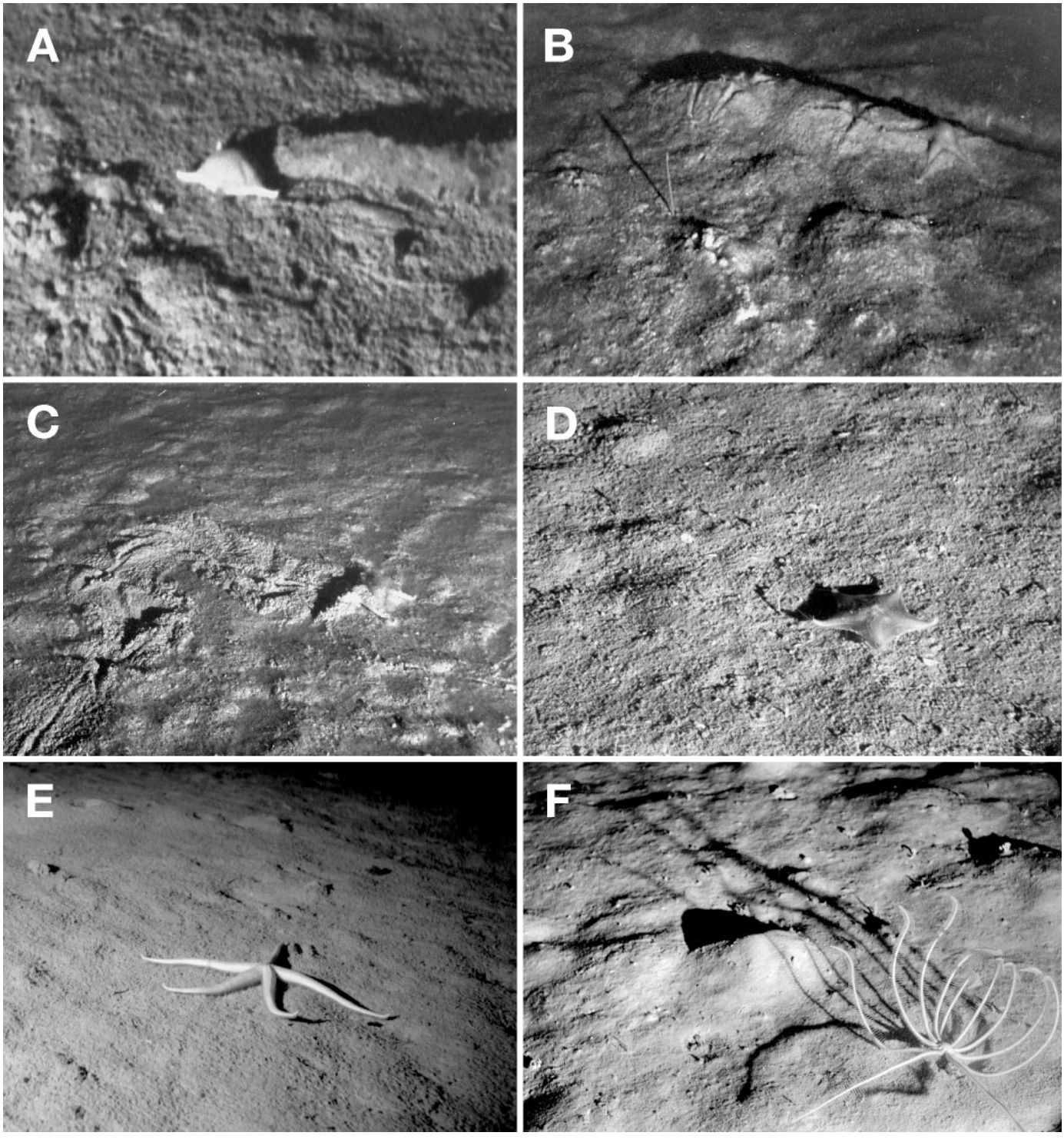

Fig. 4. Feeding behaviour of: (A) *Hyphalaster inermis*. Photograph taken by Bathysnap in the eastern North Atlantic near Great Meteor East, Stn 11262#21, depth 5376 m; (B) *Dytaster grandis grandis*. Photograph taken from a sledge mounted camera, Stn 50604#1 on the Porcupine Abyssal Plain, depth 3520 m; (C) *Bathybiaster vexillifer*. Photograph taken from a sledge mounted camera, Stn 50602#2 in the Porcupine Seabight, depth 1970 m; (D) *Hymenaster membranaceus*. Photograph taken from a sledge mounted camera, Stn 51111#1 in the Porcupine Seabight, depth 2665 m; (E) *Zoroaster fulgens* (a close relative of *Z. longicauda*). Photograph taken from a sledge mounted camera, Stn 9449#1 in the Porcupine Seabight, depth 1401 m; and (F) *Freyella elegans* in typical suspension feeding pose. Photograph taken from a sledge mounted camera, Stn 10114#1 in the mouth of the Porcupine Seabight, depth 4050 m

In contrast, stomach dissections of 52 individuals of *Freyella elegans* revealed that this species feeds almost exclusively on copepods (Table 1). The copepods taken from the stomachs of *Freyella* were in very bad condition and could not be identified. Nearly all other members of the Brisingidae, from both the Atlantic and Pacific, have been found to feed selectively on benthic copepods of the family Aetideidae (Sokolova 2000). *Novodinia antillensis*, a bathyal Atlantic brisingid, feeds on copepods of the family Metridiidae, as well as other crustaceans, including mysids and euphausids (Emson & Young 1994). Of the very limited number of stomach dissections of *Brisingella coronata* and *Brisinga endecacnemos*, only 1 individual had material in its stomach, and this consisted solely of copepods (Table 1). Jangoux (1982, and references therein) reported *B. coronata* to feed on annelids, crustaceans and detritus, although it is unspecified how these observations were made.

The high amounts of photosynthetic fatty acid biomarkers in the tissues of the suspension feeders can almost certainly be attributed to the substantial copepod component in the diet, assuming that these copepods themselves are feeding on phytodetrital food sources. Analysis of the fatty acids of benthic copepods from the abyssal BENGAL site in the NE Atlantic found high levels of both the dinoflagellate marker 22:6 (n-3) and the diatom marker 20:5 (n-3), and it was concluded that the majority of benthic copepod species fed on phytodetrital food sources (Bühring & Christiansen 2001). The high C18 ratio, which can be indicative of trophic level (Graeve et al. 1997), and higher levels of the copepod biomarkers 20:1 (n-9) and 22:1 (n-11) (Sargent & Falk-Petersen 1988) in the suspension feeders, make ingestion of copepods the more likely source of photosynthetic fatty acid biomarkers than ingestion of marine snow. It is important to remember, however, that echinoderms may have the metabolic capability to synthesise de novo various moieties of 20:1 including 20:1 (n-9) (Takagi et al. 1980, Sargent et al. 1983).

It is uncertain whether the copepods found in the stomachs of the suspension feeders were dead or alive when captured. The bathyal brisingid *Novodinia antillensis* captures its crustacean prey live using highly retentive pedicellaria on the lateral arm spines to grasp and retain prey (Emson & Young 1994). It is possible that both live and dead copepods are taken by abyssal forms as a result of the abundance of both carcasses of planktonic copepods and of living planktonic and benthopelagic crustaceans living just off the bottom in many deep-water areas (Roe 1988, Emson & Young 1994).

The clustering of the 3 suspension feeders in the MDS (Fig. 3) and cluster analysis (Fig. 2) suggests these species have very similar diets. However, there is some separation of the species in the cluster analysis. This separation may be a result of subtle differences in prey selection as a result of the prey species available at the different depths the asteroid species inhabit (Howell et al. 2002).

The predator/scavengers *Dytaster grandis grandis*, *Bathybiaster vexillifer* and *Hymenaster membranaceus* display a reliance on both pelagic-detrital and microbial-benthic food sources. This relationship to both photosynthetic and bacterial carbon sources is demonstrated by (1) the intermediate position of these species on the MDS analysis (Fig. 3) and (2) their low percentage dissimilarity with both the suspension feeders and the mud ingesters (SIMPER).

Dytaster grandis grandis, *Bathybiaster vexillifer* and *Hymenaster membranaceus* clearly separate into distinct species groups in both cluster and MDS analysis (Figs. 2 & 3). This suggests that, although all 3 species are predators or predator/scavengers, they show differences in their diet. *H. membranaceus* shows a greater dependence on the pelagic-detrital food web than either *D. grandis grandis* or *B. vexillifer*, with a high percentage composition of the dinoflagellate and copepod biomarkers present within its tissues and a lower percentage of 20:4 (n-6) (Table 5). *D. grandis grandis*, however, shows a greater reliance on bacterial carbon and the microbial benthic food web, probably through animal rather than sediment ingestion, as suggested by the high percentages of 20:4 (n-6) and 18:1 (n-9) (Table 5). *B. vexillifer* occupies a trophic position between the other 2 predator/scavenger species (Fig. 3), utilizing photosynthetic carbon to a greater extent than *D. grandis grandis* but having a greater association with the microbial-benthic food web than *H. membranaceus* (Table 5).

These results compare well with what has been found and was known already of the diet of the species from stomach content analysis. All 3 have been found to feed on a variety of benthic infaunal and epifaunal prey, as well as a certain amount of pelagic 'fall out'. *Dytaster grandis grandis* has been found to feed on echinoids, ophiuroids, sponges, bivalves, decapods, algae, foraminiferous worm tubes, a variety of infaunal macrofauna, sediment and phytodetritus (Carey 1972, Downey 1973, Khripounoff 1979, Tyler et al. 1990). There is also photographic evidence of this species ingesting a large fish bone (Bruun & Wolff 1961). Stable isotope analysis of *D. grandis grandis* has also found it to feed extensively on benthic deposit feeders (Iken et al. 2001).

Of the 30 individuals of *Dytaster grandis grandis* dissected in this study, where stomach material was present, echinoderm prey dominated (Table 1). Photographs of this species show *D. grandis grandis* leaving a trail of feeding imprints, suggesting it feeds

on macro-infauna (Fig. 4B). The ingestion of mud-consuming species is consistent with the high proportion of 20:4 (n-6) found within the tissues of this species.

Bathybiaster vexillifer has been found to take a wide variety of benthic invertebrates, in particular the irregular echinoid *Hemiaster expurgitus*. Other food items include a wide variety of gastropods, polychaetes, crustaceans and sediment (Tyler et al. 1993). Photographs of this species show *Bathybiaster* ploughing through the sediment, leaving extensive deep feeding marks (Fig. 4C) (Lampitt & Billett 1988). *B. vexillifer* and *Dytaster grandis grandis*, which both feed heavily on other echinoderms and infaunal prey, show greater similarity in their fatty acid compositions than to *Hymenaster membranaceus* (Fig. 3). The significantly greater percentage of NMIDs found in the total lipids of *Bathybiaster* may be a result of its preferential ingestion of echinoderm prey.

Hymenaster membranaceus feeds on small benthic invertebrates including foraminiferans and crustaceans, as well as surface derived matter, in particular pteropod moults and planktonic forams (Mortensen 1927, this study). Photographs of this species show it gliding across the surface of the sediment, leaving barely a trace (Fig. 4D) (Lampitt & Billett 1988). This suggests *H. membranaceus* feeds at the sediment surface on epifauna as well as planktonic detritus. The high percentage of photosynthetically derived fatty acid biomarkers in the lipids of this species is indicative of a significant input of surface-derived matter to the diet. This input of surface derived matter results in *H. membranaceus* displaying a closer relationship to the suspension feeders than either *Dytaster* or *Bathybiaster* (Fig. 3).

The diet of *Zoroaster longicauda*

Species in the genus *Zoroaster* feed extra-orally (Jangoux 1982) and so individuals rarely contain any material in the stomach. In this study only 27 of the 40 individuals examined contained any material in their stomachs and then only in very small quantities. Most had small amounts of sediment, but in some cases (12 individuals), fragments of benthic echinoderms and crustaceans were found. The indication is that species of *Zoroaster* feed on benthic fauna, as proposed by Carey (1972) for 2 Pacific species. However, the amount of material is so small that there must be some doubt over the true feeding mode of *Zoroaster* based on stomach contents alone.

The fatty acid composition of *Zoroaster* indicates a closer relationship of its diet to the suspension feeding brisingid asteroids than to other benthic predator/scavenger species. This similarity in fatty acid composition is clear in both the hierarchical cluster and MDS analyses (Figs. 2 & 3). The brisingids feed primarily on benthopelagic copepods. *Zoroaster*, however, is unlikely to be a suspension feeder in the conventional sense as it is clearly morphologically different from the brisingid asteroids (Fig. 4E,F). Its arms are not adapted to be held in the current in the way that brisingid arms are.

It is possible that *Zoroaster* captures benthopelagic prey by other means. Some forcipulatid asteroids are known for their ability to capture mobile pelagic prey using the crossed pedicellaria on their aboral surfaces (Dearborn et al. 1991, Lauerman 1998). *Z. longicauda*, however, has straight pedicellaria only (Clark & Downey 1992) and it is unknown if these are capable of capturing mobile pelagic prey. It is worth noting that we have observed that, when captured, *Z. longicauda* is coated by a mucus layer. It is possible this mucus layer has a function in capturing food items from the water column. However this is pure speculation and the mucus layer may be an artefact of the sampling process.

The specific lipid biomarkers found in *Zoroaster longicauda* indicate that it relies heavily on phytodetrital carbon to meet its nutritional requirements (Table 4). However, the presence of copepod biomarkers 20:1 (n-9) and 22:1 (n-11) and PUFAs of largely photosynthetic origin in the tube feet suggest it is also likely to be a predator of animals that, in turn, feed primarily on photosynthetic carbon (Table 2). *Zoroaster* is likely to be a specialist predator of benthic or benthopelagic crustaceans, in particular copepods. The method by which it captures its prey is, however, unknown.

CONCLUSIONS

This study has shown the overall fatty acid profile of the tube feet of asteroids to reflect the feeding mode of the species regardless of the depth range the species inhabit or the taxonomic relationships between them. Analysis of specific biomarker fatty acids has shown that the fatty acid composition of the tube feet reflected the differing diets of deep-sea asteroids. Fatty acid analysis, together with stomach content analysis and photographic observations, has revealed important information about the diets of all 9 asteroid species. *Zoroaster longicauda*, an extra-orally feeding asteroid whose diet was previously unknown, has been shown, from fatty acid analysis, to be more closely associated with the pelagic food web than would be concluded from stomach content, morphological and photographic observations alone.

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