Lipid Biomarkers in Marine Symbiotic Systems

by

J. Gregor Fullarton

NERC Unit of Aquatic Biochemistry

University of Stirling

Submitted for Degree

of

Doctor of Philosophy

(Ph.D)

to

University of Stirling

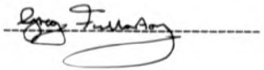
September 1993

Abstract.

Fatty acid compositional analyses of various isolated symbiotic bacteria showed all species to be rich in 16:0, 16:1(n-7) and 18:1(n-7) and depleted in PUFA. A variety of symbiont-containing marine invertebrates from a range of locations were then analysed and the majority were rich in 16:1(n-7), 18:1(n-7) and non-methylene interrupted dienoic fatty acids (NMIDs), and depleted in polyunsaturated fatty acids (PUFA), so implying that symbiotic bacteria were a major source of nutrition for these animals. It was also considered that the NMIDs are produced by the host animal in response to a relative deficiency of conventional PUFA and an extensive bacterial production of 18:1(n-7). Members of the Thyasirid family contained, in addition to the symbiont-type fatty acids, substantial proportions of fatty acids normally associated with phytoplankton, namely 18:3(n-3) and 18:5(n-3). It was deduced from the fatty acid profiles that the Thyasirids undergo a mixotrophic mode of nutrition. Fatty acid analysis was applied to three geographically distinct methane seep sites where the major trophic processes were unclear. The results revealed that at one site the symbiotic relationship dominated while at the other sites, photosynthetic processes were of greater importance for the animals' nutrition. Finally, the study examined the incorporation of radioactive bicarbonate into the lipid classes of Lucinoma borealis under a variety of environmental regimes. It was concluded that both the fatty acid biomarker and the radioactive incorporation techniques together provide a powerful tool for the detailed examination of host-symbiont relationships in the marine environment.

Declaration.

I hereby declare that this thesis has been composed entirely by myself and has not been submitted in any previous application for a degree, and the work for which it is a record has been carried out by myself. The nature and extent of any work carried out by, or in conjunction with others, has been specifically acknowledged by reference.



Acknowledgements.

The author would like to express his thanks and appreciation for help during the course of this thesis to the following people :

Prof. John R. Sargent for his supervision and helpful ideas.

Dr Paul R. Dando for his help while at the MBA in Plymouth and for organising space on research cruises, provision of samples and helpful ideas.

Prof. Alan J. Southward and Dr Eve C. Southward for providing many invertebrate samples for analyses.

Mr Sean O'Hara for help while at Plymouth and in providing samples.

Dr R. James Henderson for extensive help with laboratory procedures and organising GC-MS facilities.

Dr W.W. Christie for carrying out GC-MS analyses.

Dr Rolf Schmaljohann for providing bacterial cultures grown from the Skagerrak sediment.

Dr Ann Wood for culturing and supplying the majority of the isolated symbiotic and non-symbiotic bacteria.

Prof. Geoff Codd for supplying a symbiotic bacteria culture.

The crews of the M.V. Alkor and the R.V. Resolution.

All at the NERC Unit, University of Stirling for help in the laboratory.

Staff in the School of Natural Science, University of Stirling, for help with various problems throughout this study.

To the NERC for providing funding for this research.

Thanks to my family and friends for support during this work.

Contents.

List of Tables.

List of Figures.

List of Abbreviations.

The following abbreviations are regularly used throughout this thesis.

EFA = essential fatty acid

PUFA = polyunsaturated fatty acid

NMID = non-methylene interupted dienoic fatty acid

FAME = fatty acid methyl ester

PC = phosphatidylcholine

PS = phosphatidylserine

PI = phosphatidylinositol

CL = cardiolipin

PG = phosphatidylglycerol

PE = phosphatidylethanolamine

GLC = gas-liquid chromatography

GLC-MS = gas-liquid chromatography-mass spectroscopy

TLC = thin-layer chromatography

HP-TLC = high perfomance thin-layer chromatography

1.

Introduction to Marine Symbiotic Systems.

This section aims to outline the nature of the highly specialised and relatively recently discovered marine symbiotic systems which will be examined in this study.

1.1 Discovery of marine invertebrate-prokaryote symbiosis

For many years, biological textbooks have related the view that solar energy transformed through the photosynthetic process is the ultimate basis for all life on Earth. In the marine environment the generally accepted view is that solar energy is initially transformed into organic compounds by photosynthetic phytoplankton which in turn form the basis for the complex marine food web. The crux of this hypothesis is that, from the shallow coastal seas down to the deepest oceans, all life is dependent directly or indirectly on photosynthetically derived production. However in 1977 Ballard discovered, through his pioneering work with the submersible "Alvin", astonishing and bizzare communities clustering around deep-sea hydrothermal vents at depths of up to 2500m and so opened a new chapter in marine biology. Hot water was found to gush from the earth's crust through mid-ocean ridges at temperatures of up to 350°C (normally deep ocean temperatures are less than 5°C). The communities consisted of huge worms and massive beds of clams and mussels. This contradicted the idea that the deep ocean, because of its very limited nutritional resources, contained only sparse populations of organisms. Scientists sought to explain this paradox and eventually arrived at the conclusion that these communities survived completely independently of solar input but instead depended on a geothermally derived energy source, hydrogen sulphide, emitted in the vent fluid

and utilised by chemoautotrophic bacteria (Cavanaugh et al., 1981; Fry et al., 1983; Jannasch and Mottl, 1985). Furthermore, the apparent absence of particulate or dissolved food led to the discovery of prokaryotic, chemoautotrophic endosymbionts living in association with these animals (Cavanaugh et al., 1981; Felbeck, 1981). These bacteria seemed to provide the host with reduced organic compounds, synthesised through the fixation of carbon dioxide in the Calvin-Benson cycle (Figure 1.1), linked to the oxidation of reduced sulphur compounds (Felbeck et al., 1981; Felbeck and Somero, 1982; Cavanaugh, 1983; Jannasch and Nelson, 1984; Dando et al., 1985; Dando et al., 1986a & b; Southward et al., 1986; Spiro et al., 1986). The aforementioned chemoautotrophic process is represented in Figure 1.2. This symbiosis represents the highly efficient transfer of chemoautotrophic production to the vent animals (Jannasch and Nelson, 1984) and is considered to be responsible for the unusually high biomass found around these vents (Jannasch and Mottl, 1985). Though chemoautotrophic bacteria are the primary source of nutrition in the vent animals, it has been suggested that there may be a small input of particulate organic matter from the surface waters (de Burgh and Singla, 1984). Indeed, Le Pennec et al. (1985) found a hydrothermal vent mussel to be mixotrophic, due to the presence of diatoms in its stomach, in addition to its symbiotic bacteria.

1.2 An Explanation of Symbiosis.

The word symbiosis can be used to describe a variety of associations, although technically it refers to a mutually beneficial partnership between organisms of different kinds. Even though both partners are symbionts it has become customary to refer to the animal

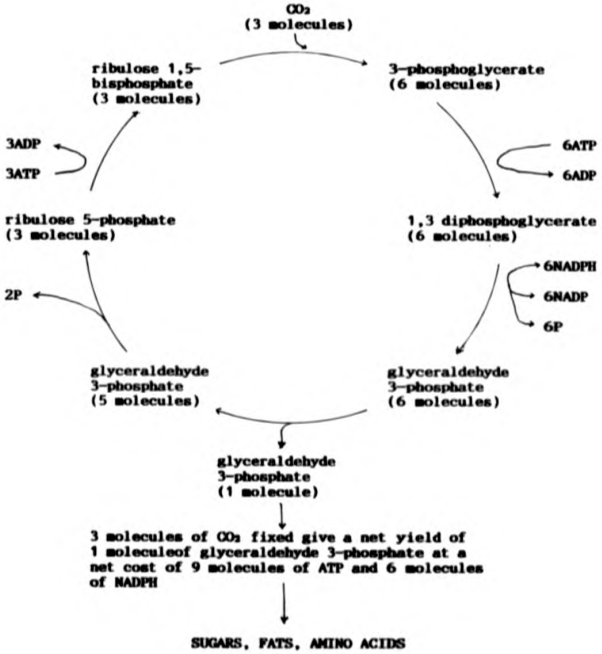


Figure 1.1 The Calvin-Benson Cycle of Carbon Fixation (the many intermediates between glyceraldehyde 3-phosphate and ribulose 5-phosphate having been omitted for clarity), Adapted from Alberts et al., 1983.

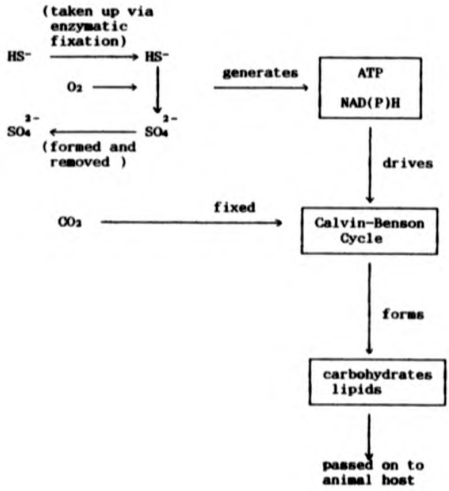


Figure 1.2 Schematic representation of the chemoautotrophic processes occurring within a hypothetical sulphur-oxidising symbiotic bacterium.

as the host and the prokaryote as the symbiont. In the symbiotic systems considered within this study, the bacterial symbionts benefit by the extension of the available habitat for bacterial oxidation of sulphide, while the animal host benefits, probably to a greater extent, by allowing its colonisation of the reducing sediment and hence nutritional radiation (Southward, 1986).

1.3 Range and Species Diversity of Invertebrate-Prokaryote Symbioses

(a) Habitats.

It soon became evident that symbiotic associations such as those described in the section 1.1 were not limited to hydrothermal vents but existed in a wide range of marine invertebrates living in reducing sediments (Cavanaugh, 1983) ranging from intertidal habitats to 1800m+ (Spiro et al., 1986). Such environments include a hypoxic basin consisting of mud close to a sewage outfall (Felbeck et al., 1981), a deep sea hydrocarbon seep (Brooks et al., 1987), reducing muds of eelgrass beds (Cavanaugh, 1983), soft mud near pulp mill effluent (Reid, 1980), coral sands (Berg and Alatalo, 1984) and seagrass beds (Fisher and Hand, 1984). However, there is a close link between all these ecotypes, namely the simultaneous access to an energy source, normally sulphide, and the oxygen required for the oxidation of sulphide to generate the ATP necessary to drive the Calvin-Benson cycle (Cavanaugh, 1983; Felbeck et al., 1983a; Fisher and Hand, 1984; Schweimanns and Felbeck, 1985; Stein et al., 1988). Several reviews have been published on such symbioses, with the majority tending to concentrate on the hydrothermal vent situation (e.g. Herrmann, 1981; Hessler et al., 1988; Jannasch, 1985; Southward, 1987, Southward, 1989).

(b) Species.

Throughout the aforementioned range of habitats, a wide number of species are involved within four major phyla. The most prominent of these species are listed in Table 1.1.

(i) Phylum Pogonophora

This phylum consists of worms lacking digestive tracts that are found at depths ranging from 20 to 9950m (Southward, 1971), although most inhabit deep water and all probably contain endosymbionts (Southward, 1987). The phylum includes the smaller, sediment living species such as Siboglinum, with long slender bodies 0.1 to 0.2 mm diameter and 50 to 500 mm long (Southward, 1987), as well as the much larger hydrothermal vent vestimentiferans, the most conspicuous of which is the red plumed, Riftia pachyptila, up to 1.5m long and 38mm diameter and lacking a mouth or gut (Felbeck et al., 1981). The latter species seems to have lost all morphologically identifiable ingestive and digestive organs. Instead its body cavity is filled with trophosome tissue which is rich in prokaryotes that make up 50 to 60% of its total wet mass (Jannasch, 1985; Jannasch and Mottl, 1985), which has its own special blood supply (Southward, 1982) and which contains several enzymes associated with a chemoautotrophic existence that are absent from the symbiont-free muscle tissue (Felbeck et al., 1981).

(ii) Phylum Polychaeta

Large numbers of symbiotic bacteria have been found attached to the body walls of the hydrothermal Pompeii worms, Alvinella pompejana and A. caudata. The animals seem to ingest the attached bacteria and in addition consume free-living, vent water bacteria (Desbruyeres et

Table 1.1 Marine Invertebrate Species Known to Contain Chemoautotrophic Symbionts.

Species	Reference
Mollusca	
Solemya velum	Cavanaugh, 1983a
S. panamensis	Felbeck et al., 1981
S. reidi	Felbeck, 1983
Codakia orbicularis	Berg and Alatalo, 1984
Lucina passula	Berg and Alatalo, 1984
Thyasira flexuosa	Southward, 1986
T. sarsi	Southward, 1986
T. gouldi	Southward, 1986
T. equalis	Southward, 1986
Myrtea spinifera	Dando et al., 1985
Loripes lucinalis	Merry et al., 1989
Lucinoma borealis	Southward, 1986
L. annulata	Vetter, 1984
L. atlantis	Kennicutt et al., 1985
L. floridana	Fisher and Hand, 1984
L. radians	Giere, 1985
L. costata	Giere, 1985
L. multilineata	Giere, 1985
L. aequizonata	Distel and Felbeck, 1987
Parvilucina tenuisculpta	Felbeck et al., 1981
Calyptogena magnifica	Cavanaugh, 1983a
C. pacifica	Cavanaugh, 1983a
C. ponderosa	Kennicutt et al., 1985
C. elongata	Vetter, 1985
Bathymodiolus thermophilus	Fiala-Medioni, 1984
Bathymodiolus species	Fiala-Medioni et al., 1986
Spisula subtruncata	Soyer et al., 1987
Tennis jenchcbromana	Distel and Felbeck, 1988a
unnamed mussels	Childress et al., 1986
undescribed gastropod	Stein et al., 1988
Polychaeta	
Alvinella pompejana	Desbruyeres et al., 1983
Alvinella caudata	Desbruyeres et al., 1983
Pogonophora	
Riftia pachyptila	Cavanaugh et al., 1981
Ridgea piscesae	DeBurgh et al., 1989
Ridgea species	DeBurgh et al., 1989
Escarpia spicata	Felbeck et al., 1981
Lamellibrachis barhami	Felbeck et al., 1981
Sibopalinum poseidoni	Schmaljohann and Flugel, 1987
S. ekmani	Southward et al., 1986
S. fiordicum	Southward et al., 1986
S. atlanticum	Southward et al., 1986
Sclerolinum brattstromi	Southward et al., 1986
Oligobrachia gracilis	Southward et al., 1986
Oligochaeta	
Phallodrilus leukodermatus	Giere et al., 1984
P. planus	Felbeck et al., 1983

al., 1983; Alayse-Danet et al., 1985, 1986). The sediment-living polychaete Astomus taenoides lacks a gut and although it is thought to take up particulate organic matter (Jouin, 1978), it may be worth further investigation for the presence of symbionts (Southward, 1987).

(iii) Phylum Oligochaeta

The majority of oligochaetes seem not to contain symbiotic bacteria. However, both Phallodrilus leukodermatus and P. planus, living within carbonate sands in Bermuda, have large numbers of symbiotic bacteria living under the cuticle of the epidermal cells. The bacteria may either be ingested whole or their excretory products could be used by the worms (Giere and Langheld , 1987; Giere et al., 1982).

(iv) Phylum Mollusca

(1) Class Gastropoda

Again, the majority of gastropods do not contain symbiotic bacteria. An undescribed hydrothermal limpet was shown to endocytose bacteria colonising its gill epithelium, but no symbiosis was demonstrated (de Burgh and Single, 1984). However Stein et al. (1988) found that a gastropod from the Mariana vent contained symbiotic chemoautotrophic bacteria, this snail being found in tight clusters around the vents with greatly enlarged gills containing high activities of CO_2-fixing enzymes and enzymes involved in sulphur-oxidation.

(2) Class Bivalvia

This class contains the widest range of symbiont-containing species and in terms of this study is the most important. The

hydrothermal species include the mussel, Bathymodiolus thermophilus, whose gills contain high densities of chemoautotrophic sulphur-oxidising bacteria, large amounts of elemental sulphur and enzymes catalysing CO_2 fixation and sulphur oxidation (Kenk and Wilson, 1985). Also containing symbionts are the hydrothermal clams, Calyptogena phaseoliformis (Chassard-Bouchaud et al., 1988) and C. magnifica (Jannasch, 1985) where no filter feeding is evident and the symbionts are thought to play an important role in its nutrition (Fiala-Medioni, 1984; Fiala- Medioni and Metivier, 1986).

In non-hydrothermal vent, sulphide-rich habitats, chemoautotrophic sulphur-oxidising bacteria have been observed inter alia in Solemya panamensis, Parvilucina tenuisculpta, Lucinoma annulata (Felbeck et al., 1981) and Codakia orbicularis (Berg and Alatalo, 1984). The gill of the king scallop, Placopecten magellanicus, contains large numbers of possibly symbiotic ciliate protozoans, which seemed to be dislodged and transported by the ciliary current to the buccal region where they were ingested (Benninger et al., 1988). Lucina floridana lives in association with oxygen-releasing root systems of seagrass beds and contains endosymbiotic bacteria and chemoautotrophic enzymes (Fisher and Hand, 1984). In the gutless bivalve, Solemya reidi, no food collecting method or digestive enzymes were found to be present and initially it was inferred to live on dissolved organic matter (Reid, 1980; Reid and Bernard, 1980). However, it is now known to obtain its nutrition from endosymbiotic bacteria (Gustafson and Reid, 1988).

It seems evident that all members of the bivalve mollusc families Lucinidae, Solemyidae and Vesicomyidae will prove to contain prokaryotic symbionts while, within the Thyasiridae, only some species contain symbionts (Southward, 1986).

1.4 Features of Symbiont-Containing Invertebrates.

A number of features are now seen to be indicative of symbiont-containing organisms. These include large fleshy gills (Dando et al., 1986a), a dark coloration, a simplified digestive tract and feeding apparatus (Schweimanns and Felbeck, 1985), gills high in elemental sulphur (Felbeck et al., 1983a; Southward, 1986; Stein et al., 1988) or, in the case of Riftia, a trophosome high in elemental sulphur (Jones, 1981). Stein et al. (1988) suggest the presence of high elemental sulphur in invertebrates to be generally indicative of prokaryotic metabolism.

1.5 The Mode of Transfer of Symbionts from Generation to Generation.

Within the bacteria-containing tissues of the host, such as the trophosome of pogonophores or the gills of bivalve molluscs, the bacteria are housed in specialised cells termed bacteriocytes (Cavanaugh et al., 1981, 1983; Wittenberg, 1985). If the bacteria are essential to the host's nutrition, this then raises the question of the mode of transfer of the symbionts from generation to generation. Southward (1982) suggested that a direct transfer of bacteria to the host takes place in its early life history. Consistent with this, Giere and Langheld (1987) observed the direct transfer of bacteria

from the parental body to the eggs via external extrusion during oviposition in Phallodrilus. However Distel and Felbeck (1987) suggested that the maintenance, growth and proliferation of the symbionts in Lucinoma species occurs completely within the bacteriocytes and is not dependent on transport of bacteria or particulate matter to or from the external environment. In Solemya reidi , bacteria develop within granular vesicles in the larval test then, following metamorphosis, the bacteria lie within the perivisceral cavity and are not seen in the gametes or gills of the juveniles. This suggests that the perpetuation of the symbiosis between generations of S. reidi occurs by transmission of the bacteria directly from the parents to the offspring (Gustafson and Reid, 1988).

1.6 Evidence for Existence of Symbiosis.

Several authors have observed Gram negative bacteria in many invertebrate species using electron microscopy (e.g. Reid and Brand, 1986; Schweimanns and Felbeck, 1985; Le Pennec and Hily, 1984; Giere, 1985b). However, this by itself does not prove that the bacteria are symbiotic. Consequently the majority of evidence presented for the existence of chemosautotrophic prokaryotes is based on the activities of enzymes of carbon dioxide fixation and sulphur metabolism (Cavanaugh, 1985). The enzyme ribulose 1,5-bisphosphate carboxylase (RuBP Case, RuBisCo etc.) is a CO_2 fixation enzyme of the Calvin-Benson cycle. The enzyme is not produced by animals but only by organisms with an autotrophic existence and it has been found by a number of authors in many symbiont-containing invertebrates (e.g. Dando and Southward, 1986; Cavanaugh, 1983, Felbeck, 1981; Tuttle, 1985; Stein et al., 1988).

The occurrence of sulphide oxidation enzymes also seems to be widespread though not consistently so in all animals studied (Table 1.2). There are five main enzymes involved in sulphur oxidation and these are adenylylsulphate reductase, sulphate adenylyltransferase, sulphate adenylyltransferase (ADP), rhodanese and sulphide oxidase (e.g. Arp et al., 1984; Fellbeck, 1983a; Fisher and Hand, 1984; Cavanaugh et al., 1981). If sulphide is, in fact, being utilised by the bacteria then the addition of sulphide compounds should stimulate CO_2 fixation and ATP production. Sulphide and thiosulphate stimulated CO_2 fixation in the isolated gills of Solemya velum (Cavanaugh, 1983), while in Sibouglinum fiordicum and Myrtea spinifera only sulphide caused an increase in CO_2 fixation (Dando et al., 1985; Southward et al., 1986). In Lucinoma borealis the greatest fixation of CO_2 occurred on the addition of sulphide to the gills of animals that had been excluded or starved of sulphide, consistent with the prokaryotes having already metabolised most of their sulphur reserve (Dando et al., 1986a). Powell and Somero (1986a) showed that reduced sulphide compounds stimulate ATP generation in homogenates of symbiont containing tissues of Riftia, Calyptogena and Bathymodiolus, while Dando et al. (1986a) demonstrated the same effect in L. borealis.

1.7 Maintenance of the Energy Source.

Since elemental sulphur is only found in symbiont-containing tissues of the animal, it has been suggested that its formation from sulphide is not part of a detoxification scheme but rather that the sulphur is used as an inorganic energy reserve that can be oxidised during the temporary absence of external sulphide, thus allowing persistence of the symbiosis (Vetter, 1985). Since hydrothermal vents

Table 1.2 Records of the Occurrence of Sulphur-Oxidising Enzymes in Symbiont-Containing Species, from Southward, 1987.

Species	Adenyl-sulphate reductase	Sulphate adenylyl-transferase	Sulphate adenylyl-transferase (ADP)	Rhodanese	Sulphide oxidase
Bivalvia					
Solemya reidi	4	4	-	4	8
Bathymodiolus thermophilus	-	-	-	-	9
Lucina floridana	7	7	-	7	-
L. tenuisculpta	-	4	-	-	-
L. annulata	-	4	-	4	-
L. borealis	2	-	-	-	-
Myrtea spinifera	1	1	1	-	-
Thyasira flexuosa	3	3	3	-	-
T. sarsi	3	3	3	-	-
Pogonophora					
Riftia pachyptila	4	4,6	-	-	9
Lamellibranchia barhami	-	4	-	-	-
Siboglinum fiordicum	10	10	10	-	-
S. ekmani	10	-	-	-	-
S. atlanticum	10	-	-	-	-
Oligobranchia gracilis	10	-	-	-	-
Sclerolinum brattstromi	10	-	-	-	-
Annelida					
Phallodrilus leukodermatus	-	5	-	-	5
P. planus	-	5	-	-	-

References : 1 - Dando et al., 1985
2 - Dando et al., 1986a
3 - Dando and Southward, 1986
4 - Felbeck et al., 1981
5 - Felbeck et al., 1983b
6 - Fisher and Childress, 1984
7 - Fisher and Hand, 1984
8 - Powell and Somero, 1985
9 - Powell and Somero, 1986a
10 - Southward et al., 1986.

may be temporary on a local basis (Grassle, 1986), this energy reserve could benefit motile species, such as gastropods, during the period when they are searching for a new vent (Stein et al., 1988). However, Felbeck et al. (1983a) cite a study where a population of Solemya reidi near a sewage outfall disappeared when current patterns changed causing oxygenation of the sediment and so removing environmental sulphide. They suggest that, though some sulphur-based energy metabolism may continue in the absence of ambient sulphide (due to the presence of sulphur as an energy reserve in the tissues), the long term maintenance of the community requires the presence of ambient sulphide for the majority of the time.

1.8 Energy Sources other than Sulphur Compounds.

More recently, methane-based symbioses have been observed. Hitherto undescribed mussels from a hydrocarbon seep in the Gulf of Mexico contain abundant intracellular bacteria and have been shown to consume methane (and oxygen) at a high rate, suggesting a methane-based symbiosis (Childress et al., 1986; Fisher et al., 1987). The pogonophore Siboglinum poseidoni has also had methanotrophic bacteria isolated from its tissues (Report to the Council of the MBA, 1987-88; Schmaljohann and Flugel, 1987). More recently, Schmaljohann et al (1990) suggested that Siboglinum poseidoni with its methane-oxidising symbionts and Thyasira sarsi with its sulphur-oxidising bacteria co-existed at the same site in the Skagerrak and furthermore postulated that the organic input driving sulphate reduction may in fact be methane diffusing up towards the surface of the sediment.

1.9 Adaptation to Sulphide Toxicity.

Sulphide is an extremely potent inhibitor of aerobic respiration, inhibiting the cytochrome-C oxidase system and so necessitating tight control of its entrance, transport and metabolism in all organisms (Arp et al., 1987; Powell and Somero, 1986a & b; Vetter, 1987). It may be asked, therefore, is it the case that the symbiotic prokaryotes are present within invertebrates solely for the host animal's nutrition or could they have a role in sulphide detoxification ? It seems that these bacteria are probably not used, or at any rate are not essential for sulphide detoxification since symbiont-free gastropods have been found grazing at a shallow water vent in the presence of high ambient sulphide concentrations (Stein, 1984). Also, in order to tolerate sulphide stress, a number of interstitial metazoans have been found with sulphide detoxification systems in their body walls and no symbiotic bacteria (Powell et al., 1980). Finally the hydrothermal vent crab Bythograea thermydron, found foraging among Riftia, Calyptogena and Bathymodiolus, encounters hydrogen sulphide concentrations up to 318μM yet contains no symbionts; it does, however, possess a similar detoxification system (Vetter et al., 1987).

1.10 Sulphide Transport to the Symbionts.

Since sulphide is a potent respiratory inhibitor, how is it transported to the symbionts in the host tissues ? Riftia pachyptila has a highly efficient blood system (Jannasch, 1985), containing an extracellular haemoglobin with a high affinity for oxygen (Arp and Childress, 1981; Cavanaugh et al., 1981; Arp et al., 1985) and a

circulating sulphide binding protein (Fisher and Childress, 1984; Arp et al., 1985). The symbiont-containing trophosome has an extended vascular system to allow good blood supply to the bacteria (Cavanaugh et al., 1981). Riftia experiences temperature fluctuations at vents from 2°C to 23°C and so it is not surprising that the oxygen affinity of its blood is relatively temperature independent (Wittenberg et al., 1981). In Calyptogena, sulphide is bound extremely tightly in the body fluids to prevent entry into the animal's cells (Felbeck et al., 1983a). Therefore the sulphide binding factor may transport sulphide to the symbiont and at the same time protect against sulphide inhibition of aerobic respiration in the animal's cells (Powell and Somero, 1986a). However, no sulphide-binding protein has been found in the Marians vent gastropod (Stein et al., 1988). Arp et al. (1984) postulate that C. magnifica takes up sulphide through its foot, which is extended into vent waters in rock fissures, and using the sulphide binding protein transports the sulphide to the bacteria in the gills, while its blood system simultaneously takes up O_2 and CO_2 through its siphon in the ambient bottom water. In small pogonophores the prime function of the haemoglobin is for oxygen transport, the worms living in tubes standing more or less vertically in the water and projecting some 20 to 40cm down into the sediment where oxygen levels are very low. Thus, the animal experiences a wide range of partial pressures of oxygen (pO_2). Since the symbiotic bacteria are present in the posterior end of the animal, the high oxygen affinity of the haemoglobin ensures that the blood is fully saturated with oxygen which is released at the posterior end of the animal where oxygen tension is low (Terwilliger et al., 1987).

Solemya reidi and Lucinoma annulata contain no sulphide-binding proteins (Felbeck et al., 1983a). In Riftia pachyptila sulphide is

oxidised by symbionts in the trophosome and also by symbiont-free animal cells on the outer body surface resulting in the direct generation of ATP (Powell and Somero, 1986a). In S. raidi. the mitochondria in the gill and foot cells show coupling of sulphide oxidation to oxidative phosphorylation resulting in ATP generation. Consequently S. raidi has the unprecedented ability to exploit sulphide directly, without the assistance of bacterial symbionts (Powell and Somero, 1986b). Powell and Somero (1986a) found sulphide oxidase systems in three hydrothermal vent invertebrates, the activities of which are correlated with the bacterial content of the tissues. They also found that sulphide was oxidised in the outer layer of symbiont-free tissue, suggesting that sulphide may be detoxified as it enters the body.

1.11 Nitrogen Assimilation.

Since many of the host animals seem not to feed at all, they must have some method of nitrogen assimilation in addition to their supply of carbon compounds. Felbeck et al. (1983a) suggested that nitrogen was fixed by the bacteria, using energy generated from sulphide oxidation, and then reduced to form essential nitrogen compounds (i.e. amino acids) required by the host animal. Fisher and Childress (1984) found no evidence of nitrogen fixation, though Brooks et al. (1987) postulated that the nitrogen requirements of some seep animals may be supported by nitrogen-fixing bacteria. Currently, it is thought that amino acids are taken up directly from the ambient seawater or from the sediment pore water, this having been demonstrated in Solemya raidi (Felbeck et al., 1983a) and Bathymodiolus (Fiala-Medioni et al., 1986a), both of which take up amino acids via the gills. Small

pogonophores have also been shown to take up amino acids from low environmental concentrations (Southward and Southward, 1980; Southward et al., 1979). Bacteria normally assimilate nitrogen as ammonia, so that, it seems likely that the symbionts can recycle ammonia produced as waste by the host animals (Southward, 1987). Additionally, sediment-living animals have access to an abundant supply of ammonia in the pore water (Dando et al., 1986b) and it seems likely that this could be an excellent source of nitrogen, although transport systems have not been fully investigated.

1.12 Transfer of Symbiont Production to the Host.

Little conclusive work has been carried out to ascertain how the fixed CO_2 is transported from the bacterial symbionts to the host. In Riftia the obtracular plume is the only part in contact with the water and it was found that radiolabelled CO_2 fixation is the major source of carbon for the animal (Felbeck, 1985a). The pogonophore, Siboglinum poseidoni, collected around methane seeps in the Skagerrak, was shown to fix carbon as formaldehyde (Report to the Council of the MBA, 1987-88). Southward (1987) cites other examples for the clam Solemya and other pogonophores where CO_2 is incorporated into a number of organic compounds, so it seems that a variety of different organic compounds are used for CO_2 transport depending on the organisms involved.

The idea of transfer of nutrients from symbiont to host is central to the symbiosis theory and a number of explanations and ideas have been advanced to account for the transfer. In Siboglinum fiordicum, 2.5mM β-aminogluteric acid was found and it was suggested that this may be involved in the transfer of fixed carbon from the

prokaryote to the host (Dando et al., 1986b). The notion of a kind of " sustained yield harvest " was suggested by de Burgh and Singla (1984) for a hydrothermal vent limpet, where the symbionts were continually endocytosed by the host cells to provide nutrition for the limpet without significantly affecting the bacterial population. In Thyasirids, the symbionts are extracellular being found in external blisters outside the cytoplasmic membrane of the bacteriocyte, and seem to be endocytosed by the host (Dando and Southward, 1986a; Southward, 1986; Southward, 1987). In the related Lucinids, the symbionts are not lysed. In this case the bacteria appear to be much more stable than in the Thyasirids, and instead seem to be " milked " of their excess nutrients by the host (Southward, 1986). However, the nutrients that are transferred have yet to be defined. In Lucinoma and Myrtea (both Lucinids), however, bacteria appear to be digested under adverse conditions (Dando et al., 1986a). In Riftia, the bacteria have been shown to be lysed, so liberating metabolites for nutrition of the worm (Bosch and Grasse, 1984).

1.13 Stable Isotope Studies : The Extent to which the Symbiont Meets the Host's Nutritive Needs.

In order to gain an insight into the impact of the the symbiont upon the host's nutrition it has proved useful to measure stable carbon isotope ratios. The ratio of the carbon isotopes ^{12}C and ^{13}C in an organism may yield useful insights into the source of primary production that an organism uses, since different CO_2-fixing reactions (and so modes of primary production) fractionate these carbon isotopes to different extents (Folbeck et al., 1983a). The major carbon isotope

within atmospheric CO_2 is ^{13}C. However, this is mixed with approximately 1.1% of the heavier isotope, ^{13}C. When plants and bacteria enzymatically fix CO_2, the ^{13}C isotope is discriminated against, the organic matter that is formed being depleted in ^{13}C (Southward, 1987). The ratio of ^{13}C:^{12}C, the $\delta[^{13}C]$, is calculated relative to an international limestone standard :

$$\delta[^{13}C] = \left(\frac{[^{13}C]/[^{12}C] \text{ sample}}{[^{13}C]/[^{12}C] \text{ standard}} - 1 \right) \times 1000$$

The ratios for biological material are normally negative, but become less so during progress through a food chain of several trophic levels (Fry et al., 1984). Figure 1.3 shows a range of $\delta[^{13}C]$ ratios commonly found within a range of marine organisms. It seems that bacteria may fractionate carbon isotopes to a greater extent than plants (Rau, 1981, 1985; Spies and DesMarais, 1983) which would account for the greater levels of isotopic depletion commonly observed within symbiont-containing animals than those found within heterotrophically feeding animals. Normally the $\delta[^{13}C]$ in tissues of benthic bivalves feeding on organic particles is about -16 to -20 ‰ (Spiro et al., 1986), while symbiont-containing bivalve gills have values of -24 to -31 ‰ and the bivalve bodies about -1 to -4 ‰ (Southward, 1987). Pogonophores show $\delta[^{13}C]$ values of -35 to -46 ‰ (Spiro et al., 1986), suggesting almost total organic dependance on symbiont production, despite the ability to take up dissolved organic carbon (Southward et al., 1986; Southward and Southward, 1987). A very negative value of -74 ‰ was reported for an undescribed seep mussel with methylotrophic symbionts, suggesting methane-based primary production was responsible for its nutrition (Cavanaugh et al., 1987;

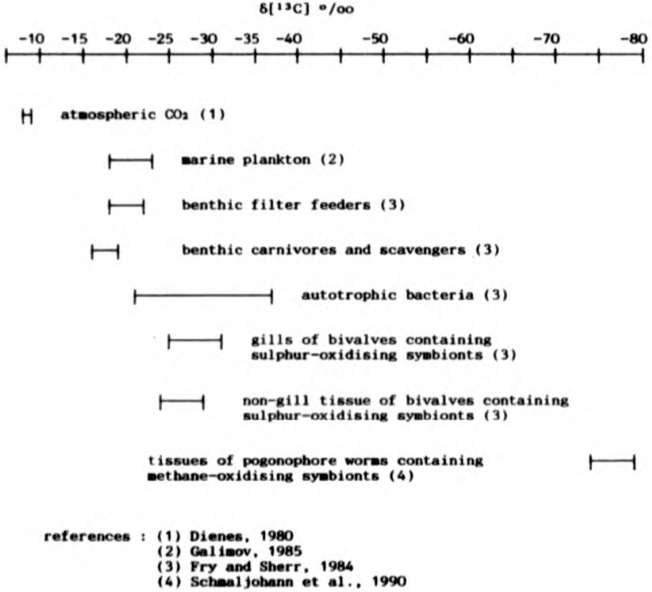


Figure 1.3 Diagramatic Representation of the Range of $^{13}C:^{12}C$ Ratios Found Throughout the Marine Environment.

Childress et al., 1986). Spiro et al. (1986) compared $\delta[^{13}C]$ values in the families Lucinidae and Thyasiridae to those of heterotrophic bivalves and suggested that the symbiont-containing species obtained a substantial proportion (50% or more) of their nutrition from the symbionts. A chemosutotrophic food source has been suggested on the basis of the $\delta[^{13}C]$ values for hydrothermal vent mytilids (Rau and Hedges, 1979) and Riftia and Calyptogena (Rau, 1981), while additional isotope ratios have also been used as evidence for chemosautotrophy in abyssal seep community invertebrates (Paull et al., 1985) and in a variety of hydrothermal vent organisms (Rau, 1985).

Ruby et al. (1987) found that laboratory grown strains of the chemosautotrophic bacteria Thiomicrospira spp. and Thiobacillus neapolitanus have $\delta[^{13}C]$ depletion values comparable to those found in organic matter produced in deep sea hydrothermal vent communities. Fry et al. (1983) used sulphur isotope ratios ($^{34}S/^{32}S$) to show that vent organisms do not use marine algae but seem to gain their nutrition from vent sulphur, i.e. the isotopic ratios were similar in the vent animal's tissues and the vent sulphur. Dando et al. (1986a) concluded from $\delta[^{13}C]$ ratios that at least half of the carbon of the non-gill tissue of L. borealis was obtained by bacterial autotrophy. Southward et al. (1987), taking into account many studies, concluded that several groups of animals obtain between 50-100% of their nutritional needs from chemosautotrophic endosymbiotic bacteria.

1.14 Aims of this Study.

All the aims of this study were intended to be carried out using lipid analysis as a tool for furthering our understanding of the

processes and interactions occurring between a range of marine invertebrates and their symbiotic bacteria.

The aims of this study were :

1. To characterise the fatty acids of isolated symbiotic bacteria obtained from marine invertebrates possessing both sulphur-based and methane-based symbiotic relationships, and to compare these results with fatty acid profiles obtained from non-symbiotic chemoautotrophic bacteria and bacteria cultured from marine sediments. The object of this section was to identify putative fatty acid "biomarkers" associated with the symbiotic bacteria.

2. To apply the results obtained from the bacterial fatty acid analyses to a wide range of marine invertebrates containing symbiotic bacteria, collected from a variety of habitats, and in particular, to examine for the presence of bacterial type "biomarkers" within the invertebrate tissue types known to be rich and poor in symbiotic bacteria.

3. To apply the experience gained from studying a range of symbiont-containing invertebrates to marine invertebrates associated with methane seeps where uncertainty exists concerning the extent to which symbiotic relationships exist in such sites.

4. To conduct a detailed study on the incorporation of carbon dioxide into the bacterial lipids of a symbiont-containing marine invertebrate, Lucinoma borealis, in order to advance knowledge of how the symbiotic process operates and may be controlled.

2.

Lipid and Fatty Acid Analytical Methodology.

Outlined in this section are the general analytical materials and methods used throughout this study. Specific techniques relating solely to subsequent chapters are detailed therein.

2.1 Lipid Extraction.

All solvents used were HPLC grade (Rathburn Chemicals Ltd., Walkerburn, Scotland) whilst all other chemicals were "Analar" grade (BDH Chemicals Ltd., Poole, Dorset, England). Samples for lipid extraction were stored at -20°C in a known volume of chloroform : methanol (2:1, v:v) and sealed under oxygen-free nitrogen until extraction using the method of Folch, Lees and Stanley (Folch et al., 1957). This methods enables the extraction of 95-99% of all lipids contained within a given sample (Christie, 1982).

Samples were kept on ice at all times during the extraction process. They were suspended in chloroform:methanol (2:1, v:v), using a ratio of 10ml of chloroform:methanol per gram of tissue to be extracted, and then homogenised for 3 minutes using a teflon-in-glass homogeniser. The resulting homogenate was then filtered through 12.5cm Whatman No. 1 filter papers (Whatman, England). The solid residue was resuspended in chloroform:methanol, homogenised for a further 2-3 minutes, filtered and combined with the initial filtrate. One quarter the volume of the filtrate of 0.88% (w:v) potassium chloride was then added and the mixture was shaken thoroughly and centrifuged for 1 minute at 2000 x rpm to achieve phase separation. The upper water-containing portion was then discarded while the remaining organic

phase was dried using a rotary film evaporator. Lipids were then redissolved in chloroform:methanol (2:1 v:v), transferred to pre-weighed glass test tubes and the solvent was removed under a steady stream of oxygen-free nitrogen. The samples were then desiccated overnight in vacuo. The lipid-containing test tubes were re-weighed the following day to enable gravimetric weight determination of the lipid extract. The amount of lipid extracted varied proportionately to the wet weight of the sample being extracted (Figure 2.1).

2.2 Separation of the Lipid Classes.

Separation of individual lipid classes was achieved by thin-layer chromatography (TLC). Depending on the desired application, TLC was carried out on either 10x10 cm high performance TLC (HP-TLC) or 20x20 cm TLC plates pre-coated with silica gel G as a binding agent (E. Merck, Darmstadt, West Germany). 20x20 cm plates were used for separating methyl esters of fatty acids for gas-liquid chromatography (GLC), or radiolabelled lipid classes for scintillation counting, while 10x10 cm plates were used effectively in the determination and quantification of lipid classes. When quantifying lipids it was necessary first to pre-wash the HP-TLC plates by developing in a single dimension with chloroform : methanol (2:1; v:v). The solvent was then allowed to evaporate from the plate and a 1cm band of silica was scraped along the upper edge of the plate to remove the impurities. The plate was then activated for 15-20 min at 110°C. The plate was rotated 90°C in a clockwise manner and the samples applied in spots or 2-3mm streaks 1.5 cm from the lower edge of the plate and 1.5cm from either side. Samples could then be applied at regular

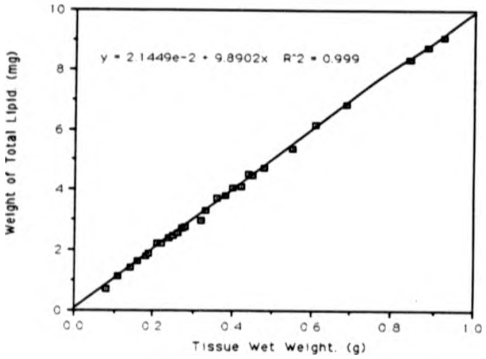


Figure 2.1 The relationship between the weight of total lipid and the wet weight of tissue from which the lipid was extracted, data taken from a number of different symbiont-containing marine invertebrate species.

(Regression based on RMS approximation)

intervals within these dimensions. A loading of 20µg lipid to each spot/streak generally provided good resolution. Samples were also applied to 20x20cm TLC plates in streaks 0.5 to 15 cm long depending on the application and the amount of lipid.

Two standard solvent systems were used throughout, both of which produced excellent TLC separation results for total lipid from a wide range of marine bivalves. For separation of methyl esters and neutral lipids, plates were developed in hexane : diethyl ether : glacial acetic acid (90:10:1 by volume), derived from Christie (1982). Polar lipids were separated by developing in methyl acetate : propanol : chloroform : methanol : 0.25% KCl (25:25:25:10:9 by vol.) , from Vitiello and Zanetta (1978). It was also possible to develop both polar and neutral lipids in a single dimension by first developing the plate halfway in the polar solvent then, after drying under vacuum, developing the plate fully in the neutral solvent.

After separation, if classes or methyl esters were to be eluted, the bands could be first sprayed with 2',7'dichlorofluorescein (DCF) (0.1% w:v in 95 % methanol) then visualised under an ultra-violet lamp. If quantification was required then the plate was first sprayed with 3% (w:v) cupric acetate in 8% (v:v) orthophosphoric acid and charred for 20 min at 160°C (Christie, 1982).

Identification of classes was carried out by examining the co-migration of authenticated lipid class standards. Quantification (in percentage terms) was carried out using a Shimadzu dual wavelength flying spot densitometer (model CS 9000) (Olsen and Henderson, 1989).

2.3 Preparation of Fatty Acid Methyl Esters.

In order to carry out gas-liquid chromatography (GLC), the total lipid extract was first converted to fatty acid methyl esters (FAME) via acid-catalysed transmethylation (Christie, 1982). The dried lipid extract was dissolved in 2ml of 1% sulphuric acid in absolute methanol and 1ml toluene, sealed under oxygen-free nitrogen and left overnight at 50°C. If an internal fatty acid standard was required it was added at this transmethylation stage. The internal standard was normally 21:0 and was added at 10% of the lipid weight. The next day, after cooling, the reaction was stopped by the addition of 5ml water and the methyl esters extracted with 5ml hexane : ether (1:1, v:v) + 0.01% (v:v) butylated hydroxytoluene (BHT), the resulting organic phase (upper phase) being removed to another test tube. The lower phase was extracted once more with 5ml hexane:ether + BHT and the organic phase added to the first test tube containing the initial organic phase extract. The combined organic phases, which contain the FAME, were then washed with 3-5ml of 2% (w:v) potassium bicarbonate. The organic solvent was evaporated under a steady stream of oxygen-free nitrogen and the methyl esters applied to a 20x20cm TLC plate for purification (see section 2.2 for details of TLC). After spraying with 2',7'dichlorofluorescein (DCF), methyl ester bands were visualised under an ultra violet lamp and the bands scraped into clean glass test-tubes. Methyl esters were eluted from the silica three times with 5ml hexane:ether + BHT (1:1) and washed with 3-5ml 2% (w:v) potassium bicarbonate to remove any DCF. The solvent was then evaporated under a steady stream of oxygen-free nitrogen and the methyl esters redissolved in hexane at a concentration of 5mg/ml. This method was

applied to both total lipid and individual lipid classes, the only difference being lipid classes were transmethylated directly on silica after class separation by TLC. Methyl esters were stored in screw top vials at -20°C until ready for GLC analysis.

2.4 Gas-Liquid Chromatography and Gas-Liquid Chromatography-Mass Spectroscopy.

Gas-liquid chromatography (GLC) is a well tried and tested technique and is covered excellently by Christie (1982). Analyses of fatty acid ethyl esters (FAME) were carried out on a Packard Model 436 gas chromatograph, fitted with a CP wax 51 column (50m x 0.32mm). A chromatogram, obtained from an isolated symbiotic bacteria sample is represented in Figure 2.2, while a representation of a typical chromatogram obtained from a symbiont-containing marine invertebrate is depicted in Figure 2.3. Gas pressures were 150 KPa for the carrier gas, hydrogen, and 200 Kpa each for air and nitrogen. At a concentration of 5mg/ml, direct on-column injection of 0.4 - 0.6 µl was sufficient to obtain a suitable loading. After injection of the sample at an oven temperature of 50°C, the oven was programmed to rise from 50°C to 150°C at 35°C per minute and then from 150°C to 225°C at 2.5°C per minute. A run would generally be stopped after 60 minutes with the data being collected and stored on a Shimadzu C-R3A recording integrator. Peaks were identified by comparison of their retention times with those of authenticated standards. If a peak had the same retention time as that of the standard then it was so named.

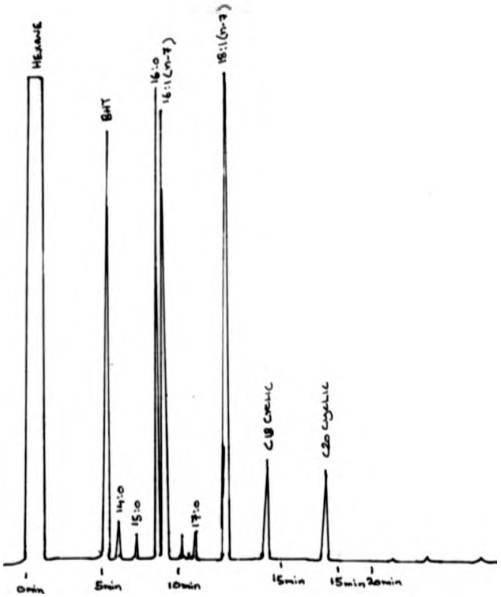


Figure 2.1 Representation of a typical gas chromatogram obtained from the total lipid of an isolated symbiotic bacterium.

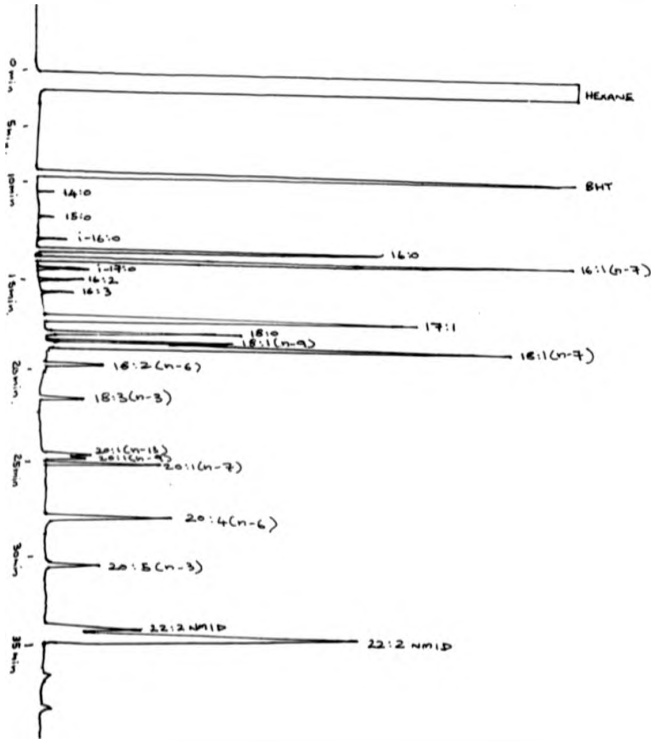


Figure 2.2 Representation of a typical gas chromatogram obtained from the total lipid of symbiont-containing marine invertebrate.

Unidentified peaks were further investigated by hydrogenation of part of the sample by bubbling hydrogen gas through the sample dissolved in hexane in the presence of Adam's catalyst. After hydrogenation the sample was then re-run to ascertain the carbon chain length of the peak. If the peak was still unidentifiable or if the identification was doubtful then samples were sent to Dr W.W. Christie at the Hannah Institute, Ayr, for precise identification using gas chromatography-mass spectroscopy (GC-MS) of picolinyl esters of the fatty acids (see Christie et al., 1988 for a description of the method). The following fatty acids were so identified by GC-MS : 16:1(n-10), 16:1(n-7), 18:1(n-13), 18:1(n-11), 18:1(n-7), 19:1, 20:1(n-13), 20:1(n-7), cyclopropane fatty acids and the non-methylene interrupted dienoic fatty acids (NMIDs). The mass spectra of these aforementioned fatty acids and the fragmentation patterns on which they were identified are represented in Figures 2.4 to 2.18.

The results of the fatty acid analyses were processed using the Supercalc 4 spreadsheet package. All fatty acid analyses were presented as percentage composition data on a weight basis.

2.5 Statistical Analysis.

All fatty acid compositional data presented within this study are means of triplicate samples. Standard deviations were less than plus or minus 5% of the mean and have been omitted from the majority of the results tables for clarity. Statistical methods employed in the analysis of the data obtained from the experimental section of this work are outlined in section 6.2.

Figure 2.6 The mass spectrum of the picolinyl ester derivative of 9,10-C16 cyclopropane.

Figure 2.5 The mass spectrum of the picolinyl ester derivative of 11,12-C18 cyclopropane.

Figure 2.6 The mass spectrum of the picolinyl ester derivative of 9,10-218 cyclopropane.

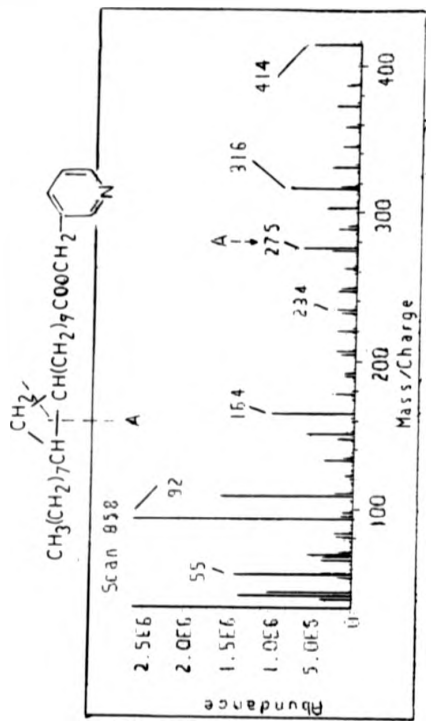


Figure 2.7 The mass spectrum of the picolinyl ester derivative of 11,12-C20 cyclopropane.

Figure 2.8 The mass spectrum of the picolinyl ester derivative of 16:1(n-10).

Figure 2.9 The mass spectrum of the picolinyl ester derivative of 16:1(n-7).

Figure 2.10 The mass spectrum of the picolinyl ester derivative of 18:1(n-13).

Figure 2.11 The mass spectrum of the picolinyl ester derivative of 18:1(n-10).

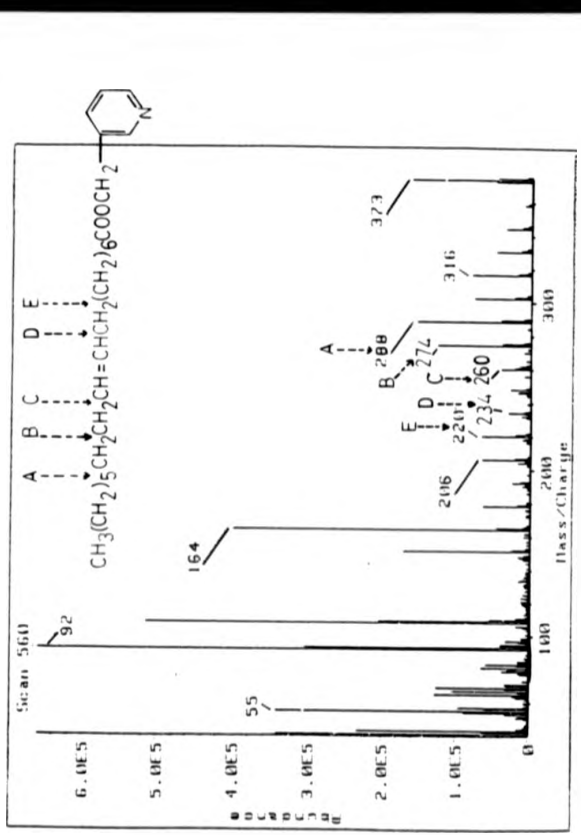


Figure 2.12 The mass spectrum of the picolinyl ester derivative of 18:1(n-9).

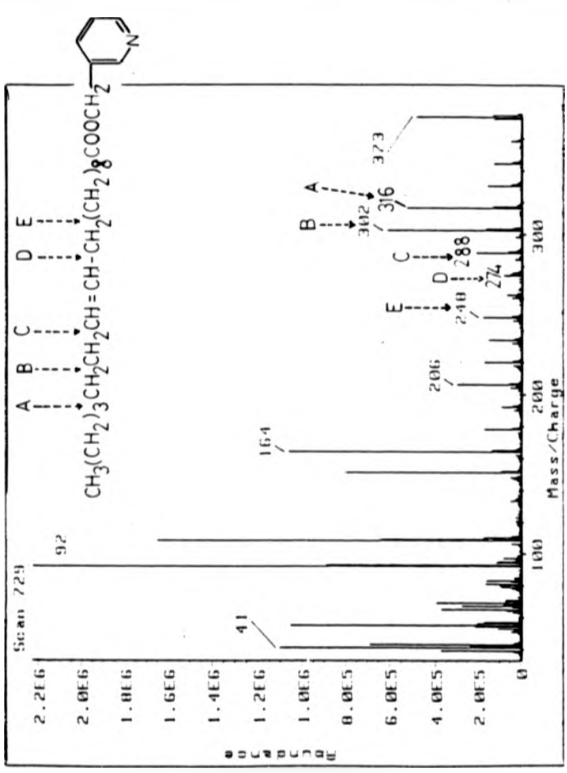


Figure 2.1 The mass spectrum of the picolinyl ester derivative of 18:1(n-7).

Figure 2.14 The mass spectrum of the picolinyl ester derivative of 19:1(n-12).

Figure 2.15 The mass spectrum of the picolinyl ester derivative of 19:1(n-7).

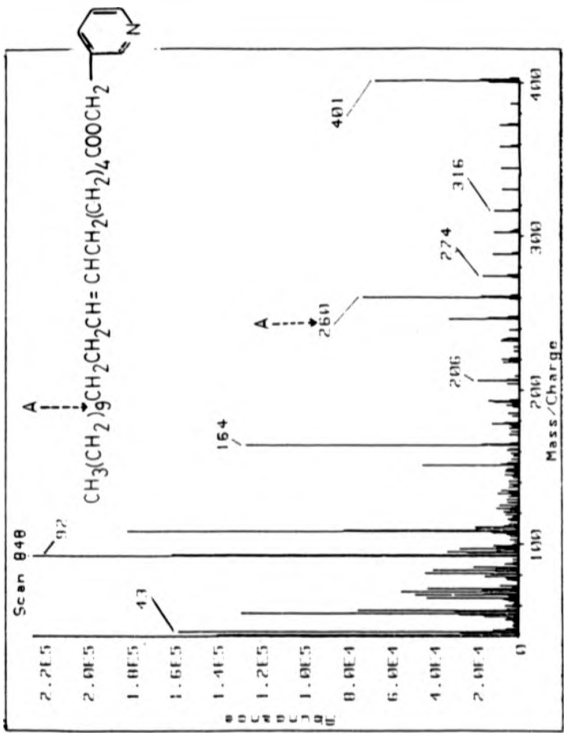


Figure 2.16 The mass spectrum of the picolinyl ester derivative of 20:1(n-13).

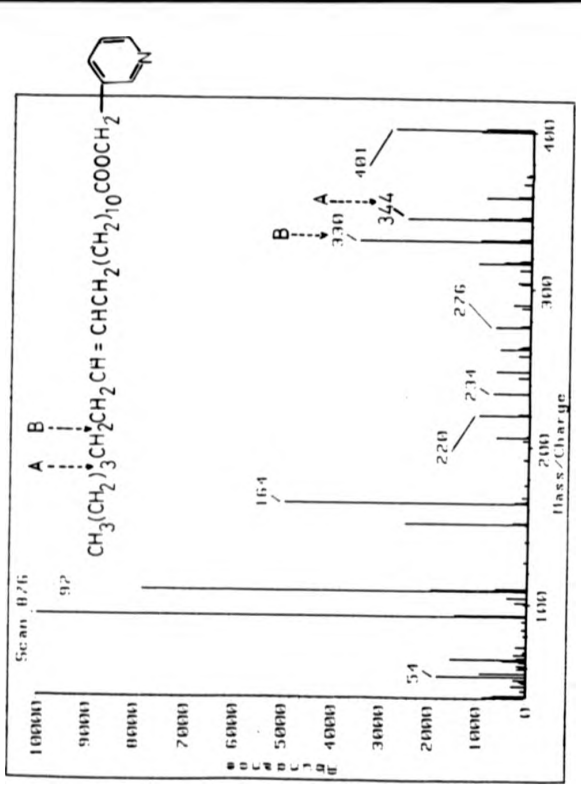


Figure 2.17 The mass spectrum of the picolinyl ester derivative of 20:1(n-7).

Figure 2.18 The mass spectrum of the picolinyl ester derivative of 7,15-22:2 NMID.

3.

Fatty Acids in Symbiotic Bacterial Isolates from Marine Invertebrates and in Related Bacteria.

3.1 Introduction.

3.1.1 Lipids as Biomarkers.

A biomarker is a chemical component of an organism which can be analysed directly from the environment (Sargent et al., 1987). Lipids are ubiquitous substances found in all organisms in the marine and terrestrial environments. Each organism or group of organisms has its own specific lipid profile, whether in terms of lipid classes or fatty acids within each given lipid class. In addition to this, lipids are relatively easy to extract, identify and quantify (Sargent et al., 1987) with respect to proteins and carbohydrates, and because the lipid content and composition of an organism invariably reflects that of its diet, they provide excellent information with regard to the trophic status of the organism. Prokaryotes have distinctive lipid profiles in comparison to eukaryotes, so that by looking for certain bacterial-type lipids within an animal, it is possible to infer and maybe to quantify its state of symbiont dependence. When considering specific fatty acids as biomarkers it should, however, be kept in mind that individual fatty acids are not generally unique to a particular organism. It is commonly necessary, therefore, to consider a combination of several fatty acids when searching for biomarkers.

The lipid biomarker technique has been well tested and its applications have included, among many others, dietary studies of zooplankton in Norwegian fjords (Sargent et al., 1985 ; Sargent and Falk-Petersen, 1981; Falk-Petersen et al., 1981), nutritional differences between a benthic filter-feeding starfish and a mud-

ingesting starfish (Falk-Peterson and Sargent, 1982), characterisation of sediment-living bacteria (Perry et al., 1979; Taylor and Parkes, 1983 and 1985) and examination for the presence and abundance of symbionts in sponges (Gillan et al., 1988).

3.1.2 Lipids in Micro-organisms.

Like eukaryotes, marine prokaryote species can be characterised by relatively specific fatty acid profiles. Bacteria commonly contain 12-20 carbon chain length saturated and monounsaturated fatty acids (MUFAs) and do not generally synthesise polyunsaturated fatty acids (PUFAs) (Harwood and Russell, 1984; Tornabene, 1985). Additionally, many bacteria are characterised by odd numbered and branched chain fatty acids as well as by hydroxy and cyclopropane derivatives. Most studies investigating marine bacterial biomarkers have examined sediment-living bacteria for the simple fact that the bacterial concentration is much higher in the sediment than in the water column (Sargent et al.,1987). Lipids and more specifically fatty acids have been used not only to suggest the presence of bacteria in marine sediment but also as a taxonomic tool and to gauge bacterial biomass. Table 3.1 represents some biomarkers used in a variety of investigations of marine bacteria.

In order to understand the significance of the use of fatty acid biomarkers presented within this study, it is helpful to consider the biosynthetic pathways of the main types of fatty acids encountered within living organisms. The next few sections provide the reader with an insight into the pathways involved.

Table 3.1 Fatty Acids as Bacterial Biomarkers (adapted from Sargent et al. 1987).

Bacteria	Fatty Acid Biomarker(s)	Reference
Sulphate-reducing		
Desulfovibrio sp. except D. gigas	i-17:1(n-7)	Taylor & Parkes (1983) Edlund et al. (1985)
Desulfobacter sp.	10Me16:0	Taylor & Parkes (1983) Dowling et al. (1986)
Desulfobulbus sp.	17:1(n-6)	Taylor & Parkes (1983) Taylor & Parkes (1985) Parkes & Calder (1985)
Bacillus sp.	branched fatty acids, usually unsaturated	Kaneda (1977)
Methane-oxidising		
Type 1	C16 monoenic fatty acids	Nichols et al. (1985)
Type 2	C18 monoenic fatty acids	
Type 1 :		
Methylomonas sp.	16:1(n-8)c, 16:1(n-8)t 16:1(n-7)t, 16:1(n-5)c 16:1(n-5)t	
Type 2 :		
Methylosinus trichosporium	18:1(n-8)c, 18:1(n-8)t, 18:1(n-7)c, 18:1(n-6)c	
Thiobacillus sp.	10-11-Me18:1(n-6), ∇19:0 (8,9), 10-11-MeO18:0, 12-13-MeO20:0, 20-OH∇16:0, 2-OH 18:0, 11-OH & 13-OH19:0	Kreger et al. (1986)
Chemotypes	Nine different types of unspecified bacteria defined by specific fatty acids	Gillan & Hogg (1984)
	Presence of cyanobacterial symbionts detected in marine sponges by specific fatty acid	Gillan et al. (1988)
Taxonomy	Hydroxy fatty acids used as part of key for identification of species of Thiobacillus	Katatama-Fujimara et al.(1982).

t = trans, c = cis, ∇ = cyclopropyl fatty acid.

3.1.3. Fatty Acid Nomenclature.

Although most fatty acids have systematic and trivial names, the standard shorthand designations have been used throughout this thesis to save confusion. The general notation takes the form of :

X:Y(n-Z)

where X is the number of carbon atoms in the molecule, Y the number of double bonds and Z is the position of the first double bond given as the number of carbon atoms from the terminal methyl end of the chain. For example, 20:5(n-3) contains twenty carbon atoms and five double bonds, the first of which is positioned three carbon atoms from the methyl end of the molecule, that is between carbon atoms 17 and 18. Occasionally a Δ^x sign is used instead of (n-Z) and this designates the position of the double bond from the carboxyl end of the carbon chain. Because adjacent double bonds are interrupted by one methylene (CH_2) group, both methods allow the exact position of all the double bonds in the molecule to be identified. For example, 18:2 $\Delta^{9,12}$ specifies that the two double bonds are between the 9th and 10th and the 12th and 13th carbon atoms numbered from the carboxyl end. The equivalent in the (n-x) nomenclature is 18:2(n-6).

3.1.4. Biosynthesis of Saturated Fatty Acids.

The general pathway of de novo saturated fatty acid biosynthesis is similar for all organisms. Acetyl CoA is first carboxylated by the enzyme acetyl CoA carboxylase the product of which is malonyl-CoA. Saturated fatty acids are then formed via a fatty acid synthetase

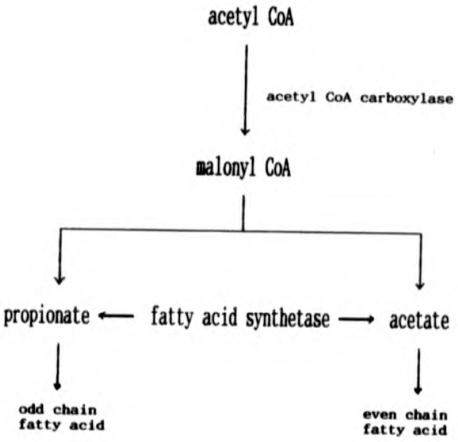
system which adds C_2 fragments from malonyl-CoA to a primer molecule (figure 3.1). The primer molecule can be acetate, in which case even chain fatty acids are formed, or propionate which results in the synthesis of odd chain fatty acids.

It should be noted that although the fatty acid synthetases of animals and bacteria are similar in their chemical reactions and metabolic function they do differ greatly in their structure (see Harwood and Russell, 1984 for further elucidation). The fatty acid synthetase reaction involves cumulative cycles of condensation of an activated acceptor with malonyl-CoA, reduction, dehydration and a secondary reduction until the required chain length is built up. The fatty acid is then released as a free acid in animals or as an acylthioester in bacteria. Generally 16:0 and 18:0 are the products of the fatty acid synthetase system, although longer chain fatty acids may be formed by the addition of further C_2 fragments.

3.1.5. Pathways of Monounsaturated Fatty Acid Synthesis.

Bacteria may synthesise monounsaturated fatty acids by one of two generally independent pathways, (Goldfine, 1972 ; Fulco, 1983) although there is evidence that that some species may use both pathways simultaneously (Wada et al., 1989). The aerobic pathway utilises a delta-9 fatty acid desaturase in the presence of oxygen to convert the products of fatty acid synthetase, chiefly 16:0 and 18:0, to their monounsaturated derivatives 16:1 delta 9 and 18:1 delta 9, i.e. 16:1(n-7) and 18:1(n-9) respectively. This pathway is present in eukaryotes, both plants and animals, as well as in prokaryotes, with 16:1(n-7) (palmitoleic acid) often being a major end product of de

Figure 3.1 Pathway for the Formation of Saturated Fatty Acids.



novo fatty acid biosynthesis in marine plants and 18:1(n-9) (oleic acid) often being a major end product in marine animals. *A priori*, the aerobic pathway operates only under aerobic conditions.

The anaerobic pathway operates only in prokaryotes and, although this pathway can readily operate in the presence of oxygen, it enables these organisms to generate monounsaturated fatty acids under anoxic conditions. In the anaerobic pathway, prokaryotes insert a double bond into the carbon-10 intermediate of fatty acid synthetase with the result that the main end product of this enzyme is, as in eukaryotes, 16:1 delta 9, i.e 16:1(n-7). This anaerobic formation of 16:1(n-7) does not preclude the formation of 16:0 and 18:0 by fatty acid synthetase. However, in the absence of an oxygen dependent conversion of 18:0 to 18:1(n-9) by delta-9 fatty acid desaturase, the only source of 18:1 is from chain elongation of 16:1 delta 9, i.e. 16:1(n-7). Therefore, when only the anaerobic pathway operates in prokaryotes, the 18:1 isomer formed is solely the (n-7) or delta 11 isomer. This fatty acid, cis-vaccenic acid, is commonly regarded as being characteristic of prokaryotes.

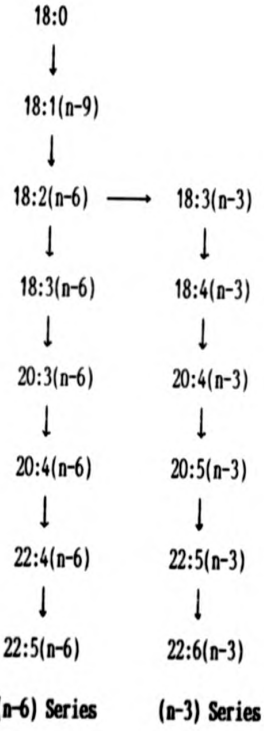
3.1.6 Polyunsaturated Fatty Acid Biosynthesis.

Although the majority of bacteria do not synthesise PUFAs, an explanation of their synthesis is helpful for the understanding of some results in this chapter and in future chapters where PUFA within animals are considered. There are two main series of PUFA, the (n-6) and the (n-3), which are deemed "essential fatty acids" (EFAs) for the long term survival and reproduction of all animals. PUFAs are formed

by the serial desaturation and chain elongation of 18:1(n-9) (figure 3.2).

It is important to note that the conversion of 18:1(n-9) to 18:2(n-6) and thence to 18:3(n-3), using sequentially delta-12 and delta-15 fatty acid desaturases, occurs only in plants. Animals require the polyunsaturated fatty acids 20:4(n-6) and, to a lesser extent, 22:5(n-6) as well as 20:5(n-3) and 22:6(n-3) for a variety of essential body functions. Some animals, especially herbivores, can convert (a) 18:2(n-6) to 20:4(n-6) and thence to 22:5(n-6), and (b) 18:3(n-3) to 20:5(n-3) and thence to 22:6(n-3), using the same enzymes principally delta-5 and delta-4 fatty acid desaturases for both the (n-6) and (n-3) conversions (Figure 3.2). In this case 18:2(n-6) and 18:3(n-3) are said to be "essential fatty acids" for these animals, meaning they are essential dietary constituents. Other animals, namely principal carnivores, cannot convert 18:2(n-6) or 18:3(n-3) to their respective C20 and C22 products in which case their essential fatty acids, in a dietary sense, are 20:4(n-6) + 22:5(n-6) and 20:5(n-3) + 22:6(n-3). It is important to note that terrestrial plants do not convert 18:2(n-6) and 18:3(n-3) to their C20 and C22 counterparts to any significant extent. However, some marine plants and phytoplankton can readily convert 18:3(n-3) to 20:5(n-3) and thence to 22:6(n-3). Thus, the latter two polyunsaturated fatty acids are abundant in phytoplankton, abundant in the zooplankton that consume the phytoplankton and abundant in the fish that consume the zooplankton. In short, marine animals, both invertebrate and vertebrate, have a luxus of both 20:5(n-3) and 22:6(n-3) in their natural diets and generally have no requirement to convert 18:2(n-6) and 18:3(n-3) to

Figure 3.2 Pathways for the Formation of Polyunsaturated Fatty Acids.



their C20 and C22 derivatives. Indeed all current experimental evidence supports the thesis that marine fish, which are invariably carnivores, have lost the ability to carry out the relevant conversions due to their lacking delta-5 fatty acid desaturase activity (Sargent et al., 1992).

3.1.7 Cyclopropane Fatty Acids.

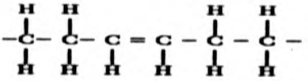
The cyclopropane fatty acids are derived from their corresponding monounsaturated fatty acid by the addition of a methylene group (CH_2) across the double bond (figure 3.3) and, although they are generally found in bacteria, they have also been found in some species of plants. It follows that, when the anaerobic pathway operates in prokaryotes, the methylene group is added to 16:1(n-7) and 18:1(n-7).

3.1.8 Branched Chain Fatty Acids.

Branched iso- and anteiso- fatty acids tend to be rare in eukaryotic organisms, although they can be substantial components of bacteria. These fatty acids are formed within bacteria via the utilisation of specific primer molecules by the fatty acid synthetase system (figure 3.4). These primer molecules take the form of the appropriate acyl-CoA compound derived from the branched chain amino acids leucine, isoleucine and valine.

Figure 3.3 Representation of Cyclopropane Formation.

a) Segment of Monounsaturate chain



b) Segment of Cyclopropane Chain

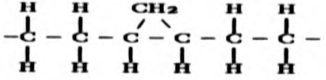


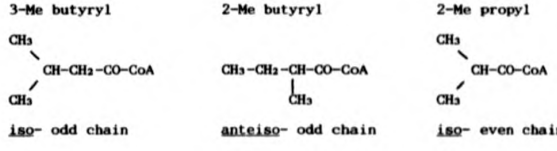
Figure 3.4 Representation of the Formation of Branched-Chain Fatty Acids.

LEUCINE ⟶ 3-Me BUTYRYL CoA ⟶ iso- ODD CHAIN FATTY ACID

ISOLEUCINE ⟶ 2-Me BUTYRYL CoA ⟶ anteiso- ODD CHAIN FATTY ACID

VALINE ⟶ 2-Me PROPYL CoA ⟶ iso- EVEN CHAIN FATTY ACID

It should be noted, that while it is possible to obtain both iso- and anteiso- odd chain (total carbon number) fatty acids from their specific primer molecules, it is only possible to obtain even chain iso- fatty acids (see below).



3.1.9. Aims of Chapter 3.

The purpose of this section was to obtain and analyse specific bacterial symbionts and to compare these with some non-symbiotic bacteria and bacteria cultured from marine sediments. These data will be built upon in future chapters when the actual host animals are analysed.

3.2 Materials and Methods.

The methods for TLC and GLC are as described in chapter 2. Prior to lipid extraction all bacterial cultures were freeze-dried for 24 hours and weighed.

3.2.1 Isolation and Culture of the Putative Symbionts and the Non-Symbiotic Bacteria.

The cultures of the putative symbionts and the non-symbiotic bacteria were all prepared and kindly donated by Dr Ann Wood of Warwick University, with the exception of TG2a which was kindly donated by Prof. Geoff Codd of Dundee University. The following method for isolation and culture of the symbionts was used (Wood and Kelly, 1989).

The host bivalves from which the symbionts were isolated and cultured were collected during 1986 from sediments at :

Jennycliff, Plymouth Sound - Thyasira flexuosa, cultures THY IA-IIIA ;

Mill Bay, Salcombe, Devon - Lucinoma borealis, cultures LUCI 5 and LUCI 7L ;

off Slemmestad, Sweden - Thyasira sarsi, culture TS2 ;

off Lysekil, Sweden - Thyasira flexuosa, culture TF1 , T. sarsi, culture TS1 , Myrtea spinifera, culture MYRTEA.

Cultures TG2a and b were of "Thiobacillus thyasirin" and were

originally derived from Thyasira flexuosa.

The bivalves were removed from the sediment, washed in running tap water before being dissected under filter-sterilised seawater. The gills were removed, washed by agitation in sterile seawater, then homogenised in sterile seawater in either a sterile mortar or hand homogeniser. The homogenates were inoculated into a wide range of media, pH 6.8-8.4, supplemented with 430mM NaCl, and incubated at 10-15°C.

Enrichment cultures for autotrophic sulphur-oxidising bacteria (and for the non-symbiotic bacteria) were made in various media suitable for the development of thiobacilli (Justin and Kelly, 1988; Wood and Kelly, 1988), using thiosulphate, tetrathionate or acetate as a substrate. Cultures were incubated under aerobic or microaerophillic conditions, or anaerobically using media supplemented with nitrate.

Methylotrophic bacteria were enriched using similar media to that used for the sulphur-oxidisers, supplemented with methanol or mono-, di- or tri-methylamines. Methylotroph enrichments were incubated under air, while sulphur bacteria enrichments were incubated both with and without 10 mM $NaHCO_3$.

Successful enrichments were sequentially subcultured three times in liquid medium before streaking the final cultures on the same medium containing 1.5% (w/v) agar. Pure cultures were obtained by repeated isolation and subculture of single colonies from agar plates.

3.2.2 Isolation and Culture of Bacteria from the Skagerrak Sediment.

The cultures obtained from the Skagerrak sediment were all obligate type I methanotrophic bacteria and were all isolated, cultured and generously supplied by Dr Rolf Schmaljohann of the Institut fur Meereskunde, University of Kiel. The cultures were enriched and subcultured in a pure mineral medium containing 1.5% (w:v) NaCl (except Methylomonas methanica) and nitrate as a source of elemental nitrogen. The only carbon source was methane, which was added to the gas phase (methane:air = 1:4) and the incubation temperature was 30°C.

Strains SK2a-4 were isolated from sediment of the central Skagerrak at a depth of approximately 300m and a temperature of 6°C. The pogonophore worm Siboglinum posedoni was found in abundance at this site. MO-BE4 was from sediment of the Kiel Bight (Baltic Sea, brackish water) and Methylomonas methanica, which was grown for comparative purposes, was from the American Type Culture Collection (strain no. 35067, fresh water). SK2b was identified as Methylococcus species and MO-BE4 as Methylobacter species, the rest being unidentified.

3.3 Results.

3.3.1 Fatty Acid Analyses of the Putative Symbionts.

Fatty acid analyses of pure cultures of symbionts, grown on a variety of media are, shown in Tables 3.2a and b. Essentially their composition was dominated by three fatty acids, namely 16:0, 16:1(n-7) and 18:1(n-7). Additionally most cultures contained two cyclopropane-type fatty acids which composed up to 18% of the total and commonly ranged between 2-8%. The putative Thyasira flexuosa symbionts grown on acetate (THY & TG2a, Table 3.2a) were dominated in composition by 18:1(n-7) (45-69%), with the rest consisting of similar proportions of 16:0 (14-20%) and 16:1(n-7) (13-28%). Cyclopropane fatty acid levels were generally less than 2% of the total, except for THY IIIA which contained about 18% of the total fatty acids as cyclopropane derivatives. The mixotrophically grown TG2b contained nearly equal proportions of all three major fatty acids (29-34%) and no cyclopropanes. The major components of the methylotroph, TF1, were 16:1(n-7) (45%) and 16:0 (29%), with 18:1(n-7) being present at a reduced level of 18%. Only trace amounts of cyclopropanes were present.

Symbionts isolated from T. sarsi, Myrtea and Lucinoma (Table 3.2b) are all methane-oxidisers grown on methylamine. In common with TF1, they all contain reduced proportions of 18:1(n-7) (12-22%), relative to the sulphur oxidisers. Levels of 16:0 (35-38%) and 16:1(n-7) (38-41%) were notably increased. The methane oxidisers also contained up to 7% 9,10- C16 Cyclopropane. All isolated symbionts in

Tables 3.2a and 3.2b were nearly completely devoid of PUFA and branched chain fatty acids.

3.3.2 Fatty Acid Analyses of some Non-Symbiotic Prokaryotes.

The prokaryotes analysed in Table 3.3, although only containing a few major fatty acids, vary considerably in composition. The main component of both the heterotrophic Thiobacillus A2i and the mixotrophic A2ii was 18:1(n-7). This was, however, depleted in A2ii which had an increased level of 16:1(n-7).

Thiobacillus tepidarus was composed mainly of 16:0, 16:1(n-7) and 18:1(n-7) in equal proportions. T. aquaesulis consisted mainly of 16:0 (45%), 16:1(n-7) (32%), and 9,10-C16 cyclopropane (11%). The level of 18:1(n-7) was greatly reduced to just 2% of the total.

Thermothrix thiopara is characterised by its lack of monoenes (only 1% 16:1 and 9% 18:1). The rest of its composition consists of equal proportions of 18:0, 9,10-C18 cyclopropane and 11,12-C20 cyclopropane.

Thiobacillus ferrooxidans consisted of equal proportions of 18:1(n-7) and 11,12-C18 cyclopropane (28% each) and equal proportions of 16:0 and 16:1(n-7) (about 13% each).

None of the bacteria analysed had detectable levels of PUFA or branched-chain fatty acids.

3.3.3 Fatty Acid Analyses of Sediment-Living Bacteria.

Although all bacteria analysed in Table 3.4 had different fatty acid compositions (which also differed from those in Tables 3.2 and 3.3) there were some features in common. The percentage of 18:1(n-7) was very low (1.7-7.7% of total) in all cases. Additionally, levels of branched chain fatty acids were negligible (less than 3% in total). There was also no evidence for the presence of any cyclopropanes. Isolates SK2A, 3A and 4 were similar in that they contained large proportions of 16:0 (42-49%) and 16:1(n-7) (23-39%) as their main constituents. SK2B contained 28% 16:0 and a diminished proportion (10%) of 16:1(n-7) which was compensated for by an increase in 18:1 moieties (both (n-9) and(n-7)), 20:1(n-7), 20:4(n-6) and 20:5(n-3). 20:5(n-3) was also found in small proportion in SK3A (3% of total) and as a major constituent (18% of total) in Methylomonas methanica which also contained about 3% 22:6(n-3). The other major components of M. methanica were 14:0 (10%), 16:0 (15%) and 16:1(n-7) (25%). The other methane oxidiser MO-BE4 contained only traces of PUFA and was composed mainly of 14:0 (6%), 16:0 (14%) and 16:1(n-7) (58%).

These results are notable for the very significant levels of PUFA in SK2B and the very substantial levels of these fatty acids in MM.

3.3.4 Lipid Class Analyses of the Putative Symbionts.

The major proportion of the total lipid extracted from the putative symbionts consisted of polar lipid (circa. 80% + of the total), the analyses of which are presented in Table 3.5 and

illustrated in Figure 3.5. All the putative symbionts contained basically the same lipid classes. With the exception of TG2, phosphatidylethanolamine (PE) constituted about 70-73% of the total, phosphatidylglycerol (PG) 19-20% of the total and cardiolipin (CL) constituted about 7% of the total. Phosphatidylserine (PS) was present in compositions ranging from trace amounts to 3.5% of the total in all symbionts except THY and TG2 where it was not detected. Although TG2 contained the same classes, its composition was different from the rest consisting of about 49% PE, 31% PG and 20% CL.

Table 3.2a The Percentage Composition of Fatty Acids of Total Lipids Extracted from Putative Symbionts of Thyasira flexuosa.

Fatty acid	THY IA	THY IIA	THY IIIA	TG2a	TG2b	TF1
14:0	0.3	0.7	0.9	0.8	1.1	1.3
14:1	0.1	0.4	0.3	0.4		
i-15:0			0.3			0.5
15:0	0.4	0.3	0.5	0.4	0.5	0.6
15:1	0.1	0.1	0.1	0.1		
16:0	14.2	20.0	18.7	15.2	28.6	29.4
16:1(n-7)	13.7	28.4	12.6	12.7	32.6	45.1
delta 9,10-C16 Cyclic	0.4	0.7	5.7	0.7		3.0
17:0	0.2	0.3	0.1	0.1	0.5	0.2
17:1		0.3	0.1	0.1		
18:0	0.9	0.9	1.4	1.3	2.2	1.3
18:1(n-9)		0.4			0.7	
18:1(n-7)	69.3	45.3	46.8	65.6	33.7	18.2
18:2(n-6)	0.4	0.4	0.5	1.1		0.2
delta 11,12-C18 Cyclic		1.8	11.9	1.2		0.3

where : THYIA, IIA and IIIA are sulphur-oxidising bacteria, isolated from bivalves collected at Jennycliff, Plymouth Sound, and were grown heterotrophically on acetate.

TG2a and b are the sulphur-oxidising bacterium, "Thiobacillus thyasiris", the putative symbiont of Thyasira flexuosa , TG2a being grown heterotrophically on acetate, while TG2b was grown mixotrophically on thiosulphate and acetate.

TF1 was a methylotroph isolated from bivalves collected off Lysekil Sweden and was grown on methylamine.

Table 3.2b The Percentage Compositions of Fatty acids of Total Lipid Extracted from the Putative Methylotrophic Symbionts Isolated from Various Bivalves.

Fatty acid	TS1	TS2	MYRTEA	LUCI 5	LUCI 7L
14:0	0.7	2.5	1.4	3.3	2.0
14:1	0.1				
i-15:0		0.1	0.2	0.1	0.2
15:0	0.5	0.7	0.4	0.8	0.4
16:0	36.1	34.7	34.8	35.6	38.4
16:1(n-7)	37.6	41.2	34.8	39.1	32.6
delta 9,10-C16 Cyclic	5.3	7.1	3.5	7.3	4.5
17:0	0.1				0.1
17:1	0.3	0.1	0.3	0.2	0.3
18:0	1.1	0.4	1.4	0.5	0.6
18:1(n-9)		0.1		0.2	
18:1(n-7)	16.9	11.9	21.7	12.1	18.8
18:2(n-6)	0.1	0.1	0.1	0.2	0.7
delta 11,12-C18 Cyclic	1.1	1.0	1.3	0.6	1.3

where : TS1 was isolated from Thyasira sarsi, collected off Lysekil, Sweden

TS2 was isolated from T. sarsi, collected from Slemmestad, Norway.

MYRTEA was isolated from Myrtea spinifera, collected off Lysekil, Sweden.

LUCI 5 and 7L were isolated from Lucinoma borealis, collected from Mill Bay, Salcombe, Devon.

Table 3.3 The Percentage Composition of Fatty Acids of Total Lipid Extracted from Pure Cultures of Non-Symbiotic Prokaryotes.

Fatty acid	A2i	A2ii	TT	TA	ThT	TFer
14:0	0.4	0.6	0.4	0.7	0.2	0.1
14:1		0.4	0.1			
i-15:0	0.1	0.1		0.1	0.1	0.1
15:0	0.2	0.4	0.2	5.7	0.1	0.1
15:1		0.2	0.1	0.6		
16:0	8.5	14.1	33.4	44.8	1.5	14.5
16:1(n-7)	1.1	13.6	29.3	32.2	1.1	12.4
delta 9,10-C16 Cyclic			0.3	10.9		3.7
17:0	0.1	0.2				1.3
17:1	0.1	0.3	0.6	2.5	0.1	0.2
18:0	1.8	0.4	3.9	0.6	28.5	2.5
18:1(n-9)		0.6				
18:1(n-7)	82.5	68.0	30.4	1.7	8.8	28.3
18:2(n-6)	0.3	0.3	0.1	0.1	0.1	0.2
delta 11,12-C18 Cyclic	4.8	0.2	0.2		2.9	28.6
delta 9,10-C18 Cyclic			0.4		27.0	0.5
delta 11,12-C20 Cyclic		0.4	0.5		29.6	7.4

where: A2i and A2ii are Thiobacillus. strain A2, grown on thiosulphate and methylamine/thiosulphate respectively.

TT is thiosulphate-grown Thiobacillus tepidarus

TA is thiosulphate-grown Thiobacillus aquaesulis

ThT is thiosulphate-grown Thermothrix thiopara

TFer is tetrathionate-grown Thiobacillus ferrooxidans.

Table 3.4 The Percentage Composition of Fatty Acids of Total Lipid Extracted from Pure Cultures of Methane-Oxidising Bacteria Isolated from Skagerrak Sediment.

Fatty acid	SK2A	SK2B	SK3A	SK4	MO-BE4	MM
10:0	1	0.6		0.5		
12:0	0.6	0.6	0.3	0.3		0.2
14:0	3.7	6.7	1.9	2.2	6.6	10.4
14:1(n-5)			0.2	0.3	0.5	1.7
i-15:0		2.6		0.2		
a-15:0	1.2	0.6	0.3	0.3	2.4	0.3
15:0	2.9	2	2.5	3.4	1.8	1.2
15:1				0.6	0.5	0.4
i-16:0	0.7	0.8	0.3	0.4	0.2	
16:0	47.2	28.4	41.6	48.7	13.5	14.7
16:1(n-7)	29.5	9.7	38.6	23	57.6	24.7
16:2	1.1	0.5	0.2	0.5		0.6
16:3		0.6	0.5	0.6	0.3	0.7
16:4(n-3)		0.4				0.9
i-17:0		0.7		0.3		
a-17:0				0.3	0.1	
17:0	2.5	3.7	0.8	1.9	1.8	1.1
17:1		1	0.5		0.5	
18:0	3.7	6.7	1.4	3.7	2.5	2.2
18:1(n-9)		6.4	1.9	4.1	3.6	5.7
18:1(n-7)	4.7	7.7	1.7	1.3	2	5
18:2(n-6)	1.2	4.6	0.4	4.2	1.7	1
18:3(n-3)		0.3			0.5	0.2
18:4(n-3)		0.5	0.2		0.3	1.3
20:0		0.7	0.1		0.3	0.2
20:1(n-11)				0.3	0.5	
20:1(n-9)			0.3			0.7
20:1(n-7)		3.6	0.3	0.4	0.2	0.9
20:2(n-9)		0.8			0.1	
20:2(n-6)		0.4				0.2
20:3(n-6)		1.2				
20:4(n-6)		4.2	0.1		0.2	0.4
20:5(n-3)		3.3	2.9		0.2	18.2
22:0			0.2	0.7	0.5	0.3
22:1(n-11)		0.7	0.5	0.8		0.2
22:3(n-3)			0.3		0.2	0.4
22:4(n-6)						0.2
22:6(n-3)			0.7		0.3	3.4
24:0			0.1		0.6	0.3
25:0			0.7			1.5
24:1			0.5	1	0.5	0.8

where : SK2A, 3A and 4 were isolated from sediment of the central Skagerrak and were all unidentified.

SK2B is *Methylococcus* species, isolated from the central Skagerrak.

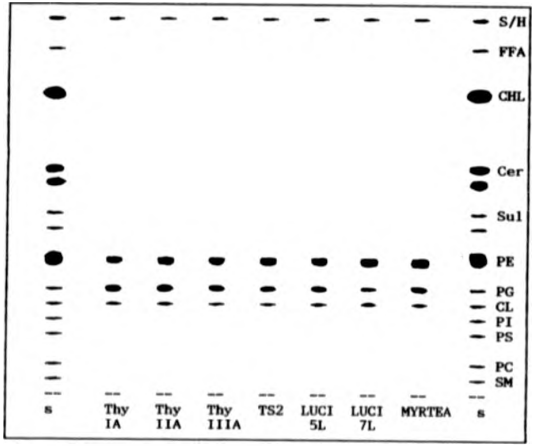
MO-BE4 is *Methylobacter* species, isolated from sediment of the Kiel Bight in the Baltic Sea.

MM is a freshwater species, *Methylomonas methanica*.

Table 3.5 The Percentage Composition of the Constituent Lipid Classes of Several Species of Isolated Putative Bacterial Symbionts (where PS is phosphatidylserine, CL is cardiolipin, PG is phosphatidylglycerol, PE is phosphatidylethanolamine; THY, TG2 and TF were all isolates from Thyasira flexuosa, TS was isolated from Thyasira sarsi and MYRTEA and LUCI were isolated from Myrtea spinifera and Lucinoma borealis respectively).

	Lipid Class			
Bacteria	PS	CL	PG	PE
THY IA	-	7.0	19.6	73.4
THY IIA	-	7.1	20.3	72.6
THY IIIA	-	6.9	20.1	73.0
TG2a	-	20.6	30.9	48.5
TG2b	-	19.8	31.2	49.0
TF1	1.9	6.8	19.0	72.3
TS1	Tr.	6.9	19.6	73.7
TS2	1.7	7.0	18.9	72.4
MYRTEA	3.5	7.0	19.1	70.4
LUCI 5L	1.8	6.5	19.0	72.7
LUCI 7L	2.0	6.7	19.3	72.0

where :
- = not detected
Tr.= trace (<1%).



where : s = standard lipid class mixture
SM = sphingomyelin PC = phosphatidylcholine
PS = phosphatidylserine PI = phosphatidylinositol
CL = cardiolipin PG = phosphatidylglycerol
PE = phosphatidylethanolamine
Cer = cerebrocytes Sul = sulphatides
CHL = cholesterol FFA = free fatty acids
S/H = sterol esters and hydrocarbons

Bacterial samples are as described in materials and methods.

Figure 3.5 Schematic Representation of Bacterial Lipid Class Separation by Thin-Layer Chromatography.

3.4 Discussion.

3.4.1 Analyses of Putative Symbionts.

The fatty acid compositions of the putative symbionts are not particularly unusual relative to those of other microbes in so much that the fatty acid profiles show only three main fatty acids with at most trace amounts of PUFA and branched chain fatty acids.

There seems to be one striking pattern in the results shown in Table 3.2a and b. This is that the fatty acid composition of the putative symbionts is not necessarily unique for a given host species but is dependent on its growth substrate and therefore its mode of chemoautotrophic nutrition. This point is demonstrated very well in Table 3.2a where all organisms are putative symbionts from one animal species, namely Thyasira flexuosa. The first four cultures, THY I-IIIA and TG2a, all similar (if not identical) autotrophic thiosulphate users, were characterised by the preponderance of cis-vaccenic acid (18:1(n-7)) and diminished amounts of 16:0 and 16:1(n-7), while TG2b was grown mixotrophically and contained more or less equal proportions of 16:0, 16:1(n-7) and 18:1(n-7). However TF1, a methylotroph, was greatly diminished in 18:1(n-7) but contained increased proportions of 16:0 and especially 16:1(n-7). These symbionts were all isolates from the same species and therefore an obvious explanation for their differences is that their fatty acid composition is dependent upon their nutritional medium.

The methylotrophic organisms featured in Table 3.2b illustrate that symbiont cultures isolated from different animal species can feature very similar fatty acid compositions not only to each other but to that of the methylotrophic T. flexuosa symbiont (TF1), namely low cis-vaccenic and high 16:0 and 16:1(n-7), relative to the acetate grown symbionts. When considering the symbiont's relationship within the host animal in terms of potential fatty acid biomarkers it is, therefore, imperative to ascertain if the system is sulphur- or methane-based.

From the results presented in Tables 3.2a and b, particularly the absence of 18:1(n-9), it would seem evident that those putative symbionts utilising sulphur-based compounds operate solely under the "anaerobic" pathway, the major end product being cis-vaccenic acid. The same appears to be true of the methylotrophs except in this case the end product is mainly 16:1(n-7).

The presence of cyclopropane fatty acids in some of the symbiont cultures is noteworthy. The cyclopropanes observed within the putative symbionts were clearly derived from 16:1(n-7), i.e 16:1 delta 9 and 18:1(n-7), i.e 18:1 delta 11. Thus 11,12 cyclic C18 is abundant in THY IIIA while 18:1 delta 11,12 is the major fatty acid (Table 3.2a). Equally 9,10 cyclic C16 is abundant in the methylotrophs where 16:1 delta 9,10 predominates (Table 3.2b). However, the reason why one isolate of symbiont contains a greater percentage of a particular cyclopropane fatty acid than a similar isolate (e.g. Thy IIIA compared to THY IA and 2A) remains unclear. If present consistently and in large percentages, cyclopropanes would make excellent symbiont

biomarkers. However, at the levels observed in this study it seems unlikely they will make suitable biomarkers since their presence within the host animal is likely to be masked by the major symbiont fatty acids.

Branched chain fatty acids are rarely encountered in higher organisms (Goldfine 1972) and their presence is generally indicative of bacterial activity, especially when branched chain amino acids are present in the growth medium. It is perhaps not surprising that branched chain fatty acids were not present in the putative symbionts because the relevant precursor amino acids are absent from the growth media. When looking at symbiont-containing animals it may be sensible to assume that the presence of significant amounts of branched chain fatty acids reflects not the symbionts per se but rather the presence of either sediment-living, free-living or other internal bacteria.

3.4.2 Analyses of Non-Symbiotic Bacteria.

These prokaryotes have been included to serve as a point of reference for the analyses of the symbionts. Considering Table 3.3, the differences in the two Thiobacillus strain A2 cultures could be accounted for by the different growth media used. The results for A2i are similar to those obtained for the same species grown autotrophically by Katayama-Fujimara et al. (1982) who found about 6% 16:0, only a trace of 16:1, 73% cis-vaccenic acid and 2% 18:1(n-7)-derived cyclopropane fatty acid. They also found about 13% of 12:1 which was not detected in this study. These comparisons indicate that environmental conditions may affect the fatty acid compositions of prokaryotes.

Thiobacillus tepidarus, T. aquaesulis and Thermothrix thiopara were all grown on the same medium as A2i but were markedly different not only from A2i but from each other. It is obvious, therefore, that the differences in fatty acid composition in these cases were not due to the growth medium or method of autotrophic nutrition but are genuine species-specific fatty acid compositions. It is interesting to note the large percentage (over 56% of the total) of cyclopropanes and the much diminished percentages of 16:1 and 18:1 within Thermothrix thiopara. Moreover, the presence of 9,10 cyclic C18, formally derived from 18:1 delta 9,10 is strong evidence for the aerobic pathway of fatty acid biosynthesis in this species. Note that 18:1 delta 9,10 can be elongated to 20:1 delta 11,12 which can be converted to 11,12 cyclic C20. These findings and considerations emphasise the important point that, although bacteria are characterised by certain types of fatty acids, it is not really possible to generalise on the composition in so much that each species must be examined individually.

This point is reiterated when considering Thiobacillus ferrooxidans which was different again in composition from the rest of the prokaryotes in Table 3.3 and contained a large proportion of 11,12 cyclic C18, clearly a product of 18:1 delta 11,12 from the anaerobic pathway. Katayama-Fujimara et al. (1982) also analysed T. ferrooxidans and, although the same major fatty acids were found as in this study, the proportions of each varied. They found 16:0 and 16:1 present in similar proportions to this study (17% and 14% of the total respectively), but they also found 18:1 at an increased level of 44% of the total and a corresponding decrease in cyclopropanes at only 11%

of the total. Presumably these difference could be accounted for by different culture procedures including possible differences in the growth phase of the cultures at harvesting.

3.4.3 Analyses of Sediment Bacteria from the Skagerrak.

These bacteria were cultured from sediment taken from the Skagerrak area of Scandinavia at a site where several of the animals analysed in further chapters were found and so act as a reference in terms of whether or not they are used in the animals' nutrition. The bacteria in question are quite different from the majority of the rest of the bacteria analysed within this chapter due to the generally diminished proportion of 18:1(n-7) within these Skagerrak bacteria. Additionally the Skagerrak bacteria contain a greater diversity of fatty acids than the others. The most interesting feature is undoubtedly the presence of PUFA within SK2B, SK3A and especially the freshwater species, Methylomonas methanica. Fulco (1974) suggests that if a bacterium contains an oxygen-dependent pathway of fatty acid biosynthesis then it is possible for it to contain PUFA. The detection of 18:1(n-9) in most of the bacteria within Table 3.4, albeit in small amounts, suggests, that the Skagerrak bacteria do indeed utilise an oxygen-dependent pathway, bond insertion occurring at the delta 9 position, in addition to an anaerobic pathway generating 18:1 delta 11. PUFA containing up to two double bonds are not particularly uncommon in bacteria (Oliver and Colwell, 1973 cite examples). In particular, Yazawa et al. (1988) found 112 different strains of marine bacteria that produced 20:5(n-3). However all these were isolated from fish intestines and no 20:5(n-3) was found within the free-living or

sediment-living bacteria isolated by these authors. Few cases of sediment or free-living bacteria containing 20:4(n-6) or 20:5(n-3) PUFA exist. However, Johns and Perry (1977) found 17.8% of 20:5(n-3) in the gliding bacterium Flexibacter polymorphus which is very similar to the proportion found in M. methanica in this study. Johns and Perry imply that the presence of 20:5(n-3) precursors, namely 18:3(n-3), 18:4(n-3) and 20:4(n-3), suggest that 20:5(n-3) is biosynthesised within Flexibacter via oxidative desaturation. Some of these fatty acids are present in small quantities in the PUFA- containing symbionts in this study, although no 20:4(n-3) was detected. It is possible that 20:5(n-3) can be biosynthesised within SK2B, SK3A and M. methanica, or it is possible that these organisms can take up and concentrate 20:5(n-3) from the ambient sediment.

DeLong and Yayanos (1986) analysed 11 strains of deep-sea bacterial isolates and found that, while two strains contained no long chain PUFA, three strains contained large proportions of 20:5 (12-37% of total) and the remaining seven strains contained 22:6 as a major constituent (10-25%). Johns and Perry (1977) suggest that 20:5(n-3) confers gliding ability within Flexibacter while de Long and Yayanos (1986) suggest an involvement of long chain PUFA in maintenance of membrane fluidity in Vibrio marinus at environmental extremes of temperature and especially pressure. It is clear that the biosynthesis and metabolic uses of long-chain PUFA within marine bacteria require further elucidation, not least for the potential nutritional value of bacteria in marine animals. Irrespective, a major conclusion from this chapter is that none of the symbionts analysed are capable of providing essential fatty acid for their animal hosts.

3.4.4 Short Note About Lipid Class Analysis.

The lipid classes present within the symbionts are common and would generally be expected within bacteria. It is unlikely, therefore, that the classes would be any use as biomarkers as they are not sufficiently specific. However the classes do have potential use in radio-incorporation experiments to characterise the host-symbiont relationship, and this is explored in chapter 6.

3.4.5. Conclusions.

1) The best potential symbiont biomarkers are a combination of 16:0, 16:1(n-7) and 18:1(n-7).

2) When considering symbiont biomarkers within animals, the symbiont energy source must be considered. If sulphur-based, then 18:1(n-7) may be the main biomarker, while a methane-based system may tend to yield 16:1(n-7).

3) Cyclopropanes and branched chain fatty acids have probably only limited potential as symbiont biomarkers.

4) The environmental regime can readily affect bacterial fatty acid profiles.

5) The presence of PUFAs in some of the sediment bacteria and their absence from symbiotic bacteria is of considerable interest.

4.

Fatty Acids in a Variety of Marine Invertebrates Containing Symbiotic Bacteria.

4.1 Introduction.

4.1.1 General Introduction to Lipids in the Marine Environment.

In the marine environment, dietary input into all eukaryotes is generally considered to be ultimately dependent on phytoplanktonic production. Phytoplankton have been described as the equivalent to grass in the sea and are characterised in lipid terms by containing high levels of polyunsaturated fatty acids (PUFAs), particularly of the (n-3) series. Marine plants, as well as terrestrial plants, are able to synthesise PUFA of both the (n-3) and (n-6) series de novo. Animals can only synthesise (n-9) series de novo and do not possess the necessary desaturases for conversion of (n-9) monounsaturates to PUFA of the (n-6) and (n-3) series, even though animals require these fatty acids for various essential functions. For a marine animal to obtain its polyunsaturated essential fatty acids (EFAs) it is therefore necessary to obtain them ready made, usually by ingesting quantities of phytoplankton or by preying upon another animal rich in PUFA that has itself obtained its EFA from its diet and ultimately from phytoplankton.

4.1.2 Algal Lipids.

Phytoplankton are the primary producers in the marine environment, possessing the ability to fix carbon dioxide using light energy and are an important food source for marine invertebrates. In terms of lipid composition, algae are important since they possess the

ability to synthesise PUFAs de novo, many of these PUFAs being essential fatty acids for marine animals.

Different algal types tend to have characteristic fatty acid compositions as can be noted in table 4.1. It should, however, be noted that fatty acid compositions vary substantially between different algal classes, although most algal types are rich in PUFA, such that a range of C16, C18, C20 and C22 PUFA is present in phytoplankton. In addition to using fatty acid compositions for the purpose of algal taxonomy, algal fatty acids have been used successfully in trophic studies in Norwegian fjords (Sargent et al., 1985; Sargent and Falk-Petersen, 1981; Falk-Petersen et al., 1981) and to assess the nutritional value of phytoplankton at various stages of the "spring bloom" (Smith and Morris, 1980; Li and Platt, 1982; Sargent et al., 1985).

4.1.3 Lipids in Marine Invertebrates.

Invertebrates are an essential component of the marine environment since they link primary production with higher trophic levels and ultimately man (Ackman, 1988). The importance of marine invertebrates as a nutritional " stepping stone " has led to a large amount of data concerning their lipid profiles being published. Table 4.2 represents fatty acid compositional data from the total lipid of a variety of marine invertebrates selected from several studies. It can be seen that, although the compositional data are different for each species in percentage terms, there are a few fatty acids constituting major elements common to all species, namely 16:0, 20:5 and 22:6(n-3).

Table 4.1 The Percentage Composition of Total Lipiid of Fatty Acids Extracted from Representatives of Various Marine Algal Classes, adapted from Sargent et al., 1987.

Fatty Acid	(a) Nostoc muscorum	(b) Chroomonas species	(c) Emiliania huxleyi	(d) Gonyaulax catenella	(e) Dunaliella tertiolecta	(f) Biddulphia sinensis
14:0	3	2	34.8	13	1.3	11.3
16:0	27	19	5.4	30	13.4	13.4
16:1(n-7)	20	2	0.3	3	1.5	37.7
16:2	-	-	0.3	-	1.2	3.1
16:3	-	-	0.1	-	6.1	1.8
16:4	-	-	-	-	24.4	2.5
18:0	3	1	0.5	4	1	1.1
18:1	16	5	14.5	7	3.4	0.9
18:2	14	3	2.3	3	5.6	0.4
18:3(n-6)	11	1	-	-	-	-
18:3(n-3)	-	23	7.2	6	28.2	-
18:4(n-3)	-	23	7.8	14	0.6	0.4
18:5(n-3)	-	-	10.1	-	-	-
20:4(n-6)	-	-	-	-	-	-
20:5(n-3)	-	14	-	1	-	24.2
22:4(n-6)	-	1	-	-	-	-
22:5(n-3)	-	-	0.6	-	-	0.9
22:6(n-3)	-	6	11.4	12	-	-

where : (a) class Cyanophyceae ; ref., Parker et al., (1967).
(b) class Cryptophyceae ; ref., Beach et al., (1970).
(c) class Haptophyceae ; ref., Volkman et al., (1981).
(d) class Dinophyceae ; ref., Harrington et al., (1970).
(e) class Chlorophyceae ; ref., Ackman et al., (1968).
(f) class Bacillariophyceae ; ref., Volkman et al., (1980).

Table 4.2 The Percentage Compositions of Fatty Acid of Total Lipid from a Variety of Symbiont-Free Invertebrates Examined by Various Authors.

Fatty acid	a	b	c	d	e	f
14:0	3.5	2.2	3.2	2.6	2.3	3.9
14:1		0.9				
16:0	28.9	12.5	15.9	14.3	8.3	6.9
16:1	4.2	4.5	7.5	5.5	3.1	2.7
18:0	3.6	3	3.7	5.6	2.9	3.3
18:1	8.2	6.2	11.1	6.1	5	7.7
18:2	2	1.9	1	3.2	2.8	0.4
18:3	3.3	1.4	1.1	2.1	0.7	0.6
18:4	2.6	1.8	1.9	2.8	2.7	0.8
20:0		0.8			0.1	
20:1	5	1.1	7.5	4.7	4	19.3
20:2 NMID			4			5.7
20:2			0.3	1.8	.4	
20:3		0.8	0.2	.9	1.3	0.3
20:4	2.3	5.6	3.8	3.8	7	4.7
20:5	11.2	11.2	10.2	15.8	11.4	18.8
22:1	0.3		1.4			1.1
22:2 NMID			2.9			1.6
22:4		3.8	0.3	3.7		0.1
22:5(n-6)		0.5		1.4	3.3	
22:5(n-3)		3.9	1.2	2.5	6.9	1.1
22:6(n-3)	9.7	13.4	13.7	18.1	11.5	10.9

Where : a = Crassostrea virginica (Watanabe and Ackman, 1974)
b = Mya arenaria (Bonnet et al., 1974)
c = Mytilus edulis (Paradis and Ackman, 1977)
d = Aquepectin irradians (Krzeczkowski et al., 1972)
e = Spisula solidissima (Bonnet et al., 1974)
f = Asterina vulgaris (Paradis and Ackman, 1977)
NMID = non-methylene interrupted dienoic fatty acid.

A major problem with much of the data already existing in the literature is that the specific isomeric position of the double bond is not generally specified. For instance 18:1 could represent several different moieties, although it is generally accepted that the (n-9) isomer is more abundant than the (n-7) form. The data available for fatty acid analyses of marine invertebrates is immense and the reader is referred to the excellent review by Ackman (1988) for further information.

4.1.4 Lipids in Symbiont-Containing Organisms.

Few studies have been carried out on the fatty acid compositions of symbiont-containing marine organisms. Gillan et al. (1988) studied five species of sponges and showed that the three containing phototrophic cyanobacterial symbionts contained much larger amounts of 16:0, 16:1(n-7), 18:0 and 18:1(n-7) than did the other two. These authors also provided estimates of symbiont abundance from the data. Taghon (1988) studied the phospholipid fatty acid composition of the deep sea hydrothermal vent polychaete, Paralvinella palmiformis, and found a significantly greater degree of fatty acid unsaturation in animals collected from 17°C water than those taken from 30-40°C. At both temperatures, the major fatty acids were 16:0 (22-27%) and 18:2 (20-23%).

4.1.5 Aims of Chapter 4.

The purpose of this section is to study the application of fatty acids as biomarkers in marine symbiotic systems. A wide range of geographical locations and invertebrate species are analysed in an attempt to identify the putative symbiotic bacterial biomarkers encountered in the previous chapter in the tissues of the host animals. The environments range from a hydrothermal vent situation to a Canadian Bay, a Norwegian fjord and various sites in the U.K. The results are discussed in relation to ecological aspects of each site.

4.2 Materials and Methods.

All methods for fatty acid analyses are as described in Chapter 2.

4.2.1 Collection of Animals and Sediment.

The deep-sea hydrothermal vent animals were collected by Prof. A.J. Southward using a submersible vehicle at a depth of approximately 1500m on the Axial Seamount in international waters west of Vancouver Island. Ridgea piscesae were taken from a vent site, while unidentified small animals were collected from a chimney site. All specimens were collected during 1986, with the exception of specimen P which was collected during 1987.

Lucinoma annulata and Parvilucina tenuisculpta were collected below the water mark from Crofton Bay, Vancouver Island, by Prof. A.J. Southward during 1987.

Lucinoma borealis was collected at two sites in the U.K. The first site was at Mill Bay, Salcombe, Devon, and its animals were collected in 1986 by Dr P.R. Dando by digging below the low water mark. Specimens of Thyasira flexuosa were also collected at this site. The second site was in upper Loch Etive and both L. borealis and Myrtea spinifera were collected here during 1989 by Prof. A.J. Southward and Dr E.C. Southward using a van Veen grab.

The remaining animal specimens and sediment samples were all collected in Norway by Dr P.R. Dando during 1988. There were two

sites involved, both lying on the same fjord. The first site, Gasevik, lies at the mouth of the fjord and reaches a depth of 30m while the other, Alsback, lies at the head of the fjord, up-fjord from a sill, and reaches of up to 150m depth. The animals collected from these sites were Thyasira equalis, T. sarsi, T. flexuosa and Myrtea spinifera.

Prior to lipid extraction, all of the above samples were stored at -20°C in glass vials containing a known volume of chloroform : methanol (2:1, v:v) and sealed under oxygen-free nitrogen. The samples were generally analysed within 1-3 months of collection and in no case longer than 12 months after collection.

Sediment samples were collected for fatty acid analysis from the Alsback and Gasevik sites by Dr P.R. Dando during October 1988. 1ml of sediment was taken from various depths of a core collected using a Van Veen grab. The sediment was then added to a glass vial with 4ml of chloroform : methanol (2:1, v:v) before being sealed under argon. The vial was then shaken for 18 hours, centrifuged and an aliquot transferred to a 4ml septum vial which was stored at -20°C until required.

4.3 Results.

4.3.1 Fatty Acid Analyses of Deep Sea Hydrothermal Vent Animals.

Presented in Table 4.3 are fatty acid analyses of Ridgea piscesae, a vestimentiferan tube worm, and unidentified "small animals" all of the same species. Specimens P, T and W represent the aggregated symbiont-containing trophosome tissue taken from an unspecified number of animals. Additionally, specimen P was collected at a different site from T and W and, after examination under a phase contrast microscope, was observed to contain few bacteria. The "small animals" were all of the same unidentified species and were collected from the same "chimney site", and represent the aggregated tissues from an unspecified number of specimens, samples 2 and 4 being symbiont-containing trophosome tissue while sample 6 was symbiont-free vestimentum tissue from the anterior end of the animals.

Animals T and W both contained large amounts of 18:1(n-7) (17%), 16:0 (11-13%) and 16:1(n-7) (9-24%). T contained a high percentage of 20:5(n-3) (14%) which only accounted for 4% in W. Specimens T and W also contained non-methylene interrupted dienes (NMIDs) (5-9%). Specimen P was considerably depleted in 18:1(n-7), 20:5(n-3) and NMIDs but was compensated for by having increased percentages of 17:1, 18:0 and 18:1(n-9). All species contained relatively high percentages of PUFA of both the (n-3) and (n-6) series.

The "small animals" contained mainly 16:0 (up to 23%), 16:1(n-7) (diminished in the vestimentum), 18:1(n-9) (10-15%), and 20:4(n-6) (greatest in the vestimentum). In comparison to Ridgea, 18:1(n-7) and 20:5(n-3) were greatly decreased in the "small animals". Only the vestimentum contained a reasonable proportion of NMIDs but these were less than 4% of the total.

4.3.2 Fatty acid analyses of two symbiont-containing, coastal-living bivalves.

Contained within Table 4.4 are fatty acid analyses of the shallow water, symbiont-containing bivalve molluscs, Lucinoma annulata and Parvilucina tenuisculpta, collected from Crofton Bay, near Vancouver Island. The material analysed consisted of prokaryote-containing gill and symbiont-free mantle and foot tissues taken from individual animals. The major fatty acid constituents of both species were 16:0 (11-13%), 16:1(n-7) (9-26%), 18:0 (5-6%), 18:1 moieties (9-18%) and 22:2 NMID (5-12%). The proportions of 16:1(n-7) and 18:1(n-7) were greater in the gill than in the mantle/foot, this being especially the case with Parvilucina whose gills, in percentage terms, contained proportionately three times more 16:1(n-7) and six times more 18:1(n-7) than in the foot/mantle tissues. Levels of PUFA were generally depleted, only 1-2% of 20:5(n-3) and up to 4% 20:4(n-6).

4.3.3 Fatty acid analyses of three Lucinacean species from Mill Bay Salcombe, Devon and Loch Etive, Argyll.

Represented in Tables 4.5 and 4.6 are the results of fatty acid analyses of total lipid extracted from individual specimens of Lucinoma borealis and Thyasira flexuosa collected from Mill Bay, Salcombe, and Loch Etive respectively.

The main constituents of Lucinoma from Mill Bay (Table 4.5) are 16:0, 16:1(n-7) (2 times more in percentage terms in the gills than in the mantle/foot), 18:1(n-7) (2.5 times more in in percentage terms in the gill than in mantle/foot), 17:1 (greater percentage in foot/mantle) and 22:2 NMID (18% in both tissues). In addition, both tissues but especially the mantle/foot contained considerable proportions of long chain PUFA, up to 8% of 20:4(n-6), and up to 3.5% each of 20:5(n-3) and 22:6(n-3).

Thyasira from Mill Bay (Table 4.5) contained, in percentage terms, proportionately 3.5 times more 16:1(n-7) (37%) and twice as much 18:1(n-7) (19%) in the gills than in the mantle/foot. The other major components were 16:0 (up to 8%), 18:5(n-3) (up to 11% in the mantle/foot) and 20:1(n-13) (up to 10% in the mantle/foot). Long chain PUFA, specifically 20:4(n-6), 20:5(n-3) and 22:6(n-3), each constituted up to 5% of the total fatty acids in the foot/mantle tissue. It is interesting to note that T. flexuosa contained no NMIDs.

The Loch Etive Lucinoma represented in Table 4.6 have similar gill compositions to those collected in Salcombe Bay (Table 4.5),

although the rest of the tissues are quite different. The main gill fatty acids are 16:0 (11%), 16:1(n-7) (18-23%), 18:1(n-7) (14%), and 22:2 NMID (13-16%). The rest of the tissues have a greatly increased proportion of 16:1(n-7) comprising 40% of the total, this being the major component by far. 18:1(n-7) and 22:2 NMID are consequently greatly decreased at 8% and 5% of the total respectively. However, 16:0 and 20:1(n-7) are nearly doubled with respect to the gill.

Analysis of Myrtea spinifera (Table 4.6) showed very little variation in composition between the gills and the rest of the tissues. The main fatty acids were 16:0 (6-10%), 16:1(n-7) (16-18%), 18:0 (8-12%), 18:1(n-7) (15- 18%), 20:4(n-6) (5-6%), and 22:2 NMID (12-13%).

4.3.4 Fatty acid analyses of sediment cores and symbiont-containing animals from two sites in a Norwegian fjord.

The results of the above analyses are represented in Tables 4.7 to 4.12. Large amounts (up to 80% of the total fatty acids) of phthalate esters from plasticisers were observed in the FAME prepared from the sediment total lipid and less so in the animals from Gasevik but not from Alsback. The Gasevik sample therefore required recalculation after analysis to omit the bias created by the phthalates.

Tables 4.7 and 4.8 represent the analyses of Alsback sediment and animals respectively. The sediment composition (Table 4.7) was variable with depth. The main fatty acids were 16:0 (16-20%),

16:1(n-7) (10-18%), 18:0 (6-8%), 18:1(n-9) (9-14%), 18:1(n-7) (3-6%), 20:5(n-3) (4-7%) and 22:6(n-3) (1-21%). The Thyasira equalis material (Table 4.8) represented the aggregated tissues from 12 specimens collected at Alsback and fatty acid analyses focussed on three tissue types : gill, mantle/foot and gonad/digestive gland. The tissues, however, were all similar in their composition, although the gill lipid did contain increased percentages of 16:1(n-7) and 18:1(n-7). Essentially, the main components were 16:0 (15-18%), 16:1(n-7) (15-22%), and 18:1(n-7) (2-7%). The following were present in the region of 2-5% of the total : 14:0, 18:0, 18:2(n-6), 18:5(n-3) and 20:5(n-3).

Tables 4.9-4.12 represent analyses from Gasevik. Sediment fatty acid composition (Table 4.9) was again variable according to depth, the main components being 16:0 (14-25%), 16:1(n-7) (10-20%), 18:0 (4-8%), 18:1(n-9) (11-14%), and 18:1(n-7) (3-6%). These observations were similar to those of the Alsback sediment with the notable exception of a marked depletion in 22:6(n-3) at Gasevik.

The symbiont-containing animals analysed from Gasevik were Thyasira sarsi, T. Flexuosa and Myrtea spinifera, all of which varied quite markedly in their compositions. The T. sarsi and M. spinifera represent fatty acid analyses of lipid extracted from individual animals, whereas the analysis of T. flexuosa was obtained from lipid extracted from the aggregated tissues of 5 animals. The two T. sarsi analysed (Table 4.10) were similar in composition, the major components of the gill being 16:0 (16%), 16:1(n-7) (22-26%), 18:1(n-11) (9-11%), 18:1(n-7) (6%) and 18:3(n-3) (11-14%). The foot contained diminished proportions of these fatty acids, except for 18:1(n-11) whose percentage was nearly twice that of the gill. The

gonad/digestive gland contained slightly less 16:0 and 16:1(n-7) and significantly less 18:5(n-3) than was present in the gill. However the 18:1 moieties were greatly increased with respect to the gill. Long chain PUFA, although a minor component, were greatest in the foot/mantle and NMIDs were totally absent.

The main fatty acids of M. spinifera (Table 4.11) were 16:0 (11-12%), 16:1(n-7) (12-21%), 18:0 (5-8%), 18:1(n-9) (7%), 18:1(n-7) (4-8%) and 22:2 NMID (8-14%). When compared to Loch Etive Myrtea (Table 4.6), the percentage of 18:1(n-7) was only around one third of that observed previously. However, PUFA levels, especially 18:3(n-3), 18:5(n-3) and 20:5(n-3) were significantly increased at Gasevik when compared to the Loch Etive Myrtea.

Analyses of T. flexuosa in Table 4.12 reveals a similar composition to the other Gasevik Thyasira. Major constituents were 16:0 (12-16%), 16:1(n-7) (10-20%), 18:0 (6%), 18:1(n-11) (6-8%) and 18:1(n-7) (6-17%). The gill also contained up to 5% each of 18:5(n-3) and 20:5(n-3). It is interesting to note that the gonad/digestive gland contained nearly twice the proportion of 18:1(n-7) as the gill. The percentage of 18:1(n-7) in the gills was half that observed for Loch Etive T. flexuosa (Table 4.6).

4.3.5 Fatty acid analyses of symbiont-free bivalve molluscs from Mill Bay, Salcombe, Devon.

The results of the fatty acid analyses of one specimen of Mya truncata and two individual specimens of Cardium edule collected from Mill Bay are shown in Table 4.13. These symbiont-free animals were all rich in (n-3) PUFA (up to 28% in total), especially 20:5(n-3) (12-14% of the total) and 22:6(n-3) (7-9% of the total) and were depleted in monounsaturates when compared to the symbiont-containing organisms. Cardium edule also contained up to 8% 22:2 NMID and substantial proportions of odd (8-9% in total) and branched (12-13% in total) chain fatty acids.

Table 4.3 The Percentage Composition of Fatty Acids of Total Lipid Extracted from Symbiont-Containing Deep Sea Hydrothermal Vent Organisms (where tr.= trophosome and vtm. = vestimentum).

	(Ridgea piscesae	)	(unknown "small animals")		
Fatty Acid	T-tr.	W-tr.	P-tr.	2-tr.	4-tr.	6-vtm.
14:0	1.2	1.0	3.6	4.6	3.6	2.9
14:1	0.6	1.6	1.8	1.6	0.7	
i-15:0		0.1	1.3			
a-15:0	0.4	0.1	1.1		0.7	
15:0	0.7	0.4	2.5	2.0	2.2	1.4
15:1	0.8	0.2	1.2	2.4	1.3	
i-16:0	0.6	0.3	2.3	1.2	1.3	1.1
16:0	11.7	13.7	17.1	22.9	19.3	16.3
16:1(n-7)	9.0	24.4	13.1	9.4	13.7	6.3
16:2	0.4	0.2	0.4			
16:3	0.3	0.3	0.9	1.3	0.9	0.7
17:0		0.1	0.4			
17:1	0.7	2.8	7.2	6.5	3.7	5.2
i-18:0	3.1		0.4	0.4	0.5	2.3
18:0	2.6	2.3	4.4	6.1	4.0	4.8
18:1(n-9)	3.1	4.9	8.8	15.1	9.1	10.9
18:1(n-7)	16.9	17.8	5.0	3.9	8.5	5.3
18:2(n-6)	3.8	3.9	3.4	5.7	3.2	4.7
18:3(n-6)		0.1				
18:3(n-3)	2.0	1.1	0.4	1.1		1.9
18:4(n-3)	0.4		1.3	1.2	1.2	1.1
18:5(n-3)	3.6	3.3	0.5			
19:0			0.6		0.9	0.7
19:1	0.5	0.5				
20:0	0.9	0.7	1.0	1.0	0.9	3.2
20:1(n-11)	2.3	2.5	0.7	0.9	1.0	3.2
20:1(n-9)			0.4	1.4	0.4	
20:1(n-7)	1.6	1.8	1.1	2.8	0.6	1.5
20:2 NMID	6.2	2.8	0.6		0.5	2.0
20:2		0.3				
20:3		0.3				
20:4(n-6)	2.0	3.1	4.4	4.7	7.2	12.7
20:4(n-3)			0.6			
20:5(n-3)	14.4	4.4	1.6	2.2	5.8	5.7
i-22:0	1.9	0.3	1.3			
22:0	1.4		1.7			
22:1		0.9	0.7			
22:2 NMID	3.1	1.9	0.4			1.9
22:3	0.9	0.5	1.4			1.6
22:5(n-6)	1.1		1.9			
22:5(n-3)	0.7	0.3	0.4	1.4	3.8	1.1
22:6(n-3)	0.9	0.7	1.3		4.9	1.5
24:0/24:1	0.4	0.2	1.8			
18:1(n-7)/(n-9)	5.5	3.6	0.6	0.3	0.9	0.5
sum of odd chain	2.7	4.0	11.9	10.9	8.1	7.3
sum of branched	6.0	0.8	6.4	1.6	2.5	3.4
sum of (n-6) PUFA	6.9	7.1	9.7	10.4	10.4	17.4
sum of (n-3) PUFA	18.0	6.5	4.3	4.7	14.5	11.3
sum of NMIDs	9.3	4.7	1.0		0.5	3.6

Table 4.4 The Percentage Composition of Fatty Acids of Total Lipid Extracted from the Tissues of Two Coastal-Living Bivalves, Lucinoma annulata and Parvilucina tenuisculpta, Collected from Crofton Bay, Vancouver Island.

	Lucinoma		Parvilucina	
Fatty acid	gill	foot/ mantle	gill	foot/ mantle
14:0	2.5	2.0	1.3	2.6
14:1	0.5	0.4	0.3	1.2
15:0	1.5	1.6	1.0	1.9
a-15:0	1.0		0.5	0.7
15:1		0.9	0.5	1.2
i-16:0	1.7	1.2	1.0	1.7
16:0	11.8	11.0	13.8	12.2
16:1(n-7)	13.3	9.0	26.0	8.7
16:2				1.0
16:3	0.4	1.1	0.9	1.2
17:1	1.9	1.5	1.4	3.7
i-18:0	3.9	4.2	3.5	2.5
18:0	4.6	5.7	6.5	6.0
18:1(n-9)	4.5	5.6	3.6	8.5
18:1(n-7)	6.6	3.9	15.9	2.5
18:1(n-5)	0.8	0.7	0.5	1.0
18:2(n-6)	1.2	3.6	1.3	3.3
18:3(n-3)		0.6	0.8	0.5
18:4(n-3)				1.0
a-19:0		1.2		
19:1		0.5	0.3	1.8
i-20:0			0.6	0.5
20:0	1.6	1.5	0.8	2.8
20:1(n-11)	1.5	1.5	0.5	0.6
20:1(n-9)			0.4	
20:1(n-7)	2.0	1.6	3.0	2.0
20:2 NMID	0.9	0.3	0.4	0.6
20:2(n-6)		0.8		
20:3(n-6)				0.4
20:4(n-6)	3.0	4.7	1.0	4.2
20:4(n-3)			0.4	1.1
20:5(n-3)	1.1	2.7	0.7	2.6
21:5	0.9			
i-22:0	6.5	3.2	3.3	4.2
22:0	3.2	2.4	1.3	3.2
22:1	6.8			
22:2 NMID	5.3	12.7	4.7	5.0
22:3	2.0	2.4	0.6	3.2
22:5(n-6)	3.6	3.1	2.2	1.2
22:6(n-3)	1.9	3.4	0.6	3.4
24:1	2.4	5.0		0.8
18:1(n-7)/(n-9)	1.5	1.7	4.4	0.3
sum of odd chain	4.2	4.5	3.2	8.6
sum of branched chain	13.1	8.6	8.9	9.6
sum of (n-6) PUFA	7.9	12.4	4.5	9.1
sum of (n-3) PUFA	3.0	6.7	2.5	8.2
sum of NMIDs	6.2	13.0	5.1	5.6

Table 4.5 The Percentage Composition of Fatty Acids of Total Lipid Extracted from the Tissues of *Lucinoma borealis* and *Thyasira flexuosa*, Collected from Mill Bay, Salcombe, Devon.

	{ *Lucinoma*		}{ *Thyasira*	}
Fatty acid	gill	foot/ mantle	gill	foot/ mantle
14:0	0.2	0.4	0.4	0.8
i-15:0		0.2		0.2
15:0	0.2	0.4	0.3	0.2
15:1	0.6	1.3		0.7
i-16:0	0.6	1.1		1.4
16:0	7.0	4.7	8.9	8.2
16:1(n-7)	16.3	8.7	36.9	10.2
16:2	1.7	3.4	0.3	0.6
16:3	0.7	1.3	0.3	0.6
i-17:0	1.8	3.3		1.9
a-17:0	1.3	1.9		0.6
17:1	7.4	12.1	0.5	1.3
i-18:0	2.4			
18:0	5.0	5.3	3.2	5.0
18:1(n-11)			2.3	4.9
18:1(n-9)	0.9	1.5		
18:1(n-7)	13.7	5.3	19.5	8.1
18:2(n-6)	1.3	2.5	3.0	5.9
18:3(n-6)		1.0	3.2	.2
18:3(n-3)	0.6	0.8	0.6	1.9
18:4(n-3)	0.6	0.3		
18:5(n-3)			4.3	11.8
19:0		0.2		
19:1	0.7	0.3		
20:0			0.7	4.1
20:1(n-13)			6.0	10.2
20:1(n-11)	1.3	1.9		
20:1(n-9)	1.2	0.9		
20:1(n-7)	3.7	1.9	1.2	3.1
20:2 NMID	1.1	0.9		
20:2(n-6)	0.3	0.4	0.3	0.6
20:3(n-6)	0.4	0.6	0.5	1.3
20:4(n-6)	4.4	8.1	1.7	4.2
20:4(n-3)			0.5	0.8
20:5(n-3)	2.2	3.5	2.9	5.1
21:5	0.3	0.5		
22:2 NMID	18.5	17.7		
22:3	0.7	1.6		
22:5(n-6)	0.2	0.6		
22:5(n-3)	0.7	1.8	0.3	1.3
22:6(n-3)	1.8	3.4	2.0	4.9
18:1(n-7)/(n-9 or 11)	15.2	3.5	8.5	1.7
sum of odd chain	8.9	14.3	0.8	2.2
sum of branched chain	3.7	6.5		4.1
sum of (n-6) PUFA	6.8	13.4	8.7	12.2
sum of (n-3) PUFA	5.9	9.8	10.6	25.8
sum of NMIDs	19.6	18.6		

Table 4.6 The Percentage Composition of Fatty Acids of Total Lipid Extracted from the Tissues of Lucinoma borealis and Myrtea spinifera , Collected from Upper Loch Etive.

Fatty acid	Lucinoma gill	Lucinoma rest	Myrtea gill	Myrtea rest	Myrtea gill	Myrtea rest
14:0	1.2	0.3	0.7	0.2	0.7	0.7
14:1		0.3		0.2	3.2	
a-15:0	1.9	0.6	3.9	0.4	0.3	2.9
15:0	0.5	0.2	0.8	0.2	0.4	0.7
15:1		0.1		0.2		
i-16:0	0.7	0.2				0.6
16:0	11.5	14.0	11.0	19.9	5.6	9.8
16:1(n-7)	18.3	42.9	23.3	41.0	18.2	16.3
16:2		0.2		0.1	0.3	0.6
16:3	1.4	0.2	0.7	0.1	1.4	
i-17:0	0.9	0.3				
a-17:0	0.6	0.2				
17:0			1.3	0.1	0.1	1.2
17:1	4.6	1.2	2.3	0.7	3.3	4.6
18:0	7.1	4.2	5.4	4.0	8.2	11.7
18:1(n-9)	2.3	0.8	1.0	0.8	1.9	2.7
18:1(n-7)	14.2	9.3	14.1	8.1	18.3	15.6
18:2(n-6)	1.9	0.6	1.9	0.1	1.1	1.6
18:3(n-6)		0.2	0.8	0.1		0.3
18:3(n-3)	1.2	0.2	0.8	0.2		
18:4(n-3)					1.3	1.4
20:0		0.3		0.2	0.4	0.6
20:1(n-11)	1.3	2.7	1.0	4.1	0.6	1.4
20:1(n-9)			0.8		1.6	
20:1(n-7)	2.4	7.6	3.4	8.7	5.9	1.9
20:2 NMID	0.6	2.3	1.4	2.2	3.6	1.0
20:2(n-6)		0.6		0.4	1.4	0.7
20:3	0.6	0.4		0.3	0.7	0.5
20:4(n-6)	4.9	2.1	3.8	1.0	4.7	6.0
20:4(n-3)	0.6	0.2	0.7	0.3	0.6	0.7
20:5(n-3)	2.6	1.0	0.8	1.0	1.0	1.5
21:5		0.3		0.1	0.7	0.3
22:2 NMID	16.4	5.6	13.2	4.7	13.6	12.3
22:3	0.6	0.3	0.7	0.1	0.9	0.8
22:5(n-6)						0.3
22:5(n-3)	0.6	0.2	3.0	0.3		0.7
22:6(n-3)	1.0	0.2	3.1	0.1		0.8
18:1(n-7)/(n-9)	6.2	11.6	14.1	10.1	9.6	7.8
sum of odd chain	4.9	1.5	4.4	1.2	3.9	6.5
sum of branched	4.0	1.3	3.9	0.4	0.3	3.5
sum of (n-6) PUFA	6.8	3.5	6.5	1.6	7.2	8.8
sum of (n-3) PUFA	6.1	1.8	8.3	1.9	2.9	5.1
sum of NMIDs	17.0	7.9	14.6	6.9	17.2	13.3

Table 4.7 The Percentage Composition of Fatty Acids of Total Lipid Extracted from a Sediment Core from Alsback.

Fatty acid	0-2cm	4-6cm	8-10cm	12-14cm	16-18cm	20-22cm
14:0	4.9	3.9	4.4	3.9	3.5	4.0
a-15:0	2.2	2.7	2.5	2.7	3.9	3.5
15:0	2.7	1.8	2.6	1.7	2.0	2.4
i-16:0	0.5	0.4	0.6	0.7		0.6
16:0	20.2	16.0	19.3	17.0	18.7	20.0
16:1(n-7)	18.5	16.8	13.7	10.4	13.5	12.5
16:2	1.4		1.4	1.1	1.1	1.6
16:3	1.9	1.2	1.8	1.1	1.4	1.6
a-17:0		0.9	0.8	0 9	1.1	1.1
17:0	1.2	1.0	1.3	1.1	1.6	1.6
18:0	6.7	6.4	7.1	6.0	8.2	8.3
18:1(n-9)	14.5	11.3	11.5	9.0	12.8	11.4
18:1(n-7)	3.3	6.0	3.0	3.4	5.2	2.5
18:2(n-6)	4.1	2.7	5.1	2.5	4.1	4.0
18:3(n-6)				1.6		1.9
18:3(n-3)	1.1	1.0	0.8	1.0	1.6	0.6
18:4(n-3)		0.5				
20:0	0.9	1.1	1.2	1.4	2.8	1.9
20:1(n-11)	0.8	1.2	0.8	1.1	1.4	0.6
20:1(n-9)	0.3	0.8				0.1
20:1(n-7)	0.4	1.6		0.7	1.4	1.0
20:2(n-6)					1.1	
20:4(n-6)	0.7	1.2	0.7	0.4	0.9	0.5
20:5(n-3)	3.8	5.1	3.9	5.3	5.3	7.0
22:1(n-11)			0.7	0.9	0.9	0.7
22:3	1.4	1.3	1.4	2.0	3.7	2.8
22:5(n-3)					1.4	
22:6(n-3)	6.3	12.6	12.5	21.1	1.6	0.8
24:0	2.1	2.6	3.2	3.1		4.6
25:0					0.8	2.2
18:1(n-7)/(n-9)	0.2	0.5	0.3	0.4	0.4	0.2
sum of odd chain	3.9	2.8	3.9	2.8	3.6	4.0
sum of branched	2.7	4.0	3.7	4.3	5.0	5.2
sum of (n-6) PUFA	4.8	3.9	5.8	4.5	6.1	6.4
sum of (n-3) PUFA	10.2	19.2	17.2	27.4	9.9	12.6

Table 4.8 The Percentage Composition of Fatty Acids of Total Lipid Extracted from the Tissues of Thyasira equalis , Collected at Alaback.

Fatty acid	gill tissue	mantle/ foot	digestive gland/ gonad
14:0	3.5	4.8	4.2
14:1	1.6	1.8	1.5
i-15:0			1.2
a-15:0	1.0	0.9	1.0
15:0	2.4	2.8	2.5
15:1	1.5	1.9	1.7
i-16:0	0.8	0.9	1.5
16:0	17.7	17.4	14.8
16:1(n-7)	21.8	15.4	16.8
16:2	1.0	0.8	1.2
16:3	1.4	1.8	1.6
i-17:0			2.5
a-17:0		1.2	2.7
17:0	1.0	1.1	1.1
17:1		0.7	
i-18:0			0.8
18:0	4.4	5.1	4.1
18:1(n-9)	10.0	11.7	10.0
18:1(n-7)	6.7	2.3	3.6
18:2(n-6)	4.3	4.8	4.3
18:3(n-6)	1.3		
18:3(n-3)	0.7	1.0	1.0
18:4(n-3)	1.8	1.3	0.9
18:5(n-3)	3.2	4.6	3.5
19:0			0.5
19:1	0.6	0.8	1.0
20:0	0.7	0.8	
20:1(n-11)	1.6	2.5	2.2
20:1(n-9)	0.4		0.5
20:1(n-7)	0.9	1.0	2.1
20:2(n-6)		0.6	0.5
20:3(n-9)	0.6	1.2	0.9
20:3(n-3)		1.3	0.4
20:4(n-6)	1.2	1.2	1.2
20:4(n-3)		0.4	
20:5(n-3)	2.7	3.1	3.8
i-22:0	0.5	0.7	0.5
22:1(n-11)		0.3	0.3
22:3	0.8		0.9
22:5(n-6)	0.7	1.1	
22:5(n-3)	0.5	0.7	0.6
22:6(n-3)	1.0	1.0	1.0
24:0	1.4	0.7	1.2
18:1(n-7)/(n-9)	0.7	0.2	0.4
sum of odd chain	5.5	7.3	6.8
sum of branched	2.5	3.7	10.3
sum of (n-6) PUFA	7.5	7.7	6.0
sum of (n-3) PUFA	9.9	13.4	11.7

Table 4.9 The Percentage Composition of Fatty Acids of Total Lipid Extracted from a Sediment Core from Ennevik.

Fatty acid	0-2cm	3-5cm	7-9cm	11-13cm	15-17cm	19-21cm	23-25cm	27-29cm
14:0	5.5	1.6	6.3	3.8	7.7	5.1	2.7	5.6
a-15:0	2.5	1.3	4.7	3.0	6.6	5.9	1.8	3.0
15:0	4.5	3.2	3.6	3.5	7.7	5.1	3.3	4.8
16:0	22.6	21.9	23.7	22.9	14.3	23.7	17.3	25.3
16:1(n-7)	19.2	20.7	19.2	20.9	11.4	17.0	10.0	18.9
16:2	0.5	1.4	1.1	1.2		0.8	0.9	0.7
16:3	2.5	2.3	1.6	2.4	0.9	1.8	1.2	2.5
16:4(n-3)	0.5	0.5			0.9			
17:0	1.0	1.6	1.4	1.4	0.5	0.8	1.2	1.4
18:0	6.6	6.7	7.4	7.1	3.9	7.7	6.8	7.6
18:1(n-9)	11.9	12.2	11.3	13.2	13.4	12.3	14.2	11.7
18:1(n-7)	6.1	6.2	5.8	5.8	2.8	3.1	6.7	5.1
18:2(n-6)	4.0	3.2	3.3	3.2	1.9	4.2	2.7	4.4
18:3(n-3)		1.2	0.9	0.6	0.9		0.6	
18:3(n-3)	0.5	0.5	0.5	0.6		0.8	3.3	0.5
19:0	2.2	0.5		0.8		0.8		0.2
20:0		1.2	1.4	1.4	0.5		2.5	
20:1(n-11)	0.5	1.4			0.9	0.8	0.4	1.7
20:1(n-9)	0.5					0.8		
20:1(n-7)	0.5	0.5					0.1	0.5
20:2(n-6)		1.2	0.9	1.2	0.5		0.2	
20:3	2.5	1.4	1.1		1.5	4.2	4.2	0.9
20:4(n-6)	0.2							0.2
20:5(n-3)	0.5	5.0	4.1	3.5	3.9		1.5	
21:5				0.6				
22:0	1.5				0.5		2.1	.9
22:2		2.3		1.2	2.9	0.8		
22:1(n-11)	1.0	0.5	0.5	0.6		2.6		1.7
22:3(n-3)	1.0			0.3	6.6	1.8	1.2	
22:4(n-6)	0.5				2.9			
22:5(n-6)	0.5	0.5	0.9	0.8			0.6	
22:5(n-3)	0.5	1.2	0.5	0.6	1.9		0.9	
22:6(n-3)					4.8		0.2	2.5
18:1(n-7)/(n-9)	0.5	0.5	0.5	0.4	0.2	0.3	0.5	0.4
sum of odd chain	7.7	5.3	5.0	5.7	8.2	6.7	4.5	6.6
sum of branched	2.5	1.3	4.7	3.0	6.6	5.9	1.8	3.0
sum of (n-6) PUFA	5.6	4.9	5.1	5.2	5.3	4.2	3.5	4.6
sum of (n-3) PUFA	3.0	8.4	6.0	5.5	14.2	2.6	7.8	3.0

Table 4.10 The Percentage Composition of Fatty Acids of Total Lipid Extracted from Various Tissues of Two Thyasira sarsi, Collected at Gasevik.

Fatty acid	animal 1			animal 2		
	gill	foot	gonad	gill	foot	gonad
14:0	2.2	2.0	6.7	3.2	2.0	2.3
14:1	0.6	1.2	1.1	0.6		0.5
a-15:0	0.6	1.1	0.3	0.4	0.6	0.6
15:0	1.0	1.5	0.7	1.6	1.5	1.3
15:1	0.4	0.8	0.2	0.5		0.4
i-16:0	0.6	0.3	0.4	0.4		0.3
16:0	16.1	11.0	15.2	16.7	7.0	13.7
16:1(n-7)	26.1	12.0	17.7	21.7	5.8	11.4
16:2	0.2	0.4	0.1			0.2
16:3	0.5	1.2	0.6	0.6	0.8	0.7
i-17:0	0.2	0.2				
a-17:0	0.1	0.3	0.3	0.2		0.2
17:0	0.8	0.7	0.4	0.4	0.6	0.1
17:1	0.3	0.4	0.2	0.8		1.2
i-18:0	1.9	0.1	0.1			0.1
18:0	1.9	3.0	3.5	3.1	3.2	3.2
18:1(n-11)	9.5	18.6	13.5	11.1	12.7	16.3
18:1(n-7)	6.2	5.3	12.5	6.1	5.5	8.6
18:1(n-5)	0.6	1.1	1.5	0.4	0.7	0.7
18:2(n-6)	2.2	3.4	2.1	2.6	3.5	3.2
18:3(n-6)		1.3				
18:3(n-3)	13.7	2.1	4.0	10.7	1.8	1.2
18:4(n-3)	0.5	0.3	0.4			
18:5(n-3)	2.5	7.2	2.6	5.8	7.5	8.5
19:0	0.4	0.2	0.3	0.2	0.6	0.1
19:1	0.7	1.1	0.7			5.5
20:0	0.5		0.6			
20:1(n-13)	2 8	5.3	4.5	2.5	6.5	4.7
20:1(n-7)	1.9	3.7	3.0	3.3	3.7	2.3
20:1(n-5)	0.4	0.6	0.3	0.3		2.4
20:2	0.1	0.1	0.1			
20:3	0.4	2.6	0.8	0.6	2.3	1.6
20:4(n-6)	0.1	0.3		0.4	2.5	
20:4(n-3)	1.0	1.4	1.9	2.0	1.0	0.6
20:5(n-3)	1.0	4.4	2.1	2.0	5.0	3.6
21:5	0.4	0.2	0.1			
22:0	0.4	0.1	0.1	0.5	2.4	0.7
22:1		0.3	0.1			1.8
22:3(n-3)					0.9	0.7
22:4(n-6)			0.6			
22:5(n-6)	0.5	0.2				
22:5(n-3)	0.1	2.3	0.3	0.3		0.3
22:6(n-3)	0.3	1.3	0.2	0.6	3.0	0.4
18:1(n-7)/(n-11)	0.7	0.3	0.9	0.5	0.4	0.5
sum of odd chain	3.6	4.7	2.5	3.5	2.7	8.6
sum of branched	3.4	2.0	1.1	1.0	0.6	1.2
sum of (n-6) PUFA	2.8	5.2	2.8	3.0	6.0	3.2
sum of (n-3) PUFA	18.6	18.7	11.1	21.4	17.3	14.6

Table 4.11 The Percentage Composition of Fatty Acids of Total Lipid Extracted from Various Tissues of Myrtea spinifera, collected at Gasevik.

Fatty acid	gill	mantle/ foot	digestive gland/ gonad
14:0	2.8	2.4	2.9
a-15:0	1.5	0.5	0.8
15:0	2.1	1.5	1.9
i-16:0	0.6		0.6
16:0	12.7	10.7	12.2
16:1(n-7)	20.9	12.1	14.0
16:2	0.2	0.4	0.6
16:3	1.6	1.9	0.6
a-17:0	1.1	1.6	1.2
17:0	1.6	1.9	1.9
17:1	3.5	5.6	4.0
18:0	4.9	6.6	8.0
18:1(n-9)	7.5	7.0	6.6
18:1(n-7)	6.5	4.0	8.0
18:2(n-6)	2.9	2.6	3.5
18:3(n-6)	0.5	0.5	0.4
18:3(n-3)	4.3	1.7	1.9
18:5(n-3)	1.3	1.4	0.4
19:0	0.5	0.4	0.4
20:0	1.4	1.4	0.9
20:1(n-11)	1.2	1.3	0.8
20:1(n-9)	0.5	0.4	0.4
20:1(n-7)	3.0	3.7	4.6
20:2	0.2		0.2
20:3	0.5	0.9	0.7
20:4(n-6)	1.6	4.8	1.8
20:4(n-3)	0.6	1.0	2.5
20:5(n-3)	3.0	5.5	7.9
22:2 NMID	9.2	14.0	7.6
22:3	0.4	1.5	0.6
22:5(n-3)	0.6	1.1	1.1
22:6(n-3)	0.7	1.6	1.1
18:1(n-7)/(n-9)	0.9	0.6	1.2
sum of odd chain	7.7	9.4	8.2
sum of branched	3.4	2.1	2.6
sum of (n-6) PUFA	5.0	7.9	5.7
sum of (n-3) PUFA	10.5	12.3	14.9
sum of NMIDs	9.2	14.0	7.6

Table 4.12 The Percentage Composition of Fatty Acids of Total Lipid Extracted from Various Tissues of Thyasira flexuosa, Collected at Gasevik.

Fatty acid	gill	foot/ mantle	digestive gland/ gonad
14:0	2.9	3.3	7.4
14:1	0.7	1.2	0.3
a-15:0	1.2	1.6	
15:0	2.1	2.3	1.4
15:1	1.1	0.6	
i-16:0	0.4	0.3	0.5
16:0	12.0	15.5	15.9
16:1(n-7)	20.3	10.6	18.6
16:2	0.7	0.6	0.3
16:3	1.0	1.2	0.3
a-17:0	0.2	0.1	
17:0	0.7	0.2	0.3
17:1		0.9	
i-18:0	0.2		0.1
18:0	5.2	6.5	5.8
18:1(n-11)	5.5	7.8	5.8
18:1(n-7)	9.8	5.9	17.2
18:1(n-5)		0.9	
18:2(n-6)	4.5	6.2	3.6
18:3(n-6)	3.1	2.1	3.7
18:3(n-3)	0.4	2.0	0.8
18:4(n-3)	0.6	0.2	
18:5(n-3)	5.2	1.8	1.4
19:0	1.1	0.2	
19:1	0.9	0.1	0.6
20:0	0.4	2.7	1.8
20:1(n-13)	2.4	4.4	1.3
20:1(n-7)	0.4	5.6	2.4
20:1(n-5)		0.7	
20:2(n-6)	3.1		
20:3	2.7	1.9	2.1
20:4(n-6)	0.2	2.3	1.0
20:4(n-3)	0.4	1.2	0.5
20:5(n-3)	4.8	2.7	2.3
21:5	0.2		
22:0	0.2	1.2	0.5
22:1	0.2	0.2	
22:3	0.7	0.6	0.3
22:4(n-6)	0.2	0.9	
22:5(n-6)	2.1	0.2	
22:5(n-3)	0.7	0.7	0.5
22:6(n-3)	0.9	2.7	2.9
18:1(n-7)/(n-11)	1.8	0.8	3.0
sum of odd chain	5.9	4.4	2.4
sum of branched	2.0	2.0	0.6
sum of (n-6) PUFA	13.2	11.7	8.3
sum of (n-3) PUFA	13.2	11.3	8.4

4.13 The Percentage Composition of Fatty Acids of Total Lipid Extracted from One Whole *Mya truncata* and Two Whole *Cardium edule*, Collected from Mill Bay, Salcombe, Devon.

Fatty acid	*Mya truncata*	*Cardium edule*	*Cardium edule*
14:0	1.3	1.3	1.4
i-15:0	0.2		0.9
a-15:0	0.1	0.7	0.8
15:0	0.6	0.7	0.8
15:1	0.6	1.4	1.5
i-16:0	0.3	0.9	0.9
16:0	20.6	10.6	10.5
16:1(n-7)	4.3	2.7	2.8
16:2		1.3	1.4
16:3	0.7	1.2	1.1
i-17:0	1.0	0.7	0.7
a-17:0	0.9	0.9	1.0
17:0	0.8		0.3
17:1	0.6	2.1	2.3
i-18:0	0.2	9.3	9.2
18:0	3.4	7.8	7.6
18:1(n-9)	2.6	1.5	1.4
18:1(n-7)	4.0	1.5	1.5
18:2(n-6)	0.4		0.4
18:3(n-6)	0.4		0.5
18:3(n-3)	0.5		0.4
18:4(n-3)	1.1	0.6	0.8
18:5(n-3)	3.2	1.3	0.9
19:0	0.3		0.8
20:0	0.1	0.4	0.9
20:1(n-13)	7.0	1.9	1.8
20:1(n-11)	2.8		
20:1(n-9)	7.8	0.9	1.8
20:1(n-7)	7.8	2.5	0.8
20:2(n-6)	1.5	0.8	0.9
20:4(n-6)	2.6	3.8	3.6
20:4(n-3)	0.4	0.6	0.7
20:5(n-3)	14.7	13.1	12.9
21:5	1.1	3.7	3.6
22:0	0.2	0.6	
22:1	0.2	0.6	0.7
22:2 NMID	1.5	8.7	8.4
22:3	1.3	2.9	2.3
22:5(n-6)	0.2	0.7	0.8
22:5(n-3)	0.8	2.4	1.9
22:6(n-3)	7.4	9.6	9.5
18:1(n-7)/(n-9)	1.5	1.0	1.1
sum of odd chain	4.0	7.9	9.2
sum of branched	2.7	12.5	13.5
sum of (n-6) PUFA	5.1	5.3	6.2
sum of (n-3) PUFA	28.1	27.4	28.1

4.4 Discussion.

4.4.1 Sources of Variation in the Data.

Measurements made in natural situations involve an inherent amount of natural variation which can be accounted for by expressing the data as a mean value plus or minus a standard deviation. Natural variation can be extreme in fatty acid analyses of organisms taken from their natural environment and, in assessing the final results, variation has to be considered from differences in individual organisms, differences in extraction technique, differences in the type of gas chromatograph and integrator used for analyses and, indeed, variation caused by the style of use by different operators. However, we can assume that the "human" element of error is minor as is the error in analytical procedures, so that most of the observed variation in fatty acid compositions are due to differences between individual animals. Variation between animals can be accounted for in three ways, either by environmental regime, i.e. temperature, pressure and light intensity, by the method of deriving its nutrition, i.e whether it feeds on sediment, algae or prokaryotes, by inherent genetic variation, or a combination all three. Irrespective, an animal's fatty acid profile will be influenced to at least some extent by the main source of its nutrition.

4.4.2 The Significance of NMIDs.

One surprising inclusion in the results of the fatty acid analyses of many of the organisms presented throughout this study is the large proportions of non-methylene interrupted dienoic fatty acids (NMIDs). These are formally polyunsaturated fatty acids, but while conventional PUFA have adjacent double bonds that are invariably separated by a single methylene group ($-CH_2-$), in NMIDs, the two double bonds are separated by more than one methylene group. NMIDs have been detected in a variety of non-symbiont containing molluscs (e.g. Ackman and Hooper, 1973 ; de Moreno et al., 1980 ; Ackman et al., 1974) and also in the symbiont-containing mollusc, Codakia orbicularis (Berg et al., 1985). Ackman and Hooper (1973) go as far as to suggest that NMIDs may be peculiar to molluscs. It would appear, therefore, that although NMIDs can be important constituents of symbiont-containing organisms, they need not be specific to animals containing symbionts. Furthermore, the fact that NMIDs were found in the hydrothermal vent worm, Riftia pisacaae (Table 4.3), proves that NMIDs are not specific to molluscs and also they are not specific to shallow waters. NMIDs were abundant in the symbiont-containing species Lucinoma but were totally absent from the symbiont-containing species from the same family, Thyasira, collected at the same site (Table 4.5). This fact was true for all analyses of Lucinoma and Thyasira presented within this chapter. It follows, therefore, that NMIDs are not present in all symbiont-containing species and are not specific to an individual site.

Figure 4.1 shows the proposed pathways for NMID biosynthesis. The major NMID detected throughout this study was 22:2 delta 7,15 ,

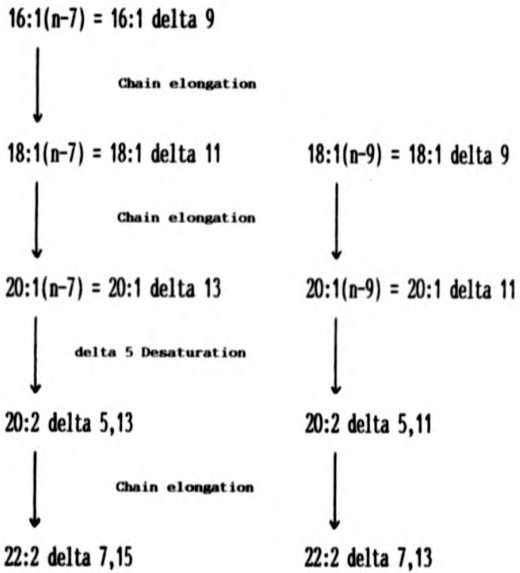


Figure 4.1 Proposed pathways for NMID biosynthesis in marine molluscs (adapted from Joseph, J.D., 1982).

entirely consistent with it being formed from a 16:1(n-7) precursor, which is elongated to 18:1(n-7) then to 20:1(n-7) before undergoing delta-5 desaturation to generate 20:2 delta 5,13. Chain elongation of the latter then yields 22:2 delta 7,15. It would seem evident therefore that, although the symbionts do not themselves produce NMIDs (see Chapter 3), they facilitate NMID production by providing the necessary precursors in the form of the potential symbiont biomarkers, 16:1(n-7) and 18:1(n-7). It is therefore evident that the NMIDs 22:2 delta 7,15 and less so 20:2 delta 5,13 , are important in assessing the assimilation of symbiont fatty acids by the host. Therefore, these fatty acids can be used in combination with 16:1(n-7) and 18:1(n-7) as biomarkers for the presence of symbionts.

The exact purpose of NMIDs is unclear. It could be that they are used as some kind of energy reserve or that they may act as replacement EFAs for long-chain PUFA, especially given a high input of 18:1(n-7) into animal tissues as is likely to occur in several of the animals studied here. Ackman (1988) suggests that specific NMIDs may have important structural, physiological or metabolic roles within molluscs but does not consider exactly what these roles might be. One possibility is that NMIDs are formed by animals in a response to a relative deficiency of dietary essential fatty acids due to the absence of PUFA in symbiotic bacterial lipids, coupled with an abundance of (n-7) monounsaturates.

4.4.3 The Hydrothermal Vent Ecosystem.

When considering deep sea ecosystems of the type encountered at the axial seamount site, it seems reasonable to assume that because of the increased pressure, reduced illumination and fluctuating temperature caused by intermittent vent discharges, an animal must not only make physical adaptations, such as its mode of nutrition, but will also compensate biochemically at a cellular level. It has been shown that animals from these hydrothermal vent sites possess specialised features such as a reduced digestive system as well as specialised haemoglobins and sulphide detoxification systems. Therefore it seems likely that their fatty acid profiles, and that of their prey, whether it be prokaryote or eukaryote should be adapted to compensate for their unusual life-style. Whether NMIDs feature in such adaptations is not known.

The above factors must therefore be kept in mind when interpreting the data for the axial seamount animals in Table 4.3. When considering Ridgea piscesae, animal P which was observed to contain few bacteria when examined microscopically resembles the unidentified "small animals" in its fatty acid composition more closely than it does the other Ridgea collected at the same site. The individual Ridgea animals, T and W, were shown by microscopy to contain large numbers of symbiotic bacteria. Therefore, it can be suggested that the large proportion of 18:1(n-7) in the Ridgea individuals T and W can be correlated with high numbers of symbionts.

The EFA 20:5(n-3) was also a major component of animal T and it is interesting to consider how the presence of this EFA might be related to symbiont activity. In such hydrothermal vent situations, many environmental parameters may change very rapidly in both a temporal and a spatial dimension (Johnson et al. 1988a & b) and so may account for the great variation in fatty acid composition. The high levels of PUFA, such as 20:5(n-3) in sample T, would immediately suggest some form of planktonic input if these were normal shallow water marine invertebrates. A deep sea holothurian living at 4400m has been shown to contain up to 23% of its total fatty acids as 20:5/22:6 (Lewis, 1967). However, it is contentious whether there is a sufficiently large or reliable planktiferous input to such a depth to maintain the PUFA content of the biota, even although it is generally accepted that loss of sedimentary organic matter from the photic zone is a limiting factor to food supply in the deep sea. It is possible that the hydrothermal vent animals either contain or are feeding upon bacteria containing PUFA similar to the deep-sea Vibrio species described by DeLong and Yayanos (1986) or to Flexibacter polymorphus described by Johns and Perry (1977).

It is presently unclear whether hydrothermal vent animals depend totally on their symbionts for nutrition or whether they are partly utilising free-living bacteria which have been shown to be abundant within the vent water (Jannasch and Wirsen, 1985; Grassle ,1986). Taghon (1988) carried out phospholipid fatty acid analyses of Paralvinella, a hydrothermal vent polychaete which does not harbour symbiotic bacteria but is instead thought to feed on free-living bacteria, and found large amounts of PUFA (up to 43% of the total fatty acids in its lipids). It is therefore a possibility that the

free-living bacteria on which these animals feed contain PUFA. It is feasible that the symbionts of Ridgea could contain PUFA. However, as of yet Ridgea symbionts have not been isolated. There is of course the possibility that the animals themselves have developed de novo PUFA synthesis. In short, the origin of PUFA, especially (n-3) PUFA in deep sea invertebrates remains a mystery. This is a critical issue in terms of the total independence of vertebrate communities from the phobic zone and will be returned to later in this thesis.

4.4.4 Fatty Acid Analyses of the Crofton Bay Bivalves.

When considering the results obtained from the two bivalves from Crofton Bay near Vancouver Island (Table 4.3), it seems that although both genera contain symbionts, Parvilucina contains a greater proportion of symbiont-type fatty acids than does Lucinoma. Since both species were collected at the same site, this suggests Parvilucina contains a greater proportion of symbionts than does Lucinoma. There seems to be no build up of long chain PUFA within Lucinoma so that it is probably not feeding in a heterotrophic state. The dependence of this species on symbiont production is further supported by the large proportion of NMIDs within the foot/mantle of Lucinoma. In both species the sum of the branched chain fatty acids totalled 8-13% suggesting the possibility that these species may also utilise sediment-living or free-living bacteria.

4.4.5 Comparison of Loch Etive and Mill Bay Lucinoma.

When considering the differences in the fatty acid compositions of animals collected from Mill Bay, Salcombe in Devon and Loch Etive in Argyll, West Scotland (Tables 4.5 and 4.6), the gill tissue that was examined from Lucinoma suggests there were few differences between the two sites, the Lucinoma from both sites being rich in 16:1(n-7), 18:1(n-7) and 22:2 NMID so suggesting healthy symbiont populations. However, the large increase in the proportions of 16:1(n-7) and the decrease in levels of 17:1 and 22:2 NMID in the foot/mantle tissue of the Loch Etive Lucinoma relative to the foot/mantle of the Mill Bay Lucinoma points towards differences in the utilisation and/or transfer of prokaryote-derived fatty acids within the animals.

If Lucinoma from either of the above sites were utilising planktonic or sediment-derived nutrition, we should see a build up of long chain PUFA which clearly is not observed.

4.4.6 Comparison of Mill Bay and Gasevik Thyasira flexuosa.

Thyasira flexuosa from both Mill Bay (Table 4.5) and the Norwegian fjord site Gasevik (Table 4.12) had different fatty acid compositions, both from each other and from the Lucinoma borealis discussed in the previous section. Perhaps the most interesting feature is that T. flexuosa from both sites contained no NMIDs whatsoever, and indeed this was the case with all Thyasirids studied at every location. Instead, the species contains moieties of 18:3(n-3) and 18:5(n-3), these fatty acids normally being associated with

plankton and so suggesting a planktonic input in the diet of T. flexuosa. The gills of the Mill Bay animals contained much larger proportions of 16:1(n-7) and 18:1(n-7) than the gills of the Gasevik animal suggesting an abundant prokaryote population in the Mill Bay animals in contrast to the Gasevik samples.

If the fatty acid composition of the gonad/digestive gland of the Gasevik T. flexuosa is considered, a high level of 16:1(n-7) and a greatly increased proportion of 18:1(n-7) were observed with respect to the gill tissue. This suggests that symbiont fatty acids are being amassed by either the reproductive or digestive tissue. It would seem that the animal is either investing a substantial part of the nutrients it derives from its symbionts into reproduction or that simply the large proportion of symbiont type fatty acids represents the processing of the symbiont fatty acids by the animal. The answer to this problem could be found by separating these tissues before analysis, but whatever the reason for this accumulation of fatty acids, it is clear that there is a transfer of bacterial symbiont production to the animal host.

4.4.7 Comparison of Loch Etive and Gasevik Myrtea.

From the fatty acid compositional data of the Loch Etive Myrtea spinifera (Table 4.6), it is evident from the large proportions of 18:1(n-7) and 16:1(n-7) that this animal appears to contain large numbers of symbionts. However, there are some important differences between this animal and the Gasevik Myrtea (Table 4.11). The Gasevik animal was depleted in the potential symbiont biomarkers, 16:1(n-7)

and 18:1(n-7), in comparison to the Loch Etive Myrtea, suggesting that the Gasevik animal contains a smaller population of symbionts. Both 18:3(n-3) and 18:5(n-3) were found in the Gasevik animal and not in the Loch Etive Myrtea, suggesting an extraneous input of algae to the Gasevik system. The above findings in combination with the greatly increased proportion of long chain PUFA in the non-gill tissue of the Gasevik animal provides evidence for Myrtea being able to switch from symbiont-based nutrition to mixotrophic or even heterotrophic nutrition when favourable condition prevail. The high levels of NMIDs in the Gasevik animal would seem to suggest that it has utilised its symbionts in the recent past.

It would seem that the presence of algae creates an unfavourable condition for the operation of the symbiont relationship in terms of increased competition for resources such as space and oxygen from heterotrophic species. Thus, the animal can either utilise the algal input, or if the symbiosis is so closely evolved that the animal is unable to feed mixotrophically, then it can either "switch off" its symbiotic relationship until conditions are favourable, otherwise it must invest its resources into gametes to enable the next generation to survive.

4.4.8 Fatty Acid Analyses of the Gasevik Thyasira sarsi and Sediment.

The Gasevik Thyasira sarsi (Table 4.10) probably rely on a combination of symbionts and phytoplankton for their nutrition. The large proportion of 18:3(n-3) present in the gills represents an input of algae, while the significant amount of 18:1(n-7) present in the

gonad/digestive gland indicates the animal's utilisation of bacterial fatty acids orginating in the gill tissue. There seems to be no direct throughput of 18:3(n-3) from the gills to the rest of the animal, although there is an increase of 18:5(n-3) in the mantle/foot. It would seem that these animals are in fact functioning mixotrophically.

Perusal of the fatty acid analyses of the Gasevik sediment reveals a composition reminiscent of the degrading remains of plant and animal detritus so that it seems unlikely that the Thyasira sarsi living within this sediment receive any substantial nutritional input from it.

The origin of the phthalate esters at the Gasevik site and their absence from Alsback deserves consideration. Phthalate esters are normally associated with plasticisers and their presence is consistent with the input of large quantities of industrial and domestic waste into the Gasevik system. Although these substance have no direct connection with the aims of this project, they may be of value as pollution indicators to other workers.

4.4.9 The Alsback Ecosystem.

From the analysis of the Alsback sediment (Table 4.7), it is evident from the large proportion of (n-3) PUFA observed that there is a substantial input of plankton-derived material. Unfortunately only one species, Thyasira equalis, was collected from this site and this species seemed to contain lower levels of symbionts than the animals from Gasevik. There could be two explanation for this. The first possibility is that T. equalis in Alsback is intrinsically less

dependent on its symbionts for its nutrition. However, the Alsback site suffers from population explosions and crashes due to severe eutrophication (P.R. Dando, personal communication). Alsback lies at the head of the same fjord in which Gasevik lies, with Alsback lying up-fjord from a sill and reaching depths of 150m. It is therefore poorly flushed and experiences eutrophication which causes periods of anoxia. At the time of sampling this site was approaching a state of total anoxia and was also greatly depleted in the iron sulphides necessary to fuel the symbiotic system. From the small amount of PUFA observed it is possible to deduce that T. equalis can utilise some planktonic matter. However, it is more likely, on balance, that these animals are much more dependent on their symbionts than on a heterotrophic mode of nutrition and due to the lack of iron sulphides in the sediment, they are in a starvation state.

4.4.10 Fatty Acid Analyses of Symbiont-Free Control Species.

The symbiont-free control species (Table 4.13) have fatty acid compositions very different from the symbiont-containing invertebrates analysed throughout this section. The main differences are that the symbiont-free animals contain low levels of the symbiont-biomarkers, 16:1(n-7) and 18:1(n-7) and are rich in (n-3) PUFA, especially 20:5(n-3) and 22:6(n-3). These fatty acid analyses are reminiscent of typical fatty acid compositions obtained from the symbiont-free invertebrates studied by other authors (Table 4.2). It is clearly evident that the Cardium and Mya in Table 4.13 undergo a "normal" plankton-based mode of nutrition and so act as an excellent reference point for comparison with the symbiont-containing species. It is interesting to note the

substantial proportions of branched and odd chain fatty acids present in Cardium (up to 13.5% and 9% in total respectively) and it may be speculated that this represents an input of non-symbiotic bacteria, either from the water column or the from the sediment. Finally, the presence of 22:2 NMID within the symbiont-free species provides further evidence that this fatty acid is not exclusive to symbiont-containing invertebrates.

4.4.11 A Note Regarding the Unsual Moities of 18- and 20- Carbon Monounsaturated Fatty Acids.

In several of the animals studied in this chapter two unusual monounsaturated fatty acids were identified with the aid of GC-MS (see Chapter 2 for mass spectra), namely 18:1(n-11) and 20:1(n-13). These fatty acids occurred in selected species, sometimes in substantial proportions (up to 18% ; see table 4.10) and seem to replace the more common isomers, 18:1(n-9) and 20:1(n-11). It seems that from all the symbiont-containing species studied so far, Thyasira sarsi and T. flexuosa are the only species to contain these fatty acids (see Tables 4.5, 4.10 and 4.12). These unusual isomers seem to occur together within the animal, that is, they are either both present or both absent.

It may be noted that both 18:1(n-11) and 20:1(n-13) are delta-7 isomers so that they could both, in principle, be accounted for by a delta-7 desaturase acting on 18:0 and 20:0 substrates. However, there is little or no evidence for the presence of such a desaturase.

Equally, they could be formed by a delta-9 desaturase acting on 20:0 and 22:0 to form 20:1 delta 9 and 22:1 delta 9 isomers respectively, followed by decarboxylation of the latter to yield 18:1 delta 7 and 22:1 delta 7 respectively. Whether or not such pathways exist in symbiont-containing animals remains to be investigated.

The collection site of the animal does not seem to have any bearing on the occurrence of 18:1(n-11) and 20:1(n-13). For example, in Table 4.5 Lucinoma borealis and Thyasira flexuosa were both taken from Mill Bay, Salcombe; Thyasira contained the unusual isomers while Lucinoma contained the more common isomers. Thyasira flexuosa collected at the Gasevik site also contained the unusual isomers, so suggesting that 18:1(n-11) and 20:1(n-13) seem to be specific to certain species, although their function remains a mystery. After investigating studies by various authors it is apparent that 18:1(n-11) and/ or 20:1(n-13) have been identified in several marine invertebrates and these include Rapana thomasiana and Mytilus galloprovincialis (Christie et al., 1988), Littorina littorea, Lunatia triseriata and Crangon septemspinosus (Ackman and Hooper, 1973), Penaeus merguiensis (Clarke and Wickins, 1980), Thyanoessa inermis and Meganyctiphanes norvegica (Ackman et al., 1970) and Strongylocentrotus droebachiensis (Takagi et al., 1980). It would seem that these two isomers may not be as unusual in the marine environment as initially suspected. Furthermore, they do not seem to be linked in any way to symbiotic activity.

4.4.12 Conclusions.

(a) the fatty acids 16:1(n-7) and 18:1(n-7) combined with low levels of long chain PUFA are useful biomarkers for determining the presence of symbiotic bacteria in the gills of marine invertebrates.

(b) high levels of 16:1(n-7) and 18:1(n-7) infer a large symbiont population.

(c) the aforementioned combination of fatty acids can also be used to infer carbon flow from the gills to the non-gill tissues.

(d) variation in fatty acid composition between symbiont-containing marine invertebrates can be large, both between species and within species.

(e) low levels of the proposed biomarkers together with elevated levels of PUFA suggest mixotrophic nutrition.

(f) elevated levels of the proposed symbiont biomarkers in the gonad/digestive gland tissue may suggest a large reproductive effort.

(g) the presence of large amounts of NMIDs may result from the processing of symbiont fatty acids by the host, so inferring symbiotic activity.

(h) certain fatty acids, particularly NMIDs, 18:3(n-3) and 18:5(n-3), found within symbiont-containing marine invertebrates are associated with a particular species or with a specific sampling site.

5.

Fatty Acids

in Marine Invertebrates

from Methane Seeps.

5.1 Introduction.

After consideration of fatty acid compositions of a variety of putative symbionts and symbiont-containing animals from a variety of locations, this section endeavours to use fatty acid analyses to make comparisons of three distinct methane seep ecosystems. The objective is to use fatty acid compositional data from a selection of symbiont-containing and symbiont-free animals, as well as sediment cores, to shed light on the major trophic processes occurring at each site. Although all three sites are associated with methane seepage, they are topographically very different.

5.1.1. The Skagerrak Methane Seep.

The Skagerrak site was the deepest of all the three sites and consisted of a soft muddy sediment through which biogenic methane was known to seep. Two symbiont-containing species were analysed from this site, namely Thyasira sarsi and Sibomlinum poseidoni. Both of these species were absent from the surrounding sediment. T. sarsi is not usually found in the open Skagerrak, the common species at 300m being T. equalis. T. obsoleta and T. ferruginea (Josefon, 1985). Furthermore T. sarsi occurs in the methane seep together with S. poseidoni which has only been identified within two very restricted areas of the Skagerrak (Flugel and Langhof, 1983). Schmaljohann et al. (1990) found that these pogonophores have a patchy occurrence within these two restricted areas of the Skagerrak with dense populations inhabiting small spots of reduced sediment not more than a few m^2 in area.

5.1.2 The Kattegat Methane Seep.

The Kattegat site was the shallowest of the three sites and consisted of a large area where methane seeped through the sea bed and could be seen on the water surface with the naked eye. This area was close to shore and had long been observed by local fishermen. A video taken by a remotely operated vehicle (ROV) showed large slabs of carbonate rock and pillars up to 4m protruding from an otherwise flat, sandy seabed. This carbonate rock seems to be associated with areas of seeping methane and it has been suggested that the rock has been formed by the utilisation of methane by methane-oxidising bacteria which then deposit carbonate to form the rock (P.R. Dando, personal communication). The rock was also observed to be covered with animal life, especially Metridium senile. No known symbiont-containing animals were obtained from this area, although a representative sample of the animal fauna was collected for comparative purposes.

5.1.3. The North Sea Pockmark.

Samples in the North Sea were taken at a pockmark with an active methane seep. It is believed that pockmark formation is the result of the displacement of fine sediment via a rapid expulsion of gas and liquid upwards through the seabed leaving the characteristic craters (Hovland and Judd, 1988). Underwater video film shows that the pockmark area has associated with it a richer fauna than that prevalent in the surrounding area. Only one known symbiont-containing animal was sampled from this site, namely T. sarsi, the rest of the samples being representative of the general fauna found within that area.

5.2 Materials and Methods.

All methods employed for routine lipid analyses are as described in chapter 2.

5.2.1. Collection of Samples.

The animal and sediment sediment samples from both the Kattegat and Skagerrak sites were all collected during October 1989 aboard the M.V. Alkor, with the exception of one Thyasira sarsi which was collected from the Skagerrak by Dr P.R. Dando during October 1987.

The samples obtained from the Kattegat were collected by divers at a depth of 10m in the waters off Laeso Island near Freidrikshavn, Northern Denmark (57°15'N; 10°50'E). Sediment samples were collected from the base of the carbonate columns.

The pogonophoran worm Siboglinum, Thyasira sarsi and the symbiont-free species were collected with a beam trawl and a van Veen grab in the Skagerrak (58°02.85'N; 9°40.04'E) at a depth of 300m. Sediment cores were obtained using a Van Veen grab.

The samples obtained around the North Sea pockmark (58°16.95'N, 00°59.20'E) were collected aboard the M.V. Resolution during 10th-13th July 1989 using a Jonasson-Olausson box corer and an Agassiz trawl at depths between 151 and 167m.

5.3 Results.

5.3.1 Fatty Acid Analysis of Skagerrak Sediment Core.

The total lipid obtained from each section of sediment core was extracted from 1cm³ of sediment. There seems to be no clear pattern of variation of fatty acid compositions within different sections of the Skagerrak sediment core (Table 5.1). The major constituent throughout was 16:0 (17-25% of total), followed by 18:1(n-9) (8-10%). Levels of 16:1(n-7) and 18:1(n-7) were generally low, between 3-6% and 3-5% of the total respectively. Branched and odd-chain fatty acids, although generally present in small amounts in an individual context, when grouped together constituted up to 19% of the total in some cases e.g. the 13-14cm section. A wide range of PUFAs were also detected and, although these were also generally present in small proportions, when the sum total was taken, they constituted a substantial proportion of the total fatty acids, in the region of 15-20% of the total.

5.3.2 Fatty Acid Analyses of Thyasira sarsi from the Skagerrak.

The fatty acid compositions of the total lipid from the Skagerrak Thyasira sarsi were remarkably similar for those animals collected during 1987 and 1989 (Tables 5.2 and 5.3) with the exception of those animals exhibiting very dark gills (Table 5.4). The fatty acid analysis shown for T. sarsi collected in 1987 (Table 5.2) was obtained from the total lipid extracted from a single individual, while the fatty acid compositions of those animals collected during 1989 (Table

5.3) represents the mean results obtained from analyses of 27 single specimens. The analyses for those animals exhibiting very dark gills were obtained from the aggregated tissues of ten individuals. Three distinct tissue groups were analysed, namely the symbiont-containing gill, the symbiont-free gonad/ digestive gland and the mantle/ foot.

The main fatty acids in the gills of the specimens in Tables 5.3 and 5.4 were as follows : 16:1(n-7) (24-35% of the total), 18:3(n-3) (12-16%), 18:1(n-11) (8-11%), 16:0 (10-11%), 18:1(n-7) (8-9%), 18:5(n-3) (4-6%), 20:1(n-11) (2-4%) and 20:1(n-7) (3-4%). Levels of 20:5(n-3) were generally low and ranged between trace amounts to 5% of the total. Proportions of branched chain fatty acids were negligible.

The fatty acids of the gonad/digestive gland were largely the same as those of the gill. However, they were present in different proportions, the main constituents being : 16:1(n-7) (12-15%), 18:1(n-11) (15%), 18:1(n-7) (11%), 16:0 (9%), 18:5(n-3) (7-9%), 18:3(n-3) (6-8%), 20:1(n-11) (5-6%) and 20:1(n-7) (4-6%). Again levels of long chain PUFA and branched chain fatty acids were low, as they also were within the mantle/foot.

The mantle/foot portion again contained different proportions of the same fatty acids found within the other tissue types. These were : 18:1(n-11) (20%), 16:1(n-7) (9-10%), 18:1(n-7) (7-8%), 16:0 (6%), 18:5(n-3) (9-15%), 20:1(n-7) (6-8%), 20:1(n-11) (7%) and 18:3(n-3) (3%).

The Thyasira sarsi exhibiting dark gills (Table 5.4) were very different in their gill fatty acid composition from the rest of the T.

sarsi. The main constituents of the gill lipids were 16:0 (19%), 16:1(n-7) (15%), 18:1(n-7) (10%) and 20:5(n-3) (12.5%). 18:3(n-3), 18:5(n-3), 20:1(n-11) and 20:1(n-7) were all very minor constituents. The other tissues were similar in composition and lay within the range of proportions encountered within the other T. sarsi specimens.

5.3.3 Fatty Acid Analysis of the Phospholipid Classes of the Gills of Thyasira sarsi.

Since the symbiotic bacteria analysed in chapter 3 were found to contain mainly phospholipid, it was decided to analyse the phospholipid classes of a single specimen of Thyasira sarsi (Table 5.5) to try to identify symbiont biomarkers within the gill. These results were obtained from the aggregated tissues of ten Thyasira sarsi collected in the Skagerrak during 1989. The main fatty acids within phosphatidylcholine (PC) were 16:0 (9%), 16:1(n-7) (22%), 18:1(n-11) (18.5%), 18:1(n-7) (13.4%) and 18:3(n-3) (6%). Within phosphatidylserine (PS) the main constituent fatty acids were 16:0 (17%), 18:0 (18%), 18:5(n-3) (9%) and 20:1(n-11) (18%). Phosphatidylinositol (PI) contained 16:0 (8%), 16:1(n-7) (7%), 18:1(n-11) (11%), 18:1(n-7) (7%), 20:1(n-9) (25%) and 20:5(n-3) (9%) as its main constituents. The major fatty acids within phosphatidylglycerol/cardiolipin (PG/CL) were 16:0 (22%), 16:1(n-7) (52%) and 18:3(n-3) (11%). The largest lipid class was phosphatidylethanolamine (PE) and its main constituent fatty acids were 16:0 (12%), 16:1(n-7) (35%), 18:1(n-11) (13%), 18:3(n-3) (9%) and 18:5(n-3) (11%). The unknown class mainly consisted of 16:0 (7%), 16:1(n-7) (7%), 18:1(n-7) (9%), 18:5(n-3) (10%), 20:0 (10%) and 20:1(n-11) (21.5%).

5.3.4 Fatty Acid Analyses of the Total Lipid of the Pogonophore Worm, Siboglinum posedoni, Collected from the Skagerrak Methane Seep.

The analyses presented in Table 5.6 show both the symbiont-containing rear portion of Siboglinum posedoni and the symbiont-free anterior section to enable comparison. These results were obtained from analysis of the total lipid extracted from the aggregated tissues of one hundred S. posedoni. These animals contained relatively few fatty acids when compared to Thyasira sarsi. There seems to be little difference in the fatty acid composition of each section of Siboglinum, although the anterior portion seems to contain a slightly greater proportion of 18:1(n-7). The main fatty acids in all sections were 16:0 (15-26% of total), 16:1(n-7) (34-45%), 18:1(n-9) (6-11%), 18:1(n-7) (10-13%) and 18:2(n-6) (3-6%). It is interesting to note the presence of three isomers of 18:1, namely (n-13), (n-9) and (n-7). Levels of branched chain fatty acids were negligible. The proportion of PUFAs was limited with 18:2(n-6) and 20:4(n-6) being the major constituents. 18:5(n-3) and 20:5(n-3) were detected in trace amounts only.

5.3.5 Fatty Acid Analyses of Symbiont-Free Skagerrak Animals.

The symbiont-free animals had fatty acid compositions different from each other (Table 5.7) and also from those of T. sarsi (with the possible exception of the terebellid polychaete). The fatty acid compositions of the symbiont-free animals were all obtained from

analysis of the total lipid extracted from individual specimens. Both gill and foot/mantle tissue were examined from the bivalve mollusc Abra nitida. The main gill fatty acids were 16:0 (11%), 18:0 (9%), 18:5(n-3) (10%), 20:1(n-11) (7%), 20:5(n-3) (12%) and 22:6(n-3) (7%). Levels of 16:1(n-7), 18:1(n-7) and 18:3(n-3) were all greatly diminished in comparison to T. sarsi. The main fatty acids detected within the foot/mantle were essentially the same as those encountered within the gill and were as follows : 16:0 (7%), 18:0 (9%), 18:5(n-3) (7%), 20:5(n-3) (15%), 22:5(n-3) (7%) and 22:6(n-3) (9%). Levels of branched and odd chain fatty acids were minimal in both tissue types.

The major fatty acids of Nereis sp. were 16:0 (12%), 18:1(n-7) (7.5%), 20:1(n-11) (7%), 20:5(n-3) (15%), and 22:6(n-3) (6%). Only trace amounts of 18:3(n-3), 18:5(n-3), branched and odd chain fatty acids were detected.

The fatty acid composition of the sipunculid was slightly different from the rest of the samples. The main constituents were : 16:0 (17%), 18:1(n-7) (7.5%), 20:4(n-6) (8%), 20:5(n-3) (8%), and 22:2 NMID (7%). 18:3(n-3), 22:5(n-3), 22:6(n-3) were only detected at very low levels.

The fatty acid composition of the terebellid polychaete was rather similar to that encountered in T. sarsi. The major constituents were 16:0 (5%), 16:1(n-7) (27%), 18:1(n-11) (11%), 18:1(n-7) (8%), 18:3(n-3) (13%) and 18:5(n-3) (11%). The levels of 20:5(n-3), 22:5(n-3) and 22:6(n-3) were all diminished compared to Abra and Nereis. Branched and odd chain fatty acids were again insignificant.

5.3.6 Fatty Acid Analysis of a Sediment Core from the North Sea Pockmark.

Presented in Table 5.8 are results of the analysis of a sediment core from the North sea pockmark. The total lipid obtained from each section of sediment core was extracted from $2cm^3$ of sediment. As with previous sediment analyses, the fatty acid composition is rather variable according to depth. The major fatty acids were 16:0 (22-28%), 16:1 moieties (up to 36%) and 18:1 moieties, especially 18:1(n-9) (up to 17%). Levels of long-chain PUFA were very low throughout the core (maximum of 3% for each fatty acid). The 13-15cm section of the core was markedly different from the rest. It contained a large proportion of 16:1(n-7) (as opposed to 16:1(n-9) in the rest of the core), at least twice the proportion of 18:1(n-7) (7%) and about 4% 22:2 NMID (absent elsewhere).

5.3.7 Fatty Acid Analyses of Animals Collected from the North Sea Pockmark.

All the results shown for the fatty acid compositions of the pockmark animals (Table 5.9) were obtained from analyses of total lipid extracted from individual specimens. The pockmark animals, although displaying rather different fatty acid profiles, all differed greatly from Thyasira sarsi in the respect that they contained large amounts of long chain PUFA, whereas Thyasira was greatly diminished in

such fatty acids. The T. sarsi analysed represents a whole animal, its main constituents being 16:0 (12%), 16:1(n-7) (11%), 18:1(n-11) (17%), and 18:5(n-3) (13%). This analysis was similar to that of the foot/mantle portion from T. sarsi collected in the Skagerrak (Tables 5.2 and 5.3).

Interesting observations may be made from the other symbiont-free animals in Table 5.7. Abra nitida was the only animal other than T. sarsi to contain a significant proportion of 18:5(n-3) (7.5%), but only a trace of 18:3(n-3). The prawn, Dichelopandalus bonnieri contains 13% 18:1(n-7).

The majority of the symbiont-free organisms were especially rich in 20:5(n-3). The seapen, Pennatula, was rich in 20:4(n-6) and contained increased proportions of 22:6(n-3) when compared to T. sarsi. The flatworm was rich in 22:5(n-3).

5.3.8 Fatty Acid Analysis of the Sediment Core from the Kattegat, Taken Near Laeso Island.

The total lipid obtained from each section of the Kattegat sediment core was extracted from 1cm³ of sediment. Although there was an element of variation in the fatty acid composition with core depth, there seemed to be no major trends apparent within the Kattegat core (Table 5.10). The main fatty acids were saturated and monounsaturated types and were as follows : 16:0 (20-28% of total), 16:1(n-7) (10-23%), 18:0 (6-8%) and 18:1(n-9) (9-15%). The major PUFA was 18:2(n-6)

(up to 7%) although levels of PUFA were otherwise generally low. The sediment was also poor in branched chain fatty acids throughout and, when summed together, these generally constituted less than 5% of the total. The 9-10cm and to a lesser extent the 10-12cm section contained the largest proportions of 16:1(n-7) and 18:1(n-7).

5.3.9 Fatty Acid Analyses of the Total Lipid of Animals and Rock from the Kattegat Methane Seep.

All the animals collected from the Laesø site were symbiont-free and their composition (Table 5.11) was quite different from the symbiont-containing animals analysed previously. The fatty acid compositions were obtained from analyses of the total lipid extracted from single specimens. The main fatty acids detected from the extract obtained from the carbonate rock (on and in which many animals lived) were : 16:0 (21%), 16:1(n-7) (5%), 18:0 (6%), 18:1(n-11) (12.5%), 20:4(n-6) (6%), 20:5(n-3) (5%) and 22:6(n-3) (5%). The main constituent of the barnacle was 20:5(n-3) making up 29% of the total. Other main constituents were 14:0 (7%), 16:0 (13%), 16:1(n-7) (14%), 18:1(n-11) (6%), 18:1(n-7) (7%) and 22:6(n-3) (6%). Metridium senile contained 13% 20:5(n-3) and its other main fatty acids were as follows : i-16:0 (5%), 16:0 (11%), 18:0 (5%), 22:1 (5%) and 22:6(n-3) (8%). Within the sponge, 20:5(n-3) was greatly depleted (only 4%) and 20:4(n-6) was the main constituent (24%). The other main fatty acids of the sponge were 16:0 (15%), 16:4(n-3) (5%), 18:0 (6%) and 18:1(n-9) (6%).

Table 5.1 The Percentage Composition of Fatty Acids of Total Lipid Extracted from a Sediment Core in the Skagerrak, Depth 300m, Collected October 1989.

Fatty acid	0-1cm	2-3cm	5-6cm	9-10cm	13-14cm	21-22cm
14:0	5.7	4.1	4.4	4.9	5.3	3.7
14:1	1.3	2.1	1.9	1.5	1.8	1.9
i-15:0	1.0	1.5	0.9	1.1	1.4	1.7
a-15:0	2.7	3.1	1.8	3.4	3.9	4.4
15:0	2.4	2.7	2.3	0.4	2.8	2.8
i-16:0	0.7	2.0	0.4	0.8	0.7	0.9
16:0	24.5	23.0	16.8	23.8	20.9	20.6
16:1(n-9)	2.6	4.3	3.8	4.2	4.6	5.7
16:1(n-7)	6.4	6.0	5.0	5.6	4.4	5.6
16:1(n-5)	2.0	2.6	1.1	2.0	1.7	1.9
16:2		0.7	0.3	0.4	0.5	0.5
16:3		1.1				
16:4(n-3)		0.2	2.2	0.5	0.3	0.3
i-17:0	0.6	0.6	0.4	1.5	0.7	0.8
a-17:0	0.5	0.7	0.2	2.0	1.5	1.2
17:0	2.7	2.8	2.2	2.4	2.5	2.1
17:1	1.8	0.7	1.0	1.0	1.6	2.0
18:0	8.1	7.9	5.5	8.0	8.0	6.6
18:1(n-9)	9.3	8.5	10.1	8.7	9.0	8.9
18:1(n-7)	4.4	4.2	4.5	3.3	3.0	3.6
18:1(n-5)		0.7	0.8	0.6	0.4	0.8
18:2(n-6)	1.4	1.6	2.3	2.0	3.3	2.0
18:3(n-6)				0.4	0.9	0.5
18:3(n-3)	0.7	1.8	1.8	0.4	0.5	0.7
18:4(n-6)		1.1				0.3
18:4(n-3)	1.6	3.3	0.9	0.4	0.3	0.5
18:5(n-3)	2.4	1.5	2.4	1.0	1.8	1.9
19:0	1.1		0.8	1.1	3.6	1.2
i-20:0		0.8	3.8		0.4	
20:0	1.1	1.2	1.0	1.9	1.3	
20:1(n-13)	2.1	0.8	1.6	1.8	0.4	1.5
20:1(n-9)	1.3	0.5	0.9	0.9	0.4	0.8
20:1(n-7)	1.6	0.6	1.5	0.4		0.3
20:2			5.4			0.4
20:3			2.0			0.5
20:4(n-6)			0.8	1.0	0.8	0.8
20:4(n-3)	0.5	0.4	1.2	0.4		0.5
20:5(n-3)	1.1	1.1	1.4	0.6	0.8	0.7
21:5	0.6	0.4	0.8	0.8	0.5	0.7
22:1(n-11)	0.6	0.4	0.3	3.8	2.5	0.3
22:2(n-6)	2.9	1.6	0.4		0.4	
22:3(n-3)	1.9	1.9			0.5	
22:4(n-6)	1.6	0.4				
22:5(n-3)		0.5	2.5	4.0	3.1	3.6
22:6(n-3)		0.2	2.7	2.7	3.4	2.3
18:1(n-7)/(n-9)	0.5	0.5	0.4	0.3	0.4	0.4
sum of odd chain	8.0	6.2	6.6	5.3	10.5	9.1
sum of branch chain	5.5	8.7	7.5	8.8	8.6	9.0
sum of (n-6) PUFA	5.9	4.7	3.5	3.4	5.4	3.6
sum of (n-3) PUFA	8.2	10.9	15.1	10.0	10.7	10.5

Table 5.2 The Percentage Composition of Fatty Acids of Total Lipid Extracted from a Specimen of Thyasira sargi, Collected in the Skagerrak, October 1987.

Fatty acid	gill tissue	digestive gland/ gonad	mantle/ foot
14:0	0.8	0.9	0.8
14:1	0.2	0.3	
a-15:0	0.1		0.6
15:0	0.4	0.5	0.7
i-16:0	0.4	0.4	0.5
16:0	10.9	9.4	6.3
16:1(n-10)	0.8	0.6	1.0
16:1(n-7)	34.9	15.1	9.8
16:2		0.2	0.3
16:3	0.4	0.5	0.8
17:0	0.7	0.5	0.5
17:1	0.3	0.2	
i-18:0			0.3
18:0	1.2	1.7	1.5
18:1(n-11)	8.4	14.8	20.0
18:1(n-7)	8.8	11.0	7.3
18:2(n-6)	0.5	1.1	1.2
18:3(n-6)		0.4	0.5
18:3(n-3)	16.2	8.3	3.4
18:4	0.4		
18:5(n-3)	4.4	8.7	15.3
19:0	0.3	0.3	
19:1	0.5	0.9	1.2
20:0	0.3	0.3	0.6
20:1(n-11)	2.3	5.3	6.7
20:1(n-7)	3.3	4.4	5.9
20:3	0.8	1.8	2.0
20:4(n-6)	0.2	0.4	0.4
20:4(n-3)	0.6	3.0	2.0
20:5(n-3)	0.3	4.4	5.3
22:3		0.2	0.7
22:5(n-6)		0.5	0.4
22:5(n-3)		0.5	0.7
22:6(n-3)		1.4	1.3
18:1(n-7)/(n-11)	1.0	0.7	0.4
sum of odd chain	2.1	2.4	2.4
sum of branched	0.5	0.5	1.4
sum of (n-6) PUFA	0.7	2.4	2.5
sum of (n-3) PUFA	21.7	26.3	28.0

Table 5.3 The Percentage Composition of Fatty Acids of Total Lipid Extracted from 27 Specimens of Thyasira sarsi Collected in the Skagerrak, October 1989. Data are expresses as means, plus or minus the standard deviation (= S.D.).

Fatty acid	gill tissue	S.D.	digestive gland/ gonad	S.D.	mantle/ foot	S.D.
14:0	0.7	0.3	2.0	1.3	0.8	0.4
14:1(n-5)	0.1	0.1	0.6	0.2	0.2	0.2
i-15:0	0.2	0.1	0.5	0.5		
a-15:0	0.6	0.5	0.5	0.2	0.6	0.8
15:0	0.5	0.1	0.4	0.1	0.6	0.3
i-16:0	0.9	0.2	0.5	0.1	0.5	0.1
16:0	9.6	1.3	8.5	1.8	6.5	1.3
16:1(n-10)	0.8	0.5	0.9	0.1	1.3	0.2
16:1(n-7)	24.0	2.8	12.0	1.2	9.1	1.1
16:1(n-5)	0.3	0.3	1.0	0.4	0.5	0.3
16:2	0.3	0	0.1	0.1	0.1	0.1
16:3	0.4	0.2	0.2	0	0.4	0
16:4(n-3)	0.1	0	0.1	0	0.2	0.1
i-17:0	0.3	0.1	0.5	0.1	0.4	0.2
a-17:0	0.1	0.1	0.2	0.1	0.2	0.2
17:0	0.4	0.1	0.4	0.1	0.5	0.1
17:1	0.6	0.3	0.8	0.1	0.0	0.4
18:0	0.7	0.2	1.5	0.3	1.5	0.8
18:1(n-11)	11.0	0.7	15.5	0.7	20.4	1.9
18:1(n-7)	8.3	0.6	10.9	1.0	7.8	0.9
18:1(n-5)	1.0	0.2	1.4	0.3	1.3	0.8
18:2(n-6)	0.5	0.1	0.5	0.2	1.0	0.4
18:3(n-6)	0.2	0.1	0.2	0.1	0.3	0.2
18:3(n-3)	12.4	1.7	5.9	1.3	3.2	0.5
18:4	0.4	0.3	0.4	0.3	0.6	0.4
18:5(n-3)	5.5	1.5	7.4	2.1	9.3	2.7
19:0	0.2	0.1	0.2	0.1	0.1	0.2
19:1	0.2	0.4	0.6	0.5	1.4	0.2
20:1(n-13)	4.4	1.5	5.9	0.3	7.2	0.7
20:1(n-7)	4.4	1.3	5.8	1.4	7.9	1.9
20:1(n-5)	0.6	0.4	0.4	0.2	0.6	0.3
20:2(n-9)	0.5	0.3	0.9	0.2	1.1	0.4
20:2(n-6)	0.2	0.1	0.3	0.1	0.2	0.1
20:3	1.0	0.3	1.5	0.5	0.9	0.2
20:4(n-6)	0.5	0.3	0.5	0.3	0.5	0.2
20:4(n-3)	2.2	0.4	2.6	0.6	2.4	0.6
20:5(n-3)	3.0	0.8	4.5	1.3	4.3	1.9
22:0	0.4	0.3	0.4	0.3	0.4	0.4
22:1(n-11)	0.6	0.4	0.5	0.3	0.8	0.3
22:5(n-6)	0.6	0.6	0.6	0.6	0.3	0.3
22:5(n-3)	0.6	0.5	0.7	0.4	0.6	0.5
22:6(n-3)	0.6	0.5	1.2	0.7	1.5	1.3
18:1(n-7)/(n-11)	0.8		0.7		0.4	
sum of odd chain	1.9		2.4		3.0	
sum of branched	1.0		1.9		2.2	
sum of (n-3) PUFA	24.8		22.8		22.1	
sum of (n-6) PUFA	2.0		2.1		2.3	

Table 5.4 The Percentage Composition of Fatty Acids of Total Lipid Extracted from the tissues of ten Thysanira sarsi, exhibiting Very Dark Gills, Collected from the Skagerrak, October 1989.

Fatty acid	gill tissue	digestive gland/ gonad	mantle/ foot
12:0		0.3	
14:0	1.5	1.3	0.7
14:1(n-5)	2.2	0.4	
i-15:0	1.2	0.1	
a-15:0	0.9	0.2	0.5
15:0	0.9	0.3	0.5
i-16:0	2.2	0.4	0.5
16:0	18.7	6.7	7.1
16:1(n-10)		0.9	1.9
16:1(n-7)	14.6	12.8	11.6
16:1(n-5)	4.5	1.1	
16:2	1.5		
16:3	0.7	0.2	0.5
16:4(n-3)	0.5	0.2	0.2
i-17:0	1.9	0.4	0.3
a-17:0	1.5	0.1	0.2
17:0	1.2	0.3	0.4
17:1	0.5	0.6	1.3
18:0	3.4	1.1	1.5
18:1(n-11)	3.5	13.5	22.5
18:1(n-7)	9.7	10.3	9.6
18:1(n-5)	0.8	1.3	1.7
18:2(n-6)	1.2	0.5	0.3
18:3(n-6)		0.5	0.7
18:3(n-3)	0.8	9.5	3.7
18:4(n-3)	0.4		
18:5(n-3)	0.5	12.5	9.6
19:0	0.4	0.2	
19:1			1.2
20:1(n-13)	1.3	4.5	6.9
20:1(n-7)	0.9	3.7	6.6
20:1(n-5)	0.4	0.3	0.5
20:2(n-9)		0.9	1.3
20:2(n-6)	0.5	0.3	0.4
20:3(n-3)		1.9	1.1
20:4(n-6)	1.0	0.2	0.2
20:4(n-3)	0.6	3.9	1.9
20:5(n-3)	12.5	6.9	4.4
22:1(n-11)	0.5	0.3	
22:5(n-6)	2.1		
22:5(n-3)	2.2		
22:6(n-3)	3.0	1.1	
18:1(n-7)/(n-11)	3.0	0.8	0.4
sum of odd chain	2.6	1.4	3.4
sum of branched	7.2	1.2	1.5
sum of (n-6) PUFA	4.8	1.5	1.6
sum of (n-3) PUFA	20.0	36.0	20.9

Table 5.5 The Percentage Composition of Fatty Acids of Individual Phospholipid Classes Extracted from the Gill Tissue of *Thynnichthys sarai* (where the percentage composition of each phospholipid class is given in brackets below each of the class headings).

Fatty acid	PC (14.8)	PS (8.1)	PI (12.6)	PG (8.5)	PE (40.3)	Unknown (3.1)
14:0	1.6	1.6	2.8	1.8	1.4	1.3
14:1					0.2	0.5
i-15:0					0.1	
a-15:0	1.4	1.5	3.0	1.5	0.9	3.8
15:0	1.4	0.9	2.0	1.0	0.6	1.0
15:1					0.1	0.6
i-16:0	0.6				1.2	
16:0	9.0	17.1	7.9	22.1	12.4	7.0
16:1(n-10)	3.3					
16:1(n-7)	22.2	3.5	6.6	52.2	35.1	7.1
16:2	0.6					
16:3	0.9	0.4			0.2	0.6
i-17:0	0.3					
a-17:0	0.2					
17:0	2.3	0.6	0.8		0.3	0.5
17:1					0.4	0.5
i-18:0	0.3					
18:0	1.2	18.3	3.5	1.9	1.2	2.8
18:1(n-11)	18.5	8.1	10.6	2.6	12.8	8.7
18:1(n-7)	13.4	1.9	7.3	3.0	3.6	2.3
18:1(n-5)	3.2	0.7	2.6		0.7	0.7
18:2(n-9)					0.1	
18:2(n-6)	0.3	0.6	1.1		0.2	0.6
18:3(n-6)	0.1					
18:3(n-3)	6.2	2.1	1.2	10.7	8.7	5.0
18:4	0.7	0.4	0.7		0.6	
18:5(n-3)		8.7			11.4	9.9
19:0	0.7	1.2				1.2
20:0	0.3					9.8
20:1(n-13)	1.7	17.6	2.6	1.0	2.8	21.5
20:1(n-9)	1.7		25.4		1.6	
20:1(n-7)	0.6	1.9	2.6		0.3	3.2
20:1(n-5)	0.6	3.2	3.6		0.5	0.7
20:2	1.0				0.1	3.7
20:3	0.6		1.0		0.3	1.7
20:4(n-6)	1.0	0.6	2.4		0.2	
20:4(n-3)	1.7	0.4	1.4		0.5	0.9
20:5(n-3)	1.2	0.9	8.9		0.8	0.7
22:0		0.6				0.5
22:1	0.2	1.2				1.6
22:5(n-6)		0.7				
22:5(n-3)	0.5	1.0	1.0		0.1	
22:6(n-3)	0.6	4.0	0.7	2.3	0.2	
18:1(n-7)/(n-11)	0.7	0.2	0.7	0.4	0.3	0.3
sum of odd chain	4.4	2.7	1.7	1.0	1.4	3.8
sum of branched	2.8	1.5	3.0	1.5	2.2	3.8
sum of (n-6) PUFA	1.4	1.9	3.5		0.4	0.6
sum of (n-3) PUFA	10.9	17.6	13.9	13.0	22.3	16.5

Table 5.6 The Percentage Composition of Fatty Acids of Total Lipid Extracted from *Sibaldinum poseidoni*, collected at a Skagerrak Methane Seep (where the rear section constituted the symbiont containing trophosome while the anterior section was symbiont-free).

Fatty Acid	rear section	anterior section	rear section	anterior section	rear section	anterior section
14:0	2.0	1.8	4.4	2.2	2.6	2.7
14:1(n-5)		0.3		0.8		
i-15:0				0.2		
a-15:0	1.0		0.6	0.4		
15:0	1.0	0.6	1.5	1.3	1.7	0.8
i-16:0			0.4	0.4		
16:0	14.5	15.8	25.7	14.9	15.6	16.8
16:1(n-7)	40.1	45.2	33.7	42.6	35.9	36.0
16:2		0.3	0.3	0.2		0.4
i-17:0			0.3	0.2		
a-17:0			0.6	0.4		
17:0	0.9	1.0	0.8	0.7	1.0	
17:1			0.9	0.8		
18:0	2.9	2.5	5.1	3.8	3.4	2.7
18:1(n-13)	4.9	2.4	4.0	1.0	3.1	2.9
18:1(n-9)	7.4	5.8	7.1	7.3	11.1	7.8
18:1(n-7)	13.1	12.2	10.3	10.4	12.1	13.3
18:2(n-6)	4.8	5.1	3.2	5.1	5.5	6.4
18:5(n-3)	1.0	0.6		0.6	1.6	0.3
20:0	0.4			0.4		0.1
20:1(n-13)	1.2	1.3		1.7	1.9	2.5
20:1(n-7)	0.9	1.1		1.4	1.8	1.9
20:4(n-6)	2.9	3.5		2.0	2.5	3.1
20:5(n-3)	1.0	0.3	1.2	1.0	1.1	1.2
18:1(n-7)/(n-9)	1.8	2.1	1.5	1.4	1.1	1.7
sum of odd chain	1.9	1.6	3.2	1.9	1.7	1.8
sum of branched	1.0		1.9	1.2		
sum of (n-6) PUFA	5.8	5.4	4.4	6.1	6.6	7.6
sum of (n-3) PUFA	3.9	4.1		2.6	4.1	3.4

Table 5.7 The Percentage Composition of Fatty Acids of Total Lipid Extracted from a Variety of Symbiont-Free Animals, Collected in the Skagerrak, October 1989.

Fatty acid	Ag	Afm	Nereis	Sip.	Pc
14:0	1.5	1.2	3.1	5.1	0.6
i-15:0/a-15:0	0.5	1.3	0.3	0.7	0.6
15:0	1.4	0.5	0.9	0.6	0.4
15:1	0.1	0.7	0.1		0.7
i-16:0	0.3	0.7	0.3	0.9	
16:0	10.9	7.4	12.2	16.9	5.2
16:1(n-7)	6.1	5.3	6.5	4.3	26.9
16:2		0.5	0.4	0.9	
16:3	0.9	0.4	0.2		0.2
16:4(n-3)	0.8	0.5	1.9	0.7	
i-17:0	0.9	0.9	0.9	0.7	0.3
a-17:0	0.8	0.4	1.0	2.1	
17:0	0.8	2.2	1.6	3.5	0.3
17:1	1.1	1.2	1.0	2.4	0.8
18:0	9.1	8.7	4.9	4.6	0.4
18:1(n-9)	4.9	2.5	3.1	5.3	10.6
18:1(n-7)	1.9	1.7	7.5	7.5	8.4
18:1(n-5)	0.6	0.7	0.9	0.6	0.9
18:2(n-6)	1.6	1.2	1.1	5.7	
18:3(n-6)	0.5	0.4	0.3		
18:3(n-3)	0.4	1.3	0.9	0.4	13.2
18:5(n-3)	10.5	6.6	0.9	4.2	10.7
19:0	0.4		0.3	0.3	0.3
19:1	0.2	1.2	0.4	0.1	0.7
i-20:0	0.3	0.8		0.7	
20:1(n-13)	7.1	3.3	6.8	0.2	3.1
20:1(n-9)			1.3		3.2
20:1(n-7)	3.6	3.4	3.4	0.9	3.4
20:2	0.6	0.7	2.0	2.0	0.6
20:3	0.5	0.5	2.6		1.0
20:4(n-6)	5.0	3.4	1.4	7.7	0.1
20:4(n-3)	0.3	0.7	0.4		2.5
20:5(n-3)	12.4	15.3	15.4	7.9	3.4
22:0	0.6	0.4	0.2	0.4	
22:1(n-11)	0.6	2.0	1.0	2.0	0.3
22:2 NMID		1.3	2.8	6.7	
22:3(n-3)	0.7	0.8	1.5	0.5	
22:5(n-6)	1.2	3.4	0.3		0.4
22:5(n-3)	2.8	7.2	3.1	0.7	0.4
22:6(n-3)	7.0	8.8	6.1	0.7	0.4
24:1	0.8	3.1			
18:1(n-7)/(n-9)	0.4	0.7	2.4	1.4	0.8
sum of odd chain	4.0	5.8	4.3	6.9	3.2
sum of branched	2.8	4.1	2.5	5.0	0.9
sum of (n-6) PUFA	8.3	8.4	3.1	13.4	0.5
sum of (n-3) PUFA	34.9	41.2	30.2	15.1	30.8

where : Ag = Abra nitida, gill tissue ; Afm = Abra nitida, foot/mantle tissue ; Nereis = Nereis species ; Sip. = Phylum Sipuncula, genus unknown. Pc = terebellid polychaete , genus unknown.

Table 5.8 The Percentage Composition of Fatty Acids of Total Lipid Extracted from a North Sea Pockmark Sediment Core, Station B8, depth 157.5m, Collected July 1989.

Fatty acid	3-5cm	8-10cm	13-15cm	23-25c	33-35cm	43-45cm
12:0		1.4		1.1	0.8	0.2
14:0	13.2	6.6	1.5	5.9	5.5	5.4
14:1(n-5)		1.9		1.1	1.0	
a-15:0	2.0	2.6	1.3	2.1	1.7	1.9
15:0	4.2	4.2	1.3	3.0	3.6	4.4
i-16:0	0.7			0.4		0.3
16:0	23.7	24.9	22.1	23.0	28.4	26.3
16:1(n-9)	9.2	13.0		9.8	15.7	
16:1(n-7)	2.4	2.7	35.6	0.7	0.8	12.2
16:1(n-5)	1.6	0.7		1.1	1.8	2.2
16:2	0.7	0.8		0.5		0.2
16:3	1.7	1.4	0.6	1.2	1.4	2.0
17:0	1.7	0.6	0.6	0.5		1.9
18:0	6.9	7.4	5.3	7.8	9.1	8.0
18:1(n-9)	12.9	14.3	4.4	16.7	14.6	16.9
18:1(n-7)	2.2	2.6	6.9	2.5	3.1	2.6
18:1(n-5)	1.1	1.8	0.3	1.2	1.3	0.9
18:2(n-6)	3.2	3.6	1.0	3.2	2.4	3.3
18:3(n-3)		0.7	0.8			0.9
18:4(n-3)	0.4	0.5		0.7	0.5	0.6
19:0	0.5					0.3
20:0	1.1	1.1	0.4	1.1	1.3	1.4
20:1(n-11)		0.5		0.5		
20:1(n-9)		0.6	3.9	0.7	1.3	1.4
20:1(n-7)	0.9	1.8	6.8	6.4	2.4	0.9
20:1(n-5)	0.4	0.5	1.8	0.4		
20:2	3.4					
20:3(n-3)	0.5					
20:4(n-6)			0.8	1.4		
20:4(n-3)		0.5				
20:5(n-3)	0.8	0.5	0.9	1.6		
22:2 NMID			3.7		0.6	
22:0	2.0	1.8		1.8	2.2	2.3
22:3(n-3)		0.5		0.4		
22:4(n-6)					0.6	0.6
22:5(n-3)				0.7		
22:6(n-3)	2.6	0.5		2.7		2.8
24:0		3.0		5.3	2.5	
24:1	1.2	3.0		3.0	4.5	2.4
18:1(n-7)/(n-9)	0.2	0.2	1.6	0.1	0.2	0.2
sum of odd chain	6.4	4.8	1.9	3.5	3.6	6.6
sum of branched	2.7	2.6	1.3	2.5	1.7	2.2
sum of (n-6) PUFA	3.2	3.6	1.8	4.6	3.0	3.9
sum of (n-3) PUFA	4.3	3.2	1.7	6.1	0.5	4.3

Table 5.9 The Percentage Composition of Fatty Acids of Total Lipid Extracted from a Variety of Animals Collected at a North Sea Pockmark, July 1989.

Fatty acid	Boghtrv	Seapen	Abra alba	Prawn	Urchin	Thyasira gouldi	Flatworm	Shrimp
14:0	4.0	0.8	2.9	4.2	7.1	3.6	0.7	1.6
14:1	0.2	0.1	2.2		0.6	.5	0.1	0.1
a-15:0	1.7	0.2		0.1	1.0	.6	0.1	0.1
15:0	1.4	0.3	1.2	0.7	1.9	1.7	0.2	0.5
i-16:0	1.2	0.1	0.6	0.2	0.9	0.2	0.2	0.1
16:0	13.7	9.2	9.9	16.7	10.6	11.6	9.0	10.0
16:1(n-9)	12.9							
16:1(n-7)	2.3	2.6	7.2	0.7	11.3	90.6	2.1	3.2
16:1(n-5)	2.4	0.2	0.8	0.3	2.7	1.2	0.3	0.3
16:2	1.3	1.3	0.3	0.2	1.7	0.6	0.5	0.2
16:3	0.1	0.8	0.4	0.6	0.8	1.0	0.7	0.6
16:4(n-3)	1.4	2.4	0.8	0.5	0.7		0.6	0.4
17:0	1.3	1.3	0.7	0.6	1.5	0.2	1.9	0.9
18:0	3.4	3.5	6.4	3.8	4.1	3.2	5.5	3.4
18:1(n-11)			0.3		2.3		1.0	
18:1(n-9)	4.2	4.7	5.8	0.2	2.6	17.2	3.0	12.6
18:1(n-7)	6.0	3.3	3.7	13.3	4.1	4.7	1.7	3.6
18:1(n-5)	0.5	0.2	0.4	3.7	0.7	0.7	1.4	1.1
18:2(n-6)	1.1	1.2	1.6	1.0	0.9	1.0	0.5	1.6
18:3(n-6)	0.7	0.6	0.3	0.3			0.1	0.1
18:3(n-3)	0.5	0.8	0.4	0.8	1.9	4.0	0.2	0.7
18:4(n-3)	0.8	1.3		0.6	1.0	0.4	0.2	0.6
18:5(n-3)	0.7	0.8	7.5	0.3		11.0	1.5	0.1
20:1(n-11)	0.1	0.8	4.4	1.6	5.1	4.6	2.1	
20:1(n-9)	2.0	2.9	1.5		1.2			2.0
20:1(n-7)	1.0	3.4	2.0	2.2	3.0	4.0	3.2	0.2
20:1(n-5)	0.3	0.2	0.6	0.2	0.6	0.5	0.2	0.2
20:2	0.7	0.9	1.3	0.6	0.8		0.2	0.5
20:3	0.2	0.8	0.5	0.5	0.5	0.4	0.2	0.2
20:4(n-6)	2.3	25.1	5.8	2.8	9.8	1.3	3.6	3.0
20:4(n-3)	0.7	1.0	0.5	0.5	0.6	2.3	0.6	0.3
20:5(n-3)	13.0	8.4	15.6	14.7	11.0	6.7	23.3	20.2
21:5	0.6	0.9	0.6	0.3	0.5		0.6	0.1
22:1	0.2	4.7		4.6	1.2		11.0	1.0
22:2(n-6)	0.5	0.2		0.3		0.5	0.6	
22:3(n-3)		2.9		0.4	0.5			
22:4(n-6)	3.9	4.8	0.6	0.1	0.2		4.6	0.2
22:5(n-6)	0.7	0.6	1.1	0.3	0.8		0.3	
22:5(n-3)	3.9	1.3	3.1	1.3	0.7	1.1	16.1	0.3
22:6(n-3)	0.4	5.6	9.3	11.7	3.5	3.1	1.6	20.2
24:1		0.9		0.6	0.8	2.9	1.0	1.3
18:1(n-7)/(n-9)	1.6	0.7	0.5	66.5	1.6	0.3	0.6	0.3
sum of odd chain	2.9	2.9	2.9	1.3	3.9	1.9	2.3	1.6
sum of branched	2.9	0.3	0.6	0.3	2.7	0.8	0.3	0.2
sum of (n-6) PUFA	9.2	33.7	8.4	4.8	11.7	2.8	9.7	4.9
sum of (n-3) PUFA	27.5	23.7	37.7	30.5	19.9	14.6	48.6	42.5

where : Boghtrv = Boghtrya hirsuticauda ; seapen = Funiculina quadrangularis ; prawn = Pandalus montagui ; urchin = Echinocardium flavescens ; flatworm and shrimp are both unidentified.

Table 5.10 The Percentage Composition of Fatty Acids of Total Lipid Extracted from a Sediment Core from the Kattegat, Collected near Laesø Island.

	sediment depth					
Fatty acid	0-1cm	2-3cm	4-5cm	6-7cm	9-10cm	11-12cm
14:0	4.3	5.0	3.8	4.5	3.6	4.4
14:1	2.1	0.8	1.3	1.5		1.0
i-15:0	0.6	0.4	0.6	0.9	2.9	1.3
a-15:0	1.4	0.9	1.9	1.6	2.1	1.8
15:0	2.1	2.0	2.8	3.1	2.6	3.4
15:1	0.6	0.5	1.1	0.8		0.8
i-16:0	0.5	0.3	0.3			
16:0	28.2	27.5	20.3	24.6	20.8	21.8
16:1(n-9)	4.0					
16:1(n-7)	11.8	10.3	16.5	15.9	22.5	19.0
16:2		0.3	0.3	0.8		
16:4(n-3)	0.6	0.8	1.2			
i-17:0		0.4	0.7	3.1		
a-17:0			0.6	0.7		
17:0	1.9	1.4	1.6	1.6	1.1	1.4
17:1	1.2	1.5	2.0	2.0	2.9	2.0
18:0	8.1	8.3	7.8	6.7	6.2	6.8
18:1(n-9)	12.3	11.5	10.7	14.7	8.8	12.3
18:1(n-7)	4.2	4.8	5.3	4.1	7.6	6.7
18:2(n-6)	4.2	2.5	5.5	6.6	7.2	6.8
18:3(n-3)	0.3	0.5		0.6	0.9	0.5
20:0	1.0	0.2			1.4	1.3
20:1(n-13)	1.9	3.1	0.9		0.9	0.7
20:1(n-7)	0.7	3.7	2.3			
20:2	0.5	0.2	0.7			
20:4(n-6)		1.1	2.1		2.2	1.4
20:4(n-3)		0.9		1.7		
20:5(n-3)	1.0	2.4	1.8	1.5	5.0	1.0
22:0			1.4	1.5		2.4
22:1		2.9	3.1		1.1	3.2
22:2		3.5				
22:5(n-3)		1.1	1.2	0.6		
22:6(n-3)	6.6	0.9	2.2	1.2		
18:1(n-7)/(n-9)	0.3	0.4	0.5	0.3	0.9	0.5
sum of odd chain	5.8	5.4	7.5	7.5	6.6	7.6
sum of branched	2.5	2.0	4.1	6.3	5.0	3.1
sum of (n-6) PUFA	4.2	3.6	7.6	6.6	9.4	8.2
sum of (n-3) PUFA	7.9	6.6	6.3	5.6	5.9	1.5

Table 5.11 The Percentage Composition of Fatty Acids of Total Lipid Extracted from Rock and Symbiont-Free Animals, Collected at Lasaso, Kattegat, October 1989.

Fatty acid	carbonate rock	barnacle	Metridium senile	sponge
14:0	3.3	6.6	0.9	2.7
i-15:0/a-15:0	1.1	0.1	0.3	1.3
15:0	1.1	0.3	0.3	0.8
15:1	0.3		0.1	0.4
i-16:0	0.5	0.4	5.1	2.3
16:0	21.1	12.9	11.4	14.8
16:1(n-7)	5.1	13.8	3.6	4.4
16:1(n-5)	2.2		0.9	0.3
16:2	0.8	0.8	0.2	2.2
16:3	1.4	0.7	0.8	1.2
16:4(n-3)	0.5	1.4	3.5	5.3
i-17:0/a-17:0	2.1	0.1	3.4	1.2
17:0	0.5	0.1	1.1	0.8
17:1	1.2	1.1	0.6	1.2
i-18:0		0.1	0.9	
18:0	6.3	1.7	5.1	6.0
18:1(n-11)	12.5	6.4	0.2	
18:1(n-9)			4.7	5.7
18:1(n-7)	3.7	7.2	2.4	2.3
18:1(n-5)	0.5		3.2	0.5
18:2(n-6)	4.1	1.2	1.1	1.5
18:3(n-3)	1.5	0.2	0.6	0.8
18:4(n-3)	1.2	2.0	0.2	0.6
18:5(n-3)	0.7		0.6	0.8
19:0	0.2	0.2	0.3	0.5
20:0	0.2	0.1	0.2	0.5
20:1(n-13)	2.3	0.7	0.5	0.3
20:1(n-9)			0.9	1.9
20:1(n-7)	1.8	1.3	4.6	1.3
20:2	1.0	0.1	1.7	0.4
20:3	0.5	0.2	0.8	0.8
20:4(n-6)	5.6	0.5	1.6	24.1
20:4(n-3)	0.2	0.3	0.9	0.4
20:5(n-3)	5.2	28.9	13.0	3.6
21:5	0.2	0.5	0.5	
22:0	1.1		0.3	0.3
22:1	0.2	0.2	5.4	3.5
22:2(n-6)	0.3	0.7	0.6	
22:3(n-3)			2.7	0.9
22:5(n-6)	0.6		0.4	0.4
22:5(n-3)	0.6	0.2	5.1	0.4
22:6(n-3)	5.2	6.4	7.6	2.6
24:1	2.1	1.7	1.4	
18:1(n-7)/(n-11 or 9)	0.3	1.1	0.5	0.4
sum of odd chain	3.3	1.7	3.4	3.7
sum of branched	3.7	0.7	9.7	4.8
sum of (n-6) PUFA	11.3	3.0	3.2	26.6
sum of (n-3) PUFA	15.3	39.4	33.7	15.4

5.4 Discussion.

The results obtained for this section were derived from three distinct marine ecosystems. Each system will be discussed individually before comparisons are made.

5.4.1. The Skagerrak Methane Seep Ecosystem.

The sediment in which the Thyasira sarsi live resembles the degraded remains of plants and animals since large proportions of saturated and monounsaturated fatty acids were detected. However the presence of long-chain PUFA, especially below 5cm depth, suggests an input of dead animal and planktiferous material in the past. This area was sampled in October and, although sedimentation rates are unknown, it seems probable that this input was due to the "spring bloom". It is also interesting to note the presence of 18:3(n-3) and 18:5(n-3) within the sediment, albeit only detected at low levels. These fatty acids were important components of Thyasira sarsi and it would be interesting to ascertain whether they arose from T. sarsi or from planktonic input. If one reconsiders the analyses of bacteria cultured from this sediment and the putative symbionts cultured from T. sarsi (Tables 3.4 and 3.2b respectively) then it seems unlikely that these fatty acids were derived from the bacteria. Additionally, the analyses of the sediment bacteria would also suggest that these bacteria were not present in the Skagerrak sediment in great numbers since their main fatty acids were not prevalent within the sediment. Therefore, it seems unlikely they would be able to constitute a sustainable food

source for the population of T. marsi. When summed together, branched and odd-chain fatty acids constituted 13-19% of the total sediment fatty acids and suggests a bacterial presence different from those isolated and analysed in Chapter 3, i.e. not sulphur-oxidising or methylotrophic bacteria.

The fact that the Thyasira marsi collected in October 1987 and October 1989 were very similar in their fatty acid compositions indicates that the community is rather stable and has continued to utilise the same food source, at least during the autumn months. From the general lack of long-chain PUFAs and the substantial proportion of 16:1(n-7) present in the gills of T. marsi, it is obvious that these animals use a different source of nutrition from the majority of the other marine bivalves (see Table 4.1 for a comparison). The low levels of the potential symbiont biomarker, 18:1(n-7), could suggest that symbiont activity is not important at this site and that the fatty acid composition encountered in T. marsi was simply a product of the non-symbiont based trophic processes that generally occur in the Skagerrak. However, if one considers the symbiont-free animals taken from the same site, this argument is invalidated. All the animals in question, excepting the terebellid polychaete, have fairly typical compositions for marine animals, i.e. a high level of long-chain PUFA and a low level of 16:1(n-7). They most certainly derive their nutrition via a different method from T. marsi. Abra nitida is a filter-feeding bivalve mollusc and in those terms is most closely related to T. marsi. Although greatly different from T. marsi in its general composition, the two bivalves did have one important common feature, namely substantial levels of 18:5(n-3). This fatty acid is not usually found in marine animals and tends to be associated with

certain species of plankton, especially Phaeocystis pouchetti (Sargent et al., 1985). The fatty acid probably originates from specific plankton associated with this area. Since Abra is planktonivorous by nature this implies that T. sarsi must also utilise plankton to a certain extent. The remarkable similarity in fatty acid composition between T. sarsi and the terebellid polychaete implies the polychaete may also contain symbionts similar to those of Thyasira.

The low levels of 18:1(n-7) in the gills of T. sarsi can be accounted for in terms of their symbionts' fatty acid composition. Considering the composition of the putative methylotrophic symbionts of T. sarsi (Table 3.2b) where levels of 18:1(n-7) were low, then there seems no reason why 18:1(n-7) should be present in elevated levels within the gills of T. sarsi. This would further suggest that the symbionts utilise biogenic methane released at the seep where T. sarsi were collected. However, enzymatic and stable isotope work carried out on T. sarsi from the same site by Schmaljohann et al. (1990) suggests otherwise. They found no evidence of methanol dehydrogenase activity, although they did not assay for hexulosephosphate synthetase, these two enzymes being characteristic of bacterial C1-metabolism. Additionally they obtained ^{13}C isotope ratios of -39‰ for the gills and -37.4‰ for the rest of the body and concluded that the methanotrophic bacteria isolated from the gills of T. sarsi by Wood and Kelly (1989), the same as those analysed in Chapter 3 of this study, are probably not the main symbiont species. This presents a dilemma in terms of the results of this study and those of Schmaljohann et al. (1990). Although it seems that the gills of T. sarsi contain both sulphur-oxidising and methanotrophic bacteria, it is not plausible that T. sarsi can switch to using a

different type of bacteria depending on the available energy source, since the animals analysed within this study and by Schmaljohann et al. (1990) came from the same sample.

The answer to the above dilemma seems to lie with the carbon isotope ratios. Generally the gills of marine bivalves containing chemoautotrophic bacteria utilising sulphur compounds have ^{13}C ratios of -24 to -31 $^{o}/_{oo}$ (see chapter 1). It is generally accepted that the greater the depletion of carbon isotopes, the greater the dependance on symbionts. However, the T. sarsi analysed in the study by Schmaljohann et al. (1990) have ratios even more depleted than those that would be normally expected so suggesting a great dependence upon their symbionts. In contrast, the large proportion of PUFAs detected within this study would suggest that T. sarsi do not depend to such a great extent upon their symbionts but in fact also utilise planktonic food. However, if biogenic methane diffusing up to the surface of the sediment acted as an organic input driving sulphate reduction (as suggested by Schmaljohann et al., 1990), then methane-derived carbon would become incorporated with the sulphur-derived carbon causing a greater isotopic depletion. The role of the methylotrophic symbionts could be to fix methane which they utilise for their own devices, while at the same time providing energy for the reduction of sulphate by the sulphur-oxidising symbionts. This effectively creates a symbiosis within a symbiosis. This process may involve different enzymes from those assayed for by Schmaljohann et al. and would necessitate the re-examination of the use of carbon isotope depletion to gauge symbiont contribution to the host's nutrition.

The T. sarsi exhibiting dark gills are interesting since the dark coloration suggests a healthy symbiont population. However, the reduced level of 16:1(n-7) and the increased proportion of 20:5(n-3) within the gills of these animals, in relation to the T. sarsi analysed in Table 5.3, would point to a reduced symbiotic activity and an increase in the dietary contribution of filter feeding. The proportion of the bacterial fatty acid 18:1(n-7) remains similar to, if not slightly higher than, the other T. sarsi while levels of planktonic derived 18:3(n-3) and 18:5(n-3) are negligible. It could be that the symbionts of T. sarsi with dark gills are suffering the effects of competition for methane, such that the population is dying leaving the sulphur-oxidising symbionts that are richer in 18:1(n-7) and poor in 16:1(n-7). Furthermore, if the methane-oxidising bacteria are somehow integral in the overall symbiotic situation, then it follows that the sulphur-oxidisers would be unable to meet their host's nutritive needs, although they could sustain themselves. The increased levels of 20:5(n-3) could be represented by the conversion of 18:3(n-3) and 18:5(n-3). Alternatively, these T. sarsi may selectively feed on a different type of plankton rich in 20:5(n-3) and the dark colour of the gills could very well be due to extensive ingestion of phytoplankton. It is interesting to note that the other two tissue types, namely the foot/mantle and the gonad/digestive gland, have fatty acid compositions similar to the other T. sarsi suggesting that the event causing the decline in symbiont activity has occurred recently and may even be short term.

The fatty acid compositions of the lipid classes were determined in an effort to place certain fatty acids within certain classes which are specific to either prokaryotes or eukaryotes. Generally PC, PS,

PI, CL and PE would be indicative of eukaryotes while PG, CL and PE are the main bacterial classes (see Chapter 3). PG is, therefore, the only truly bacterial class and it is rich in bacterial-type fatty acids, 16:0 and 16:1(n-7). The high proportion of 18:3(n-3) could be accounted for in two ways : either it is produced by the bacteria or the symbionts can utilise animal type fatty acids, which would in turn imply an element of parasitism. PE contains both bacterial and animal fatty acids and, although the large proportion of 16:1(n-7) is almost certainly symbiont-derived, it would seem that 18:5(n-3) is animal derived. PC is probably the only truly animal derived class and its large proportions of 16:1(n-7) and 18:1(n-7) have interesting implications. These two fatty acids are almost definitely bacterial fatty acids showing a substantial utilisation of bacterial fatty acids for animal membrane lipid formation.

Siboglinum poseidoni would seem to be highly dependent on symbionts for their nutrition due to the distinct depletion of PUFAs in its lipids, especially 18:3(n-3) and 18:5(n-3), both of which were prevalent in all other animals analysed from this site. Furthermore, the fact that bacterial type markers, 16:1(n-7) and 18:1(n-7), were ubiquitous throughout both sections of the animal suggests a large translocation of products of bacterial chemoautotrophy from the symbiont-containing rear section to the symbiont-free anterior portion. These results complement those of Schmaljohann et al. (1990) who carried out stable isotope analyses of Siboglinum poseidoni from the same batch of animals analysed within this study. They obtained ^{13}C values of -73.6‰ for the symbiont-free anterior part and -78.3‰ for the symbiont-containing rear section, suggesting a high nutritional dependence upon their methanotrophic symbionts and a large

translocation of autotrophically derived carbon throughout the animal.

Schmaljohann et al (1990) suggest the coexistence of both sulphur- and methane-based symbioses at this Skagerrak site and the results of this study would seem to complement their idea. However, although S. posedoni is highly dependent upon its symbionts for nutrition, it would seem that T. marai is dependent also upon an external planktonic food source and does not operate solely in symbiotic mode.

5.4.2. The North Sea Pockmark Ecosystem.

Although there is variation in fatty acid compositions within the pockmark sediment, the most striking feature of this analysis is the difference in composition between the 13-15cm section and the rest of the core. Most of the core resembles the degraded remains of plants of animals, being rich in saturates and monounsaturates and low in PUFAs. However the 13-15cm section has a fatty acid profile resembling that of a symbiont-containing animal, namely increased proportions of 18:1(n-7) and especially 16:1(n-7). It is thought this section is rich in free-living methane-oxidising bacteria since Dando et al. (1991) found elemental sulphur concentrations of 0.10 mg-atom S dm^{-3} and 0.6 mg-atom S dm^{-3} total reduced sulphur compounds at 15cm depth for the same core. These values are very low and, although methane was not measured for this core, it was measured for a core taken on the opposite side of the pockmark from the one in this study, where methane was shown to be present in concentrations in excess of 200 nmoles dm-3 at 25cm depth, although it could not be detected above

10cm depth (Dando et al., 1991). If the core in this study is similar, then it may be that the bacterial activity at 13-15cm is due to methane-oxidising bacteria. The bacteria probably favour this section of the sediment rather than that of the methane maximum, as it may allow access to oxygen for methane oxidation which would not be available in deeper anoxic layers. The bacterial presence would also explain why methane is not found above 10cm.

The fatty acid compositions of the symbiont-free animals are typical of heterotrophically feeding organisms, namely a preponderance of long-chain PUFAs such as 20:4(n-6), 20:5(n-3), 22:5(n-3) and 22:6(n-3). It is interesting to note the unusually large proportion of 18:1(n-7) within the prawn, Dichelopandalus bonnieri. Dando et al (1991) obtained a carbon isotope ratio of -17.0‰ for this species and so it does not evidently use symbiotic nutrition. The large proportion of 18:1(n-7) may be due to either mutualistic or parasitic prokaryotes living within its intestine or between the body and the exoskeleton.

Although Thyasira sarsi does not contain large proportions of 16:1(n-7) or 18:1(n-7), its levels of 20:4(n-6) and 20:5(n-3) are very low when compared to the symbiont-free animals, so suggesting that T. sarsi does utilise an alternative form of nutrition. This conclusion is supported by carbon isotope ratio analyses performed on T. sarsi collected at the same site by Dando et al. (1991) which suggest a degree of nutritional dependence upon symbionts. It would seem that the large proportion of 18:5(n-3) is probably derived from plankton.

5.4.3. The Kattegat Ecosystem.

It seems that the increased proportion of 16:1(n-7) and 18:1(n-7) between 9-12cm in the Kattegat sediment core reflects a population of free-living bacteria that most likely utilise the methane supply as an energy source. The three animals analysed from this ecosystem all have fatty acid compositions that would be expected of heterotrophic organisms. The barnacle and Metridium are both rich in 20:5(n-3), so obtaining their nutrition from planktonic filter feeding, while the encrusting sponge was rich in 20:4(n-6) and probably fed upon detritus. The carbonate rock was interesting due to the great diversity of fatty acids it contained. Although visible animal life within the rock was removed before extraction, it is probable that these fatty acids are the result of planktonic particles becoming lodged within the porous rock. This probably explains the myriad of animals found tunnelling within this honeycombed rock. It is evident that this methane seep is home to a diverse range of animals, although the complete lack of symbiont-containing animals and the lack of characteristic bacterial-type fatty acids suggests that methane/sulphur processes are not at all important in a nutritional context at this site, which seems to be a normal photosynthetically-dependent ecosystem.

5.4.4 Comparison of the Three Sites and Conclusions.

Although the three sites were all associated with methane production, fatty acid analyses reveals they are all distinctly different ecosystems. Symbiotic processes are important at the

Skagerrak site but the patchy distribution of the animals suggests a localised phenomenon. Siboglinum poseidoni also certainly relies heavily on methanotrophic symbionts for its nutrition, while Thyasira sarsi is evidently subject to dual nutrition and, additionally, seems to contain both sulphur- and methane-oxidising symbionts.

At the North Sea pockmark, fatty acid analyses were not sufficient to prove the existence of symbiotic nutrition within T. sarsi. However, stable isotope work by Dando et al. (1991) suggests a reliance by T. sarsi upon autotrophically-derived carbon at this site. The sediment at this site seems to have a substantial free-living bacterial population at 13-15cm depth which probably utilise biogenic methane. However, fatty acid analyses showed the symbiont-free animals were all dependent upon a photosynthetic food web.

At the Kattegat site there was no evidence for any symbiotic activity and this ecosystem seems to be primarily photosynthetically dependent. However, there was some evidence of free-living bacterial activity within the sediment, and this bacterial population probably utilises the abundant methane supply.

6.

The Incorporation of Carbon Dioxide into the Lipids of the Symbiont - Containing Marine Invertebrate, *Lucinoma borealis*.

6.1 Introduction.

The use of labelled bicarbonate to measure the accumulation and metabolism of ^{14}C is well documented for symbiont-containing species, e.g. Cavanaugh (1983), Dando et al. (1985, 1986a), Felbeck (1983, 1985), Felbeck et al. (1983b), Fisher and Childress (1986), Southward et al. (1986) and Tuttle (1985). Generally CO_2 fixation in the presence of reduced sulphur is measured as a marker for symbiotic activity. Experiments have included using $^{14}CO_2$ to show incorporation into the gills of Bathymodiolus (Fiala-Medioni et al., 1986a), the effect of thiosulphate and sulphide upon CO_2 fixation in Riftia and Bathymodiolus (Belkin et al., 1986) and the effects of sulphide and/or thiosulphate upon CO_2 fixation in various symbiont-containing shallow water bivalves such as Solemya velum (Cavanaugh,1983), Lucinoma borealis (Dando et al., 1986a) and Myrtea spinifera (Dando et al., 1985 ; Southward et al., 1986). Fisher and Childress (1986) showed by liquid scintillation counting and autoradiography that, in Solemya reidi. ^{14}C from bicarbonate was first fixed in the gills before being later transferred to the rest of the body.

Labelled bicarbonate has not yet been used to examine the incorporation and accumulation of ^{14}C into the lipids of symbiont-containing animals. Techniques for radio-labelling lipids are well established and could be an important method for further elucidating the relationship between the symbionts and their invertebrate hosts. It was decided that Lucinoma borealis should be used in this range of experiments, the reasons being that Lucinoma has a distinctive lipid profile, it is of relatively large size and so is easier to work with

and yields sizeable amounts of lipid and, third, it is readily accessible around the U.K. coastline. Hammen and Osborne (1959) showed that several symbiont-free marine invertebrates have the ability to fix CO_2 into the various acids of the Krebs cycle. Therefore, it is essential for symbiont-free species from the same habitat to be used as comparative controls during radioactive incorporation experiments with Lucinoma.

Supplementary to the ^{14}C incorporation work it was planned also to identify and isolate a second form of PE that was specifically bacterial. PE is the major lipid class of both Lucinoma and its symbionts. However, since the symbionts were found to be devoid of PUFA (chapter 3) whereas the animals contain PUFA and these fatty acids are commonly abundant in PE, it follows that bacterial and animal PE are likely to have different polarities and so should separate when run on a TLC plate. Additionally, by studying PG, which is absent from the animal tissues, direct measurement of radioactivity into this bacterial phospholipid should give a direct measure of bacterial lipid formation.

6.2 Materials and Methods.

The methods for carrying out gas-liquid and thin-layer chromatography were as described in Chapter 2.

6.2.1 Collection and Storage of Lucinoma.

All specimens of Lucinoma borealis were collected at Mill Bay, Salcombe, Devon, during October 1990 and May 1991 with aid from and grateful thanks to Dr Paul Dando, Mr Sean O'Hara and Prof. A.J. Southward. The method of collection was to dig a trench below the low water mark in areas of black, sulphide sand and then to sieve out the Lucinoma. The distribution of Lucinoma was highly sporadic.

The Lucinoma were initially kept in beakers of seawater in the University of Stirling aquarium in a constant temperature room at 10°C (unless they were to be acclimated at a specific temperature). The seawater was changed daily to prevent a build up of toxic wastes. The beakers containing the Lucinoma were devoid of sulphide due to the fact that Dando et al. (1986) had observed that the greatest fixation of carbon dioxide within the gills of Lucinoma occurred when the animals were kept in a sulphide-free state prior to commencement of experimentation. The animals used in this study were kept for 2-3 days before being used in an experiment.

6.2.2 Experimental Conditions.

All radioactive incorporation experiments were carried out in 0.5 litre capacity glass jars containing 50ml of filtered seawater with a large airspace and sealed with a screw-top lid. Each jar contained up to five animals (depending upon their size). Care was taken to cause as little disturbance as possible when starting the experiment and throughout its course. Approximately 0.5mCi $NaH^{14}CO_3$ (Amersham, Batch 205) was added to each container at the start of the experiment. The water was not changed or topped up and no other additions were made to the jars after an experiment was started. Unless otherwise stated, the incubation temperature was 10°C.

(i) Conditions and treatments used in the initial experiment.

During the first experiment, five jars each containing five Lucinoma borealis were incubated with ^{14}C-bicarbonate for 48h. The contents of each jar were as follows :

1. Lucinoma with added sulphide and sediment.
2. Lucinoma with no sulphide but added sediment.
3. Lucinoma with no sediment but added sulphide.
4. Lucinoma with no sulphide or sediment.
5. Control animals with no sulphide or sediment.

Where applicable, sediment was added to a depth of 5cm, while sulphide was added in the form of an initial concentration of 40µM sodium sulphide (Na_2S). The control animals were Venus striatula and Cardium edule, both symbiont-free animals collected from the same site as Lucinoma.

(ii) Conditions and treatments used in the second experiment.

During the course of this experiment 10 jars containing Lucinoma borealis and one control jar were incubated with ^{14}C-bicarbonate. The conditions were as follows :

1-5. Each contained 4 Lucinoma incubated with 30µM Na_2S, for 8h, 24h, 48h, 72h and 96h respectively.

6. Contained 4 Lucinoma incubated for 96h with no sulphide.

7-8. Each contained 3 Lucinoma incubated with antibiotics and 30µM Na_2S for 24h and 96h respectively. The antibiotics used were a 50:50 (w:w) mix of penicillin / streptomycin (5000U/5000µM) and were added 24h prior to starting the experiment with the addition of ^{14}C-bicarbonate to ensure the effect of the antibiotics would be observed during the course of the experiment.

9. Contained 4 Lucinoma incubated for 96h with the algae, Ochromonas danica, and no sulphide, to determine whether Lucinoma would feed on algae. 24h prior to the start of the experiment, 5ml of O. danica were added to 20ml of filtered seawater with labelled bicarbonate being added at this stage so that the algae would incorporate ^{14}C. The experiment was then started by the addition of the algae/bicarbonate to the Lucinoma that were already in 25ml filtered seawater.

10. Contained 3 Lucinoma collected 140 days prior ("aged" Lucinoma) and kept in a starvation state (i.e. free of added sulphide or algae). This was incubated for 96h with 30µM Na_2S.

11. Symbiont-free bivalve molluscs incubated for 96h with no sulphide and containing 3 specimens of Venus striatula and 1 of Mya truncata, taken from the same site as Lucinoma, to act as controls.

(iii) Conditions and treatments used in the third experiment.

Ten jars containing L. borealis and one jar containing symbiont-free control bivalves were incubated with ^{14}C-bicarbonate under the following conditions :

1-4. Each contained 3 Lucinoma incubated with 30µM sodium thiosulphate for periods of 24h, 48h, 72h and 96h respectively, to ascertain whether or not thiosulphate was a useful energy source.

5-7. Each contained 4 Lucinoma and were incubated for 72h with 30µM, 100µM and 250µM Na_2S respectively.

8. Incubated for 72h with 30µM Na_2S but contained 3 Lucinoma collected at Dunstaffnage Bay, near Oban, to determine whether geographical location of the specimens influenced carbon dioxide fixation.

9-10. Two jars incubated for 72h with 30µM Na_2S, one being acclimated to 0°C and one to 20°C, containing 3 and 4 Lucinoma respectively.

11. Incubated for 72h with no sulphide or thiosulphate and contained four symbiont-free Venus striatula to act as a control.

6.2.3 Analysis Techniques.

At the end of each experiment 1ml of water was taken from each jar from which 10µl was used to determine the level of ^{14}C remaining in the seawater. The bivalves were then immediately placed on ice where they were dissected and divided into symbiont-containing gill tissue and symbiont-free non-gill tissue, the latter basically comprising the remainder of the tissues. The tissues were carefully

washed three times with filtered seawater before being immersed for 1h in seawater acidified to pH 2.5 with hydrochloric acid, to remove bicarbonate. After another rinse with filtered seawater, excess water was removed with absorbent paper before the tissues were weighed and placed in screw-top vials containing 5ml chloroform:methanol (2:1, v:v), sealed under nitrogen and stored at -20°C until lipid extraction. Lipid extraction was carried out as per the method of Folch et al. (1957) and is described in Chapter 2. The total lipid weight was determined and 0.5mg total lipid was streaked on a 20x20cm TLC plate precoated with silica gel 60 (without fluorescent indicator) to enable the separation of polar lipids as described in Chapter 2. The plates were stained lightly with iodine and the lipid classes were scraped off in a fume cupboard and transferred into scintillation mini-vials to which 2.5ml "Ecoscint" scintillation fluid was added. Radioactivity was determined using a Packard 2000CA Tri-Carb liquid scintillation analyser, vials being counted for 10 min each. The results were corrected against a blank, converted to cpm/mg lipid and then nCi ^{14}C incorporated / mg lipid. Statistical analysis of the results was carried out on an OPUS V computer using the Statgraphics package (Statistical Graphics Corporation, 1988). The tests used were one-way analysis of variance (ANOVA), unequal variance being detected by Bartlett's test and transformed logarithmically, and multiple-range tests to show where differences lie within each group.

6.3 Results.

6.3.1. Incorporation of $NaH^{14}CO_3$ into the lipid classes of Lucinoma borealis under varying environmental conditions.

Table 6.1 represents the incorporation of labelled bicarbonate into the lipid classes of the gills of Lucinoma borealis. Incorporation was highly variable between individual animals subjected to the same environmental conditions but some clear trends emerged. For all conditions tested, the greatest incorporation occurred in PE and PG with some into CL. Incorporation into other classes tended to be at a basal level, although occasionally substantial levels of fixation were observed in the neutral lipids. Substantial incorporation rates were usually never obtained in more than two of the replicate Lucinoma within each condition. Although statistically significant differences were not observed between the various treatments, general trends did occur. Thus, the most favourable conditions for incorporation seemed to be those of added sulphide and no sediment, followed by added sulphide and added sediment. Addition of sediment with no sulphide seemed to have a slightly deleterious effect when compared to incorporation with neither sediment nor sulphide. It was interesting to note that two of the control animals fixed appreciable amounts of ^{14}C. However, fixation in Cardium occurred in PC, PS, unknown and total neutral lipids as well as into PE. In Venus fixation occurred into PC and total neutrals.

Table 6.2 shows the incorporation of labelled bicarbonate into the non-gill, symbiont free tissues of L. borealis. It is difficult to notice any distinct trends within these tissues, and again there were no statistically significant differences between treatments. However, in no case did the total level of fixation approach those of the high values frequently observed within the corresponding gill tissue. For the non-gill tissue, the bulk of fixed carbon tended to appear in PE, the neutral lipids and to a lesser extent PC. Although the control animals generally fixed less carbon, the bulk also appeared within PC, PE and the neutrals.

6.3.2 Incorporation of $NaH^{14}CO_3$ into the lipid classes of Lucinoma borealis following incubation over a time course and varying environmental conditions.

Presented in Tables 6.3 and 6.4 are the results of the incorporation of labelled bicarbonate into the lipid classes of the gill and the non-gill tissues respectively. Tables 6.5 and 6.6 represent the mean values for the gills and non-gill tissues respectively. In these latter tables, letters are used to indicate differences and overlap between the different treatment groups, e.g. group "a" is statistically significantly different from "b". However groups designated ab and bc or a and ab show overlap and are not significantly different. These difference or overlaps were established using multiple range tests. When compared to the results in section 6.3.1., incorporation into the lipid classes was generally diminished

under the same environmental conditions. However, variation between replicates was notably less and one-way ANOVA established that there were significant differences within all lipid classes attributable to the various treatments ($p<0.01$).

Incorporation in the gills (Tables 6.3 and 6.5) was generally greatest in classes PE(1) and PG, with PE(1) incorporating a slightly greater amount. Incorporation into these classes was similar between 8h and 24h before increasing significantly after 48 hours incubation. The level increased up to 72 hours then decreased slightly after 96 hours although this latter change was not statistically distinct.

When no sulphide was added, after 96 hours incubation a slight but not statistically significant decrease in fixation into PE(1) and PG was observed, with respect to the same time point in the presence of sodium sulphide.

Addition of antibiotics seemed to cause little detectable effect after 24 hours incubation. However after 96 hours, incorporation into PE(1), PG and PI/CL was greatly increased in the presence of antibiotics, although results of the multiple range test show overlap between this and the incubation with Na_2S for 96h.

Animals offered algae showed a very low basal level of incorporation with no notable differences between the lipid classes. Nonetheless, incorporation levels were significantly different for all classes (except the unknown) from those animals sampled after 96h without the introduction of algae.

Lucinoma collected 140 days prior to incubation ("aged" Lucinoma) showed an incorporation into classes PG and PE(1) of only about half that observed with "fresh animals", although the difference was found not to be significant. The control, symbiont-free animals showed a low level of incorporation, mainly into PE, PC and the neutral lipids, although a discernible trend was difficult to identify. This low level of incorporation was found to be statistically significant from that found after 96h incubation of "fresh" Lucinoma with Na_2S within PC, PG, PE(2) and the neutrals.

Incorporation into the non-gill tissues (Tables 6.4 & 6.6) was very different from that observed in the gills. Statistically significant differences were observed within all classes with $p < 0.01$. The majority of incorporation was into the classes PC and PE. PC showed an almost linear increase in incorporation over the time course from 8 hours to 96 hours incubation with sodium sulphide. In PE , incorporated ^{14}C was low between 8 and 24 hours, then increased suddenly between 48-96 hours incubation. Fixation into PG was only about half the level of PC, although there was a more or less linear increase from 8 hours to 72 hours, which then decreased slightly between 72-96 hours. When no sulphide was added, incorporation was generally decreased when compared to the same time point with added sulphide, although a large incorporation into PC was observed. These differences caused by the omission of sulphide were generally not statistically significant, with the exception of the neutral lipids.

The addition of antibiotics caused little effect after 24 hours in the non-gill tissues, but after 96 hours incubation when compared

to the same time point with only Na_2S added, incorporation was reduced into PC, PG and PE, although this reduction was not statistically distinct.

When incubated with algae, there was very little incorporation into the non-gill tissues, as occurred in the gill, and within all classes there were significant differences when compared to the 96h incubation with only Na_2S added. Additionally, the "aged" Lucinoma showed a general decrease in incorporation when compared to the "fresh" Lucinoma at the same time point, the difference being statistically significant within PS, PI/CL, PG, PE and the neutral lipids. The control animals showed a very low level of fixation, about the same as that observed in those animals offered algae, the difference between the same time-point for Lucinoma being significantly different within all classes with the exception of the unknown.

6.3.3 Incorporation of $NaH^{14}CO_3$ into the Lipid Classes of Lucinoma borealis while varying Na_2S Concentration and Varying Incubation Time with Sodium Thiosulphate.

The results presented in Tables 6.7-6.10 represent the incorporation of radiolabelled bicarbonate into L. borealis under a variety of parameters. Significant differences were found between the treatment means in all lipid classes, with the exception of PE(1) in the gill, using one-way ANOVA (Tables 6.9 and 6.10). The addition of sodium thiosulphate to the gills (Tables 6.7 and 6.9) seems to have

little or no stimulatory effect within PG and PE when compared to the addition of varying concentrations of sulphide. However the bulk of incorporation of carbon in the presence of thiosulphate seems to occur within PC and the neutral lipids. There seems to be a very slight increase in carbon incorporation within the gill by increasing the incubation time with thiosulphate though this difference was not statistically significant.

Increasing the concentration of sodium sulphide seems to make little difference to the incorporation of ^{14}C into the gill lipid classes and no significant differences were evident.

When incubated at 0°C and 20°C in the presence of 30µM Na_2S, large differences in the amounts of incorporated ^{14}C were observed. Incorporation into the gill was similar, if not slightly diminished at 0°C when compared to that observed at 10°C, i.e. the temperature at which the rest of these experiments were carried out. When incubated at 20°C, incorporation of ^{14}C into the gills with respect to incubation at lower temperatures, was greatly increased and significant differences were observed in all classes except PG and PE(1).

Incorporation into the gills of the control, symbiont-free animals was negligible and was significantly different from that encountered within Lucinoma at the same time point for all lipid classes (except PC).

The results for the non-gill tissues (Tables 6.8 and 6.10) were greatly different from those of the gills. Firstly, the non-gill

tissue did not contain the class designated PE(1). When the non-gill tissue was incubated with thiosulphate, the bulk of the incorporation appeared in PC, PE(2) and the neutrals. It also seems that, if these main classes are considered, then increasing incubation time with thiosulphate increases the incorporation of labelled bicarbonate, although significant differences were only found between 24h and 96h incubation. If these classes are considered again, when looking at the effect of increasing sulphide concentration then it is evident that there was no real difference in incorporation between incubation with 30μM and 100μM Na_2S. However, incubation with 250μM Na_2S seemed to cause an increase in incorporation within the main classes, although this difference was only significant within PC. No significant differences were found when the animals collected from the Oban site were compared.

With the exception of PG and the neutrals, incubation at 20°C produced a significant increase in bicarbonate incorporation when compared with incubation at 0°C (and 10°C).

Incorporation within the control animals was generally similar or slightly less than that observed within Lucinoma under the same conditions and the only significant difference was found within CL.

6.3.4 Fatty Acid Analyses of the Gill Lipid Classes of Starved and Unstarved Lucinoma borealis and Total Lipid of Lucinoma Offered Algae.

Tables 6.11 and 6.12 represent the fatty acid analyses of the lipid classes of gills taken from starved and unstarved Lucinoma

borealis respectively. The proportion of individual fatty acids varies greatly within each class. Considering first the starved Lucinoma (Table 6.11), the major fatty acids within each lipid class were as follows :

	PC	PS/PI	PG	PE/CL	Unknown	Neutrals
16:0			15.3%		12.4%	21%
16:1(n-7)	16.6%		11.8%	15.4%		10.7%
18:0			10.5%			
18:1(n-7)	10.8%					
22:2 NMID		19.2%		22.4%		

The main fatty acids within the lipid classes of the unstarved Lucinoma (Table 6.12) were as follows :

	PC	PS	PI/CL	PG	PE(1)	PE(2)	Unknown	Neutral
16:0	13.7%	16.2%	17.3%	18%	17.4%		15.3%	20.2%
16:1(n-7)	14%	11.9%	19.1%	18.7%	26.5%		12.6%	13.6%
18:0	10.6%	12.9%	15.8%			12.6%		
18:1(n-7)	13.3%		11.6%		15.8%			
22:2 NMID		13.9%			12.6%	29.7%		

Table 6.13 represents the fatty acid composition of Lucinoma borealis subsequent to being offered the algae, Ochromonas danica, for 96 hours. The gill and non-gill tissues were analysed from 3 animals, all of which had fairly similar compositions. The main fatty acids of the gill tissue were as follows : 16:0 (10-18%), 16:1(n-7) (15-16%),

18:0 (7-10%), 18:1(n-7) (8-13%), 20:4(n-6) (3-6%) and 22:2 NMID (13-17%). The main fatty acids of the non-gill tissues were 16:0 (14-21%), 16:1(n-7) (30-40%), 18:0 (2-7%), 18:1(n-7) (7-8%) and 22:2 NMID (6-9%).

Table 6.1 The Incorporation of Radioactive $NaH^{14}CO_3$ into the Lipid Classes of the Gill Tissue of Lucinoma borealis (nCi ^{14}C/mg lipid).

condition	replicate no.	LIPID CLASS PC	PS	PI	PG	PE	CL	Unknown	Neutral
+sulphide	2	0.054	0.029	0.021	0.006	0.07	0.018	0.016	0.044
	3	0.051	0.029	0.023	0.073	0.085	0.068	0.033	0.163
-sediment	4	0.393	0.112	0.922	0.854	5.022	0.005	0.996	1.185
	5	0.21	0.064	0.286	3.582	3.063	0.594	0.437	0.635
-sulphide	1	0.057	0.016	0.045	0.065	0.131	0.053	0.019	0.086
	2	0.038		0.004		0.226	0.128	0.018	0.135
-sediment	3	0.002	0	0.006	0.004	0.035	0.012	0	0.01
	4	0.194	0.095	0.179	0.282	1.87	1.732	0.067	0.41
	5	0.179	0.029	0.138	0.496	0.635	0.345	0.112	0.334
+sulphide	1	0.042	0.01	0.09	0.026	0.522	0.054	0.028	0.14
	2	0.169	0.083	0.11	0.093	1.002	0.001	0.079	0.416
+sediment	3	0.002	0	0.02	0	0.304	0.037	0	0.1
	4	0.333	0.115	0.528	2.1	3.31	0.715	0.297	0.754
	5	0.046	0.005	0.036	0.191	0.311	0.047	0.063	0.172
-sulphide	1	0.024	0.022	0.014	0.155	0.155	0.038	0.121	0.198
	2	0.176	0.033	0.214	0.876	0.988	0.209	0.064	0.764
+sediment	3	0	0	0.002	0	0.144	0.024	0.026	0.133
	4	0.017	0.04	0.043	0.109	0.132	0.044	0.008	0.106
	5	0.038	0.137	0.082	0.564	0.624	0.16	0.119	0.587
controls	Cardium	0.592	0.635	0.28	0.006	0.405	0.062	0.486	0.474
	Cardium	0.053	0.001	0	0	0.008	0	0.017	0.074
	Venus	0.158	0	0.04	0	0.037	0.001	0.034	0.543
	Venus	0.096	0.004	0.03	0	0.072	0.027	0.054	0.202

where : PC = phosphatidylcholine PS = phosphatidylserine

PI = phosphatidylinositol PG = phosphatidylglycerol

PE = phosphatidylethanolamine CL = cardiolipin

Table 6.2 The incorporation of Radioactive $NaH^{14}CO_3$ into the lipid classes of the Mantle/Foot Tissue of Lucinoma borealis (nCi ^{14}C/mg lipid).

condition	replicate no.	LIPID CLASS PC	PS	PI	PG	PE	Unknown	Neutral
+sulphide +sediment	1	0.456	0.098	0.178	0.029	0.784	0.213	0.687
	3	0.058	0.073	0.016	0.025	0.179	0.023	0.256
	4	0.016	0.04	0	0.013	0.073	0.41	0.107
	5	0.503	0.338	0.164	0.035	0.655	0.223	0.623
-sulphide -sediment	1	0.01	0.045	0.005	0	0	0.05	0.09
	2	0.136	0.001	0.05	0.034	0.247	0.044	0.356
	4	0.289	0.103	0.05	0.032	0.322	0.077	0.233
	5	0.23	0.283	0.269	0.476	0.764	0.188	0.562
+sulphide +sediment	1	0.19	0.057	0.162	0.079	0.271	0.124	0.190
	2	0.131	0.079	0.223	0.323	0.275	0.1	0.13
	3	0.526	0.261	0.087	0.305	1.19	0.169	0.409
	4	0.314	0.24	0.379	0.394	1.38	0.582	0.517
	5	0.528	0.444	0.341	0.606	0.61	0.22	0.437
-sulphide +sediment	1	0.39	0.18	0.142	0.152	0.495	0.433	0.399
	2	0.096	0.214	0.169	0.116	0.21	0.107	0.274
	3	0.251	0.234	0.193	0.094	0.343	0.096	0.134
	4	0.052	0.003	0.084	0.02	0.134	0.028	0.086
	5	0.066	0	0.012	0.007	0.153	0.021	0.163
controls	Cardium	1.1	0	0.012	0.007	0.153	0.021	0.163
	Cardium	0.005	0.01	0.434	0.007	0.169	0.028	0.133
	Venus	0.023	0	0.138	0	0.076	0.003	0.061
	Venus	0.128	0.007	0.049	0.013	0.781	0.056	0.177

Table 6.3 Incorporation of $NaH^{14}CO_3$ into the gill tissue of Lasionema hermilis (nCi ^{14}C incorporated per mg lipid).

Time/ condition	replicate no.	LIPID CLASS PC	PS	PI/CL	PG	PA(1)	PA(2)	Unknown	Neutral
time = 0h	1	0.477	0.176	0.344	0.732	0.97	0.317	0.134	0.468
	2	0.509	0.248	0.197	0.203	0.107	0.11	0.224	0.677
	3	0.415	0.172	0.203	0.33	0.378	0.124	0.118	0.396
	4	0.203	0.08	0.196	0.074	0.075	0.076	0.151	0.099
time=24h	1	0.194	0.103	0.303	0.227	0.382	0.127	0.128	0.139
	2	0.476	0.127	0.682	0.446	0.163	0.112	0.174	0.772
	3	0.183	0.152	0.175	0.251	0.275	0.139	0.217	0.164
	4	0.23	0.149	0.302	0.238	0.311	0.122	0.152	0.125
time =24h +antibiotics	1	0.565	0.511	0.121	0.168	0.236	0.169	0.083	0.202
	2	2.41	0.169	0.207	0.275	0.455	0.183	0.122	0.989
	3	0.237	0.101	0.473	0.386	0.351	0.182	0.147	0.286
time=48h	1	0.774	0.166	0.903	1.25	1.94	0.553	0.159	0.442
	2	0.593	0.255	0.957	1.63	2.1	0.391	0.426	0.489
	3	0.3	0.137	0.709	0.899	1.32	0.236	0.189	0.346
	4	0.386	0.23	0.531	1.04	1.25	0.376	0.281	0.442
time=72h	1	0.28	0.162	0.293	0.785	0.776	0.23	0.222	0.293
	2	0.459	0.24	0.257	0.672	0.553	0.258	0.378	0.344
	3	0.287	0.293	0.228	0.39	0.533	0.16	0.257	0.581
	4	0.612	0.404	2.28	0.16	6.86	0.589	0.842	0.497
time=96h	1	0.735	0.246	0.956	1.1	1.14	0.358	0.308	0.315
	2	0.476	0.225	0.68	0.414	0.69	0.32	0.354	0.931
	3	0.762	0.183	0.789	0.951	1.09	0.334	0.233	0.978
	4	0.834	0.303	1.15	0.834	1.66	0.454	0.548	1.35
time=96h -sulphide	1	0.698	0.361	0.411	1.5	1.26	0.463	0.206	0.316
	2	0.379	0.19	0.387	0.129	0.204	0.275	0.365	0.219
	3	0.804	0.298	0.756	0.35	0.583	0.355	0.378	0.308
	4	0.411	0.193	0.709	0.736	1.02	0.218	0.385	0.405
time=96h +antibiotics	1	0.366	0.213	1.64	3.61	3.01	0.401	0.45	0.28
	2	0.433	0.314	1.34	2.76	2.53	0.583	0.471	0.255
	3	0.877	0.416	0.77	1.21	1.3	0.484	0.632	0.419
time=96h +algae	1	0.151	0.08	0.156	0.082	0.092	0.085	0.098	0.105
	2	0.146	0.073	0.07	0.091	0.105	0.075	0.087	0.102
	3	0.096	0.07	0.097	0.085	0.067	0.084	0.082	0.081
	4	0.302	0.121	0.182	0.232	0.135	0.093	0.197	0.131
time=96h + aged Lasionema	1	0.273	0.108	0.418	0.501	0.641	0.146	0.157	0.076
	2	0.32	0.102	0.195	0.254	0.32	0.12	0.157	0.399
	3	0.281	0.107	0.257	0.454	0.652	0.233	0.211	0.297
time=96h Vegas & Uro	1	0.13	0.066	0.11	0.105	0.167		0.105	0.202
	2	0.127	0.066	0.064	0.075	0.08		0.078	0.176
	3	0.279	0.16	0.22	0.123	0.328		0.239	0.769
	4	0.326	0.17	0.251	0.427	0.702		0.295	0.669

Table 6.4 Incorporation of $NaH^{14}CO_3$ into the non-gill tissue of Lucinoma borealis ($uCi\ ^{14}C$ incorporated per ng lipid).

Time/ condition	replicate no.	LIPID CLASS PC	PS	PI/CL	PG	PE	Unknown	Neutral
time = 0h	1	0.293	0.151	0.169	0.147	0.172	1.53	0.421
	2	0.146	0.13	0.072	0.084	0.159	0.08	0.32
	3	0.147	0.125	0.163	0.123	0.104	0.115	0.335
	4	0.172	0.08	0.13	0.094	0.128	0.118	0.102
time=24h	1	0.379	0.124	0.19	0.222	0.198	0.481	0.377
	2	0.351	0.148	0.213	0.149	0.323	0.22	0.409
	3	0.275	0.178	0.162	0.224	0.586	0.162	0.276
	4	0.394	0.175	0.208	0.434	0.449	0.217	0.385
time =24h +antibiotics	1	0.72	0.213	0.328	0.218	0.294	0.135	0.258
	2	0.522	0.091	0.393	0.108	0.127	0.142	0.311
	3	0.523	0.157	0.229	0.175	0.313	0.16	0.533
time=48h	1	0.533	0.171	0.345	0.334	0.715	0.302	0.526
	2	0.446	0.142	0.203	0.301	0.691	0.203	0.622
	3	1.15	0.308	0.573	0.538	1.92	0.304	0.768
	4	0.563	0.288	0.265	0.365	0.77	0.215	0.546
time=72h	1	0.854	0.229	0.331	0.802	0.837	0.338	0.408
	2	1.1	0.328	0.43	0.572	1.05	0.354	0.503
	3	0.849	0.296	0.292	0.785	1.05	0.31	0.683
	4	1.34	0.368	0.603	0.607	1.56	0.496	0.6
time=96h	1	1.47	0.567	0.838	0.682	1.53	0.615	1.63
	2	0.885	0.261	0.605	0.296	0.892	0.427	1.51
	3	1.07	0.244	0.622	0.644	0.823	0.287	1.12
	4	0.942	0.381	0.651	0.364	1.03	0.426	0.692
time=96h +sulphide	1	0.718	0.289	0.186	0.295	0.506	0.125	0.294
	2	1.39	0.309	0.306	0.402	0.753	0.244	0.361
	3	4.22	0.491	0.502	0.311	0.767	0.624	0.598
	4	6.67	0.623	0.663	0.304	0.469	0.647	0.368
time=96h +antibiotics	1	0.531	0.259	0.346	0.472	0.674	0.385	0.308
	2	0.725	0.241	0.352	0.272	0.602	0.369	0.378
	3	1.08	0.24	0.565	0.389	0.804	0.37	0.605
time=96h +algae	1	0.21	0.064	0.16	0.101	0.09	0.103	0.11
	2	0.184	0.073	0.094	0.1	0.12	0.115	0.108
	3	0.208	0.091	0.081	0.081	0.097	0.081	0.077
	4	0.281	0.116	0.159	0.162	0.207	0.129	0.135
time=96h aged Lucinoma	1	0.237	0.117	0.161	0.131	0.309	0.151	0.264
	2	1.2	0.113	0.181	0.213	0.342	0.156	0.231
	3	0.567	0.122	0.178	0.193	0.278	0.162	0.409
time=96h Tonne & Rho	1	0.151	0.01	0.136	0.119	0.121	0.104	0.144
	2	0.127	0.066	0.064	0.075	0.08	0.078	0.176
	3	0.229	0.098	0.169	0.129	0.176	0.109	0.203
	1	0.444	0.214	0.37	0.211	0.969	0.406	0.276

Table 6.5 The mean incorporation of $NaH^{14}CO3$ into the lipid classes of the gill of Lucinoma borealis (nCi ^{14}C incorporated/mg lipid ; where Hom.Gps. indicates homogeneous groups as determined by a multiple range test, confidence interval 95% and the p value determined by one-way analysis of variance).

	PC	Hom.Gps.	PS	Hom.Gps.	PI/CL	Hom.Gps.
	(p=0.0026)		(p=0.0006)		(p=0.0000)	
T=8h	0.401	abc	0.169	abcd	0.235	abc
T=24h	0.271	abcd	0.133	abcd	0.366	bcd
T=48h	0.498	bcd	0.197	bcd	0.773	cd
T=72h	0.410	abcd	0.275	cd	0.765	abc
T=96h	0.702	d	0.239	bcd	0.794	cd
T=96h -sulphide	0.573	cd	0.256	cd	0.566	bcd
T=24h+ Antibiotics	1.071	cd	0.260	bcd	0.267	abc
T=96h+ antibiotics	0.552	bcd	0.314	d	1.250	d
T=96h + algae	0.174	a	0.086	a	0.126	a
T=96h aged Lucinoma	0.291	abcd	0.106	ab	0.290	abc
T=96h controls	0.216	ab	0.121	ab	0.161	abc

	PG	Hom.Gps.	PE(1)	Hom.Gps.	PE(2)	Hom.Gps.
	(p=0.0000)		(p=0.0000)		(p=0.0000)	
T=8h	0.335	abc	0.383	ab	0.157	bcd
T=24h	0.291	abc	0.283	ab	0.125	ab
T=48h	1.203	de	1.653	d	0.389	e
T=72h	2.502	cde	2.181	bcd	0.359	de
T=96h	0.825	cde	1.095	cd	0.367	e
T=96h -sulphide	0.679	bcd	0.767	bcd	0.328	cde
T=24h+ Antibiotics	0.276	abc	0.347	abc	0.178	ab
T=96h+ antibiotics	2.527	e	2.280	d	0.489	e
T=96h + algae	0.123	a	0.099	a	0.084	a
T=96h aged Lucinoma	0.403	abcd	0.538	bcd	0.166	abc
T=96h controls	0.183	ab			0.319	ab

	Unknown	Hom.Gps.	Neutral	Hom.Gps.
	(p=0.0000)		(p=0.0000)	
T=8h	0.157	ab	0.410	bc
T=24h	0.168	ab	0.300	ab
T=48h	0.314	bc	0.430	bc
T=72h	0.400	bc	0.404	bc
T=96h	0.361	bc	0.894	d
T=96h -sulphide	0.376	bc	0.311	ab
T=24h+ Antibiotics	0.117	ab	0.292	ab
T=96h+ antibiotics	0.520	de	0.318	bc
T=96h + algae	0.116	a	0.105	a
T=96h aged Lucinoma	0.175	ab	0.357	ab
T=96h controls	0.179	ab	0.405	bc

Table 6.6 The Mean Incorporation of $NaH^{14}CO3$ into the lipid classesof Non-Gill Tissue of Lucinoma borealis ($nCi^{14}C$ incorporated/mg lipid; where the p value was determined by a one-way analysis of variance and where Hom.Gps. indicates homogenous groups determined by a multiple range test, confidence interval 95%).

	PC	Hom.Gps.	PS	Hom.Gps.	PI/CL	Hom.Gps.
	(p=0.0000)		(p=0.0001)		(p=0.0000)	
T=8h	0.189	a	0.122	abcd	0.134	ab
T=24h	0.350	abc	0.156	abcde	0.188	abcd
T=48h	0.668	cd	0.227	bcde	0.347	cde
T=72h	1.036	de	0.305	cde	0.414	de
T=96h	1.292	de	0.358	de	0.579	e
T=96h -sulphide	3.200	e	0.378	e	0.414	de
T=24h+ antibiotics	0.588	bcd	0.154	abcde	0.317	bcde
T=96h+ antibiotics	0.779	cd	0.247	bcde	0.414	de
T=96h + algae	0.201	ab	0.087	ab	0.124	a
T=96h aged Lucinoma	0.668	bcd	0.117	abc	0.167	abcd
T=96h controls	0.238	ab	0.097	a	0.184	abc

	PG	Hom.Gps.	PE	Hom.Gps.	Unknown	Hom.Gps.
	(p=0.0000)		(p=0.0000)		(p=0.0000)	
T=8h	0.112	a	0.141	a	0.461	ab
T=24h	0.257	abc	0.389	bcd	0.270	ab
T=48h	0.385	cd	1.024	de	0.256	ab
T=72h	0.692	e	1.124	e	0.374	b
T=96h	0.497	de	1.069	e	0.439	b
T=96h -sulphide	0.328	bcd	0.624	cde	0.310	ab
T=24h+ antibiotics	0.167	abc	0.245	ab	0.146	ab
T=96h+ antibiotics	0.378	cd	0.693	de	0.375	b
T=96h + algae	0.111	a	0.129	a	0.107	a
T=96h aged Lucinoma	0.179	abc	0.243	abc	0.156	ab
T=96h controls	0.134	ab	0.237	ab		

	Neutral	Hom.Gps.
	(p=0.0000)	
T=8h	0.295	bc
T=24h	0.362	bcd
T=48h	0.616	de
T=72h	0.499	cd
T=96h	1.188	e
T=96h -sulphide	0.355	bcd
T=24h+ antibiotics	0.367	bcd
T=96h+ antibiotics	0.364	bcd
T=96h + algae	0.108	a
T=96h aged Lucinoma	0.301	bc
T=96h controls	0.200	ab

Table 6.7 The incorporation of $BaH^{14}CO_3$ into the Gill Tissues of *Lariosoma luvialis* ($dCi^{14}C$ incorporated/ug lipid).

condition	replicate no.	LIPID CLASS PC	PS	PI	CL	PG	PA(1)	PA2	Unknown	Control
time = 24h	1	2.820	0.797	0.240	0.252	0.213	0.324	0.284	0.362	0.562
+ thiosulphate	2	0.178	0.086	0.215	0.198	0.551	0.459	0.634	0.124	0.364
	3	0.097	0.069	0.131	0.084	0.047	0.127	0.066	0.081	0.160
time = 48h	1	0.686	0.096	0.101	0.127	0.463	0.202	0.211	0.120	0.229
+ thiosulphate	2	0.938	0.219	0.262	0.305	0.430	0.535	0.432	0.593	1.700
	3	0.133	0.068	0.099	0.109	0.136	0.165	0.093	0.077	0.229
time = 72h	1	0.547	0.600	0.124	0.278	0.099	0.416	0.263	0.222	0.398
+ thiosulphate	2	0.501	0.301	0.133	0.192	0.532	0.5	0.272	0.201	0.431
	3	0.650	0.4	0.172	0.143	0.681	0.453	0.331	0.257	0.523
time = 96h	1	1.040	0.275	0.362	0.301	0.912	0.715	0.696	0.305	0.630
+ thiosulphate	2	0.651	0.510	0.268	0.231	0.697	0.795	0.619	0.430	0.555
	3	0.593	0.254	0.115	0.181	0.229	0.251	0.294	0.276	0.320
	1	0.112	0.071	0.077	0.198	0.113	0.073	0.087	0.103	0.171
time = 72h	2	0.915	0.369	0.380	0.311	0.513	0.359	0.370	0.536	0.597
+ 30pM Ba_2S	3	0.590	0.236	0.238	0.398	0.944	1.440	0.373	0.584	0.365
	4	0.389	0.196	0.162	0.171	0.583	0.447	0.189	0.270	0.361
	1	0.351	0.195	0.167	0.218	0.458	0.232	0.173	0.229	0.375
time= 72h	2	0.577	0.426	0.210	0.358	0.606	0.286	0.258	0.418	0.726
+ 100pM Ba_2S	3	0.188	0.172	0.150	0.176	0.347	0.407	0.214	0.127	0.332
	4	0.226	0.109	0.154	0.122	0.160	0.156	0.229	0.163	0.308
	1	0.935	0.735	0.477	0.309	0.477	0.484	1.100	0.914	1.560
time = 72h	2	0.353	0.231	0.200	0.174	0.282	0.308	0.244	0.197	0.398
+ 250pM Ba_2S	3	0.673	0.431	0.226	0.478	1.210	1.260	0.545	0.301	0.761
	4	0.639	0.167	0.114	0.094	0.269	0.269	0.245	0.157	0.192
time = 72h	1	0.258	0.217	0.1	0.238	0.101	0.109	0.213	0.182	0.521
+ 30pM Ba_2S	2	0.080	0.456	0.162	0.499	0.347	0.366	0.390	0.490	0.809
(40hm)	3	0.325	0.273	0.153	0.345	0.178	0.182	0.284	0.219	0.465
	1	0.412	0.100	0.178	0.181	0.294	0.412	0.147	0.189	0.673
time = 72h	2	0.278	0.200	0.151	0.156	0.509	0.697	0.238	0.196	0.638
0°C	3	0.524	0.140	0.206	0.320	0.478	0.519	0.256	0.196	0.790
	1	4.820	1.560	3.560	1.580	0.981	1.160	4.790	4.010	3.110
time = 72h	2	2.110	0.797	1.970	0.749	0.946	1.280	3.040	3.300	1.870
20°C	3	1.410	0.388	0.620	0.634	0.296	0.416	0.645	1.200	0.844
	4	2.700	0.660	0.532	1.990	0.261	1.390	2.760	2.100	1.360
	1	0.072	0.033	0.036	0.027	0.032		0.051		0.057
time = 72h	2	0.083	0.030	0.043	0.030	0.064		0.100		0.070
controls	3	0.092	0.033	0.032	0.032	0.032		0.064		0.071
	4	0.155	0.070	0.109	0.215	0.075		0.190		0.349

Table 6.8 The Incorporation of $NaH^{14}CO_3$ into the Non-Gill Tissues of Lasionas borealis (nCi^{14}C incorporated/ mg lipid).

Time/ condition	replicate no.	LIPID CLASS							
		PC	PE	PI	CL	PS	PE(2)	Unknown	Neutral
time = 24h	1	0.166	0.086	0.424	0.211	0.174	0.174	0.104	0.389
+ thiosulphate	2	0.910	0.300	0.203	0.143	0.192	0.217	0.103	0.395
	3	0.104	0.099	0.176	0.178	0.187	0.100	0.093	0.188
time = 48h	1	0.190	0.086	0.149	0.103	0.184	0.313	0.156	0.365
+ thiosulphate	2	0.383	0.121	0.272	0.267	0.531	0.702	0.236	0.164
	3	0.154	0.062	0.133	0.097	0.137	0.103	0.067	0.177
time = 72h	1	0.941	0.342	0.158	0.277	0.116	0.463	0.259	0.916
+ thiosulphate	2	0.620	0.201	0.230	0.210	0.232	0.501	0.250	0.670
	3	0.701	0.297	0.301	0.193	0.210	0.459	0.239	0.602
time = 96h	1	2.450	0.387	0.441	0.192	0.309	0.819	0.281	2.450
+ thiosulphate	2	0.613	0.400	0.205	0.127	0.141	0.473	0.233	0.715
	3	0.921	0.174	0.220	0.141	0.251	0.623	0.335	0.806
	1	0.147	0.073	0.159	0.101	0.068	0.062	0.070	0.099
time = 72h	2	0.304	0.298	0.100	0.184	0.199	0.294	0.344	0.497
+ 30pM Na_2S	3	0.362	0.161	0.168	0.162	0.175	0.274	0.291	0.459
	4	0.372	0.171	0.176	0.163	0.210	0.286	0.372	0.677
	1	0.388	0.239	0.265	0.213	0.207	0.355	0.133	0.916
time= 72h	2	0.342	0.213	0.266	0.233	0.237	0.286	0.384	0.665
+ 100pM Na_2S	3	0.339	0.102	0.184	0.139	0.148	0.318	0.156	0.443
	4	0.302	0.125	0.123	0.100	0.220	0.226	0.150	0.442
	1	2.220	0.496	0.776	0.503	0.663	2.150	1.010	2.680
time = 72h	2	0.555	0.264	0.177	0.138	0.210	0.499	0.251	0.739
+ 250pM Na_2S	3	0.957	0.258	0.220	0.169	0.234	0.628	0.278	0.826
	4	2.300	0.327	0.461	0.192	0.283	0.725	0.353	0.712
time = 72h	1	0.291	0.184	0.176	0.155	0.116	0.135	0.135	0.681
+ 30pM Na_2S	2	0.513	0.209	0.184	0.318	0.221	0.500	0.290	0.583
(0hm)	3	0.525	0.168	0.150	0.276	0.182	0.315	0.216	0.500
	1	0.416	0.139	0.169	0.263	0.459	0.671	0.192	0.559
time = 72h	2	0.392	0.184	0.147	0.306	0.685	0.305	0.302	0.880
0°C	3	0.496	0.263	0.300	0.354	0.515	0.380	0.186	0.706
	1	4.780	0.781	2.990	0.830	0.907	3.680	2.490	13.900
time = 72h	2	2.670	0.860	1.330	0.568	1.690	1.780	3.360	0.824
20°C	3	3.280	0.850	0.377	1.630	0.337	2.000	1.650	1.630
	4	5.560	1.200	0.736	2.170	0.476	6.210	2.570	2.500
	1	0.156	0.079	0.125	0.063	0.000	0.194		0.253
time = 72h	2	0.268	0.086	0.157	0.064	0.150	0.315		0.613
controls	3	0.361	0.079	0.181	0.076	0.133	0.256		0.178
	4	0.522	0.217	0.419	0.067	0.205	0.714		1.090

Table 6.9 The mean incorporation of $NaH^{14}CO3$ in the lipid classes of the gill tissues of Lucinoma borealis (nCi ^{14}C incorporated/mg lipid ; where the p value was determined by one-way analysis of variance and where Hom.Gps. indicates Homogenous groups determined by a multiple range test, confidence interval 95%).

	PC (p=0.0006)	Hom.Gps.	PS (p=0.0003)	Hom.Gps.	PI (p=0.0000)	Hom.Gps.
t=24h + thiosulphate	1.032	ab	0.317	ab	0.195	b
t=48h + thiosulphate	0.493	ab	0.127	ab	0.197	ab
t=72h + thiosulphate	0.566	bc	0.460	bc	0.143	ab
t=96h + thiosulphate	0.761	bc	0.346	bc	0.242	b
t=72h + 30µM sulphide	0.502	ab	0.213	b	0.214	b
t=72h + 100µM sulphide	0.336	ab	0.226	bc	0.170	b
t=72h + 250µM sulphide	0.650	bc	0.386	bc	0.254	b
t=72h (Oban) +30µM Sulphide	0.301	ab	0.315	bc	0.138	b
t=72h 0°C	0.405	ab	0.147	ab	0.205	b
t=72h 20°C	2.760	c	0.828	c	1.616	c
t=72h controls	0.101	a	0.044	a	0.055	a

	CL (p=0.0000)	Hom.Gps.	PG (0.0034)	Hom.Gps.	PE(1) (p=0.1045)	Hom.Gps.
t=24h + thiosulphate	0.178	ab	0.270	ab	0.303	ab
t=48h + thiosulphate	0.180	ab	0.345	b	0.294	ab
t=72h + thiosulphate	0.204	b	0.411	b	0.456	ab
t=96h + thiosulphate	0.238	b	0.613	b	0.560	ab
t=72h + 30µM sulphide	0.270	b	0.538	b	0.580	ab
t=72h + 100µM sulphide	0.219	b	0.343	b	0.250	a
t=72h + 250µM sulphide	0.264	b	0.560	b	0.580	ab
t=72h (Oban) +30µM Sulphide	0.361	bc	0.209	ab	0.219	a
t=72h 0°C	0.218	b	0.427	b	0.609	ab
t=72h 20°C	1.238	c	0.621	b	1.041	b
t=72h controls	0.076	a	0.046	a		

	PE(2) (p=0.0001)	Hom.Gps.	Unknown (p=0.0001)	Hom.Gps.	Neutral (p=0.0006)	Hom.Gps.
t=24h + thiosulphate	0.301	ab	0.189	a	0.349	ab
t=48h + thiosulphate	0.245	ab	0.197	a	0.746	b
t=72h + thiosulphate	0.289	ab	0.227	a	0.451	ab
t=96h + thiosulphate	0.536	b	0.337	a	0.502	b
t=72h + 30µM sulphide	0.255	ab	0.373	a	0.374	ab
t=72h + 100µM sulphide	0.219	ab	0.334	a	0.435	ab
t=72h + 250µM sulphide	0.534	b	0.387	a	0.723	b
t=72h (Oban) +30µM Sulphide	0.269	ab	0.297	a	0.598	b
t=72h 0°C	0.214	ab	0.194	a	0.687	b
t=72h 20°C	2.804	c	2.713	b	1.796	c
t=72h controls	0.096	a			0.137	a

Table 6.10 The mean incorporation of $NaH^{14}CO3$ into the lipid classes of the non-gill tissue of Lucinoma borealis ($nCi\ ^{14}C$ incorporated/mg lipid; where the p value was determined by a one way analysis of variance and where Hom.Gps. indicates homogenous groups determined by a multiple range test, confidence interval 95%).

	PC (p=0.0000)	Hom.Gps.	PS (p=0.0000)	Hom.Gps.	PI (p=0.0010)	Hom.Gps.
t=24h + thiosulphate	0.393	a	0.164	ab	0.268	a
t=48h + thiosulphate	0.312	a	0.090	a	0.184	a
t=72h + thiosulphate	2.262	abc	0.840	b	0.689	a
t=96h + thiosulphate	1.328	bc	0.387	bc	0.289	a
t=72h + 30µM sulphide	0.296	a	0.176	ab	0.171	a
t=72h + 100µM sulphide	0.343	ab	0.170	ab	0.200	a
t=72h + 250µM sulphide	1.508	c	0.336	b	0.409	a
t=72h (Oban) +30µM sulphide	0.443	abc	0.167	ab	0.170	a
t=72h $0^{o}C$	0.435	abc	0.195	ab	0.205	a
t=72h $20^{o}C$	4.408	d	0.923	c	1.208	b
t=72h controls	0.248	a	0.075	a	0.154	a

	CL (p=0.0000)	Hom.Gps.	PG (p=0.0006)	Hom.Gps.	PE(2) (p=0.0000)	Hom.Gps.
t=24h + thiosulphate	0.177	b	0.184	ab	0.164	a
t=48h + thiosulphate	0.156	b	0.284	ab	0.373	abc
t=72h + thiosulphate	0.680	b	0.558	ab	1.423	abc
t=96h + thiosulphate	0.153	b	0.234	ab	0.638	bc
t=72h + 30µM sulphide	0.153	b	0.168	a	0.229	ab
t=72h + 100µM sulphide	0.171	b	0.226	ab	0.296	abc
t=72h + 250µM sulphide	0.251	b	0.348	abc	1.001	bc
t=72h (Oban) +30µM sulphide	0.250	b	0.173	a	0.317	abc
t=72h $0^{o}C$	0.274	b	0.486	bc	0.385	abc
t=72h $20^{o}C$	1.297	c	0.853	c	3.348	d
t=72h controls	0.054	a	0.121	a	0.225	abc

	Unknown (p=0.0000)	Hom.Gps.	Neutral (p=0.0021)	Hom.Gps.
t=24h + thiosulphate	0.100	a	0.324	a
t=48h + thiosulphate	0.153	a	0.229	ab
t=72h + thiosulphate	0.748	ab	2.188	abc
t=96h + thiosulphate	0.283	ab	1.324	bc
t=72h + 30µM sulphide	0.369	ab	0.433	ab
t=72h + 100µM sulphide	0.255	ab	0.617	ab
t=72h + 250µM sulphide	0.473	b	1.239	bc
t=72h (Oban) +30µM sulphide	0.216	ab	0.591	ab
t=72h $0^{o}C$	0.227	ab	0.688	abc
t=72h $20^{o}C$	2.518	c	4.684	c
t=72h controls			0.281	ab

Table 6.11 The Percentage Composition of Fatty Acids of the Lipid Classes Extracted from the Gill Tissue of Lucinoma borealis. Collected from Mill Bay, Salcombe, Devon, and Following 8 Weeks Starvation.

Fatty acid	PC	PS/PI	PG	PE/CL	Unknown	Neutral
14:0	0.7	0.7	1.3	0.4	2.1	1.9
14:1			1.3	0.7		0.9
i-15:0	0.4	0.7	0.7	0.2		0.7
a-15:0	2.1	2.9	5.2	1.7	4.3	1.7
15:0	1.6	0.5	1.2	0.7	1.6	1.5
15:1	2.0	0.7	3.9	2.5		1.6
i-16:0	1.4	1.2	2.0	0.6	3.7	1.0
16:0	8.2	3.8	15.3	4.5	10.4	20.6
16:1(n-7)	16.0	3.8	11.8	15.3	6.2	10.5
16:2	6.2	6.4	14.4	1.9	19.5	5.6
16:3	1.8	0.9	1.1	1.2		1.2
16:4	0.4					
i-17:0		0.6	1.9	0.4		0.9
a-17:0		1.4	1.5	2.4		1.3
17:0	2.0	1.0	3.3	0.8		1.1
17:1	1.7	1.2	4.1	3.5		1.9
18:0	5.7	6.2	10.5	4.5	5.5	5.0
18:1(n-9)	4.7	1.8	3.6	1.4	6.8	5.6
18:1(n-7)	10.4	2.4	5.3	5.5	1.5	5.5
18:1(n-5)	1.1	0.3		0.2		0.3
18:2(n-9)	0.6	0.4		0.2		
18:2(n-6)	1.9	1.1	1.5	1.3	6.3	3.5
18:3(n-6)		0.3		0.6		0.9
18:3(n-3)	0.7			0.8		0.5
18:4	0.4	0.4				0.8
19:0	0.4					0.7
20:0		1.9	1.6	0.4	2.8	0.8
20:1	3.1	3.9		1.0		3.2
20:2	1.9	1.9		1.1		3.7
20:3		0.3		0.6		0.8
20:4(n-6)	6.4	6.1		3.1	5.1	1.2
20:4(n-3)			0.7	0.3		0.7
20:5(n-3)	2.7	1.0		2.2		0.9
21:5				0.5		
22:0		0.7			2.8	
22:1						0.4
22:2 NMID	1.9	18.9	1.1	22.2	1.1	6.7
22:4	2.2	0.3		1.2		0.5
22:5(n-6)	0.5			0.5		0.8
22:5(n-3)	2.7			0.6		0.5
22:6(n-3)	2.4			1.8		0.7
24:1	2.2	8.8	6.5	2.4	4.5	1.0

Table 6.12 The Percentage Composition of Fatty Acids of Lipid Classes Extracted from the Gills of Unstarved Lucinoma borealis, Collected from Hill Bay, Salcombe, Devon.

Fatty acid	PC	PS	PI/CL	PE	PE(1)	PE(2)	Unknown	Control
14:0	1.1	1.2	1.1	1.3	0.8	1.7	1.7	2.1
14:1	0.6	1.2	0.5	0.6	0.4	0.6	0.5	
i-15:0	0.3		0.4	0.3	0.6	0.7		0.7
a-15:0	0.6	1.1	0.6	0.7	0.5	0.9	0.6	0.6
15:0	1.3	1.3	1.1	1.0	1.1	0.9	3.0	3.2
15:1	1.0	1.7	0.9	1.6	0.6	1.4	1.1	1.6
i-16:0	1.5	1.3	1.1	1.4	0.7	1.7	1.2	3.6
16:0	13.7	16.2	17.3	10.0	17.4	7.4	15.3	20.2
16:1(n-7)	14.0	11.9	10.1	10.7	26.5	7.7	12.6	13.6
16:2	0.2	1.1		0.6			10.5	1.1
16:3	0.9	1.5		1.3	0.7		2.4	
16:4				0.6			0.9	1.9
i-17:0	1.9	1.3	0.7	1.3	0.6	0.9	1.9	1.6
a-17:0	2.0	1.1	0.4	1.2	0.6	0.7	1.9	1.5
17:0	3.1	2.6	3.1	1.7	1.3	3.3	2.6	2.0
17:1	1.9	1.3	2.7	3.7	1.8	2.6		7.4
i-18:0	0.8	1.9		0.9		2.9	0.8	1.1
18:0	10.6	12.9	15.8	7.8	5.6	12.6	9.9	9.6
18:1(n-9)	3.3	3.1	5.5	5.4	2.4	3.8	5.5	7.9
18:1(n-7)	13.3	6.5	11.6	9.4	15.8	3.9	6.4	5.6
18:2(n-6)	0.7		2.5	0.7	0.4	0.8	2.8	0.7
18:3(n-6)	1.3			3.7	0.7			1.4
18:3(n-3)			1.3	0.9		0.8	1.0	1.4
18:4			0.9	2.4				
19:0					0.6			
19:1	0.9					0.9		
20:0			0.7	0.7			0.6	
20:1(n-11)	3.0		0.9	0.8	0.9	0.9	0.5	1.1
20:1(n-9)	0.3	1.3	0.8	1.5	0.4		0.6	1.4
20:1(n-7)	1.8	1.0	2.6	1.2	0.5	0.6	1.1	1.7
20:2			0.9	0.5	0.9	0.9	0.4	
20:3	0.8		0.8	0.7	1.8	1.8	0.5	
20:4(n-6)	1.8		1.2	0.5	0.6	0.7	0.4	
20:4(n-3)				0.6		0.9	0.6	
20:5(n-3)	0.6		0.6	0.8	0.7	1.9		1.0
21:5		4.0	0.7		1.3	4.6		
22:0							0.6	0.8
22:1				0.9				
22:2 NMID	0.6	13.9	2.7	2.1	12.6	29.7	9.7	3.5
22:1	0.5							
22:6								
22:5(n-6)				0.6				
22:5(n-3)	0.4		0.5	0.5				
22:6(n-3)	0.3			0.9				0.6
23:0							1.2	
24:0		3.2		1.0	1.6	0.8	0.6	
24:1	1.6		0.6	0.9			0.6	

Table 6.13 The Percentage Composition of Fatty Acids of Total Lipid Extracted from the Tissues of Lucinoma borealis Offered the algae, Ochromonas danica.

Fatty acid	Animal 1 Gill	Animal 1 Rest	Animal 2 Gill	Animal 2 Rest	Animal 3 Gill	Animal 3 Rest
14:0	0.9	0.7	1.0	0.7	1.9	1.2
14:1			0.5	0.3	0.9	
i-15:0				0.2	0.3	1.2
a-15:0			0.3	0.2	1.2	
15:0	1.2	0.8	1.3	0.8	0.8	1.2
15:1	0.5		1.2	0.4	0.6	0.9
i-16:0	0.9	0.7	0.9	0.6	0.5	1.0
16:0	12.6	21.5	10.6	14.5	16.5	18.0
16:1(n-7)	15.7	33.4	16.5	29.6	15.7	39.8
16:2	2.1	0.7	0.5	0.4	1.8	1.2
16:3		0.4	0.3	0.6	1.3	
16:4	1.6	0.4	0.7	0.3	0.6	
i-17:0	0.8	0.6	0.6	0.8	0.6	0.4
a-17:0		0.8	0.8	0.7	0.4	0.5
17:0	2.3	0.7	1.8	0.9	2.1	0.7
17:1	3.0		6.4	0.8	0.5	
18:0	7.7	7.0	6.6	6.3	9.8	2.2
18:1(n-9)	2.2	1.3	2.9	1.6	6.3	2.7
18:1(n-7)	12.8	7.2	9.1	8.7	5.4	8.0
18:2(n-6)	2.3	2.3	3.2	2.2	2.8	1.9
18:3(n-6)	0.6		0.5	0.3		
18:3(n-3)	1.1	0.9	1.2	0.9	0.3	0.7
19:0				0.3	0.3	
19:1				0.6		
20:0	0.6	0.7	0.4	0.4	0.7	
20:1(n-11)	1.1	0.4	1.2	0.9	0.9	
20:1(n-9)	0.6	2.5	0.6	1.8	1.2	2.1
20:1(n-7)	1.8	2.9	1.7	4.0	1.9	3.0
20:2	0.6	1.0	0.6	1.1	0.9	
20:3	0.8	0.4	0.8	0.7	1.0	
20:4(n-6)	5.6	2.7	4.7	3.5	3.3	3.2
20:4(n-3)			0.4	0.2		
20:5(n-3)	1.2	1.1	1.3	1.4	0.7	
21:5			0.4	0.3	0.2	
22:0	0.6		0.6	0.3	1.0	
22:1				0.3	0.5	
22:2 NMID	13.9	6.3	17.2	9.5	13.1	8.2
22:3	0.8	0.5	0.9	0.7	0.8	
22:5(n-6)				0.1	0.3	
22:5(n-3)	0.7	0.4	0.7	0.8	0.4	
22:6(n-3)	0.9	0.5	0.8	0.7	0.6	
24:1	2.5	1.1	0.9	0.6	1.9	1.8

6.4 Discussion.

6.4.1 Discussion of Initial Incorporation Experiment Involving the Omission and Addition of Sulphide and Sediment.

The purpose of this initial experiment was to test whether it was feasible to use radiolabelled carbon isotope uptake within lipid classes to study carbon flow within Lucinoma borealis. Although it was not possible to observe statistically significant trends between the the various treatments, this experiment did show that not only is the technique feasible but that it is also possible to ascertain the main lipid classes into which radioactive incorporation occurs. Within the gills of Lucinoma, when incorporation did occur, it was generally into CL, PG , PE and to a lesser extent the neutral lipids. The former three classes are major constituents of the bacterial symbionts (see Chapter 2) and hence it can be suggested that the symbiotic bacteria do indeed incorporate radiolabelled bicarbonate into the lipids. It is not surprising that the control animals also incorporate bicarbonate if one considers the findings of Hammen and Osborne (1959) that several symbiont-free marine invertebrates have the ability to fix CO_2 via more conventional biochemical pathways (see section 6.1).

Although there seems to be a general trend that sulphide causes a stimulatory effect and sediment seems to have little effect on incorporation, it is not possible to make any conclusions from this experiment due to the lack of significant differences between treatments. It seems that statistical differences could not be found due to the small sample size (Zar, 1984). It is interesting to note

the great variation in carbon incorporation between individual animals within treatments. This could be accounted for either by some of the Lucinoma not being properly acclimated or too short an incubation time or, more likely, to inter-animal variation. It is striking that some Lucinoma should incorporate large amounts of ^{14}C while others incorporate virtually none. This demonstrates the great capacity for variability within this species, as already encountered from fatty acid compositional data.

The fact that the non-gill tissue also incorporated ^{14}C is consistent with there being a transfer of carbon products from the gill symbionts to the rest of the body. The level of incorporation was generally smaller within the non-gill tissue suggesting that carbon transfer was still taking place or that transfer had not had time to occur fully. It therefore is important to consider incorporation over a time-scale.

6.4.2 Incorporation of ^{14}C Into the Lipid Classes of Lucinoma borealis Over a Time Scale and with the Addition of Antibiotics and Algae.

In this experiment it was possible to separate, within the gills, PE attributable to the symbionts, namely PE(1) and that attributable to Lucinoma, namely PE(2) (see section 6.4.4 for discussion of this point). The high labelling of PE(1) is consistent with the bulk of the incorporation taking place in the symbionts. Equally, the fact that there was some incorporation into PE(2) suggests that there is a

transfer of carbon products from the symbionts to the gill tissue per se of Lucinoma.

It is clear from this experiment that incorporation of ^{14}C increases with the time of incubation, the peak being reached within the gills after 72h, as shown in Figure 6.4.1. Incorporation in the gills is mirrored by a near linear increase in incorporated ^{14}C within the main classes of the non-gill tissue (i.e. PC, PG and PE(2)) with the latter lagging behind the former. This is consistent with a cumulative transfer of ^{14}C compounds from the gill symbionts to the rest of the tissues. However, these results shed no light on whether the symbionts are digested whole or whether it is only their digestion products that are utilised by Lucinoma.

When sulphide was omitted from the incubation medium there was only a slight but statistically insignificant drop in incorporation, suggesting that the symbionts of Lucinoma may be able to utilise an energy reserve during times of sulphide absence from their environment. This energy reserve may take the form of elemental sulphur which has been frequently observed within the gills of symbiont-containing animals (Dando et al., 1985).

It was decided to add antibiotics to try to inhibit the symbiotic bacteria, in the expectation that bacterial fixation would cease and that any incorporation left would be due to fixation by Lucinoma itself. It was, therefore, surprising to find that 24h incubation with antibiotics made no difference to the level of incorporation within the gill, while after 96h there was a large increase in the amount of

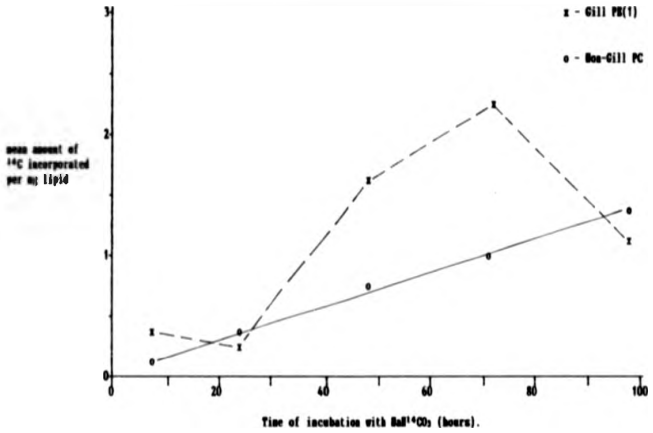


Figure 6.4.1 The mean amount of ^{14}C incorporated into two lipid classes, representative of gill and non-gill lipids extracted from *Lepidonta borealis* following incubation with $NaH^{14}CO_3$ for up to 96 hours (standard deviation bars have been omitted for clarity).

^{14}C incorporated into the main gill lipid classes. This large increase in incorporation, however, did not materialise within the non-gill tissue and, although there were no significant differences, there was a tendency for a lower level of incorporation into the non-gill tissue after 96h incubation with antibiotics than that encountered after 96h without antibiotics. It would seem, therefore, that adding antibiotics stimulates incorporation within the gill and causes a slight inhibition within the non-gill tissue.

Figure 6.4.2 attempts to provide an explanation for these findings. Here the overall symbiotic process is broken into three distinct stages, firstly the fixation of CO_2, secondly the assimilation and utilisation of the fixed carbon by the bacteria, then finally the export of carbon from the symbiont to the host. The antibiotics used in this case were a penicillin/streptomycin mix. Penicillin is specific against gram positive bacteria and since the symbionts seem to be gram negative (Southward, 1987) then it is unlikely that they would be affected by this antibiotic. Streptomycin does not kill bacteria but instead inhibits the production of messenger ribonucleic acid (mRNA) which is necessary to direct protein synthesis. Carbon fixation within the symbionts is an enzymatic process, so that it must follow that the addition of streptomycin will prevent carbon-fixing enzyme activity, at least in the long term. Since ^{14}C still accumulates within the gills of *Lucinoma* after the addition of streptomycin then there are two possibilities. The first is that carbon-fixing enzymes are produced by the animal and utilised by symbionts. This seems very unlikely. The more plausible explanation is that the symbionts are using enzymes synthesized prior to the

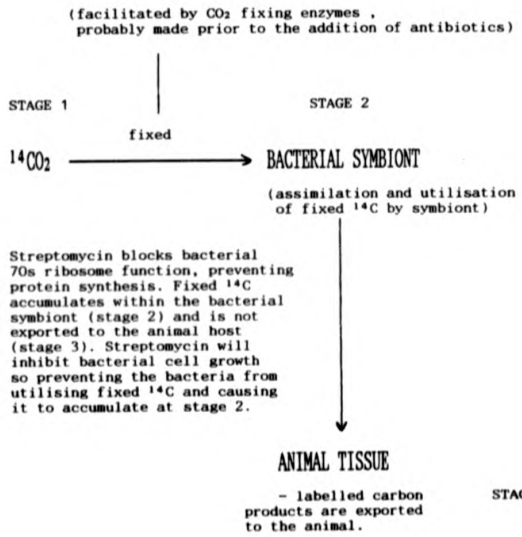


Figure 6.4.2 Diagramatic Representation of the Effect of Antibiotics Upon the Relationship between Lucinoma borealis and its Symbionts.

addition of antibiotics. Since there was no increase in incorporated ^{14}C within the non-gill tissues, then it is evident that Lucinoma is unable to utilise the store of fixed carbon within the symbionts in the presence of streptomycin, i.e. the antibiotic has blocked the transfer of fixed carbon from the symbiont to the animal. One implication of this interpretation is that Lucinoma do not engulf their symbionts whole in order to obtain their nutrition, otherwise we would expect to see a large amount of incorporated carbon within the non-gill tissue. The carbon incorporated within the non-gill lipid classes could either be due to fixation by the animal, carbon being translocated before the antibiotics took effect, or as the result of the Lucinoma "harvesting" some of its symbionts in an attempt to obtain nutrition. If this hypothesis is correct then it must demonstrate a highly evolved form of symbiotic relationship between Lucinoma and its symbionts in relation to the transfer of nutrients from the latter to the former.

When algae were offered to Lucinoma there was virtually no incorporation of ^{14}C. This could be accountable for in three ways. Firstly it could be that all the ^{14}C had been used by the algae leaving none for symbiotic fixation. This seems unlikely since a water sample taken at the end of the experiment yielded 40 000 cpm in 10µl of water. A second possibility is that bacterial fixation is "switched off" in favour of an algal diet. The incorporation of ^{14}C into the algae would probably be low on an individual cell basis and hence would be low within algae ingested by Lucinoma. The final possibility is that instead of switching to an algal based diet, the addition of algae has in some way created unfavourable environmental conditions

for Lucinoma and/or its symbionts, causing carbon fixation to temporarily shut down in the symbionts. This final hypothesis is strengthened when one considers the fatty acid analyses of Lucinoma that have been offered algae (Table 6.13). The general lack of algal type fatty acids such as 20:5(n-3) strongly suggests that Lucinoma were not feeding upon algae to any great extent. Rather, the fact that they retain a fatty acid composition similar to those Lucinoma encountered in chapter 3, may suggest that the symbiotic relationship is still working, possibly running off reserve compounds stored within the symbionts instead of freshly fixed carbon. The small amount of ^{14}C incorporated into Lucinoma offered algae was not significantly different from that observed within the symbiont-free control animals and it can, therefore, be suggested that this was due to residual CO_2 fixation by Lucinoma.

The Lucinoma that had been starved for 140 days ("aged" Lucinoma) had an incorporation level that was generally between the levels encountered with the control animals and the "fresh" Lucinoma. implying that even after nearly 4 months starvation the symbiotic relationship still functioned, albeit at reduced capacity. This shows a great capacity for Lucinoma (and its symbionts) to be able to survive adverse conditions and also favours the possibility that Lucinoma may be able to adapt to a state of basal metabolic activity or even semi-hibernation in order to survive long-periods of adverse environmental conditions.

6.4.3 Incorporation of ^{14}C after incubation with thiosulphate, varying sulphide concentration and varying ambient temperature.

It had been suggested by Dando (pers. comm.) that thiosulphate may in fact be a major energy source for the symbionts of Lucinoma borealis. It was decided to test this idea by incubating the animals with sodium thiosulphate over a time-course of up to 96h. The fact that there was only a slight and generally statistically insignificant increase in ^{14}C incorporation over the time-scale suggests either that reserve compounds are utilised to drive carbon fixation, similar to the situation in the previous experiment where sulphide was omitted, or that varying the time of incubation with thiosulphate makes little difference to the incorporation of ^{14}C. Levels of incorporation found with thiosulphate were not significantly different from those observed with the same concentration of sulphide at the same time point in both gill and non-gill tissue. It remains feasible, therefore, that in terms of driving sulphur based symbiotic relationships, thiosulphate is as good an energy source as sulphide and may act more quickly than sulphide in terms of driving carbon fixation.

It appears that increasing the concentration of sulphide may have a slightly positive, although statistically insignificant effect on incorporation within gill and non-gill lipid classes. There was certainly no inhibitory effect at high concentrations as might be expected. Lucinoma is, therefore, either able to survive high ambient sulphide concentrations by some form of sulphide detoxification system, or more likely by its symbionts acting to oxidise any excess sulphide which could be stored in the form of elemental sulphur.

The fact that there was no difference in incorporation of ^{14}C between Lucinoma collected at Dunstaffnage Bay, near Oban, and those taken at Mill Bay, Salcombe, Devon, suggests that the mechanism for carbon fixation is fairly ubiquitous and is not affected by geographical origin. It is clear, however, from the animals incubated at different temperatures that temperature is an important factor in influencing incorporation of ^{14}C and again underlines the importance of environmental regime in affecting the symbiotic process. At 0°C and 10°C (the temperature at which all the other treatments were carried out) there seemed to be relatively little difference. However, the marked increase in ^{14}C incorporation at 20°C was probably largely due to metabolic adaptation. This illustrates a capacity for Lucinoma and its symbionts to survive elevated temperatures. It is not clear however, how long the symbiotic relationship would be able to function at elevated temperatures, as it is possible it would "burn out" after an extended period of greatly increased metabolic activity. The fact that ^{14}C incorporation was greatly increased in the non-gill tissues by elevated temperatures shows not only the transfer of carbon from the symbionts to the host tissues, but also demonstrates the closeness of the metabolic coupling that operates between Lucinoma and its symbionts.

6.4.4 Fatty acid analyses of gill lipid classes of starved and unstarved Lucinoma borealis.

The unstarved Lucinoma are probably of greater interest since better lipid class separation was achieved in this case. It can be clearly seen that PE(1) is largely bacterially derived due to the

preponderance of 16:1(n-7) and 18:1(n-7), further confirming the finding of the incorporation experiments. The largely animal lipid class PC, also contains a large proportion of 18:1(n-7) and it is thought that this reflects the transfer of fatty acids from bacteria to animal. PE(2) was thought to be largely animal-derived and this is verified by the low proportion of bacterial type fatty acids contained therein. It is interesting, however, to note the large proportion of 22:2 NMID within this class which seems to be related to the animal although it is probably formed as a result of processing bacterial lipids (see chapter 4 for details). The NMID found within PE(1) was most likely due to contamination caused by incomplete separation from PE(2). It should be recalled that it was predicted in section 4.4.2 that the absence of PUFA from the symbiotic bacteria, may lead to the formation of NMIDs from (n-7) fatty acids by the animal, in response to a relative deficiency of dietary EFAs. The relative lack of PUFA within the lipid classes associated with Lucinoma, ie. PC, PS, PE(2) and the neutrals, or in the bacterial classes, combined with the fact that bacteria do not possess a delta-5 desaturase, suggests that it is highly unlikely that the symbionts synthesise NMIDs, that is, these fatty acis are animal-derived. This is virtually akin to the animal exploiting prokaryotes as a source of PUFA, albeit non-conventional PUFA.

Although the separation of lipid classes was not so complete within the starved Lucinoma (Table 6.11) some interesting comparisons to the unstarved animals may be drawn. Bacterial type fatty acids are generally present in lower proportions than in the unstarved animals, suggesting that either the bacterial population has died off, or that Lucinoma has engulfed some of its symbionts, or simply that particular

fatty acids have been catabolised.

6.4.5 Conclusions.

(a) measuring ^{14}C incorporation into lipid classes is a useful method for examining symbiotic relationships;

(b) uptake of ^{14}C highly variable within individual animals;

(c) time-course measurements of incorporation into gill and non-gill tissues is consistent with a transfer of carbon from the gills to the non-gill tissue;

(d) the peak of incorporation into the gills occurs after about 72h;

(e) Lucinoma and its symbionts may have substantial energy reserves, from results when sulphide was omitted and from using animals starved for 140 days;

(f) the addition antibiotics appears to cause an accumulation of fixed carbon in the symbionts;

(g) although Lucinoma do not seem to feed on algae under the conditions used, the addition of algae causes the shut down of symbiotic activity;

(h) thiosulphate may provide an energy source for the maintenance of the symbiosis;

(i) Lucinoma has a high sulphide tolerance as it is possible to add at least 250µM Na_2S without causing any deleterious effect in fixation of $^{14}CO_2$;

(j) geographical location seems to have no bearing upon symbiont activity;

(k) ambient temperature is an important factor affecting symbiotic activity;

(l) fatty acid analyses suggests the idea that class PE(1) is largely bacterially derived and that class PE(2) is largely animal derived. The animal-derived PE(2) is rich in NMIDS.

7.

Overall Discussion and the Implications for Future Work.

The results of this study clearly established the value of using lipids and fatty acids as biomarkers in marine symbiotic systems. From the fatty acid composition of a given marine invertebrate, it is possible to infer whether or not it contains symbiotic bacteria or if the invertebrate utilises a mixotrophic mode of nutrition. Isolated symbiotic bacteria were shown to be rich in 16:1(n-7) and 18:1(n-7) and totally devoid of polyunsaturated fatty acids (PUFA). Typically, a symbiont-containing marine invertebrate, which gains a substantial portion of its nutrition from the symbionts, was rich in 16:1(n-7), 18:1(n-7) and non-methylene interrupted dienoic fatty acids (NMIDs) and is depleted in conventional PUFA. Furthermore, the presence of such a fatty acid profile in the non-gill symbiont-free tissue demonstrates the transfer of symbiont production to the host animal. Excellent examples of such animals are the specimens of Lucinoma borealis in Table 4.6. None of the symbiont-containing species contained large proportions of 20:5(n-3) or 22:6(n-3), although some species, especially Thyasira sarsi (e.g. Table 5.3) , contained substantial proportions of 18:3(n-3) and less so 18:5(n-3), coupled with a relative depletion in 18:1(n-7) and 16:1(n-7). Such animals as the aforementioned, although they evidently contain symbionts, would also seem to ingest phytoplankton and so are mixotrophic in their nutrition.

None of the Thyasirid family contained NMIDs but they did contain increased proportions of PUFA relative to the Lucinids. Therefore, it would seem likely that NMIDs are only formed in animals that are depleted in PUFA and that have a large available source of 18:1(n-7). Klingensmith et al. (1982) found an inverse relationship between 22:2

NMID and levels of the PUFAs 20:5(n-3) and 22:6(n-3) in the symbiont-free clam Mercenaria mercenaria. The aforementioned facts provide further evidence for ideas introduced in Chapter 4 and developed in Chapter 6, that the NMIDs are produced by the host animal from symbiont precursor (n-7) fatty acids, in response to a relative depletion of conventional PUFA.

The foregoing raises two issues, the first of which is whether NMIDs can fulfil at least partially the physiological functions of essential fatty acids or whether they are formally equivalent to the "Mead acid" in mammals, 20:3(n-9), that is formed from 18:1(n-9) as a response to (n-6) essential fatty acid deficiency in an unsuccessful effort to replace arachidonic acid, 20:4(n-6). In the latter case, NMIDs like the Mead acid would be an indicator of essential fatty acid deficiency in the animals studied here. The second and perhaps more important issue is that, if an excess production of 18:1(n-7) together with a relative deficit of (n-3) PUFA causes an essential fatty acid deficiency in marine invertebrates, then what is the normal source of (n-3) PUFA in deep-sea invertebrates with highly evolved symbiosis, particularly those associated with hydrothermal vents ? (n-3) PUFA are currently thought to be essential for brain function and vision and for sperm production in all animals studied so far. It is possible, as is considered in Chapter 4, that (n-3) PUFA in vent animals are obtained from (n-3) PUFA - containing, free-living bacteria present in their immediate environment. An alternative possibility is that the (n-3) PUFA of vent invertebrates are obtained during the free-living larval and early developmental stages of the animals as these stages migrate upwards to meet material sedimenting from the photic zone.

which is known to be rich in (n-3) PUFA. The latter possibility is not incompatible with current views that vent communities have a limited life span of the order of 20-30 years. It is not impossible, therefore, that their life span is limited by their having an eventual requirement to access the photic zone to obtain essential nutrients that are necessary for reproduction in the long term but not necessarily for non-reproductive growth. These considerations are speculative but they do point to the importance of exploring the nutrition of vent communities in much more detail than has been possible in the present study. These communities may yet surprise us with hitherto unsuspected aspects of their metabolism.

The fatty acid biomarker technique was also successfully applied to three geographically distinct methane seep ecosystems to determine the extent of symbiont activity at each site (Chapter 5). In all cases the results illuminated the trophic relationships of the different sites, demonstrating that fatty acid biomarkers show great potential in providing a quick and powerful tool for determining the major trophic processes occurring at a previously unstudied site.

One unknown factor, as yet, is the actual extent of such symbiotic relationships in the marine environment. So far, over 40 symbiont-containing species have been identified (Table 1.1) and it seems certain that many more species will be found to contain symbionts in the future. The fatty acid biomarker technique would provide a fast and effective method for screening large numbers of marine invertebrates for the presence of symbiotic bacteria.

It also remains unclear how exactly the symbiotic relationship has evolved. Many of the symbiont-containing bivalves are prevalent in the fossil record and it is interesting to consider whether they have always contained symbionts or whether this has been a relatively recent development. The results of this study suggest a highly evolved and efficient relationship between the host and its symbionts, so that it is probable that this relationship has evolved over a relatively long period. Whether or not the relationship extends back to the first appearance of marine invertebrates on the planet is a different matter. In the absence of fossil evidence it is impossible to tell how far back such symbioses date. However, it is probable that symbioses started in invertebrates as a loose association that evolved in the direction of increasing intimacy. Once established, natural selection would operate to increase the efficiency of the symbioses.

Adaptation to one highly specialised environment implies a decreased ability to survive in other environments. This idea is emphasised when one considers the fact that the Lucinoma borealis in this study were unable to feed on algae when it was offered, the presence of algae seemingly causing a "switching off" of the symbiotic process. The fact that some species in this study seem to feed on both phytoplankton and on their symbionts, e.g. the Thyasird family, would suggest that these species are still undergoing evolution of their symbioses.

Another question that requires clarification is how can the symbiont-containing animal survive if the symbiont's energy source is removed ? The answer to this question is as yet unclear, although the

results of this study provide some interesting possibilities. The fact that Lucinoma borealis could function with an ambient sulphide concentration of 250μM (Chapter 6) would suggest that this animal has the ability to oxidise excess sulphide to elemental sulphur which can then be stored in the gills as an energy reserve for its symbionts. This deduction is supported by the fact that several authors have found elemental sulphur deposits in the gills of many symbiont-containing bivalves (Southward, 1987). Combined with this, the finding that Lucinoma borealis starved for 140 days were still able to fix significant amounts of ^{14}C-bicarbonate (Chapter 6) implies that the symbiont population was still active. It would seem likely, therefore, that the symbioses was maintained by reserves of elemental sulphur in the gills of the host. This area requires further work using lipid biomarkers to provide clarification.

Although sulphidic conditions occur naturally in marine sediments, it is evident that the increasing burden on the sea over the last century, in terms of pollution, has created large areas of "man-made" sulphidic sediments which are highly toxic to symbiont-free invertebrates. It follows, therefore, that many species have evolved symbiotic relationships to enable them to inhabit areas that would otherwise be inaccessible and, in the process, reducing their competition for space and nutrients.

Because many symbiont-containing species are poor in conventional PUFA, it would seem likely that they are of poor nutritional value to predators, which may confer a further advantage of reducing predation pressure. It would, therefore, be interesting to carry out a

controlled study on the predation of Lucinoma to see if predators selectively avoid them.

Since initiation of this study, the symbiont-containing bivalve, Solemya velum, has been investigated by Conway and McDowell Capuzzo (1990, 1991) and Conway (1990) using the lipid biomarker technique. The results of their study complement the results presented here, these authors finding the fatty acid composition of S. velum to be characterised by large amounts of 18:1(n-7) (up to 35% of the total fatty acid content), 16:0 and 16:1(n-7) and generally low concentrations of PUFA. No NMIDs were found, although up to 14% 20:4(n-6) was observed which suggests that this fatty acid acts as an essential fatty acid (EFA) in S. velum. Zhukova et al. (1992) studied the symbiont-containing Lucinid, Pillucina pisidium, collected from Vostok Bay in the Sea of Japan. This study also complements the results presented within this thesis, these authors finding an abundance of 18:1(n-7) (14.2%) combined with low levels of 20:5(n-3) (3.8%) and 22:6(n-3) (2.2%). It is interesting to note that Zhukova et al. also detected NMIDs (5.6% in total) in the tissues of P. pisidium.

The lipid biomarker technique was successfully extended within this study to show radioactive incorporation into the lipid classes associated with symbiotic bacteria in the gill tissue of Lucinoma borealis. Furthermore, radioactivity in the symbiont-free, non-gill tissue of Lucinoma demonstrated the transfer of symbiont production to the host. It is, therefore, important to separate tissues into symbiont-containing and symbiont-free types in order to demonstrate a transfer of production in terms of lipid biomarkers. This technique

could be easily utilised in the future to suit any symbiont-containing marine invertebrate.

The present work remains inconclusive on whether thiosulphate is an effective energy source for the symbionts of Lucinoma and this area requires further investigation, using the incorporation of radiolabelled carbon into the lipid classes as a tool.

Another important finding of this study was achieving the separation of phosphatidylethanolamine (PE) associated with the bacteria and PE associated with the host, PE(1) and PE(2) respectively (Chapter 6). Measurement of high levels of radioactivity in PE(1) clearly demonstrated that this lipid class is largely bacterial. Fatty acid composition obtained from class analyses do suggest, however, that separation of the two PE types was not totally complete. In order to further develop the use of this class separation in studying the symbiotic relationship, it would be desirable to develop a TLC solvent system that achieves full class separation.

To conclude, it is evident from the results presented within this study that fatty acid biomarkers provide an excellent tool for studying symbiotic relationships and show great potential in screening marine invertebrates for the presence of symbionts. Coupled with this, radioactive incorporation into the lipid classes of symbiont-containing bivalves allows the relationship to be studied in much greater depth. Together, these techniques can be used not only to infer an animal's mode of nutrition, but also to establish whether the nutrition is symbiont-based. They allow deductions to be made

concerning the flow of carbon from the symbiont to the host and also permit investigations of the optimal conditions for perpetuation of the symbiosis, in terms of the energy source and its optimal concentration and the most favourable environmental regime.

REFERENCES

Ackman, R.G. 1988. Marine Biogenic Lipids, Fats, and Oils. Vol. II. CRC Press, Inc. Boca Raton, Florida. 143pp.

Ackman, R.G. and Hooper, S.N. 1973. Non-methylene-interrupted fatty acids in lipids of shallow-water marine invertebrates: a comparison of two molluscs (Littorina littorea and Lunatia triseriata) with the sand shrimp (Crangon septemspinosus). Comp. Biochem. Physiol. B. 46: 153.

Ackman, R.G., Tocher, C.S., McLachlan, J. 1968. Marine phytoplankter fatty acids. J. Fish. Res. Board. Can., 25: 1603-1610.

Ackman, R.G., Epstein, S., Kelleher, M. 1974. A comparison of lipids and fatty acids of the ocean quahuag, Artica islandica, from Nova Scotia and New Brunswick. J. Fish. Res. Board Can. 31: 1803.

Ackman, R.G., Eaton, C.A., Sipos, J.C., Hooper, S.N. and Castell, J.D. 1970. Lipids and fatty acids of two species of north Atlantic Krill (Meganyctiphanes norvegica and Thysanoessa inermis) and their role in the aquatic food web. J. Fish. Res. Board Can. 27: 513-521.

Alayse-Danet, A.M., Gaill, F. and Desbruyeres,D. 1985. Preliminary studies on the relationship between the pompeii worm, Alvinella pompejana (Polychaeta: ampharetidea) and its epibiotic bacteria. In Proceedings of the 19th European Marine Biological Symposium (ed. P.E.Gibbs), pp. 167-172. Cambridge University Press, Cambridge.

Alayse-Danet, A.M., Gaill, F. and Desbruyeres, D. 1986. In situ bicarbonate uptake by bacteria - Alvinella associations. Marine Ecology, Naples, 7: 233-240.

Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., Watson, J.D. 1983. Molecular biology of the cell. Garland Publishing, Inc., New York and London, p 515.

Allen,J. 1958. On the basic form and adaptations to habitat in the Lucinacea (Eulamellibranchia). Phil.Trans.R.Soc. B., 684:421-484

Anderson, A.E., Childress,J.J. and Favuzzi, J.A. 1987. Net uptake of CO_2 driven by sulphide and thiosulphate oxidation in the bacterial symbiont containing clam Solemya reidi. J.Exp. Biol. 133: 1-31.

Arp, A.J. and Childess, J.J. 1981. Blood function in the hydrothermal vent vestimentiferan tube worm. Science, 213: 342-344.

Arp, A.J. and Childress, J.J. 1983. Sulphide binding by the blood of the hydrothermal vent tube worm, Riftia pachyptila. Science, 219: 295-297.

Arp, A.J., Childress, J.J. and Fisher Jr., C.R. 1984. Metabolic and blood gas transport characteristics of the hydrothermal vent bivalve, Calyptogena magnifica. Physiol.Zool., 57: 648- 662.

Arp, A.J., Childress, J.J. and Fisher Jr., C.R. 1985. Blood gas transport in Riftia pachyptila. Bull. Biol. Soc. Wash., 6: 289-300.

Arp, A.J., Childress, J.J. and Vetter, R.D. 1987. The sulphide binding protein in the blood of the vestimentiferan tube worm , Riftia pachyptila, is the extracellular haemoglobin. J.Exp.Biol., 128: 139-158.

Austin, B. 1988. Deep-sea bacteria. In Methods in Aquatic Bacteriology (ed. B. Austin). John Wiley and Sons Ltd.

Ballard, R.D. 1977. Notes on a major oceanographic find. Oceanus, 20: 35-44.

Beach, D.H., Harrington, R.W., Holtz, G.G., Jr. 1970. The polyunsaturated fatty acids of marine and freshwater cryptomonads. J. Protozool., 17: 501.

Bell, M.V. and Sargent, J.R. 1985. Fatty acid analyses of phosphoglycerides from tissues of the clam Chlamys islandica (Muller) and the starfish Ctenodiscus crispatus (Retzius) from

Balsfjorden, Northern Norway. J. Exp. Mar. Biol. Ecol. 87: 31-40.

Belkin, S., Nelson, D.C., Jannasch, H.W. 1986. Symbiotic assimilation of CO_2 in two hydrothermal vent animals, the mussel Bathymodiolus thermophilus and the tube worm Riftia pachyptila. Biol. Bull. Mar. Biol. Lab. Woods Hole. 170: 110-121.

Benninger, P.G., Le Pennec, M. and Salaün, M. 1988. New observation of the gills of Placopecten magellanicus (Mollusca: Bivalvia) and implications for its nutrition. Mar. Biol., 98: 61-70.

Berg, C.J. and Alatalo, P. 1984. Potential of chemosynthesis in molluscan mariculture. Aquaculture, 39: 165-179.

Berg Jr., C.J., Krznowek, J., Alatalo, P. and Wiggin, K. 1985. Sterol and fatty acid composition of the clam Codakis orbicularis, with chemoautotrophic symbionts. Lipids, 20: 116-120.

Blackburn, H.T. 1987. Microbial food webs in sediments. In Microbes in the Sea (ed. M.A. Sleigh). pp39-58. Keynote papers from the 3rd European Microbiology Symposium, Bangor, N. Wales, 14th-18th September 1987. Ellis Horwood Ltd., Chichester.

Bonnet, J.C., Sidwell, V.D., and Zook, E.G. 1974. Chemical and Nutritive values of several fresh and canned finfish, crustaceans and molluscs. II Fatty acid composition. Mar. Fish. Rev., 36 : 8-19.

Bosch, C. and Grasse, P.P. 1984. Cycle partiel des bacteries chimioautotrophiques symbiotiques et leurs ropports avec les bacteriocytes chez Riftia pachyptila Jones (Pogonophore : Vestimentifere). II. L'evolution des bacteries symbiotiques et des bacteriocytes. Compte rendu hedomadaires des seances de L'Academie des Sciences. (Ser.III), 299: 613-619.

Brault, M., Marty, J.C., Saliot, A., Laubier, L. 1985. Biogeochemical markers (hydrocarbons and fatty acids) in sea water surrounding animal communities of the East Pacific Rise, near 13 N. Compte

rendu hedomaires des seances de l'Academie des Sciences (ser. III), 301: 1-8.

Brooks, J.M., Kennicutt II, M.C., Fisher, C.R., Macko, S.A., Cole, K., Childress, J.J., Bidigare, R.R. and Vetter, R.D. 1987. Deep-sea hydrocarbon seep communities: evidence for energy and nutritional carbon sources. Science,238 : 1138-1142.

de Burgh, M.E. and Singla, C.L. 1984. Bacterial colonisation and endocytosis on the gill of a new limpet species from a hydrothermal vent. Mar. Biol., 84: 1-6.

Cary, S.C., Felbeck, H., Holland, N.D. 1989. Observations on the reproductive biology of the hydrothermal vent tube worm Riftia pachyptila. Mar. Ecol. Prog. Ser. 52: 89-94.

Cavanaugh, C.M. 1983. Symbiotic chemoautotrophic bacteria in marine invertebrates from sulphur rich habitats. Nature, London, 302: 58-61.

Cavanaugh, C.M. 1985. Symbioses of chemoautotrophic bacteria and marine invertebrates from hydrothermal vents and reducing sediments. Bull. Biol. Soc. Wash., 6: 373-388.

Cavanaugh, C.M., Gardiner, S.L., Jones, M.L., Jannasch, H.W., Waterbury,J.B. 1981. Prokaryotic cells in the hydrothermal vent tube worm Riftia pachyptila Jones: possible chemoautotrophic symbionts. Science, 213: 340-342.

Cavanaugh, C.M., Levering, P.R., Maki, J.S., Mitchell, R., Lidstrom, M.E. 1987. Symbioses of methylotrophic bacteria and deep sea mussels. Nature, London, 325: 346-348.

Chassard-Bouchaud, C., Fiala-Medioni, P.B., Escaig, F., Kleinbauer, F., Brissard, J. and Galle, T. 1988. Calyptogena phaseoliformis (Mollusque bivalve) indicateur biologique des phenomenes

geochemiques associes aux zone de subduction situee all large du Japon. Compte rendu hedomadaire des sceances de l'academie des Sciences (Ser.III), 306: 237-244

Childress, J.J., Fisher, C.R., Brooks, J.M., Kennicutt, M.C., Bidigare, R. and Anderson, A.E. 1986. A methylotrophic marine molluscan (Bivalvia: Mytilidea) symbiosis : mussels fuelled by gas. Science, 233: 1306-1308.

Christie, W.W. 1982. Lipid Anlysis, 2nd edition. Pergamon Press, Oxford.

Christie, W.W., Brechany, E.Y. and Stefanov, K. 1988. Silver ion high-performance liquid chromatography and gas chromatography - mass spectroscopy in the analysis of complex fatty acid mixtures: application to marine invertebrates. Chemistry and Physics of Lipids, 46: 127-135.

Clarke, A. and Wickins, J.F. 1980. Lipid content and composition of cultured Penaeus merguiensis fed with animal food. Aquaculture, 20: 17-22.

Conway, N. 1990. The nutritional role of endosymbiotic bacteria in animal-bacterial symbioses: Solemya velum, a case study. MIT/WHOI Ph.D. Thesis.

Conway, N. and Mc Dowell Capuzzo, J. 1990. The use of biochemical indicators in the study of trophic interactions in animal- bacteria symbioses: Solemya velum, a case study. In Trophic Relationships in the Marine Environment, Proc. 24th Europ. Mar. Biol. Symp. Eds. M. Barnes and R.N. Gibson, Aberdeen University Press, pp553-564.

Conway, N and McDowell Capuzzo, J. 1991. Incorporation and utilisation of bacterial lipids in the Solemya velum symbiosis. Mar. Biol. 108: 277-291.

Dando, P.R. and Southward, A.J. 1986. Chemoautotrophy in bivalve molluscs of the genus Thyasira. J. Mar. Biol. Ass. UK., 66: 915-929.

Dando, P.R., Southward, A.J., Southward, E.C., Terwilliger, N.B. and Terwilliger, R.C. 1985. Sulphur oxidising bacteria and haemoglobin in gills of the bivalve mollusc Myrtea spinifera. Marine Ecology - Progress Series, 23: 85-98.

Dando, P.R., Southward, A.J. and Southward, E.C. 1986a. Chemoautotrophic symbionts in the gills of the bivalve mollusc, Lucinoma borealis and the sediment chemistry of its habitat. Proc. R. Soc. B., 227: 227-247.

Dando, P.R., Southward, A.J., Southward, E.C. and Barrett, E.T. 1986b. Possible energy source for chemoautotrophic prokaryotes, symbiotic with invertebrates from a Norwegian fjord. Ophelia, 26: 135-150.

Dando, P.R., Austen, M.C., Burke Jr., R.A., Kendall, M.A., Kennicutt II, M.C., Judd, A.G., Moore, D.C., O'Hara, S.C.M., Schmaljohann, R., Southward, A.J. 1991. Ecology of a North Sea pockmark with an active methane seep. Mar. Ecol. Prog. Ser. 70: 49-63.

DeLong, E.F. and Yayanos, A.A. Biochemical function and ecological significance of novel bacterial lipids in deep-sea prokaryotes. Applied and Environmental Microbiology, 51(4): 730-737.

Desbruyeres, D., Gaill, F., Laubier, L., Prieur, D. and Rau, G.H. 1983. Unusual nutrition of the "Pompeii worm" Alvinella pompejana (polychaetous annelid) from a hydrothermal vent environment: SEM, TEM, ^{13}C and ^{15}N evidence. Mar. Biol., 75: 201-205.

Dienes, P. 1980. The isotopic composition of reduced organic carbon. In Handbook of environmental isotope chemistry, Vol. 1. The terrestrial environment (eds. P. Fritz and J.C. Fontes), pp.328-406. Elsevier, Amsterdam.

Distel, D.L. and Felbeck, H. 1987. Endosymbiosis in the lucinid clams Lucinoma aequizonata, Lucinoma annulata and Lucina floridana: a re-examination of the functional morphology of the gills as bacteria bearing organs. Mar. Biol., 96: 78-86.

Dowling, N.J., Widdel, F., White, D.C. 1986. Comparison of the phospholipid-linked fatty acid biomarkers of acetate-oxidising sulphate-reducers and other sulphide-forming bacteria. J. Gen. Microbiol., 132: 1815-1825.

Edlund, A., Nichols, P.D., Roffey, R., White, D.C. 1985. Extractable and lipopolysaccharide fatty acid and hydroxy acid profiles from Desulfovibrio species. J. Lipid Res., 26: 982-988.

Falk-Petersen, I.B. and Sargent, J.R. 1982. Reproduction of astroids from Balsfjorden, northern Norway: analysis of lipids in the gonads of Ctenodiscus crispatus, Asterias lincki and Pteraster militaris. Mar. Biol., 69: 291-298.

Falk-Petersen, S., Gatten, R.R., Sargent, J.R. and Hopkins, C.C.E. 1981. Ecological investigations on the zooplankton community in Balsfjorden, northern Norway: seasonal changes in the lipid class composition of Meganyctiphanes norvegica (M. Sars), Thysanoessa raschi (M. Sars) and T. inermis (Kroyer). J. Exp. Mar. Biol. Ecol. 54: 209-224.

Felbeck, H. 1981. Chemoautotrophic potential of the hydrothermal vent tube worm, Riftia pachyptila Jones (Vestimentifera). Science, 213: 336-338.

Felbeck, H. 1985. CO_2 fixation in the hydrothermal vent tube worm Riftia pachyptila Jones. Physiol. Zool., 58: 272-281.

Felbeck, H. and Somero, G.N. 1982. Primary production in deep-sea hydrothermal vent organisms: role of sulphide-oxidising bacteria. Trends Biochem. Sci., 7: 201-204.

Felbeck, H., Childress, J.J. and Somero, G.N. 1981. Calvin-Benson cycle and sulphide oxidation enzymes in animals from sulphide rich habitats. Nature, London, 293: 291-293.

Felbeck, H., Childress, J.J. and Somero, G.N. 1983a. Biochemical interactions between molluscs and their algal and bacterial symbionts. In The Mollusca, Volume 2, Environmental Biochemistry and Physiology (ed. P.W. Hochachka), pp. 351- 358. Academic Press, New York.

Felbeck, H., Liebezeit, G., Dawson, R. and Giere, O. 1983b. CO_2 fixation in the tissues of marine oligochaetes (Phallodrilus leukodermatus and P. planus) containing symbiotic, chemoautotrophic bacteria. Mar. Biol., 75: 187- 191.

Felbeck, H., Powell, M.A., Hand, S.C. and Somero, G.N. 1985. Metabolic adaptations of hydrothermal vent animals. Biol. Soc. Wash. Bull. 6: 261-272.

Fiala-Medioni, A. 1984. Mise en evidence par microscopic electronique a transmission de l'atondance de bacteries symbiotiques dans la brachie de mollusque bivalves de sources hydrothemales profondes. Compte rendu hedomadaire des seances de l'Academie des Sciences (Ser.III), 298: 487- 492.

Fiala-Medioni, A. and Metivier, C. 1986. Ultrastructure of the gill of the hydrothermal vent bivalve Calyptogena magnifica with a discussion of its nutrition. Mar. Biol., 90: 215-222.

Fiala-Medioni, A., Alayse, A.M. and Cahet, G. 1986. Evidence of in situ uptake and incorporation of bicarbonate and amino acids by a hydrothermal vent mussel. J. Exp. Mar. Biol. Ecol., 96: 191-198.

Fisher, C.J. and Childress, J.J. 1986. Translocation of fixed carbon from symbiotic bacteria to host tissues in the gutless bivalve Solemya reidi. Mar. Biol., 93: 59-68.

Fisher, C.R. and Hand, C. 1984. Chemosautotrophic symbionts in the bivalve Lucina floridana from seagrass beds. Biol. Bull., 96: 59-72.

Fisher, C.R., Childress, J.J., Oremland, R.S. and Bidigare, R.R. 1987. The importance of methane and thiosulphate in the metabolism of the bacterial symbionts of 2 deep sea mussels. Mar. Biol., 96: 59-72.

Fisher, C.R., Childress, J.J., Minnich, E. 1989. Autotrophic carbon fixation by the chemoautotrophic symbionts of Riftia pachyptila. Biol. Bull. 177(3) : 372-385.

Flugel, H.J., Langhof, I. 1983. A new hermaphroditic pogonophore from the Skagerrak. Sarsia, 68: 131-138.

Folch, J., Lees, M, Sloane-Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497-509.

Francis, T. et al. 1988. Fatty acids as biological markers for bacterial symbionts in sponges. Lipids, 23: 1139-1145.

Fry, B. and Sherr, E.B. 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Contributions in marine science, 27: 49-63.

Fry, B., Gest, H., Hayes, J.M. 1983. Sulphur isotopic composition of deep-sea hydrothermal vent animals. Nature, London, 306: 51-52.

Fry, B., Anderson, R.K., Entzeroth, L., Bird, J.L. and Parker, J.L. 1984. ^{13}C environment and oceanic food web structure in the north-western Gulf of Mexico. Contributions in Marine Science, 27: 49-63.

Fulco,A.J. 1974. Metabolic alterations of fatty acids. Ann. Rev. Biochem. 43: 215-241.

Fulco, A.J. 1983. Fatty acid metabolism in bacteria. Prog. Lipid Res. 22: 130-160.

Galbraith, L. and Wilkinson, S.G. 1991. Polar lipids and fatty acids of Pseudomonas carvophylli, Pseudomonas gladioli and Pseudomonas pickettii. J. Gen. Microbiol. 137: 197-202.

Galimov, E.M. 1985. The biological fractionation of isotopes. Academic Press, New York.

Giere, O. 1985. Stucture and position of a bacteria and symbionts in the gill filaments of Lucinidae from Bermuda. Zoomorphology, 105: 296-301.

Giere, O. 1989. Meiofauna and microbes - the interactive relations of annelid hosts with their symbiotic bacteria. Proc. Biol. Soc. Wash. 102(1) : 109-115.

Giere, O. and Langheld, C. 1987. Structural organisation, transfer and biological fate of endosymbiotic bacteria in gutless oligochaetes. Mar. Biol., 93: 641-650.

Giere, O., Liebzeit, G. and Dawson, R. 1982. Habitat conditions and distribution pattern of the gutless oligochaete Phallodrilus leukodermatus. Marine Ecology - Progress Series, 8: 291-299.

Gillan, F.T. and Hogg, R.W. 1984. A method for the estimation of bacterial biomass and community structure in mangrove-associated sediments. Journal of Microbiological Methods, 2: 275-293.

Gillan, F.T., Stoilov, I.L., Thompson, J., Hogg, R.W., Wilkinson, C.R. and Djerassi, C. 1988. Fatty acids as biological markers for bacterial symbionts in sponges. Lipids 23(12): 1139-1145.

Goldfine, H. 1972. Comparative aspects of bacterial lipids. Adv. microb. Physiol. 8: 1-58.

Grassle, J.F. 1986. The ecology of deep-sea hydrothermal vent communities. Advances in Marine Biology, 23: 301-362.

Gustafson, R.G. and Reid, R.G.B. 1988. Association of bacteria with larvae of the gutless protobranch bivalve Solemya reidi (Cryptodonta

: Solemyidae). Mar. Biol., 97: 389-401.

Hammen, C.S. and Osborne, P.J. 1959. Carbon dioxide fixation in marine invertebrates: a survey of major phyla. Science, 130 : 1409-1410.

Harrington, G.W., Beach, D.H., Dunhan, J.E., Holz, J.J., Jr. 1970. The polyunsaturated fatty acids of marine dinoflagellates. J. Protozool., 17: 213-222.

Harwood, J.L. and Russel, N.J. 1984. Lipids in plants and microbes. 162pp. George Allen and Unwin, London.

Herrmann,L. 1981. Alien worlds on the ocean floor. Science Digest, April: 52-57, 122.

Herry, A., Diouris, M., LePennec, M. 1989. Chemoautotrophic symbionts and translocation of fixed carbon from bacteria to host tissues in the littoral bivalve Loripres lucinalis (Lucinidae). Mar. Biol. 101: 305-312.

Hessler, R., Lonsdale, P. and Hawkins, J. 1988. Patterns on the ocean floor. New Scientist, 1605: 47-57.

Hovland, M and Judd, A.G. 1988. Seabed pockmarks and seepages. Graham and Trotman Ltd., London.

Huber, R., Kurr, M., Jannasch, H.W. and Stetter, K.O. 1989. A novel group of abyssal methanogenic archaebacteria (Methanopyrus) growing at 110°C. Nature, 342: 833-834.

Jannasch, H.W. 1985. The chemosynthetic support of life and the microbial diversity at the deep-sea hydrothermal vents. Proc. R. Soc. Lond. B., 225: 227-297.

Jannasch, H.W. and Mottl, M.J. Geomicrobiology of deep sea hydrothermal vents. Science, 229: 717-725.

Jannasch, H.W. and Nelson, D.C. 1984. Recent progress in the microbiology of hydrothermal vents. In current perspectives in microbial ecology (eds. M.J. King and C.A. Reddy), pp. 170-176.

American Society for Microbiological Publications, Washington, D.C.

Jannasch, H.W., Nelson, D.C. and Wirsen, C.O. 1989. Massive natural occurence of unusually large bacteria (Beggiatoa sp.) at a hydrothermal deep-sea vent site. Nature, 342: 834-836.

Jannasch, H.W., and Wirsen, C.O. 1985. The biochemical versatility of chemosynthetic bacteria at deep-sea hydrothermal vents. Bull. biol. Soc. Wash., 6: 325-334.

Johns, R.B. and Perry, G.J. 1977. Lipids of the marine bacterium Flexibacter polymorphus. Arch. Microbiol. 114 : 267-271.

Johnson, K.S., Childress, J.J., Beehler, C.L. 1988a. Short-term variability in the Rose Garden hydrothermal vent field: an unstable deep-sea environment. Deep Sea Research, 35: 1711-1721.

Johnson, K.S., Childress, J.J., Hessler, R.R., Sakamoto-Arnold, C.M., Beehler, C.L. 1988b. Chemical and biological interactions in the Rose Garden hydrothermal vent field, Galapagos spreading centre. Deep Sea Research, 35: 1723-1744.

Jones, M.L. 1981. Riftia pachyptila Jones: observation on the vestimentiferan worms from the Galapagos rift zone. Science, 213: 333-336.

Josefon, A.B. 1985. Distribution of diversity and functional groups of marine benthic infauna in the Skagerrak (eastern North Sea) - can larval availability affect diversity ? Sarsia, 70: 229-249.

Joseph, J.D. 1982. Lipid composition of marine and estuarine invertebrates. Part II: Mollusca. Prog. Lipid Res. 22: 109-153.

Jouin, C. 1978. Description of a free-living Polychaete without a gut: Astomus taenoides n. gen., n.sp. (Protodrillidae, Archiannelida). Can. J. Zool., 57: 2448-2456.

Justin, P. and Kelly, D.P. 1978. Growth kinetics of Thiobacillus denitrificans in anaerobic and aerobic chemostat culture. J. Gen. Microbiol. 107: 123-130.

Kaneda, T. 1977. Fatty acids of the genus Bacillus: an example of branched chain preference. Bacteriological Reviews, 41: 391-418.

Karl, D.M. 1987. Bacterial production at deep-sea hydrothermal vents and cold seeps: evidence for chemosynthetic primary production. In Ecology of Microbial Communities, Symposium of the Society of General Microbiology, Cambridge, 41: 319-360.

Katyama-Fujimura, Y., Tsuzaki, N. and Kuraishi, H. 1982. Ubiquinone, fatty acid and DNA base composition determination as a guide to taxonomy of the genus Thiobacillus. J. Gen. Microbiol., 128: 1599-1611.

Kenk, V.C. and Wilson, B.R. 1985. A new mussel (Bivalvia, Mytillidae) from hydrothermal vents in the Galapagos rift zone. Macalogia, 26: 253-271.

Kennicutt II, M.C., Brooks, J.M., Bidigare, R.R., Fay, R.R., Wade, T.L., McDonald, T.J. 1985. Vent-type taxa in a hydrocarbon seep region on the Louisiana slope. Nature, 317: 351-352.

Klingensmith, J.S. 1982. Distribution of methylene and non-methylene dienoic fatty acids in polar lipids and triacylglycerols of selected tissues of the hardshell clam (Mercenaria mercenaria). Lipids, 17: 976-988.

Kreger,R.D., Nichols, P.D., Antworth, C.P., Sand, W, Bock,E., Cox, J.C., Langworthy, T.A., White, D.C. 1986. Signature fatty acids in the polar lipids of acid producing Thiobacillus sp.: Methoxy, cyclopropyl, alpha-hydroxy-cyclopropyl and branched and normal monoenoic fatty acids. FEMS Microbiological Ecology, 32.

Krzeczkowski, R.A., Tenney, R.D. and Hayes, M.L. 1972. Fatty acid content and proximate analysis of bay, calico, sea and weathervane scallop adductor muscle. J. Food Sci., 37 : 300-311.

Le Pennec, M., and Hily, A. 1984. Anatomie, structure et ultrastructure de la brachie d'un Mytillidae des sites hydrothermaux du Pacifique oriental. Oceanologica Acta, 7: 517-523.

Le Pennec, M., Prieur, D. and Lucas, A. 1985. Studies on the feeding of a hydrothermal vent mytilid from the East Pacific Rise. In Proceedings of the 19th Marine Biology Symposium (ed. P.E. Gibbs). pp. 159-166. Cambridge University Press, Cambridge.

Lesser, M.P. and Blakemore, R.P. 1990. Description of a novel symbiotic bacterium from the brittle star Amphipholis squamata. Applied and Environmental Microbiology, 56(8): 2436-2440.

Lewis, R.W. 1967. Fatty acid composition of some marine animals from various depths. J. Fish. Res. Board Can., 24: 1101-1115.

Li, W.K.W. and Platt, T. 1982. Distribution of carbon among photosynthetic end-products in phytoplankton of the eastern Canadian artic. Journal of Phycology, 18: 466-471.

de Moreno, J., Pollero, A.E., Moreno, V.J., Brenner, R.R. 1980. Lipids and fatty acids of the mussel (Mytilus platensis d'Orbingy) from south Atlantic waters. J. exp. mar. Biol. Ecol. 48: 263-276.

Nichols, P.D., Smith, G.A., Antworth, C.P., Hanson, R.S., White, D.V. 1985. Phospholipid and polysaccharide normal and hydroxy fatty acids as potential signatures for methane-oxidising bacteria. FEMS Microbiological Ecology, 31: 327-335.

O'Brien, J. and Vetter, R.D. 1990. Production of thiosulphate during sulphide oxidation by mitochondria of the symbiont-containing bivalve Solemya reidi. J.exp.Biol. 149: 133-148.

Olsen, R.E. and Henderson, R.J. 1989. The rapid analysis of neutral and polar lipids using double-development HPTLC and scanning densitometry. J. exp. mar. Biol. Ecol. 129: 189-197.

Paradis, M and Ackman, R.G. 1977. Potential for employing the distribution of anomalous non-methylene interrupted dienoic fatty acids in several marine invertebrates as part of food web studies. Lipids 12 : 170-182.

Okuyama, H., Yamada, K., Kameyama, Y., Ikezawa, H., Akamatzu, Y., Nojima, S. 1977. Regulation of membrane lipid synthesis in Escherichia coli after shifts in temperature. Biochem. 16: 2668-2673.

Oliver, J.D. and Colwell, R.R. 1973. Extractable lipids of gram-negative marine bacteria: fatty acid composition. Int. J. Syst. Bacteriol. 23: 442-458.

Parker, P.L., Van Baalen, C, and Maurer, C. 1968. Fatty acids in eleven species of blue-green algae; geochemical significance. Science, 155: 707-719.

Parkes, R.G. and Calder, A.J. 1985. The cellular fatty acids of three strains of Desulfobulbus, a proprionate utilising sulphate-reducing bacterium. FEMS Microbiological Ecology, 31: 361-363.

Paull, L.K., Jull, A.J.T., Toulin, L.J. and Linick, T. 1985. Stable isotope evidence for chemosynthesis in an abyssal seep community. Nature, London, 317: 709-711.

Perry, G.J., Volkman, J.K., Johns, R.B. and Bavor, H.J. 1979. Fatty acids of bacterial origin in contemporary marine sediments. Geochimica et Cosmochimica Acta, 43: 1715-1725.

Powell, M.A. and Somero, G.N. 1986a. Adaptations to sulphide by hydrothermal vent animals: sites at mechanisms of detoxification and metabolism. Biol. Bull., 171: 274-290.

Powell, M.A. and Somero, G.N. 1986b. Hydrogen sulphide oxidation is coupled to oxidative phosphorylation in mitochondria of Solemya reidi. Science 233: 563-566.

Powell, E.N., Crenshaw, M.A. and Reiger, R.M. 1980. Adaptations to sulphide in sulphide system meiofaunna. End products of sulphide detoxification in three turbellarians and a gastrotrich. Marine Ecology - Progress Series, 2: 169-177.

Prieur, D., Chamroux, S., Durand, P., Erauso, G., Fera, Ph., Jeanthon, C., LeBorgne, L., Mevel, G., Vincent, P. 1990. Metabolic diversity in epibiotic microflora associated with the Pompeii worms Alvinella pompejana and A. caudata (Polychaetae: Annelida) from deep-sea hydrothermal vents. Mar. Biol. 106: 361-367.

Prieur, J.E., Nichols, P., White, D.C., Porter, T., Geesey, C.G. 1989. Microscopic examination and fatty acid characterisation of filamentous bacteria colonising substrata around sub-tidal hydrothermal vents. Archives of Microbiology, 152(1) : 64-71.

Ratledge, C. and Wilkinson, S.G. 1988. An overview of microbial lipids. In Microbial Lipids, Volume 1. Academic Press Ltd.

Rau, G.H. 1981. Hydrothermal vent clam and tube worm $^{13}C/^{12}C$: further evidence of non-photosynthetic food sources. Science, 213: 338-340.

Rau, G.H. 1985. $^{13}C/^{12}C$ and $^{15}N/^{14}N$ in hydrothermal vent organisms: ecological and biogeochemical implications. Bull. Biol. Soc. Wash., 6: 243-247.

Rau, G.H. and Hedges, J.I. 1979. Carbon-13 depletion in a hydrothermal vent mussel: suggestion of a chemosynthetic food source. Science, 203: 648-649.

Reid, R.G.B. 1980. Aspects of the biology of a gutless species of Solemya (Bivalvia: Protobranchia). Can. J. Zool., 58: 386-393.

Reid, R.G.B. and Bernard, F.R. 1980. Gutless bivalves. Science, 208: 609-610.

Reid, R.G.B. and Brand, D.G. 1986. Sulphide oxidising symbiosis in lucinaceans: implications for bivalve evolution. Veliger, 29: 3-24.

Report to the council of the Marine Biological Association of the United Kingdom. 1987-1988. Benthic Studies, pp. xvii- xviii.

Ruby, E.G., Jannasch, H.W. and Deuser, W.G. 1987. Fractionation of stable carbon isotopes during chemoautotrophic growth of sulphur oxidising bacteria. Appl. Environ. Microbiol., 53: 1940-1943.

Sargent, J.R. and Falk-Petersen, S. 1981. Ecological investigations on the zooplankton community in Balsfjorden, northern Norway: lipids and fatty acids in Meganyctiphanes norvegica, Thysanoessa raschi and T. inermis during mid-winter. Mar. Biol. 62: 131-137.

Sargent, J.R., Falk-Petersen, I.B. and Calder, A.G. 1983. Fatty acid composition of neutral glycerides from the ovaries of the asteroids Ctenodiscus crispatus, Asterias lincki and Pteraster militaris from Balsfjorden, Northern Norway. Mar. Biol. 72: 257-264.

Sargent, J.R., Bell, J.G., Bell, M.V., Henderson, R.J., Tocher, D.J. 1993. The metabolism of phospholipids and polyunsaturated fatty acids in fish. Symp. 5th Europ. Soc. Comp. Bioche. Physiol. Antibes, October 1991. In Press.

Sargent, J.R., Eilertsen, H.C., Falk-Petersen, S. and Taasen, J.P. 1985. Carbon assimilation and lipid production in phytoplankton in northern Norwegian fjords. Mar. Biol., 85: 109-116.

Sargent, J.R., Parkes, R.J., Muller-Harvey, I, Henderson, R.J. 1987. Lipid biomarkers in marine ecology. In Microbes in the Sea (ed. M.A. Sleigh). pp119-138. Keynote papers from the 3rd European Marine Microbiology Symposium, Bangor, N. Wales, 14th-18th September 1987. Ellis Horwood Ltd., Chichester.

Schmaljohann, R., Faber, E., Whiticar, M.J., Dando, P.R. (1990). Co-existance of methane- and sulphur- based endosymbioses between bacteria and invertebrates at a site in the Skagerrak. Mar. Ecol. Prog. Ser. 61: 119-124.

Schmaljohann, R. and Flugel, H.J. 1987. Methane oxidising bacteria in Pogonophora. Sarsia 72 : 91-98.

Schmaljohann, R. 1991. Oxidation of various potential energy sources by the methanotrophic endosymbionts of Siboglinum poseidoni (Pogonophora). Mar. Ecol. Prog. Ser. 76: 143-148.

Schweimanns, M. and Felbeck, H. 1985. Significnce of the occurrence of chemoautotrophic bacterial endosymbionts in the lucinind clams from Bermuda. Mar. Ecol. - Prog. Ser. 24: 113-120.

Smith, A.E. and Morris, I. 1980. Synthesis of lipid during photosynthesis by phytoplankton of the southern ocean. Science, 207: 197-199.

Southward, A.J. and Southward, E.C. 1980. On the value od dissolved organic matter as food for Siboglinum ekmani and other small pogonophores. J. Mar. Biol. Ass. U.K., 60: 1005-1034.

Southward, A.J., Southward, E.C., Brattegard, T., Bakke, T. 1979. Further experiments on the value of dissolved organic matter as food for Siboglinum fiordicum (Pogonophora). J. Mar. Biol. Ass. U.K., 59: 133-148.

Southward, A.J., Southward, E.C., Dando, P.R., Barrett, R.L., Ling, R. 1986. Chemoautotrophic function of bacterial symbionts in small Pogonophora. J. Mar. Biol. Ass. U.K., 66: 415-437.

Southward, A.J. 1989. Animal communities fuelled by chemosynthesis: Life at hydrothermal vents, cold seeps and in reducing sediments. J. Zool., Lond., 217: 705-709.

Southward, E.C. 1971. Recent researchers on the Pogonophora. Oceanography and Marine Biology, an Annual Review, 9: 193-200.

Southward, E.C. 1982. Bacterial symbionts in Pogonophora. J. Mar. Biol. Ass. U.K., 62: 899-906.

Southward, E.C. 1986. Gill symbionts in thyasirids and other bivalve molluscs. J. Mar. Biol. Ass. U.K., 66: 889-914.

Southward, E.C. 1987. Contribution of symbiotic chemoautotrophy to the nutrition of benthic invertebrates. In Microbes in the Sea (ed. M.A. Sleigh). pp84-118. Keynote papers from the 3rd European Microbiology Symposium, Bangor, N. Wales, 14th-18th September 1987. Ellis Horwood Ltd., Chichester.

Spiro, R, Greenwood, P.B., Southward, A.J., Dando, P.R. 1986. $^{13}C/^{12}C$ ratios in marine invertebrates from reducing sediments: confirmation of nutritional importance of chemoautotrophic endosymbiotic bacteria. Mar. Ecol. - Prog. Ser., 28: 233-240.

Stein, J.L. 1984. Subtidal gastropods consume sulphur-oxidising bacteria: evidence from coastal hydrothermal vents. Science, 233: 696-698.

Stein, J.L., Cary, S.C., Hessler, R.R., Ohta, S, Vetter, R.D., Childress, J.J., Felbeck, H. 1988. Chemoautotrophic symbiosis in a hydrothermal vent gastropod. Biol. Bull., 174: 373-378.

Taghon, G.L. 1988. Phospholipid fatty acid composition of the deep-sea hydrothermal vent polychaete Paralvinella palmiformis (polychaeta-ampharetidae): effects of thermal regime and comparison with two shallow water confamilial species. Comp. Biochem. Physiol., 91B(3): 593-596.

Takagi, T., Eaton, C.A., and Ackman, R.G. 1980. Distribution of fatty acids in lipids of the common Atlantic sea urchin,

Stromylocentrotus droebachiensis. Can. J. Fish. Aquat. Sci. 37: 195-206.

Taylor, J. and Parkes, J. 1983. The cellular fatty acids of the sulphate-reducing bacteria, Desulfobacter sp., Desulfobulbus sp. and Desulfovibrio desulfuricans. J. Gen. Microbiol., 129: 3303-3309.

Taylor, J. and Parkes, J. 1985. Identifying different populations of sulphate-reducing bacteria within marine sediment systems, using fatty acid biomarkers. J. Gen. Microbiol., 131: 631-652.

Terwilliger, R.C., Terwilliger, N.B., Hughes, G.M., Southward, A.J., Southward, E.C. 1987. Studies on the haemoglobins of the small pogonophora. J. Mar. Biol. Ass. U.K., 67: 219-234.

Tornabene, T.G. 1985. Lipid analysis and the relationship to chemotaxonomy. In Methods in Microbiology (ed. G. Gottschalk), 18: 209-234.

Tuttle, J.H. 1985. The role of sulphur-oxidising bacteria at deep-sea hydrothermal vents. Bull. Biol. Soc. Wash., 6: 335-343.

Vetter, R.D. 1985. Elemental sulphur in the gills of three species of clam containing chemoautotrophic bacteria: a possible inorganic energy storage compound. Mar. Biol., 88: 33-42.

Vetter, R.D., Wells, M.E., Kurtsman, A.L., Somero, G.N. 1987. Sulphide detoxification by the hydrothermal vent crab Bythograea thermydon and other decapod crustaceans. Physiol. Zool., 60: 121-137.

Vitiello,F and Zanetta, J.P. 1978. Thin-layer chromatography of phospholipids. J. Chromatogr. 166: 637-640.

Volkman, J.K., Eglinton, G, Corner, E.D.S. 1980. Sterols and fatty acids of the marine diatom Biddulphia sinensis. Phytochemistry, 19: 1809-1813.

Volkman, J.K., Smith, D.L., Eglinton, G, Forsberg, T.E.V., Corner, E.D.S. 1981. Sterol and fatty acid composition of four marine haptophycaean algae. J. Mar. Biol. Ass. U.K., 61: 509-527.

Wada, M., Fukunaga, N., Sasaki, S. 1989. Mechanism of biosynthesis of unsaturated fatty acids in Pseudomonas sp. strain E-3, a psychotrophic bacterium. J. Bacteriol., 171:4267-4271.

Watanabe, T and Ackman, R.G. 1974. Lipids and fatty acids of the American (Crassostrea virginica) and European flat (Ostrea edulis) oysters from a common habitat and after one feeding with Dicrateria inornata or Isochrysis galbana. J. Fish. Res. Board Can., 31: 403-412.

Wittenberg, J. 1985. Oxygen supply to intracellular bacterial symbionts. Bull. Biol. Soc. Wash., 6: 301-310.

Wittenberg, J.B., Morris, R.J., Gibson, Q.H., Jones, M.L. 1981. Haemoglobin kinetics of the Galapagos rift vent tube worm Riftia pachyptila Jones (Pogonophora : Vestimentifera). Science, 213: 344-346.

Wood, A.P. and Kelly, D.P. 1988. Isolation and Physiological characterisation of Thiobacillus aquaesulis sp. nov., a novel facultively autotrophic moderate thermophile. Archives of Microbiology, 149: 339-343.

Wood, A.P. and Kelly, D.P. 1989. Methylotrophic and autotrophic bacteria isolated from lucinind and thyasirid bivalves containing symbiotic bacteria in their gills. J. Mar. Biol. Ass. U.K., 69: 165-179.

Yazawa, K, Araki, K, Watanabe, K, Ishikawa, C., Inoue, A, Kondo, K, Watabe, S., Hashimoto, K. 1988. Eicopentaenoic acid productivity of the bacteria isolated from fish intestines. Nippon Suisan Gakkaishi, 54(10): 1835-1838.

Zar, J.H. 1984. Biostatistical analysis. 2nd ed. Prentice-Hall, Englewood Cliffs, New Jersey.

Zhukova, N.V., Kharlamenko, V.I., Svetashev, V.I., Rodionov, I.A. 1992. Fatty acids as markers of bacterial symbionts of marine bivalve molluscs. J. Exp. Mar. Biol. Ecol., 162: 253-263.