J. Mar. Biol. Ass. U.K. (2001), 81, 817–821
Printed in the United Kingdom

Lipid composition of the hydrothermal vent clam *Calyptogena pacifica* (Mollusca: Bivalvia) as a trophic indicator

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Specimens of the chemoautotrophic symbiont-bearing hydrothermal vent clam *Calyptogena pacifica* were collected from hydrothermal vents at the Endeavour segment of the Juan de Fuca Ridge. Total lipid was extracted from gill, foot and mantle tissues, and lipid class and fatty acid composition determined by thin layer chromatography with flame ionization detection (TLC FID), gas chromatography (GC) and gas chromatography with mass spectrometry (GC MS). An abundance of n–7 monounsaturated fatty acids (MUFA), especially in the gill, reflected the large contribution of chemoautotrophic symbiotic bacteria to the nutrition of this clam. The absence of n–8 MUFA suggests that *C. pacifica* does not contain methanotrophic symbiotic bacteria. Low levels of highly unsaturated fatty acids (HUFA) such as 20:5 n–3 and 22:6 n–3 were detected in *C. pacifica* and their presence is attributed to a source other than chemoautotrophic symbiotic bacteria. Significant levels of non-methylene interrupted dienoic fatty acids and eicosatrienoic acid (20:3) were also detected in *C. pacifica* and it is suggested that these fatty acids are synthesized from n–7 MUFA as alternatives to HUFA. In contrast to shallow water bivalves, elevated levels of triglyceride were detected in the gills compared to the mantle.

INTRODUCTION

The fatty acid composition of a marine organism is an amalgamation of fatty acids assimilated and fatty acids produced either *de novo*, or by elongation and desaturation of assimilated fatty acids. Therefore, within certain limitations, the fatty acid composition of an animal can be used as an indication of its trophic ecology (Sargent et al., 1987). The rich source of organic carbon provided by bacteria at deep-sea hydrothermal vents supports a variety of organisms by means of both conventional trophic interactions and chemoautotrophic symbiotic relationships (reviewed by Childress & Fisher, 1992). Different biosynthetic pathways used by chemoautotrophs and photoautotrophs can result in the production of distinctive fatty acids that can distinguish the ultimate source of organic carbon.

The fatty acid composition of hydrothermal vent organisms is characterized by high concentrations of n–7 monosaturated fatty acids (MUFA) and considerable amounts of non-methylene interrupted dienoic fatty acids (NMID; Ben-Mlih et al., 1992; Rieley et al., 1995; Pranal et al., 1997; Pond et al., 1998; Allen Copley et al., 1998). Bathymodiolid mussels from the Mid-Atlantic Ridge, known to contain both thiotrophic and methanotrophic symbionts, contain n–8 MUFA characteristic of methanotrophs, in addition to the fatty acids found in bivalves with only thiotrophic symbionts (Pond et al., 1998). Highly unsaturated fatty acids (HUFA) such as 20:5 n–3 and 22:6 n–3 are present only at low levels (Pranal et al., 1997; Pond et al., 1998) if at all (Rieley et al., 1995) in bathymodiolid mussels and were not detected in vesicomyid clams (Ben-Mlih et al., 1992).

Deep-sea hydrothermal vent clams and mussels rely primarily on chemoautotrophic symbionts situated in bacteriocyte cells in the gills for their nutrition (Childress & Fisher, 1992). Bulk stable isotope data for vesicomyids also confirm that these organisms derive their organic carbon primarily from a chemoautotrophic source (Fisher et al., 1994; Van Dover & Fry, 1994). However vent bivalves do possess a reduced gut, and may obtain some of their nutrition by filter feeding (Nelson & Fisher, 1995). This explains the presence of low levels of putatively phototrophically-derived HUFA in the tissues of some vent bivalves (Pranal et al., 1997; Pond et al., 1998). It has been suggested that the hydrothermal vent clam *Calyptogena pacifica* may possess methanotrophic as well as thiotrophic symbiotic bacteria (Gal'chenko et al., 1988), although it is generally believed that such vesicomyids contain only thiotrophic symbionts (Scott & Fisher, 1995). If *C. pacifica* does possess methanotrophic bacterial symbionts in addition to thiotrophs, this would be reflected in its fatty acid composition (Pond et al., 1998).

Shallow water bivalves tend to rely primarily on glycogen for energy storage, but triglyceride can also be an important energy reserve (Beninger & Lucas, 1984). Elevated levels of triglyceride in the gill of bathymodiolid mussels from the south-west Pacific (compared with shallow water mytilid mussels) may be evidence of an

adaptation that allows vent bivalves to withstand periods of hydrothermal quiescence (Pranal et al., 1997).

The aim of this study was to determine fatty acid and lipid class composition of *C. pacifica* and relate the composition to the trophic ecology of this species. In particular we aimed to discover whether *C. pacifica* contained fatty acid biomarkers for methanotrophic bacteria and if levels of storage lipid were elevated in the gill tissue of *C. pacifica* compared with mantle tissue.

METHODS

Specimen collection

Calyptogena pacifica were collected during the High Rise 1995 cruise to the Endeavour segment of the Juan de Fuca Ridge, at the clam bed south of the High Rise vent field (47°57.7′N 129°05.6′W; ~2200 m). Individual clams were carefully collected using the manipulators of the US Navy Advanced Tethered Vehicle, deployed from DSVSS 'Laney Chouest'. On recovery five clams were dissected into gill, mantle and foot tissue and frozen to −20°C. Frozen samples were returned to the UK and frozen to −70°C within one month of collection. Tissues were lyophilized and finely ground before lipid extraction.

Lipid extraction

Lyophilized tissue was weighed, re-hydrated to ensure efficient triglyceride extraction and extracted with 2:1 chloroform/methanol as previously described (Allen et al., 2000). Total lipid extracts were evaporated to dryness under nitrogen, re-dissolved in a small amount of chloroform containing 0.01% BHT (butylated hydroxytoluene—an antioxidant) and stored under nitrogen at −20°C until analysis.

Lipid class analysis

Lipid class composition was determined by thin layer chromatography with flame ionization detection (TLC–FID) as previously described (Allen et al., 2000).

Fatty acid analysis

Aliquots of total lipid were used to prepare and analyse fatty acid methyl esters (FAME) as previously described (Allen et al., 2000). Fatty acid methyl esters were identified by reference to a cod liver oil standard and FAME samples of composition previously determined by GC–MS (Allen Copley et al., 1998; Pond et al., 1998) and (as far as possible without further derivatization) by GC–MS.

RESULTS

Lipid class analysis

Mean lipid class composition was determined as a percentage dry weight for gill, foot and mantle tissues (Table 1). Whole animal lipid composition was calculated from the sum of lipid extracted from each tissue of an individual and divided by the total dry weight of tissue for that individual (Table 1). The highest mean total lipid, as a percentage dry weight was found in gill and mantle. Foot and mantle comprised mostly polar lipid, while gill lipids contained also a significant proportion of triglycerides. Triglycerides were not detected in mantle tissue and were present at a low level in foot tissue. Similar proportions of free fatty acids and sterols were detected in all tissues.

There was also considerable variation in the amount of triglyceride in the gills among individuals. In the gill, triglyceride comprised almost 50% of total lipid in two individuals, but less than 10% of total lipid in the other three (Table 3).

Fatty acid analysis

The most abundant fatty acids detected in the tissues of *Calyptogen pacifica* were 16:0, 16:1 n−7, 20:1 n−7, 20:1 n−9, 20:2 NMID, 20:3 and 22:1 (Table 2). Overall MUFA comprised the largest proportion of fatty acids in all tissues. Non-methylene interrupted dienoic fatty acids constituted >20% of fatty acids in mantle and foot and >15% in gill. Only small amounts of the HUFA 20:5 n−3 and 22:6 n−3 were detected in all tissues. However, the polyunsaturated fatty acid (PUFA) 20:3 comprised >5% of total fatty acids in foot and mantle.

Gill contained a higher proportion of MUFA than foot and mantle as a consequence of higher levels of 16:1 n−7 in gill (Table 2). Foot and mantle fatty acids comprised a higher proportion of NMID and HUFA than gill fatty acids.

Because there were significant differences in triglyceride composition of gills of individual *C. pacifica*, the fatty acid composition of individual gills requires consideration (Table 3). Sample size was too small to allow statistical comparisons, but it appears that individuals with high triglyceride concentrations (2 & 5) comprised a higher proportion of MUFA and a lower proportion of NMID than individuals with low triglyceride concentrations (1, 3 & 4). The ratio of n−7 to n−9 fatty acids was also higher in individuals with high triglyceride concentrations.

Table 1. *Lipid class composition of* Calyptogena pacifica *as a percentage dry weight tissue ±SE.*

Tissue	Triglycerides	Free fatty acids	Sterols	Polar lipids	Total lipids
Foot (N=5)	0.10 ±0.08	0.23 ±0.07	0.17 ±0.17	2.22 ±0.20	2.72 ±0.20
Gill (N=5)	1.19 ±0.62	0.36 ±0.05	0.38 ±0.10	2.90 ±0.48	4.84 ±0.55
Mantle (N=5)	0.00	0.56 ±0.14	0.39 ±0.39	4.72 ±0.64	5.67 ±0.81
Whole animal (N=5)	0.66 ±0.33	0.35 ±0.04	0.43 ±0.20	3.00 ±0.44	4.41 ±0.33

Table 2. *Percentage fatty acid composition (as fatty acid methyl esters) of* Calyptogena pacifica *±SE.*

	Foot	Gill	Mantle	Whole
14:0	1.35 ±0.08	4.13 ±0.72	1.49 ±0.13	3.31 ±0.44
14:1	0.62 ±0.09	0.95 ±0.28	0.43 ±0.04	0.78 ±0.17
15:0	0.77 ±0.12	0.38 ±0.09	0.77 ±0.18	0.47 ±0.08
16:0	7.00 ±0.56	7.54 ±1.39	6.97 ±0.22	7.55 ±1.12
16:1 n–7	14.7 ±0.32	41.4 ±2.52	18.7 ±0.92	35.8 ±2.96
17:0	0.37 ±0.10	0.17 ±0.06	0.52 ±0.04	0.23 ±0.05
18:0	2.03 ±0.12	0.87 ±0.10	1.61 ±0.06	1.07 ±0.09
18:1 n 9	3.05 ±0.17	1.14 ±0.20	2.34 ±0.14	1.47 ±0.13
18:1 n 7	2.50 ±0.38	3.59 ±1.22	2.35 ±0.16	3.50 ±1.00
18:2 n–6	0.29 ±0.08	0.14 ±0.08	0.30 ±0.07	0.20 ±0.07
18:2	0.65 ±0.04	0.29 ±0.04	0.68 ±0.01	0.38 ±0.04
18:3	0.12 ±0.03	0.08 ±0.03	0.13 ±0.02	0.10 ±0.02
20:1 n 9	15.0 ±0.14	9.42 ±1.18	14.0 ±0.31	10.4 ±1.12
20:1 n–7	10.3 ±0.50	6.01 ±1.08	8.74 ±0.51	6.91 ±0.75
20:2 Δ5,11	5.19 ±0.56	4.32 ±2.37	4.29 ±1.13	4.76 ±2.12
20:2 Δ5,13	16.4 ±0.32	8.25 ±2.24	16.1 ±0.92	9.48 ±2.19
20:3	9.76 ±0.41	3.52 ±0.41	7.50 ±1.81	4.81 ±0.61
20:4	0.21 ±0.04	0.20 ±0.06	0.27 ±0.06	0.20 ±0.05
20:5 n–3	1.33 ±0.12	0.69 ±0.08	1.18 ±0.27	0.81 ±0.09
22:1	7.60 ±0.76	4.21 ±1.05	10.4 ±0.68	5.41 ±0.96
22:2 Δ7,13	0.25 ±0.06	2.28 ±0.91	0.59 ±0.17	1.78 ±0.65
22:2 Δ7,15	0.20 ±0.09	0.41 ±0.20	0.16 ±0.05	0.34 ±0.16
22:6 n 3	0.28 ±0.08	0.07 ±0.04	0.49 ±0.12	0.14 ±0.06
SFA	11.5 ±0.64	13.1 ±0.78	11.4 ±0.11	12.7 ±0.70
MUFA	53.7 ±0.76	66.7 ±2.18	56.9 ±1.82	64.3 ±2.26
NMID	22.1 ±0.41	15.3 ±2.59	21.2 ±1.57	16.4 ±2.27
HUFA	1.82 ±0.08	0.97 ±0.13	1.94 ±0.28	1.16 ±0.13
18:1 n–7/n–9	0.81 ±0.09	3.33 ±0.94	1.01 ±0.06	2.46 ±0.71

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; NMID, non-methylene interrupted dienoic fatty acids; HUFA, highly unsaturated fatty acids (⩾4 double bonds). Foot, N=5; gill, N=5; mantle, N=5; whole organism, N=5.

Table 3. *Triglyceride content (percentage dry weight tissue) and percentage fatty acid composition (as fatty acid methyl esters) of individual* Calyptogena pacifica *gills.*

Individual	1	2	3	4	5
Triglyceride	6.13	50.0	5.61	4.60	47.9
SFA	13.0	15.5	11.6	11.3	14.0
MUFA	63.6	73.1	61.9	64.2	70.5
NMID	17.5	7.75	22.1	18.0	11.0
HUFA	1.83	0.66	0.71	0.98	1.16
18:1 n–7/n–9	0.96	5.40	3.35	1.48	5.45

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; NMID, non-methylene interrupted dienoic fatty acids; HUFA, highly unsaturated fatty acids (⩾4 double bonds).

DISCUSSION

The high proportion of n 7 MUFA in all *Calyptogena pacifica* tissues is a strong indication of the reliance of this species on symbiotic chemoautotrophic bacteria. A higher proportion of these fatty acids, in particular 16:1 n–7, in the gills reflects the presence of symbiotic bacteria in this tissue. The ratio of 18:1 n–7 to 18:1 n–9 fatty acids has been used as an indicator of the contribution of bacterially derived lipid to total lipid (Sargent et al., 1987). It is therefore not surprising that the highest ratio of 18:1 n–7/n–9 fatty acids was detected in gill tissue that harbours symbiotic bacteria. The ratio of ~3 determined for *C. pacifica* gill is somewhat lower than that of ~7 reported for the gill of the congeneric clam *C. magnifica* from the Galapagos Rift (Ben-Mlih et al., 1992). However, the ratio lies within the range (2–11) reported for vent bathymodiolid gill (Ben-Mlih et al., 1992; Rieley et al., 1995; Pranal et al., 1997). Fatty acid biomarkers of methanotrophy such as n–8 MUFA (Jahnke et al., 1995; Pond et al., 1998) were not detected in any tissues. This is contrary to the assertion (Gal'chenko et al., 1988) that vesicomyids harbour methanotrophic as well as thiotrophic bacteria but agrees with other studies that detected only thiotrophs in vesicomyids (reviewed by Scott & Fisher, 1995).

Non-methylene interrupted dienoic fatty acids and 20:3 constituted the dominant PUFA in *C. pacifica*. These fatty acids can be produced by elongation and desaturation of n–7 MUFA (Zhukova et al., 1991; Pond et al., 1998) and although it is possible that they are produced by bacteria, bivalve tissues have been shown to produce them from the aforementioned precursors (Zhukova et al., 1991). As the highest levels of NMID and 20:3 were detected in symbiont-free foot and mantle, it seems plausible that they are synthesized by the clam alone.

Although HUFA such as 20:5 n–3 and 22:6 n–3 (or their precursors) are considered essential fatty acids for most marine animals (reviewed by Sargent et al., 1995), it is not clear whether these fatty acids are a nutritional requirement for bivalves. Several studies have suggested that in the absence of HUFA or their precursors, NMID and trienoic fatty acids act as substitutes (e.g. Zhukova et al., 1991; Pranal et al., 1997; Pond et al., 1998). The levels of NMID in the tissues of *C. pacifica* are in the same region as those detected in bathymodiolids (Ben-Mlih et al., 1992; Rieley et al., 1995; Pranal et al., 1997; Pond et al., 1998) that also lack HUFA and this would support this theory.

Without compound-specific stable isotope analysis, it is not possible to confirm whether the small amounts of HUFA detected in *C. pacifica* originated in the photic zone or at the vents. However, as these fatty acids are not known to be synthesized *de novo* by bivalves or by their symbiotic thiotrophic bacteria, it is possible that they were obtained by the organism filter-feeding on particulate organic matter. In addition, the bulk stable isotope composition of bathymodiolid mussels suggests that at least some of their organic matter is obtained from a source other than chemoautotrophic bacteria (Dubilier et al., 1998). If HUFA are indeed required by the clams, this may be a reason for the retention of a filter feeding mechanism.

Bathymodiolid mussels with symbiotic bacteria contain approximately equal levels of triglyceride in mantle and gill (Pranal et al., 1997), in contrast to bivalves without symbiotic bacteria that do not usually store triglyceride in the gill. Triglyceride was detected in the gill but not in the mantle of *C. pacifica*. The lack of triglyceride in mantle is perhaps most likely a result of the detection limits of the method used in this study, which equated to 0.25–1% dry tissue weight. It has been suggested that use of the gills for energy storage allows bathymodiolids to withstand periods of hydrothermal quiescence (Pranal et al., 1997) and it seems likely that this may also be the case for vesicomyids.

High standard errors of mean values for free fatty acid and sterols may be attributed to the fact that these lipid classes occurred only at low levels and, as a consequence of method detection limits, were not detected in all samples. The variation in gill triglyceride levels among individuals was perhaps more significant. The two gills with the highest triglyceride content also contained the highest proportion of MUFA and the highest ratios of 18:1 n–7/n–9. This suggests that there may be a larger bacterial biomass in the gills of these individuals. The sample size is small and there is no information on the microenvironment from which these clams were collected, or the reproductive state of the individuals. However, the greater levels of triglyceride in the gills of two clams might indicate a more favourable microenvironment for these individuals or a difference in reproductive state.

Thanks to the Captain and crew of the DSVSS 'Laney Chouest' and the US Navy's Deep Submergence Unit, operators of DSVs 'Seacliff' and Advanced Tethered Vehicles. Thanks also for the comments and suggestions of two anonymous referees. I would also like to acknowledge David Pond and colleagues at the University of Stirling for their help with the identification of fatty acids. C.E.A. was supported by a University of Southampton PhD Studentship during this work and the cruise to collect the specimens was funded by NOAA's West Coast & Polar Regions Undersea Research Center.

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Submitted 3 January 2001. Accepted 18 May 2001.